Standard Operating Procedure (SOP) for Inbound Shipments

I. SCOPE AND PURPOSE

The Biospecimen Core Resource (BCR) receives a variety of inbound packages including FedEx Padded Paks, Styrofoam insulated boxes, and liquid nitrogen dry vapor shippers (cryoports). These packages contain an assortment of specimen types, including ambient paraffin blocks and whole blood, to frozen tissues and nucleic acids. This procedure applies to BCR Logistics laboratory personnel, though some steps may include or reference procedures in Clinical Outreach or the Molecular Genetics Laboratory (MGL) for shipment receipt.

Navigation

II. PROCEDURE

A. Safety Precautions

1. Liquid nitrogen (LN_2) and dry ice can cause asphyxiation and severe burns. Always wear the appropriate personal protective equipment (PPE) required when working with these substances, including an impermeable lab coat, goggles, closed toe shoes, nitrile gloves, and thermal insulated gloves. A full face shield is required in lieu of goggles when working with LN_2.
    a. Refer to BPC-SAF-004 “Compressed Gases Hazards and Safety” for additional handling precautions.
2. Assume all samples are infectious. Refer to the following documents for guidance:
    a. BPC-SAF-011 “Microbiological Hazards and Safety”
    b. BPC-SAF-010 “Laboratory Exposure Plan”
    c. NCH IC-VII-1 “Exposure Control Plan for OSHA Blood-borne Pathogen”
3. If disposable items (such as packing material, shipping documents, or dry ice) become contaminated during this process they must be discarded in a biohazard bin.

B. Quality Control

1. All BCR groups are responsible for ensuring that Logistics is made aware of expected incoming shipments whenever possible. Any shipment that is expected but was not received is reported to the Logistics Supervisor and any other responsible individual(s) (e.g., Clinical Outreach Representative). The Logistics Supervisor will work with the relevant people (e.g., Clinical Outreach Representative, shipper, etc.) to resolve shipping problems.

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2. Logistics will be notified when packages have been received in WA1340. Sign and date the BPC Forwarding Materials Log when retrieving packages.
3. For quality control purposes use BCR-FRM-015 "Inbound Shipment Verification and Quality Control Form" to document receipt of all incoming specimen shipments.
4. Several monitors are in place to ensure adequate shipment tracking:
   a. The technician assigned to the shipping rotation tracks expected packages using the courier's website to ensure timely delivery and to correct any issues or delays as quickly as possible. Packages are tracked at the end of the work day in which they are shipped from sites, and throughout the following work day(s) until a confirmation of delivery is received. **Any shipment that is experiencing unexpected delays requires immediate action from Logistics, including involving the Logistics Supervisor. The courier should be contacted for resolution and Clinical Outreach, as well as BCR leadership, should be notified.**
   b. When a package has been received at the BCR, an "expected package" is created in LabVantage (BCR-SOP-A005) to reflect this.
   c. In the Logistics Lab (W230) a whiteboard is used to track outstanding cryoports and is updated daily. Once an expected cryoport has been returned to the BCR, the shipment can be removed from the whiteboard.
5. Sample handling must occur in teams of two, each individual will independently quality control the sample identifier and placement of samples throughout the process. Work with only one sample at a time in order to reduce risk of sample swaps.
6. Specimens must be placed in appropriate temporary storage locations and temperatures to ensure specimen integrity (e.g. specimens received in cryoports are placed in a cryocart until they can be entered into LabVantage).
7. All specimens are labeled with a unique identifier, which is compared to the provided shipping manifest. All sample labels are visually inspected by both individuals to ensure that correspond with the manifest and original specimen label.
8. Any discrepancies found (e.g. between the specimens received and manifest) must be resolved before the specimen can be processed or sent to another area within the BCR.
9. Specimens labeled inappropriately (e.g. containing PHI) must be reported to the Clinical Outreach Representative, as well as documented in an incident report.

C. Inbound Ambient Packages

1. Slides

a. Handle slides with care due to sharp edges and fragile nature.
b. Compare the physical slide label to the identifier(s) on the shipping manifest to ensure there are no discrepancies. Quality control for slide images (received on a hard drive or other digital storage) is completed by Virtual Microscopy (VM) team members. Should an issue arise, such as discrepancies or slide/image quality, then the QCing technician contacts a member of Clinical Outreach. Slides will be held from accessioning and distribution until all issues are resolved.

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c. Complete BCR-FRM-015 with package and specimen information.
d. Accession slides following steps outlined in II. F and perform a Custodial Domain Transfer (CDT) in LabVantage to Virtual Microscopy (VM), and physically distribute the slides.

2. Unstained Slides

a. Create a spreadsheet of all slides received with each slide having a unique row. Group slides from the same TSS together.
b. The spreadsheet headers are:
   a) TSS ID
   b) TSS Alias
   c) Slide Identifier
   d) Parent Tissue Type (Primary Tumor, Metastatic, Normal, etc.)

Table 1: Example spreadsheet and data for unstained slides.

TSS ID	TSS Alias	Slide Identifier	Tissue Type

c. Submit a BCR Informatics JIRA ticket with the completed spreadsheet attached. The ticket title must include "Import of Unstained Slides." BCR Informatics will convert the spreadsheet into an import file, and import the file into LabVantage. Logistics is notified when this import has been completed.
d. Create an expected package for the unstained slides (refer to BCR-SOP-A005).
e. Receive the package and take custody of the slides imported by BCR Informatics.
f. Using the Data Entry tab, QC the slides imported to confirm that all slides received appear in LabVantage.
g. "Approve" all slides.
h. Do not verify the Unstained Slides. Notify Informatics when Approval is complete via email or by submitting a second JIRA ticket. Informatics will add back barcodes that were lost during approval process and notify Logistics when the barcodes are complete.
i. In LabVantage, select all slides to be labeled.
j. Click "Print Label" and select "Histology Label" and "Histology Printer 1". Print one copy of the label.
k. Using the TSS shipping manifest and the barcode data in LabVantage, two technicians must perform QC prior to removing TSS identifiers on physical slides.
l. Using a black permanent marker or razor blade, obscure all TSS identifiers from the physical slide.
m. Apply the BCR Histology slide label to the slide.

3. Formalin-Fixed Paraffin-Embedded (FFPE) Blocks

a. Quality control is performed by a Logistics Technician and a Clinical Outreach Representative by comparing the specimen labels to the information on the

received manifest. Any discrepancies will be noted and addressed by Clinical Outreach.

b. Accession specimens following steps outlined in II.F. Follow the appropriate Logistics Working Documents for specific project guidelines (BCR SharePoint / Projects / <Project folder>.

c. Label the samples, and create a CDT to Histology in LabVantage. Compare the specimens to the CDT with another technician, and distribute FFPE blocks to Histology.

d. If specimens are not ready for distribution, and overnight storage is needed, then place in the Histology refrigerator in W240.

4. FFPE Scrolls

a. Quality Control is performed by a Logistics Technician and a Clinical Outreach Representative by comparing the specimen labels to the information on the received manifest. Any discrepancies will be noted and addressed by Clinical Outreach.

b. Accession specimens following steps outlined in II.F. Follow the appropriate Logistics Working Documents for specific project guidelines (BCR SharePoint / Projects / <Project folder>.

c. After a single FFPE Scroll is accessioned, a portion will have to be created for the sample (if there are multiple tubes for a single patient, then each tube will have to be brought in as a separate portion of the Primary Tumor).
   a.) In LabVantage, select the parent FFPE Scroll and click Add Derivative
   b.) Check the "Mark Consumed" box
   c.) Sample Template: FFPE PORTION / Number of Derivatives: 1 / Sample Type: SCROLLS / Prep Type: SCROLLS / Quantity: 0
   d.) Save and confirm the new scroll portion.

d. If a slide was received with the scroll, then the slide will need to be linked to the portion created in LabVantage.
   a.) Under Admin Samples select the portion and click Slide Assignment
   b.) In the "Top Slide" box select in the drop down menu the LabVantage ID of the appropriate slide.
   c.) If there are 2 slides, then enter the second slide's LabVantage ID into "Bottom Slide".
   d.) On the occasion that more than 2 slides are received, contact the Logistics Supervisor or the Clinical Outreach Coordinator for instructions.
   e.) Click Save.

e. Label the samples, and create a CDT to the Molecular Genetics Lab (MGL). Compare the specimen labels with the CDT with another technician, and distribute scrolls to MGL.

f. If specimens are not ready for distribution, and overnight storage is needed, then they are placed in the Histology refrigerator in W240.

5. Whole Blood

a. Quality control is performed by a Logistics Technician and a Clinical Outreach Representative by comparing the specimen labels to the information on the

received manifest. Any discrepancies will be noted and addressed by Clinical Outreach.

b. Accession specimens following steps outlined in II.F. Follow the appropriate Logistics Working Documents for specific project guidelines (BCR SharePoint / Projects / <Project folder>.
c. Label the samples. If there are multiple tubes with the same anticoagulant and collection time point for a single patient and the tubes are labeled identically, then the order of the tubes (which is the first) is not significant. **Only CDT the first tube (NB1-A) to MGL.** The remaining tubes will be banked using LabVantage into a -80°C freezer in W234.
d. Create a CDT to MGL. With a second technician, compare the specimens with the CDT, and distribute the blood to MGL.
e. If specimens are not ready for distribution, and overnight storage is needed, then they are placed in the Histology refrigerator in W240.

D. Inbound Dry Ice

1. Quality control is performed by a Logistics Technician and a Clinical Outreach or MGL Representative by comparing the specimen labels to the information on the manifest received. Any discrepancies will be noted and addressed by the representative.
2. After quality control is completed, specimens are placed inside a dry ice bin and buried in dry ice for short-term storage, or into a -80°C freezer in W234 if samples will be stored overnight.
3. Accession specimens following steps outlined in II.F. Follow the appropriate Logistics Working Documents for specific project guidelines (BCR SharePoint / Projects / <Project folder>.
4. Label the samples and bank (BCR-SOP-A005) in the appropriate Logistics freezer, or perform a CDT and distribute the samples, if applicable. Frozen samples should be distributed in a 9x9 tissue box, placed inside a biohazard-labeled Styrofoam box completely covered (above and below) in an appropriate amount of dry ice.

E. Inbound Cryoport

1. Logistics will be notified when an inbound cryoport has been delivered to WA1340. A cart should be used to retrieve the cryoport.
2. The temperature of the cryoport upon receipt is recorded on the Cryoport Temperature Log (BCR-FRM-011) inside the cryoport (if returned) and on the Inbound Shipment Verification and Quality Control Form. Note whether the site completed their portion of the temperature log and if the cryoport lid was "locked" correctly with cable ties.
3. Stop the data logger and download the temperature data.
   a. If using a T-TEC Logger™:
      a) Connect the data logger to the computer with the provided cable, and open the T-TEC Logger™ tracking system program. Select Menu under USB-COM:4, then select Stop Logger.

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b) Under Menu select Download Data. Save the file to the Shared Drive (RESQualman G Drive > BCR > BCR Cryoport Data > Year > Month) using the "Recipient, Cryoport #, Date, R" naming format.
c) Once saved, open and view the temperature graph to ensure that an appropriate temperature was maintained.

b. If using a Libero™ Data Logger:
a) Stop the logger by holding the Arrived button until the screen display changes from "Transit" to "Arrived". Remove the logger from the bracket and insert into the USB computer port.
b) Open the pdf to view the temperature graph and ensure that an appropriate temperature was maintained. Save the file to the Shared Drive using the "Recipient, Cryoport #, Date, R" naming format.

4. If discrepancies in temperature occurred during transit (note: not during sample loading/removal at the site), then email the Logistics Supervisor, Program Director, Project Manager, the Clinical Outreach Representative, and/or the MGL Technical Director with an attached pdf of the temperature graph. If the graph displays an extended period of time at which the cryoport remained warmer than -185°C, then the Logistics Supervisor will investigate sample integrity and cryoport functionality and will create an incident report describing the issue.
5. **If the cryoport is empty:**
   a. Ensure that any boxes returned inside of the cryoport are empty.
   b. In LabVantage create, then receive and empty, an expected package (BCR-SOP-A005).
6. **If the cryoport contains frozen specimens:**
   a. Following the initial Logistics QC of samples, Clinical Outreach is emailed a list of the projects and specimen types received.
   b. Inbound sample shipments should include a specimen manifest. A copy of this form and any other project-specific paperwork is given to the Clinical Outreach Representative during the QC process.
   c. Specimens must be verified by the Clinical Outreach Representative and a Logistics technician by comparing the actual specimens received with what is indicated on the manifest.
   d. Once quality control has been completed, specimens are placed into a cryocart (excluding glass collection tubes, which are stored on dry ice) or an appropriate freezer (-80°C or LN_2) until ready to process, per project guidelines.
   e. Accession specimens following steps outlined in II.F. Follow the appropriate Logistics Working Documents for specific project guidelines (BCR SharePoint / Projects / <Project folder>.
   f. Label the samples and bank (BCR-SOP-A005) in the appropriate Logistics freezer, or label and process following BCR-SOP-L020 and Working Document guidelines.
7. **Analytes**

a. Following the initial Logistics QC of samples (number of plates compared to manifest), MGL is emailed when analytes are received and when they have been distributed.
b. Quality control of individual samples/wells is completed by MGL once the analytes have been delivered on dry ice by Logistics.
c. Returned processed analytes (e.g., amplified analytes from Qiagen) are received per SOP L012, "Receiving WGA Plates From Qiagen".
d. In LabVantage, create an expected package (BCR-SOP-A005) reflecting what was received. Change the default content option from Sample to Box on the "Package Content" drop down during package creation.
e. When applicable, boxes in LabVantage that match the physical plates received are placed into the created package; which is then received and custody is taken of the boxes to empty the package. The boxes are then CDT's to MGL to reflect the physical distribution of the analyte plates.

F. Accessioning

1. After performing quality control, the Clinical Outreach representative will work with Clinical Data and IT to import and allocate all specimens. Logistics will be notified via email once allocation has occurred.
2. Reference project specific guidelines found in Logistics Working Documents.
3. For the steps below, refer to BCR-SOP-A005 "LabVantage User Manual" to complete in LabVantage.
4. In LabVantage under Packages & CDTs, add expected packages reflecting what Logistics received.
    a. Description: "Project, Sample Types, Shipping Site, Date (00-00-00)
    b. Shipped & Expected Date (expected date is the date received)
    c. Study ID & Origination ID
    d. Carrier & Tracking Number, **with spaces**
    e. Cryoport Number and Receipt Temperature, or check the No Cryoport box
    f. Click Save
5. For shipments from one site, but with multiple study/origination IDs, create a separate package for each ID.
6. Select the package and use Manage Contents to place the allocated samples into their appropriate package. Receive the package and take custody of the samples.
7. Navigate to Data Entry Samples in LabVantage
    a. Verify the specimen IDs match the manifest.
    b. Check that all appropriate data fields are filled in.
    c. If the sample is DNA, ensure that the "Purified DNA Flag" drop down is set to "Y" (Yes) before approving samples.
    d. Save changes and approve samples.
    e. Ask a second technician to verify your samples in Data Entry.
8. Once verified, the samples are now accessioned and in circulation.

III. REFERENCES

Standard Operating Procedure (SOP) for Inbound Shipments

IV. COMPREHENSIVE REVISION HISTORY

A. Version **1**, Effective Date **8/16/2016**

1. This SOP combines previous SOPs
   a. L013, Top and Diagnostic slides
   b. L021, Accessioning FFPE blocks
   c. L022, Shipping and Receiving Cryoports
   d. L023, Receipt and Distribution of Unstained Slides

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

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Standard Operating Procedure (SOP) for Shipping an Outbound Package

I. SCOPE AND PURPOSE

The Biospecimen Core Resource (BCR) ships a variety of outbound packages, including FedEx Padded Paks, Styrofoam insulated boxes, and liquid nitrogen dry vapor shippers (cryoports). Selecting the most appropriate shipment conditions and packaging is essential to ensure specimen integrity and compliance with regulations. This procedure applies to BCR Logistics laboratory personnel, though some steps may include or reference related procedures in Clinical Outreach, Histology, Virtual Microscopy, and the Molecular Genetics Laboratory (MGL) for shipment preparation.

Navigation (skip to section)

II. PROCEDURE

A. Safety Precautions

1. Liquid Nitrogen (LN_2) and dry ice can cause asphyxiation and severe burns. Always wear the appropriate personal protective equipment (PPE) required when working with these substances, including an impermeable lab coat, goggles, closed toe shoes, nitrile gloves, and thermal insulated gloves. A full face shield is required in lieu of goggles when working with LN_2.
   a. Refer to BPC-SAF-004 “Compressed Gases Hazards and Safety” for additional handling precautions.
2. Assume all samples are infectious. Refer to the following documents for guidance:
   a. BPC-SAF-011 “Microbiological Hazards and Safety”
   b. BPC-SAF-010 “Laboratory Exposure Plan”
   c. NCH IC-VII-1 “Exposure Control Plan for OSHA Blood-borne Pathogen”
3. If disposable items (such as packing material, shipping documents, or dry ice) become contaminated during this process, then they must be discarded in a biohazard bin.

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B. Quality Control

1. For quality control purposes use BCR-FRM-016 "Outbound Shipment Verification and Quality Control Form" to document all outgoing shipments.
2. All BCR personnel responsible for shipping must complete IATA/Saf-T-Pak Compliance Training before they can prepare, create, or handle a shipment. Trained employees must maintain this certification to continue to ship specimens.
3. Shipping addresses should be located using the calendar located on BCR SharePoint. A representative from Clinical Outreach or MGL will confirm this address as correct, or will provide the corrected shipping information. The address will be verified for typographical errors by an additional Logistics Tech, after an airbill has been generated, during the QC process.
4. Several monitors are in place to ensure adequate shipment tracking:
   - **a.** The technician assigned to the shipping rotation is responsible for tracking packages to ensure timely delivery and to correct any issues or delays as quickly as possible. The technician checks the designated courier's website (e.g. FedEx) at the end of the work day in which they are shipped, and throughout the following work day(s) until a confirmation of delivery is received (via email or status on the website). **Any shipment that is experiencing unexpected delays requires immediate action from Logistics, including involving the Logistics Supervisor. The courier should be contacted for resolution and Clinical Outreach, as well as BCR leadership, should be notified.**
   - b. When packages have been delivered to the recipient, they are proxy received in LabVantage (BCR-SOP-A001). A custom data audit tracks packages that have been created or shipped in LabVantage that have not been proxy received. These data audits are sent to the Program Director, Project Manager, Logistics Manager, and Logistics Supervisor.
   - c. In the W230 Logistics Lab, a shipping whiteboard is used to track outstanding cryoports; this is updated daily. If a cryoport is not returned in an appropriate time frame (typically within 7 days), then Clinical Outreach should be notified to contact the site.

C. Ambient Temperature Shipments (e.g. slides, FFPE blocks)

1. Shipping Slides

- a. If the slides to be shipped are not currently in Logistics' custody, then Virtual Microscopy (VM) will be notified. VM will pull the slides from storage and send them to Logistics, accompanied by a Custodial Domain Transfer (CDT) report that reflects this physical transfer in LabVantage (BCR-SOP-A001). Logistics will then verify the slides they received against this report before accepting slide custody in LabVantage.
- b. Generate a shipping manifest from LabVantage (refer to BCR-SOP-A005, LabVantage User Manual).

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i. The manifest should contain the following information: site contact name and address, tracking number (once generated), total number of slides in the package, and the specimen/barcode information for all slides being shipped.
   (a) Note: When applicable, the BCR must act as an honest broker when distributing slides. No TSS identifiers can be present or viewable on slides or manifest distributed from the BCR to a different site.
ii. A copy of this manifest is emailed to the designated Clinical Outreach (CO) representative and shipment confirmation is requested.
iii. Once shipping address confirmation is received, an airbill can be created (see II.C.3. below). The tracking number is added to the shipping manifest, which is then saved on the BCR SharePoint (Shared documents > Logistics > Shipping Logs > Return of Slides) and an updated copy is emailed back to the CO Representative.
iv. A hard copy of the manifest is used by Logistics for QC purposes and will also be included in the outbound package.

c. Create a FedEx airbill (refer to section C.3. below)
d. A second Logistics technician must verify that the physical slides being shipped match those listed on the manifest created in II.C.1.b. Once verified, the technician signs and dates BCR-FRM-016 "Outbound Shipment Verification and QC".
e. Once verified, the glass slides can be placed into an appropriate slide case which is then taped shut and bubble wrapped to ensure safety during transit.
f. If size allows, place the shipping manifest and protected slides into a padded FedEx Pak, then close and seal it following the instructions on the outside of the Pak. Place the airbill pouch on the designated area on the front of the Pak. For larger packages, use an appropriately-sized cardboard box.
g. Complete the following in LabVantage (BCR-SOP-A005):
   i. Receive CDT and take custody of the slides.
   ii. Create a package for the shipment.
   iii. Place virtual slides into the LabVantage package.
   iv. "Ship" the package.
h. Physically take the package to the designated FedEx pick-up location for shipment. Track the package as specified in section II.B.4 above.

2. Shipping Formalin-Fixed Paraffin-Embedded (FFPE) Blocks

a. If the blocks to be shipped are not currently in Logistics' custody then Histology will be notified. Histology will pull the blocks from storage and send them to Logistics, accompanied by a Custodial Domain Transfer (CDT) report that reflects this physical transfer in LabVantage (BCR-SOP-A001). Logistics will then verify that the blocks they received against this report before accepting custody of them in LabVantage.
b. Create a shipping manifest as outlined above in II.C.1.b.
c. Create a FedEx airbill for FFPE blocks (refer to section C.3 below).
d. A second Logistics technician must verify, and initial BCR-FRM-016, that the physical blocks being shipped match those listed on the shipping manifest.

e. Once verified the blocks can be placed into an appropriate container. During warmer months the blocks may be packaged with an ice pack to prevent melting of the paraffin.
f. If size allows, place the shipping manifest and blocks into a padded FedEx Pak, then close and seal it following the instructions on the outside of the Pak. Place the appropriate airbill pouch on the designated area on the front of the Pak. For larger packages use an appropriately sized cardboard box. For shipments requiring use of an ice pack during warmer months, use an appropriately sized insulated box.
g. Complete the following in LabVantage (BCR-SOP-A005):
    i. Receive CDT and take custody of the blocks.
    ii. Create a package for the shipment.
    iii. Place virtual blocks into the LabVantage package.
    iv. "Ship" the package.
h. Physically take the package to the designated FedEx pick-up location for shipment. Track the package as specified in section II.B.4 above.

3. Creating FedEx Airbills for slides or FFPE blocks

a. Log in to the FedEx website. Under the "Ship" header, select "Create a Shipment".
b. In the "To" section, enter the confirmed address and contact information.
c. In the "Package & Shipment Details" section, enter the following:
    i. Service Type: Standard or 2 Day shipping.
    ii. Package Type: Your Packaging or FedEx Pak (as applicable)
    iii. No. of Packages: 1
    iv. Weight: Weight of package (scale located in W230)
    v. Declared value:
        (a) Domestic shipments have a declared value of 0 USD
        (b) International shipments have a declared value of 10 USD
d. In the "Billing Details" section, enter the following:
    i. Bill transportation to: BCR-662
    ii. Your reference: Project, Receiving Site, and Sample Type.
e. In the "E-mail Notifications" section, expand the "Edit" link. Enter an email address for both the shipper and the receiver; then select "Tendered", "Exception" and "Delivery" in the "Notification type" column.
f. In the "Complete your Shipment" section, click "Ship".
g. Print the FedEx generated airbill.
h. For International Shipments:
    i. In the "Commodity Information" section select "Add new commodity". In the drop down menu under "Commodity", enter the following:
        (a) Commodity description
            (i) Slides: "This package contains # glass slides used for pathology. The slides are non-hazardous and are for research/analysis purposes only."
            (ii) Blocks: "This package contains # fixed blocks used for pathology. The samples are non-hazardous and are for research/analysis purposes only."

(b) Unit of measure: pieces
(c) Quantity: 1
(d) Commodity weight: weight (from C.3.c.iv above)
(e) Customs value: 10 USD
(f) Country of manufacture: United States
(g) Shipment weight: same as commodity weight
(h) Total value: 10 USD

ii. Click "Add this commodity".
iii. In the "Customs Documentation" section, enter the following:
(a) Select "Commercial Invoice"
(b) Terms of sale: Free Carrier (FCA/FOB)
iv. In the "Complete your Shipment" section, click "Ship".
v. Print the Commercial Invoices and FedEx generated airbill.
vi. International shipments must also include three copies of the customs declaration letter found on Q-Pulse (BCR-TMP-003).
vii. Sign and place the Commercial Invoice and Customs Letter copies in a re-sealable airbill pouch along with the airbill.

4. Miscellaneous Ambient Packages

a. Follow all IATA/Saf-T-Pak guidelines and regulations for shipping.
b. Consult the Logistics Supervisor regarding packaging types and shipping rates.

D. Shipping on Dry Ice

1. Project guidelines (e.g. proteomics) and/or sample types (e.g. glass collection tubes) may require specimens to be shipped on dry ice in lieu of a cryoport. International dry ice shipments are not recommended. Contact the Logistics Supervisor for guidance if a dry ice package must be shipped outside the United States.
2. If the samples to be shipped are not currently in Logistics' custody then the department with custody will be notified. They will pull the samples from storage and send them to Logistics, accompanied by a Custodial Domain Transfer (CDT) report that reflects this physical transfer in LabVantage (BCR-SOP-A001). Logistics will then verify the samples they received against this report before accepting sample custody in LabVantage.
3. Generate a shipping manifest from LabVantage (refer to BCR-SOP-A005, LabVantage User Manual).
   a. The manifest should contain the following information: destination contact name and address, tracking number (once generated), total number of samples in the package, and the specimen/barcode information for all samples being shipped.
   b. A copy of this manifest is emailed to the designated CO Representative (and MGL representative if applicable) and shipment confirmation is requested.
   c. Once shipping address confirmation is received, an airbill can be created and the newly generated tracking number added to the manifest. The updated manifest is then saved on the BCR SharePoint (Shared documents > Logistics >Return Manifests) and a copy is emailed back to the Clinical Outreach representative.

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   d. A hard copy of the manifest is used by Logistics for QC purposes and will also be included in the outbound package.
4. A second Logistics technician must verify, and initial BCR-FRM-016 "Outbound Shipment Verification and QC", that the physical samples being shipped match those listed on the shipping manifest.
5. Place sample(s) in a Saf-T-Pak Biohazard bag with an absorbent sheet and seal completely. Place the sealed bag inside an insulated foam shipping container, working quickly to avoid thawing the sample(s). Using the shipping station scale, weigh and record the package weight prior to dry ice. Remove the sealed biohazard bag containing the samples and return to the cryocart or dry ice chest.
6. Fill the insulated container with enough dry ice to cover the bottom of the package. Place the sealed biohazard bag on top of this dry ice layer, then fill the rest of the box with dry ice.
   a. **Note: To ensure that samples remain frozen at all times, specimens must be completely buried in enough dry ice to still be covered upon arrival at the destination.
   b. A minimum of 4 pounds of dry ice must be used per package.
7. Weigh the full insulated container and subtract the weight of the insulated container and samples to calculate the weight of dry ice used.
8. **Never use tape to close the inner insulated container; dry ice must be allowed to ventilate as it evaporates to prevent an explosion.**
9. Place the insulated foam container into a secondary shipping cardboard box.
10. Place the shipping manifest in a plastic bag on the outside of the inner insulated foam shipping container, but inside the outer cardboard box.
11. Tape the secondary shipping cardboard box shut.
12. Fill out and attach a Dry Ice UN 1845 label to the package.
   a. **Note: Dry Ice UN 1845 labels require the shipper and recipient address to be durably marked on the package (in addition to the FedEx shipping label). The recipient address should be clearly marked and placed directly next to the dry ice label.
13. Create a FedEx Priority Overnight dry ice shipping label and air bill.
   a. Log in to the FedEx website, www.FedEx.com with assigned user name and password and go to "Create Shipment".
   b. In the "To" section, enter the confirmed address and contact information.
   c. In the "Package & Shipment Details" section, enter the following:
      i. Weight: Total weight of package (including the dry ice)
      ii. Declared value: 0 U.S. Dollars
      iii. Service Type: Priority Overnight
      iv. Package Type: Your Packaging
   d. In the "Billing Details" section, enter the following:
      i. Bill transportation to: BCR-662, FedEx Account # 491048662
      ii. Your reference: Project, Receiving Site, Dry Ice

e. In the "Special Services" section, expand the "Edit" link. Select "Dry Ice" and enter the weight of the dry ice only in the "Total dry ice weight" field.
f. In the "E-mail Notifications" section, expand the "Edit" link. Enter an email address for the shipper and the receiver. For both, select "Tendered", "Exception" and "Delivery" in the "Notification type" column.
g. In the "Complete your Shipment" section, click "Ship".

14. Print the FedEx shipping label and place inside an airbill pouch attached to the package.
15. Complete the following in LabVantage (BCR-SOP-A005):
    a. Create a package for the shipment.
    b. Place virtual samples into the LabVantage package.
    c. "Ship" the package.
16. Physically take the package to the designated FedEx pick-up location for shipment. Track the package as specified in section II.B.4 above.

E. Outbound Cryoport Shipments

1. Clinical Outreach and Molecular should notify Logistics at least 24 hours prior to shipping. The BCR staff member requesting a cryoport is responsible for arranging and confirming the shipping date with the destination institution. For cryoports sent to TSSs, Clinical Outreach is responsible for confirming that the TSS meets all project requirements per BCR-SOP-C005 "Establishing New Tissue Source Sites and Communicating Project Requirements", and that all relevant shipping details are entered into the BCR SharePoint calendar prior to requesting a cryoport from Logistics.
2. **Charging Cryoports**:
    a. Place the cryoport beside the liquid nitrogen source and remove the data logger lid.
        i. If using the W230 fill station:
            (a) Connect the LN_2 Diffusion Stone to the fill station's LN_2 hose.
            (b) Place the stone end of the hose into the bottom of the cryoport and close the cryoport shell over the hose to subdue LN_2 splash.
            (c) Scan your Employee ID to activate the fill station and depress the green button to start the flow of LN_2.
            (d) Periodically lift the shell lid to visually check fill progress. Stop the LN_2 once it has reached the bottom of the cryoport neck and remove the hose.
            (e) Release the red button to stop the flow of LN_2.
        ii. If using a LN_2 dewar (when the fill station is unavailable):
            (a) Cryoports should be filled close to air vents to ensure that excess nitrogen is expediently removed from the area.
            (b) Secure the LN_2 transfer hose to the spout on the dewar labeled "liquid".
            (c) Place the Diffusion Stone end of the hose into the bottom of the cryoport and close the cryoport shell over the hose to subdue LN_2 splash.
            (d) Slowly turn open the liquid valve to begin filling the cryoport.
            (e) Periodically lift the shell lid to visually check fill progress. Stop the LN_2 once it has reached the bottom of the cryoport neck and remove the hose.
            (f) Turn valve in the opposite direction to shut off LN_2 flow.

iii. Replace the data logger lid and label the cryoport shell with initials and the charge date.
iv. Allow the cryoport to properly charge (i.e. allow liquid nitrogen to fully saturate the absorbent material in the outer walls of the cryoport):
(a) If the cryoport is newly filled, the LN_2 must absorb for a minimum of 4 or up to 24 hours. The cryoport must then be refilled and the LN_2 absorbed for an additional 1-3 days.
(b) For all subsequent uses (i.e. when the cryoports is -80°C or colder from a previous use) of the cryoport, the LN_2 must absorb for 1-3 days to fully charge.
(c) Any deviations are noted on BCR-FRM-016 "Outbound Shipment Verification and QC".

3. Charged cryoports are prepared for shipment by pouring off the excess liquid nitrogen into an empty cryocart.
   a. Use the dated label on the cryoport shell to verify that an adequate charge time has elapsed (24 – 72 hours) and remove the data logger lid.
   b. With at least 2 technicians present, position the cryocart near the air vent and remove the lids. CAUTION – The cryocart must be positioned near the air vent during pour off. Do not prepare the cryoports when in close proximity to the O2 monitors as this may cause a centralized pocket of gaseous nitrogen to trigger a false low oxygen level alarm.
   c. Lift the cryoport using the top metal handle on the inner dewar, and position it over the cryocart until the flow of liquid nitrogen stops.
   d. Lower the cryoport to the ground, check for appropriate temperature probe length, and then replace the data logger lid.
4. Cryoports must maintain a temperature of -185°C or below to be shipped from the BCR. A one-hour temperature log is recorded using the data logger lid to confirm that the cryoport is holding temperature and functioning properly.
   a. To use the T-TEC Logger™ on an outbound cryoport:
      i. With the provided cable, connect the data-logger to the computer and open the T-TEC Logger™ tracking system program. Select Menu under USB-COM:4, then select Start Logger.
      ii. Enter: "Recipient, Cryoport #, Date, S" (S is for shipped) on Line 1 of the comments section.
      iii. Set the Logging Interval to 2 minutes for domestic and Canada shipments. Select 5 minutes for all international shipments.
      iv. Wait a minimum of 1 hour.
      v. Reconnect the data-logger to the computer and open T-TEC Logger™ software. Under Menu select Stop Logger.
      vi. Under Menu select Download Data. Save the pdf to the Shared Drive (RESQualman G Drive > BCR > BCR Cryoport Data > Year > Month) using the "Recipient, Cryoport #, Date, S" naming format. Once saved, open and view the temperature graph to ensure that the probe is working properly.

vii. If the cryoport and logger are functioning properly, then restart the logger following the steps above but substituting the "S" for shipped with an "R" for received in the T-TEC™ comments section.

b. To Use the Libero™ Data Logger on an outbound cryoport:

i. Remove the data-logger USB from its bracket and simultaneously press the Transit and Arrived buttons until the screen says "Conf" (configure). Insert the logger into a USB port on the W230 shipping computer and open the Libero™ Configuration Utility program, selecting either the gear icon or Device > Configuration.

ii. In the left hand column, select Description and enter: "Recipient, Cryoport #, Date, S". Enter the same for the .pdf file name. **Note: This field has a character limit; the file name may need to be shortened.

iii. In the left hand column, select Logging and set the interval length to 2 minute for domestic shipments and Canada, and 5 minutes for international shipments. Maintain all other default start options and select "Apply".

iv. Remove the USB and replace the logger in the cryoport bracket. Depress the Transit button on the logger until the temperature displays.

v. After one hour, stop the logger by holding the Arrived button until the screen display changes from "Transit" to "Arrived". Remove the logger from the bracket and insert into the USB computer port.

vi. Open the .pdf to view the temperature graph and ensure that the probe is working properly. Save the file to the Shared Drive using the "Recipient, Cryoport #, Date, S" naming format.

vii. If the cryoport and logger are functioning properly, restart the logger following the steps above but substituting the "S" for shipped with an "R" for received in the Libero™ Configuration Utility "Description" section.

c. If the cryoport is not holding an acceptable temperature and the data logger is the suspected issue, then the one-hour test is repeated using a new logger.

d. If a temperature of -185°C or below was not maintained for at least one hour, then the cryoport CANNOT be used to ship, and a note is entered on the Cryoport and Data Logger Incident spreadsheet on the BCR SharePoint (REX/BPC/TCGA-BCR > Shared Documents > Logistics > Cryoport and Logger Issues). If the cryoport is suspected to be malfunctioning, then it is taken to Biomedical Engineering and another cryoport is selected for the shipment. The one hour temperature test is repeated on the new cryoport.

5. Returning specimens to sites:

a. If not provided by the CO representative, generate a shipping manifest from LabVantage (refer to BCR-SOP-A005, LabVantage User Manual).

i. A copy of this manifest is emailed to the designated Clinical Outreach (CO) representative and shipment confirmation is requested.

ii. Once shipping address confirmation is received, an airbill can be created, and the newly generated tracking number added to the manifest. The updated manifest is saved on the BCR SharePoint (Shared documents > Logistics >

Shipping Logs > Return of Specimens) and a copy is emailed back to the CO representative.

iii. A hard copy of the manifest is used by Logistics for QC purposes and will also be included in the outbound package.

b. Pull specimens from freezer and place in a properly filled cryocart.

c. After the manifest has been confirmed by the CO representative, two technicians must verify that the specimens pulled match what is on the manifest.

d. Place samples into 9x9 specimen boxes and load them into a cryoport rack. Then insert the rack into the prepared outbound cryoport.

6. **Creating FedEx airbills for cryoports**:

a. Domestic Shipments

i. Go to "Ship" > "Create Shipment".

ii. In the "To" section, enter the address and contact information. Verify address and contact information from Clinical Outreach Coordinator before shipping a package (refer to BCR-SOP-C030 Customer Relationship Management (CRM) Software in Clinical Outreach).

iii. In the "Package & Shipment Details" section, enter the following:

(a) Weight: 51 lbs.
(b) Declared value: 2000 U.S. Dollars
(c) Service Type: Priority Overnight
(d) Package Type: Your Packaging

iv. In the "Billing Details" section, enter the following:

(a) Bill transportation to: BCR-662, FedEx Account # 491048662
(b) Your reference: Project, Destination, Outbound Cryoport

v. In the "E-mail Notifications" section, expand the "Edit" link. Enter an email address for the shipper and the receiver. For both, select "Tendered", "Exception" and "Delivery".

vi. In the "Complete your Shipment" section, click "Ship" then print the receipt and airbill.

vii. Create an inbound airbill following the steps outlined above ensuring that the BCR address is used for the destination and your reference: Project, Site, Inbound Cryoport.

b. International Shipments

i. Go to "Ship" > "Create Shipment".

ii. In the "To" section, first select the "Country/Location" to which you are shipping. Then enter the address and contact information. Always verify the address and contact information from Clinical Outreach Coordinator before shipping a package, if applicable (refer to BCR-SOP-C030, "Customer Relationship Management (CRM) Software in Clinical Outreach").

iii. In the "Package & Shipment Details" section, enter the following:

(a) Weight: 51 lbs.
(b) Declared value: $25
(c) Service Type: International Priority

Standard Operating Procedure (SOP) for Shipping an Outbound Package

(d) Package Type: Your Packaging
(e) Package contents: Products/Commodities
(f) Total customs value: $25.00 USD.

iv. In the "Billing Details" section, enter the following:
(a) Bill transportation to: BCR-662, FedEx Account # 491048662
(b) Bill duties/taxes/fees to: BCR-662, FedEx Account # 491048662
(c) Your reference: Project, Destination, Outbound Cryoport

v. In the "E-mail Notifications" section, expand the "Edit" link. Enter an email address for the shipper and the recipient. For both, select "Tendered", "Exception" and "Delivery" in the "Notification type" column.

vi. Select "Continue Your Shipment".

vii. In the "Commodity Information" section, select "Add new commodity" in the drop down menu, under "Commodity", enter the following:
(a) If the cryoport is empty: This package is an empty dry vapor shipper. The package contains an aluminum dewar inside of a plastic shell.
(b) If samples are being shipped: This is a dry shipper that contains n# sample(s) of n# human exempt samples. These samples are non-infectious and non-hazardous, for research/analysis purposes only
(c) If analyte plates are being shipped: "This is a dry shipper that contains n# plate(s) of n# human genomic DNA/RNA samples. These samples are non-infectious and non-hazardous, for research/analysis purposes only."
(d) Unit of measure: pieces
(e) Quantity: 1
(f) Commodity weight: 51 lbs.
(g) Customs value: $25
(h) Country of manufacture: United States
(i) Shipment weight: 51 lbs.
(j) Total carriage value: Same value entered in steps II. C. 4.f.
(k) Click "Add this commodity".

viii. In the "Customs Documentation" section, enter the following:

ix. Select "Commercial Invoice"

x. Terms of sale: Free Carrier (FCA/FOB)

xi. Click "Ship".

xii. Print receipt, commercial invoice, and airbill. Sign all 3 invoice copies that automatically print for international shipments.

xiii. Complete an international customs letter (SharePoint>Logistics Forms> Shipping Documents> International Customs Letter)
(a) Select ONE of the following on the letter and delete the other.
(i) This package is an empty dry vapor shipper. The package contains an aluminum dewar inside of a plastic shell.
(ii) This is a vapor shipper that has no restrictions or regulations. It contains n# plate(s) of _ human genomic RNA samples. These

samples are non-infectious and non-hazardous, for research/analysis purposes only.

(b) Print and sign 4 copies of the international customs letter on Nationwide Children's Hospital letter head.

(c) Consult with Logistics Supervisor to determine if additional customs documentation is needed.

xiv. International sites must generate their own return airbill to ensure all appropriate customs paperwork is completed. The BCR FedEx account number is provided to these sites for this purpose.

7. **Preparing for Shipment**:
   a. The cryoport lid must be securely labeled with the destination contact name, street address, and telephone number.
   b. The outbound shipping label (and commercial invoice, if applicable) is placed in an airbill pouch attached to an airbill tie-on tag. The tie-on tag must be securely attached to a handle on the outer shell of the cryoport with a zip tie. See Figure 1.
   c. The following items are placed into a 9x12 inch plastic bag:
      i. The return shipping label and tie-on tag for domestic sites; or an empty airbill pouch and tie-on tag for international sites.
      ii. Completed BCR-FRM-011 "Cryoport Temperature Log"
      iii. Return Address Form (with FedEx account # for international sites)
      iv. Exempt Human Specimen sticker if the returning cryoport will contain human specimens.
      v. 8 Cable Ties
      vi. For analyte shipments, also include the 96-well plate sample information form(s) and matrix well plate data form(s) provided by MGL.
   d. Tape the 9x12 bag to the inner lid of the cryoport shell using packing tape.
   e. The 9x9 specimen boxes are placed into a cryoport rack which may be pre-chilled in a cryocart before shipping (recommended to more rapidly achieve the required shipping temperature rebound). The specimen boxes will be shipped empty for sample collections, will contain specimens for sample returns, or will contain matrix well plates for analyte shipments.
   f. The cryoport temperature probe must remain exposed, not retracted into the Styrofoam portion of the lid.
   g. The Libero™ data loggers must be secured to in their bracket with a red zip tie. See Figure 2.
   h. The cryoport shell lid is closed and the locking mechanism fastened with two cable ties, which must be place through the locking mechanism per Figure 3.
8. Complete the following in LabVantage (BCR-SOP-A005):
   a. Create a package for the shipment.
   b. Place virtual samples into the LabVantage package if applicable.
   c. "Ship" the package.
9. Complete the appropriate information on BCR-FRM-016.

a. The tracking numbers are e-mailed to the BCR Logistics group for verification. Once verified they are e-mailed out to the relevant BCR departments.
b. Two Logistics technicians must review and initial that the shipment information is correct. For Molecular analyte shipments the Plate ID section is filled in and reviewed by one Logistics technician and one Molecular technician.

10. Add the following information for all cryoport shipments to the whiteboard before taking an outbound cryoport to the dock: ship date, destination, estimated arrival date, comments, tracking number, and shipper initials.
11. Physically take the cryoport to the designated FedEx pick-up location for shipment. Track the package as specified in section II.B.4 above.

F. Documentation and Shipment Tracking

1. The technician on the shipping rotation will check the FedEx site during the workday as well as at least once daily after shipment to ensure that there are no delays or issues with the shipments.
   a. If a shipment is delayed, then notify the appropriate Clinical Outreach representative and the Logistics Supervisor.
2. Upon confirmation of receipt (via automated email from FedEx or a "Delivered" status on the FedEx website), the package is proxy received in LabVantage (refer to A005) and the whiteboard is updated with the site received date.
3. All outstanding cryoport shipments on the whiteboard are reviewed. If the return shipment is overdue (7 or more days past the original ship date), then notify the appropriate Clinical Outreach representative. Cryoport shipments are removed from the whiteboard only when they have been returned to the BCR.

II. REFERENCES

None

II. COMPREHENSIVE REVISION HISTORY

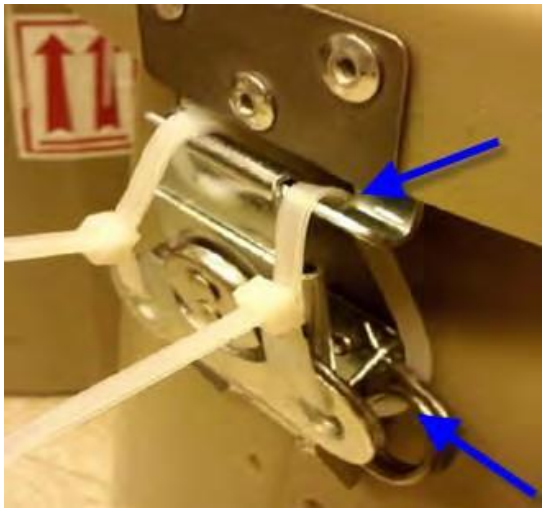
A. Version 1, effective date **8/16/2016**- New
   1. Combined SOP BCR L007, L010, the returning portion of L013, L019, and the outbound section of L022
   2. Changed all titles in Clinical Outreach to Clinical Outreach Representative

Standard Operating Procedure (SOP) for Shipping an Outbound Package

Figure 1

Figure 2

Figure 3



Standard Operating Procedure (SOP) for Shipping an Outbound Package

Signatures

Approved By: **Signature on file**

Julie M. Gastier-Foster, Ph.D., FACMG, Principal Investigator

Date: **Date on file**

Standard Operating Procedure (SOP) for DNA/RNA Extraction with Allprep (DNA) and MirVana (Total RNA with Small RNA)

I. SCOPE AND PURPOSE

Qiagen AllPrep kits are designed to isolate DNA and total RNA from small quantities of starting material. In addition, they provide a fast and simple method for the preparation of DNA and column purified RNA from human tissues. The purified DNA and RNA are ready for use in standard downstream applications such as DNA amplification and expression arrays.

For micro RNA analysis, the downstream characterization centers require a representative sampling of the total RNA content within the tumor tissue homogenate, most notably the low molecular weight species. Therefore, the flow through from the AllPrep DNA column is taken and the total RNA is isolated with the *mir*Vana kit from Applied Biosystems (Life Technologies). The AllPrep kit utilizes the RNeasy prep which excludes small RNAs.

The *mir*Vana™ miRNA Isolation Kit was designed for purification of RNA suitable for studies of both siRNA and miRNA in natural populations. The kit employs an organic extraction followed by immobilization of RNA on glass-fiber filters to purify total RNA. The *mir*Vana miRNA isolation procedure combines the advantages of organic extraction and solid-phase extraction, while avoiding the disadvantages of both. High yields of ultra-pure, high quality, small RNA molecules can be prepared in less than two hours.

II. PROCEDURE

A. Safety Procedures

1. Wear Personal Protective Equipment (PPE), including a lab coat, goggles or face shield, closed-toe shoes, and nitrile gloves. Liquid nitrogen and dry ice are extremely cold and may cause 'burns'. Wear cryogenic gloves designed to withstand extremely cold temperatures when handling samples stored in liquid nitrogen and large quantities of dry ice.
2. Bloodborne pathogens can be present in the unfixed frozen tissue (refer to SOP S009, "Bloodborne Pathogen and Exposure Control Plan" found in the BCR Safety Manual). Use all universal precautions.
3. Liquid nitrogen is an asphyxiate; all work should be conducted in a well-ventilated room.
4. 2-mercaptoethanol (2-ME) is toxic. The stock solution should be opened in a fume hood only. Solutions containing 1% or less of 2-ME may be used outside of a fume hood. PPE must be used when handling any solution containing 2-ME.
5. Buffer RLT Plus and Buffer AW1 contain a guanidine salt which is not compatible with disinfectants containing bleach.
6. miRNA Wash Solution included in the *mir*Vana kit contains guanidinium thiocyanate; this is a potentially hazardous substance and should be used with appropriate caution.
7. Acid-phenol:chloroform contains phenol, which is a poison and an irritant. Use gloves and personal protective equipment when working with this reagent.

Standard Operating Procedure (SOP) for DNA/RNA Extraction with Allprep (DNA) and MirVana (Total RNA with Small RNA)

8. Buffer AW1 contains guanidine thiocyanate. PPE must be used when handling this reagent.

B. Quality Control

1. The incoming tissue samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID; the human readable has the internal LabVantage ID, CCG BCR barcode, and TSS identifier.
2. Working labels (containing the internal LabVantage ID, CCG BCR barcode, and scannable 2D barcode) are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original LabVantage ID (to follow the subportion) and the corresponding newly created (DNA or RNA) LabVantage IDs. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels (internal LabVantage ID (DNA or RNA), CCG BCR barcode, TSS identifier, and 2D barcode) are printed for storage in Matrix 2D barcode tubes.
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Samples are tracked in LabVantage. Every subportion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample, LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.
6. DNA and RNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. RNA quality is measured by RIN using Agilent Bioanalyzer (see SOP M002, "RNA Nano Assay") and quantified by Spectrophotometer (see SOP BCR-MGL-EQP-1 "BIO-MATE UV-Visible Spectrophotometer"). DNA quality is evaluated for integrity by agarose gel electrophoresis (see SOP M003, "Gel Electrophoresis with the E-gel System"), quantified by PicoGreen Assay (see SOP M017, "Picogreen DNA Quantification Manual") and genotypic identity using SNP loci (see SOP M010 "Tissue Matching by SNP Analysis"). Primary stock aliquots should not be subject to numerous freeze thaw cycles.
7. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
8. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
9. The isolation kit is tested against predetermined specifications to ensure consistent product quality.

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10. All new lots of reagents are tested in parallel with the one in current use before being put into use. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeable between kit lot numbers. Results are recorded on positive control extraction worksheets. All QC results are recorded in the Quality Control notebook.
11. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.
12. RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.
    a. Bench space is wiped down at the beginning of each extraction session with RNase Zap. Pipettes are wiped down with RNase Zap once a week or as needed.
    b. Gloves should always be worn throughout the process and should be changed frequently.
    c. Only sterile, disposable plasticware and pipettes that are dedicated strictly to RNA work should be used to prevent cross-contamination with RNases from shared equipment.
    d. Containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNase-free.

C. Specimen Information:

1. Type: Frozen tissues or control cell line cultured cells.
2. Handling Conditions: Follow standard precautions when handling all tissues or cultured cells. Samples should be stored in liquid nitrogen vapor phase until analytes can be isolated.
3. Sample Preparations: Tissues are prepared by Logistics by cutting a piece (typically 10-30 mg) of frozen tissue and placing into a 2-mL Eppendorf safe-lock tube.
4. Indications for Study: This procedure should be used when DNA and RNA are needed from the same piece of tissue. DNA is isolated from the AllPrep DNA column and total RNA including small RNAs is derived from the *mir*Vana isolation kit.

D. Required Equipment, Supplies and Reagents:

1. Equipment

a. UV visible spectrophotometer
b. Capsule centrifuge
c. Digital dry bath
d. Liquid nitrogen freezer
e. Microcentrifuge
f. Multi-channel and single channel pipettes
g. Qiagen TissueLyser
h. Vortexer

2. Supplies

a. AllPrep DNA/RNA Mini Kit (50) (Qiagen, 80204)

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b. *mir*Vana™ miRNA Isolation Kit (Applied Biosystems, AM1560)
c. Filtered, sterile pipette tips, assorted sizes
d. 1.5 mL Eppendorf tubes (Fisher, #05-408-137)
e. 0.5 mL tubes (Fisher, #05-408-128)
f. 2 mL screw cap tubes (Fisher, 02-707-355)
g. 2 mL SafeLock Eppendorf tubes (Fisher, 022363352)
h. Wet and dry ice
i. Insulating trays for dry ice
j. Personal protective equipment (PPE), including insulated gloves
k. Stainless steel beads, 5 mm (Qiagen, 69989)

3. Reagents

a. 2-mercaptoethanol (2-ME), 100% (Sigma, M3148)
b. Absolute ethanol, molecular grade (Sigma, E7023)
c. Diethylpyrocarbonate (DEPC)-treated water (Invitrogen, 750023)
d. Tris-EDTA Buffer (100X) (Sigma, T9285)
e. Sodium hydroxide, 5M (Sigma, S8263)
f. Reagent DX (Qiagen, 19088)
g. RNase Zap (Ambion, M9780)
h. Water nuclease-free (Sigma, # W4502-6X1L)

Notes: It is possible to substitute disposable materials and certain equipment from other vendors as long as they are the equivalent of the item described above.

Products and disposable materials used need to be RNase-free, and handled only with gloved hands in order to prevent contamination with skin RNases.

All reagents must be made with RNase-free materials and chemicals, and containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNase-free.

In the event that a reagent or disposable item either becomes contaminated or is even suspected to be contaminated, it must be discarded.

E. Reagent Preparation (including storage conditions):

1. 2-mercaptoethanol (2-ME) – must be added to Buffer RLT Plus before use (final 1% 2-ME). Add 450 µL of 2-ME to an unopened stock bottle of Buffer RLT Plus (45ml). Buffer RLT Plus is stable at room temperature for 1 month after the addition of 2-ME.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle, to obtain a working solution. Add 25 mL EtOH to an unopened bottle of Buffer AW1 to obtain a 44 mL total volume. Add 30 mL EtOH to an unopened bottle

of Buffer AW2 to obtain a 43 mL total volume Buffer AW1 and Buffer AW2 are stable for 1 year at room temperature.

3. Add 21 mL 100% ethanol to miRNA Wash Solution 1 before use. Add 41 mL 100% ethanol to miRNA Wash Solution 2/3 to obtain a working solution.
4. miRNA Wash Solution can be stored at room temp for up to 1 month. For longer term storage, store in the refrigerator, but warm to room temperature before use.
5. An aliquot of DEPC water needs to be heated to 95ºC for the elution step.
6. To prepare 50 mM NaOH: dilute 10 mL of stock 5M NaOH with 990 mL deionized water. This reagent may be stored at room temperature for up to one year.
7. 0.1X TE is made by diluting a stock solution of 100X TE. Add 1 mL of 100XTE to 999 mL of deionized water. This reagent may be store at room temperature for up to one year.

F. Homogenization

1. Remove tissues from liquid nitrogen storage and place in dry ice.
2. Add 600 µL Buffer RLT Plus containing 1% 2-ME to each 2 mL safe lock Eppendorf tube containing 25-30 mg of tissue and immediately place in a rack at room temperature.
3. Add 3 µL Reagent DX and one 5 mm stainless steel bead to each tube.
4. Place the tubes (up to 48) in the TissueLyser Adapter Set (see MGL-EQP-21: Qiagen TissueLyser II), making certain the machine is balanced, operate for 2 minutes at 20 Hz.

   Note: Prepare a maximum of eight tubes and begin homogenization in under five (5) minutes from thawing to minimize RNA degradation. Excursions above five minutes should be noted on the isolation worksheet.
5. Disassemble the adapter set. Remove tubes from adapter and observe for homogenization.
6. If samples are not completely homogenized, then rotate the rack of tubes so that the tubes nearest the TissueLyser are now outermost and reassemble the adapter set. Rearranging the tubes ensures uniform disruption and homogenization.
7. Operate the TissueLyser for another 1 minute at 20 Hz.
8.
9. If samples are still not completely homogenized, operate for another 1 minute at 20 Hz. The duration of disruption and homogenization depends on the tissue being processed. If processing fiber-rich tissues, then complete disruption and homogenization may not be possible. Homogenization should not be attempted for more than 4 minutes total at 20 Hz, as this may result in shearing the nucleic acids.
10. Remove tubes from TissueLyser.
11. Spin tubes down briefly in microcentrifuge and transfer homogenate to a clean, labeled 1.5 mL Eppendorf tube. Do not reuse the stainless steel beads; discard in biohazard waste.
12. Centrifuge the homogenate at room temperature for 3 minutes at maximum speed (16,100 x g).

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13. Carefully remove the supernatant from each sample by pipetting, and transfer it to the AllPrep DNA spin column placed in a 2 mL collection tube (supplied in the AllPrep kit). Avoid aspirating any solids or debris.
14. Close the lid gently, and centrifuge at room temperature for 30 seconds at 8,000 x g.
15. Place the AllPrep DNA spin column into a new, 2 mL collection tube. At this point, the DNA isolation can continue with step II.H.1 or the columns can be stored in the refrigerator for up to 18 hours for later isolation. Use the flow-through for the RNA purification by proceeding with step II.G.1.

G. Total RNA purification using the mirVana kit

1. Transfer the flow-through from each DNA column into a separate labeled 2 mL screw cap tube. Adjust the volume of each sample to 600 µL with Buffer RLT Plus containing 1% 2-ME.
2. Add 60 µL (1/10 volume) of miRNA Homogenate Additive to each flow-through, and mix well by vortexing or inverting the tube several times.
3. Place mixture on ice for 10 minutes. Co-isolation for the DNA could be completed during this incubation.
4. Add 600 µL of acid-phenol:chloroform to each flow through (volume equal to the lysate volume before addition of the miRNA Homogenate Additive). Withdraw from the bottom phase in the bottle of acid-phenol:chloroform because the upper phase consists of an aqueous buffer.
5. Vortex for 30-60 seconds to mix.
6. Centrifuge for 10 minutes at 10,000 x g at room temperature to separate the aqueous and organic phases. After centrifugation, the interphase should be compact; if it is not, repeat the centrifugation.
7. Begin heating DEPC treated water for elution now.
8. Carefully remove the aqueous (upper) phase without disturbing the lower phase or interphase layers, and transfer it to a fresh labeled 1.5 mL tube. Note the volume removed.
9. Add 1.25 volumes of room temperature 100% ethanol to each aqueous phase, mix thoroughly by vortexing and spin briefly to collect. For example, for a 600 µL aqueous phase, add 750 µL of 100% ethanol.
10. For each sample, place a mirVana RNA filter cartridge into one of the collection tubes supplied.
11. Pipet each lysate/ethanol mixture onto a filter cartridge. Up to 700 µL can be applied to a filter cartridge at a time. For larger samples, apply the mixture in successive applications to the same filter.
12. Centrifuge at room temperature for 15 seconds at 10,000 x g. Warning: Spinning faster than this may damage the filters.
13. Discard the flow-through, and repeat steps 11 and 12 until all of the lysate/ethanol mixture is through the filter. Reuse the collection tube for the washing steps.
14. Apply 700 µL miRNA Wash Solution 1 (working solution mixed with ethanol) to each filter cartridge and centrifuge at room temperature for 5-10 seconds at 10,000 x

g. Discard the flow-through from the collection tubes, and replace the filter cartridge into the same collection tube.

15. Apply 500 µL Wash Solution 2/3 (working solution mixed with ethanol) and centrifuge the filter cartridge for 5-10 seconds at 10,000 x g (room temperature).
16. Discard the flow-through from the collection tubes, and replace the filter cartridge into the same collection tube and repeat step II.G.15.
17. After discarding the flow-through from the last wash, replace the filter cartridge in the same collection tube and spin the assembly at 10,000 x g for 2 minutes to remove residual fluid from the filter.
18. Transfer the filter cartridge into a fresh collection tube (provided with kit). Apply 100 µL preheated (95º C) DEPC water to the center of the filter, and close the cap. Centrifuge at room temperature for 20-30 seconds at 10,000 x g to recover the RNA.
19. If multiple columns were required for a sample, combine all eluates into a single tube.
20. Place all samples on wet ice immediately and proceed to RNA quantification step before freezing.

H. Genomic DNA Extraction

1. Add 500 µL Buffer AW1 to the AllPrep DNA spin column (prepared in 2.F.15). Close the lid gently and centrifuge for 1 minute at 14,000 x g at room temperature. Transfer the column to a clean 2mL collection tube.
2. Add 500 µL Buffer AW2 to the AllPrep DNA spin column. Close the lid gently and centrifuge for 2 minutes at 14,000 x g at room temperature to wash the spin column membrane.
3. Place AllPrep DNA spin column in the previously prepared 1.5 mL collection tube. Add 100 µL 0.1X TE (10 mM Tris: 1 mM EDTA, pH 8.0) directly to the spin column membrane and close the lid. Incubate at room temperature for 1 minute and then centrifuge at room temperature for 1 minute at 14,000 x g to elute the DNA.
4. If multiple columns were required for a sample, combine all eluent into a single tube with a single channel pipette. Discard the empty tubes.
5. Check the samples into the designated PicoGreen plate in LabVantage and place in the corresponding PicoGreen plate in the refrigerator. The physical sample should be accompanied by an empty labeled matrix tube with final storage label for final transfer by the technician completing the picogreen quantification. Proceed to DNA quantification and normalization steps. Do not store in the refrigerator for more than one week.

I. Quantification and Normalization of RNA Samples

1. Measure and record the volume of the RNA from the extraction procedure.
2. Prepare a set of 0.5 mL tubes with the unique RNA sample identifier. Add 98 µL of 50 mM sodium hydroxide to each tube.
3. Add 2 µL of the concentrated stock RNA sample to the sodium hydroxide. Vortex for at least 5 seconds to ensure that the diluted sample is well mixed and briefly spin down. Read and record the absorbance for 260, 280 and 320 nm in a spectrophotometer using a quartz cuvette.

Standard Operating Procedure (SOP) for DNA/RNA Extraction with Allprep (DNA) and MirVana (Total RNA with Small RNA)

4. Desired sample concentration may vary among projects.
   a. If the concentration is above the desired concentration, use the known volumes and concentrations to calculate the amount of DEPC-treated water to add to the sample to yield a final desired concentration as shown below in Example 1.
   b. If the concentration of the RNA sample is less than the desired concentration, use the known volumes and concentrations to calculate the amount to concentrate the sample to the desired volume as shown below in Example 2. This should be accomplished by the use of a speed vac (see MGL-EQP-23: DNA Centrifugal Concentrators) with no heat.
5. After diluting or concentrating samples, repeat step 3 to confirm that the sample is within the target concentration range. NOTE: The starting volume of RNA must be known to complete all calculations.
6. Once the samples are at target concentration, transfer the liquid to a labeled matrix tube (Primary sample aliquot) for final storage. Create a 2.5 µL aliquot for subsequent sample quality control assay (see SOP M002, "RNA Nano Assay").

J. Quantification and Normalization of DNA Samples - Refer to SOP M017 for DNA quantification and normalization by PicoGreen.

K. Sample Storage

1. RNA samples should be stored in a liquid nitrogen freezer.
2. DNA samples should be stored in a -80ºC freezer.

L. Sample Calculations

1. Samples with concentrations > 0.165 µg/µL need to be diluted using:

Normalization to 0.165 µg/µL

((current concentration/desired concentration) x current volume) – current volume

Example:

((0.21 µg/µL/0.165 µg/µL) x 18 µL) – 18 µL

= 4.9 µL (Volume of diluent to add)

Final sample volume = 22.9 µL

2. Samples with concentrations < 0.165 µg/µL need to be concentrated using:

Normalization to 0.165 µg/µL

((current concentration/desired concentration) x current volume) – current volume

Example:

((0.08 µg/µL/0.165 µg/µL) x 18 µL) – 18 µL

= - 9.3 µL (Volume to be removed during speedvac concentration)

Final sample volume = 8.7 µL

Standard Operating Procedure (SOP) for DNA/RNA Extraction with Allprep (DNA) and MirVana (Total RNA with Small RNA)

III. REFERENCES

A. Allprep DNA/RNA Mini Kit Handbook (November 2005)
B. mirVana miRNA Isolation Kit Handbook (2011)
C. BCR-REF-001, "BCR Acronym List"
D. BCR-SOP-M002, "RNA Nano Assay"
E. BCR-SOP M003, "Gel Electrophoresis with the E-gel System
F. BCR-SOP M010 "Tissue Matching by SNP Analysis"
G. BCR-M017, "Picogreen DNA Quantification Manual"
H. BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan"
I. SOP MGL-EQP-6 "BIO-MATE UV-Visible Spectrophotometer"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 4, Effective Date 4/27/2016
1. Made title not all capitalize
2. Updated equipment SOP number for BioMate UV Spec.
3. Specimen Information - added that tissue pieces are "typically" 10 -30 mg. and that procedure is used when "specimen" DNA and RNA are needed from the same piece of tissue.
4. All centrifugation steps are completed at room temperature.
5. Procedure 13. Corrected numbers or steps to repeat for washing (11 and 12)
6. Procedure 20. specified that purified RNA is put on "wet" ice "Immediately" after eluted.
7. H.1. Specified step where DNA column is taken.
8. Added reference for DNA concentrators.
9. Added NOTE that "Starting volume of RNA must be known to complete all calculations.

B. Changes made in Version 3, Effective Date 09/04/2014
1. In sections II.F.8. and II.G. 16., clarified the steps to follow
2. In section II. F.15., updated the steps to follow

C. Changes made in Version 2, Effective Date 09/02/2014
1. New format used
2. Removed any reference to TCGA
3. Removed any reference to concentration range
4. Removed the step of adjusting volume with RLT before the sample goes through the DNA spin column.
5. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
6. Removed the reference to pooling samples and re-extracting samples.

D. Version 1, Effective Date 9/14/2012 - New

Standard Operating Procedure (SOP) for DNA/RNA Extraction with Allprep (DNA) and MirVana (Total RNA with Small RNA)

Signatures

Approved By: Signature on file

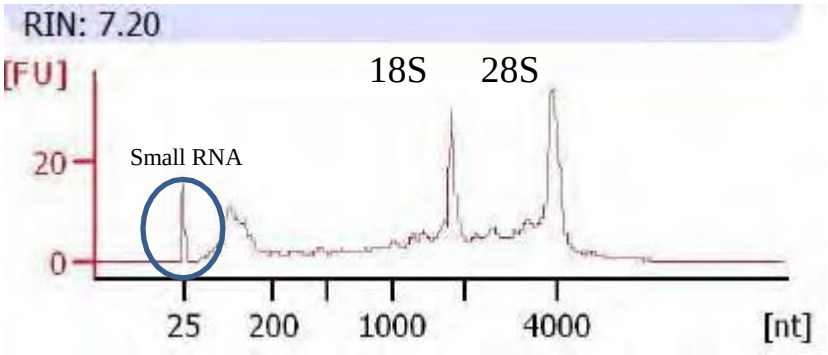
Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Date: Date on file

Standard Operating Procedure (SOP) for RNA Nanoassay

I. SCOPE AND PURPOSE

RNA samples frequently contain low levels of degradation that are difficult to detect by standard gel electrophoresis methods, but which may hinder downstream applications. Such degradation can be detected and analyzed by the Agilent Bioanalyzer. This instrument estimates the concentration of RNA and calculates the amount of 18S and 28S ribosomal RNA in each sample. In addition, the Bioanalyzer calculates the RNA integrity number (RIN), from the electrophoretic trace of the RNA sample, which includes the presence of degradation products. The assigned RIN is independent of sample concentration, instrument, and analyst, therefore becoming a de facto standard for measuring RNA integrity. Small RNAs are apparent when using this kit, see diagram below. This assay should be used to determine the overall integrity of RNA following nucleic acid extraction.



Any deviation from this Standard Operating Procedure will be noted on the Agilent printout; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety Precautions

1. Use Standard Precautions when handling all body fluids, tissues and cell cultures. Refer to the Specimen Collection and Handling procedure, GEN-1, for guidelines specific for the Molecular Genetics Laboratory and samples.
2. The RNA Nano dye binds to RNA and contains dimethysulfoxide (DMSO), which is known to facilitate the entry of organic molecules into tissues. The dye should be treated as a potential mutagen and used with appropriate care. Wear nitile gloves (latex gloves may not be substituted) and follow good laboratory practices when preparing and handling reagents and samples.
3. RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA, special precautions are needed. Records are maintained to show that RNase-free conditions (i.e. wiping the lab areas with RNaseZAP) are met, with corrective action if conditions are not met.

Standard Operating Procedure (SOP) for RNA Nanoassay

a. In addition, the Agilent RNA Nano Assay uses pre-made aliquots that are disposed after use. The use of these pre-made aliquots prevents the need to thaw the stock RNA vial for RIN testing (in most instances)**.**

B. Equipment and materials

1. Agilent RNA 6000 NanoChip kit (Agilent, 5067-1511)
   a. 25 RNA Nano Chips
   b. 2 electrode cleaners
   c. 1 syringe
   d. RNA Nano Dye Concentrate
   e. RNA Nano Marker
   f. RNA 6000 Nano Gel Matrix
   g. Agilent RNA Nano 6000 molecular weight ladder
   h. 4 spin filters
2. 0.5 mL RNase-free microcentrifuge tubes
3. Sterile, nuclease free water (Fisher, BP-2484-50)
4. RNase Zap (Ambion, AM9780)
5. Thermocycler or dry heat block
6. Microcentrifuge
7. Pipette man
8. Pipette tips, assorted sizes
9. Wet ice
10. Vortex mixer
11. Agilent 2100 Bioanalyzer
12. Agilent IKA vortex mixer
13. Universal Human Reference RNA (Agilent, 740000)

It is possible to substitute disposable materials and certain equipment from other vendors, as long as they are the equivalent of the items described above.

Products and disposable materials used need to be RNAse-free and handled only with gloved hands in order to prevent contamination with RNAses.

All reagents must be made with RNAse-free materials and chemicals, and containers and tubes for sample handling must be covered whenever possible during the entire procedure to ensure that they remain dust- and RNAse-free. Before beginning the procedure wipe down the bench with RNase Zap.

In the event that a reagent or disposable item either becomes contaminated or is suspected of being contaminated, it must be discarded.

C. Quality Control

1. This quantitative assay includes a reportable range of RIN values between 0 and 10.

Standard Operating Procedure (SOP) for RNA Nanoassay

2. All chips must include a reference control RNA with a known average RIN to monitor assay performance on each run. This control sample should have a RIN of ± 0.5 of its average RIN. For example, control AV-AB03-20A-01R has an average RIN of 9.3, so it's acceptable range would be 8.8 to 9.8. If control RNA produces a RIN outside of this range, the entire chip will be repeated.

3. Some common situations that may cause analytically inaccurate results would be improperly loaded gel matrix, RNase contamination, and/or bubbles. Control RIN values will be monitored monthly to document and follow up on outliers, trends or omissions. This monthly review must include calculations for standard deviation (SD) and coefficient of variability (CV).
4. This SOP includes a process for automatic import of Bioanalyzer test data into the LIMS system. Imported data is cross checked against printed Agilent reports in an effort to provide an additional level of control over data integrity.
5. All new lots of reagents are tested in parallel with the one in current use before being put into use. Results are recorded in QC log. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be interchanged between kit lot numbers.

D. Preparing the RNA ladder

1. Upon receipt, thaw ladder on wet ice. Heat denature the ladder at 70°C for 2 minutes. Immediately cool the vial on wet ice and aliquot 5 µL of the RNA ladder into 0.5 mL RNase-free microcentrifuge tubes (provided by the Agilent kit) on wet ice. Store the aliquots in a -80°C freezer.
2. Before use, thaw an aliquot of the ladder on wet ice (avoid extensive warming of the ladder during the thawing process).

E. Preparing the gel

1. The Agilent LabChip kit reagents are stored refrigerated and used before the manufacturer's expiration date (the physical ships are stored at room temperature). Allow the RNA gel matrix to equilibrate to room temperature for 30 minutes.
2. Pipette 550 µL of RNA gel matrix to the spin filter (provided).
3. Place the spin filter in a microcentrifuge and spin for 10 minutes at room temperature at 1,500 x g.
4. Aliquot 65 µL of the filtered gel into 0.5 mL RNase-free microcentrifuge tubes (provided). Label the tube with the date of preparation, lot number, and date of expiration. Store the filtered gel in a refrigerator and use within 4 weeks of preparation.

F. Preparing the gel/dye mix

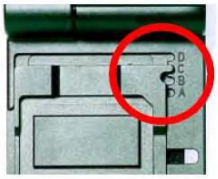
1. Allow the RNA dye concentrate (blue-capped tube) and RNA filtered gel to equilibrate to room temperature for 30 minutes. Protect the dye concentrate from light at all times. (Reference the kit instructions.)
2. Vortex the RNA dye concentrate for 10 seconds and centrifuge briefly.
3. Pipette 1 µL of the dye concentrate into a RNA filtered gel tube.

Standard Operating Procedure (SOP) for RNA Nanoassay

4. Cap the tube, vortex for 10 seconds, then centrifuge at 13,000 x g for 10min at room temperature. Visually inspect for homogenous mixing of gel and dye.
5. The prepared gel/dye mix can be stored for up to 8 hours in a refrigerator, and then must be discarded.

G. Preparing the priming station

1. Make sure the base plate is in position C as shown below. Using a screwdriver, open the screw at the underside of the base plate.



2. Make sure the syringe clip is at the top position by releasing the lever of the clip and sliding it up.

H. Heat denaturation of the RNA

1. Thaw the Agilent aliquot (test samples and controls) and a previously prepared aliquot of ladder on ice.
2. Place the tubes containing the ladder and each of the test samples and control in a thermocycler or heat block set to 70°C.
3. Heat the samples for 2 minutes while preparing the gel and chip.

I. Loading the gel/dye mix

1. Allow the gel/dye mix to equilibrate to room temperature for 30 minutes before use. Protect the gel/dye mix from light during this time. If the gel/dye mix is freshly prepared and has not been placed in the refrigerator, this step may be skipped.
2. Remove the RNA chip from its sealed bag.
3. Pipette 9.0 µL of the gel/dye mix at the bottom of the well marked G. Do not introduce air bubbles into the well when dispensing.
4. Place the RNA chip on the chip priming station. Set the timer for 30 seconds, make sure that the plunger is positioned at 1 mL, and then close the chip priming station. The lock of the latch will click when the chip priming station is closed properly.
5. Press the plunger of the syringe down until it is held by the clip.
6. Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.
7. Visually inspect that the plunger moves back to at least the 0.8 mL mark.
8. Wait for 5 seconds and then slowly pull back the plunger to the 1 mL position.
9. Open the chip priming station.

Standard Operating Procedure (SOP) for RNA Nanoassay

10. Pipette 9.0 µL of the gel/dye mix in each of the wells marked G.
11. Protect the gel/dye mix from light and store in the refrigerator when not in use for more than one hour. The gel/dye mix has a maximum shelf life of 8 hours.

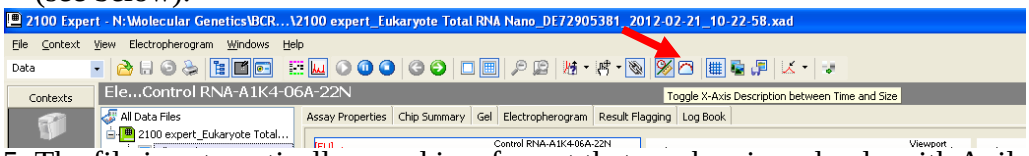
J. Loading the Marker - Pipette 5 µL of the RNA 6000 Nano Marker into the well marked with the ladder symbol and into each of the 12 sample wells. If not running a full chip (12 samples), pipette 6ul of the RNA 6000 Nano Marker into any unused sample wells. Avoid introducing air bubbles into the wells when dispensing.

K. Loading the Ladder and Samples

1. Pipette 1 µL of the heat-denatured RNA ladder into the well marked with the ladder symbol. Avoid introducing air bubbles into the wells when dispensing.
2. Add 1 µL of heat-denatured test sample to the appropriate well. Avoid introducing air bubbles into the wells when dispensing.
3. Place the chip horizontally in the adapter of the IKA vortex mixer.
4. Vortex for 60 seconds at 2,400 rpm. NOTE: If the vortexing speed is too high, liquid may spill out of the channels and interfere with the analysis.
5. Run the chip in the Agilent 2100 Bioanalyzer within 5 minutes of loading.
6. Record the number of samples run on each machine on the equipment log.

L. Running the chip

1. Turn the Bioanalyzer unit on (switch is located on the back of the instrument).
2. Clean the Bioanalyzer probes (see section J) with RNase ZAP and Nuclease free water.
3. Place the chip in the Bioanalyzer. Close lid.
4. Start the software "2100 Expert".
5. Select Assay->RNA->"Eukaryote Total RNA Nano Series II"
6. Verify that the chip image displayed matches the chip being used.
7. Enter sample names on the instrument tab under the chip summary section.
8. Chip lot number and Reagent Kit lot number should also be recorded in this section.
9. Save file with the default date and time only.
10. Select the number of wells to be analyzed (e.g. 1-12).
11. Press "Run".
12. Select the pull down window labeled "Instrument" and select "Data".
13. After the run is complete, make sure the x-axis on the electropherogram is labeled with nt (nucleotide) units. If the x-axis is labeled with sec (seconds) click the Axis button at the top of the screen to change it back to nt.
14. If running the RNA Nano Assay for FFPE RNA samples, perform Smear Analyis (see below).



15. The file is automatically saved in a format that can be viewed only with Agilent software. To make a PDF copy, select file->print. Select "PDF", choose the correct path (e.g., external USB drive), verify that the following are checked: electropherogram, gel like, result tables, and include ladder, then press "save".

16. Save the file as an XML. Select File. Click on Export. Check the box that says "Export to XML". Under Export Directory, highlight "Custom". Select the "Export RESLABVANTAGE" drive. Click Export.
17. Print a copy of the results: Select File, Print. Verify that the following options are checked: run summary, result tables, and include ladder. Click Print.
18. Refer to A005: LabVantage Manual section II.H.10 for importing results into LabVantage.

M. FFPE Smear Analysis

1. Highlight sample(s) to be analyzed.
2. At the right border of the software window, there are two tabs for additional options: Local and Global. Local is used for the individual sample selected. Global is used to apply the option to all samples.
3. Use the drop down menu to select "Advanced Settings."
4. Scroll down to "Smear Analysis" and check the box for "Smear Analysis."
5. Double click on row "Regions" to add table.
6. Add the following four smear reasons:
   Low: base pair 35 – 235
   Medium: base pair 500 – 1280
   High: base pair 1800 – 3440
   DV200: base pair 200 to max
7. Click "OK" to initiate calculation. The smear analysis will provide the total peak heights in the defined base pair ranges.

III. REFERENCES - None

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 3, Effective Date 6/6/2016

1. Made title not all capitalized
2. Updated safety information section.
3. Quality Control - removed RIN Control table. Any chip with failed control will be fully repeated. Imported data checked against printed agilent report. All kits are QC'd together and used together as kits.
4. Ladder Prep - thawed and held on wet ice while prepared.
5. Preparing gel - reagents are stored refrigerated, used by mfg exp date.
6. Preparing gel/dye mix - protect from light, mix dye before use, gel/dye should be mixed to homogenous.
7. NEW - FFPE Smear Analysis (how to perform, what is recovered).

B. Changes made in Version 2, Effective Date **9/11/2014**

1. New format used
2. Added flow chart for repeating samples
3. Added section of preparing the priming station
4. Updated materials by removing PCR strip tubes and adding 0.5mL tubes

Standard Operating Procedure (SOP) for RNA Nanoassay

5. Updated disclaimer
6. Deleted section for cleaning electrodes before use/after use (this is part of equipment maintenance), as this information is found in MGL-EQP-9, "Agilent 2100 Bioanalyzer"

C. Version 1, Effective Date 9/14/2012 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

I. SCOPE AND PURPOSE

Agarose gel electrophoresis is a rapid technique used to resolve nucleic acids and to estimate their molecular weight. DNA molecules are negatively charged due to their phosphate backbone. During electrophoresis they migrate toward the positively charged electrode. Small DNA fragments migrate more rapidly in the gel matrix compared to large fragments due to size-dependent sieving effects of the gel matrix. As a result, DNA molecules are separated based on their size.

E-Gels are self-contained bufferless, pre-cast agarose gels designed to provide fast, convenient, and easy electrophoresis. Each E-gel contains agarose, electrodes, and ethidium bromide all packaged inside a dry, disposable, UV-transparent cassette eliminating the need to weigh, melt, and pour agarose and to dispose of liquid waste containing ethidium bromide. They offer excellent resolution of up to 96 samples at a time in as little as 12 minutes.

The E-Editor software allows for quick reconfiguration of digital E-Gel images for analysis and documentation. All gel photographs must be of sufficient resolution and quality (low background, clear signal, absence of bubbles) to permit accurate interpretation.

Any deviation from this Standard Operating Procedure will be noted in LabVantage and with an incident report; the number of the samples affected by the deviation will be noted as well.

This procedure establishes the process for performing gel electrophoresis of DNA samples using the E-Gel system by trained lab personnel. It is applicable to native, genomic DNA and is used to determine the overall integrity of genomic DNA following nucleic acid extraction.

II. PROCEDURE

A. Safety Procedures

1. Wear personal protective equipment (lab coat and gloves).
2. Ethidium bromide is a potential carcinogen and known mutagen (GHS WARNING! Mutagenic (Category 2)). Nitrile gloves and lab coat must be worn when handling anything that has come into contact with ethidium bromide. **Latex gloves will not provide protection**.
3. Discard gels containing ethidium bromide in the appropriate biohazard waste container.
4. This SOP involves the use of an ultraviolet (UV) light source. An overexposure to UV radiation can cause sunburn, some forms of skin cancer, and eye damage. Proper protective shielding should be used anytime UV light sources are in use.

B. Equipment and Materials

1. PPE (lab coat, gloves)
2. E-Base (Invitrogen, EB-M03, no substitution)

Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

3. 96-well plate (USA Scientific, 1402-9700)
4. E-Gel 48 1% agarose gels (Invitrogen, G8008-01)
5. Aerosol-barrier pipette tips
6. 10X Blue Juice (Invitrogen, 10816-015)
7. Nuclease-free water (Fisher, BP2484-50)
8. 0.1X Tris-EDTA (TE) Buffer (1 mM Tris, 0.1 mM EDTA; 100X Concentrate, Sigma, T9285)
9. UVP gel imaging system
10. Vortexer
11. Adhesive foil for 96 well plates (BioRad, PMSF1001)
12. E-Gel 1Kb Plus DNA Ladder (Invitrogen, 10488-090)
13. Centrifuge capable of holding 96-well plates

It is possible to substitute disposable materials and certain equipment from other vendors as long as they are equivalent to the item described above.

In the event that a reagent or disposable item either becomes contaminated or is suspected of being contaminated, it must be discarded.

C. Quality Control

1. The loading dye is colored and easily identifiable for sample loading and tracking. It includes a visual marker to indicate where the endpoint of electrophoresis occurred.
2. Ladder of known molecular weight is run in every row with extracted and normalized DNA to estimate the size range of DNA fragments.
3. All gel photographs must be labeled adequately so that each sample can be identified and cross-reference the LIMS system.
4. All new lots of reagents are tested in parallel with the one in current use before being put into use. Results are recorded in QC log.
5. Some common situations that may cause analytically inaccurate results would include, but are not limited to: the gel ran for too long, the gel is improperly inserted into the E-base, or the gel was not examined within 30 minutes after it was run.

D. Reagent Preparation

1. Preparing 0.1X TE Buffer from 100X Concentrate:

(1) To make a 50 mL aliquot: add 5 mL 100X TE to 45 mL sterile water.

(2) 0.1X TE can be stored at room temperature and expires one year from date prepared. The container should be labeled with the stock 100X TE log number, date prepared, date of expiration, applicable hazard warnings, preparer initials, and storage requirements.

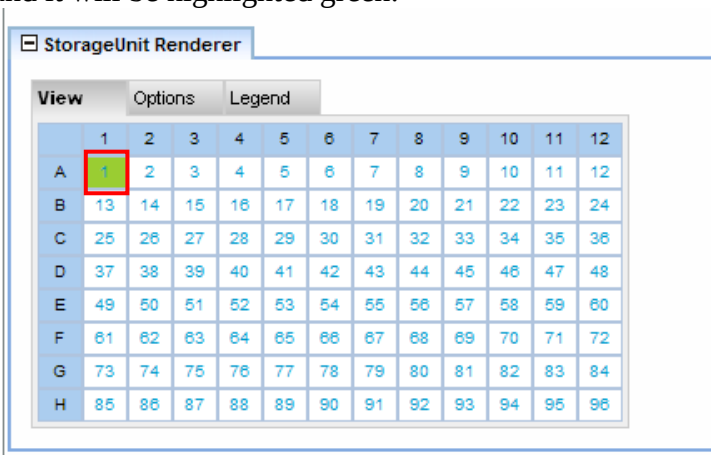
(3) Record reagent information into lab QC book and enter results once QC has passed.

E. Creating A Genomic Gel in LabVantage

1. Log into LabVantage
2. Under Storage, click on "File Samples."

Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

3. Click on magnifying glass. Click on "Sorted Box." Click on "MyBoxes."
4. Click on an empty Matrix Rack box.
5. Place the cursor on the spot that corresponds with where the first sample will be filed. Click on the spot and it will be highlighted green.



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

6. Place the cursor on the TrackItem Management box. Now the barcode on the sample can be scanned to file it in LabVantage.

7. Click "Save."
8. Under Molecular Analyte, click on "Create Robot Rack."
9. Select a Matrix Rack, and click on "Create Gel."
10. Click on the magnifying glass to choose the Transfer Mode.
11. Click on T-00019 - PCR Plate to 48 Row Gel - Top Half.
12. Type in 1 for the Aliquot Volume (uL).
13. Click "OK."
14. Select the Gel, and click "Print Gel." Print the gel layout.
15. Under Molecular Analyte, click on "Create Robot Rack."
16. Select the Matrix Rack again, and click on "Create Gel."
17. Click on the magnifying glass to choose the Transfer Mode.
18. Click on T-00021 - PCR Plate to 48 lane gel bottom half.
19. Type in 1 for the Aliquot Volume (uL).
20. Click "OK."
21. Select the Gel, and click "Print Gel." Print the gel layout.

F. Prepare samples as follows for native DNA:

1. Add 17 µL nuclease-free water to each well of a 96-well plate that will receive sample.
2. Visually inspect that there is liquid in each well.
3. Add 2 µL of Blue Juice to each well.

Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

4. Add 1 µL of DNA (normalized to 0.165 µg/µL ± 0.015 µg/µL) to each well.
5. Visually inspect that there is dye in each well.
6. Cover the plate with an adhesive foil and hold down uniformly while vortexing until dye is evenly dispersed. Spin down the plate for 1 minute at 2200 rpm (room temperature).

G. Setting up the E-Gel

1. Plug the E-base into the wall and ensure that the display reads "EG." Push the "pwr/prg" button to change the program if required.
2. Remove the gel from the packaging and insert it into the E-base. If properly inserted, a red light illuminates and the display reads "20." **The electrophoresis run must be started within 15 minutes of opening the gel**.
3. Load samples individually, adding 15 µL total prepared sample per well. NOTE: Use caution to ensure each sample is placed into the correct well.
4. Load 15 µL of ladder in appropriate wells (one at each end of each row).
5. Load all remaining empty wells with 15 µL of water. The gel will not run properly without liquid in all empty wells.
6. Press the "pwr/prg" button to begin electrophoresis within 1 minute of loading samples. The red light changes to green to indicate the run is in progress.
7. Completion of electrophoresis is indicated by a flashing red light and beeping sound after 20 minutes. The gel should be analyzed within 30 minutes.
8. Remove the gel from the base and capture an image of the gel with the gel imaging system (see MGL-EQP-32 or MGL-EQP-36 for UVP instruction).

H. Gel Imaging

1. Turn on the UVP.
2. Turn the UV Trans switch on. The transilluminator switch at the bottom also needs switched on. Place the gel in the UVP and close the door.
3. View the gel in LIVE mode.
4. Press the Time Stamp button to add the UVP gel number to the image.
5. Press the SNAP button to take a picture.
6. Press the PRINT button to print the gel.
7. Press the Save button to save the gel image on the USB drive.
8. Transfer the gel image file to the BCR/Gels/UVP Images folder.
9. From LabVantage, copy the Gel Report (created in step A14 above) into Excel. Paste into Excel; be sure to select Paste Options to "Use Text Import Wizard." Click Next twice. Click Finish. The samples from the gel file will now be in the correct format to paste into the gel template.
10. Highlight samples 1 through 24. Copy. Paste using the "Transpose" option. Now the sample ID's will be listed from left to right. Copy this transposed version of samples.
11. Open the Power Point file "Template for Labeling Gels." This template has a top and bottom gel to represent the physical gel that was run.

Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

12. On the top gel paste the first 24 samples copied from Excel. Under the Home tab, under Paragraph in Excel click on "Text Direction." Choose the option to "Rotate all text 270°."
13. Go back to Excel. Highlight samples 25-48. Copy. Paste using the "Transpose" option. Now the sample will be listed from left to right. Now copy this transposed version of samples.
14. Paste this set of samples on the bottom gel of the template. Under the Home tab, under Paragraph in Excel click on "Text Direction." Choose the option to "Rotate all text 270°."
15. Now insert the gel image twice. Open the gel image saved on the USB and crop the gel image to display the top gel and align it with the appropriate labels. Crop the gel image again, to display the bottom gel and align it with the appropriate labels.
16. Any lanes that do not have a sample should be labeled as "Blank."
17. Save the published file as "Genomic Gel ### UVP### MM-DD-YY." The genomic gel number is taken from LabVantage, the UVP is from the time stamp that is displayed on the gel image, and the date is the date the gel was run.
18. Print a copy of the labeled gel and staple the gel image from the UVP.

I. Interpretation:

1. Review gel to determine the overall qualitative level of sample integrity.
   a. **High:** A single high molecular weight band or a small smear of genomic DNA is present with all or most of the band/smear above 2,000 bp.
   b. **Medium:** A smear of genomic DNA is present with most of the smear lying between 500 and 2,000 bp.
   c. **Low:** A smear of genomic DNA is present with most of the smear below 500 bp.
   d. **Indeterminate:** If the sample's concentration is so low that it limits the visibility of any possible band being present.
2. After scoring sample integrity, update Gel Status in LabVantage using the 4 integrity categories listed above.
3. Place the printed gel file/gel image in the Genomic Gels binder.

III. REFERENCES

A. *E-Base Electrophoresis Device User Guide (Invitrogen)*

B. BCR-REF-001, "BCR Acronym List"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 4, Effective Date 4/27/2016

1. Made title not all capitalized
2. Safety - added GHS warning for ethidium bromide; noted that UV can also cause eye damage.
3. EQP and Materials: added PPE, 0.1X TE, and plate centrifuge.
4. Reagent Prep - added details for making 0.1X TBE
5. F. 6. added instruction to spin plate down after mixing.
6. G.3. Added note to use caution/add sample to correct well.

Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

7. G.8. added EQP SOP numbers for UVP's.
8. Removed details about "graphics editing software" (this is not required - per change request).
9. Gel Interpretation Section: re-written; now describes High, Medium, Low, and Indeterminate categories for DNA bands on gels.

B. Changes made in Version 3, Effective Date 09/02/2014

1. New format used
2. Updated LabVantage screenshot
3. Removed Tris-EDTA from materials
4. Updated volume of water added when preparing the samples

C. Version 2, Effective Date 10/2/2012

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for Normalization of Whole Genome Amplification Samples

I. SCOPE AND PURPOSE

Whole genome amplified (WGA) samples are shipped from Qiagen at varying concentrations. Because the samples contain the raw components of the amplification reaction, they cannot be accurately quantified via spectrophotometry and the normalization procedure must be modified accordingly.

Any deviation from this Standard Operating Procedure (SOP) will be noted on the WGA documentation sheet, normalization sheet, and the sample record in LabVantage. The number of the samples affected by the deviation will be noted as well.

This procedure is meant to establish a protocol to accurately and reproducibly normalize the whole genome amplification products received from Qiagen. There are two methods; an automated method and a manual method to normalize the samples. If the robot is down for repair the manual method may be used to normalize and dilute the samples.

II. PROCEDURE

A. Safety precautions

1. Use universal safety precautions, including the use personal protective equipment (PPE).

B. Required Equipment, Supplies and Reagents

1. "WGA plate" - WGA plate received from Qiagen
2. Water, RNase- and DNase-free (Sigma, #W4502)
3. Centrifuge capable of spinning 96 well plates.
4. Non-skirted 96-well plate (Fisher, AB-0600L)

For the Automated method:

5. Biomek FXp
6. Tip barrier Span8 P250 (VWR, #379503)
7. Tip barrier Span8 P20 (VWR, # 379506)
8. Greiner UV-Star® 96 well plates (no substitution, Fisher, #NC0532986)

For the Manual Method:

9. Pipettes
10. Pipette tips, assorted sizes

Notes:
It is possible to substitute disposable materials and certain equipment from other vendors, as long as they are equivalent to the items described above.

In the case that a reagent or disposable item either becomes contaminated or is suspected of being contaminated, it must be discarded.

Standard Operating Procedure (SOP) for Normalization of Whole Genome Amplification Samples

III. PROCEDURE STEPWISE

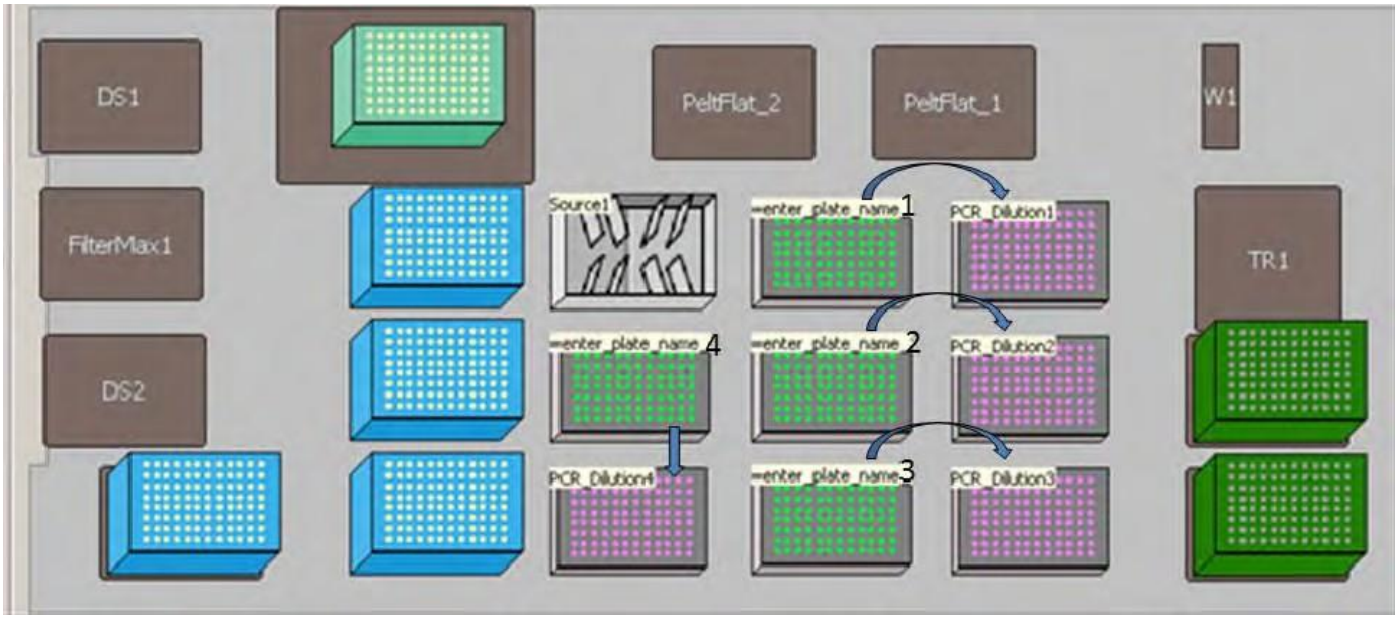
A. Calculations

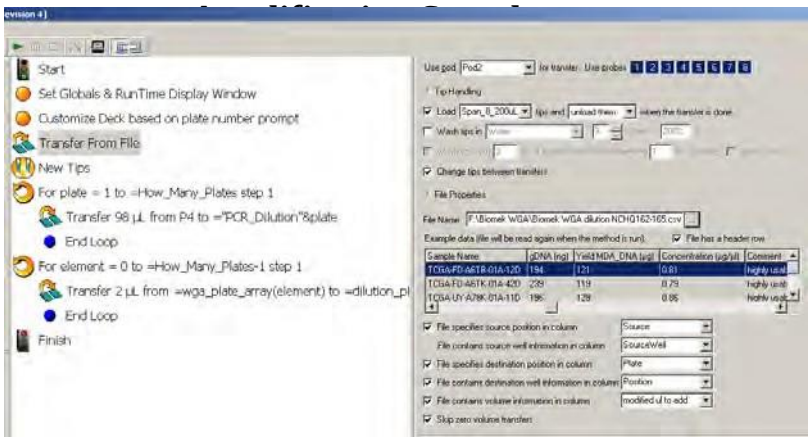
1. Insert the CD received from Qiagen. Click on "My Results" and open the Excel file. Print the "Report" tab on one or two pages (all columns for each sample should be included on a single page). Write the Plate ID, Batch number, and shipping location on the print-out (this print-out is the Repli_g_report).
2. Save the excel file as "Repli_g_report_#_#" in this folder: \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen\Repli-g-reports.
3. Open the excel document and click on the Report_Table tab.
4. This information needs to be transferred to a dilution template file and saved on the Biomek. Open the Biomek WGA dilution template form on Sharepoint. (\\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen > Biomek WGA dilution template.xlsx)
5. Starting with cell A4 on the Repli_g_report, highlight up to four plates of data (columns A through G). If there are more than four plates on the Qiagen form, the additional plates will need to be saved to a separate file. The Biomek can only accommodate four plates at a time.
6. Paste this information "as values" into cell A2 of the Biomek WGA Dilution template.
7. An equation "F4/0.5*150-150" [(actual concentration/desired concentration)*sample volume-sample volume] has been added to column H named "µL to Add" for 0.5µg/µL. **The volumes calculated in row H cannot exceed 120µL**.
   (1) The plates can only accommodate 280µL of liquid, and the samples come back from Qiagen at 150µL each. To avoid any spillover and contamination, any sample that needs more than 120µL, will be adjusted to receive only 120µL maximum for normalization. A formula has been added to column K to change any volume greater than 120µL to 120µL maximum and any volume less than 0µL to 0µL (=IF((H2>120),120,IF(H2>0,H2,IF(H2<0,0))).
8. Save the Biomek WGA Dilution file in the folder on the SharePoint site as an Excel File and a CSV File under the name "Biomek WGA dilution NCHQXXX.xlsx" where the plate ID is inserted for the X's. (\\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen\Biomek WGA dilution data forms).
9. Print the Excel file with all columns on one page. This dilution document will be kept with the Excel report document printed in 1.
10. Open the CSV file created in Step 8. Filter by Sample (Column C) and filter the "QC", "0", negative values, or blank names. Delete all rows that have those sample names. Unfilter all columns. Click Save. Note the sample ID (Column C) and well position (Column B) of the first sample on the spreadsheet for comparison to the file that is in the program.

11. Copy this file onto a flashdrive and take to the Biomek computer. *Note: If performing the manual method, just print the file and continue to "Section C Manual Fluid Handling".*

B. Automated Fluid Handling Method

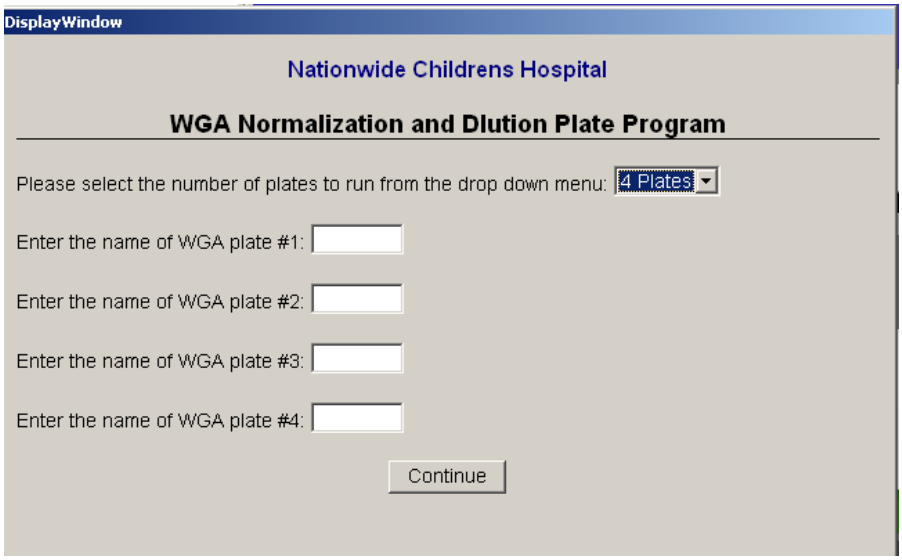
1. Remove the WGA Plate from the freezer and allow it to thaw at room temperature, checking every 20 minutes. Proceed with normalization as soon as samples are thawed.
2. Once thawed, spin the plate at 2000 x g for 1min.
3. Turn on the Biomek and Home All Axes.
4. Open the Biomek program "WGA Dilution HTML Prompt" and click on the Transfer File step. Open the correct WGA dilution plate file from the flashdrive.
   (1) The sampleID and well position noted in step 10 above should match the information shown in the Biomek software.
5. Make sure the correct drop-downs are selected by confirming with the picture below.
6. Very carefully, remove the heat sealed foil from each WGA plate without splashing and place in the appropriate position on the Biomek deck as prompted by the Biomek software. The picture below is an example of what the deck setup would look like if you had to dilute 4 WGA plates.
   a. The green and gray plates labeled "enter_plate_name_1 (through 4)" represent the WGA plates that need to be diluted.
   b. The pink and gray plates labeled "PCR_Dilution1 (through 4)" represent the SNP dilution plates being created for each WGA plate.
   c. Source 1 should be filled with H_2O up to the designated black mark on the reservoir.



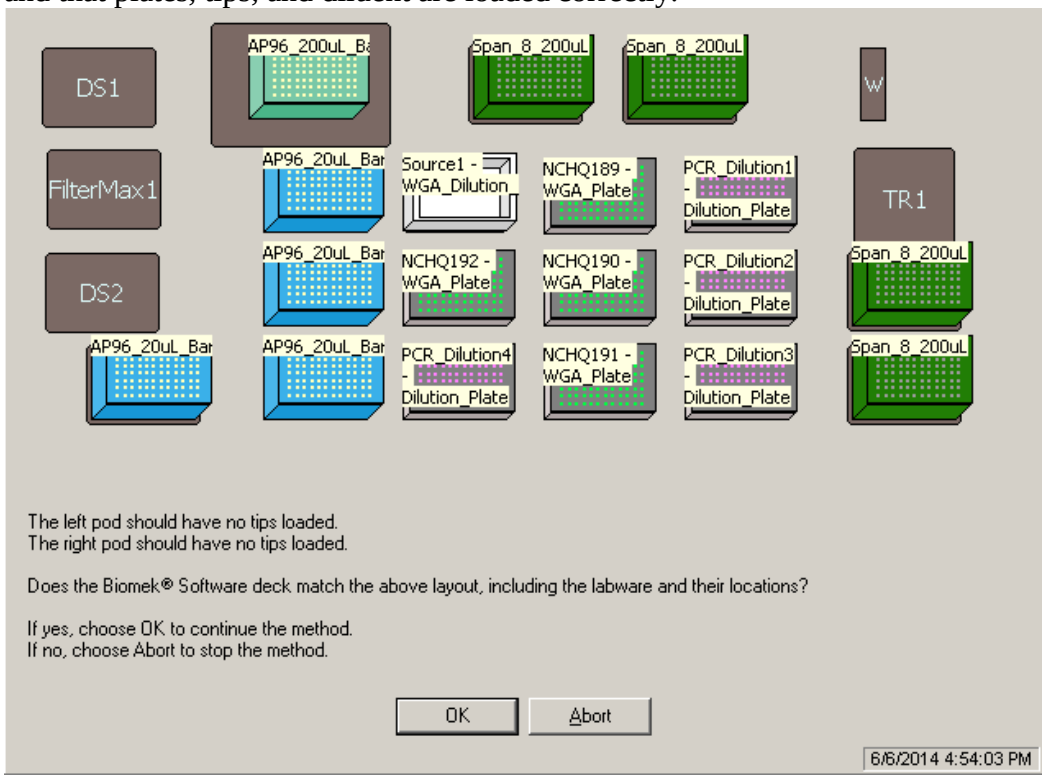


d. The dilution plate for each specific WGA stock plate must be positioned adjacent to the stock plate as shown by arrows in figure D above. This dilution plate will be a 96-well non-skirted plate labeled with the WGA plate ID, date (of dilution), and dilution value (1:50). Using the Repli_g_report, outline the plate to correspond with the wells containing samples on the WGA plate. The 96-well plate should be nestled and taped into a UV-Star 96-well plate.
e. The blue boxes represent the P250 barrier tips.
f. The green boxes represent the P20 barrier tips.

7. After the deck has been set up and all plates and tips are in the proper location, start the program by hitting the green "Play" button.
8. A pop-up window will prompt the tech to name each plate that will be normalized. On the drop down, select the number of plates to run. Enter each plate name to match the plate name on the WGA plate. For example "nchq131".



9. Click continue.
10. The user is now prompted to review and confirm the deck configuration. The plate names are now visible on the deck. Confirm that all positions were accurately named, and that plates, tips, and diluent are loaded correctly.



11. Click OK to confirm and start the dilution run: the program will execute two basic steps. First, it will normalize the concentration of the stock WGA plates by adding the volume of water specified in WGA Dilution Template column K. Then it will create a 10 ng/µL dilution plate for SNP genotype testing.
12. Once the program is complete, seal all plates with adhesive foil and store in a -80^{o}C freezer. Normalized stock WGA and 10 ng/ µL dilution plates are currently stored in the -80 Freezer BONO. Dilution plates can be prepared for shipping using SOP M018 "Shipping Analytes to Downstream Centers."
13. The 10 ng/µL dilution plates will undergo Tissue Matching by SNP Analysis (SOP M010). Passing samples are eligible to be called up to shipping (SOP M018 Shipping Analytes to Downstream Centers) if needed for the project.
14. File the Excel documents printed into the appropriate binders.

C. Manual Fluid Handling Method

1. Remove the WGA Plate from the freezer and allow it to thaw at room temperature, checking every 20 minutes. Proceed with normalization as soon as samples are thawed.
2. Once thawed, spin the plate at 2000 x g for 1min.

3. Using the "modified µL to Add" column K of the Excel spreadsheet as a reference, transfer the correct volume of RNase- and DNase-free water to the appropriate wells of the WGA Plate by piercing the film cover of the plate with the pipet tip.
4. Seal the plate with a second, new adhesive foil and mix by gentle inversion. Spin the plate at 2000 x g for 1 min.
5. Take an empty 96-well non-skirted plate and label it with the WGA plate ID, date, and dilution value (1:50). Using the Repli_g_report, outline the plate to correspond with the wells containing samples on the WGA plate.
6. Add 99 µL of RNase- and DNase-free water to each well in the dilution plate.
7. Add 1 µL of DNA from the WGA plate to the dilution plate.
8. Seal all plates with adhesive foil and store in a -80^{o}C freezer. Normalized stock WGA and 10 ng/µL dilution plates are currently stored in the -80 Freezer BONO. Dilution plates can be prepared for shipping using SOP M018 Shipping Analytes to Downstream Centers.
9. The 10 ng/µL dilution plates will undergo Tissue Matching by SNP Analysis (SOP M010). Passing samples are eligible to be called up to shipping (SOP M018 Shipping Analytes to Downstream Centers) if needed for the project.
10. File the Excel documents printed into the appropriate binders.

II. REFERENCES

A. BCR SOP M018 "Shipping Analytes to Downstream Centers"
B. BCR SOP M010 "Tissue Matching by SNP Analysis"

III. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date **8/16/2016**
1. Made title not all capitalized
2. Updated two instances where "non-skirted" PCR plate needed to be specified.

B. Changes made in Version 2, Effective Date 12/31/2014
1. Updated the location of where the Qiagen file is saved.
2. Updated the dilution template to have a fixed equation in place to eliminate typing in the equation each time it is used.
3. Updated formatting.
4. Added section "Automated Fluid Handling Method"
5. Added equipment, supplies, and reagents
6. Updated catalog numbers

C. Version 1, Effective Date 9/11/2012 - New

Standard Operating Procedure (SOP) for Normalization of Whole Genome Amplification Samples

Signatures

Approved by:	**Signature on file**	**Date on file**
	Julie M. Gastier-Foster **Principal Investigator**	**Date**

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

I. SCOPE AND PURPOSE

Sequenom's MassARRAY™ system and iPLEX™ chemistry are used to generate SNP genotypes for the purposes of tracking samples from sample extraction/ normalization through amplification as a quality control tool. Sample validations will be conducted on two levels: first, genotypes from patient tumor and normal samples will be compared to verify a patient match and second, genotypes will be compared against the entire database to identify duplicate patient samples.

1. The purpose of this Standard Operating Procedure (SOP) is to establish a protocol for the Biospecimen Core Resource (BCR) to provide high quality and reproducible genotype data on both the normal and tumor DNA samples as well as normal and tumor WGA samples.
2. This procedure applies to all trained BCR laboratory personnel.

II. PROCEDURE

A. Safety Procedure

Wear Personal Protective Equipment (PPE) including lab coats and gloves.

B. Quality Control

1. Worksheets are used for cocktail component calculations. Lot numbers, concentrations and expiration dates of reagents used are recorded where applicable. Unusual observations in set up of assays are also noted on these worksheets.
2. This PCR assay requires separate areas for set up: The DNA extraction is performed in the Pre-PCR room. PCR reactions are set-up in a separate room from the thermal cycler and post-PCR analysis area. No amplified products are allowed in the PCR set-up area. Aerosol barrier pipet tips are used for the set-up of all PCR reactions to prevent cross contamination. The mass spectrometry is done in the Post-PCR room.
3. Dedicated equipment (pipettes), filtered tips and other supplies are used in each area. Powder-free gloves are always used.
4. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
5. All new lots of reagents are tested in parallel with the one in current use before being put into use. Results are recorded in QC log. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be interchanged between kit lot numbers.
6. For all assays, specimens should be ordered in the following sequence: participant samples, positive controls, negative controls. This is done to minimize the chance for cross contamination of patient samples while providing the greatest chance for detecting contamination in the negative control.
7. Analytical Controls: Two controls are used for the Sequenom Genotyping System All control samples are tested at the same time, in an identical manner, and by the same technologist as the patient samples included with each assay group:

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

a. Negative Control: Nuclease Free-Water. This blank control should contain no peaks in the markers being analyzed. Some background signal can be tolerated, but no more than ten calls per blank control will be permitted on a passing run.
b. Positive Control: DNA from a control cell line sample (-07-0227-20A-01D) is set up with each run to ensure reproducibility of the Sequenom System and to ensure that mass spectrometry and analysis software are functioning correctly. The SNP profile of the positive control is known and available for comparison to confirm the results of each positive control run.
c. Internal negative and sensitivity controls are not required in this SOP.
d. Sequenom software interprets peak heights to determine the quality of signal. These categories are Conservative, Moderate, Aggressive, Low Probability, or No-Alleles. SNP allele calls are only made if the signal is classified as conservative or moderate. No reaction (positive control or patient sample) can have more than 10 loci with no SNP value. Patient samples with greater than 10 no calls should be repeated. Positive control samples with greater than 10 no calls requires that the entire plate of samples be repeated.
e. The results of controls are verified for acceptability before the data is released.
    i. Annotations will be added to the setup worksheet for assays with unacceptable analytical controls and a link to the repeat test. For example, if BCRPL192 demonstrated amplification from the negative control, the assay would be repeated as BCRPL192b. The BCRPL 192 assay worksheet would be annotated to convey the assay failed for amplification of negative control and repeated as BCRPL 192b.
f. Some common situations that may cause analytically inaccurate results are: inadequate spotting from the nanodispenser, low humidity in the room, and/or PCR reagent contamination.

C. Equipment and Materials

1. DNA source (Native DNA or WGA DNA), 10 ng per reaction
2. Nuclease free water (Fisher, BP2484-50)
3. Sequenom PCR Accessory Set (Sequenom# 11327)
    a. PCR buffer, 10x
    b. $MgCl_2$, 25 mM
    c. dNTP mix, 25 mM
    d. PCR enzyme, 5 U/µL
4. PCR primer mix, 1 µM (stock from IDT)
5. Sequenom iPlex Gold Reagent Kit(Sequenom, #10165)
    a. SAP buffer plus, 10x
    b. SAP enzyme, 1.7 U/µL
    c. iPLEX buffer
    d. iPLEX Termination mix, 1X
    e. iPLEX enzyme

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

f. Calibrant

6. Extend Primer mix, 7/9.3/11.6/14 µM (stock from IDT)
7. ThermoSequenase DNA Polymerase (Sequenom, 10186)
8. Sequenom SpectroCHIP II G96 and Resin Kit(Sequenom #10163)
   a. SpectroCHIP II
   b. Clean Resin
9. 5M Sodium Hydroxide (Sigma Cat# S8263-150ML)
10. 96 well Non-Skirted Reaction Plate (Fisher, Catalog #AB-0600L)
11. Microseal 'B' Adhesive Seals (BioRad, #MSB1001)
12. Microseal 'F' Foil (BioRad, MSF1001)
13. 1.5 mL tubes (Direct resource, 05-408-137)
14. Reagent reservoirs (Thermoscientific, 95128095)
15. Pipettors and Multi-Channel Pipettors
16. P-200ul Filtered Tips (Rainin, Catalog #RT-L200F)
17. P -10ul Pipette Tips (Rainin, # RT-L10F)
18. Metal Spatula/spoon (Not disposable)
19. Resin Dimple plate (Not disposable)
20. Resin scraper (Not disposable)
21. OpticalSealing Roller Tool
22. Centrifuge for 96 well plate
23. Vortex
24. Standard thermocycler for 96 well non-skirted plates
25. MassARRAY TM system
26. Rotator, capable of rotating 360° on PCR plate long axis
27. Cap-it-All

Notes:

1. Seal Plates with clear, adhesive PCR sealer; NOT foil.
2. The PCR, SAP, and Extension reactions build on each other, use the SAME reaction plate throughout!

D. SNP Loci Analyzed and Oligonucleotide primer sequences (human)

SNP_ID	Forward Primer	Reverse Primer	Extension Primer
rs1005533	ACGTTGGATGTGCCCCCAAGTTTAACATCG	ACGTTGGATGTTATGCCTCCCCTGAACAGC	TGAACAGCGGCGACT
rs914165	ACGTTGGATGGGATGCTGATGGGCACCAAA	ACGTTGGATGAATCACGGAGTGCAGACCAG	GCTGCCTGAGGGTGT
rs1382387	ACGTTGGATGAATAAGACGCTGCATCTGCC	ACGTTGGATGTGTTGTGTACACGAAACGGC	ACACCTGAACTTTCAA
rs901398	ACGTTGGATGGCATCTGGAATGTACTAGGC	ACGTTGGATGATGTCAGTCTGGGTGCAAAC	cGCTGAATATCAGCCC
rs727811	ACGTTGGATGTCTCTTACCGGAACTTCAAC	ACGTTGGATGCATGAGATTGCTGGGAGATG	GGAGATGCAGATGATT
rs1360288	ACGTTGGATGTCCATCTTTCAGGTCTGGAG	ACGTTGGATGACTCTCTGTGTGTGGCTTTG	AGGAGACCTGGGAAGA
rs2831700	ACGTTGGATGGCTCCTTCCAACAATTTCCC	ACGTTGGATGGACCTATGCTGTGCAGTATC	AAACTATTGCCGGAGAT
rs1413212	ACGTTGGATGCATTCCATTATCCAGGAGAC	ACGTTGGATGCCTCCTTTGGAAACACTGAC	gcGTGGTGGAGCATGGG
rs876724	ACGTTGGATGTATGAGCAGGCAGTTAGCAG	ACGTTGGATGGCAGGCTCCATTTTTATACC	TGCACTGAAGTATAAGTA

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

rs2056277	ACGTTGGATGTCTCGTCATACTTCCCTGTC	ACGTTGGATGATGTGTCAACCGCCAAACTG	ttACAGGCATGAATGAGA
rs2107612	ACGTTGGATGTGGGAGAGCATTCTCTTCTG	ACGTTGGATGCATTATTCAACTGTTTTGG	AACTGTTTTGGAGAAAAG
rs1357617	ACGTTGGATGAGGGATAGCTGATAAGAAAC	ACGTTGGATGATGCAGACCACTTCACCCTC	gggAAGCTGAATTTGGGG
rs1028528	ACGTTGGATGGATGAAGGTTAGAGCCAGAC	ACGTTGGATGTGGTCTTGGCAAGCACAATG	cctGTCCTTACTCGACATC
rs2040411	ACGTTGGATGTTTCACCACCAATGGTGCTC	ACGTTGGATGTGTGTGCTGAGTGTTCTCTG	ATTTTCTTACTCTAAGTGC
rs729172	ACGTTGGATGTCTACAGATGGCAAGCTTGG	ACGTTGGATGCATTTCCCTCTTGCGGTTAC	aTTGCGGTTACATACATTC
rs1031825	ACGTTGGATGTGCTGCCTTATCTTTCCCAC	ACGTTGGATGATCACTGCTTTCAAGTATGC	aTTTCAAGTATGCTCGGGG
rs891700	ACGTTGGATGTGTGTGAGCTATGACACTCC	ACGTTGGATGGTGGTATTTCTAGCTGAGTG	TTCTTTTTTTTTTGAAGCCT
rs1024116	ACGTTGGATGCGTCCAGGGAGTGAAAAATC	ACGTTGGATGCGGTGGGAAGTGAGCAAAAG	cAGGCATACTTTATTAAGTG
rs737681	ACGTTGGATGGGTCACATGTGAGGCCATC	ACGTTGGATGCTGGAAATCCAGAGGCTTCG	ggaaCTGGGCCCAGGGATGA
rs1463729	ACGTTGGATGCATGTGCATGCTTTTGGGTG	ACGTTGGATGCAGTCTCTGCCCTTATTCTG	tcatCATACACTCATAGCCAC
rs2076848	ACGTTGGATGTACACAGGTATCCTGGCCTC	ACGTTGGATGTTTTCAACTCTCCTACCGCC	tggcCCGCTTCAGGTCCATCA
rs1528460	ACGTTGGATGGTATGTTTGGCAAATGCAGG	ACGTTGGATGCAAACTGCATCTTCCTCCTG	TTAGCCTTAACATATTTAAGA
rs1029047	ACGTTGGATGTTGTGGAATAAACTGAAGGC	ACGTTGGATGCATAACGTGGATTTGTCAGC	TAAGAATTCAAGATGGTATTT
rs8037429	ACGTTGGATGACTTTGCTACACCTCCATAG	ACGTTGGATGGCTACGTAAGAGGTCATTGC	ccctAAATAACTCTTGCGCTAC
rs907100	ACGTTGGATGCTGAAGGAAAATCTGGAGGC	ACGTTGGATGCCAAGCCTTGGAAAACACAG	ggggCCAGTTGGAGCCTTCTTT
rs2016276	ACGTTGGATGAGTATTTGCATCCCAGCCTC	ACGTTGGATGTTTGTGTGGCTGAGAGAGAG	tcttAGAGAGAGAAAAATGTGC
rs1490413	ACGTTGGATGACTTCTCACTAGTGTCCCTG	ACGTTGGATGTCTGACAGCTCAGAACTGCC	gggaTGATGTGGGTTCTTTGCA
rs10495407	ACGTTGGATGCTTCCTCTTGGTTGCATTGG	ACGTTGGATGTTGCCACTGAAATGAGTCTG	gAAACAACAGAATGGAATAGGA
rs2830795	ACGTTGGATGCTATAGACATAGGACACACC	ACGTTGGATGAGGCTCTGAATCAGGATGAG	GCAATATAATAGGACTTCTTTGC
rs722098	ACGTTGGATGGGAAGGGAAGTACACATCTG	ACGTTGGATGGGGTAAAGAAATATTCAGCAC	GCACATCCAAAATTTAAATCCTTA
rs717302	ACGTTGGATGCCAAACACAGAAAGAGGTTC	ACGTTGGATGAGCTTTAGAAAGGCATATCG	gAAGGCATATCGTATTAACTGTGT
rs826472	ACGTTGGATGGATGCTGAATTTTGTCTCTG	ACGTTGGATGGAAATTTGTAGGCAATAGAC	ttctACTAATTAATCTGCTATCCTG
rs873196	ACGTTGGATGCAGGAGTTGGAGTCAATCAG	ACGTTGGATGTCAGAATCAGATAGCCCTGC	tgacTTCAAATCCCAAGTGCTGCCC
rs763869	ACGTTGGATGTATGATGTGGCTACTCCCTC	ACGTTGGATGAGCCAAACCATATCAAGTGC	gTTGTTTATATTATTTCTAACTCAA
rs1454361	ACGTTGGATGATAGAGTCTACGACCCCTAC	ACGTTGGATGGTCCATCATCAGTAAGACAC	tgTCAGTATCCATTTAGAAACAACA
rs1335873	ACGTTGGATGCAGGGTGCAGGTATGTATTG	ACGTTGGATGACAAACGTGTGATGCTCTCC	cagtTACCTAGCTATGTACTCAGTAT
rs2046361	ACGTTGGATGGGTCATTGTTGACACTTCAC	ACGTTGGATGCTGCCAAATGTATCCTTACC	GAACGATCATTTTCAAAATTAAAATTA
AMEL_XY	ACGTTGGATGGGCACCCTGGTTATATCAAC	ACGTTGGATGCACTATTCTTTACAGAGC	gggatgTTCTTTACAGAGCCCAGGG
HSFY-2	ACGTTGGATGATCTACATCTTCAAGTCTG	ACGTTGGATGCCATTTTTCAGTGGTCTGTG	cttcAAATTGGCCACAGACATCTAACC
XKRY-1	ACGTTGGATGTCCCTCTGTGGTACAGAAAC	ACGTTGGATGATGACCCCAAAAGCACAGAC	ctccAACAATTGGATCACATTACATCAAA
rs7483	ACGTTGGATGCATCAACAACAAGATGGCCC	ACGTTGGATGACCCCTTACGGACAGGATGA	CCTCCTGCTCAGCATA
rs2070235	ACGTTGGATGAAGAGGCGTGTGGCTCTGTC	ACGTTGGATGTCTTGGGCGTGAGGCTGTTA	AAGGACAGACTGGTGC
rs1950902	ACGTTGGATGAGATTGACTAGCATCAATG	ACGTTGGATGTTCCAAGCATCCCTTAGGCG	GGTCACCTCTAGCAAGT
rs2228001	ACGTTGGATGTGGTGGGTGCCCCTCTAGT	ACGTTGGATGTGGGCCCAAGAAGACCAAAA	gACCTGTTCCCATTTGAG
rs3218536	ACGTTGGATGAGTGTGAACTTACAGGAGTC	ACGTTGGATGCTGCATTATAGTTTGTGTCG	GTTGCAAAAAGAACCAGG
rs10380	ACGTTGGATGGATGAGTTAAGATCCCATGC	ACGTTGGATGTGACAACCTTTTAGTGATCC	TTTCAGAAAAGAGCTCAGA
rs25640	ACGTTGGATGCCTCCTTTTACCCTATACAAC	ACGTTGGATGCCAGTCTTCATCACTTATCC	ATCACTTATCCTAGCAAAGGAA
rs486907	ACGTTGGATGTCTGGAAGCGTGTTTGGATG	ACGTTGGATGCCACAGGACAAGTGTAGTTC	TAAATATAGATGACAGGACATTT

rs1801516	ACGTTGGATGGTCAGACTGTACTTCCATAC	ACGTTGGATGCGAGAAGTGTCGAAGACAGC	GATTTCTCCATGATTCATTTGTAT
rs689647	ACGTTGGATGGTTGAGGCTTGTGGTGATAC	ACGTTGGATGAGAAGGAGGAGAGCCTTTTC	gctgTTCAGAAGAAACTTCAAAGT

E. Reagent Preparation (including storage conditions)

1. Primer mix
   a. Prepare PCR primer mix at a final concentration of 1 µM, this should contain both the forward and reverse primer for each assay in the corresponding "plex."
   b. Prepare Extend primer mix containing Low, Med1, Med2, High mass primers to a final concentration of 7, 9.3, 11.6, 14 µM, respectively, using nuclease free water. Mass Spec the extend primers to verify intensities are roughly equal across masses and make adjustments accordingly. Refer to step H in the Procedure section below for instructions of spotting the chip with the primer mix.
2. All reagents are vortexed and centrifuged before use or cycling.
   a. Enzymes are not vortexed, but will be centrifuged before use.
3. All reagent stocks are stored in a -20°C freezer.
   a. It is acceptable to thaw reagents at room temperature and store refrigerated when in use.
   b. Enzymes are *not* thawed and should always be stored in a -20°C freezer.
4. Calibrant should be reused as long as possible and should be stored in a refrigerator.

III. PROCEDURE-STEPWISE

A. Creation of a SNP/SSTR Plate in LabVantage – If Applicable

1. Log into LabVantage.
2. Go to File Samples under Storage.
3. Find an 8x12 Robot Rack to file samples.
   a. Click on the magnifying glass.
   b. Click on Storage Unit.
   c. Click on My samples.
   d. Click on an 8x12 Robot Rack
4. Place cursor in the "Track item Management" space.
5. Scan samples into the box.
6. Click on "Create Robot Rack" under Molecular Analyte.
7. Type in the box number in the search field.
8. Highlight the Robot Rack and select "Create SSTR Plate." Select the transfer mode T-00018. The description will auto-populate based on the transfer mode selected.
9. Type in 1 for Aliquot volume (µL).
10. Highlight the newly created plate and click the print wells button.
11. This will display the location of each sample in the plate. Print the plate layout.
12. Leave this open to create the PCR worksheet.

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B. Creation of PCR Worksheet

1. Open the PCR worksheet N:\MolecularGenetics\BCR\Sequenom\ Sequenom_Multiplex_PCR-worksheet.xlsx.
2. If a SNP plate was not created in LabVantage, type in the sample ID's in their respective wells.
3. If a SNP plate was created go to the Plate Layout screen.
    a. Copy and paste all the wells onto the tab labeled "Plate Layout LV."
    b. Under the paste options in Excel use the Text Import Wizard.
    c. Select Delimited.
    d. Click Next.
    e. Make sure "space" is selected as the delimiter.
    f. Click Next. Click Finish.
    g. Adjust columns 9-12 to be in line with columns 1-8 from the plate.
    h. Copy and paste this on the tab labeled "PCR."
4. Print the PCR worksheet to record lot numbers during the rest of the procedure.

C. Creating the dilution plate

1. Remove the Matrix rack containing the stock samples from the liquid nitrogen, -80°C freezer, or the refrigerator.
2. When the samples are completely thawed, centrifuge the stock samples briefly to collect all the liquid at the bottom of the tube.
3. Label a 96-well unskirted PCR plate with the plate ID, 10 ng/µl, and the date.
4. Calculate the amount of water and DNA to add to create a 10 ng/µl dilution. This would be 14 µl of nuclease-free water and 1 µl of DNA if the stock DNA was at 150 ng/µl.
5. Add the nuclease-free water to each well.
6. Use the Cap-It All to remove the caps of the tubes in the Matrix rack containing the stock samples. Samples will be kept uncapped only long enough to remove the sample.
7. Transfer the stock samples immediately to the appropriate wells of the labeled 96-well unskirted PCR plate using a multi-channel pipet.
8. Use the Cap-It All to recap the stock sample Matrix rack.
9. Seal the dilution plate with a sheet of adhesive foil.
10. Vortex the plate for 5 seconds, while holding firmly at the edges.
11. Briefly centrifuge the plate in a bench top centrifuge at 2200 rpm for 30 seconds.

D. PCR Amplification

The following steps are for PCR amplification of 96 reactions, adjust accordingly if more or less reactions are needed.

1. Vortex and centrifuge samples and reagents to mix.
2. Prepare PCR master mix in a 1.5 mL tube (recommended volumes account for a 20% overhang).

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Reagent	Final conc. in 5 µL rxn	Volume of reagent in 5 µL [µl]	Volume (20% overhang)* [µL] Number of reactions : 96 (+20%) =115
Water, HPLC grade	N/A	0.8	92
10 x PCR Buffer with 20 mM $MgCl_2$	2 mM $MgCl_2$	0.5	57.5
25mM $MgCl_2$	2 mM	0.4	46
25 mM dNTP Mix	500 µM	0.1	11.5
1 µM Primer Mix	0.1 µM	1	115
5 U/µl PCR Enzyme**	1 U/rxn	0.2	23
100ng/µL DNA (not added to mix)	10 ng/rxn	2	n/a
Total volume [µL]	n/a	5.000	575

3. Vortex master mix thoroughly, briefly spin down, and distribute 3 µL into a Non-skirted PCR plate.
4. Add 2 µL from the 10 ng/µL DNA dilution plate to the appropriate wells. Add all samples first, then positive control samples, and finally negative control samples last. (The dilution plate may be sealed with a new sheet of adhesive foil and stored in a -80ºC freezer).
5. Seal the plate (now referred to as the sample plate) with a clear adhesive seal.
6. Vortex plate and then centrifuge (2000 g for 5 sec).
7. Cycle the 96-well sample plate on a standard thermocycler with the following program:

1. **94°C for 2 min**
2. **45 Cycles:**
   a. **94°C for 30 seconds**
   b. **56°C for 30 seconds**
   c. **72°C for 60 seconds**
3. **72 for 5 min**
4. **4 degree hold**

E. SAP Treatment

1. Retrieve sample plate from thermocycler following the PCR reaction and briefly centrifuge to collect reagents in the bottom of the plate.
2. Prepare SAP master mix in 1.5 mL tube.

Reagent	Final C in 7 µL reaction volume	Volume of reagent in 2 µL of SAP	Volume (38% overhang) [µL] Number of reactions :

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	(PCR + SAP)	cocktail [µL]	96 (+38%) =132
Nanopure Water, Autoclaved	**n/a**	**1.53**	**201.96**
SAP Buffer	**0.243x**	**0.17**	**22.44**
SAP Enzyme (1.7 U/µl)	**0.5 U**	**0.30**	**39.6**
Total volume [µL]	n/a	**2**	**264**

3. Vortex master mix thoroughly, briefly spin down.
4. Distribute 2 µL of SAP mix into all 96- wells of the sample plate (Total volume after addition: 7 µL).
5. Seal plate, vortex lightly and then centrifuge (2000 g for 5 sec).
6. Run the 96-well sample plate on a standard thermocycler with the following program:

37° C	**40 min**
85° C	**5 min**
4° C	**∞**

F. Extension reaction

1. Retrieve plate from thermocycler following the SAP reaction and briefly centrifuge to collect reagents in the bottom of the plate.
2. Prepare Extend master mix in 1.5 mL tube.
3. Vortex master mix thoroughly, briefly spin down.
4. Distribute 2 µL of Extend mix into all 96- wells of the sample plate (Total volume after addition: 9 µL).

Reagents	Final C in 9 µL reaction volume	Volume of reagent in 2 µL cocktail [µL]	Volume (38% overhang) [µL] Number of reactions : 96 (+38%) =132
Nanopure water	**N/A**	**0.619**	**81.7**
iPLEX Buffer	**0.222X**	**0.200**	**26.4**
iPLEX Termination mix	**1X**	**0.200**	**26.4**
Extend Primer Mix 8/10/15 µM or linear regression mixture	**0.84/1.04/1.57 µM**	**0.94**	**124.1**
iPLEX Enzyme	**1X**	**0.041**	**5.4**
Volume [µL]	**n/a**	**2.000**	**264**

5. Seal plate, vortex lightly and then centrifuge (4000 x g for 5 sec).
6. Cycle the 96-well sample plate on a standard thermocycler with the following program:

1. **94°C for 30 seconds**

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2. **40 Cycles:**
   - **94°C for 5 seconds**
   - a. **5 Cycles:**
     - **52°C for 5 seconds**
     - **80°C for 3 seconds**
3. **72 °C for 3 min**
4. **4 °C hold**

G. Samples conditioning

1. Spread out clean Resin onto dimple plate (15 mg) and let dry for at least 5 minutes. (Resin color will lighten once dry).
2. While drying, add 41 µL of Nuclease Free H_2O to sample plate and centrifuge briefly.
3. Add the 15 mg clean Resin to the sample plate by gently turning the sample plate over and putting it on top of the dimple plate. Then turn the dimple plate with sample plate over and let resin fall into the wells.
4. Seal the plate and rotate for at least 30 minutes.
5. Centrifuge the plate at minimum 2000 x g for 5 minutes.

H. Dispensing the chip

1. Place the non-skirted sample plate in a skirted 96-well plate and load onto the Nanodispenser.
2. Perform a volume check on the sample plate to find the optimum dispensing speed.
   a. **Note**: Sequenom recommends dispensing 8-12 nL of sample.
3. Load the calibrant reservoir with 60 µL of 3pt calibrant and dispense with a speed of at least 140 mm/sec.
4. Dispense samples onto new SpectroCHIP TM II.
5. Refer to procedure MGL-EQP-27 Sequenom Nanodispenser RS1000 for more detailed instruction.

I. Running the chip on the Mass Spec

1. Set up plate in typer software and link the plate and chip.
2. Load chip(s) onto scout plate and into the mass spec using the Target In/Out button.
3. Start RT processes and turn High Voltage "On."
4. Run the SpectroCHIP using the Autostart button in the Acquire program
5. Refer to procedure MGL-EQP-29 Sequenom MassArray Analyzer for more detailed instruction.

J. Post-process analysis

1. Export modified data from the Plate Data Pane and place in Sequenom data import drop box created by research informatics (\\resbcr01\Sequenom_DROPBOX). Genotyping data are automatically imported into the SNP database and homology calculations are performed immediately after the plate is dropped in the DROPBOX. An email is sent to Molecular technologists trained on this procedure once the import and scoring is complete.

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

2. To retrieve aligned SNP results, access the plate of interest through the application portal. Review the cell line control calls to ensure there were no greater than 10 loci without nucleotide calls. If there are 11 or more no-calls, the plate must be re-run.
3. Access the report under TCGA BCR →Reports→snpReportNewAssay (http://resshare01/BPC/TCGA-BCR/Reports/snpReportNewAssays.aspx) on SharePoint and retrieve the genotyping plate of interest.
4. Export the result to excel and print in color to review the genotyping results.
   a. Pairwise comparisons will be conducted using the 'Best' normal as defined primarily by analyte type; blood takes precedent over any other normal analytes. Second, if two or more 'Best' normals exist the one with the highest quality will be used. Quality will be determined by the sample with the least number of aggressive or low confidence calls.

K. Review Results

1. Any sample with greater than 10 no calls must be repeated or the case identity validated by SSTR.
2. The SNP report groups samples based on two variables: CCG BCR ID and sequence homology ≥ 80%. Therefore, each group on a SNP report is comprised of the genotyped samples within a case and any other samples from different cases with homology ≥ 80%.
3. To pass the identity metric, tumor, normal, and WGA samples within the same case must have matching genotypes that are unique from any other case in the SNP database.
4. Any deviations in genotype alignment within a case, aside from LOH (Loss of Heterozygosity, the loss of heterozygous alleles in the tumor compared to the normal) in tumor samples, are indicative of a mismatch and should be investigated per SOP M021, "Genotyping Failures."
5. All instances of ≥ 80% sequence alignment between two different cases must be evaluated. The presence of loci with homozygous mismatches between cases is the strongest evidence to refute case duplication. The presence of tumor heterozygosity at loci where normal controls are homozygous can also serve as evidence to refute case duplication.
6. A column labeled "Number of '00'" calculates the number of no calls present in the sample.
7. A column labeled "EZ Score" calculates the number of homozygous mismatches and tumor heterozygosity. Homozygous mismatches are counted as 100. Tumor heterozygosities at a loci are counted as one. For example, if a sample has two homozygous mismatches and one tumor heterozygosity at a loci the EZ Score would be 201.
8. Any deviations in Gender alignment within a case are indicative of a mis-match and should be investigated per SOP M021, "Genotyping Failures." The column labeled "Gender" is the clinical gender and must match the actual Gender SNP results for the sample.

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

IV. REFERENCES

1. This SOP reflects customization of Sequenom's suggested instruction for Multiplexed Genotyping Analysis using iPLEX in a 96-well format on the MassARRAY.
2. MGL-EQP-27 Sequenom Nanodispenser RS1000
3. MGL-EQP-29 Sequenom MassArray Analyzer
4. BCR-SOP-M021 Genotyping Failures

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 2/5/2015

1. Updated formatting
2. Removed TCGA references
3. Added catalog numbers for materials
4. Updated concentration of template DNA from 5ng/µL to 10ng/µL
5. Added description of results from the new SNP report
6. Changed the number of acceptable no calls from 8 to 10
7. Added section on creating the SNP plate in LabVantage
8. Added section on creating the PCR worksheet.
9. Added section on creating the dilution plate.
10. Updated disclaimer
11. Moved a few points from the notes section to the reagent preparation section
12. Former SOP M005, Creating and Handling of DNA Dilution Plate for Downstream Assays, combined with this procedure.

B. Version 1, Effective Date 9/11/201 - New

STANDARD OPERATING PROCEDURE (SOP) FOR TISSUE MATCHING BY SNP ANALYSIS

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR POOLING OF MOLECULAR ANALYTES FROM DIFFERENT TISSUE PORTIONS

I. SCOPE AND PURPOSE

The ideal, standard BCR sample set for analysis is one consisting of high purity DNA and RNA co-isolated from the same portion of a tissue sample. Occasionally, the quantity of analyte obtained from one portion/subportion of a tissue sample is insufficient for case qualification. This procedure describes the steps required to obtain permission and pool analytes to obtain a sample which passes case metric requirements.

This procedure applies to all trained Biospecimen Core Resource (BCR) laboratory and non-laboratory personnel.

II. PROCEDURE

A. SAFETY PRECAUTIONS:

1. Follow universal precautions; wear appropriate personal protective equipment (PPE) when handling biological specimens.

B. SPECIMEN INFORMATION:

1. Purified DNA and/or RNA.

C. QUALITY CONTROL:

1. Any deviation from the established sample standard (DNA and RNA co-isolated from the same portion of a tissue sample) that produces a mixed, non-standard sample from multiple tissue portions requires prior discussion with and/or specific direction from the Program Office.
    a. This applies to pooling tissue from different pathology-qualified portions.
    b. Approval from the Program Office is not required when pooling from the same portion.
2. Production and distribution of non-standard samples should occur only under special circumstances such as a; e.g. unique, special specific request from SC/PI; sample types for which it is hard to get 'good' tissue samples; and sample types for which it is hard to get good RNA from the sample, etc.
3. All pooled samples should be clearly identified and tracked in the BCR Laboratory Information Management System. The potential consequences of using such sample sets should be unambiguously communicated to all parties involved in the production, disbursement and use of such samples.
    a. The nomenclature for pooled samples involves labeling the resulting analyte with a new LabVantage ID. The BCR ID will be retained from the sample with the most recent LabVantage ID number.
4. If one analyte is pooled from multiple portions/subportions, the other analytes from those portions/subportions must be pooled as well. Due to this requirement, consideration must be given to the consequence of pooling on all analytes. For example, if DNA yield requires pooling but the RIN from one subportion has failed, the subportions cannot be pooled. QC is repeated after samples are pooled.

STANDARD OPERATING PROCEDURE (SOP) FOR POOLING OF MOLECULAR ANALYTES FROM DIFFERENT TISSUE PORTIONS

5. Barcode readers are used to confirm the analyte tubes pulled from storage are the specimens selected to be pooled in the LabVantage software and to scan labels between sample transfers where possible.

D. Procedure- Stepwise

1. Using the list provided by a senior technician or supervisor, obtain all analytes that have been approved for pooling. Thaw as appropriate for sample type. RNA samples will be thawed on wet ice. DNA samples will be thawed at room temperature.
2. Scan each sample into a folder in LabVantage. Using these samples, create the new pooled analyte in LabVantage (see SOP A005 LabVantage User Manual, Molecular section: Pooling a Sample); print labels and prepare tubes as instructed.
3. Spin down all samples and combine the complete volume from each analyte into the matrix tube labeled with the new pooled LabVantage ID. Always pool the same sample type together. For example, combine DNA and DNA or RNA and RNA. Never combine DNA with RNA or vice versa.
4. Mix sample well. (Do not vortex RNA.)
5. Perform all QC procedures for the pooled sample as required by analyte type.
   (1) DNA is normalized using SOP M017 "PicoGreen DNA Quantification (Manual)", quality checked using SOP M003 "Gel Electrophoresis with E-Gel System", and identity checked by using SOP M010 "Tissue matching by SNP Analysis".
   (2) RNA is normalized using a UV Spectrophotometer and quality checked by SOP M002 "RNA Nano Assay".

III. REFERENCES

A. BCR SOP A005 "LabVantage User Manual"
B. BCR SOP M003 "Gel Electrophoresis with E-Gel System"
C. BCR SOP M010 "Tissue matching by SNP Analysis"
D. BCR SOP M017 "PicoGreen DNA Quantification (Manual)"
E. BCR SOP M002 "RNA Nano Assay"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 12/31/2014

1. Updated formatting
2. Removed any TCGA reference
3. Updated disclaimer
4. Added Step-wise direction for pooling
5. Added clarification language for pooling subportions from the same vs. different portion

B. Version 1, Effective Date 9/14/2014 - New

STANDARD OPERATING PROCEDURE (SOP) FOR POOLING OF MOLECULAR ANALYTES FROM DIFFERENT TISSUE PORTIONS

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

I. SCOPE AND PURPOSE

Isolation of large quantities of DNA from blood presents a unique challenge. Blood contains few nucleated cells in a relatively large volume. Qiagen QIAamp Kits are designed to isolate high molecular weight DNA from up to 2 mL of blood or buffy coat. They provide a fast and simple method for the preparation of up to 60 µg of DNA. The purified DNA is ready for use in standard downstream applications such as DNA amplification and microarray.

Any deviation from the Standard Operating Procedure (SOP) will be noted in LabVantage and on the isolation worksheet; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety Procedure

1. Wear appropriate personal protective equipment (PPE), including lab coats, goggles, face shield, closed toe shoes and gloves.
2. Bloodborne pathogens can be present. Follow universal precautions per BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan." All blood samples are opened behind a face shield.
3. Liquid nitrogen and dry ice are extremely cold and can cause 'burns.' Wear cryogenic gloves designed to withstand extremely cold temperatures when handling samples stored in liquid nitrogen and large quantities of dry ice (see BPC-SAF-004).
4. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
5. Buffer AW1 contains guanidine thiocyanate. Buffer AL contains guanidine hydrochloride. PPE must be used when handling this reagent; do not mix these reagents with bleach (produces cyanide gas)

B. Quality Control

1. The incoming samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID;
2. Working labels are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original parent portion, sub-portion, and newly created DNA portion LabVantage IDs. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels are printed for storage in Matrix 2D barcode tubes.
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Only one blood sample is opened at a time during the initial transfer of blood into the centrifuge tube.
6. Samples are tracked in LabVantage. Every portion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample,

Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.

7. DNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. DNA quality is evaluated for integrity by agarose gel electrophoresis per BCR-SOP-M003, "Gel Electrophoresis with the E-Gel System" and genotypic identity using SNP loci per BCR-SOP-M010, "Tissue Matching by SNP Analysis". DNA is quantified by PicoGreen Assay per BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)". Primary stock aliquots should not be subject to numerous freeze thaw cycles.
8. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
9. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
10. The isolation kit is tested against predetermined specifications to ensure consistent product quality.
11. All new lots of reagents are tested in parallel with the one in current use before being put into use; results are recorded in the QC book. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
12. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.

C. Specimen Information

1. Type: Blood or buffy coat.
2. Handling Conditions: Follow standard precautions when handling all blood or buffy coats. Samples should be stored in liquid nitrogen vapor phase until isolation can occur.

D. Required Equipment, Supplies and Reagents

1. PPE: Lab coat, gloves, insulated gloves
2. Biological Safety Cabinet or Splash Guard
3. 1.5 mL microcentrifuge tubes (Fisher, #05-408-137)
4. 15 mL Falcon tubes (Fisher, catalog# 14-959-49B)
5. Pipettes, 50 µL – 1,000 µL, single or multichannel
6. Filtered tips for pipettes
7. Absolute ethanol, molecular grade (Sigma, E7023)
8. QIAamp DNA Blood Midi Kit (no substitution, Qiagen, #51185). Do not use Protease supplied with kit.

Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

9. Proteinase K (10mL) (Qiagen, #19133)
10. 70°C water bath
11. 37°C Water bath
12. RNase A, 10 mg/mL (Qiagen , #158924)
13. 1X Phosphate-buffered saline (PBS)(Invitrogen, #10010-031)
14. Tris-EDTA Buffer (100X) (Sigma, T9285)
15. Water nuclease-free (Sigma, # W4502-6X1L)
16. Centrifuges for 15 mL tubes (swinging bucket rotor)
17. Vortex Mixer
18. Capsule Centrifuge
19. Wet and dry ice
20. Insulating trays for dry ice
21. Racks to hold 15mL conical tubes and microtubes
22. Serological pipets for large volumes (5, 10, 25 mL)
23. Pipet-Aid (or equivalent)Colored labels: THT, B, WHT 1x0/5”W0.375”DIA: Blue (Y1439894), Red (Y1439892), Green (Y1439893), Yellow (Y1439895) (Brady)
24. Thermal Transfer Printer Labels (Freezerbondz, # THT-152-492-3)

Notes: It is possible to substitute disposable materials and certain equipment from other vendors as long as they are the equivalent of the item described above.

In the event that a reagent or disposable item either becomes contaminated or is even suspected to be contaminated, it must be discarded.

E. Reagent Preparation (including storage conditions)

1. All reagents will be labeled with name and concentration, date opened (prepared), expiration date, preparer’s initials (if applicable), storage conditions, and appropriate hazard labeling.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%), as indicated on the bottle, to obtain a working solution. For example, add 200 mL EtOH to an unopened bottle of Buffer AW1 to obtain a 298 mL total volume; add 190 mL EtOH to an unopened bottle of Buffer AW2 to obtain a 271 mL total volume. Buffer AW1 and Buffer AW2 are stable for one year at room temperature.
3. 0.1X TE is made by diluting a stock solution of 100X TE. To prepare 1 liter: add 1 mL of 100XTE to 999 mL of deionized water. This reagent may be store at room temperature for up to one year.

F. Genomic DNA Extraction

1. Remove the samples from the storage freezer. Large bloods in EDTA tubes can be thawed at room temperature; buffy coats in cryovial tubes should be thawed quickly in a 37°C water bath. Check frequently for presence of solids and proceed to the next step immediately after thawing.

Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

a) When working with granulocyte samples, begin with the MGL Reference Lab Thawing Procedure (MGL-REF-48 Reference lab Specimen Processing and Banking, Thawing Cryopreserved Cells section). The cell pellets will be resuspended in 2 mL of 1x PBS before proceeding to step 3 (addition of RNase A).

2. Add up to 2 mL of sample (blood or buffy coat) to a 15 mL centrifuge tube. If less than 2 mL of sample is available, bring the volume of the sample up to 2 mL with 1X PBS.
3. Add 4 µL of RNase A and mix briefly by inversion (Note: do not vortex enzymes).
4. Add 200 µL of QIAGEN Proteinase K and mix thoroughly by repeated inversion.
5. Add 2.4 mL of Buffer AL and mix thoroughly by inverting the tube 15 times followed by additional vigorous shaking for at least 1 minute.
6. Incubate in a 70°C water bath for 10 minutes. (Incubation for longer than 10 minutes will not adversely affect sample quality/yield, but should not exceed 1 hour.)
7. Add 2 mL of ethanol (96% – 100%) to the sample and mix by inverting the tube 10 times followed by additional vigorous shaking for 10 seconds.
8. Carefully transfer one half of the solution from step II.F.7. onto the QIAamp Midi column by carefully dispensing the liquid into the column, taking care not to moisten the rim.
9. Close the cap and immediately centrifuge at 3,000 rpm (1610 x g) for 3 minutes (room temperature).
10. Remove the QIAamp Midi column and place the QIAamp Midi column into a new 15 mL centrifuge tube. Load the remainder of the solution from step II.F.7. onto the QIAamp Midi column. Close the cap and centrifuge again at 3,000 rpm (1610 x g) for 3 minutes (room temperature).
11. If necessary, repeat step II.F.10. to obtain a dry column.
12. Remove the QIAamp Midi column and place the QIAamp Midi column into a new, labeled 15 mL centrifuge tube.
13. Add 2 mL of Buffer AW1 to the QIAamp Midi column. Close the cap and centrifuge at 5,000 rpm (4472 x g) for 1 minute (room temperature). Do not discard the flow-through, but do not allow it to come in contact with the column.
14. Carefully, without moistening the rim, add 2 ml Buffer AW2 to the QIAamp Midi column. Close the cap and centrifuge at 5,000 rpm (4472 x g) for 15 min (room temperature).
15. Carefully transfer the QIAamp Midi column to a clean, labeled 15 mL centrifuge tube. Do not allow the flow-through to come in contact with the column during transfer.
16. Pipet 300 µL of 0.1X TE buffer directly onto the membrane of the QIAamp Midi column and close the cap. Incubate at room temperature for 5 minutes and centrifuge at 5,000 rpm (4472 x g) for 2 minutes (room temperature).

Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

17. Transfer the flow-through from the 15 ml conical tube into a labeled 1.5 mL microcentrifuge tube. The label will contain the unique sample identifier of the DNA analyte.

G. Quantification and Normalization of Products – Refer to BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)".

H. Sample Storage – Store the samples in the -80ºC freezer after normalization.

III. REFERENCES

A. QIAamp DNA Midi Blood Handbok June 2012

IV. RELATED PROCEDURES

A. BCR-SOP-M003, "Gel Electrophoresis with the E-Gel System"
B. BCR-SOP-M010, "Tissue Matching by SNP Analysis"
C. BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)"
D. BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan"
E. BPC-SAF-004: "Compressed Gases Hazards and Safety"
F. MGL-REF-48: Reference Lab Processing

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version **3**, Effective Date **8/16/2016**
1. Made title not all capitalized
2. Removed normalization calculations

B. Changes made in Version **2**, Effective Date 12/17/2014
1. New format used
2. Minor word and grammatical changes made throughout
3. Removed any reference to TCGA
4. Removed any reference to concentration range
5. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
6. Removed the reference to pooling samples and re-extracting samples.
7. Updated reagents and vendor catalog numbers
8. Added instruction for processing granulocyte samples.

C. Version 1, Effective Date 9/11/2014 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

I. SCOPE AND PURPOSE

Qiagen QIAamp kits provide a fast, easy, and reliable method to purify total DNA from small quantities of human tissue or blood. The purified DNA is up to 50 kb in size, with fragments of 20 – 30 kb predominating. This length of DNA readily amplifies with high efficiency and is ready for use in standard downstream applications such as sequencing.

Any deviation from this Standard Operating Procedure (SOP) will be noted on the extraction sheet and in LabVantage; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety procedure

1. Wear appropriate personal protective equipment (PPE) including lab coats, goggles, face shield, closed toe shoes and gloves.
2. Bloodborne pathogens can be present (refer to SOP S009, "Bloodborne Pathogen and Exposure Control Plan"). Use universal precautions. All blood samples are opened behind a face shield.
3. Liquid nitrogen and dry ice are extremely cold and can cause 'burns.' Wear cryogenic gloves designed to withstand extremely cold temperatures when handling samples stored in liquid nitrogen and large quantities of dry ice (see BPC-SAF-004).
4. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.

1. Buffer AW1 contains guanidine thiocyanate. Buffer AL contains guanidine hydrochloride. PPE must be used when handling this reagent; do not mix these reagents with bleach (produces cyanide gas).

B. Quality Control

1. The incoming samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID.
2. Working labels are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original parent portion, subportion and newly created DNA analyte LabVantage ID. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels are printed for storage in Matrix 2D barcode tubes.
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Only one blood sample is opened at a time during the initial placement of the blood into the centrifuge tube.
6. Samples are tracked in LabVantage. Every portion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample,

Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.

7. DNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. DNA quality is evaluated for integrity by agarose gel electrophoresis (see SOP M003) and genotypic identity using SNP loci (see SOP M010). DNA is quantified by PicoGreen Assay (see SOP M017, "Picogreen DNA Quantification (Manual)"). Primary stock aliquots should not be subject to numerous freeze thaw cycles.
8. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
9. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
10. The isolation kit is tested against predetermined specifications to ensure consistent product quality.
11. All new lots of reagents are tested in parallel with the one in current use before being put into use; results are recorded in the QC book. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
12. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.

C. Specimen Information

1. Type: blood, buffy coat, or skin punches. The Qiagen QIAamp Mini Kit is to be used with samples that contain low volume bloods, i.e. ≤ 200 µl or skin biopsy punches. For larger volumes, use SOP M014.
2. Handling Conditions: Follow standard precautions when handling all blood or buffy coats. Samples should be stored in liquid nitrogen vapor phase until isolation can occur.

D. Required Equipment, Supplies and Reagents

1. PPE: Lab coat, gloves, insulated gloves
2. Biological Safety Cabinet or Splash Guard
3. Insulating trays for dry ice (styrofoam or plastic)
4. 1.5 mL microcentrifuge tubes (Fisher, #05-408-137)
5. QIAamp DNA mini kit (Qiagen 51304 or 51306, no substitution)
    a. Do not use the Protease that comes with the kit.
6. Proteinase K (Qiagen, 19133)
7. Micro-Pipettes to cover a range of 0.5 µL – 1,000 µL

Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

8. Filtered tips for the Micro-Pipettes
9. Pipettor
10. Tris-EDTA Buffer 100X Concentrate (Sigma, #T9285)
11. Ethanol (absolute) (Sigma, E7023)
12. 37°C water bath
13. 56°C water bath
14. Freezer (-80°C)
15. Microcentrifuge
16. Minifuge
17. Vortex
18. Centrifuge for microtubes
19. Racks to hold microcentrifuge tubes
20. Dry Ice
21. Water nuclease-free (Sigma, # W4502-6X1L)
22. 1X Phosphate-buffered saline (PBS)(Invitrogen, #10010-031)
23. Colored labels: THT, B, WHT 1x0/5"W0.375"DIA: Blue (Y1439894), Red (Y1439892), Green (Y1439893), Yellow (Y1439895) (Brady)
24. Thermal Transfer Printer Labels (Freezerbondz, # THT-152-492-3)

It is possible to substitute disposable materials and certain equipment from other vendors as long as they are equivalent to the item described above.

In the case that a reagent or disposable item becomes contaminated or is suspected of being contaminated or reaches its expiration date, it must be discarded.

E. Reagent Preparation (including storage conditions)

1. All reagents will be labeled with name and concentration, date opened (prepared), expiration date, preparer's initials (if applicable), storage conditions, and appropriate hazard labeling.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%), as indicated on the bottle, to obtain a working solution. For example; add 25 mL EtOH to an unopened bottle of Buffer AW1 to obtain a 44 mL total volume; add 30 mL EtOH to an unopened bottle of Buffer AW2 to obtain a 43 mL total volume. Buffer AW1 and Buffer AW2 are stable for 1 year at room temperature.
3. 0.1 X TE is made by diluting a stock solution of 100 X TE. To prepare 1 liter: add 1 mL of 100 X TE to 999 mL of deionized water. This reagent may be store at room temperature for up to one year.

III. PROCEDURE-STEPWISE

A. Initial preparation

Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

1. If working with low volume blood that has been frozen in liquid nitrogen**,** quick thaw in 37°C water bath before proceeding with lysis. Check frequently for presence of solids and proceed to the next step immediately after thawing.
2. When extracting from skin punches, mince the tissue into smaller pieces to optimize the digestion process. Add 180 µL of ALT buffer to the sample and homogenize in the TissueLyser (MGL-EQP-21: Qiagen Tissuelyser) for 20 seconds at 15 Hz before proceeding directly to DNA Extraction Step 1.
3. When working with buccal swab samples that have been frozen back in freezing media, proceed first with the MGL Reference Lab Thawing Procedure (see MGL-REF-48: Reference Lab Specimen Processing and Banking – Thawing Cryopreserved Cells section). The cell pellet will be resuspended in 200 µL 1x PBS before proceeding to Step 1 of DNA Extraction.

B. DNA Extraction

1. Pipet 20 µL QIAGEN Proteinase K into the bottom of a 1.5 mL microcentrifuge tube.
2. Add up to 200 µL of sample (whole blood, plasma, serum, buffy coat, or homogenized skin punch) to the microcentrifuge tube. If the sample volume is less than 200 µL, add the appropriate volume of PBS to bring the volume up to 200 µL.
3. Add 200 µL Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.
4. Incubate in a 56°C water bath for 10 min. (Incubation for longer than 10 minutes will not adversely affect sample quality/yield, but should not exceed 1 hour.)
5. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
6. Add 200 µL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column without wetting the rim. Close the cap, and centrifuge at 6000 x g for 1 min (room temperature).
8. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided).
9. Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g for 1 min (room temperature).
10. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided).
11. Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g) for 3 min (room temperature).
12. Place the QIAamp Mini spin column in a clean, labeled 1.5 mL microcentrifuge tube.
13. Carefully open the QIAamp Mini spin column and add 30 µL of 0.1X TE buffer directly onto the membrane of the QIAamp mini column and close the cap.
14. Incubate at room temperature (15–25°C) for 1 min.
15. Centrifuge at 6,000 x g for 1 min (room temperature).

C. Quantification and Normalization of Samples:

Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

1. Refer to SOP M017 for DNA quantification and normalization by PicoGreen.

D. Sample Storage

1. Store the samples in a -80ºC freezer.

IV. REFERENCES

E. QIAamp DNA Mini and Blood Mini Handbook 11/2007

V. RELATED PROCEDURES

E. BCR-SOP-M003, "Gel Electrophoresis with the E-Gel System"
F. BCR-SOP-M010, "Tissue Matching by SNP Analysis"
G. BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)"
H. BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan"
I. BPC-SAF-004: "Compressed Gases Hazards and Safety"
J. MGL-EQP-21: Qiagen Tissuelyser
K. MGL-REF-48: Reference Lab Processing

VI. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 3, Effective Date **8/16/2016**

1. Made title not all capitalized
2. Deviations will also be noted in LV.
   a. Added reference for SAF-004; LN2 and dry ice safety
   b. Added Buffer AL contains guanidine compound; don't mix AL and AW1 with bleach
   c. Added that kits are QC'd and used together thereafter
   d. Added reagents/equipment/supplies: PPE, splash guard, microtubes, racks, labels
   e. Added what must be included on reagent labels
   f. Added reference for Tissuelyser; centrifugations are performed at RT
   g. Added "related procedures"

B. Changes made in Version 2, Effective Date 12/31/2014

1. Updated formatting
2. Removed any reference to TCGA
3. Removed any reference to concentration range
4. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
5. Removed the reference to pooling samples and re-extracting samples.
6. Updated reagents and vendor catalog numbers
7. Updated DNA extraction steps to reflect Qiagen Mini Kit handbook
8. Added a reagent preparation section.
9. Added instruction for processing buccal cells.

C. Version 1, Effective Date 9/14/2012 - New

Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

I. SCOPE AND PURPOSE

PicoGreen is an ultra-sensitive fluorescent nucleic acid stain used for quantifying double-stranded DNA (dsDNA). Unlike many other DNA quantitation methods, PicoGreen is minimally affected by the presence of lipid, protein, carbohydrates and other cellular components, making it an ideal method to quantify dsDNA in cell or tissue lysates or other unfractionated matrices.

This SOP establishes a procedure for determining the concentration of double-stranded DNA in a sample and applies to all laboratory personnel. Any deviation from this SOP will be noted on the PicoGreen assay worksheet; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety Procedures

1. Wear personal protective equipment (PPE), which includes lab coats and gloves.
2. Picogreen is a potential carcinogen. It is dissolved in DMSO, which is known to facilitate movement of organic molecules through the skin. Wear nitrile gloves. Do not wear latex gloves.
3. Wash hands after handling PicoGreen.

B. Quality Control

1. All new lots of reagents are tested in parallel with the one in current use before being put into use. Results are recorded in QC log. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
2. Positive and negative controls are run with each assay for quality control. If either control fails (positive is outside of the acceptable range or negative has a concentration higher than “0”), the results will not be used and the entire assay will be repeated.
3. Samples which are within the acceptable DNA concentration range, but have a CV of greater than 0.1 between duplicate readings will be repeated using the same prepared dilution.
4. Worksheets are used to prepare each assay. Lot numbers, concentrations and expiration dates of reagents used are recorded where applicable. Unusual observations in set up of assays are also noted on these worksheets. All control samples are tested at the same time, in an identical manner and by the same technologist as the samples included with each assay group.

C. Reagents, Equipment, And Supplies

1. Single channel pipettes
2. Multi-channel pipettes (only to be used where noted)
3. Pipet-Aid

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4. Pipette tips, assorted sizes
5. Serological pipettes, assorted sizes
6. Sample to be analyzed
7. Picogreen assay kit (Invitrogen, P7589)
   a. Picogreen Reagent
   b. Tris-EDTA Buffer (10 mM tris, 1 mM EDTA), pH8.0, 20X
   c. 100 ng/µL of Lamba (λ) DNA, sheared by intensive vortexing
8. 50 mL conical tube
9. 1.5 mL micro-centrifuge tubes
10. Water nuclease-free (Sigma, W4502-6X1L)
11. FilterMax F5 Multi-Mode Microplate Reader (Molecular Devices)
12. Excitation Filter – EXP1 F535/25, F485/20, F485/20, F370/TRF
13. Emission Filter – EMP1 F595/35, F535/25, F535/25, F535/25, F625/35
14. Grenier Bio-one black flat bottom 96 well plate (Bio-Express, T-3025-16)
15. Foil seal (BioRad, MSF-1001)
16. ABgene 96-well PCR Plate, Non-Skirted (Fisher, AB-0600L)
17. Reservoirs, Sterile, 25 mL with divider (Diversified Biotech, RESE-2000)
18. CentriVap DNA Concentrator (Labconco, or equivalent)
19. Vortex Mixer
20. Minifuge Centrifuge
21. Centrifuge for spinning 96-well plates

D. Dilution of Reagents

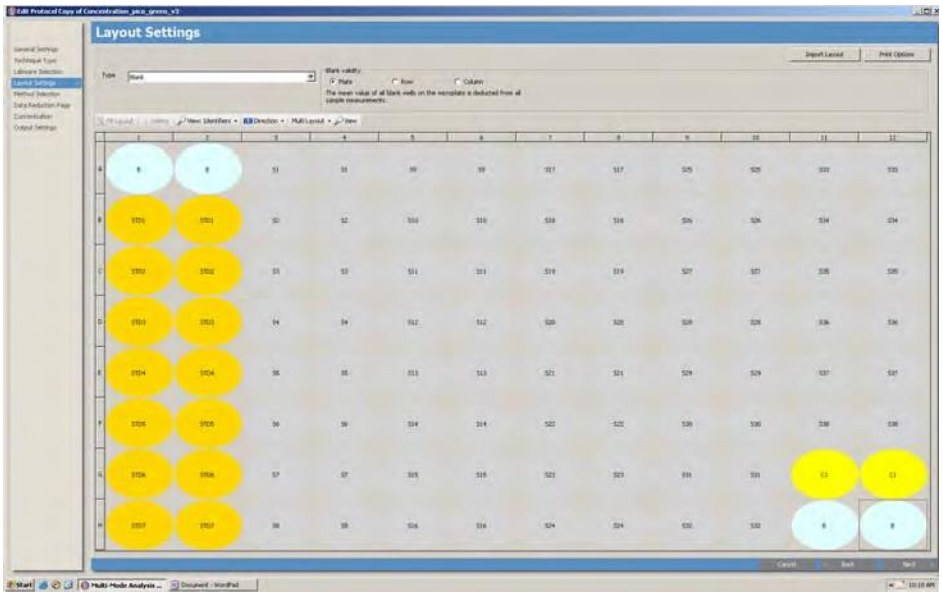
1. Preparing 1X TE from 20X TE Stock:
   a. To make a 50 mL aliquot: add 2.5 mL 20X TE to 47.5 mL sterile water.
   b. To make 1 Liter: add 50 mL of 20X TE to 950 mL of sterile water.
   c. 1X TE can be stored at room temperature and expires one year from date prepared. The container should be labeled with the Stock 20X TE lot number, date prepared, date of expiration, applicable hazards warnings, and preparer initials.
2. Dilute the PicoGreen reagent 1:200 by adding 5 µL of stock dye per 1 mL 1X TE. PicoGreen is a light sensitive dye and should be protected from light when possible. Keep this reagent in the dark until ready for use.
   a. Each well used will need 100 µL of the diluted PicoGreen reagent; prepare enough 1:200 PicoGreen for number of samples to be analyzed (including controls and calibration standards).
   b. The PicoGreen reagent is stored refrigerated and expires one year from date opened.
3. 100 ng/mL DNA standard is stored refrigerated and expires one year from date opened.

III. PROCEDURE STEPWISE

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

A. Preparation of Samples

1. Each sample is run in duplicate (two separate dilutions, both run once on the same plate). For example, sample #1 (S1) will be run in wells A3 and A4, sample 2 (S2) in wells B3 and B4, and so on throughout the plate up to 38 samples per plate. A duplicate reading of the control DNA used to make the standard curve is run in wells G11 and G12. This control comes with the kit at a concentration of 100 ng/µL. If the average value of these duplicates falls outside 15% of that (85-115 ng/µL) the plate must be repeated. A negative control is also read in wells H11 and H12, where no control DNA or sample is added. See the example below.



2. All samples to be run will be filled in on a Sample PicoGreen Worksheet (Located: N:\Molecular Genetics\BCR\Picogreen\CCG BCR\Blank worksheet for Fridge, example shown below). All sample IDs and volumes must be documented before beginning.

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	BCR PicoGreen:												
2	Date:												
3	Initials:												
4		1	2	3	4	5	6	7	8	9	10	11	12
5		Standard Curve		Sample	Vol	Sample	Vol	Sample	Vol	Sample	Vol	Sample	Vol
6	A	STD Blank	STD Blank										
7	B	STD 15.625 ng/ul	STD 15.625 ng/ul										
8	C	STD 31.25 ng/ul	STD 31.25 ng/ul										
9	D	STD 62.5 ng/ul	STD 62.5 ng/ul										
10	E	STD 125 ng/ul	STD 125 ng/ul										
11	F	STD 250 ng/ul	STD 250 ng/ul										
12	G	STD 500 ng/ul	STD 500 ng/ul									control	control
13	H	STD 1000 ng/ul	STD 1000 ng/ul									0	0
14													
15				Reagent		Lot #			Exp date				
16				1XTE								Manage Quantity	
17				DNA Standard (100ng/ul)									
18				PicoGreen Reagent									
19													

3. Make a DNA dilution plate according to the prepared plate layout using a 96-well PCR plate. Add 49 µL of 1X TE and 1 µL of stock sample DNA or control DNA for each dilution as appropriate. Control DNA is placed in positions G11 and G12. Pipet all dilutions up and down to mix.
4. Cover the plate with a foil seal, vortex and spin down.

B. Preparation of Standards

1. The first two columns of each plate are used to make a serial dilution in duplicate of the standard curve. Add 100 µL 1X TE into all wells of columns 1 and 2 (A1 through H1 and A2 through H2) of a black flat bottom Greiner plate (PicoGreen plate).
2. Add an additional 96 µL 1X TE into wells H1 and H2 of the same plate.
3. Pipette 4 µL of standard DNA (100 ng/µL) into wells H1 and H2 and mix by pipetting. Wells H1 and H2 will be the highest points of the standard curve.
4. Serial dilutions are then prepared by transferring 100 µL of standard beginning from well H1 to G1, and H2 to G2 using a multichannel pipet. Pipet up and down to mix.
5. Continue the serial dilution up the plate transferring 100 µL from G1 to F1, F1 to E1, E1 to D1, D1 to C1 and C1 to B1 (same steps with column 2), mixing by pipetting in between each transfer. At this point take the extra 100 µL from wells B1 and B2 and discard, leaving A1 and A2 as blanks. Now all wells in the first two columns of the PicoDNA plate should have a volume of 100 µL.
6. This set of dilutions will give you a standard curve from 0-1,000 pg/µL. Adding the subsequent dilutions made to the samples and plates, another 1:1,000 dilution (1:50 dilution for the stock DNA plate, 1:10 dilution when that stock DNA plate is added to

the pico plate, and an additional 1:2 dilution when the pico green is added to the plate) is applied and adjusts the standard curve to read from 0-1000 ng/µL.

a. H1/H2 – 1,000 ng/µL
b. G1/G2 – 500 ng/µL
c. F1/F2 – 250 ng/µL
d. E1/E2 – 125 ng/µL
e. D1/D2 – 62. 5ng/µL
f. C1/C2 – 31.2 5ng/µL
g. B1/B2 – 15.625 ng/µL
a. A1/A2 – blank

NOTE: The software used to analyze the data is programmed with the necessary factors to correct for concentration and dilutions applied to samples/controls on the bench.

C. Addition of Diluted DNA samples to prepared Black flat-bottom 96-well "PicoGreen" Plate

1. To the black flat bottom PicoGreen plate (containing prepared standard serial dilutions), add 90 µL of 1X TE to all wells that will have samples or controls.
2. Add 10 µL of the diluted DNA from the 96-well PCR plate to the black flat bottom PicoGreen plate according to the plate layout. Pipet up and down to mix.
3. Add 100 µL of the prepared PicoGreen reagent to all samples, controls, and standards. Pipet up and down to mix, avoiding the addition of bubbles.
4. Immediately proceed to Section D (assay is time sensitive once PicoGreen has been combined with DNA samples).

D. Load samples into the Fluorimeter plate

NOTE: the FilterMax should be turned on and the software opened prior to adding PicoGreen to samples so the incubation time of the PicoGreen plate can be controlled.

1. Preparing FilterMax 5 Software:
   a. Samples will be measured using the excitation at 485 nm and the emission at 535 nm with the Molecular Devices FilterMax F5 Fluorimeter (see SOP MGL-EQP-31).
   b. Turn on the power to the FilterMax F5.
   c. Once the FilterMax F5 has completely powered on, double-click on the Multi-Mode Analysis Software.
   d. Select the protocol "Concentration_pico_green_v3."
   e. Click Run.
2. Click "Eject Plate Carrier."
3. Load prepared PicoGreen plate (step C.4) onto the FilterMax F5 to be read with position A1 in the upper right hand corner.
4. Click "Close Plate Carrier."

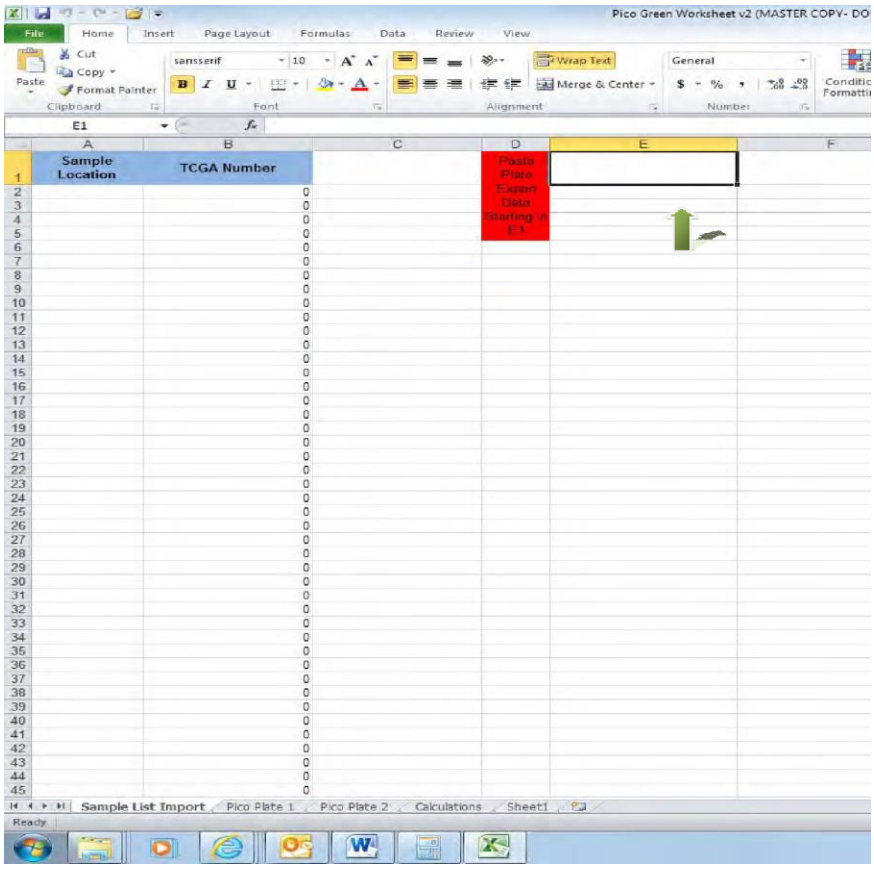
STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

5. Click "Run." The program is designed to incubate the samples for 5 minutes before reading. (If the plate has already incubated for the appropriate time, this step can be skipped by clicking the next/skip step button in the software.)
6. Once the program is completed, record the "Correlation" and "R^2" values on the worksheet. Verify that the standard curve fits a linear model. If not, the entire plate will need to be repeated.
7. Four files will appear after the run. Save the file labeled "Sheet 2" to a flash drive, naming with the date and the number of the run for that day (i.e. 031415_1). This file includes the concentration data for each sample. Use the flash drive to open the data file on a networked computer. Save a copy of the raw data in the file folder LabShared\Molecular Genetics\BCR\Picogreen\RAW PICO Data.

E. Data Analysis

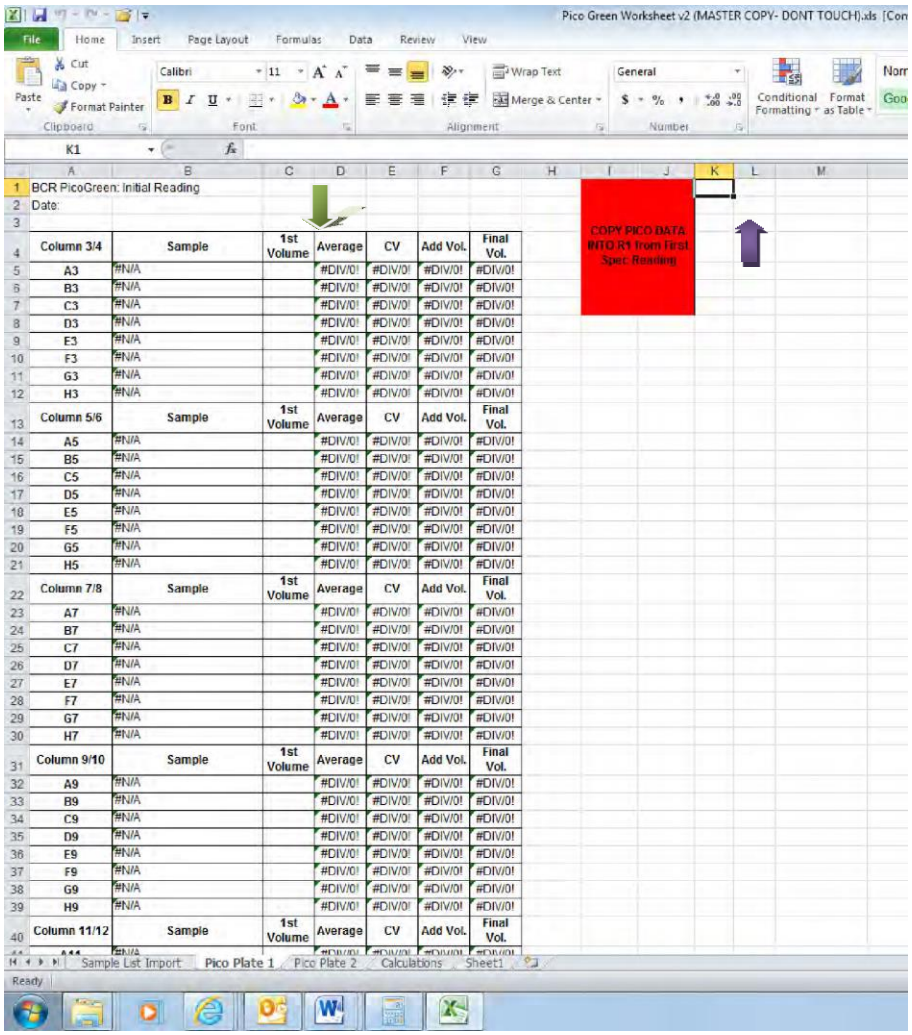
1. A spreadsheet has been created that will calculate the average concentration values, and normalization volume needed for each sample. This can be found on the Molecular Genetics Shared Drive at N:\Molecular Genetics\BCR\Picogreen\CCG BCR\PICO GREEN WORKSHEET v2.xls.
2. Log into LabVantage.
3. Under Sample Management, click on Lab Operations.
4. Under the SearchQuery, click on SamplesByBoxSorted.
5. Type in the box number of the PicoGreen plate.
6. Select all the samples and place them into a new folder.
7. Select all the samples and click on "View as Excel" on the far left side. Click Open.
8. Copy all data and paste into E1 (as instructed by the template) on the Sample List Import tab.

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)



9. The sample information is automatically transferred to the next tab in the spreadsheet. Confirm that all sample ID's were assigned to the correct position on the "Pico Plate 1" tab and that these positions correspond to the position of the physical tubes in the plate. Enter the beginning volume for each sample in the appropriate cell in column C of "Pico Plate 1" tab using the Sample PicoGreen Worksheet.

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)



10. Copy the raw data saved in Step D7 and paste into cell K1 (as instructed by the template) on the Pico Plate 1 tab (example shown above). The template also includes a second tab (Pico Plate 2) that will allow documentation for the second round of PicoGreen results after normalizing.
11. If samples are outside the standard curve (concentration greater than 1,000 ng/ µL) they will need to be diluted and run again to obtain an accurate concentration.
12. This worksheet uses the average concentration value from the duplicate dilutions for each sample to normalize the sample to 160 ng/ µL ± 15 ng/ µL using the following calculation:

((current concentration/desired concentration) x current volume) – current volume

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

a. This calculation will give the amount of diluent (0.1X TE) to be added for samples that are ≥ 175 ng/ µL, or the target volume for concentrating samples that are ≤ 145 ng/ µL.
b. Concentration ranges may vary between projects, and as such the target concentration and range used for calculations can be adjusted if required.

13. After adjusting the volume to normalize the sample, record the adjusted volume. Repeat this procedure to confirm that the target concentration has been achieved.
14. A printed copy of all assay data sheets is kept in the most recent Picogreen Data Binder in the lab. An electronic copy is kept in the Molecular Genetics Share Drive under N:\Molecular Genetics\BCR\Picogreen\CCG BCR named with the run date and the plate number(s) included.

IV. REFERENCES -None

V. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version # 3 Effective Date 7/28/2015

a. Updated formatting
b. Updated disclaimer
c. Removed TCGA reference
d. Updated location of Pico Green Worksheet
e. Added reagent preparation of 1 liter of 1X TE
f. Added description on creating the Pico Green Worksheet in the data analysis section.
g. Updated loading the plate into fluorimeter to include instruction on using the software.

B. Changes made to Version # 2 Effective Date _____

1. New Format Used
2. Minor word changes made throughout
3. In section II.F.1.
   a. Number of separate dilutions each sample is run was changed from triplicate to duplicate
   b. Average value is now used to determine repeat rather than median value
4. Section II.F.2.: An excel file directly from LabVantage is used to fill in the names of the samples run on the pico plate.
5. Section II.I.3: Concentration ranges were changed.

C. Version #1, Effective Date 10/2/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

Signatures

Approved By: Signature on file **Date:** Date on file

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

I. SCOPE AND PURPOSE

Molecular analytes derived from tumor and normal control samples within a project are provided to Characterization and Sequencing Centers in Matrix Well Plate tubes. These tubes are shipped with a human readable wrap-around label that includes all components of the sample identifier established by the Characterization and Sequencing Centers of the project. The Matrix Well Plate tube also has a unique two dimensional barcode (RVSI Number) on the bottom of the tube. In preparation for dispensing the analytes into the Matrix Well Plate tubes, the barcodes are scanned to identify their respective locations in the plate and then the tubes labeled by a Scinomix robot. After the tubes are labeled, the barcodes are scanned again to ensure proper placement. If applicable the samples are also shipped to Qiagen for Whole Genome Amplification (WGA). The samples for Qiagen are aliquoted in a hard shell 96-well plate.

Whole genome amplification (WGA) is a technique employed to increase the amount of limited DNA samples. This is particularly useful for genetic disease research where DNA quantities are limited but many downstream analyses are required. Qiagen provides a custom WGA service (REPLI-g) that accurately copies the original source DNA, without bias, and produces amplified DNA that can be directly used in a range of genetic analyses.

The BCR submits DNA samples derived from tumor and normal control tissues to Qiagen for WGA. Qiagen employs proprietary internal quality control testing to enable reliable prediction for the success of the targets in downstream applications. If needed, the WGA sample can be shipped to downstream centers.

This procedure is used to establish quality control measures for accurate distribution of analytes derived from the project. This procedure applies to all trained laboratory personnel.

II. PROCEDURE

A. Safety Procedures

1. Use universal safety precautions, including the use of personal protective equipment (PPE).

B. Quality Control

1. Sample barcodes will be verified by two individuals prior to plating and shipping.
2. Two technicians will confirm the configuration and set-up of robots used for labeling or transferring samples.
3. When analytes are transferred between containers, there is a 100% quality control check by a second technologist who observes all sample transfers are made to the proper final tube or plate location.

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

4. Any deviation from this Standard Operating Procedure will be noted on the Shipping Layout Checklist.

C. Equipment and Materials

1. Tube labels(need vendor, and catalog #)
2. Empty Matrix Well Plates (Matrix, catalog# 4900, no substitution)
3. Matrix Rack with 0.5 mL Screw Tubes with caps (Thermo, catalog# 3744, no substitution)
4. Matrix Rack with 0.5 mL Screw Tubes without caps (Thermo, catalog# 3745, no substitution)
5. Visionmate96 Scanner (Matrix #3111)
6. Visionmate 96 scanner software programs
7. Computer with Excel, TechPrinter Software, and Visionmate 96 scanner software programs installed
8. Scinomix software and robot
9. 8-Multi-Channel Pipetman
10. Single-channel pipette
11. Cap-It All
12. Hard shell skirted 96-well plate (Biorad, catalog # HSP9631)
13. Aerosol barrier pipette tips
14. 0.1X Tris-EDTA (TE) buffer (1 mM Tris, 0.1 mM EDTA; 100x concentrate, Sigma catalog #T9285)
15. Foil adhesive heat seals (Eppendorf, catalog# E0030127854)
16. Heat sealer
17. Biomek FXp
18. Robot Tips P250 Conductive Barrier (VWR, catalog# 394627)
19. Greiner UV-Star® 96 well plates (no substitution, Fisher, catalog# NC0532986)
20. TempPlateIII, 96-well plates (USA Scientific, #1402-9700, no substitution)
21. Personal Protective Equipment
22. Cap-it-All(Thermo Scientific)
23. Centrifuge
24. Repli-g Order Form (available: www1.qiagen.com)
25. SOP A005 LabVantage Manual. Specific MGL sections necessary include: Creating shipping layouts, Approving shipping layouts, Creating WGA shipping layouts from Qiagen plates, CDT Shipping Aliquot Boxes to Logistics
26. SOP M024: Scinomix Tube Labeler

It is possible to substitute disposable materials and certain equipment from other vendors as long as they are equivalent to the item described above.

III. PROCEDURE-STEPWISE

A. Creating a Shipping List/ QC Shipping List

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

1. Open Excel and click on the Data Tab. Click on "From Other Sources." Click on "From SQL Server."
2. Under Server Name type: **resbcrdb**. Click on the drop down and select "BCR."
3. Double click on "vwQCSampleSummaryMGL" and click "Finish." Click "Yes."
4. View the data as a table and click "OK."
5. Filter the data by Batch and select the pseudobatches being shipped. Copy this information into a new Excel File. Save the file to \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Shipping layout checklist under a folder labeled with the date of the shipment. Name the Shipping Layout Checklist file with the date and "shipment." This is the MGL Shipping Review File.
6. The Clinical Outreach Shipping Review File must be created from the MGL Shipping Review File. Save only the following columns from the MGL file:

BatchID	tcgaBarcode	sampleID	studyname	studysubtypeName	organ site	NormalAnatomicSiteOfCase	tssSubjectID	ocStudySubjectID	tssID

7. Save the file to \\rex\BPC\TCGA-BCR\Clinical Outreach\Pre-shipping Review.
8. Email Clinical Outreach to begin their QC of each case (SOP C032 "Quality Control of Case Qualification Metrics for BCR Analyte Shipments").
9. Email BCR Informatics for a request of Batch Numbers. Give them the name of the studies that will be shipped.
10. While Clinical Outreach QC's the samples, Molecular will QC simultaneously using the MGL Shipping Review File created in step 5. Sort this file accordingly to examine yields and RIN's for samples in the Pseudobatches.
11. After Molecular and Clinical outreach review to confirm all analytes meet metrics for distribution per specific project requirements (project specific metrics are found on the BCR SharePoint under the Project and QMS folder), the Pseudobatch can be renamed with the Batch Number as assigned by Informatics.
12. Open the MGL Shipping Review File and copy all Sample IDs into a new tab. Save the tab as a CSV file to be imported into LabVantage.
13. Log into LabVantage.
14. Under Sample Management, click on "Lab Operations Samples."
15. Click on the "Import List" button. Click on "browse" and select the CSV file. Click on "Open."
16. Refresh the page and the list will appear in a new folder. Click on the folder.
17. Sort the folder by Study. Select one study at a time to change the batch number.

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

18. Click on "Update Batch Number." Type in the new Batch Number for that specific study given by Informatics. Click "OK."

B. Setting up Matrix Plates

1. Export the imported list from LabVantage by selecting all the samples and clicking "Export to Excel" on the left. Sort the Excel file to have all DNA on one tab and all RNA on another tab. Arrange the samples by study and characterization center.
2. Retrieve DNA/RNA from their storage locations; arrange the RNA in an empty Matrix Rack and the DNA in another Matrix rack.
    a. For smaller shipments, DNA tumors are arranged in columns 1-6 in groups by study/sequencing center and normals are arranged in columns 7-12 that correspond to the tumor.
    b. For larger shipments, normals are placed on one plate and all tumors on another plate with corresponding positions.
    c. If possible keep row H empty so the same shipping layout may be used to create the Qiagen plate (if applicable).
    d. Note any discrepancies in storage location, tube condition, or other abnormalities on the shipping checklist. Discrepancies must be reconciled prior to distribution. If a specimen is missing, LabVantage must be updated to reflect that the specimen cannot be located.
3. Confirm there is enough volume to send each analyte to the recipients as required by the project metrics (located on the BCR SharePoint in the Project and QMS folder). Make sure the quantity (volume) of each sample is sufficient for shipping to all required centers. Document gross discrepancies between volumes recorded in LabVantage and the physical tube on the shipping checklist.
4. Once all DNA/RNA samples are arranged in Matrix Plates, begin the shipping process in LabVantage.
5. Go to the BCR Home Page in SharePoint. Click on Shared Document on the left. Click on document labeled "NCH BCR Sequencing and Characterization Center Plating Checklist-v3."
6. Click "OK." Document should open in Word. Print document and close.
7. Create shipping layouts for the shipment by referencing the LabVantage Manual in the section "Creating shipping layouts."
8. If applicable create shipping layouts for WGA samples that will be shipped to downstream centers by referencing the LabVantage Manual in the section "Creating WGA shipping layouts from Qiagen plates."
9. Email the senior laboratory technologist ("QC Person") who will be verifying and approving the shipment with notification that the shipping layout is ready for review. Specify the shipping layout number. The QC Person may approve the layouts by referencing the LabVantage Manual in the section "Approving shipping layouts."

C. Creating Shipping Aliquots

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

1. Once the shipping layout is approved in LabVantage, reference the section in the LabVantage Manual "Creating Shipping Aliquots" or "Creating WGA shipping layouts from Qiagen plates", whichever is applicable.
2. Set up the empty matrix rack with the tubes in the appropriate locations based on the Aliquot Box created from the Shipping Layout.
3. Label the matrix rack with "NCH Plate ID XXXX, Batch XXX"
4. Create a folder in N:\Molecular Genetics\BCR\Matrix Tube Barcode Scans labeled with the batch numbers. (i.e. Batch 1).
5. Open up the Visionmate 96 software. Make sure it is in "rack mode." Go to the excel tab and change the directory to your batch folder created above.
6. Scan the matrix plate. A pop up will appear asking how to name the file. Use the plate ID written on the rack (i.e. A235). Open the excel file that is created with the RVSI barcode positions and select "text tab delimited" file extension and save on the Import drive on 'reslabvantage.'
7. After Matrix racks have been scanned and saved in the Labvantage Import folder, RVSI numbers are added to the Aliquot boxes:
    a. Log on to LabVantage. Under Storage, click on the "Boxes" page. Find your Aliquot Box, check the box, and click on the "Add RVSI Alias" button. This will link the unique RVSI barcode to a specific aliquot of a sample being shipped.
    b. Find the .txt tab delimited file corresponding to the Aliquot Box. Click "Open."
    c. If import was unsuccessful double check that your tubes are in the correct orientation. Repeat steps 6/7 if necessary.
    d. Select the Aliquot Box. Click on "Update TCGA Number." This will complete the process of attaching the PlateID at the end of the CCG BCR ID. This can only be done one Aliquot Box at a time. *NOTE: Only click this once! If you click more than once the TCGA number will be updated that many times.*
8. Repeat for all remaining aliquot boxes.

D. Printing Labels on Aliquot Matrix Tubes

1. See Scinomix Tube Labeler SOP (M024: Scinomix Tube Labeler) for details on labeling the tubes.
2. Once the tubes are labeled, the labels are QC's by visually verifying that each of the tube labels are clearly printed.
    a. If a tube label is not printed completely, reprint the label per SOP M024.
3. Once the visual QC step is complete and labels are re-printed if necessary, scan the rack with the Visionmate software (as stated in step 6 under the Creating Shipping Aliquots in LabVantage section).
4. Open the excel file. Copy the barcodes from the second tab and paste it in column E of the first tab of the initial scan. In column F enter the formula "= E=C". Drag this formula down and make sure it covers all of the barcodes. All of the values for this column should be TRUE. If any value displays FALSE, check for swapped matrix tubes and rescanning the matrix rack to resolve.

E. Print Paperwork for Downstream Centers

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

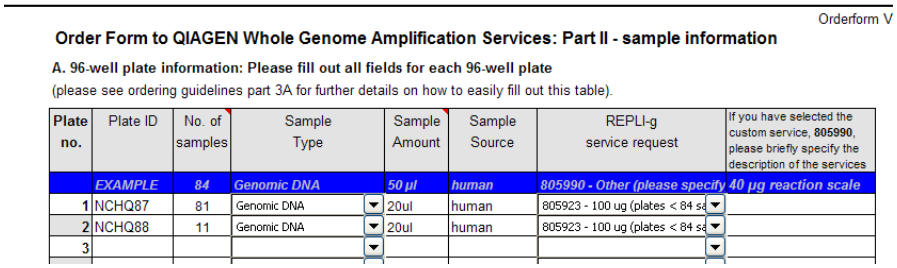
1. Samples need to be created as aliquots in LabVantage one day before paperwork can be printed.
2. Go to BCR SharePoint.
3. Go to Reports, Click on "shippingforms."
4. Type in the PlateID. Click on the Calendar and select the date of shipment.
5. Click the Apply button.
6. Click on the "Actions" drop-down. Highlight over Export and click on "Excel."
7. Click on Sheet 5.
8. Print on 11x17 paper to a colored printer.
9. Create a folder in \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Shipping Forms labeled with the Batch Numbers. Save the EXCEL file as "Location (shipping destination) and Batch Number."
10. Return to the webpage that has the shipping form.
11. Click on the "Actions" drop-down. Highlight over Export and click on "PDF."
12. Print the pages containing the Audit Checklists, QC Checklists, and the Matrix Well Plate Form. If there are no problems with the Audit, these pages will appear on Page 1, 5, 9, 13, 17.
13. Save the PDF file as "Location (shipping destination) and Batch Number" in the folder created in \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Shipping Forms.
14. QC Shipping forms using the Shipping Layout Checklist printed from the PDF. Date and initial the checklist if correct. The Shipping Layout Checklist defines all items specifically reviewed prior to shipping.
15. Give the checklist and shipping forms to Quality Management.
16. Quality Management will review and give the checklist and shipping forms to Informatics.
17. Once all shipping documents have been approved, the paperwork is returned to Molecular for release with final signature.

F. Print Paperwork for Qiagen – if applicable

1. Open the file "Template for Creating Qiagen Shipping Form.xlsx" which can be found at \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen
2. Log-in to LabVantage.
3. Use the Shipping Layout Checklist to retrieve the Aliquot Box number for the Qiagen plates.
4. Under LabOperations, search for bxSorted.
5. Type in the Aliquot Box number. Click OK. All the samples will appear.
6. Select all the samples. On the left, click on "Export to Excel."
7. Delete all the columns between TCGA ID and Location.
8. Copy the columns TCGA ID and Location. Paste this into columns A and B (respectively) on the Template for Creating Qiagen Shipping Formopened in step 1.
9. Paste Column A into Column C.

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

10. Update the Box Number in Column F.
11. All the samples will appear in the correct location in Column J.
12. Open the "Repli_g_Orderform0907_template.xls" found on \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen.
13. Click on the tab titled "PartII Samples."
14. Enter the appropriate information under the "A. 96-well Plate Information."
    a. The Plate ID and number of sample will always be different.
    b. Sample Type – Genomic DNA
    c. Sample Amount – enter appropriate volume
    d. Sample Source – human
    e. REPLI-g service request – select appropriate request
    f. Last column is always left blank



Orderform V

Order Form to QIAGEN Whole Genome Amplification Services: Part II - sample information

A. 96-well plate information: Please fill out all fields for each 96-well plate

(please see ordering guidelines part 3A for further details on how to easily fill out this table).

Plate no.	Plate ID	No. of samples	Sample Type	Sample Amount	Sample Source	REPLI-g service request	If you have selected the custom service, **805990**, please briefly specify the description of the services
	EXAMPLE	*84*	*Genomic DNA*	*50 µl*	*human*	*805990 - Other (please specify*	*40 µg reaction scale*
1	NCHQ87	81	Genomic DNA	20ul	human	805923 - 100 ug (plates < 84 s	
2	NCHQ88	11	Genomic DNA	20ul	human	805923 - 100 ug (plates < 84 s	
3							

15. Copy Column J from the "Template for Creating Qiagen Shipping Form.xlsx" file into the Sample Names column for that specific plate on "Repli_g_Orderform0907_template.xls" file.
16. The number of samples for each Plate can be found in LabVantage when you selected the samples.
17. Save file as "Repli_g_Orderform0907_BatchXXX,XXX.xls"
18. Click on tab Part I. Add the Purchase Order number provided by Program Office. Print this page.
19. Click on tab PartII. Print page 1-3 if you only have one Qiagen plate. Print pages 1-5 if you have two Qiagen plates (and so on).
20. Submit the excel form to a senior technologist for QC and form approval. Inform logistics you are ready to QC the shipment.

G. Aliquot the Stock DNA/RNA into Aliquot Matrix Racks

If aliquots are needed for shipping Stock DNA to Qiagen (described in H below) the Qiagen plates can be made simultaneously when aliquoting DNA/RNA into Matrix Racks.

1. Remove the Matrix rack containing the stock shipping samples from the liquid nitrogen or -80°C freezer. Keep RNA on wet ice to thaw; immediately transfer the RNA to dry ice when aliquoting is finished. DNA samples can be thawed at room temperature.
2. When the samples are completely thawed, centrifuge the stock shipping samples briefly to collect all the liquid at the bottom of the tube.

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

3. Use the Cap-it-All to de-cap the Matrix rack containing the empty aliquot tubes. Re-cap the caps from the aliquot tubes onto a clean, empty Matrix tube rack for storage while aliquoting is being performed.
4. Use the Cap-It-All to remove the caps of the tubes in the Matrix rack containing the stock shipping samples. All samples will be kept uncapped long enough to aliquot the samples. These caps are kept on the Cap-it-All until aliquoting is complete and the stock tubes can be recapped.
5. Transfer the stock samples to the appropriate wells of the aliquot Matrix rack. One technologist transfers the analytes using a multichannel pipet, one column at a time, while the second one observes. The second quality control technologist will confirm that the height of the volume aspirated is the same in all of the multichannel tips and that all liquid has been dispensed from each tip into the appropriate well.
6. If applicable – continue to step H "Aliquot the stock DNA for Qiagen."
7. Use the Cap-It-All to recap the stock sample Matrix rack, confirming correct matrix plate orientation before recapping.
8. Use the Cap-It-All to de-cap the caps from the empty Matrix tube rack. Recap these caps onto the aliquot Matrix rack.
9. In the event that there is insufficient sample to meet the requested volume, the actual volume provided will be entered in LabVantage.
10. Spin down the aliquot Matrix rack before freezing in a -80°C freezer, where the aliquot Matrix rack will be stored until the entire shipment is ready.
11. Return RNA and DNA stock tubes to appropriate storage location.

H. Aliquot the Stock DNA for Qiagen – if applicable

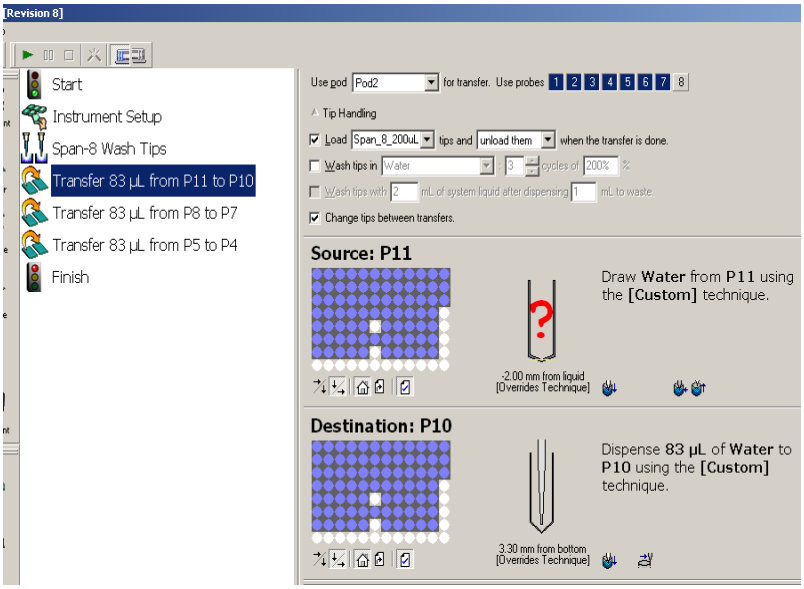
1. Label a skirted 96-well plate with the NCHQXXX identifier.
2. Transfer the stock samples to the appropriate wells of the skirted 96-well plate. One technologist transfers the analytes, one column at a time, while the second one observes. The second quality control technologist will confirm that the height of the volume aspirated is the same in all of the multichannel tips and that all liquid has been dispensed from each tip into the appropriate well. Note: Unless requested otherwise by the Project Sponsor, 2uL of DNA at 160ng/uL ± 15ng/uL plus 18uL of distilled H2O will be shipped to Qiagen. Qiagen suggests sending 250ng at 5ng/uL but will accept anything from 1ng/uL to 100ng/uL.
3. Use the Cap-It-All to recap the stock sample Matrix rack.
4. Cover the Qiagen plate with a foil adhesive and heat seal. NOTE: Samples shipped under liquid nitrogen vapor require heat-sealable foil adhesives.
5. Spin down the skirted 96-well plate before freezing in a -80°C freezer, where the plate will be stored until the entire shipment is ready.

I. Aliquot the WGA DNA into Aliquot Matrix Racks – if applicable

1. Open the Biomek Software.
2. Open the File "WGA Transfer 1 Plate Span 8."

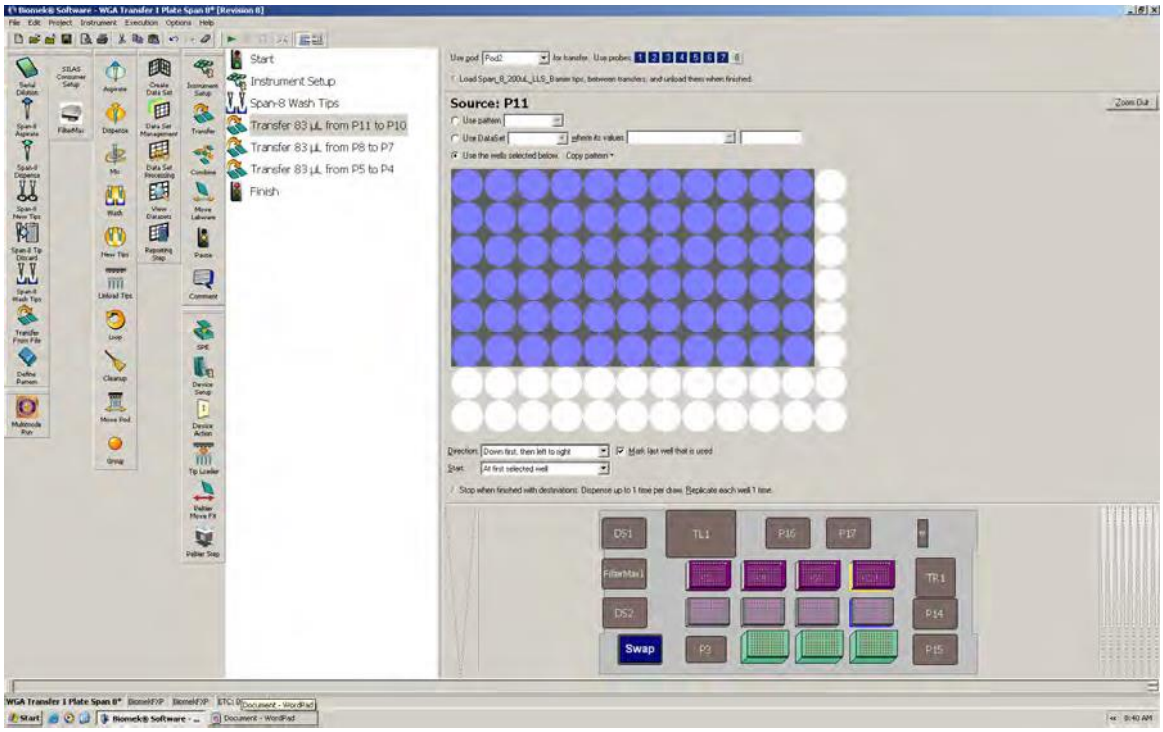
STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

3. Only three WGA plates may be aliquoted at one time. Disable the step "Transfer 83 µL from P5 to P4" if there are only 2 WGA plates to aliquot. Also, disable "Transfer 83 µL from P8 to P7" as well if there is only 1 WGA plate to aliquot.
4. Click on "Transfer 83 uL from P11 to P10."



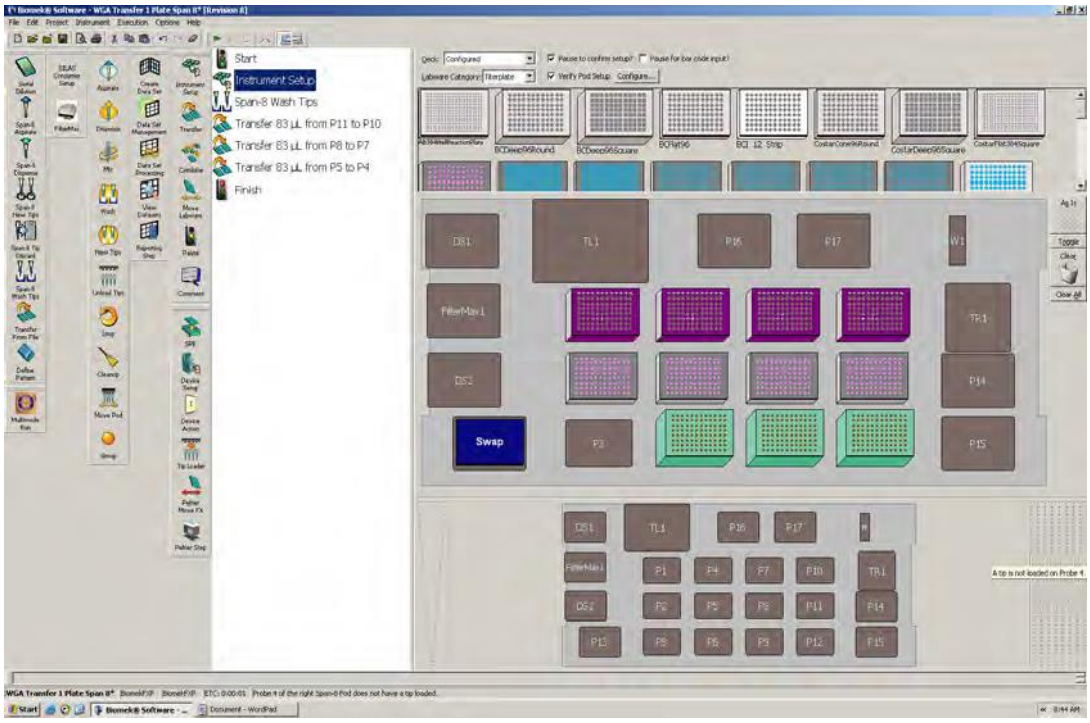
5. Double-click on the Source: P11 Plate. This will allow you to select the wells intended for aliquoting. Use the Qiagen Form printout to determine which wells to select. Drag and hold to select. If you hold the Shift Key, you can select other wells. If hold the Ctrl Key, you can deselect the wells.
6. Click on Zoom Out.

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS



7. Double-click on the Destination: P10 Plate. Use the Qiagen Form printout to determine which wells to select. The same wells should be selected as on the Source: P11 plate.
8. Click on Zoom Out.
9. Repeat Steps 5-8 as needed for additional plates (Transfer 83 µL from P8 to P7 and Transfer 83 µL from P5 to P4).
10. Centrifuge stock WGA plates. Use the Cap-It-All to decap the matrix rack (and recap these tops onto an empty matrix rack).
11. Open the WGA plate carefully, without splashing.
12. Set up the deck to reflect the layout below.
    i. Dark purple plate – Represents the Matrix Aliquot plate
    ii. Light purple/gray plate – Represents the WGA plate nestled into a UV-Star 96 well plate and held into place with tape
    iii. Green tip box – Represents the Conductive Barrier P250 tips

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS



13. Once the deck is set up to reflect the number of plates being aliquoted, a senior technologist will QC the Well Layouts for each transfer step.
14. After the deck has been QC'd by a second technician, click on Instrument > Home All Axes. Follow the prompts and make sure there are no bubbles present in the tubing connected to the Span 8 head.
15. Click on the "Play" button at the top of the screen to initiate transfer program.
16. When the program has finished, re-seal the WGA plate and recap the matrix tubes.
17. The aliquot matrix rack is labeled with a Tech label and includes NCH PlateID XXXX and Batch XXX. The WGA plate seal is initialed and dated by both the plating and QC technicians.
18. Aliquoting can also be performed by hand while observed by a second QC technologist.

J. CDT Shipping Aliquot Boxes to Logistics

1. Reference the LabVantage Manual section "CDT Shipping Aliquot Boxes to Logistics."

K. Communication with Sites Receiving Samples

1. GCC/GSC Sites
   a. Prior to shipping samples, the GCC/GSC coordinators will be contacted to confirm their facility is available to accept delivery.
   b. On the day of shipment, the GCC/GSC coordinators will contacted to notify them that samples are being shipped. This notification will include relevant package

tracking information and a shipping manifest to document the contents of the shipment and the unique identifiers on each sample. This includes the human readable CCG BCR ID printed on the sample tube label (side) as well as the RVSI barcode (bottom). The RVSI is a unique identifier that links to a specific tube (aliquot) shipped to a center on a specific date.

c. To maintain a feedback loop from investigators regarding the QC/QA of specimens produced and stored at the BCR, the following language will be included in the shipping notification email:
"Please let us know if you have any questions or feedback on the samples after they arrive."

d. When feedback is received from recipients, the GCC/GSC coordinators will forward the information to the BCR Quality Manager and, if appropriate, initiate a full investigation into the matter.

e. Receiving institutions are required to confirm receipt by faxing the BCR a copy of the signed Matrix Well Form.

2. Qiagen – If applicable

a. On the day of shipment, the CCG BCR shipping coordinator will contact Qiagen to notify them that samples are being shipped. This notification will include relevant package tracking information and a shipping manifest to document the contents of the shipment and the unique identifiers on each sample.

b. Qiagen will return the cryoport empty and ship the WGA samples to the BCR on dry ice or via a cryoport according to their standard protocol.

c. Qiagen will provide a "Whole Genome Amplification Service Report" indicating that the samples passed their internal quality control criteria. This report will be used in BCR SOP M0006 "Normalization of Whole Genome Amplification Samples"

d. The Project Sponsors will be notified via email if WGA is deemed "unusable" or "synthesis failures" by Qiagen standards for any samples. In this event the BCR will send a replacement sample to Qiagen in the next shipment.

e. If Qiagen deviates from their SOP in the whole genome amplification or in the receipt, handling, and/or shipping of the samples, then they will provide a deviation report detailing the deviation and the number of samples affected will be noted.

f. After normalization (using SOP M006 "Normalization of Whole Genome Amplification Samples"), SNP analysis is performed (refer to SOP M010 "Tissue Matching by SNP Analysis"), and passing WGA samples are shipped to the centers of interest.

IV. REFERENCES

A. BCR SOP M024 "Scinomix Tube Labeler"

B. BCR SOP C032 "Quality Control of Case Qualification Metrics for BCR Analyte Shipments"

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

C. BCR SOP "LabVantage Manual"
D. BCR SOP M0006 "Normalization of Whole Genome Amplification Samples"
E. BCR SOP M006 "Normalization of Whole Genome Amplification Samples"
F. BCR SOP M010 "Tissue Matching by SNP Analysis"

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 1/7/2015

1. Updated formatting
2. Updated disclaimer
3. Added SOP M009 (Whole Genome Amplification) to this procedure
4. Added SOP M007 (Quality Control Plating) to this procedure
5. Removed any reference to TCGA
6. Minor word changes
7. Updated LabVantage images
8. Updated locations of files used throughout the procedure
9. Add the section "Aliquot the WGA DNA into Aliquot Matrix Racks – if applicable"
10. Removed LabVantage steps where applicable and placed them in the LabVantage Manual SOP A005.
11. Added to the Materials section to include the SOP's that go along with this SOP
12. Included language of where to find project specific metrics

B. Version 1, Effective Date 9/14/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR SHIPPING ANALYTES TO DOWNSTREAM CENTERS

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA Extraction with AllPrep (DNA) and mirVana (Total RNA with small RNA) 3 Column-Modified Melanoma Protocol

I. SCOPE AND PURPOSE

Qiagen AllPrep kits are designed to isolate DNA and total RNA from small quantities of starting material. In addition, they provide a fast and simple method for the preparation of DNA and column purified RNA from human tissues. The purified DNA and RNA are ready for use in standard downstream applications such as DNA amplification and expression arrays.

A representative sampling of the total RNA content within the tumor tissue homogenate, most notably the low molecular weight species, can be used for micro RNA analysis. Therefore, the flow through from the AllPrep DNA column is taken and the total RNA is isolated with the *mir*Vana kit from Life Technologies.

The *mir*Vana™ miRNA Isolation Kit was designed for purification of RNA suitable for studies of both siRNA and miRNA in natural populations. The kit employs an organic extraction followed by immobilization of RNA on glass-fiber filters to purify total RNA. The *mir*Vana miRNA isolation procedure combines the advantages of organic extraction and solid-phase extraction, while avoiding the disadvantages of both. High yields of ultra-pure, high quality, small RNA molecules can be prepared in less than two hours.

This protocol has specifically been prepared to reduce melanin associated with RNA isolated from melanoma tumor samples. Melanin is a naturally occurring cation exchange material known to intercalate with DNA and crosslink with RNA under oxidative conditions (White, 1958; Geng, 2010; Wilkins, 2006). Removal of melanin from RNA derivatives is important as melanin interferes with mRNA/miRNA analysis by reversibly binding to DNA Polymerase and inhibiting RT-PCR (Eckhart, 2000). Furthermore, melanin complicates quantitative approaches for determining RNA concentration by interfering with UV absorbance and fluorescence emissions.

This protocol varies from the standard AllPrep *mir*Vana protocol in that it includes an overnight incubation of the mirVana eluted RNA with the cationic detergent cetyl-trimethylammonium bromide (CTAB)-urea. CTAB is widely used to separate proteins and polysaccharides from nucleic acids, as it selectively forms complexes with anionic nucleic acids (Lagonigro, 2004). This results in solution where charged nucleic acids can be purified from neutral proteins and polysaccharides. Urea is included in the solution as it has been found to enhance the specificity of the reaction (Lagonigro, 2004). In this protocol, melanotic RNA is complexed with CTAB-urea to facilitate isolation of charged RNA from the insoluble uncharged melanin.

II. PROCEDURE

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA Extraction with AllPrep (DNA) and mirVana (Total RNA with small RNA) 3 Column-Modified Melanoma Protocol

A. Safety precautions

1. Wear Personal Protective Equipment (PPE), including a lab coat, goggles or face shield, closed-toe shoes, and nitrile gloves. Liquid nitrogen and dry ice are extremely cold and may cause 'burns.' Wear cryogenic gloves designed to withstand extremely cold temperatures when handling samples stored in liquid nitrogen and large quantities of dry ice.
2. Bloodborne pathogens can be present in the unfixed frozen tissue (refer to SOP S009, "Bloodborne Pathogen and Exposure Control Plan" found in the BCR Safety Manual). Use all universal precautions.
3. Liquid nitrogen is an asphyxiate; all work should be conducted in a well-ventilated room.
4. 2-mercaptoethanol (2-ME) is toxic. The neat stock solution should be opened in a fume hood only. Solutions containing 1% or less of 2-ME may be used outside of a fume hood. PPE must be used when handling any solution containing 2-ME.
5. Buffer RLT Plus and Buffer AW1 contain a guanidine salt which is not compatible with disinfectants containing bleach.
6. miRNA Wash Solution included in the *mir*Vana kit contains guanidinium thiocyanate, this is a potentially hazardous substance and should be used with appropriate caution.
7. Acid-phenol:chloroform contains phenol, which is a poison and an irritant. Use gloves and personal protective equipment when working with this reagent.
8. CTAB solubility: 0.3 g/100 mL dH_2O at 20°C, up to 10% by heating to 65°C.

B. Quality Control:

1. The incoming tissue samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID
2. Working labels are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original portion and subportion LabVantage ID and the corresponding newly created DNA or RNA LabVantage IDs. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels are printed for storage in Matrix 2D barcode tubes..
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Samples are tracked in LabVantage. Every portion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample, LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.
6. DNA and RNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. RNA quality is measured by RIN using Agilent Bioanalyzer (SOP M002, "RNA Nano Assay") and quantified by Spectrophotometer (see SOP MGL-EQP-6 "BIO-MATE UV-Visible

Spectrophotometer). DNA quality is evaluated for integrity by agarose gel electrophoresis (SOP M003, "Gel Electrophoresis with the E-gel System"), quantified by PicoGreen Assay (see SOP M017, "Picogreen DNA Quantification Manual") and genotypic identity using SNP loci (SOP M010, "Tissue Matching by SNP Analysis"). Primary stock aliquots should not be subject to numerous freeze thaw cycles.

7. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
8. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
9. The isolation kit is tested against predetermined specifications to ensure consistent product quality.
10. All new lots of reagents are tested in parallel with the one in current use before being put into clinical use. Results are recorded on positive control extraction worksheets.
11. The CTAB-UREA solution is made fresh with each extraction. This solution must be used on a control melanoma sample once a week when any melanoma extraction is completed. The control melanoma samples are highly pigmented samples, therefore after using the CTAB-UREA one must document the change in color of the flow-through from Day 1 and Day 2 of the procedure. Due to the limited number of control melanoma samples, it is not necessary to run a control each time an extraction is performed.
12. At each step in the RNA and DNA isolation, the supernatant or pellet that should not contain the RNA or DNA is retained until after isolation and quantitation is completed.
13. RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.
    a. Bench space is wiped down at the beginning of each extraction session with RNase Zap. Pipettes are wiped down with RNase Zap once a week or as needed.
    b. Gloves should always be worn throughout the process and should be changed frequently.
    c. Only sterile, disposable plasticware and pipettes that are dedicated strictly to RNA work should be used to prevent cross-contamination with RNases from shared equipment.
    d. Containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNase-free.

C. Specimen information:

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1. Type: Frozen Melanoma tumor or adjacent normal tissues.
2. Handling Conditions: Follow standard precautions when handling all tissues. Samples should be stored in liquid nitrogen until analytes can be isolated.
3. Sample Preparations: Tissues are prepared by Logistics by cutting 25-30 mg pieces of frozen tissue and placing into a 2-mL Eppendorf lock tube.
4. Indications for Study: This procedure should be used when DNA and RNA are needed from the same piece melanoma tissue. DNA is isolated from the AllPrep DNA column and Total RNA including small RNAs is derived from the *mir*Vana isolation kit.

D. Required Equipment, Supplies and Reagents:

1. Equipment

UV visible spectrophotometer
Capsule centrifuge
Digital dry bath
Liquid nitrogen freezer
Freezer (-20^{o}C)
Microcentrifuge, Eppendorf 5415 R
Multi-channel and single channel pipettes
Refrigerator (4^{o}C)
Qiagen TissueLyser
Vortex
Water bath

2. Supplies

AllPrep DNA/RNA Mini Kit (Qiagen 80204)
*mir*Vana™ miRNA Isolation Kit (Ambion, Cat# AM1560)
Filtered, sterile pipette tips, assorted sizes
1.5 mL Eppendorf tubes (Fisher, 05-408-137)
0.5ml tubes (Fisher, #05-408-128)
2 mL screw cap tubes (Fisher, Cat# 02-707-355)
2 mL SafeLock Eppendorf tubes (Fisher, Cat#022363352)
Wet and dry ice
Insulating trays for dry ice
Personal protective equipment (PPE) including insulated gloves
Stainless steel beads, 5 mm (Qiagen, Cat# 69989)

3. Reagents

2-mercaptoethanol (2-ME), 100% (Sigma M3148-100 mL)
Absolute ethanol, molecular grade (Sigma, E7023)
Diethylpyrocarbonate (DEPC)-treated water (Invitrogen, 750023)
Tris-EDTA Buffer (1X) (Sigma T9285, 100 mL)
Sodium hydroxide (Sigma S8263, 150 mL)
Reagent DX (Qiagen 19088, 1 mL)

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RNase ZAP (Ambion, M9780)
5M NaCL (Ambion AM9760G, 100mL)
CTAB, Hexadecyltrimethylammonium bromide, BioUltra, >=99.0%, (Sigma 52365-50G, 50g)
Urea, Ultrapure MB grade (Ambion AM9902, 1kg)
0.5M EDTA pH 8.0 (Ambion AM9260G, 100mL)
1M Tris:Cl pH 7.0 (Ambion AM9850G, 100mL)
Water, Molecular Biology Reagent, DNase/RNase Free (Sigma W4502, 1L)

Notes: It is possible to substitute disposable materials and certain equipment from other vendors as long as they are the equivalent of the item described above.

In the event that a reagent or disposable item either becomes contaminated or is even suspected to be contaminated, it must be discarded.

E. Reagent preparation (including storage conditions):

1. 2-mercaptoethanol (2-ME) – must be added to Buffer RLT Plus before use (final 1% 2-ME). Buffer RLT Plus is stable at room temperature (15-25°C) for 1 month after addition of 2-ME.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%), as indicated on the bottle, to obtain a working solution. Buffer AW1 and Buffer AW2 are stable for one year.
3. Add 21 mL 100% ethanol to miRNA Wash Solution 1 before use. Add 41 mL 100% ethanol to miRNA Wash Solution 2/3.
4. miRNA Wash Solution can be stored at room temp for up to 1 month. For longer term storage, store at 4ºC, but warm to room temperature before use.
5. Store Elution Solution at -20ºC, 4ºC or room temp.
6. An aliquot of DEPC water needs to be heated to 95ºC for elution step.
7. 10 M Urea solution: Make at least 1 day prior to homogenizing the specimen(s): 30.035 g Urea, bring up to 50 mL with RNAse-free dH_20; Sterile filter 0.2 μM; store at room temperature. Heat to 37°C to re-solubilize. It is stable up to 6 months.
8. 10% CTAB solution: Make at least 1 day prior to homogenizing the specimen(s): 5.0 g CTAB, bring up to 50 mL with RNAse-free dH_20, heat to 37°C to dissolve, sterile filter 0.2 μM store at room temperature; stable up to 6 months. Heat to 65°C to re-solubilize.
9. CTAB-UREA solution (50 mM Tris-HCl pH 7.0, 1% CTAB, 4 M Urea, 1mM EDTA): Make fresh same day. Add together in sterile conical tube: 1.25 mL 1 M Tris-HCl pH 7.0; 2.50 mL 10% CTAB; 10.00 mL 10 M Urea; 0.05 mL 0.5 M EDTA and 11.20 mL RNAse-free water to obtain a final volume of 25 mL working solution. Keep at 25°C -30°C (to avoid precipitation) until just before use. It is stable less than 3 days.

F. Homogenization

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1. Remove tissues from liquid nitrogen storage and place in dry ice.
2. Add 600 µL Buffer RLT Plus containing 1% 2-ME to each 2 mL safe lock Eppendorf tube containing 25-30 mg of tissue and immediately place in a rack at room temperature.
3. Add 3 µL Reagent DX and one 5 mm stainless steel bead to each tube.
4. Place the tubes (up to 48) in the TissueLyser Adapter Set, making certain the machine is balanced, and operate for 2 min at 20 Hz.

   Note: Prepare a maximum of eight tubes per laboratory technician and begin homogenization in under five (5) minutes to minimize RNA degradation. Excursions above five minutes should be noted on the isolation worksheet.
5. Disassemble the adapter set. Remove tubes from adapter and observe for homogenization.
6. If samples are not completely homogenized, rotate the rack of tubes so that the tubes nearest the TissueLyzer are now outer most and reassemble the adapter set. Rearranging the tubes ensures uniform disruption and homogenization.
7. Operate for another 1 min at 20 Hz.
8. Repeat step 5.
9. If samples are still not completely homogenized, operate for another 1 min at 20 Hz. The duration of disruption and homogenization depends on the tissue being processed. If processing fiber-rich tissues, complete disruption and homogenization may sometimes not be possible.
10. Remove tubes from TissueLyser.
11. Spin tubes down briefly in microcentrifuge and transfer homogenate to a clean, labeled 1.5 mL Eppendorf tube. Do not reuse the stainless steel beads.
12. Centrifuge the homogenate for 3 min at maximum speed (16,100 x g).
13. Carefully remove the supernatant from each sample by pipetting, and transfer it to the AllPrep DNA spin column placed in a 2 mL collection tube (supplied in the AllPrep kit). Avoid aspirating any solids or debris.
14. Close the lid gently, and centrifuge for 30 seconds at 8000 x g.
15. Place the AllPrep DNA spin column into a new, 2 mL collection tube. At this point, the DNA isolation can continue with step I1, or the columns can be stored in the refrigerator for up to 18 hours for later isolation. Use the flow-through for the RNA purification.

G. Total RNA purification using the *mir*Vana kit

1. Transfer the flow-through from each DNA column into a separate labeled 2 mL screw cap tube. Adjust the volume of each sample to 600 µL with Buffer RLT Plus containing 1% 2-ME.
2. Add 60 µL (1/10 volume) of miRNA Homogenate Additive to each flow-through, and mix well by vortexing or inverting the tube several times.
3. Leave the mixtures on ice for 10 min.

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4. Add 600 µL of acid-phenol: chloroform to each flow through (volume equal to the lysate volume before addition of the miRNA Homogenate Additive). Be sure to withdraw from the bottom phase in the bottle of acid-phenol:chloroform, because the upper phase consists of an aqueous buffer.
5. Vortex for 30-60 seconds to mix.
6. Centrifuge for 10 minutes at 10,000 x g at room temperature to separate the aqueous and organic phases. After centrifugation, the interphase should be compact; if it is not, repeat the centrifugation.
7. Begin heating DEPC-Treated water to 95°C.
8. Carefully remove the aqueous (upper) phase without disturbing the lower phase or interphase layers, and transfer it to a fresh labeled 1.5 mL tube. Note the volume removed.
9. Add 1.25 volumes of room temperature 100% ethanol to each aqueous phase and mix thoroughly by vortexing. Spin tubes down briefly in microcentrifuge.
10. For each sample, place a filter cartridge into one of the collection tubes supplied.
11. Pipet each lysate/ethanol mixture onto a filter cartridge. Up to 700 µL can be applied to a filter cartridge at a time. For samples larger than this, apply the mixture in successive applications to the same filter.
12. Centrifuge for 15 seconds at 10,000 x g. **Warning:** Spinning faster than this may damage the filters.
13. Discard the flow-through, and repeat until all of the lysate/ethanol mixture is through the filter. Reuse the collection tube for the washing steps.
14. Apply 700 µL miRNA Wash Solution 1 (working solution mixed with ethanol) to each filter cartridge and centrifuge for 5-10 seconds at 10,000 x g. Discard the flow-through from the collection tubes, and replace the filter cartridge into the same collection tube.
15. Apply 500 µL Wash Solution 2/3 (working solution mixed with ethanol) and centrifuge the filter cartridge for 5-10 seconds at 10,000 x g.
16. Discard the flow-through from the collection tubes, replace the filter cartridge into the same collection tube, and repeat step 15.
17. After discarding the flow-through from the last wash, replace the filter cartridge in the same collection tube and spin the assembly at 10,000 x g for 2 minutes to remove residual fluid from the filter.
18. Transfer the filter cartridge into a fresh collection tube (provided with the kit). Apply 100 µL preheated (95ºC) DEPC water to the center of the filter, and close the cap. Spin for 20-30 seconds at 10,000 x g to recover the RNA.
19. Bring the volume up to 100 µL with DEPC water.

H. CTAB-urea separation of RNA from Melanin

1. Set the filter cartridge aside and add 32.5 µL 5 M NaCl to the eluent and mix gently by inversion.
2. Add 400 µL CTAB-UREA (4 volumes) to the sample and mix gently by inversion.

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3. Incubate sample overnight at 4°C; slow rotation may help the detergent break up and capture the melanin. Make sure cap is tight. Place Parafilm around cap to keep it sealed.
4. The next day, retrieve the sample from the refrigerator and add 53 µL miRNA Homogenate Additive. Mix well by vortexing or inverting the tubes several times.
5. Incubate the mixtures on ice for 10 min.
6. Pipet each mixture onto a filter cartridge. Up to 700 µL can be applied to a filter cartridge at a time. For samples larger than this, apply the mixture in successive applications to the same filter.
7. Begin heating DEPC-Treated water to 95°C.
8. Centrifuge for 15 seconds at 10,000 x g. **Warning:** Spinning faster than this may damage the filters.
9. Discard the flow-through, and repeat until all of the mixture is through the filter. Reuse the collection tube for the washing steps.
10. Apply 700 µL miRNA Wash Solution 1 (working solution mixed with ethanol) to each filter cartridge and centrifuge for 5-10 seconds at 10,000 x g. Discard the flow-through from the collection tubes, and replace the filter cartridge into the same collection tube.
11. Apply 500 µL Wash Solution 2/3 (working solution mixed with ethanol) and centrifuge the filter cartridge for 5-10 seconds at 10,000 x g.
12. Discard the flow-through from the collection tubes, replace the filter cartridge into the same collection tube, and repeat step 11.
13. After discarding the flow-through from the last wash, replace the filter cartridge in the same collection tube and spin the assembly at 10,000 x g for 2 minutes to remove residual fluid from the filter.
14. Transfer the filter cartridge into a fresh collection tube (provided with the kit). Apply 100 µL preheated (95ºC) DEPC water to the center of the filter, and close the cap. Spin for 20-30 seconds at 10,000 x g to recover the RNA.
15. Remove the filter cartridges and place the samples on ice before proceeding to the RNA quantification step.

I. Genomic DNA Extraction

1. Add 500 µL Buffer AW1 to the AllPrep DNA spin column. Close the lid gently and centrifuge for 1 minute at 14,000 x g. Transfer the column to a clean 2 mL collection tube.
2. Add 500 µL Buffer AW2 to the AllPrep DNA spin column. Close the lid gently and centrifuge for 2 minutes at 14,000 x g to wash the spin column membrane.
3. Place one AllPrep DNA spin column per sample in the previously prepared 1.5 mL collection tube. Add 100 µL 0.1 X TE (10 mM Tris: 1 mM EDTA, pH 8.0) directly to the spin column membrane and close the lid. Incubate at room temperature for 1 minute and then centrifuge for 1 minute at 14,000 x g to elute the DNA.

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4. If multiple columns were required for a sample, combine all eluent into a single tube with a single channel pipette. Discard the empty tubes.
5. Check the samples in the designated PicoGreen plate in LabVantage and place in the corresponding PicoGreen plate in the refrigerator (4°C). The physical sample should be accompanied by an empty labeled matrix tube with final storage label for final transfer by the technician completing the picogreen quantification. Proceed to DNA quantification and normalization steps after multiple DNA samples are ready for quantification.

J. Quantification and Normalization of RNA Samples

1. Measure and record the volume of the RNA from the extraction procedure.
2. Prepare a set of 0.5 mL tubes with the unique RNA sample identifier. Add 98 µL of 50 mM sodium hydroxide to each tube.
3. Using a single channel micro-pipette, add 2 µL of the concentrated stock sample to the sodium hydroxide. Vortex for at least 5 seconds to ensure that the diluted sample is well mixed and briefly spin down. Read the absorbance for 260, 280 and 320nm in a spectrophotometer using a quartz cuvette.
4. Desired sample concentration may vary among projects.
    a. If the concentration is above the expected concentration, use the known volumes and concentrations to calculate the amount of DEPC-treated water to add to the sample to yield a final desired concentration as shown below in Example 1.
    b. If the concentration of the RNA sample is less than the desired concentration, use the known volumes and concentrations to calculate the amount to concentrate the sample to the desired volume shown below in Example 2. This should be accomplished with the use of a speed vac with no heat.
5. After diluting or concentrating samples, repeat step 3 to confirm that the sample is within the target concentration range.
6. Once the samples are at target concentration, transfer the liquid to a labeled matrix tube (Primary sample aliquot) for final storage. Create a 2.5uL aliquot for subsequent sample quality control assay (SOP M002, "RNA Nano Assay").

K. Quantification and Normalization of DNA Samples

1. Refer to SOP M017 for DNA quantification and normalization by PicoGreen.

L. Sample Storage

1. RNA samples should be stored in a liquid nitrogen freezer.
2. DNA samples should be stored in a -80°C freezer.

M. Sample Calculations

Example 1: Samples with concentrations > 0.165 µg/µL need to be diluted using:

<u>Normalization to 0.165 µg/µL</u>

((current concentration/desired concentration) x current volume) – current volume

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= ((0.21 µg/µL/0.165 µg/µL) x 18 µL) – 18 µL
= 4.9 µL (Volume of diluent to add)

Final sample volume = 22.9 µL

Example 2: Samples with concentrations < 0.165 µg/µL need to be concentrated using:

Normalization to 0.165 µg/µL
((current concentration/desired concentration) x current volume) – current volume
= ((0.08 µg/µL/0.165 µg/µL) x 18 µL) – 18 µL
= - 9.3 µL (Volume to be removed during speedvac concentration)

Final sample volume = 8.7 µL

III. REFERENCES

A. Allprep DNA/RNA Mini Kit Handbook (November 2005)
B. mirVana miRNA Isolation Kit Handbook (2011)
C. Lagonigro M, De Cecco L, Carninci P, Di Stasi D,Ranzani T, Rodolfo M, and Gariboldi M. CTAB–Urea Method Purifies RNA from Melanin for cDNA Microarray Analysis. Pigment Cell Research 2004; 17:3: 312–315.
D. Eckhart L, Bach J, Ban J, Tschachler E. Melanin Binds Reversibly to Thermostable DNA Polymerase and Inhibits Its Activity. Biochemical and Biophysical Research Communications 271, 726–730 (2000)

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 12/31/2014
1. Updated format
2. Removed any reference to TCGA
3. Removed any reference to concentration range
4. Removed the step of adjusting volume with RLT before the sample goes through the DNA spin column.
5. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
6. Removed the reference to pooling samples and re-extracting samples.
7. Added a reference section

B. Version 1, Effective Date 9/14/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA Extraction with AllPrep (DNA) and mirVana (Total RNA with small RNA) 3 Column-Modified Melanoma Protocol

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR NINE MARKER MICROSATELLITE INSTABILITY (MSI) ANALYSIS BY POLYMERASE CHAIN REACTION

I. SCOPE AND PURPOSE

Microsatellites are short, tandem repeat (STR) DNA sequences with repeating units from 1-6 base pairs in length. Microsatellites are distributed throughout the human genome, and individual repeat loci often vary in length from one individual to another. Microsatellite instability (MSI) is the change in length of a microsatellite allele due to either insertion or deletion of repeating units and a failure of the DNA mismatch repair (MMR) system to fix these replication errors. This genomic instability arises in a variety of human neoplasms where tumor cells have a decreased ability to faithfully replicate DNA. MSI is particularly associated with colorectal cancer where 15-20% of sporadic tumors show MSI, in contrast to the more common chromosomal instability (CIN) phenotype (seen in 65-70% of sporadic colorectal cancer tumors), with MSI status being an independent prognostic indicator. MSI analysis is also clinically useful in identifying patients at increased risk of hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch Syndrome, where a germline mutation of a MMR gene causes a familial predisposition to colorectal cancer. MSI analysis alone is not sufficient to make a diagnosis of a germline MMR mutation given the high rate of sporadic MSI positive colorectal tumors, but a positive result is an indication for follow-up genetic testing and counseling.

DNA from tumor tissue and corresponding adjacent normal tissue or normal blood is subjected to multiplex PCR using fluorescently-labeled primers for co-amplification of nine markers, including 4 mononucleotide and 3 dinucleotide repeat markers for MSI determination and two pentanucleotide markers (Penta D and Penta E) for establishing sample identity. The resulting PCR fragments are separated using capillary electrophoresis. Allelic profiles of normal versus tumor tissue are compared by two independent reviewers, and MSI is scored as the presence of novel microsatellite lengths in tumor DNA compared to normal DNA.

II. PROCEDURE

A. Safety Procedure

1. Use universal safety precautions when handling when handling all body fluids, tissues, and cell cultures; wear personal protective equipment (PPE).

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B. Quality Control

1. STR analysis is subject to contamination by very small amounts of non-template human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, setting up amplification reactions and analyzing amplification products.
2. Worksheets are used for cocktail component calculations. Lot numbers, concentrations and expiration dates of reagents used are recorded where applicable. Unusual observations in set up of assays are also noted on these worksheets. All control samples are tested at the same time, in an identical manner and by the same technologist as the CCG BCR samples included with each assay group.
   Aerosol barrier pipet tips are used for the set-up of all PCR reactions to prevent cross contamination.
3. PCR assays require separate areas for set-up and amplification. The DNA extraction is performed in the Pre-PCR room. PCR reactions are set-up in a separate room from the thermal cycler and post-PCR analysis area. No amplified products are allowed in the PCR set-up area.
4. Dedicated equipment (pipettes), filtered tips and other supplies are used in each area. Powder-free gloves are always used and goggles are used as needed.
5. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
6. All new lots of reagents are tested in parallel with the one in current use before being put into use; results are recorded in QC log. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
7. The threshold values (MSI-H, MSI-L, MSS) of a known case will be verified every six months if a new lot of reagents has not been tested within that time period.
8. For all assays, specimens should be ordered and set up in the following sequence: participant samples, positive controls, negative controls. This is done to minimize the chance for cross contamination of patient samples while providing the greatest chance for detecting contamination in the negative control.
9. Analytical Controls: Two controls are used for the MSI Analysis System:
   a. Negative Control: Nuclease Free Water. This blank control should contain no peaks in the markers being analyzed.
   b. Positive Control: DNA from the control cell line HCT 116 (positive for MSI) is set up with each run to ensure reproducibility of the MSI Analysis System and to ensure that capillary electrophoresis and analysis software are functioning correctly.
10. Amplification from any loci in control or patient samples must have amplification height greater than 100 intensity units. If the intensity if less than 100 units, the sample will be repeated.

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11. The control results are verified for acceptability before the data are released.
    a. Reactions that do not have acceptable results from analytical controls must be repeated.
    b. Annotations will be added to the setup worksheet for assays with unacceptable analytical controls and a link to the repeat test. For example, if MSI-92 demonstrated amplification from the negative control, the assay would be repeated as MSI-92b. The MSI-92 assay worksheet would be annotated to convey the assay failed for amplification of negative control and repeated as MSI-92b.
12. Some common situations that may cause analytically inaccurate results are PCR reagent contamination, capillary malfunction, and/or decreased fluorescent intensity.
13. Primers are light sensitive. All reagents, reactions, and products containing labeled primers should be protected from light when possible.

C. Specimen information

DNA isolated from matched normal and tumor samples.

E. Required equipment, supplies, and reagents

1. Equipment

Capsule Centrifuge
PCR Hood
Pipettes – adjustable
Thermal Cycler
Vortex Mixer
ABI PRISM 3730XL Genetic Analyzer
Heat Block (95°C)

2. Supplies

Aerosol Barrier Pipet Tips
Personal Protective Equipment (PPE)
MicroAmp Optical 96-well reaction plate (AB, N801)
Plate Septa-96 well (AB, 4315933)
ABI 3730XL 48-Capillary Array (AB, 4331247)
Ice
Microcentrifuge tubes, 1.5 mL

3. Reagents

Nuclease-Free Water (Fisher, BP2484-50)
HCT 116 Genomic DNA (10 ng/µL) (ATCC, CCL-247)
LIZ Size Standard (Applied Biosystems, Cat# 4322682)

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Matrix Standards Dye Set G5 (Applied Biosystems, Cat# 4345833)
Amplitaq Gold DNA Polymerase (Applied Biosystems Cat# N808-0249)
Hi-Di Formamide (Applied Biosystems Cat# 4311320)
ABI 3730XL Pop-7 Polymer (Applied Biosystems Cat# 4332241)
dNTPs (Roche, Catalog# 11-969-064-001)
50 mM $MgCl_2$ (Invitrogen Life Technologies, catalog# 10966-034)
10X PCR Buffer (Invitrogen Life Technologies, catalog# 10966-034)
10X Genetic Analyzer Buffer (Applied Biosystems, catalog# 402824)
GeneScan 500 LIZ internal lane standard

F. Reagent preparation (including storage conditions)

1. Oligonucleotide Primers for Human Loci amplified by the MSI Analysis System:

Marker Name	GenBank Number	Major Repeat Sequence	Size Range (bp)	Primer Dye
BAT-40	M38180	$(A)_x$	85-140	PET
BAT-26	U41210	(A)26	90-135	NED
BAT-25	L04143	(A)25	99-130	6-FAM
TGFBRII	Unigen HS: 82028		60-80	VIC
D5S346	181171	CA repeat	85-135	VIC
D17S250	177030	CA repeat	130-185	6-FAM
D2S123	187953	CA repeat	175-250	NED
Penta D	AC000014	$(AAAAG)_{2-17}$	376-449	6-FAM
Penta E	AC027004	AAAGA	379-474	VIC

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Marker Name	Forward Primer Sequence	Reverse Primer Sequence
BAT-40	PET-ATT AAC TTC CTA CAC CAC AAC	GTAGAGCAAGACCACCTTG
BAT-26	NED-TGACTACTTTTGACTTCAGCC	AACCATTCAACATTTTTAACCC
BAT-25	6FAM - TCGCCTCCAAGAATGTAAGT	TCTGCATTTTAACTATGGCTC
TGFBRII	VIC-CTTTATTCTGGAAGATGCTGC	GAAGAAAGTCTCACCAGGC
D5S346	VIC-ACTCACTCTAGTGATAAATCGGG	AGC AGA TAA GAC AGT ATT ACT AGT T
D17S250	6FAM-GGAAGAATCAAATAGACAAT	GCTGGCCATATATATATTTAAACC
D2S123	NED-AAACAGGATGCCTGCCTTTA	GGACTTTCCACCTATGGGAC
Penta D	GAAGGTCGAAGCTGAAGTG	ATTAGAATTCTTTAATCTGGACACAAG
Penta E	ATTACCAACATGAAAGGGTACCAATA	TGGGTTATTAATTGAGAAAACTCCTTACAATTT

III. PROCEDURE-STEPWISE

A. Polymerase Chain Reaction Set-Up:

1. Obtain the appropriate worksheet for the NCH 9 Marker MSI Assay.
2. Clean work area.
3. Prepare enough Primer master mix (PMM) for the plates to be run plus an additional 15%. Place primer master mix on ice.

Primer (Forward, Reverse)	Stock Concentration	Final Concentration	Volume (1ml total)
BAT25	100 µM	0.4 µM	4 µL F + 4 µL R
D17S250	100 µM	2.0 µM	20 µL F + 20 µL R
TGFBR2	100 µM	0.2 µM	2 µL F + 2 µL R
D5S346	100 µM	0.5 µM	5 µL F+ 5 µL R
BAT26	100 µM	0.36 µM	3.6 µL F+ 3.6 µL R
Penta E	100 µM	1.0 µM	10 µL F + 10 µL R
Penta D	100 µM	1.0 µM	10 µL F + 10 µL R
BAT40	100 µM	0.75 µM	7.5 µL F + 7.5 µL R
D2S123	100 µM	0.4 µM	4 µL F + 4 µL R
			867.8 µL water

4. Label one 1.5 mL microfuge tube with the name of the MSI master mix (MMM) reaction to be set up, i.e. MSI xx.
5. Using the calculated volumes from the worksheet, add each component of the master mix to the MMM 1.5 mL microfuge tube. Mix gently and spin down briefly to collect liquid at the bottom of the tube.

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Component	Volume Per Sample
Nuclease-Free water	2.80 µL
10X PCR Buffer II	1.20 µL
25 mM $MgCl_2$	1.20 µL
2.5 mM dNTPs	1.40 µL
Primer Master Mix (PMM)	3.00 µL
Amplitaq Gold DNA polymerase	0.40 µL
Total reaction volume	**10.0 µL**

6. Add 10 µL of the MMM cocktail to each well of an appropriately labeled 96 well PCR Plate.
7. Prepare a dilution plate for the template DNA so that the final concentration is 10 ng/µL.
8. Add 2 µL of each diluted sample DNA (10 ng/µL) to the appropriate well containing the master mix cocktail, pipet up and down several times to mix.
9. Add 2 µL of the HCT 116 (10 ng/µL) control DNA to its appropriate tube.
10. Add 2 µL of nuclease free water to the negative/blank control tube.
11. Place the tubes in the MJ thermal cycler and run method "MSI":

95°C for 7 minutes	
94°C for 60 seconds 58°C for 30 seconds 72°C for 45 seconds	2 cycles
93°C for 45 seconds 54°C for 30 seconds 72°C for 40 seconds	41 cycles
72°C for 5 minutes 4°C hold indefinitely	

12. When the program is complete, remove the tubes from the thermal cycler. The PCR products can be stored at 4°C until ready for electrophoresis.
13. Remove an aliquot of Hi-Di formamide and vial of LIZ size standard from the –20°C freezer and thaw at room temperature. Combine 9 µL of formamide with 0.5 µL of LIZ size standard for each sample and control to be run in a 1.5 mL microfuge tube. Vortex briefly and spin down to collect liquid.
14. In a 96-well plate, pipet 9.5ul of formamide/LIZ mixture into separate wells of a 96 well plate.

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15. Prepare a dilution plate (1:5) of the PCR product by adding 1 µL PCR product to 4 µL Nuclease-Free water. Mix by pipetting, and then add 0.5 µL of the dilute PCR product to the appropriate wells of the plate prepared in the previous step (Step 14).
16. Place a 96-well plate septa on the plate, making sure it is seated properly on the wells. Centrifuge plate briefly.
17. Place the plate on the 95°C heat block to denature for 3 minutes. Immediately chill on ice for 3 minutes.
18. Prepare the plate assembly by placing the reaction plate into the plate base.
19. Snap the plate retainer onto the reaction plate and base. Verify that the holes of the plate retainer and the plate septum are aligned.

B. Create a Plate Record:

1. In the Data Collection director, click on Plate Manager. Click on "New" to define a new run. A dialog box for plate description will open.
2. Complete the dialog box by entering a name for the plate "date of run (MMDDYY) and MSI run number." A description of the plate is not necessary.
3. In the "Application" pull –down menu, select GeneMapper-DGMXVHL1.
4. In the [Plate Type] pull-down menu, select "96-well."
5. In the [Plate Sealing] pull-down menu, select "Septa."
6. Enter initials for the owner and operator.
7. Click OK and the Plate Editor opens.
8. In the Sample Name column of each row, enter the sample ID and in the Sample Type column, select the appropriate sample type from the pull down list.
9. In the Size Standard column, select "-250 LIZ" from the pull-down menu.
10. In the Panel column, select "None."
11. In the Analysis Method column, select "G5insstd."
12. In the Results Group column, select "Molecular Genetics_BCR" from the pull-down menu.
13. In the Instrument Protocol 1 column, select "G5" from the pull-down menu.
14. Click "OK" to save.

C. Running the Sample Plate:

1. In the Data Collection software directory, click on "Run Scheduler." This page displays all sample plates that have been created and run on the ABI 3730XL.
2. Select "Search All" to find the Plate Record that was just created. The plate can be found by typing in the name of the plate and clicking on Search, or by selecting Find All. All plates will be in order of date. Choose the plate you want to run, click "Add" and "Done".

3. In the top left corner, a small green arrow will become highlighted to indicate that the instrument is ready to begin the run. Click on the green arrow. A dialog box will open and ask if the run is ready. Click on "yes" and the run will begin.

E. Data Analysis

When the electrophoresis run is complete, the data must be analyzed with the GeneMapper ID software program.

ABI 3130XL Data Analysis:

1. Launch the GeneMapper program by clicking on the icon in the desktop. Log onto the program, using the supplied password.
2. Once the software is launched, it will open to an empty Project page. To add the sample data files collected, go to the "File" menu and select "Add samples to project". This opens a new window.
3. Locate the run folder then click on "Add to List" at the bottom of the window. This will move the run including all sample files to the right side of the screen for analysis in the Project window. Click "Add".
4. Next, the analysis parameters must be defined for the software to perform the correct analysis on the data files. The analysis parameters should be the same as what was defined in the Plate Manager when setting up the ABI run with the following changes: set the Analysis Method column to "Microsatellite Default," and the Panel column to "New Panel" under MSI Folder.
5. Click on the green "Analyze" button at the top of the page. The "Save Project" box will appear. Enter the project name as "MSI" followed by the date and initials. Click OK, and the samples will be analyzed using the parameters specified above. After analysis is complete, the "Status" column (first column on the left side of the project window) should change from showing a green arrow to being empty.
6. On the right side of the project table are several columns with letters as the header. These are PQV (Process Quality Values) values that flag problem samples. As the samples are analyzed, they are subjected to specific criteria defined within the PQV. If the sample passes a specific PQV, a green square will be visible in that column. If the sample data is questionable and should be reviewed by the technologist, a yellow triangle will be visible in that column. If a red octagon is visible in that column, that sample has failed. Consult the supervisor for direction on investigation of the specific PQV problem and solution.
7. All assay controls must be examined prior to interpretation of sample results. If the controls do not yield the correct results, the assay is not valid and the samples should not be interpreted. Consult the supervisor or director for further instruction.
   a. The following describes the analysis of each of the controls and the decisions necessary based upon the results of the capillary electrophoresis. All must be true before continuing to patient analysis:

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i. **Blank Control (Acceptable Negative):** Shows no peaks greater than 100 Fluorescent Units (FU) in the blue, green, red or black channels.

ii. **Blank Control (Unacceptable Positive):** Shows peaks greater than 100 Fluorescent Units (FU) in the blue, green, red or black channels. This result indicates possible contamination of all PCR amplification reactions. Prepare fresh master mix and repeat amplification of all samples and controls or consult with supervisor or director if unsure on how to proceed.

iii. **HCT 116 Positive Control (Acceptable Positive):** Allele peaks should be present for all loci and sizes should be plus or minus 1bp from values seen on previous runs.

iv. **HCT 116 Positive Control (Unacceptable Negative):** Allele peak(s) are not present (i.e. low amplification). This indicates there may be a dilution error, reagent problem or thermal cycling issue. If allele peaks are present but allele(s) are not within 1 bp of posted size, there may be a problem with the capillary electrophoresis or LIZ size standard.

8. For each patient case, select both the normal and tumor file and go to the "Analysis" menu. Select "Display Plots" or click the display plots icon. This will open a new window with the analyzed data displayed graphically. Ensure that Tumor and Normal pairs appear on the same page with Tumor always residing above Normal. Go to the "Plot Settings" menu in the upper left corner of the Samples Plot window and select "MSI" from the pull down menu. Markers in the blue channel will be displayed and can be analyzed first.
9. Adjust the scale of the X-Axis of each plot to exclude any low molecular weight background signal if present.
10. Adjust the scale of the Y-Axis of each plot to allow easy viewing of the associated peaks by positioning the cursor over the Y axis scale and right clicking the mouse. Select "Zoom To..." and enter the appropriate axis scale values.
11. Ensure Page Layout is set to “Landscape” before printing the chromatograms for all blue markers.
12. Repeat Steps 8-10 for remaining color channels.
13. Repeat Steps 7-11 for all patient cases. For the HCT116 control and blank you can select peaks and print with all colors (blue, green, and yellow) selected/displayed.
14. Attach the printed electropherograms to the worksheet. Proceed to the next section of the procedure.

F. Data Interpretation

Patient sample interpretations should be completed as follows.

1. Compare allelic profiles of the Penta D and Penta E markers for a given sample. Normal and tumor tissue for a patient should display the same profile. If they do not, a sample mix up may have occurred. Stop with interpretation of the sample and

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consult with supervisor or director for further action. Similarly, if both Penta D and E markers fail to amplify, stop with interpretation of the sample and consult with supervisor or director for further action. Cases with high MSI may also show MSI at Penta D and E. An alternative identity check (e.g. SNPs) may be necessary.

2. Compare allelic profiles for the 7 MSI markers (all but Penta D + E) between the normal and tumor tissue of a sample. Alleles present in the tumor sample that are not present in the corresponding normal tissue indicate MSI.
3. Consider LOH when comparing allelic profiles. LOH is defined by the presence of an Allelic Index (AI) that is <0.7 or >1.6. The calculation for AI is as follows.

$$AI = \frac{\text{(Ht Normal Allele 1 / Ht Normal Allele 2)}}{\text{(Ht Tumor Allele 1 / Ht Tumor Allele 2)}}$$

4. Use the following numeric code when recording the allelic profile for each MSI marker. This is considered Level 2 data. (Shift = new allele)
    a. 0= Not Interpretable
    b. 1= Shift, Uninformative for LOH
    c. 2= Shift, with LOH
    d. 3= Shift without LOH
    e. 4= No Shift, Uninformative for LOH
    f. 5= No Shift, with LOH
    g. 6= No Shift, without LOH.
5. Determine the overall MSI status of the case as follows. This is considered Level 3 data. MSI status is determined by the number of altered or additional alleles found in the tumor. A single marker found to be "not interpretable" can be tolerated in MSI cases if the marker does not influence the overall call for the case.

    a. MSI-H (high) = ≥40% (≥3/7 markers altered)
    b. MSI-L (low) = 0<40% (1-2 of 7 markers altered)
    c. MSS (stable) = 0% (0/7 markers altered)

6. For samples with low or no amplification, repeat assay with more DNA. Consult with supervisor or director if required action is unclear.
7. Complete the MSI Interpretation Worksheet, initial and date.
8. The MGL Technical Director must review all results.
9. Example of MSI positive and negative mononucleotide markers are in Figure 1 below:

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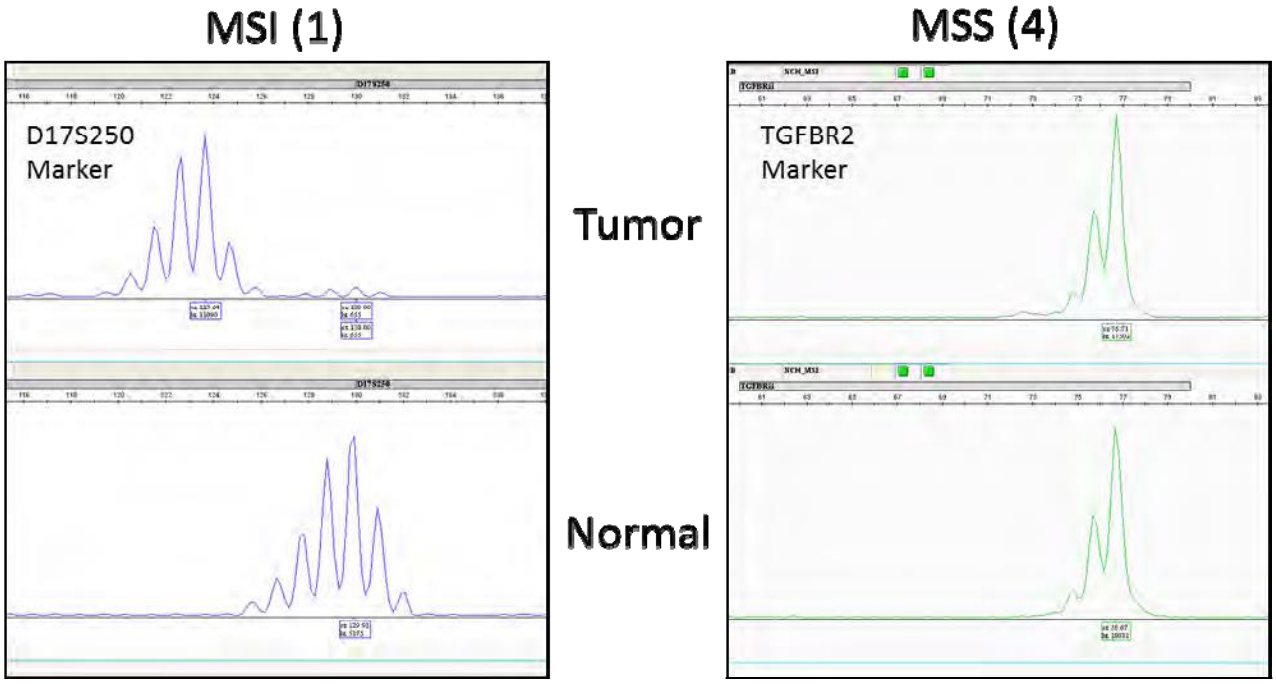

Figure 1. Representative MSI data from case TCGA-AX-A2HD (FSA files 2012-02-28018/018). Two markers are shown from an endometrial case tested from TCGA for MSI. The D17S250 marker on the left is an example of a MSI shift that is uninformative for LOH. The TGFBR2 marker on the right is an example of a marker that does not shift and is uninformative for LOH.

G. Reporting of Results

1. Upon completion of MSI testing for a designated Tumor Study, the Level 2 and 3 results will be summarized in a table.
2. This table will also provide a link to the raw FSA files that gave rise to the Level 2 and 3 data for each case. The FSA files are considered Level 1 data.
3. Prior to finalization of the data set, the table will be spot checked for data entry errors. Cases will be selected at random and the level 2 data confirmed against the original scored FSA printout. All Level 3 calls will be confirmed to have the appropriate number of altered or additional alleles.
4. Level 1 files for each case will be transferred to a folder designated to contain the finalized raw data set. This folder will include the Level 2 and 3 Data file and a description file that defines the assay, scoring criteria, and interpretation key.
5. All finalized Level 1, 2, and 3 data along with the description file will be transferred to BCR informatics for upload to the Data Coordinating Center (DCC).

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IV. REFERENCES

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V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 1/16/2015
1. Updated formatting
2. Removed any TCGA reference
3. Updated disclaimer
4. Updated reagent catalog numbers

B. Version 1, Effective Date 10/2/2012 - New

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Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR MOLECULAR IDENTITY INVESTIGATIONS

I. SCOPE AND PURPOSE

Genotyping issues can be detected when a case enters the identity testing stage of the BCR pipeline. These issues can involve either (1) an identity mismatch between the tumor and normal samples submitted for the case or (2) a positive identity match to an existing case. Because genotyping issues are rare, the following steps should be taken and documented on the "Identity Investigation Checklist" to verify the result.

This procedure applies to all trained Biospecimen Core Resource (BCR) laboratory personnel. The purpose of this Standard Operating Procedure (SOP) is to establish a protocol for the BCR to follow when questions arise around the identity of cases passing through the BCR pipeline. This SOP covers genotyping issues due to two main reasons:

1. SNP genotyping mismatches that arise from sample swaps (patient normal and/or tumor samples) at the Tissue Source Site (TSS) or BCR which results in a failure to qualify for CCG BCR due to "Identity."
2. SNP genotyping matches to a historical sample. These are not considered genotyping failures. However, additional communication with the TSS and the Project Sponsor are required if a historical match is found.

In addition to establishing a protocol, this SOP also establishes the minimum datasets required to qualify cases that have genotype mismatches.

II. PROCEDURE

A. Specimen Information

1. SNP results from tumor tissue and blood and/or normal tissue

B. Required Equipment

1. Computer
2. BCR Identity Mismatch Checklist (http://rex/BPC/TCGA-BCR/Shared%20Documents/Forms/Identity%20View.aspx)

III. PROCEDURE-STEPWISE

A. For identity mismatch:

1. Isolate DNA and RNA from the following sources and complete a SNP analysis for each.
   - **a)** If tumor or normal tissue is part of the investigation, isolate DNA/RNA from a second subportion of the original portion, as well as a subportion from a second portion of tumor sample (when possible).
   - **b)** If a normal blood sample is part of the case, re-isolate DNA from the original TSS normal blood vial using the residual sample left in the tube. In addition, compare the information on the TSS label with the CCG BCR label to ensure that appropriate identifiers match. The Logistics department is usually involved in

STANDARD OPERATING PROCEDURE (SOP) FOR MOLECULAR IDENTITY INVESTIGATIONS

this step and can reference the original shipping manifest to reference the correct identifiers. If a TSS isolated normal blood DNA sample is part of the case, recover residual DNA from the original TSS DNA vial. In addition, compare the information on the TSS label on the TSS vial with the CCG BCR label to ensure that appropriate identifiers match.

2. Compare the genotype profile from all available samples in the case to each other and to all existing genotype profiles to confirm or resolve a mismatch and identify if any component of the case matches the genotype of another existing case in the CCG BCR SNP database.

B. For positive match to an existing case:

a) Compare all available paperwork between the matching cases for evidence that suggests the genetic match is due to either the submission of a duplicate sample or a new tumor from the same patient. This includes the following:

a. Pathology Reports- Compare the hardcopy diagnostic pathology report, making sure to discriminate between reports for cancer resection, cancer biopsy, and reports that have been abbreviated. Samples from matching patients will likely have identical (photocopy) pathology reports. If the overall appearance of the two pathology reports is not identical, compare the content of the data fields to determine if the form has been structurally reformatted or transposed into a spreadsheet. Note whether the tumor types between the two cases match.

b. Case Quality Control Form (CQCF) - Compare the consent dates between the two cases involved to see if they match.

c. Enrollment Form- If this form is available, check for matching dates of birth between the two cases involved.

C. Interpretation, documentation, and corrective action

Upon completion of the steps above, several scenarios may be identified that require interpretation and individualized corrective action. These scenarios include, but are not limited to the following:

a) Identity Mismatches

a. Extraction of DNA from residual material or secondary portions may reveal that the original contents of TSS vials do not match the initial extraction, and now result in matching case samples. This would suggest that a sample swap has occurred somewhere within the BCR. If this occurs, then laboratory personnel should first complete an incident report fully documenting the steps that were taken to verify the identity failure and the result. After the incident report has been reviewed and signed by managers, the identity failure may be resolved and the case passed by relabeling per relabeling SOP A018 "Relabeling Samples".

b. Extraction of DNA from residual material or secondary subportions/portions may reveal that the original contents of TSS vials match the initial extraction, yet still do not contain matching case samples.

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In this situation, results from the comparison made in Section III.A.2 may identify the nature of the mismatch.

b) Gender Mismatches

a. When the gender from the SNP does not match the gender from the OpenClinica data, a second review of the pathology data will be done and the TSS will be contacted to review the data that was entered for the case in OpenClinica.

c) Reciprocal Case Sample Swap

a. The comparison to historical cases reveals that there are two or more (even numbered) cases where Tumor and Normal samples have a reciprocal match. If this occurs, then it may be possible to rescue the case by re-assigning sample ID's to the genetically matched samples. However, without independent verification of sample identity using DNA extracted from paraffin embedded tumor (PET) used for the original patient diagnosis for <u>all</u> cases involved, it is not possible to determine if the reciprocal swap involves the Tumor or Normal sample. Therefore, resolution of reciprocal case sample swaps requires additional procedural steps.

b. Request PET from Tissue Source Site for each case involved in the reciprocal swap. Paraffin scrolls (15 µm sections, 10 sections for each PET) or paraffin blocks are acceptable.

c. Isolate genomic DNA from paraffin tissue for all cases involved and complete a SNP genotyping comparison between PET and samples in question.

d. If genotyping comparison reveals a match between PET, Tumor, and Normal samples, the case may be resolved by following the relabeling SOP A018 "Relabeling Samples" so that samples with genotypes matching the PET DNA receive the PET CCG BCR ID.

d) Orphan case sample swap

a. The comparison to historical cases reveals that Tumor and Normal samples do not match anything else in the database. If this occurs, nothing more will be done to rescue the case unless the TSS takes the initiative to prove case identity by providing BCR with diagnostic PET and matching Tumor/Normal samples.

e) Sample swap with duplication

a. The comparison to historical cases reveals that one or both of the samples from the mismatched case independently match different cases previously entered into the database. If this occurs, nothing more will be done to rescue the case unless the TSS takes the initiative to prove case identity by providing BCR with diagnostic PET and matching Tumor/Normal samples. Depending on the circumstances, the identity of the existing case found to match the mismatched case should be reviewed.

STANDARD OPERATING PROCEDURE (SOP) FOR MOLECULAR IDENTITY INVESTIGATIONS

f) Case Duplication

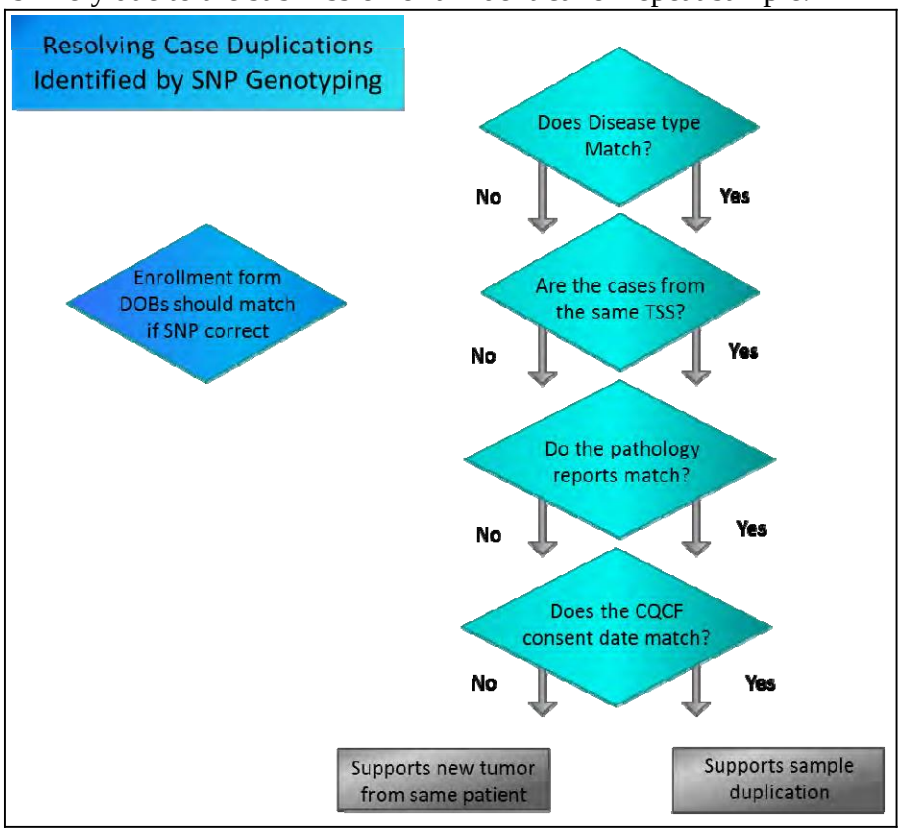
Cases with identical SNP profiles but different ID's must be reviewed further to determine if they can qualify for inclusion. The BCR program director and Project Sponsor should be notified to discuss for resolution. Below are few circumstances that may arise.

a. If two cases, submitted by either the same or different TSSs, have identical genotypes but different patient information (i.e. pathology report, CQCF, Enrollment Form), then both cases may be disqualified. If the first case has already shipped from the BCR, the project sponsor must be notified for directive on case redaction. This will be brought to leadership for resolution.
b. If two cases submitted by the same TSS have identical genotypes and all patient information matches (i.e. pathology report, CQCF, Enrollment Form), then the case submitted second is considered a duplicate and may be disqualified. If the first case did not qualify, the second case may be shipped to the characterization centers, however this will be brought to leadership for resolution.
c. If two different TSSs submit cases that have identical genotypes and patient information (i.e. pathology report, CQCF, Enrollment Form), then both cases may qualify but maybe only one case should ship. If neither case meets all qualifying metrics for yield and integrity, the project sponsor may authorize creating a qualified chimeric case that results from combining individual qualifying components provided from the different TSSs.

g) Case Duplication Discovery

a. In the event that a case duplication is discovered, the source and nature of the duplication should be identified. There are essentially two means by which duplication can occur; (1) the same sample is inadvertently submitted twice by the same TSS or different TSS, or (2) the patient associated with the case has a second tumor type submitted to the study.
b. The flow diagram below represents one approach to reviewing case documentation to determine the source of the duplication. If the duplicate case is not of the same tumor type, does not come from the same TSS, does not have matching pathology reports, and does not have matching consent dates, then the duplication is likely due to the submission of a new tumor from an existing patient. However, if the duplicate case is the same tumor type, comes from the same TSS (or from a tissue bank), has matching pathology reports and matching consent dates, then duplication

is likely due to the submission of an identical or repeat sample.



c. In either situation, special consideration should be given to the identity of the existing case. For example, if the pathology reports do not match, additional steps must be taken. These include contacting the TSS for investigation into the source of the discrepancy and completion of diagnostic PET genotyping to prove that the clinical data is associated with the correct case. Depending on the circumstances, consideration should also be given to redaction of the existing case if it has already shipped. Alternatively, if the pathology reports are an identical match, the Project Sponsor may accept the identity of the existing case and give permission to transfer genetically identical samples from different cases into a single case if it adds value to the project.

IV. REFERENCES

A. BCR SOP A018 "Relabeling Samples"

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 1/12/2015

1. Updated formatting

STANDARD OPERATING PROCEDURE (SOP) FOR MOLECULAR IDENTITY INVESTIGATIONS

2. Updated disclaimer
3. Removed any TCGA reference
4. Updated Program Office with Project Sponsor
5. Include language for gender mismatch

B. Version 1, Effective Date 9/18/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR MOLECULAR IDENTITY INVESTIGATIONS

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

I. SCOPE AND PURPOSE

This SOP is specific to leukemia specimens.

Qiagen AllPrep kits are designed to isolate DNA and total RNA from small quantities of starting material. In addition, they provide a fast and simple method for the preparation of DNA and column purified RNA from human tissues. The purified DNA and RNA are ready for use in standard downstream applications such as DNA amplification and expression array.

A representative sampling of the total RNA content within the tumor tissue homogenate, most notably the low molecular weight species, can be used for micro RNA analysis. Therefore, the flow through from the AllPrep DNA column is taken and the total RNA is isolated with the *mir*Vana kit from Life Technologies.

The *mir*Vana™ miRNA Isolation Kit was designed for purification of RNA suitable for studies of both siRNA and miRNA in natural populations. The kit employs an organic extraction followed by immobilization of RNA on glass-fiber filters to purify total RNA. The *mir*Vana miRNA isolation procedure combines the advantages of organic extraction and solid-phase extraction, while avoiding the disadvantages of both. High yields of ultra-pure, high quality, small RNA molecules can be prepared in less than two hours.

II. PROCEDURE

A. Safety Procedures

1. Wear Personal Protective Equipment (PPE), including a lab coat, goggles or face shield, closed-toe shoes, nitrile gloves and a pair of insulated gloves (when handling LN_2 samples).
2. Bloodborne pathogens can be present in the unfixed frozen tissue (refer to SOP S009, "Bloodborne Pathogen and Exposure Control Plan" found in the BCR Safety Manual). Use all universal precautions.
3. Liquid nitrogen and dry ice are extremely cold and may cause 'burns.' Wear cryogenic gloves designed to withstand extremely cold temperatures.
4. Liquid nitrogen is an asphyxiate; all work should be conducted in a well-ventilated room.
5. 2-mercaptoethanol (2-ME) is toxic. The neat stock solution should be opened in a fume hood only. Solutions containing 1% or less of 2-ME may be used outside of a fume hood. PPE must be used when handling any solution containing 2-ME.
6. Buffer RLT Plus and Buffer AW1 contain a guanidine salt which is not compatible with disinfectants containing bleach. PPE must be used when handling this reagent.

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

7. miRNA Wash Solution included in the *mir*Vana kit contains guanidinium thiocyanate, this is a potentially hazardous substance and should be used with appropriate caution.
8. Acid-phenol:chloroform contains phenol, which is a poison and an irritant. Use gloves and personal protective equipment when working with this reagent.

B. QUALITY CONTROL:

1. The incoming tissue samples have a printed label with a 2D barcode and a human readable format.
2. Working labels are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original portion and subportion identifiers and the corresponding newly created DNA or RNA analyte identifiers. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels are printed for storage in Matrix 2D barcode tubes.
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Samples are tracked in LabVantage. Every portion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample, LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.
6. DNA and RNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. RNA quality is measured by RIN using Agilent Bioanalyzer (see SOP M002, "RNA Nano Assay") and quantified by Spectrophotometer (see SOP MGL-EQP-6 "BIO-MATE UV-Visible Spectrophotometer"). DNA quality is evaluated for integrity by agarose gel electrophoresis (see SOP M003, "Gel Electrophoresis with the E-gel System"), quantified by PicoGreen Assay (see SOP M017, "Picogreen DNA Quantification (Manual)"), and genotypic identity using SNP loci (see SOP M010, "Tissue Matching by SNP Analysis). Primary stock aliquots should not be subject to numerous freeze thaw cycles.
7. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
8. The isolation kit is tested against predetermined specifications to ensure consistent product quality.

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

9. All new lots of reagents are tested in parallel with the one in current use before being put into clinical use. Results are recorded on positive control extraction worksheets.
10. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.
11. RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.
    a. Bench space is wiped down at the beginning of each extraction session with RNase Zap. Pipettes are wiped down with RNase Zap once a week or as needed.
    b. Gloves should always be worn throughout the process and should be changed frequently.
    c. Only sterile, disposable plasticware and pipettes that are dedicated strictly to RNA work should be used to prevent cross-contamination with RNases from shared equipment.
    d. Containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNase-free.

C. Specimen Information

1. Type: Liquid AML tumor samples provided by the Tissue Source Site.
2. Handling Conditions: Follow standard precautions when handling all tissues or cultured cells. Samples should be stored in liquid nitrogen until analytes can be isolated.
3. Indications for Study: This procedure should be used when DNA and RNA are needed from the same liquid tumor sample. DNA is isolated from the AllPrep DNA column and Total RNA including small RNAs is derived from the *mir*Vana isolation kit.

D. Required equipment, supplies, and reagents

1. Equipment

UV visible spectrophotometer
Capsule centrifuge
Digital dry bath
Liquid nitrogen freezer
-20^{o}C freezer
Microcentrifuge
Multi-channel and single channel pipettes
Refrigerator
Qiagen TissueLyser
Vortex
Water bath
Cytospin (if applicable)
Microscope (if applicable)
Serological pipette

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2. Supplies

Allprep DNA/RNA mini Kit (Qiagen 80204)
*mir*Vana™ miRNA Isolation Kit (Applied Biosystems, Cat# AM1560)
Filtered, sterile pipette tips, assorted sizes
1.5 mL Eppendorf tubes
0.5 ml tubes (Fisher, #05-408-128)
2 mL screw cap tubes (Fisher, Cat# 02-707-355)
2 mL SafeLock Eppendorf tubes (Fisher, Cat#022363352)
Wet and dry ice
Insulating trays for dry ice
Personal protective equipment (PPE), including insulated gloves
Stainless steel beads, 5 mm (Qiagen, Cat# 69989)
Hemacytometer
Coverslips
Cytofunnels (ThermoScientific, #5991040) (if applicable)
Cytoslides (ThermoScientific, #5991051) (if applicable)
15mL tubes (Fisher, #50-869-570)
Filtered serological pipets assorted sizes

3. Reagents

2-mercaptoethanol, 100% (Sigma M3148-100ml)
Absolute ethanol, molecular grade (Sigma, E7023)
Diethylpyrocarbonate (DEPC)-treated water (Invitrogen, 750023)
Tris-EDTA Buffer - 100X (Sigma T9285, 100ml)
Sodium hydroxide – 5M (Sigma S8263, 150 mL)
Reagent DX (Qiagen 19088, 1mL)
Hanks Balanced Salt Solution (HBSS) (Sigma, #H8264)
Dulbecco Modified Eagle Medium (DMEM) (Sigma, #D5796)
0.4% Trypan Blue (Sigma, Catalog# T8154)
Fetal Bovine Serum (FBS) (Sigma, #F2442)

It is possible to substitute disposable materials and certain equipment from other vendors as long as they are the equivalent of the item described above.

In the event that a reagent or disposable item either becomes contaminated or is even suspected to be contaminated, it must be discarded.

E. REAGENT PREPARTION (INCLUDING STORAGE CONDITIONS):

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

1. 2-mercaptoethanol (2-ME) – must be added to Buffer RLT Plus before use (final 1% 2-ME). Buffer RLT Plus is stable at room temperature (15-25°C) for 1 month after addition of 2-ME.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%), as indicated on the bottle, to obtain a working solution. Add 25 mL EtOH to an unopened bottle of Buffer AW1 to obtain a 44 mL total volume. Add 30 mL EtOH to an unopened bottle of Buffer AW2 to obtain a 43 mL total volume Buffer AW1 and Buffer AW2 are stable for 1 year at room temperature.
3. Add 21 mL 100% ethanol to miRNA Wash Solution 1 before use. Add 41 mL 100% ethanol to miRNA Wash Solution 2/3 to obtain a working solution.
4. miRNA Wash Solution can be stored at room temp for up to 1 month. For longer term storage, store at 4ºC, but warm to room temperature before use.
5. An aliquot of DEPC water needs to be heated to 95ºC for elution step.
6. Defrosting Media is 80% DMEM and 20% FBS. A 12 mL aliquot is necessary for each sample. To make 12 mL stock, add 9.6 mL of DMEM and 2.4 mL of FBS.
7. Cytospin Media is 95% DMEM and 5% FBS. For a 30 mL stock add 28.5 mL of DMEM and 1.5 mL of FBS.
8. To prepare 50 mM NaOH: dilute 10 mL of stock 5 M NaOH with 990 mL deionized water. This reagent may be stored at room temperature for up to one year.
9. 0.1X TE is made by diluting a stock solution of 100X TE. Add 1 mL of 100XTE to 999 mL of deionized water. This reagent may be store at room temperature for up to one year.

III. PROCEDURE-STEPWISE:

A. Thawing

1. Place 12 mL of defrosting media in a 15 mL conical tube and place in 37°C water bath.
2. Remove the specimen vial from the freezer and place immediately in the 37°C water bath. Constantly shake the vial until the sample is completely thawed. Make sure the cap does not get immersed in water to avoid contamination. It is very important to thaw cells quickly, but gently.
3. Once completely thawed, spray ethanol on tube, wipe off residual ethanol and open the vial. Drop in a few drops of warm defrosting media. Using a sterile pipette, immediately take out all the cells and gently mix them into the defrosting media. Place the tip of the dispensing pipette into the defrosting media and gently move it up and down while dispensing the cell suspension. Do not drop cells through the air or create too many air bubbles while mixing.
4. Centrifuge at 1000 rpm for 8 minutes at room temperature. Pour off supernatant and use finger to tap loose cell pellet in the residual supernatant. Bring volume up to **2.5 mL** with HBSS and take 10 µL of the cell suspension for counting.

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5. Place cell suspension into ice when not in use.

B. Cell Counting

1. Approximate a dilution ratio (cell suspension: 0.4% Trypan blue) that is adequate for counting 10-100 cells per square on a hemacytometer.
2. Load 10 µL of the cell suspension mixture into one side of a hemacytometer with a proper cover slip. Do not overload.
3. Count the number of live cells and then dead cells in one large corner square (divided into 16 smaller ones). Repeat with another corner square. If the cells are too numerous to count, they can be further diluted with Trypan blue.
   a. Be sure to note the ratio of cell suspension to Trypan blue used so that the dilution factor is known for the calculation of the cell concentration.
   b. If the cell counts in the two large squares are highly disparate, count additional squares and use the average.
4. Rinse the hemacytometer with isopropanol and wipe with a Kimwipe between each use. Ensure that the isopropanol is dry before adding a new sample, as residual alcohol may kill viable cells.
5. Enter data into LabVantage to get the cell volume needed for cytospin slides.

C. Cytospin Slides

1. Print 2 slide labels from LabVantage using the "Histology Label" label, Molecular Printer2 printer and label the cytoslides. Place the slides into a cytoclip with the cytofunnel correctly positioned and place the whole unit into the barrel. Be sure the barrel is balanced.
2. Put the LabVantage specified volume of cell suspension for 70,000 cells depending on the cell concentration into two labeled 0.5 mL Eppendorf tubes.
3. Add cytospin media to the cell suspension so that the final volume is 150 µL and mix by pipetting.
4. Load the entire sample into its corresponding cytofunnel. Secure the lid on the barrel, and place barrel back into the cytocentrifuge.
5. Centrifuge at 750 rpm for 3 minutes with medium acceleration on the ThermoShandon Cytospin 4 centrifuge.
6. Place the slides in a histology transport box and deliver to histology for staining.

D. Cell Prep

1. Centrifuge cell suspension for 7 minutes at 400g at 4°C.
2. Pipette off supernatant and keep cell pellets on ice until ready for homogenization.

E. Homogenization

1. Add 600 µL Buffer RLT Plus containing 1% 2-ME to the cell pellet and transfer to a 2 mL safe lock Eppendorf tube and immediately place in a rack at room temperature.
2. Add 3 µL Reagent DX and one 5 mm stainless steel bead to each tube.

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3. Place the tubes (up to 48) in the TissueLyser Adapter Set, making certain the machine is balanced, operate for 30 seconds at 20 Hz.
   NOTE: Prepare a maximum of eight tubes per laboratory technician and begin homogenization in under five (5) minutes to minimize RNA degradation. Excursions above five minutes should be noted on the isolation worksheet.
4. Disassemble the adapter set. Remove tubes from adapter and observe for homogenization.
5. If samples are not completely homogenized, rotate the rack of tubes so that the tubes nearest the TissueLyser are now outer most and reassemble the adapter set. Rearranging the tubes ensures uniform disruption and homogenization.
6. Operate for another 30 seconds at 20 Hz.
7. Repeat step 4.
8. If samples are still not completely homogenized, operate for another 30 sec at 20 Hz.
9. Remove tubes from TissueLyser.
10. Spin tubes down briefly in microcentrifuge and transfer homogenate to a clean, labeled 1.5 mL Eppendorf tube. Do not reuse the stainless steel beads.
11. Centrifuge the homogenate for 3 min at maximum speed (16,100 x g).
12. Carefully remove the supernatant from each sample by pipetting, and transfer it to the AllPrep DNA spin column placed in a 2 mL collection tube (supplied in the AllPrep kit). Avoid aspirating any solids or debris.
13. Close the lid gently, and centrifuge for 30 seconds at 8000 x g.
14. Place the AllPrep DNA spin column into a new, 2 mL collection tube. At this point, the DNA isolation can continue with step G1, or the columns can be stored in the refrigerator for up to 18 hours for later isolation. Use the flow-through for the RNA purification and proceed with step F1.

F. Total RNA purification using the *mir*Vana kit

1. Transfer the flow-through from each DNA column into a separate labeled 2 mL screw cap tube. Adjust the volume of each sample to 600 µL with Buffer RLT Plus containing 1% 2-ME.
2. Add 60 µL (1/10 volume) of miRNA Homogenate Additive to each flow-through, and mix well by vortexing or inverting the tube several times.
3. Leave the mixtures on ice for 10 min. Co-isolation for the DNA could be completed during this incubation.
4. Add 600 µL of Acid-phenol:chloroform to each flow through (volume equal to the lysate volume before addition of the miRNA Homogenate Additive). Be sure to withdraw from the bottom phase in the bottle of Acid-phenol:chloroform, because the upper phase consists of an aqueous buffer.
5. Vortex for 30-60 seconds to mix.

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6. Centrifuge for 10 minutes at 10,000 x g at room temperature to separate the aqueous and organic phases. After centrifugation, the interphase should be compact; if it is not, repeat the centrifugation.
7. Begin heating DEPC treated water for elution now.
8. Carefully remove the aqueous (upper) phase without disturbing the lower phase or interphase layers, and transfer it to a fresh labeled 1.5 mL tube. Note the volume removed.
9. Add 1.25 volumes of room temperature 100% ethanol to each aqueous phase and mix thoroughly by vortexing.
10. For each sample, place a filter cartridge into one of the collection tubes supplied.
11. Pipet each lysate/ethanol mixture onto a filter cartridge. Up to 700 µL can be applied to a filter cartridge at a time. For samples larger than this, apply the mixture in successive applications to the same filter.
12. Centrifuge for 15 seconds at 10,000 x g. **Warning:** Spinning faster than this may damage the filters.
13. Discard the flow-through, and repeat until all of the lysate/ethanol mixture is through the filter. Reuse the collection tube for the washing steps.
14. Apply 700 µL miRNA Wash Solution 1 (working solution mixed with ethanol) to each filter cartridge and centrifuge for 5-10 seconds at 10,000 x g. Discard the flow-through from the collection tubes, and replace the filter cartridge into the same collection tube.
15. Apply 500 µL Wash Solution 2/3 (working solution mixed with ethanol) and centrifuge the filter cartridge for 5-10 seconds at 10,000 x g. Discard the flow-through from the collection tubes, and replace the filter cartridge into the same collection tube. Repeat one time for a total of 2 washes with Wash Solution 2/3.
16. After discarding the flow-through from the last wash, replace the filter cartridge in the same collection tube and spin the assembly at 10,000 x g for 2 minutes to remove residual fluid from the filter.
17. Transfer the filter cartridge into a fresh collection tube (provided with the kit). Apply 100 µL preheated (95º C) DEPC water to the center of the filter, and close the cap. Spin for 20-30 seconds at 10,000 x g to recover the RNA.
18. If multiple columns were required for a sample, combine all eluates into a single tube.
19. Place all samples on ice and proceed to RNA quantification step before freezing.

G. Genomic DNA Extraction

1. Add 500 µL µl Buffer AW1 to the AllPrep DNA spin column. Close the lid gently and centrifuge for 1 minute at 14,000 x g. Transfer the column to a clean 2 mL collection tube.
2. Add 500 µL Buffer AW2 to the AllPrep DNA spin column. Close the lid gently and centrifuge for 2 minutes at 14,000 x g to wash the spin column membrane.

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3. Place one AllPrep DNA spin column per sample in the previously labeled 1.5 mL collection tube. Add 100 µL 0.1X TE (10 mM Tris: 1 mM EDTA, pH 8.0) directly to the spin column membrane and close the lid. Incubate at room temperature for 1 minute and then centrifuge for 1 minute at 14,000 x g to elute the DNA.
4. If multiple columns were required for a sample, combine all eluent into a single tube with a single channel pipette. Discard the empty tubes.
5. Check the samples into the designated PicoGreen plate in LabVantage and place in the corresponding PicoGreen plate in the refrigerator. The physical sample should be accompanied by an empty labeled matrix tube with final storage label for final transfer by the technician completing the picogreen quantification. Proceed to DNA quantification and normalization steps after multiple DNA samples are ready for quantification.

H. Quantification and Normalization of RNA Samples

1. Prepare a set of 0.5 mL tubes with the appropriate LabVantage labels. Add 98 µL of 50 mM sodium hydroxide to each tube.
2. Using a single channel micro-pipette, add 2 µL of the RNA stock sample to the sodium hydroxide. Vortex for at least 5 seconds to ensure that the diluted sample is well mixed and spin briefly. Read the absorbances for 260, 280 and 320nm in a spectrophotometer using a quartz cuvette.
3. Desired sample concentration may vary among projects.
   a. If the concentration is above the desired concentration use the known volumes and concentrations to calculate the amount of DEPC-treated water to add to the sample to yield a final desired concentration as shown below in Example 1.
   b. If the concentration of the RNA sample is less than the desired concentration, use the known volumes and concentrations to calculate the amount to concentrate the sample to the desired volume as shown below in Example 2. This should be accomplished by the use of a speed vac with no heat.
4. After diluting or concentrating samples, repeat step 2 to confirm that the sample is within the target concentration range.
5. Once the samples are at target concentration, transfer the liquid to a labeled matrix tube (Primary sample aliquot). Create a second aliquot for subsequent sample quality control assay (see SOP M002, "RNA Nano Assay").

I. Quantification and Normalization of DNA Samples

1. Refer to SOP M017 for DNA quantification and normalization by PicoGreen.

J. Sample Storage

1. RNA samples should be stored in a liquid nitrogen freezer.
2. DNA samples should be stored in a -80^{o}C freezer.

K. Sample calculations

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

Example 1: Samples with concentrations > 0.165 µg/µL need to be diluted using:

Normalization to 0.165 µg/µL
((current concentration/desired concentration) x current volume) – current volume
= ((0.21 µg/µL/0.165 µg/µL) x 18 µL) – 18 µL
= 4.9 µL (Volume of diluent to add)

Final sample volume = 22.9 µL

Example 2: Samples with concentrations < 0.165 µg/µL need to be concentrated using:

Normalization to 0.165 µg/µL
((current concentration/desired concentration) x current volume) – current volume
= ((0.08 µg/µL/0.165 µg/µL) x 18 µL) – 18 µL
= - 9.3 µL (Volume to be removed during speedvac concentration)

Final sample volume = 8.7 µL

IV. REFERENCES

A. Allprep DNA/RNA Mini Kit Handbook (November 2005)
B. mirVana miRNA Isolation Kit Handbook (2011)
C. BCR SOP S009, "Bloodborne Pathogen and Exposure Control Plan"
D. BCR SOP M002 "RNA Nano Assay"
E. SOP MGL-EQP-6 "BIO-MATE UV-Visible Spectophotometer"
F. BCR SOP M003 "Gel Electrophoresis with the E-Gel System"
G. BCR SOP M017 "PicoGreen DNA Quantification (Manual)"
H. BCR SOP M010 "Tissue Matching by SNP Analysis"

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 12/31/2014

1. Updated formatting
2. Removed any reference to TCGA
3. Removed any reference to concentration range
4. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
5. Removed the reference to pooling samples and re-extracting samples.
6. Updated reagents and vendor catalog numbers
7. Updated reference section
8. Removed the step of adjusting volume with RLT before the sample goes through the DNA spin column.

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

9. Indicated that this SOP is specific to leukemia
10. Removed specifics of barcodes

B. Version 1, Effective Date 9/14/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR DNA/RNA EXTRACTION WITH ALLPREP (DNA) AND MIRVANA (TOTAL RNA WITH SMALL RNA) - MODIFIED LEUKEMIA PROTOCOL

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR CASE METRICS REVIEW

I. SCOPE AND PURPOSE

Characterization and Sequencing Centers request a specific amount and quality of each analyte associated with a case. These metrics may be different between projects and current metrics are located on the BCR SharePoint's "Project and QMS" folder. Once the analytes are created from each of these specimens, the case may be reviewed for qualification potential of being shipped out to the Characterization and Sequencing Centers.

This procedure is for evaluating case metrics prior to and after SNP analysis. The SOP applies to all laboratory personnel.

II. PROCEDURE

A. Quality Control

1. After the data on the isolation worksheets is complete, a second technologist performs a quality review to verify that the data recorded on isolation worksheets matches the data that was entered into LabVantage by the isolating technologist. The data are "released" in LabVantage only if the information recorded on extraction worksheets matches what has been entered into Labvantage. If the data does not match, the case is reviewed by a senior technologist in the lab and appropriate corrective action is taken. A summary of the corrective action will be included in an incident report.
2. The Biospecimen Core Resource does not have a requirement that restricts the depletion of Tissues or Analytes. Therefore, there is no warning mechanism in place that signals when vial or volume quantities fall below a defined threshold.

B. Specimen Information

1. A case includes analytes from tumor tissues, normal blood and/or normal tissues.

C. Required Equipment

1. Computer

III. PROCEDURE-STEPWISE:

A. PRE-SNP Analysis

1. The isolation worksheets, pico green worksheets, and Agilent documentation contain critical quality control information regarding extractions performed on cases processed by the molecular group. These include, in part, DNA Yield, RNA Yield, RNA RIN, Analyte Volumes, and Analyte Concentrations.
2. After the quality control process has been complete and all data are entered into LabVantage, the case is reviewed to determine if a patient status update is required.
3. This process utilizes the following chart:

STANDARD OPERATING PROCEDURE (SOP) FOR CASE METRICS REVIEW

	Case includes?	Processed?	Does **DNA concentration** meet Project guidelines?	Does **DNA yield** meet Project guidelines?	Does **RNA concentration** meet Project guidelines?	Does **RNA yield** meet Project guidelines?	Does **RNA RIN** meet Project guidelines?
Tumor Tissue							
Normal Blood					n/a	n/a	n/a
Normal Tissue							

4. For many projects, a case must have a tumor and normal. The normal may be blood or normal tissue or both.
   a. If a case has only normal tissue as the qualifying normal, then the RNA may not need to meet metrics for the case to qualify.
5. If it is discovered that any component of a case has not been processed, the necessary samples will be requested from Logistics, extracted, and QC'd.
6. Criteria for making patient status updates:
   a. If all the components of a case have been processed and are passing metrics, then the status should be changed to Pass-Pending Genotyping.
   b. If one or multiple components do not pass metrics, check LabVantage to see if more specimens are available. If available, change the subject status to MGL Re-eval New Portion and send an email with subject "MGL Tissue Request" to Logistics specifying which CCG BCR ID to send to molecular for isolation. After extraction of the re-eval tissue is complete, repeat the review of quality control information.
   c. Special situations for re-evaluating tissues include:
      (a) If a sample fails for RNA RIN two times, the status may be changed to Fail-Pending Genotyping.
      (b) If a sample fails for yield, the specimen must be re-isolated until all sub-portions have been exhausted. Repeat case evaluation (steps 3-6)
      (c) If the sample can be pooled from the same or multiple portions to meet metrics and if allowed for that project, pool the sample, and go back to step six. Repeat case evaluation (steps 3-6). Special permission may be needed from the Project Sponsor. Contact the appropriate Director to create a request for pooling samples. (See BCR M011 Pooling of Molecular Analytes from Different Tissue Portions.)

STANDARD OPERATING PROCEDURE (SOP) FOR CASE METRICS REVIEW

(d) If there is no specimen remaining to re-isolate, then the status may be changed to Fail-Pending Genotyping.

B. POST-SNP Analysis

1. Upon completion of the SNP assay, review all quality control information using the following chart:

	Case includes?	Processed?	Does **DNA concentration** meet Project guidelines?	Does **DNA yield** meet Project guidelines?	Does **RNA concentration** meet Project guidelines?	Does **RNA yield** meet Project guidelines?	Does **RNA RIN** meet Project guidelines?	Does the Genomic Gel Pass?	Do all components of a case match each other by SNP analysis?
Tumor Tissue									
Normal Blood					n/a	n/a	n/a		
Normal Tissue									

2. All components to a case must meet yield, integrity, and identity metrics defined by the Project guidelines and SOP M010 (Tissue Matching by SNP Analysis) before the case status can be changed from Pass-Pending to Pass or Pass-Shipping On Hold.
3. When case status is changed to Pass, the individual components that allowed the case to qualify are assigned an appropriate pseudobatch for shipping identification.
4. In any case, if the SNP Analysis reveals a mismatch between all components within a case or a positive match to another case in the SNP database, leave the status as Pass/Fail Pending Genotyping is and contact the appropriate Director to discuss further. SOP M021 contains instructions on how to resolve mismatch cases.

IV. REFERENCES

A. BCR SOP M011 "Pooling of Molecular Analytes from Different Tissue Portions"
B. BCR SOP M021 "Molecular Identity Investigations"
C. BCR SOP M010 "Tissue Matching by SNP Analysis"

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 1/7/2015
1. Updated formatting
2. Removed any TCGA reference
3. Updated the chart for quality control metrics
4. Removed references to Rosetta Stone and wrote to say "project"

STANDARD OPERATING PROCEDURE (SOP) FOR CASE METRICS REVIEW

5. Included location of where to find current project metrics

B. Version 1, Effective Date 9/18/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR CASE METRICS REVIEW

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR MONITORING MOLECULAR LAB QUALITY INDICATORS

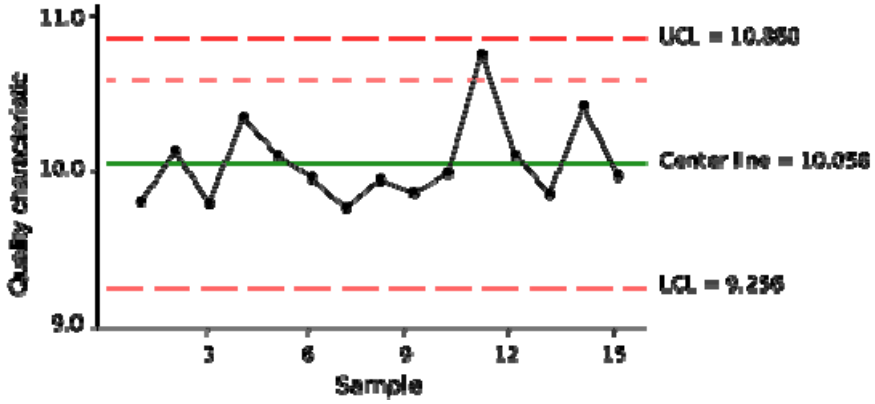
I. SCOPE AND PURPOSE

This SOP defines a process by which operational quality in the lab is monitored. This includes surveillance of (1) PCR amplification failures; (2) PCR analytical control failures; (3) Internal controls from Bioanalyzer tests; and (4) the result from nucleic acid extractions using statistical process control (SPC).

SPC is a mathematical method for reviewing and monitoring process quality. The goal of this method is to ensure that a process is operating at its full potential. SPC can be applied to the BCR's molecular extraction protocols using nucleic acid yield and integrity metrics as indicators of protocol performance. A central component of SPC is the control chart. Control charts allow SPC users to evaluate if a process is in a state of statistical control by differentiating between natural variability in a process and significant unanticipated change. Control charts typically consist of the following:

- Individual data measurements of quality characteristics (DNA Yield, RNA Yield, RNA RIN) in samples taken from the process at different times [the data].
- The mean and standard error (e.g., standard deviation/sqrt(n) for the mean) of the quality characteristics for (1) all samples being evaluated or (2) from a set of samples defined as a point of reference for process performance.
    - The reference data set used is the data compiled from all previous extractions of the same extraction type as the specimen(s) of interest.
- Graphical presentation of individual data measurements on a chart overlaid with lines drawn at the value for the 'historical' mean as well as two and three standard deviations from the mean (i.e. control warning and control limit respectively).
- Control warning and control limits have both upper and lower values (Upper Control Limit= UCL; Upper Control Warning= UCW; Lower Control Limit= LCL; Lower Control Warning= LCW) and indicate the threshold at which the process output is considered statistically 'unlikely'. Therefore, individual data points that fall above upper- or below lower-control warnings or limits are "out of control". Out of control data points can be indicators that a process may not be performing as expected. The severity of this potential process defect is relative to the number of consecutive 'out of control' data points.
- An example control chart is shown below:

STANDARD OPERATING PROCEDURE (SOP) FOR MONITORING MOLECULAR LAB QUALITY INDICATORS



The CCG BCR has engineered a similar form of control chart that allows large amounts of information to be presented efficiently. It is known that numerous variables will influence the outcome of nucleic acid extraction from biobanked human tissue. These factors include, but are not limited to tissue type, procurement method, frozen storage conditions and duration, and laboratory processing and handling. Therefore, to better monitor the performance of the molecular process at CCG BCR, control charts are created that take these variables into consideration. Quality metrics from samples are aggregated into predefined groups representing select variables known to influence process outcome (i.e. prep type, TSS ID, tumor type, and molecular technician). The standard deviation from the mean is calculated for samples within the predefined group for each data point and plotted on a control chart. Trends are visualized through lines connecting sequential data points (i.e. Breast, Endometrial, Thyroid, and Bladder are represented by separate lines on a control chart for DNA yield). With this approach, multiple variables can be examined on a single chart (i.e. All TSS processed in a given month). CCG BCR will investigate individual data points that exceed the LCW (2 standard deviations) on an individual basis. A sequential series of LCW data points will be investigated for a potential generalized processing issue.

This Standard Operating Procedure (SOP) applies to the molecular area of the BCR and establishes a procedure for periodic (monthly) review of quality indicators from PCR amplifications and molecular nucleic acid extractions. This assessment covers PCR amplification or analytical control failures, internal controls from Bioanalyzer tests, and the quality of tissue extractions using nucleic acid (DNA & RNA) yield and RNA integrity (RIN) as endpoints.

II. PROCEDURE

A. Quality Control

All Molecular Lab Quality summaries will be reviewed for accuracy by a senior technician in the molecular lab and the BCR quality management team. Once finalized, summaries will be submitted to senior management for review. The Technical Director

STANDARD OPERATING PROCEDURE (SOP) FOR MONITORING MOLECULAR LAB QUALITY INDICATORS

of the BCR Molecular Lab will review and sign the monthly quality summary. The Principal Investigator will review and sign quarterly quality summaries. Any individual sample with Yield or Integrity metrics that fall below the lower control warning threshold (i.e. 2 standard deviations below the average) will be investigated for a quality issue. More general processing issues will be investigated if a consecutive series of 5 samples or more fall below the lower control warning.

B. Specimen Information

This SOP applies to any CCG BCR specimen processed by the CCG BCR.

III. PROCEDURE-STEPWISE

A. Prepare Control Chart Summary

1. SPC should be completed once per month for the processing that occurred during the previous month. It is preferred that this review take place within the first week of each month.
2. Open SPC spreadsheets "MGL SPC – DNA RNA Yield – Updated Data Connections.xls" and "MGL SPC – RIN – Updated Data Connections.xls".
3. These spreadsheets have data connections that pull in QC data directly from the BCR's Data Warehouse. To ensure that QC data has been synchronized with the data warehouse, "refresh" all tabs with datasets for SPC. These tabs include "mglTech", "StudyName", "TSSID".
   (1) To refresh a dataset for the tab being viewed, select "Data" option at the top of the Excel window and click on "refresh all".
4. Update graphs on "Dashboard" tab to select the current month on the "month created" filter on the graph. Note, only one month's worth of data can be viewed at a time.
5. For Yield SPC, select DNA for sample type ID in the box to the right of each graph.
6. Copy and paste each graph as an image into the appropriate page of the SPC summary template. Each graph should be placed on its own page. This should be done for both RIN and Yield SPC charts.
7. Note any samples that fall below the LCW and add their LabVantage ID below the control chart pasted into the summary.
   (1) The LabVantage IDs for samples below the LCW can be found for each of the groups (mglTech, StudyName, TSSID) by double clicking on the grand total cell on the respective data set tab. This creates a new tab with detailed sample information that can be sorted in ascending order by qcValStdDeviation. This will bring the samples with the lowest standard deviation to the top of the spreadsheet. Highlight all rows with values greater than -2 standard deviations from the mean and copy/paste into a new tab for samples to investigate on the MGL SPC excel spreadsheet.
8. For Yield SPC, repeat steps 6 and 7 after changing sample type ID to RNA (see Step 5).

B. Investigate Out-of-Control Samples

1. Open the MGL QC table linked to Reslimsdb | BCR |vwQCSampleSummaryMGL.

STANDARD OPERATING PROCEDURE (SOP) FOR MONITORING MOLECULAR LAB QUALITY INDICATORS

2. Remove all filters and refresh dataset.
3. Filter dataset to "Analyte" under Sample Category.
4. Sort Data by "CCG BCR Barcode".
5. Lookup the CCG BCR ID for all samples that require investigation using the sample IDs collected on the investigation tab created in Section A Prepare Control Chart Summary, Step 7, Subpart 1.
6. Copy and paste all rows from the QC table for the cases involved into the MGL SPC investigation tab. There will be two investigation tabs: Yield and RIN.
7. After information for all cases that require investigation have been pulled into the investigation tab, review the metrics for each case individually for signs of abnormal processing. Use the logic below as a guide to this investigation.
    - Has a second subportion been extracted for the sample flagged by SPC?
        (a) If no, process an additional subportion and evaluate the resulting QC metrics as done below.
        (b) If yes, were the QC metrics comparable between the two extractions?
            (i) If no and the second portion qualifies by CCG BCR standards, no further action is required.
            (ii) If no and the second portion still doesn't qualify, process an additional subportion and evaluate the resulting QC metrics. This step may be repeated multiple times until the sample either qualifies, results in consistent QC metrics, or is depleted.
            (iii) If yes, then no further action is required. Independent extractions that result in failing metrics suggest the sample has poor starting quality.

C. Investigate Out-of-Control Process

1. If SPC suggests there is an ongoing processing issue, halt processing of any new CCG BCR cases until the issue has been identified and resolved.
2. Repeat Section B for all samples involved to determine if secondary extractions are associated with improved QC metrics.
3. Review information on samples involved in an effort to identify common factors that may have influenced QC metrics.
4. Replace reagents or conduct personnel training as needed. Prior to resuming processing of CCG BCR cases, conduct extractions on control tissue to confirm that processing issue has been remedied.

D. Review PCR Reaction and Control Failures

1. Contact a senior technician in the lab and review the worksheets from the previous months PCR reactions.
2. Document the following any failures in amplification or analytical controls:
    (1) Date of reaction, reaction type (ie. MSI, SSTR, SNP), name of technician, nature of failure, and plate or sample ID's.
    (2) Nature of corrective action taken.
    (3) Result from corrective action.

E. Review Bioanalyzer Internal Control

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1. All Bioanalyzer chips must include a reference control RNA to monitor assay performance on each run.
2. This control sample has an established RIN value that is acceptable within ±0.5.
3. If control RNA gives a RIN outside of the established range, it is expected that all samples on the chip will be repeated.
4. Control RIN values will be monitored monthly to document and follow up on outliers, trends or omissions.
5. This monthly review must include calculations for standard deviation (SD) and coefficient of variability (CV).

IV. INTERPRETATION/ANALYSIS/DOCUMENTATION

A. Documenting Molecular Lab Quality Reviews

1. A summary of the quality review should be prepared and submitted to Quality Management and Senior Management for review.
2. Summaries should include statistics on PCR reaction/control failures, Bioanalyzer internal control failures, and control charts that aggregate samples by Study, Extraction Technician, and Tissue Source Site.
3. SPC should be used to monitor RIN, DNA Yield, and RNA Yield when possible.
   (1) SPC Summaries should include:
   (a) A table of samples flagged during the review
   (b) Notes from the investigation
   (c) Action required for follow up.
4. Monthly quality reviews will be stored as follows:
   (1) Electronic copies (no signatures) will be on the CCG BCR Internal SharePoint site (\\rex\DavWWWRoot\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Monthly SPC Review).
   (2) Signed paper copies will be stored in a "Molecular Monthly Quality Review" binder in the lab.
5. Quarterly quality reviews will be submitted to the BCR Quality Manager as part of the "Quality Monitoring" process as defined in the Quality Manual.

V. REFERENCES

A. Deng, H; Runger, G; Tuv, Eugene (2012). System monitoring with real-time contrasts, Journal of Quality Technology, 44(1), pp. 9-27.
B. Oakland, J (2002) *Statistical Process Control* ISBN 0-7506-5766-9
C. Wheeler, D J & Chambers, D S (1992) *Understanding Statistical Process Control* ISBN 0-945320-13-2
D. *NIST/SEMATECH e-Handbook of Statistical Methods*, http://www.itl.nist.gov/div898/handbook/7.10.2012.

VI. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 1/12/2015

STANDARD OPERATING PROCEDURE (SOP) FOR MONITORING MOLECULAR LAB QUALITY INDICATORS

1. Updated formatting
2. Removed any TCGA reference
3. Updated disclaimer

B. Version 1, Effective Date 9/18/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR MONITORING MOLECULAR LAB QUALITY INDICATORS

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for FFPE RNA/DNA Co-Isolation Using the Allprep FFPE Kit and the Highpure miRNA Kits

I. SCOPE AND PURPOSE

Tissues that have been formalin-fixed and paraffin embedded (FFPE) present a challenge to molecular biology. Formalin fixation causes a wide variety of chemical modifications and damage to nucleic acid, including crosslinking and strand breaks. Paraffin embedding renders tissues insoluble in common molecular biology buffers and aggregates nucleic acid in a manner that lowers quality and further complicates efficient extraction.

This protocol uses two technologies: the AllPrep FFPE kit (Qiagen) which is well-suited for purification of DNA, and the Highpure miRNA kit, which is well-suited for purification of RNA (including miRNA). The initial preparation section of this procedure is responsible for deparaffinization and decrosslinking specific to FFPE-derived tissues. The RNA Prep and DNA Prep sections are similar to standard column-based extraction protocols used on fresh or frozen tissues.

The purpose of this SOP is to establish a procedure for co-isolating DNA and RNA from FFPE specimens and applies to all trained Biospecimen Core Resource laboratory personnel.

II. PROCEDURE

A. Safety Precautions

1. Wear personal protective equipment such as lab coats, goggles and gloves.
2. Bloodborne pathogens can be present in the unfixed frozen tissue (refer to SOP S009, "Bloodborne Pathogen and Exposure Control Plan" found in the BCR Safety Manual). Use all universal precautions.
3. Buffers AL and AW1 contain guanidine hydrochloride, which can form reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a laboratory detergent and water; if the liquid contains potentially infectious agents, follow with 1% sodium hypochlorite to disinfect.

B. Quality Control

1. The incoming tissue samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID; the human readable has the internal LabVantage ID, CCG BCR barcode, and TSS identifier.
2. Working labels (containing the internal LabVantage ID, CCG BCR barcode, and scannable 2D barcode) are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original LabVantage ID (to follow the subportion) and the corresponding newly created (DNA or RNA) LabVantage IDs. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels (internal LabVantage ID (DNA or RNA), CCG BCR barcode, TSS identifier, and 2D barcode) are printed for storage in Matrix 2D barcode tubes.

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NOTE: For BLGSP FFPE analytes, DNA is denoted with an "E" and RNA is denoted with an "S". All other projects use "D" for DNA and "R" for RNA.

4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Samples are tracked in LabVantage. Every subportion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample, LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.
6. DNA and RNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. RNA quality is measured by RIN/DV200 using Agilent Bioanalyzer (see SOP M002, "RNA Nano Assay") and quantified by Spectrophotometer (see SOP BCR-MGL-EQP-1 "BIO-MATE UV-Visible Spectrophotometer"). DNA quality is evaluated for integrity by agarose gel electrophoresis (see SOP M003, "Gel Electrophoresis with the E-gel System"), quantified by PicoGreen Assay (see SOP M017, "Picogreen DNA Quantification Manual") and genotypic identity using SNP loci (see SOP M010 "Tissue Matching by SNP Analysis"). Primary stock aliquots should not be subject to numerous freeze thaw cycles.
7. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
8. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
9. The isolation kit is tested against predetermined specifications to ensure consistent product quality.
10. All new lots of reagents are tested in parallel with the one in current use before being put into use. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeable between kit lot numbers. Results are recorded on positive control extraction worksheets. All QC results are recorded in the Quality Control notebook.
11. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.
12. RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.
    a. Bench space is wiped down at the beginning of each extraction session with RNase Zap. Pipettes are wiped down with RNase Zap once a week or as needed.

Standard Operating Procedure (SOP) for FFPE RNA/DNA Co-Isolation Using the Allprep FFPE Kit and the Highpure miRNA Kits

b. Gloves should always be worn throughout the process and should be changed frequently to prevent cross-contamination and transfer of ubiquitous RNases.
c. Only sterile, disposable plasticware and pipettes that are dedicated strictly to RNA work and certified RNase-free should be used to prevent cross-contamination with RNases from shared equipment.
d. Containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNase-free.
e. All reagents must be made with RNase-free materials and chemicals, and containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNase-free.

C. Specimen Information –

1. Type: FFPE tissue (blocks or unstained slides)
2. Handling Conditions: Follow standard precautions when handling all tissue samples. Samples should be stored refrigerated until isolation is commenced.
3. Sample Preparations: FFPE samples are prepared for isolation by Histology; blocks are cut into 10 µM scrolls and placed into a 2-mL safe-lock tube. Optimally, scrolls should be isolated within 24 hours fromtime cut. FFPE unstained slides are scraped by the Histology department and can be stored in the refrigerator until ready for isolation.
4. Indications for Study: This procedure is used when DNA and RNA are needed from the same piece of FFPE tissue. DNA is isolated from the AllPrep FFPE kit and RNA is isolated using the HighPure miRNA kit.

D. Required Equipment, Supplies and Reagents

1. Equipment

a. PPE (Gloves, Lab coat)
b. UV visible spectrophotometer
c. Capsule centrifuge
d. Digital dry bath
e. Microcentrifuge
f. Multi-channel and single channel pipettes
g. Vortexer
h. DNA/RNA Concentrator

2. Supplies

a. Roche Highpure miRNA kit (Roche #05080576001; no substitution)
b. AllPrep FFPE kit (Qiagen #80234; no substitution)
c. 1.5 mL microcentrifuge tubes (Fisher, 05-408-137)
d. 2.0 mL Safe-lock tubes (Fisher, 05-402-7)
e. 0.5 mL microcentrifuge tubes (Fisher, 05-408-128)
f. Matrix 0.5 mL ScrewTop Tube (ThermoScientific #3745)
g. Matrix ScrewCap (Direct Resource #4477 [RED, BLUE, GRE, PUR])
h. Pipettes, 10 µL – 1000 µL, single or multichannel

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i. Filtered tips for pipettes
j. Sodium dodecyl sulfate (SDS) (Fisher #BP166-100)

k. Wet and dry ice
l. Insulating trays for dry ice

3. Reagents

a. Heptane (VWR #BDH1127-4LP; no substitution)
b. Methanol (VWR #BDH1135-4LP; nosubstitution)
c. 100% molecular grade ethanol (Sigma # E7023-6X500mL)
d. RNase-Free DNase (Qiagen #79254)
e. PureLink RNase A (Invitrogen #12091-021)

4. Water, Molecular Biology Reagent (Sigma #W4502)Tris-EDTA Buffer, 100X (Sigma #T9285)
   a. Sodium Hydroxide, 5.0 M (Sigma #S8263)
5. Colored labels: THT, B, WHT 1x0/5”W0.375”DIA: Blue (Y1439894), Red (Y1439892), Green (Y1439893), Yellow (Y1439895) (Brady)
6. Thermal Transfer Printer Labels (Freezerbondz, # THT-152-492-3)
   a.

Notes:
It is possible to substitute disposable materials and certain equipment from other vendors, unless otherwise noted, as long as they are the equivalent to the item described above.

In the event that a reagent or disposable item either becomes contaminated or is suspected of being contaminated, it must be discarded.

E. Reagent Preparation

Note: All reagents are labeled with reagent name, concentration, date opened/prepared, expiration date, storage conditions, and appropriate hazard labeling.

1. 10% SDS: Dissolve 5 g of SDS in 40 mL of Sigma water, and then add Sigma water to 50 mL. This stock solution is stable for 6 months at room temperature.
2. DNase stock solution is prepared by dissolving lyophilized DNase 1 enzyme in 550 µL water that is provided by the kit. Mix gently by inverting the vial. Do not vortex reconstituted DNase 1. DNase 1 is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial. For long-term storage of DNase 1, remove the stock solution from the vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

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3. The Proteinase K provided from the Roche Highpure miRNA kit must be dissolved prior to use. Dissolve the Proteinase K in 4 mL Elution buffer, aliquot and store at -15 to -25°C for up to one year after preparation.
4. Wash Buffer is provided in the Roche HighPure miRNA kit. Before using for the first time, add 40 mL 100% ethanol to the Wash Buffer bottle. Store at room temperature for up to 6 months after adding ethanol.
5. Buffers AW1 and AW2 are supplied as a concentrate in the AllPrep FFPE kit. Before use for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle; for example, add 25 mL of ethanol to the unopened bottle of AW1 to obtain a total of 44 mL, or add 30 mL of ethanol to the unopened bottle of AW2 to obtain a total volume of 43 mL. Write the open and expiration dates; both buffers are stable at room temperature for up to one year after adding ethanol.
6. 0.1X TE is made by diluting a stock solution of 100X TE. Add 1 mL of 100XTE to 999 mL of deionized water. This reagent may be stored at room temperature for up to one year.
7. To prepare 50 mM NaOH: dilute 10 mL of stock 5M NaOH with 990 mL deionized water. This reagent may be stored at room temperature for up to one year.
8. All solutions in the Roche HighPure miRNA kit should be clear. If any solution contains a precipitate, warm the solution prior to use at 37°C to dissolve the precipitate.
9. Before starting the procedure, check whether precipitate has formed in Buffer AL and ATL in the AllPrep FFPE Kit. If necessary, dissolve by heating to 70°C with gentle agitation.
10. The QIAamp MinElute spin columns from the AllPrep FFPE Kit should be immediately stored at 2–8°C upon receipt in the lab. The buffers can be stored at room temperature (15–25°C). Under these conditions, the kit components can be kept for at least 9 months without any reduction in performance.
11. The Proteinase K supplied in the AllPrep FFPE Kit is in a specially formulated storage buffer and is stable for at least 1 year after delivery when stored at room temperature (15–25°C).
12. Buffer AW1 contains guanidine thiocyanate. PPE must be used when handling this reagent.

F. Deparaffinization and Cell Lysis

5. Add 8 x 10 µM scrolls with ~150 mm^2 tissue surface area each to a 2 mL safe lock tube. The number of scrolls can be adjusted to obtain a total tissue surface area of ~1,200 mm^2 (e.g., 16 scrolls with ~75 mm^2 surface area or 12 scrolls with ~100 mm^2 surface area).
6. In the fume hood add 1.4 mL **heptane**, vortex for 10 seconds, and incubate at room temperature for 10 minutes.
7. In the fume hood add 70 µL **methanol**, vortex for 10 seconds, and centrifuge for 2 minutes at 9,000 x g.

Standard Operating Procedure (SOP) for FFPE RNA/DNA Co-Isolation Using the Allprep FFPE Kit and the Highpure miRNA Kits

8. Remove the supernatant via pipette without disturbing the pellet. This waste contains heptane and methanol. The supernatant is kept in a 50 mL conical tube at the bench and poured into the glass waste bottle (located in the hood) after the isolation is complete.
9. Add 1 mL 100% **ethanol** to each pellet and mix by brief vortexing.
10. Centrifuge for 2 minutes at 14,000 x g.
11. Remove the supernatant without disturbing the pellet. Retain the supernatant until after the isolation is complete.
12. Leave the cap open and incubate the tissue pellet for 12 minutes at 37°C in the CentriVap.
13. Add 300 µL of **Paraffin Tissue Lysis Buffer** (Highpure miRNA kit), 48 µL **10% SDS** and 120 µL **Proteinase K** working solution (Highpure miRNA kit) to each pellet. Pulse vortex 3 x 5 seconds to mix.
14. Incubate the tubes on a dry block at 55°C for 45 minutes.
15. Centrifuge the samples for 15 minutes at 16,000-20,000 x g. Remove from the centrifuge carefully, insuring the pellet is not disturbed.
16. Transfer the supernatant to a new 2 mL safe-lock tube and continue with the **RNA Extraction** below with the supernatant.
17. Close the tube with the pellet. Continue with the **DNA Extraction** below with the pellet. Note that the RNA and DNA isolation can be conducted simultaneously as there are two long incubation steps in the DNA protocol.

G. **DNA Extraction (Qiagen AllPrep FFPE reagents and columns) –** *Note that the first two steps should be performed before starting the RNA Extraction if performing simultaneously. The RNA Extraction can be completed during step 2 and 3.*

1. Using the pellet from Deparaffinization and Cell Lysis section, re-suspend in 180 µL of **Buffer ATL** (AllPrep FFPE Kit) and 40 µL of **proteinase K** (AllPrep FFPE Kit). Mix by vortexing.
2. Incubate at 56°C in a water bath for 1 hour.
3. Incubate at 90°C in a heat block for 2 hours.
4. Briefly centrifuge at 2,000 x g to remove drops from the inside of lid.
5. Allow sample to cool for 2 minutes at room temperature.
6. Add 4 µL **RNase A** (20 mg/mL). Invert 5 times. Incubate for 2 minutes at room temperature. Briefly spin down.
7. Add 200 µL of **Buffer AL** and vortex for 5 seconds. Briefly spin down.
8. Add 200 µL of 100% **ethanol** and vortex for 5 seconds. Briefly spin down.
9. Transfer entire sample to a QIAamp MinElute spin column (AllPrep FFPE kit) place in a 2 mL collection tube. Centrifuge for 1 minute at 8,000 x g.
10. Inspect columns. If liquid has not passed through the column, then centrifuge at 16,000 x g for 2 minutes and re-inspect. Repeat this step until all liquid has passed through the column and record how many times this step was conducted on the isolation worksheet.
11. Place column in a new collection tube.

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12. Add 700 µL of **Buffer AW1** to the column. Centrifuge for 30 seconds at 16,000 x g. If the entire sample does not go through, then repeat at 16,000 x g for 2 minutes. Repeat again if necessary at 16,000 x g for 1 minute. Record on the isolation worksheet. Place column in a new collection tube.
13. Add 700 µL of **Buffer AW2** to the column. Centrifuge for 30 seconds at 16,000 x g. If the entire sample does not go through, then repeat at 16,000 x g for 2 minutes. Repeat again if necessary at 16,000 x g for 1 minute. Record on the isolation worksheet. Place column in a new collection tube.
14. Add 700 µL of 100% **ethanol** to the column. Centrifuge for 30 seconds at 16,000 x g. If the entire sample does not go through, then repeat at 16,000 x g for 2 minutes. Repeat again if necessary at 16,000 x g for 1 minute. Record on the isolation worksheet.
15. Place the column in a new 2 mL collection tube. Centrifuge for 5 minutes at 16,000 x g to dry the column.
16. Place the column in a clean labeled 1.5 mL microcentrifuge tube. Add 100 µL of 0.1X TE to the column membrane and incubate at room temperature for 1 minute.
17. Centrifuge for 1 minute at 16,000 x g. Discard the spin column and store the sample at in a -80°C freezer or proceed with analysis immediately.

H. RNA Extraction (HighPure miRNA reagents/columns)

1. Take the supernatant from the Deparaffinization and Cell Lysis section.
2. In the fume hood, add 800 µL of **Binding Buffer** (Highpure miRNA kit) to each sample tube and vortex 15 seconds. Briefly spin down the sample.
3. In the fume hood, add 544 µL **Binding Enhancer** (Highpure miRNA kit) to each tube. Vortex 15 seconds. Briefly spin down the sample.
4. Add 700 µL of sample to the High Pure Filter and centrifuge for 30 seconds at 13,000 x g. Discard the flow through in biohazardous waste. Repeat three times with the remaining sample.
5. Centrifuge filter at 14,000 x g for 30 seconds to dry the filter and discard the flow through in biohazardous waste.
6. Add 500 µL of **Wash Buffer** working solution (HighPure miRNA kit) and centrifuge for 30 seconds at 13,000 x g. Discard the flow through in biohazardous waste.
7. Add 300 µL of **Wash Buffer** working solution and centrifuge for 30 seconds at 13,000 x g. Discard the flow through in biohazardous waste.
8. Centrifuge the filter for 1 minute at 14,000 x g to dry the filter.
9. Place the filter into a fresh 1.5 mL Eppendorf tube. Add 100 µL of **Elution Buffer** (HighPure miRNA kit) and incubate for 1 minute at room temperature. Centrifuge for 1 minute at 13,000 x g. Place sample on ice until ready to add the working DNase solution.
10. Make the working DNase solution. Note: DNase I is supplied lyophilized and should be reconstituted as described in "Reagent Preparation Section" before making the working DNase solution. The working DNase solution should be made fresh just prior to use.

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a. 1st Dilution: 1 µL DNase 1 (1500 Kunitz units) + 10 µL Buffer RDD; Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.
b. Working dilution: 1 µL of 1st dilution DNase 1 + 19 µL water (provided by DNase Kit); mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.
c. Place the working dilution on ice until ready for use.

11. Add 1 µL of working DNase solution to the eluant. Gently mix by inverting the tube and incubate 30 min in a 37°C water bath.
12. After incubation, briefly spin down the sample.
13. In the fume hood, add 325 µL of **Binding Buffer,** briefly vortex, and briefly spin down the sample.
14. In the fume hood, add 210 µL **Binding Enhancer**, vortex 15 seconds, and briefly spin down the sample.
15. Add the entire sample to a new filter tube and centrifuge for 30 seconds at 13,000 x g. Discard the flow through in biohazardous waste.
16. Centrifuge the filter tube at 14,000 x g for 30 seconds to dry the filter. Discard the flow through in biohazardous waste.
17. Add 500 µL of **Wash Buffer** working solution and centrifuge for 30 seconds at 13,000 x g. Discard the flow through.
18. Add 300 µL of **Wash Buffer** working solution and centrifuge for 30 seconds at 13,000 x g. Discard the flow through in biohazardous waste.
19. Centrifuge the filter tube for 1 minute at 14,000 x g to dry the filter.
20. Place the filter into a labeled 1.5 mL microcentrifuge tube. Add 100 µL of **Elution Buffer** incubate for 1 minute at room temperature.
21. Centrifuge for 1 minute at 13,000 x g. Discard the column and store sample in a liquid nitrogen freezer or proceed with QC immediately.

I. Quantification and Normalization of RNA Samples

1. Prepare a set of 0.5 mL tubes with the unique sample identifier and portion number followed by R (for RNA). Add 98 µL of 50 mM sodium hydroxide to each tube.
2. Add 2 µL of the concentrated stock sample to the sodium hydroxide. Vortex for at least 5 seconds to ensure that the diluted sample is well mixed and briefly spin down.
3. Read the absorbance at 260 nm, 280 nm, and 320 nm in the spectrophotometer using a quartz cuvette.
4. Desired sample concentration may vary among projects.
   a. If the concentration is above the desired concentration use the known volumes and concentrations to calculate the amount of DEPC-treated water to add to the sample to yield a final desired concentration as shown below.
   b. If the concentration of the RNA sample is less-than the desired concentration, concentrate to the desired volume (see example below) using the speedvac with no heat.

Standard Operating Procedure (SOP) for FFPE RNA/DNA Co-Isolation Using the Allprep FFPE Kit and the Highpure miRNA Kits

5. After diluting or concentrating samples, repeat steps 1-3 to confirm that the sample is within the target concentration range.
6. When the samples are at the target concentration, transfer to a labeled matrix tube (this is the primary sample aliquot). The matrix tube has a max capacity of 500 µL. If the sample has a volume larger than 500 µL, the sample must be split into multiple matrix tubes and aliquots must be created in LabVantage per BCR-SOP-A005, "LabVantage User Manual". Create a second aliquot for RIN/DV200 determination per BCR-SOP-M002, "RNA NanoAssay".

J. Quantification and Normalization of DNA Samples – Refer to BCR-SOP-M017, "PicoGreen DNA Quantification (Manual).

K. Sample Storage – PRNA samples are stored in the liquid nitrogen freezer; DNA samples are stored in a -80°C freezer.

L. Sample Calculations

1. Example: Samples that need to be diluted (concentrations >0.165 µg/µL)

[(current concentration/desired concentration) x current volume] – current volume
= [(0.21 µg/µL / 0.165 µg/µL) x 18 µL] - 18 µL
= 4.9 µL (volume of diluent to add)

Final sample volume: 22.9 µL (18 µL + 4.9 µL)

2. Example: Samples that need to be concentrated (concentrations <0.165 µg/µL)

[(current concentration/desired concentration) x current volume] – current volume
= [(0.08 µg/µL / 0.165 µg/µL) x 18 µL] - 18 µL
= 9.3 µL (volume to remove during concentration)

Final sample volume: 8.7 µL (18 µL – 9.3 µL)

III. REFERENCES

A. BCR-REF-001, "BCR Acronym List"
B. BCR-REF-002, "BCR Glossary"
C. BCR-SOP-A005, "LabVantage User Manual"
D. BCR-SOP-M002, "RNA NanoAssay"
E. BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)
F. HighPure miRNA Isolation Kit, Roche, March 2013
G. AllPrep DNA/RNA FFPE Handbook, Qiagen, June 2012

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made the Version **2**, Effective Date 8/16/2016
1. Minor word, spelling, and grammatical changes made throughout
2. Updated section II.E., including:

Standard Operating Procedure (SOP) for FFPE RNA/DNA Co-Isolation Using the Allprep FFPE Kit and the Highpure miRNA Kits

a. Added specific storage temperatures
b. Changed the stability of buffers from 6 months to up to one year
c. Added instructions to wear PPE when handling guanidine thiocyanate

3. Updated section II.F., including
   a. Added Cell Lysis
   b. Clarified contents and disposal of supernatant
4. Reorganized flow of procedure so that DNA Extraction is now before RNA Extraction.
5. In the RNA Extraction section, clarified the procedure for making the working DNase solution.
6. Updated the procedure in section II.I. for Quantification and Normalization of RNA Samples
7. Removed the details of section II.J. for Quantification and Normalization of DNA samples and left reference to BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)
8. Removed the Criteria for Repeating Extraction and Sample Pooling in section II.K.

B. Version 1, Effective Date 08/04/14 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for Confirming Specimen Identity Using The Identifier Sequence-Specific Tandem Repeat (SSTR) Multiplex

I. SCOPE AND PURPOSE

The AmpF*l*STR® Identifiler® PCR Amplification Kit is a short sequence-specific tandem repeat (SSTR) multiplex assay that co-amplifies 15 SSTRs and the Amelogenin marker in a single PCR amplification reaction. The Amelogenin locus is used for gender identification as PCR amplification products of different lengths are generated from the X and Y chromosomes. The multiplex produces quality results necessary to uniquely identify a sample. The average match probability of identity profiles for 2 randomly selected unrelated Caucasian individuals is 5.01×10^{18}. The widely accepted tetranucleotide SSTRs co-amplified in the Identifilier Kit include the thirteen core SSTRs as required for sample entry into the Combined DNA Index System (CODIS) (Budowle et al., 1998). The data generated from these loci also satisfy the recommendations for several worldwide human identification databases including the European Network of Forensic Science Institutes (ENFSI) and Interpol organizations.

This procedure is used as a back-up method to SOP M010, "Tissue Matching by SNP Analysis" to confirm that normal and tumor samples are from the same patient as a quality control metric. It may also be used for cases in which a sample swap is suspected or to confirm whether DNA from two subjects are co-mingled in one sample.

II. PROCEDURE

A. Safety Procedures

1. Use Standard Precautions when handling all body fluids, tissues and cell cultures.
2. Wear Personal Protective Equipment (PPE), including a lab coat and nitrile gloves.

B. Reagent Preparation

1. AmpF*l*STR PCR reaction mix, Identifiler® Primer Set, and Identifiler® Allelic Ladder are received frozen. They are stored in a -20°C and refrozen after each use.
2. AmpliTaq Gold® DNA Polymerase is stored in a -20°C and refrozen after each use.
3. AmpF*l*STR Control DNA 9947A is stored in a -20°C and refrozen after each use.
4. IMPORTANT: The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpF*l*STR Identifiler Primer Set, Allelic Ladder, GeneScanTM-500 LIZTM Size Standard and amplified, fluorescently labeled PCR products from light.

C. Quality Control

1. Any deviations from the protocol as written should be documented at the sample level in LabVantage. Protocol deviations that have the potential to compromise sample quality or results should also be documented with an incident report.
2. Patient cases for which the genetic profile of the tumor tissue does not correlate with that of the normal control will be considered genotyped mismatches.
3. All new lots of reagents are testing in parallel with the one in current use before being put into use. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used

interchangeable between kit lot numbers. All QC results are recorded in the Quality Control notebook.

4. Separate designated areas will be used for pre- and post-PCR to avoid amplicon contamination of new reactions. The thermal cycler and ABI 3730 are located in the post-PCR area. Filtered pipet tips and pre-PCR-only designated pipettes will be used to set up all PCRs.
5. Aerosol-resistant tips are used for reaction assembly and sample analysis to prevent possible contamination.
6. Controls:
   a. Negative controls (e.g., a sample containing nuclease-free water with all reaction components except DNA) are run with each assay to detect potential contamination
   b. A single master mix is made containing all reagents necessary for the run except the template to control for individual well contamination.
   c. The cell line DNA, TCGA-AV-A03D-20A-01D is used as a positive control.
   d. The accuracy and batch-to-batch reproducibility of the assay is assessed by confirmation of the positive control genotype calls.

D. Required Equipment, Supplies, and Reagents

1. Equipment

a. 96-well formatted centrifuge
b. Vortex
c. ABI 3730 Genetic Analyzer
d. Genotyper® software
e. 96-well formatted Thermal cycler
f. Pipettors (2 µL, 20 µL, 200 µL, 1,000 µL)
g. Cap-It-All
h. Heat Block (95°C)

2. Supplies

a. ABI 3730 48 capillary array, 36 cm (Applied Biosystems, catalog# 4331247)
b. Filtered pipet tips
c. Labeling tape
d. Personal Protective Equipment (PPE)
e. Microseal F film (Biorad, catalog# MSF-1001)
f. Microseal B film (Biorad, catalog# MSB-1001)
g. Microcentrifuge tubes, 1.5 mL
h. Microcentrifuge tubes, 2.0 mL
i. MicroAmp optical 96 well reaction plate (Applied Biosystems, catalog# N801)
j. Plate Septa-96 Well (Applied Biosystems, Inc., Catalog# 4315933)

3. Reagents

a. Water, Molecular Biology Reagent (Sigma #W4502)
b. Deionized, Distilled Water – Type I (AquaSolutions)

Standard Operating Procedure (SOP) for Confirming Specimen Identity Using The Identifier Sequence-Specific Tandem Repeat (SSTR) Multiplex

c. 1X or 0.1X Tris-EDTA (TE), pH 8, diluted from 100X TE (Sigma, catalog# T9285)
d. 10X Genetic Analyzer Buffer (Applied Biosystems, catalog# 402824)
e. POP7 Polymer (Applied Biosystems, catalog# 4335615)
f. Hi-Di ™ formamide (Applied Biosystems, catalog# 4311320)
g. AmpF*l*STR® Identifiler® kit (Applied Biosystems, catalog# 4322288)
h. GeneScan 500 LIZ internal lane standard

E. Procedure

1. Tumor, normal, and control DNA are diluted to a final concentration of 10 ng/µL. following the instructions below.
    a. Remove the Matrix rack containing the stock samples from the liquid nitrogen, -80°C freezer, or the refrigerator.
    b. When the samples are completely thawed, centrifuge the stock samples briefly to collect all the liquid at the bottom of the tube.
    c. Label a 96-well unskirted PCR plate with the plate ID, 10 ng/µl, and the date.
    d. Calculate the amount of water and DNA to add to create a 10 ng/µl dilution. This would be 14 µl of nuclease-free water and 1 µl of DNA if the stock DNA was at 150 ng/µl.
    e. Add the nuclease-free water to each well.
    f. Use the Cap-It All to remove the caps of the tubes in the Matrix rack containing the stock samples. Samples will be kept uncapped only long enough to remove the sample.
    g. Transfer the stock samples immediately to the appropriate wells of the labeled 96-well unskirted PCR plate using a multi-channel pipet.
    h. Use the Cap-It All to recap the stock sample Matrix rack.
    i. Seal the dilution plate with a sheet of adhesive foil.
    j. Vortex the plate for 5 seconds, while holding firmly at the edges.
    k. Briefly centrifuge the plate in a bench top centrifuge at 2200 rpm for 30 seconds.
2. Prepare the master mix, taking care to avoid exposing fluorescent dyes to light.
    a. Determine the total number of samples, including controls (one positive and one negative) and 10% extra to account for pipetting error.
    b. Vortex the following reagents for 5 seconds to mix and briefly centrifuge: AmpF*l*STR PCR Reaction Mix, and AmpF*l*STR Identifiler Primer Set.
        i. NOTE: Do not vortex AmpliTaq Gold DNA Polymerase enzyme.
    c. Calculate the required amount of components as follows:
        i. Number of samples x 5.25 µL of AmpF*l*STR PCR Reaction Mix
        ii. Number of samples x 0.25 µL of AmpliTaq Gold DNA Polymerase
        iii. Number of samples x 2.75 µL of AmpF*l*STR Identifiler Primer Set
        iv. Number of samples x 4 µL of Nuclease-free Water
    d. Vortex the master mix at medium speed for 5 seconds.
    e. Dispense 12.5 µL of master mix per PCR tube.

Standard Operating Procedure (SOP) for Confirming Specimen Identity Using The Identifier Sequence-Specific Tandem Repeat (SSTR) Multiplex

3. Add 1 µL of sample, previously diluted to the appropriate PCR wells.
4. Add 1 µL of control DNA to the appropriate well.
5. Add 1 µL of Nuclease Free water to the negative control well.
6. Cover the plate with an adhesive clear plastic cover, vortex slightly and spin down.

F. PCR Amplification

1. Select program "SSTR" on the thermal cycler:

Initial Incubation	Denature	Anneal	Extend	Final Extension	Final Step
HOLD	CYCLE (28 cycles)			HOLD	HOLD
9°C 11 minutes	94°C 1 minute	59°C 1 minute	72°C 1 minute	60°C 60 minutes	4°C (forever)

2. Place the tray in the thermal cycler and close the heated cover.
3. Start the thermal cycler.
4. Remove the tubes from the instrument block after the PCR is complete.
5. Store the amplified DNA in a -20°C freezer, or proceed directly to the ABI for genotyping.
   IMPORTANT: Protect the amplified products from light.

G. ABI 3730 Set-up

1. Dilute PCR products 1:10 with Nuclease-free Water.
2. Combine 0.5 µL of diluted PCR product with 9 µL Hi-Di™ Formamide and 0.5 µL GS500 LIZ (internal lane standard). Cover with plate septa and spin down briefly in a plate centrifuge.
3. Heat denature for 3 minutes at 95°C, and immediately chill on ice for 2 minutes.
4. Assemble plate, tray cover, and plate base. Place on autosampler.
5. Follow the AB 3730 Genetics Analyzer procedure (EQP-35) for instrument preparation and use.
6. Complete plate record and link to plate assignment.
7. Press the green arrow.
8. Review extracted sample files in Genotyper software.
9. Print results from each case. Normal DNA should be on one page and tumor DNA on another for comparison. Each color is represented on its own line, and peaks should be labeled with base pair size. The base pair size peaks for each marker should match between the normal and the tumor. If a sample is mixed (more than two alleles for each marker), or if the tumor does not match the normal, then the case will fail for identity.

H. Amplified Loci

Locus Designation	Chromosome Location	Dye Label
D8S1179	8	6-FAM

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D21S11	21q11.2 – q21	
D7S820	7q11.21 – 22	
CSF1PO	5q33.3 – 34	
D3S1358	3p	VIC
TH01	11p15.5	
D13S317	13q22 – 31	
D16S539	16q24 – qter	
D2S1338	2q35 – 37.1	
D19S433	19q12 – 13.1	NED
vWA	12p12 – pter	
TPOX	2p23 – 2per	
D18S51	18q21.3	
Amelogenin	X: p22.1 – 22.3 Y: p11.2	PET
D5S818	5q21 – 31	
FGA	4q28	

III. REFERENCES

A. AmpF*l*STR® Identifiler PCR Amplification kit instructions. August 2012.
B. Application Note for AmpF*l*STR® Identifiler PCR Amplification Kit
C. Budowle, B. et al. 1998a. CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools. Second European Symposium on Human Identification. 73-88.

IV. COMPREHENSIVE REVISION HISTORY

A. Version 2, Effective Date 4/25/2016
1. Made title lower case
2. Added wording to clarify how profiles are compered ("average probability of identity profiles").
3. Reagents are stored in a "-20°C freezer"
4. Added wording for QC and use of reagents that come in a kit.
5. Added heat block as required equipment
6. Added steps for creating dilution plate.
7. Added precautionary statement: do not vortex enzyme.
8. Added step to spin down plate after adding sample and reagents.

B. Version 1, Effective Date **08/26/2014** - New

Standard Operating Procedure (SOP) for Confirming Specimen Identity Using The Identifier Sequence-Specific Tandem Repeat (SSTR) Multiplex

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR DATA ENTRY OF PATHOLOGY REVIEW FORMS

I. SCOPE AND PURPOSE

A pathologist reviews all cancer patient cases to ensure high quality of specimens for all Nationwide Children's Hospital (NCH) Biospecimen Core Resource (BCR) projects. Hemotoxylin and eosin (H&E)-stained tissue sections are provided to the pathologist on glass slides or as digital images generated by a whole slide imager (such as the Aperio Scanscope XT). The pathologist reviews the slide(s) or images, completes the appropriate Pathology Review form and forwards the information to the BCR. These data are entered into LabVantage either by a qualified BCR staff member or automatically via the LabVantage-VIPER integration process.

This procedure applies to all BCR personnel responsible for entry of the Pathology Review data. The purpose of this Standard Operating Procedure is to describe the method for completing the entry of the pathology review data into the LabVantage database.

II. PROCEDURE

A. Equipment And Materials

1. VIPER Digital Pathology Review System
2. Aperio AT
3. Aperio Scanscope XT
4. Hamamatsu Nanozoomer, 2.0HT
5. NCH BCR Review Form
6. LabVantage

B. Data Entry from paper review form

1. When a Pathologist completes pathology review form, the VIPER System notifies appropriate BCR staff that electronic review form(s) have been submitted. The forms are then printed and distributed to data entry personnel. Each form is specific to a unique BCR case or slide.
2. The BCR staff member logs onto LabVantage using his/her assigned username and password to open the database.
3. Navigate to "Admin Samples" and search by "TCGA Patient ID".
4. Enter the unique NCH BCR Patient ID (example as underlined: TCGA-BS-A0G7-01A-01-TS1)
5. Hit the "OK" Button and this will generate an onscreen list of slides that have already been created for the requested case.
6. Select the entry that matches the accession number on the respective "NCH BCR Review Form" form by checking the box next to all slides and potions for this case and hit the "Modified Data Entry" button at the top of the screen.
7. Under "Choose a View" select "Gridview Test Results Across".
8. All data from the respective "Pathologist Review of H&E Slide" form (as follows) is entered into LabVantage by :
   a. Entering the numeric percentages for each data entry field

STANDARD OPERATING PROCEDURE (SOP) FOR DATA ENTRY OF PATHOLOGY REVIEW FORMS

b. Adding the reviewing Pathologist's name
c. Entering the date the review was completed
d. Entering the portion status (pass or fail determination based upon the rules for the particular tumor type)
e. Changing the slide status from "Pending" to "Complete" and the process status from "In Progress" to "VM-Complete"
f. Hit the "Save" button at the top of the screen
g. Stamp review paper form with "Entered Stamp"
h. Hit the "Return to List" button at top of screen to proceed to next case

9. Other requirements for data entry (Please note that this information may not be applicable for all tissue types):
   a. Make sure values add to 100% where required (% Tumor Nuclei and % All Other Nuclei) and (% Cellular Tumor, % Normal, % Stroma and % Necrosis)
   b. If portion is not cut, enter "Not Used" as portion status
   c. Enter any comments from the review pathologist in the "Comments" field. If the Pathologist does not provide any information for the cellular components (lymphocytes, macrophages, endothelial cells and neutrophils), ask the Pathologists if these numbers should be zero and if he/she agrees, enter zero for these fields in LabVantage. The values for lymphocytes, macrophages, endothelial cells and neutrophils do not need to add to one hundred, but ensure they do not exceed 100%.
10. When all data for the specific slide has been entered in LabVantage, save the data.
11. Return to Step II.B.4. and enter the unique BCR Patient ID to continue entering data into LabVantage for the next slide.

C. Automated LabVantage Data Entry

1. Daily, an integration service running across LabVantage and VIPER will run at 7:00 am.
2. Data from any case that has been reviewed and submitted in VIPER during the previous 24 hours is automatically entered into the LabVantage database.
3. An audit is automatically generated and sent to biomedical imaging coordinators with the results of the data transfer, including the cases that were entered and the % Tumor Nuclei and % Necrosis for each slide.
4. Using the data provided in the audit, imaging coordinators select the appropriate status for each portion from the dropdown list (pass or fail determination based upon the rules for the particular tumor type).
5. If a completed review is submitted after 7:00 am but prior to the close of the business day, imaging coordinators can manually initiate the LabVantage-VIPER integration process. (Per BCR-SOP-A005, "LabVantage User Manual").
6. After data has been entered into the LabVantage database, the imaging coordinator updates the slide status from "pending" to "complete" in the dropdown list.
7. Click "save" and "return to list" (per BCR-SOP-A005, "LabVantage User Manual").
8. Other requirements

STANDARD OPERATING PROCEDURE (SOP) FOR DATA ENTRY OF PATHOLOGY REVIEW FORMS

a. If portion does not have a slide cut, enter "Not Used" as portion status
b. Check comments from the review pathologist in the "Comments" field. If there is a comment that causes concern, notify the Program Director or the Lead Pathologist and copy the BCR PI to assist in decision making.
c. If portion 3 is sent for pathology review from a Molecular re-evaluation and portion 3 fails pathology, please contact Logistics via email to see if more tissue is available to process more portions to be reviewed. If so have additional slide(s) sent for pathology review. If not, fail the case at the subject level (per BCR-SOP-A005, "LabVantage User Manual") and leave the Molecular fail reasons.
d. If project requires use of multiple slides, an average of path metrics may be used. The BCR staff member will add path metrics for each slide used and take the average. Qualifying path metrics may be different per project. Obtain the qualifying project path metrics from the BCR Program Director, a BCR Project Manager, or the VM manager.

D. Quality Control - After the Pathology Review data are entered into the LabVantage database, the data are independently reviewed by a second qualified member of the BCR staff. Any discrepancies identified are discussed with the originating staff member and either consensus is reached, or the discrepancy is referred to the BCR Program Director and Lead Pathologist and copy the BCR PI for further clarification with the Pathologist.

III. REFERENCES

A. BCR-REF-001, "BCR Acronym List"
B. BCR-REF-002, "BCR Glossary"
C. BCR-SOP-A005, "LabVantage User Manual"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version **5**, Effective Date 4/27/2015
1. Renumbered must of the formatting
2. Added the option to use multiple slides and use the average of path metrics when determined by the project.
3. Added references
4. Removed "4-digit" from BCR Patient ID number
5. Replaced "Sapphire" with "LabVantage"

B. Changes made to Version 4 Effective Date 04/22/2014
1. Used new format
2. Minor word changes made throughout, including changing "Helpful Hints" to "Other requirements"
3. An additional section regarding "Other requirements" was included
4. Removed TCGA labels and made the SOP more general to cover all NCH BCR projects.
5. The link to Sapphire/LabVantage was removed since an error message appeared when used

STANDARD OPERATING PROCEDURE (SOP) FOR DATA ENTRY OF PATHOLOGY REVIEW FORMS

C. Changes made to Version 3 Effective Date 7/30/2012
1. Formatting and minor word changes made throughout
2. Removed statement in the Introduction that the pathologist will directly enter data
3. Section II.A. was clarified with an additional step to search
4. Section II.A.8.d. was clarified with the rules for pass/fail determination
5. In section II.A.9., steps c and d were recombined
6. Section II.B. was added regarding automated LabVantage data entry

D. Changes made to Version 2 Effective Date 7/10/2012
1. Minor word changes made throughout
2. Title changed from "Data Entry of Pathology of Quality Control" to "Data Entry of Pathology Review Form"
3. References to the Sapphire database updated to LabVantage to reflect the new name for the same database
4. Procedures related to ICG were removed
5. Section II.8. was clarified to state that the values for lymphocytes, macrophages, endothelial cells and neutrophils do not need to add to one hundred, but should not exceed 100%

E. Version 1 Effective Date 11/14/2011 - New

STANDARD OPERATING PROCEDURE (SOP) FOR DATA ENTRY OF PATHOLOGY REVIEW FORMS

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie M. Gastier-Foster, Ph.D.,
FACMG, Principal Investigator

Standard Operating Procedure (SOP) for Quality Control of Pathology Paper Forms

I. SCOPE AND PURPOSE

The paper "NCH-BCR Review Form" and a rubber "ENTERED" stamp are used as communication tools to document the completion status of pathology-related data entry.

This procedure applies to all Biospecimen Core Resource (BCR) personnel responsible for filing the paper "NCH-BCR Review Form" and the data entry processing for the portions and the corresponding results of the quality control completed by the pathologist. BCR pathologists submit completed pathology review forms with specific pathology metrics that are used to determine qualification of the tissue. When a review form is unable to be electronically submitted to the LabVantage system, the review form must be printed and manually entered into the system.

The stamped paper "NCH-BCR Review Form" reflects the work status of each case. The use of this form with entered stamp ensures that all pathology related data is filed and entered into the LabVantage system in a timely, accurate and consistent manner.

II. PROCEDURE

A. Pathology metric data is entered into the LabVantage system per SOP P001, "Data Entry of Pathology Review Forms".

B. After data have been entered in LabVantage from the paper "NCH-BCR Review Form", the paper form is stamped with a rubber "ENTERED" stamp.

C. The entered and stamped "NCH-BCR Review Form" is passed to another qualified BCR staff member to verify that the paper "NCH-BCR Review Form" matches LabVantage. This staff member documents this verification by initialing inside the box of the rubber "Entered Stamp".

D. The QC'd paper "NCH-BCR Review Form" will go into the appropriate binder for filing.

E. Any discrepancies identified are discussed and consensus is reached or the discrepancy is referred to BCR Management for further clarification.

III. REFERENCES - SOP P001, "Data Entry of Pathology Review Forms"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 3, Effective Date 8/25/2015
1. Minor formatting

B. Changes made in Version 2, Effective Date 1/3/2014
1. New format used
2. References to TCGA and International Genomics Consortium removed
3. Title modified
4. Minor word and grammatical changes made throughout

C. Version 1, Effective Date 11/14/2011 - New

Standard Operating Procedure (SOP) for Quality Control of Pathology Paper Forms

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for Virtual Imaging for Pathology Education and Research (VIPER) Quality Control

I. SCOPE AND PURPOSE

The daily created Nationwide Children's Hospital Biospecimen Core Resource (NCH BCR) case list spreadsheet and the Virtual Microscopy (VM) document titled "VIPER Case QC Record" on the Biospecimen Core Resource (BCR) SharePoint site are the tools used for communication for this process.

This procedure applies to all BCR personnel responsible for entering data into the VIPER system for NCH BCR Pathology review. Biomedical Imaging Coordinators (BIC) will enter all information into the VIPER system and a Biomedical Imaging Technician (BIT), or designee; will Quality Control (QC) all data that has been entered.

All QC updates are entered into the VIPER QC log on SharePoint to ensure that cases have been reviewed for accuracy. Should imaging staff notice any discrepancies or have any questions, the BIC who entered the information will be notified of these issues and the SharePoint site will be updated.

II. PROCEDURE

A. After new NCH BCR cases have been entered in VIPER by a BIC (per BPC SOP VMI-005 "Central Reviews"), the Coordinator will assign the cases to an account used for internal viewing (per BPC SOP VMI-005 "Central Reviews").

B. The BIC will update the BCR SharePoint with the daily case list of cases that need to be QC'd. The cases that need to be QC'd will be highlighted yellow so the BIT knows what cases to look for in the VIPER account. The most recent VIPER Case QC Record is located in VM's Shared Documents folder on the BCR SharePoint site.

C. The BIT will be notified by the BIC that cases are on the BCR SharePoint site ready for QC.

D. The BIT will go to the latest VIPER Case QC Record located in VM's Shared Documents folder on BCR SharePoint and pull up the case list to be QC'd. On Sheet 1 the cases to be QC'd will be highlighted in yellow

E. Log into VIPER as a reviewer

1. Open the review form for each case and ensure all data are accurate. QC to ensure correct specific project form is attached to the case. Make sure case numbers match on slides and the correct number of slides are present. Also look to make sure the subtype listed on the review form matches the subtype in the case QC record.

F. The BIT will then highlight and cut the case list from Sheet 1 of the SharePoint spreadsheet and paste the information into Sheet 2 of the SharePoint spreadsheet. All cases that have been QC'd without issue will then be highlighted green. Cases with issues will be left on Sheet 1 and highlighted red

Standard Operating Procedure (SOP) for Virtual Imaging for Pathology Education and Research (VIPER) Quality Control

G. The Technician will also put their initials and the date the QC was performed on the list on Sheet 2 and will hit "Save" to save all new information.

H. The BIT will inform the BIC that VIPER QC has been completed.

I. Quality Control

1. Slides and project specific review forms are checked for accuracy of information and to ensure the number of slides is correct, and forms function properly for the reviewing pathologist.
2. Case must be QC'd using the daily NCH BCR case list.
3. Any discrepancies identified are discussed and a decision is reached by either the Program Director, Pathology Director, or VM Manager

III. REFERENCES

- BPC SOP VMI-005 "Central Reviews"

IV. COMPREHENSIVE REVIEW HISTORY

A. Changes made to Version 3, Effective Date **1/21/2016**

1. Minor changes to process to accommodate VIPER upgrade
2. Made title not all capitalized

B. Changes made to Version 2, Effective Date **4/18/2014**

1. New format used
2. Minor word and grammatical change made throughout
3. Removed minor steps on what to click on when in SharePoint and in the VIPER application
4. References to TCGA were removed throughout so the SOP can apply to all BCR projects
5. In the Quality Control section, added that the Program Director, Pathology Director, or VM Manager will make the final decision regarding any discrepancies

C. Version 1, Effective Date 9/18/2012 - New

Signatures

Approved By: **Signature on File** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

STANDARD OPERATING PROCEDURE (SOP) FOR DAILY CASE CREATION LIST IN VIRTUAL MICROSCOPY (VM)

I. SCOPE AND PURPOSE

The daily case list spreadsheet, LabVantage, the "PackageTransferCDT" report, and the "PathologyDataForVIPER" report are the tools used for this process.

This procedure applies to all Biospecimen Core Resource (BCR) Biomedical Imaging Coordinators responsible for creating cases in the VIPER system. The procedure describes the process for the creation of a case list for entering new cases into the VIPER system.

II. PROCEDURE

A. After taking custody of slides per SOP A005, "LabVantage User Manual", BCR biomedical imaging coordinators utilize the "PackagetransferCDT" report (http://rex/BPC/TCGA-BCR/Reports/Forms/Category%20Grouped.aspx) to generate an electronic list of the received slides and their subtypes.

B. The Custodial Domain Transfer (CDT) number found at the top of the CDT Package Transfer Report accompanying the glass slides is selected from the "CDT" dropdown box, the "Status" dropdown box is set to "empty" and the "apply" button is clicked

C. After the report displays, select "Export" > "Excel" from the "Actions" dropdown menu in the top left-hand portion of the screen.

D. Copy all slide barcodes and subtypes in the excel spreadsheet as a block of cells.

E. Select the first cell under the "Case" column of the Daily Case List and paste the block of cells copied in the previous step

F. The alphanumeric slide names (i.e. TSA, S1 etc.) for the slides present for each unique case are entered into the "Slides" column next to the first slide barcode of each unique case. The rows containing subsequent slide barcodes (TS2, S2, etc.) for each case are deleted.

G. Using the find function, the suffixes containing the portion numbers and slide names (i.e., -01-TS1) are removed from the slide barcodes, leaving the "case name" to be entered into VIPER (e.g., TCGA-AA-A000-01A, ALCH-ABBY-TTP1-A-1-0-S1).

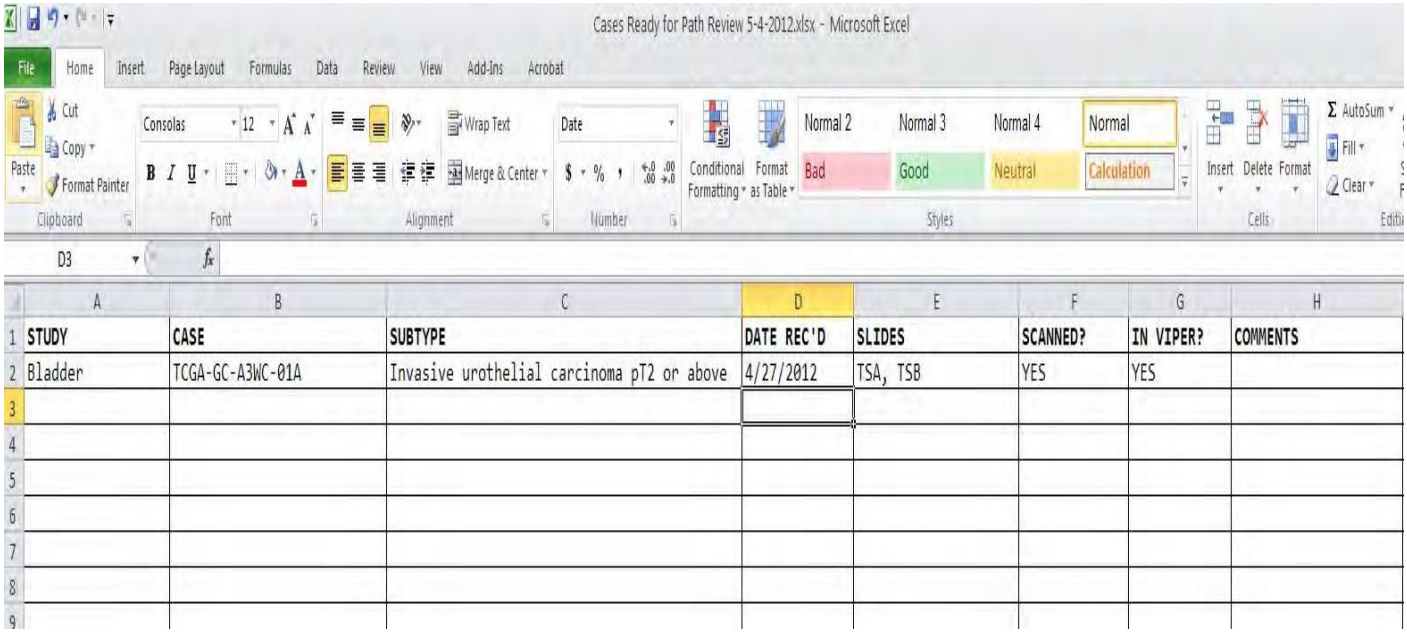
H. Enter the study for each case under the "STUDY" column.

STANDARD OPERATING PROCEDURE (SOP) FOR DAILY CASE CREATION LIST IN VIRTUAL MICROSCOPY (VM)

I. Indicate whether the case has been scanned by typing "YES," "NO," or "IN PROGRESS" into the "SCANNED?" column.

J. Type "NO" next to each case under the "IN VIPER?" column. Once cases have been created in VIPER, "Yes" may be entered in the column before the list is sent for QC.

K. For each case, enter the date the case was received into the BCR under the "DATE REC'D" column.



	A	B	C	D	E	F	G	H
1	STUDY	CASE	SUBTYPE	DATE REC'D	SLIDES	SCANNED?	IN VIPER?	COMMENTS
2	Bladder	TCGA-GC-A3WC-01A	Invasive urothelial carcinoma pT2 or above	4/27/2012	TSA, TSB	YES	YES	
3								
4								
5								
6								
7								
8								
9								

L. **QUALITY CONTROL:** Imaging technicians QC the daily case list by checking the information contained in the list against the reports used to generate the list, as well as the cases created in VIPER.

III. REFERENCES

A. BCR-REF-001, "BCR Acronym List"

B. BCR-REF-002, "BCR Glossary"

C. BCR-SOP-A005, "LabVantage User Manual"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version 4, Effective Date 6/17/2015

1. Removed step to select barcodes from "Case" column and copy/paste into "Slides" column in "Slides" sheet of Daily NCH BCR Case List
2. In Section II.J., added option to enter "Yes" into column before sending list for QC.
3. Added new references

B. Changes made to Version 3, Effective Date 4/17/2014

1. New format used

STANDARD OPERATING PROCEDURE (SOP) FOR DAILY CASE CREATION LIST IN VIRTUAL MICROSCOPY (VM)

2. Minor word and grammatical changes made throughout
3. Title updated
4. All references to TCGA were removed to reflect the use of this SOP for all BCR projects
5. Removed section to delete columns from "CDT Package Transfer Report" to make "TCGA Number" and "Subtype" columns adjacent to one another

C. Changes made to Version 2, Effective Date 9/11/2012
1. Some minor word changes made throughout
2. Screen shots were changed
3. Significant changes made to the step-by-step instructions

D. Version 1, Effective Date 9/14/2012 - New

STANDARD OPERATING PROCEDURE (SOP) FOR DAILY CASE CREATION LIST IN VIRTUAL MICROSCOPY (VM)

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

I. SCOPE AND PURPOSE:

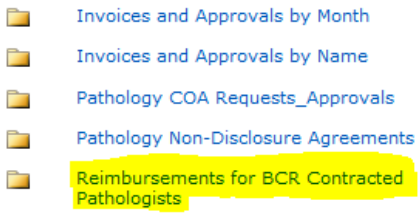
The purpose of this Standard Operating Procedure (SOP) is to provide guidelines to process payments for contracted pathologists who review pathology slides for the Biospecimen Core Resource (BCR) Projects. This procedure applies to the BCR Clinical Outreach (CO) team and Virtual Microscopy (VM) team. A monthly report is generated listing pathologists and their assigned slides per project that have been reviewed the previous month. The pathologists are paid for each slide they have reviewed.

II. PROCEDURE:

A. Clinical Outreach Representative (COR) receives email from VM with the reimbursement report attached.

1. Monthly generation of reimbursements is created by a VM team member who sends it to the COR via email.
2. COR saves the reimbursement report to the BCR SharePoint page by opening the "Shared Documents" folder located on the left hand side of the screen.
    a. Open the "Pathology" folder.

    b. Open the "Reimbursements for BCR Contracted Pathologists" folder.



    c. Save the current month's spreadsheet created by the VM team member.
3. COR checks the total number of slides for each pathologist.
    a. Each pathologist is listed with a list of slides that he/she reviewed per project.

B. COR inputs slide count numbers into "*Pathologist Details of Payments – All Inclusive*" spreadsheet found on SharePoint.

1. COR opens spreadsheet named "*Pathologist Details of Payments – All Inclusive*" located in the "Pathology" folder on SharePoint.
2. COR inputs information into "*Pathologist Details of Payments – All Inclusive*" spreadsheet in two locations of tabs located at the bottom on the spreadsheet:
    a. <u>By [Project] [Year] Tab (</u>updated by creating a new tab per project each year)
        i. Locate the pathologist's name and input in the total number of slides reviewed under the field "# Slides Reviewed"

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

Color Key:
No Slides Reviewed
Slides Reviewed - Pending Payment
Slides Reviewed - Payment Complete
General Information

Pathologist Name 1	# Slides Reviewed	# Hours	Amount Owed	Amount Paid	PO Amount Available "PO NUMBER"	Invoice #	Check Sent	NOTES
					$ 10,000.00			
Jan-16	0	0	$ -		$ 10,000.00			
Feb-16	0	0	$ -		$ 10,000.00			
Mar-16	0	0	$ -		$ 10,000.00			
Apr-16	50	12.5	$ 1,200.00	$ 1,200.00	$ 8,800.00	BCR###	MM/DD/YYYY	
May-16	0	0	$ -		$ 8,800.00			
Jun-16	2	0.5	$ 48.00		$ 8,800.00			
Jul-16		0	$ -		$ 8,800.00			
Aug-16		0	$ -		$ 8,800.00			
Sep-16		0	$ -		$ 8,800.00			
Oct-16		0	$ -		$ 8,800.00			
Nov-16		0	$ -		$ 8,800.00			
Dec-16		0	$ -		$ 8,800.00			
Total	52	13	$ 1,248.00	$ 1,200.00				

ii. Spreadsheet will calculate out the number of hours and amount owed to each pathologist at rate of $24/slide and 4 slides/hour. E.g.: 32 slides = 32/4 = 8 hours and 32 x $24 = $768

iii. Invoice number will be generated in the next step. The invoice number should be copied and pasted to "Invoice #" field.

iv. Highlight the row orange to indicate that it is a pending payment. Once payment has been issued, the row is highlighted green to indicate completion.

b. <u>By Invoice No. Tab</u>

i. An invoice number is predetermined in the spreadsheet field called "Invoice No."

(a) The invoice number's format is BCR### with ascending numbers starting from BCR001.

(b) The last listed invoice number is dragged down the column in order to generate more invoice numbers if needed.

(c) Assign each pathologist to an invoice number by inputting the pathologist name in each available field.

Invoice No.	Pathologist	P.O. #	Description	Amount	Slide Count	Hours	Date Emailed Pathologist	Date Approval	Date to Accounts Payable	Date of Check Sent
BCR001	Pathologist Name 1	PO 1	Reviews for June 2016	$ 48.00	2	0.5	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR002	Pathologist Name 2	PO 2	Reviews for June 2016	$ 96.00	4	1	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR003	Pathologist Name 3	PO 3	Reviews for June 2016	$ 120.00	5	1.25	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR004				$ -		0				
BCR005				$ -		0				
BCR006				$ -		0				

(d) Copy and paste the invoice number to the "By [Project] [Year]" Tab to the corresponding pathologist's name and to the month of reimbursement.

ii. Verify that each pathologist has the correct Purchase Order (P.O.) number.

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

(a) The P.O. number is generated by Nationwide Children's Hospital Accounting Department and is unique for each pathologist and project.

(i) P.O. numbers can be found by searching for the name of the pathologist in the tab "By Name [Year]" in the "*Pathologist Details of Payments – All Inclusive*" Spreadsheet. This is where the P.O. numbers are stored for easy look up.

Color Key:
No Slides Reviewed
Slides Reviewed - Pending Payment
Slides Reviewed - Payment Complete
General Information

Pathologist Name 1	# Slides Reviewed	# Hours	Amount Owed	Amount Paid	PO Amount Available "PO NUMBER"	Invoice #	Check Sent	NOTES
					$ 10,000.00			
Jan-16	0	0	$ -		$			
Feb-16	0	0	$ -		$			
Mar-16	0	0	$ -		$			
Apr-16	50	12.5	$ 1,200.00	$ 1,200.00	$ 8,800.00	BCRnnn	MM/DD/YYYY	
May-16	0	0	$ -		$ 8,800.00			
Jun-16	2	0.5	$ 48.00		$ 8,800.00			
Jul-16		0	$ -		$ 8,800.00			
Aug-16		0	$ -		$ 8,800.00			
Sep-16		0	$ -		$ 8,800.00			
Oct-16		0	$ -		$ 8,800.00			
Nov-16		0	$ -		$ 8,800.00			
Dec-16		0	$ -		$ 8,800.00			
Total	52	13	$ 1,248.00	$ 1,200.00				

(ii) A formal way to view the P.O. number is by searching in the Navision Web Report Center (addressed in II.K.1-9).

(b) Copy and paste the P.O. number next to the pathologist's name in the field "P.O. #."

Invoice No.	Pathologist	P.O. #	Description	Amount	Slide Count	Hours	Date Emailed Pathologist	Date Approved	Date to Accounts Payable	Date of Check Sent
BCR001	Pathologist Name 1	PO 1	Reviews for June 2016	$ 48.00	2	0.5	7/5/2016	7/5/2016	7/6/2016	7/11/2016
BCR002	Pathologist Name 2	PO 2	Reviews for June 2016	$ 96.00	4	1	7/5/2016	7/5/2016	7/6/2016	7/11/2016
BCR003	Pathologist Name 3	PO 3	Reviews for June 2016	$ 120.00	5	1.25	7/5/2016	7/5/2016	7/6/2016	7/11/2016
BCR004				$ -		0				
BCR005				$ -		0				
BCR006				$ -		0				

iii. Type out the description for the invoice in the field "Description."

(a) Format: "Reviews for [Month, Year]"

Invoice No.	Pathologist	P.O. #	Description	Amount	Slide Count	Hours	Date Emailed Pathologist	Date Approved	Date to Accounts Payable	Date of Check Sent
BCR001	Pathologist Name 1	PO 1	Reviews for June 2016	$ 48.00	2	0.5	7/5/2016	7/5/2016	7/6/2016	7/11/2016
BCR002	Pathologist Name 2	PO 2	Reviews for June 2016	$ 96.00	4	1	7/5/2016	7/5/2016	7/6/2016	7/11/2016
BCR003	Pathologist Name 3	PO 3	Reviews for June 2016	$ 120.00	5	1.25	7/5/2016	7/5/2016	7/6/2016	7/11/2016
BCR004				$ -		0				
BCR005				$ -		0				
BCR006				$ -		0				

iv. Type in the total number of slides reviewed under the field "Slide Count."

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

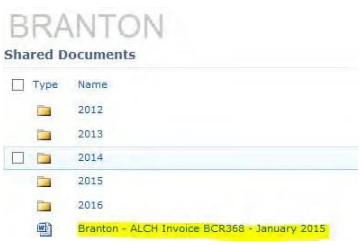
v. The fields of "Amount" and "Hours" will calculate automatically after the number of slides has been entered.

3. COR QC's the two tabs (By [Project] [Year] & By Invoice No.) in the "*Pathologist Details of Payments – All Inclusive*" spreadsheet.
   a. Check that the pathologist name, P.O. number, slide count, reimbursement amount, hours, and invoice number are correct and matching in both tabs for all pathologists.

C. COR generates a PDF invoice for pathologist's reimbursement.

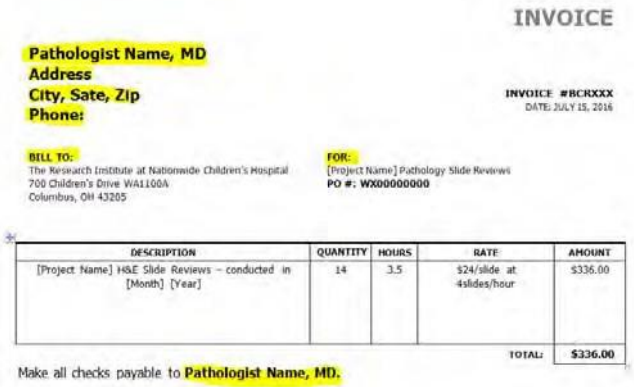
1. If a pathologist reviews slides for more than one project, the COR must complete a separate invoice per project.
2. Open the "Pathology" folder on SharePoint under "Shared Documents".
3. Open the "Invoices and Approvals by Name" folder.

4. Folders are listed out by the last name of each pathologist.
5. Open a folder for a pathologist.
6. Locate the word document of the invoice template.



Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

7. Open the word document of the invoice.
   a. Items that will generally stay the same with no updates include the name and contact of the pathologist, bill to institution, P.O. number, rate, and name of the person that the check will be made out to.



INVOICE

Pathologist Name, MD
Address
City, Sate, Zip
Phone:

INVOICE #BCRXXX
DATE: JULY 15, 2016

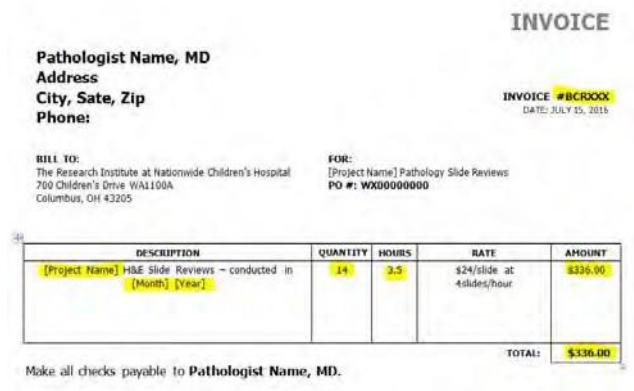
BILL TO:
The Research Institute at Nationwide Children's Hospital
700 Children's Drive WA1100A
Columbus, OH 43205

FOR:
[Project Name] Pathology Slide Reviews
PO #: WX00000000

DESCRIPTION	QUANTITY	HOURS	RATE	AMOUNT
[Project Name] H&E Slide Reviews – conducted in [Month] [Year]	14	3.5	$24/slide at 4slides/hour	$336.00
			TOTAL:	$336.00

Make all checks payable to **Pathologist Name, MD.**

8. Fill out each column of invoice using the information from the "*Pathologist Details of Payments – All Inclusive*" spreadsheet.



INVOICE

Pathologist Name, MD
Address
City, Sate, Zip
Phone:

INVOICE #BCRXXX
DATE: JULY 15, 2016

BILL TO:
The Research Institute at Nationwide Children's Hospital
700 Children's Drive WA1100A
Columbus, OH 43205

FOR:
[Project Name] Pathology Slide Reviews
PO #: WX00000000

DESCRIPTION	QUANTITY	HOURS	RATE	AMOUNT
[Project Name] H&E Slide Reviews – conducted in [Month] [Year]	14	3.5	$24/slide at 4slides/hour	$336.00
			TOTAL:	$336.00

Make all checks payable to **Pathologist Name, MD.**

   a. Description: [Project Name] H&E Slide Reviews – conducted in [MONTH/YEAR]
   b. Quantity: number of slides
   c. Hours: hours of service
   d. Rate: $24/slide at 4 slides/hour (keep as written)
   e. Amount: calculated amount owed and total at bottom
   f. Invoice #: change to match the current invoice number
   g. Review invoice to make sure information is correct.
   h. Save invoice as a word document, named in the format [Pathologist's Last Name] [Project Name] – Invoice BCR[###] – [Month/Year] (i.e. Smith - ALCH Invoice BCR100 – June 2016).
   i. Save invoice again as a PDF with the same name as the word document to the desktop.

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

D. COR emails invoices to individual pathologists.

1. The contact information for the individual pathologist, including his/her email can be found in the "Contacts" tab in the "*Pathologist Details of Payments – All Inclusive*" spreadsheet.
2. Open a new email window.
3. Click on "Attach file".
4. Search for the pathologist's invoice. Attach invoice to email.
5. Complete the subject line as: "[Project Name] Invoice To Review – Invoice BCR-[###]-[MONTH/YEAR]" (i.e. TCGA Invoice to Review - BCR100 - June 2014)
6. Write out the email body using this email template:

   Dear Dr. [Name],

   I have attached the [PROJECT NAME] invoice for your services from [MONTH/YEAR]. Please take a look and reply back with your approval and I will submit the paperwork to get your check issued. Please let me know if you have any questions. Thank you.
7. QC email for correct pathologist's email, invoice attached, subject line, and body of email before sending email.
8. Repeat these steps for all pathologists receiving reimbursements for the month.
9. Send a separate email for each project a pathologist completes slide reviews for.
10. Update the "*Pathologist Details of Payments – All Inclusive*" spreadsheet to include the date on which the email sent to the pathologist.

Invoice No.	Pathologist	P.O. #	Description	Amount		Slide Count	Hours	Date Emailed Pathologist	Date Approval	Date to Accounts Payable	Date of Check Sent
BCR001	Pathologist Name 1	PO 1	Reviews for June 2016	$	66.00	7	8.5	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR007	Pathologist Name 2	PO 2	Reviews for June 2016	$	96.00	4	1	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR003	Pathologist Name 3	PO 3	Reviews for June 2016	$	126.00	5	1.25	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR004				$	-		0				
BCR005				$	-		0				
BCR006				$	-		0				

E. COR waits for approval from pathologist that the invoice he/she received is correct and saves that approval email as a PDF.

1. Pathologists will reply to email sent stating that they approve or do not approve of the invoice.
2. If pathologist approves the invoice:
   a. Open email in a new window.
   b. Save the email as a PDF to the desktop with the same name as the invoice and add "Approval" at the end.
      i. Format of file: [Pathologist's Last Name] – Invoice BCR[###] – [Month/Year] – [Project Name] Approval (i.e. Smith - Invoice BCR100 - June 2016 – ALCH Approval).
   c. Update the "*Pathologist Details of Payments – All Inclusive*" spreadsheet to include the date of the approval from the respective pathologist.

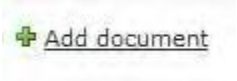
Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

3. If pathologist does not approve of invoice:
   a. Pathologists will often send a list of slides they have reviewed.
   b. Reconcile the list from the pathologist to the list which VM uploaded from II.A.3.
   c. Work with VM staff to identify any discrepant slides.
   d. Make necessary changes to the "*Pathologist Details of Payments – All Inclusive*" spreadsheet.
   e. Respond to the pathologist with a new invoice to approve by repeating steps II.C.1-7 and II.D.1-7.
      i. Verify that the invoice reflects the updated change(s).
      ii. Receive confirmation of approval and follow steps II.E.2a-f.

F. COR uploads PDF invoices and approval emails into SharePoint folders.

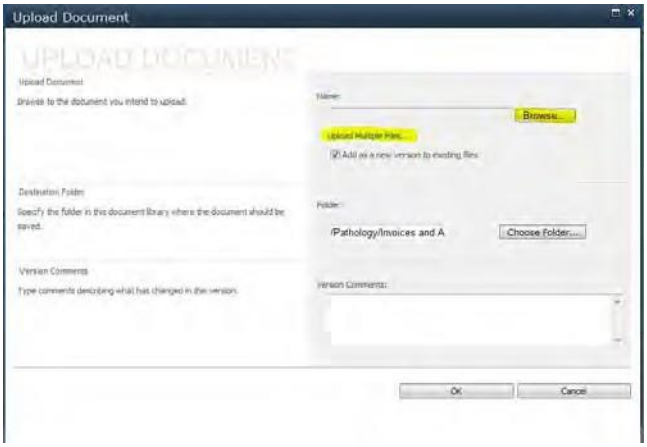
1. Invoices and approval emails are to be uploaded to two different folders:
   a. Invoices and Approvals by Name
   b. Invoices and Approvals by Month
2. Upload PDF's into "Invoices and Approvals by Name".
   a. Enter the BCR SharePoint page and open up the "Shared Documents" folder located on the left hand side of the screen.
   b. Open the "Pathology" folder.
   c. Open the "Invoices and Approvals by Name" folder.

   d. Locate and open the folder of the correct pathologist.
   e. Select the folder with the current year to upload the files.
   f. Click on "Add document" button.



   g. Click on the "Browse" button to upload one file at a time or click on the "Upload Multiple Files" link to upload more than one file.

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments



h. Locate the PDF invoice and the PDF approval email on the desktop and click "OK".
i. Upload is complete when you see both the PDF invoice and PDF approval email on the screen.
j. Repeat these steps with the remaining pathologists receiving reimbursements for the month.

3. Upload PDF's into "Invoices and Approvals by Month".
   a. Enter the BCR SharePoint page and open the "Shared Documents" folder located on the left hand side of the screen.
   b. Open the "Pathology" folder.
   c. Open the "Invoices and Approvals by Month" folder.
   d. Select the folder for the month during which the pathology reviews occurred.
   e. Click on "Add document" button.
   f. Click on the "Browse" button to upload one file at a time or click on the "Upload Multiple Files" link to upload more than one file.
   g. Locate the PDF invoice and the PDF approval emails and upload.
   h. Upload is complete when you see both the PDF invoice and PDF approval email on the screen.
   i. Repeat these steps with the remaining pathologists receiving reimbursements for the month.

G. COR creates spreadsheets to send to the BCR Program Director for approval so that invoices may be processed for payment by the Research Accounts Payable Department.

1. Send an email to the BCR Program Director and the Research Accounts Payable Office to let them know you have completed invoicing for the current month.
   a. Email should be titled "[Month] Invoices to Approve" (i.e. June Invoices to Approve)
2. Email will include the following charts illustrating invoicing information:
   a. Separate the charts by project.

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

b. The first chart summarizes the slide total and dollar amount owed to each pathologist.

Pathologist	Invoice #	PO#	Slide Count	Hours	Amount
Name	BCR###	WX00*****	5	1.25	$120.00

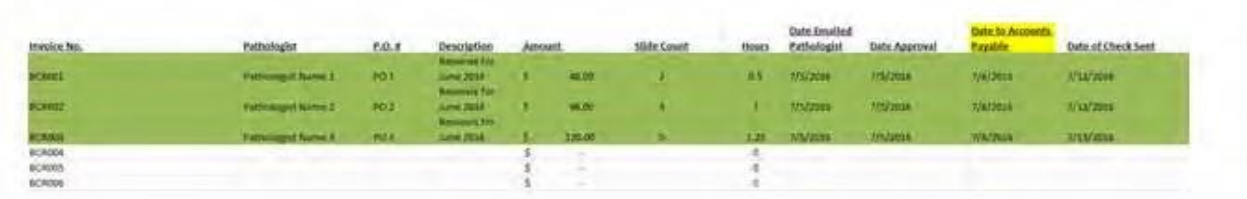
c. The second chart summarizes the average spending per month to give an idea of when the pathologist's current P.O. amount will be depleted, so their contract can be amended if necessary.
   i. Use the equations found in the table below to include a row for each pathologist.

Pathologist	Average $ per Month	Amount remaning after (this month's) Payment	Number of months until out of funds at current rate
Name	(=average(all numbers in amount owed column in "By Name [Year]" tab in "Pathologist Details of Payments – All Inclusive" spreadsheet)	Amount that will remain in account after the current month's payment	**(Amount remaining after payment/Average $ per month)**

3. Once the BCR Program Director approves the invoices by responding to the email, follow the steps below.

H. COR prints out invoices with approval emails and delivers them to the Research Accounts Payable Office.

1. Print and match invoices and approval emails.
2. Invoice page is placed on top of the approval email page and both are clipped together.
3. Arrange invoices in numerical order using the invoice number and separate by project.
4. Place in a folder and take to the Accounts Payable Office.
5. Update the "*Pathologist Details of Payments – All Inclusive*" spreadsheet to include the date the invoices were taken to Accounts Payable.



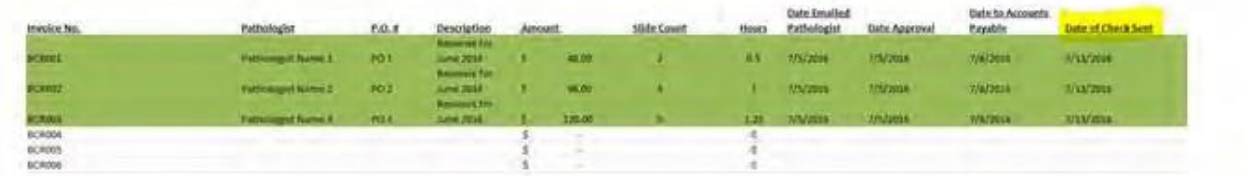
Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

I. COR responds to pathologists to inform them of the date that the reimbursement check will be mailed.

1. All checks are sent from Accounts Payable on Wednesdays as long as invoices are received on Monday at noon of the same week.
2. Notify the pathologist of date the check will be sent using the following email template:

   Dear Dr. [Name],

   The paperwork for your [project name] reimbursement has been submitted for processing and your payment will be mailed on [MM/DD/YYYY]. Thank you.
3. COR updates the "*Pathologist Details of Payments – All Inclusive*" spreadsheet to include the date the check will be sent.
4. Highlight the row green to indicate that the invoice is complete and processing for payment.

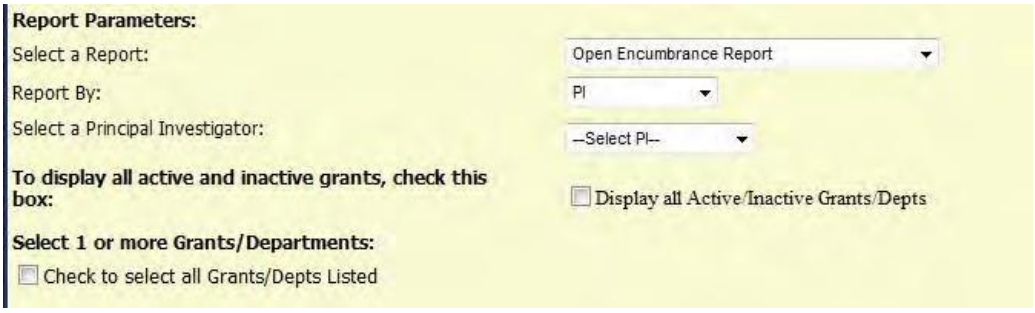


Invoice No.	Pathologist	P.O. #	Description	Amount	Slide Count	Hours	Date Emailed Pathologist	Date Approval	Date to Accounts Payable	Date of Check Sent
BCR001	Pathologist Name 1	PO 1	Reviewed for June 2016	$ 40.00	2	0.5	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR002	Pathologist Name 2	PO 2	Reviewed for June 2016	$ 80.00	4	1	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR003	Pathologist Name 3	PO 3	Reviewed for June 2016	$ 120.00	6	1.25	7/5/2016	7/5/2016	7/6/2016	7/13/2016
BCR004				$ -		0				
BCR005				$ -		0				
BCR006				$ -		0				

J. COR reviews and saves the "*Pathologist Details of Payments – All Inclusive*" spreadsheet onto the BCR SharePoint page.

1. Review all information and make sure all fields are completed.
2. Double check that fields are highlighted in green to indicate processing for payment is in progress.
3. Save the spreadsheet (it is automatically uploaded to SharePoint).

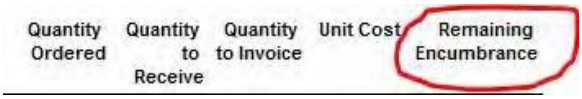
K. COR checks the Open Encumbrance Report in the Navision Web Report Center to confirm that the correct amount of reimbursement money has been deducted from the P.O. for each pathologist.



1. Open the Navision Web Report Center (http://resnaviportal/default.aspx).
2. Click the "Reports" button at the top of the screen.

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

3. From the "Select a Report" line, use the drop down button to select "Open Encumbrance Report".
4. From the "Report By" line, use the drop down button to select "PI".
5. From the "Select a Principal Investigator" line, use the drop down button to select the name of the principal investigator for the grant/contract the reimbursements are issued from.
6. A selection of the grant number and name will appear.
7. Select the correct grant/contract number by checking the box next to the grant.
8. Click the button "Generate Report".
9. Locate the name of the pathologist or the P.O. number.
10. Check that the amount listed in the far right column called "Remaining Encumbrance" matches the amount in the spreadsheet column titled "P.O. Amount Available" in the "*Pathologist Details of Payments – All Inclusive*" spreadsheet.



   a. If the amounts match, the pathologist has been reimbursed the correct amount.
   b. If the amounts do not match, contact Accounts Payable to determine the reason for the variance.

L. Adding a New Pathologist to "*Pathologist Details of Payments – All Inclusive*" spreadsheet.

1. Create new folders in SharePoint to house all reimbursement invoices and approval emails for new pathologist.
   a. Open "Pathology Review Reimbursements" folder in SharePoint.
   b. Open Invoices and Approvals by Name folder.
   c. Select "New Folder" button.
   d. Name the folder the last name of the new pathologist.
2. Create new fields in the spreadsheet for the new pathologist.
   a. Set up required information in the fields.
      i. Copy and paste another pathologist's information field(s), delete out data, and use the fields as a template.
   b. Look up P.O. number for pathologist and input the P.O. amount.
3. Email introduction to pathologist and request reimbursement name and address for checks to be mailed.
   a. Email template:

   Dear Dr. [Name],

   My name is [your name], Clinical Research Assistant for the Biospecimen Core Resource (BCR) at Nationwide Children's Hospital Research Institute. I will be your contact for pathology reimbursements for the [Name] Project. I would like to begin the process by updating our database and request that you send me the address to which you would

Standard Operating Procedure (SOP) for Pathology Review Invoicing Payments

like the reimbursement checks sent and to whom the checks should be made payable. I will also need the information of your institution, address, phone number, and point of contact for our records. Please feel free to contact me by phone [phone number] or email [email address] if you have any questions. Thank you and I look forward to working with you on this exciting project.

4. Log information received from pathologist into the "*Pathologist Details of Payments – All Inclusive*" spreadsheet in the "Contacts" tab.
5. Use information to create an invoice template to be used for II.C.

III. REFERENCES - None

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version #3, Effective Date 8/16/2016
1. Made title not capitalized
2. Changed "CRA" to "COR"
3. Updated pictures
4. Updated wording to account for multiple projects

B. Changes made in Version #2, Effective Date 12/13/2014
1. New format used
2. Minor word and grammatical changes made throughout
3. Remove TCGA
4. Remove unnecessary steps
5. Remove duplicate pictures
6. Added section (G.) on sending email with spreadsheet updates to BCR Program Director.

C. Version #1, Effective Date 9/3/2012 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) for Receiving Whole Slide Images

I. SCOPE AND PURPOSE

This procedure establishes the process for Clinical Outreach, Logistics, and Virtual Microscopy (VM) personnel to follow to receive whole slides images into the Biospecimen Core Resource (BCR) submitted by a Tissue Source Site (TSS). These images are submitted through a variety of methods including a BCR FTP server at the Ohio Supercomputer Center (OSC) or NCH, USB flash drives, DVDs, CDs, and external hard drives.

II. PROCEDURE

A. FTP server

1. The Lead Clinical Outreach Coordinator (LCOC) is responsible for initiating image transfer from FTP servers.
2. The LCOC e-mails VM at VMWorkingGroup@nationwidechildrens.org to request a unique username and password for the TSS to access the server. Each username and password is assigned to only one TSS, to prevent multiple TSSs from having access to the same folder (which would allow others to view these files).
3. The LCOC provides the TSS with instructions for using the FTP server and a list of acceptable compatible scanners (provided by VM). The instructions are found on: BCR SharePoint site>Shared Documents>VM Folder>Training Materials>FTP Documents
4. After the LCOC receives an e-mail from the TSS that the slides have been uploaded and have submitted a shipping manifest, they inform Logistics and VM via e-mail using the distribution lists BCR_Logistics@nationwidechildrens.org and VMWorkingGroup@nationwidechildrens.org and attach the shipping manifest.

B. DVD, CD, and USB drives

1. Logistics receives the DVDs, CDs, and USBs.
2. Once checked in, they e-mail VM via VMWorkingGroup@nationwidechildrens.org and the Clinical Outreach Coordinators via BCRClinicalOutreachCoordinators@nationwidechildrens.org and include the shipping manifest
3. The LCOC e-mails the TSS informing them that the images were delivered and works with them if any issues are found.

C. BCR-provided external hard drives

1. The LCOC signs the hard drive out from the designated CRA and ships it to the TSS. Prior to this release, the CRA is responsible for ensuring that the hard drive does not contain any data or images.
2. The LCOC informs the TSS to contact them when they are ready to return the hard drive to the BCR. When contacted, the LCOC sends the TSS an air bill and shipping manifest.
3. When the hard drive arrives at the BCR, the LCOC e-mails Logistics and VM and attaches the shipping manifest
4. Once the images have been uploaded to the Ohio Supercomputer Center (OSC), VM returns the hard drive to the LCOC

Standard Operating Procedure (SOP) for Receiving Whole Slide Images

5. The LCOC completely erases all data and images from the drive, including from the Recycle Bin, and signs it back into the CRA.

D. VM verifies image quality and usability

1. Prior to any image processing, VM uses the shipping manifest to confirm the correct images were received and that they are usable for pathology review in the Virtual Image for Pathology Education and Research (VIPER) system.
2. VM e-mails the LCOC and Logistics to let them know that the images are usable or if there are any issues.

E. Image processing

1. Logistics brings all images into LabVantage per SOP L013, "Top and Diagnostic Slides"
2. Once Logistics receives notification that the images are usable, a Custodian Domain Transfer (CDT) with the images is created and custody is transferred to VM
3. Once VM receives the CDT and shipping manifest, they take custody and copy the images to a local computer hard drive
4. VM digitally revises the image label and file name with the appropriate project numbers
5. VM uploads the relabeled slide images to the appropriate BCR OSC storage space and then files in LabVantage per A005, "LabVantage User Manual"

III. REFERENCES

A. BCR-REF-001, "BCR Acronym List"
B. BCR-SOP-L013, "Top and Diagnostic Slides"
C. BCR-SOP-A005, "LabVantage User Manual"

IV. COMPREHENSIVE REVISION HISTORY

A. Version 2, Effective Date 5/13/2016
   1. Made title not all capitalized
   2. Removed language regarding alternative FTP instructions
B. Version 1, Effective Date **08/04/14** - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Standard Operating Procedure (SOP) For Expert Pathology Committee (EPC) Workflow

I. SCOPE AND PURPOSE

This procedure applies to all Nationwide Children's Hospital (NCH) Biospecimen Core Resource (BCR) personnel responsible for organizing and administering EPCs. NCH BCR personnel assist with pathology review form mock up, build the final approved pathology review form in VIPER, provide whole slide images to reviewers, assign cases to reviewers, collect and organize reviews, and send the collected pathology review data to the EPC. All EPC information will be stored on the BCR SharePoint which contains records of case assignments, pathology review results, and other pertinent documents for the EPC.

II. PROCEDURE

A. Slides, cases and electronic review forms are checked for accuracy of information and to ensure all slides, forms and/or pathology reports function properly for the reviewing pathologist.
   a. Any discrepancies identified are discussed and consensus is reached.

B. When a new EPC is implemented, Biomedical Imaging Coordinator works on identifying the case list for pathology review by using the Informatics report available on the BCR SharePoint. The informatics report pulls all qualified cases that have been uploaded to Data Coordinating Center (DCC)/Genomics Data Commons (GDC). The Biomedical Imaging Coordinator ensures that all slide images and/or pathology reports exist for qualified cases are pulled into corresponding EPC folder.

C. The EPC group will provide a draft form for pathology review data collection.

D. Biomedical Imaging Coordinator will mockup the review form in VIPER, a web-based pathology review application based on the draft received.

E. The mocked up review form will be sent to the EPC group for approval. Once the group approves the form, the Biomedical Imaging Engineer and NCH Research Informatics Core (RIC) will complete the final build and testing on the approved VIPER form. Since this process may take several weeks, it is important to discuss potential deadline and concerns with the EPC group prior to requesting a form.

F. The Imaging team will be responsible for testing review form before it is released to EPC reviewers.

G. VIPER accounts are created for each reviewer of the EPC group and are trained via a video link.

H. Cases are assigned based on discussed case assignment for the EPC.

I. Once the reviews are complete, RIC will create a report that pulls all VIPER collected data for the EPC.

J. Combined reviews will be presented to the EPC group via a spreadsheet.

III. REFERENCES - None

IV. COMPREHENSIVE REVISION HISTORY

A. Version 1, Effective Date 3/3/2016 - New

Standard Operating Procedure (SOP) For Expert Pathology Committee (EPC) Workflow

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Biopathology Center
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700 Children's Drive, WA1300
Columbus, Ohio 43205
BPCBank@nationwidechildrens.org

Responsibility and Overview of the Biopathology Center (BPC) and Biospecimen Core Resource (BCR) Safety

Procedure Number: SAF-001		
Supersedes Procedure Number: N/A		
Date Adopted: 9/10/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/5/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
6/19/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
8/8/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: This document serves as an introduction to the responsibility and overview of the Biopathology Center (BPC) and Biospecimen Core Resource (BCR) safety processes.

The following individuals serve as the designated contacts for the Research Institute:

Chemical Spill	5-2776 (Research Safety) 2-3333 (Emergency Command Center)
Chemical Exposure	5-4135 (Employee Health) 5-2776 (Research Safety)
Chemical Waste Disposal	5-2776 (Research Safety)
Radiation Spill	2-2338 (Radiation Safety, Nancy Joy Mackenzie, RSO)
Radiation Exposure	5-4135 (Employee Health) 2-2338 (Radiation Safety, Nancy Joy Mackenzie, RSO)
Biohazard Spill	5-2776 (Research Safety)
Biohazard Exposure	5-4135 (Employee Health)
Water Spill	5-1801 (Environmental Services)
Fire	2-3333 or 2-2130 (Safety and Security)
Unauthorized Personnel	2-2130 (Safety and Security)

Facility Safety Concerns	5-3415 or 270-5007 (Facilities Manager, Phil Bowers)

The following telephone numbers should be available on all phones in the BPC and BCR:

Code Blue & Stat	2-2233
Routine Security	2-2128
All Other Emergencies	9-911 (x2-3333 Emergency Command Center)

Important Medical Attention Contact Numbers

Poison Control Center	1-800-222-1222 or 5-0436
Employee Health	5-4135
Emergency Command Center	9-911 (x2-3333)

BPC and BCR Safety Policies and Procedures are located electronically and in hard copy form. It is the responsibility of BPC and BCR management to ensure that these documents are easily accessible to the personnel in their areas and is reviewed and signed off by all personnel.

I. Responsibility and Overview of BPC Safety Program

A. BPC Safety Representative(s): The BPC and BCR Safety Representative(s) is/are responsible for the overall safety program in the BPC/BCR. Their main function is to provide guidance to BPC/BCR directors and managers who have direct responsibility for providing a safe workplace for all employees. The Safety Representative(s) attend all Research Safety meetings and assist in the development and monitoring of the BPC/BCR safety programs. They additionally provide safety expertise in conjunction with other lab professionals with specific experience; oversee and assess safety orientation and training for new employees, as well as continuing education programs; inspect/survey work areas for appropriate location of the safety manual and any safety deficiencies; review the safety manual periodically. The Safety Representative(s) is/are not, however, solely responsible for safety. Safety is a combined responsibility of management and personnel alike. The Safety Representative(s) is/are expected to make safety recommendations and is/are authorized to stop activities that are clearly unsafe.

1. Safety policies and procedures comprise a section in the Biopathology Policy and Procedure Manual located in the office area of WA1320 and WA1100A and electronically on Q-Pulse.

B. Safety Inspections: An ongoing program to review safe operations and equipment occurs quarterly in the BPC and BCR. First, the designated BPC Safety Representatives conduct an internal survey to inspect all work sites to ensure all safety policies and procedures are being followed in each laboratory area. Inspections are documented showing the items that were checked and the questions staff was asked. Any issues found during the safety inspection will be communicated to staff via email and/or meetings. The College of American Pathologist (CAP) general checklist may be used as a guideline during the survey. The audits serve to remind staff of potential hazards, ensure employee compliance, and remind managers and directors of their responsibility. Secondly, the Biomedical Engineering Department conducts annual electrical safety checks of all instruments and devices for proper grounding and shock hazard. Lastly, the Research Safety Office of the Research Institute at Nationwide Children's Hospital conducts an annual inspection of the institute, including the BPC and BCR. These Lab Safety Reviews satisfy institutional and National Institutes of Health guidelines. Lab safety reviews conducted in 2013 will be considered training. Official reviews with documented deficiencies will take place from 2014, and on.

C. **BPC and BCR Safety Manual**: This safety section of the BPC and BCR Policy and Procedure manual is available in all work areas. The manual is general in nature but each laboratory area may develop specific additional procedures for their own laboratory's needs. Safety policies are reviewed at least annually by the BPC Director and BCR Principal Investigator to ensure they are up-to-date. Annual safety presentations and employee orientation cover policy content.

D. **Compliance with Governing Bodies:** The safety program at the Research Institute at Nationwide Children's Hospital provides a framework, in addition to accreditation regulations enforced by CAP, under which the BPC and BCR operates and complies. Safety policies and procedures defined by the Research Institute are followed by the BPC and BCR and information is incorporated into BPC and BCR documents. The BPC and BCR adhere to and ensure the most strict regulations of any governing body under which the BPC and BCR fall serve as the minimum requirement for BPC and BCR operations. Hard copies and electronic links to institutional safety documents are made available to BPC and BCR staff for their reference.

II. BPC Safety Records

A. **Identification of Hazards:** Hazardous areas are clearly identified using appropriate signs. Fire hazard materials to be used within the laboratory are properly identified and all doors leading into these areas are marked as to the hazards present within. The BPC and BCR Safety Representative(s) is/are responsible for periodically reviewing and updating this hazard identification system to ensure its relevance to the hazards known to be present.

B. **Reporting Incidents, Accidents and Occupational Illness:** The BPC and BCR have a program for reporting laboratory incidents and potential hazards. "Stars" (formally CS Stars) is the Hospital's event reporting system. The icon for Stars can be found on the desktop of all BPC and BCR computers. If an employee needs medical attention, he/she should be sent to Employee Health or the Emergency Department. Once the employee receives necessary treatment, the incident should be filed in the CS Stars system as well as documented with an internal BPC and BCR incident report form. All reports include a full description of the incident, an assessment of the cause, recommendations for preventing similar incidents, and actions taken to implement the recommendations. Incident reports regarding safety will be discussed at a BPC and BCR monthly meeting when deemed necessary by BPC and BCR management and in conjunction with the presentation of Quarterly Quality Indicators. Additional information regarding reporting can be found in "ADM-009 Incident, Feedback, and Event Reporting" , "BCR A013 Incidents and Reporting" and "SAF-012 Reporting Accidents, Illnesses, and Incidents".

C. **Education and Training**: Training in safe work practices is always emphasized and ongoing. A training program begins with new employees as part of their new employee orientation (New employee Hospital and Departmental Checklists). Present employees are required annually, during their anniversary month, to do a computer based safety review program offered by CHEX. This program covers review on all areas of safety. Employees are required to show proof of course completion prior to their annual review. Safety topics may also be presented at BPC and BCR meetings, as well as mandatory annual presentations covering Safety and Ergonomics.

III. References

A. N/A

Biopathology Center
The Research Institute at
Nationwide Children's Hospital
700 Children's Drive, WA1300
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BPCBank@nationwidechildrens.org

Chemical Hazards and Safety

Procedure Number: SAF-002		
Supersedes Procedure Number: BPC Safety Manual		
Date Adopted: 9/17/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
3/6/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The Biopathology Center (BPC) and Biospecimen Core Resource (BCR) maintain policies regarding chemical hazards that are, or may be, present in the lab in addition to preventative measures to avoid incident. The purpose of this procedure is to provide education on chemical hazards and chemical safety.

I. **General Information:** Laboratories may contain many caustic and corrosive, as well as toxic, flammable and unstable, reagents. All containers of hazardous chemicals must be clearly labeled. NOTE: prepared reagents (Example: lysing media) or kit systems (Example: Qiagen kits) may contain hazards. These contents should be reviewed and appropriately labeled.
   - **A.** BPC and BCR managers are responsible for informing employees of significant chemical hazards. Material Safety Data Sheets (MSDS) display the characteristics of hazardous chemicals. MSDS's can be found on the Research Institute Safety Page through MSDSonline. Copies of MSDS's may be found on each department's SharePoint page and/or printed copies are kept in each lab. For safety, employees are responsible for learning these hazards and following safe practices. Employees are trained upon hire. Any changes or additional chemicals added will be reviewed with applicable staff. New chemicals or any review determined to be needed will be discussed with management. A list of chemicals within the BPC department has been developed and is located BPC SharePoint > BPC Documents-Forms > Safety > Chemical Inventory. A list of chemicals within the BCR laboratories may be kept in the MSDS binder or within the research safety chemical inventory online. Refer to "SAF-003 Chemical Hygiene" for additional details regarding the Chemical Inventory.
   - **B.** Employees are to be aware of *Code of Federal Regulations*. Title 29, part 1910.1450 and its appendices. This document outlines the Occupational Safety and Health Administrations (OSHA) regulations for toxic and hazardous substances, and exposure to hazardous chemicals in laboratories. Access can be obtained through the hyperlink, above.
   - **C.** MSDS sheets for all chemicals can be found at http://anchor/msds-online. This site may serve as a useful reference should one come in contact with a new or unfamiliar chemical.
   - **D.** No container should be accepted without an adequate identifying label. The label should include, at minimum, the substance name, an appropriate hazard warning, and specific target organs. Labels cannot be removed or defaced. Additional information regarding chemical storage container labeling requirements can be found in "SAF-014 Warning Signs and Labels".

II. **Classification:** Hazardous chemicals may be grouped into the following categories:

A. ***Corrosive*** on a reagent label refers to any substance that causes visible destruction or irreversible alterations in human tissue at the site of contact.
   1. When applied to chemical waste, the term implies a pH <2.1 or >12.5.

B. ***Toxic*** is a term that can be applied to almost any substance in large quantity. For laboratory purposes, a substance is considered toxic if serious biological effects can follow inhalation, ingestion or skin contact with relatively small amounts.

C. ***Carcinogen*** is a particularly difficult term because of the wide variety of test systems in which the ability of a chemical to induce a malignant tumor can be defined. The most practical labeling system confines the term to the OSHA-defined chemicals regulated under 45 *Federal Register* 5.001-5.296 and 45 *Federal Register* 53.572-53.579.

D. ***Ignitable*** may relate to any chemical that can burn. The term includes both "combustible" and "flammable."

E. **Flammable Liquids** (those that have a flash point "FP" below 100°F; 38°C) may be subdivided into the following classes;
   1. Class 1A:FP<73°F (22°C) and boiling point (BP) < 100° F (38°C)
   2. Class 1B:FP<73°F (22°C) and BP>100°F (38°C)
   3. Class 1C:FP<72°F (21°C) and BP<100°F (38°C)

F. **Combustible Liquids** (those that have a FP at or above 100°F; 38°C but below 140°F; 60°C) are subdivided into classes as follows:
   1. Class IIIA: FP>140°F (60°C) and <200°F (94°C)
   2. Class IIIB: FP>200°F (94°C)

G. ***Explosive Chemicals*** are those reactive and unstable substances that readily undergo violent chemical change. Explosive decomposition can occur at normal temperatures and pressures.

III. Labels: A Material Safety Data Sheet (MSDS) as required by law is available for purchased materials that might be hazardous. Under OSHA's Laboratory Standard, all hazardous chemicals are required to be labeled with information, in an easy-to-understand format that will warn both knowledgeable and unknowledgeable persons of potential hazards. Labeling may be in the form of text, pictures, standardized code or most commonly, some combination of these.

A. The BPC and BCR use the common **NFPA**® approach labeling system, which was developed by the National Fire and Protection Agency. This label uses a numerical rating and color-coding to indicate the levels and nature of the hazard. The label assigns a numerical rating (e.g., 0-4) to the hazards presented by health (blue field), flammability (red field) and reactivity (yellow field); the higher the numerical rating, the greater the potential hazard. If the NFPA ratings are not available in the MSDS sheet, the BPC and BCR will use the available system and note the system utilized accordingly on the chemical inventory.

B. Laboratories may also use the GHS® labeling system. The label assigns a numerical rating (e.g. 1-5) to the hazards presented by health (blue field), flammability (red field) and reactivity (yellow field); the lower the numerical rating, the greater the potential hazard. If GHS ratings are not available in the MSDS sheet, the BPC and BCR will use the available system and note the system utilized accordingly on the chemical inventory.

C. Additional information regarding warning signs and labels can be found in "SAF-014 Warning Signs and Labels".

IV. Storage and Handling of Corrosives

A. **Storage:** Corrosive materials must be stored near the floor to minimize danger of falling or in a cabinet under the hood and in resistant trays or catch basins/containers.
   1. **Acid Bottle Carriers:** Acid bottle carriers must be used for containers of concentrated reagents over 500mL.
   2. **Incompatible Chemicals:** Care should be taken not to store mutually incompatible chemicals in the same area. For example, organic acids, such as acetic acid or acetic anhydride should be stored separately from strong oxidizers, such as sulfuric, nitric or perchloric acids. Also, store concentrated acids separately from concentrated bases. Do not store corrosives with flammable liquids.

3. **Personal Protective Equipment (PPE):** Personnel in areas where corrosives are being used should wear impermeable lab coats, nitrile gloves, safety glasses and other personal protective garments rated for minimal penetration by the specific corrosive.
4. **Spills:** Any chemical spill in the laboratory will be cleaned up according to MSDS on that particular chemical. Employees should be familiar with each type of chemical they use and what needs to be done in the event of a spill. Refer to the "SAF-013 Biohazard and Chemical Waste Disposal" SOP. Research Safety may be consulted for spill clean-up instructions, as needed.
5. **Emergency Devices:** Areas in which corrosives are being used have adequate emergency shower facilities and eye washing stations.
6. **Chemical Fume Hood:** All corrosives with vapors that represent a health hazard must be handled in a chemical fume hood.

B. Storage and Handling of Flammables: Flammable and combustible liquids are stored in approved containers. Glass containers larger than one pint must be stored in a Flammable Cabinet and away from heat or ignition sources. Such liquids are dispensed in accordance with NFPA 30, *Flammable and Combustible Code*. Working supplies of flammable and combustible liquids are minimal.

1. The total capacity of flammable or combustible liquids outside of approved storage cabinets is in compliance with NFPA 30 for the specific laboratory conditions.
2. **Special Considerations for Diethyl Ether:** Diethyl Ether is extremely volatile and flammable. Substitute compounds, such as ethyl acetate should be used whenever possible.
3. **Special Safety Considerations for Formaldehyde:** Formaldehyde poses special safety considerations because of its potential carcinogenicity and toxic properties. OSHA governs the concentration of formaldehyde in the workplace.
   - **a.** The term "formalin" is applied to the commercially stabilized aqueous solution of formaldehyde containing 37% formaldehyde. The solution is ordinarily diluted (1:10) for use in the laboratory to approximately 4% in buffered water. Formaldehyde gas is combustible and can form explosive mixtures in air when the concentration exceeds 7%. This concentration of formaldehyde gas can be exceeded in the event of a fire in a room in which formalin is stored. Therefore, formalin should be stored in a well-ventilated area away from oxidizing agents.
   - **b.** Because of the danger of a chemical reaction that would produce bischloromethyl ether, which is a potent carcinogen, formalin should not be stored in the same cabinet with, or mixed with, hydrochloric acid or chloride-containing solutions, such as sodium hypochlorite (bleach). As with other dangerous chemicals, the amounts in storage should be kept at a minimum consistent with rate of use.
   - **c.** Disposal of formalin and formaldehyde-containing solutions and mixed specimens is governed by state and local ordinances, by the EPA and the Department of Transportation (DOT). In Ohio, disposal is permitted via the sanitary sewer system after neutralization with appropriate reagents. Refer to "SAF-013 Biohazard and Chemical Waste Disposal" for institutional disposal instructions.
   - **d.** In the event of a major formalin or formaldehyde spill, which may produce air concentrations in excess of the OSHA-permissible exposure limit, or in which the air concentration is unknown, respiratory protection is required during clean up. In the event of a major spill, evacuate the area and contact the Research Institute Facilities Manager, the Research Institute Biosafety Officer, and NCH Environmental Services from outside the affected area. Those personnel responsible for containment and cleanup must wear a self-contained breathing apparatus (SCBA). Appropriate respiratory protection devices and procedures are listed in the OSHA *Standard for Formaldehyde*.
   - **e.** For minor spills, particularly in locations such as hoods, in which the danger of excessive respiratory exposure is unlikely, follow spill-containment and disposal procedures for formaldehyde. In general, small spills should be handled by making absorbent material available and providing for the disposal of the formaldehyde waste as required by the hospital.
   - **f.** Formaldehyde should be used only in a well-ventilated area that draws fumes away from the operator's face. Personnel using formaldehyde should wear clothing that is impervious to formaldehyde. Recommended materials include nitrite rubber, butyl rubber, Teflon and

polyethylene for aprons and protective clothing. Polyvinyl chloride (PVC) and neoprene are recommended for short exposures.

g. When using formalin, eye protection is important and should take the form of goggles, full-face shields or barrier protection (hoods). Where a face shield is worn, chemical safety glasses are also required if there is a danger of formaldehyde reaching the area of the eye. Easily accessible eyewash stations are required.

h. The accidental or deliberate ingestion of formalin is a medical emergency because it may be lethal in amounts as small as 30mL to 40mL. Medical attention should always be sought in the case of accidental ingestion of formalin. Contact the Central Ohio Poison Center at extension 22636 immediately for instructions.

C. Storage and handling of oxidizers, water reactive and pyrochloric material, and peroxidizable chemicals:

1. The BPC does not have chemicals that fall under these classifications. Information regarding the handling and storage of these chemicals can be found in the RINCH Chemical Hygiene Plan.

2. The BCR does have chemicals that fall under the oxidizer classification.

a. All oxidizers must be stored away from combustible and flammable materials.

b. All oxidizers must be stored in a cool, dry place.

c. All oxidizers must be kept away from reducing agents such as zinc, alkaline metals, and formic acids.

D. Emergency Response: The possibility of spills and releases of hazardous chemicals may occur in the laboratory. OSHA's standard covering hazardous waste operations and emergency response (HAZWOPER) mandates that all employees receive at least awareness training in dealing with spills and releases. This training is covered in the lab employee's mandatory safety training. It includes the recognition of releases of hazardous materials, mechanisms that have been adopted by the BPC and BCR for reporting them to appropriate supervision and steps that should be taken for the employee's own safety. In the event of a major spill or release, the hospital's Emergency Command Center (ECC) must be notified (extension 23333). The ECC may ask that the area be evacuated and call in an outside emergency response firm or have trained in-house emergency response personnel deal with the situation. Where there is the possibility of a release of hazardous chemicals, the hospital has developed an emergency response plan. This plan includes the following items:

1. Pre-emergency planning and coordination with outside agencies
2. Personnel roles, lines of authority, training and documentation
3. Emergency recognition and prevention
4. Safe distances and places of refuge
5. Site security and control
6. Evacuation routes and procedures
7. Decontamination
8. Emergency medical treatment and first aid
9. Emergency alerting and response procedures
10. Critique of response actions and provisions for follow-up of any incidents
11. Personal Protective Equipment and emergency equipment

E. Chemical Spills: The BPC and BCR should have adequate spill-response equipment readily available, including appropriate PPE. Research Safety additionally supplies spill pillows (located on top of the ice machines nearest the freight elevators on all floors) and Mercury Spill kits (located on top of the ice machines nearest the freight elevators on all floors) To use the equipment properly, laboratory personnel who will respond to a spill or release of hazardous material must have appropriate training. Information is provided during employee orientation and a procedure documenting spill clean-up is available. Refer to "SAF-018 Biohazard and Chemical Spill Clean-up". As a general rule, implement the CLEAN plan:

- C Contain the spill
- L Leave the Area
- E Emergency eyewash, shower, medical care

- A Access MSDS
- N Notify lab manager, safety officer and/or Safety and Security.

1. **Minor Chemical Spill:** Less than one gallon, low toxicity, and/or poses no fire or life threat
    a. Alert people in the immediate area of the spill.
    b. Wear chemical protective equipment (chemical goggles, gloves, lab coat, etc.).
    c. Avoid breathing vapors, open the fume hood.
    d. Confine spill to small area using spill pillows.
    e. Access the MSDS information on the chemical – kept in the lab, in the Poison Control Center 1-800-222-1222 or x50436, or various online locations.
    f. Use the appropriate spill kit to neutralize and absorb chemicals.
    g. Collect the residue/contaminated items and place in a container/bag. Place in the fume hood for disposal. Label the bag as defined in "SAF-013 Biohazard and Chemical Waste Disposal" and contact Research Safety (x52776) for pick-up.
    h. Clean spill area with water or appropriate solvent. Call environmental services (x51801) for assistance, if needed.
2. **Major Chemical Spill:** More than one gallon, high toxicity, and/or is a fire or life threat.
    a. Attend to injured or contaminated persons and remove them from exposure.
    b. Alert people in the laboratory to evacuate.
    c. If spilled material is flammable, turn off ignition and heat sources.
    d. Open fume hood and close the doors to the affected area.
    e. Call the Emergency Command Center (x23333), Research Safety (x52776), and notify BPC and/or BCR management from outside the affected area.
    f. Access the MSDS information on the chemical – kept in the lab, in the Poison Control Center 1-800-222-1222 or x50436, or various online locations.
    g. Have person knowledgeable of incident and laboratory procedures available to assist emergency personnel.
3. **Recommended Sequence of Actions:** A recommended sequence of actions to be followed in the event of a spill or release of a large amount of hazardous material is shown below. All of the steps might not be applicable or necessary and each spill or release incident is different.
    a. Determine the hazard potential of the spill. What was spilled and does it pose a fire or explosion emergency or health hazard?
    b. If it is an emergency, evacuate the area. If it is a fire hazard, eliminate sources of ignition.
    c. Establish site security to prevent access by and harm to others.
    d. Determine the extent of the spill and start any appropriate notification procedures.
    e. Obtain assistance, i.e. trained persons, emergency services and equipment.
    f. Establish what PPE is needed. If unsure, use a self-contained breathing apparatus.
    g. Establish back-up personnel for rescue/help of spill responders.
    h. Rescue injured/endangered personnel, decontaminate and provide medical attention.
    i. Control the spill.
    j. Conduct perimeter monitoring and; revise isolation area and required PPE as necessary.
    k. Establish a decontamination station as necessary.
    l. Clean up the spill using appropriate equipment and decontaminate the area.
    m. Monitor the effectiveness of the cleanup procedure.
    n. Properly dispose of all materials associated with the spill according to the MSDS.
    o. Make all necessary corrections/revisions to reports.
    p. Demobilize.
    q. Prepare an incident report. BPC: Refer to "ADM-009 Incident, Feedback, and Event Reporting". BCR: Refer to "A013 Incidents and Reporting".
    r. Critique the response to the spill.

F. Distribution

1. Chemicals should not be distributed to other labs without approval from the Chemical Hygiene Officer, Management, or other designee of both the donating and receiving laboratories.
2. No chemicals should be distributed to minors or non-RINCH employees.
3. Redistributed chemicals should be removed from the donating lab's chemical inventory and transferred to the receiving lab's chemical inventory.

G. Transportation

1. Chemicals must be properly labeled.
2. Secondary containment vessels are to be utilized and large enough to hold the contents in the event of a breakage.
3. Appropriate spill supplies should accompany the transported chemicals and sufficient for chemical type and amount.
4. Carts should be used when transporting multiple containers.
5. If transporting compressed gas cylinders, do so only with caps in place and secured to a cylinder cart.

H. Decontamination and Disposal: The BPC and BCR laboratories are responsible for routine decontamination. Decontamination can be required after work, at the completion of a work shift or in the event of a chemical spill. Equipment should not be removed from the laboratory for repair or servicing until a BPC and/or BCR manager certifies that the equipment is free of chemical hazards. Directions for proper handling of hazardous chemical waste are as follows:

1. **Waste Chemicals:** Presume waste chemicals are hazardous unless identified otherwise.
2. **Containers:** Place chemical waste in containers with tight fitting, screw-cap lids or rubber stoppers.
3. **Labeling:** See "SAF-013 Biohazard and Chemical Waste Disposal"
    a. **Hazardous Waste:** All materials, absorbents and neutralizers used during the cleanup of a spill of hazardous materials are considered hazardous wastes. They must be treated as such. See "SAF-013 Biohazard and Chemical Waste Disposal" for further information
4. **Disposal:** Follow MSDS information for proper disposal or contact Research Safety for verification or with any questions.

V. References

A. N/A

Biopathology Center
The Research Institute at
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Chemical Hygiene Plan

Policy Number: SAF-003		
Supersedes Procedure Number: N/A		
Date Adopted: 9/10/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/4/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
3/6/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
8/15/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The following Chemical Hygiene Plan has been adopted and is in place in the Biopathology Center (BPC) and Biospecimen Core Resource (BCR), Nationwide Children's Hospital, Columbus, Ohio. The purpose of this document is to outline the plan the BPC will follow to reduce exposure limits to chemicals.

The Research Institute at Nationwide Children's Hospital (RINCH) has available an institute-wide Chemical Hygiene Plan. This document is electronically available on REX > Support Resources > Research Safety > Lab Safety Book Components > "RINCH Chemical Hygiene Plan". The Biopathology Center and Biospecimen Core Resource will adhere to the requirements of the Research Institute and maintain a hard copy in laboratory areas. Information found in the Research Institute's Chemical Hygiene Plan is found throughout several BPC and BCR policies and procedures. BPC and BCR policies and procedures are internally maintained in addition to the Research Institute's documentation to clarify information specific to BPC and BCR operations and make material more readily accessible and easily findable to staff. Updates to Research Institute policies will be compared against BPC and BCR policies and procedures to ensure congruency.

OSHA Laboratory Standard

29 CFR 1910.1450

I. **General Information:** OSHA's standard governing safety in the laboratory mandates that each laboratory using hazardous chemicals prepare a written, readily available plan that delineates how the laboratory will ensure applicable chemical exposure limits. Following are the basic elements that must be contained in the plan:
   - **A.** Designation of a Chemical Hygiene Officer.
     1. The BPC utilizes BPC Safety Representatives to serve this role.
     2. The BCR utilizes the Safety Officer or the Principal Investigator to serve this role.
   - **B.** SOPs for operations involving the use of hazardous chemicals.

- **C.** Criteria that will be used to determine and implement controls to limit exposures to hazardous chemicals including: engineering controls, personal protective equipment and personal hygiene practices.
- **D.** How the laboratory will ensure that fume hoods and other protective devices are functioning properly and measures to be taken to ensure their upkeep and maintenance.
- **E.** Provisions of mandated employee information and training programs.
- **F.** Circumstances under which a laboratory procedure or activity must have prior approval from designated supervision before being undertaken.
- **G.** Provisions for required medical consultations and examinations.
- **H.** Provision for additional employee protection for work with very hazardous substances, reproductive toxins and chemicals that have a high degree of acute toxicity including designated work areas, use of contaminated devices such as fume hoods, safe handling and disposal of contaminated wastes and well documented contamination procedures.
- **I.** Develops and maintains a laboratory-wide chemical inventory.

II. Housekeeping

- **A.** Lab areas and aisles are to be kept clean and uncluttered
- **B.** Contaminated glassware is to be cleaned daily
- **C.** Spills of water or ice are to be cleaned immediately
- **D.** Floors must be dry at all times
- **E.** Doorways, walkways, and corridors shall not be blocked or used as storage
- **F.** Access to exits, emergency equipment, and utility controls shall never be blocked
- **G.** Windows in laboratory doors shall remain unobstructed
- **H.** Do not put dry ice in laboratory epoxy sinks.
- **I.** Visitors to the laboratory are to be escorted and are the responsibility of the employee. All safety regulations must be observed.
- **J.** When using hazardous materials or performing hazardous procedures, working alone is not allowed.

III. Avoid Unnecessary Exposure to Chemicals

- **A.** *Do not* smell or taste chemicals. Vent any apparatus that can discharge toxic chemicals into local exhaust devices.
- **B.** Inspect gloves before use.
- **C.** Do not allow release of toxic substances in cold rooms and warm rooms, since these have contained circulated atmospheres.
- **D.** Use only those chemicals for which the available ventilation system is appropriate.
- **E.** Eating, drinking, gum chewing or applying of cosmetics and lip balm in areas where laboratory chemicals is strictly prohibited.
- **F.** Avoid storing, handling or consuming food and/or beverages in technical areas, storage areas, refrigerators or use glassware or utensils that are also used for laboratory operation.
- **G.** Handle and store laboratory glassware with care to avoid damage.
- **H.** Wash areas of exposed skin thoroughly before leaving the laboratory.
- **I.** Avoid practical jokes or other behavior that might confuse, startle or distract another worker.
- **J.** Do not use mouth suction for pipetting or starting a siphon.
- **K.** Confine long hair and loose clothing.
- **L.** Wear closed-toed shoes.
- **M.** Keep the work area clean and uncluttered, with chemicals and equipment properly labeled and stored. Clean up the work area on completion of an operation or at the end of each day.
- **N.** Ensure that appropriate eye protection, where necessary, is worn by all persons, including visitors, in areas where chemicals are stored or handled.
- **O.** Wear appropriate gloves when the potential for contact with toxic materials exists. Remove gloves and wash hands before exiting the laboratory.
- **P.** Use any other protective and emergency apparel and equipment as appropriate.
- **Q.** Avoid the use of contact lenses in the laboratory unless necessary. If they are used, inform your supervisor so special precautions can be taken, if needed.

R. Remove laboratory coats immediately upon significant contamination.
S. Seek information and advice about hazards, plan appropriate protective procedures and plan positioning of equipment before beginning any new operation.
T. Leave lights on, place appropriate sign on the door and provide for containment of toxic substances in the event of failure of a utility service, (such as cooling water) in an unattended operation.
U. Use a hood for operations that might result in release of toxic chemical vapors or dust. As a rule of thumb, use a hood or other ventilation device when working with any appreciably volatile substance with a Threshold Limit Value (TLV) of less than 50 ppm. Confirm adequate hood performance before use: keep hood closed at all times except when adjustments within the hood are being made. Keep materials stored in hoods to a minimum and do not allow materials to block vents or airflow.
V. Be aware of unsafe conditions and see that they are corrected when detected.

IV. Procurement: All chemicals in the laboratory must have prior approval from the BPC Director, a BPC Program Manager, BCR Principal Investigator, BCR Program Director, or designee. Prior to ordering, current stock will be checked, handling, storage, and disposal procedures will be reviewed, facility adequacy will be reviewed, training will occur (if necessary), and registration requirements will be checked. IBCSC policies will be followed as written in regards to chemicals meeting the institution's definition of hazardous. Per the IBCSC Hazardous Chemical Policy, all hazardous chemicals:

A. Will be added to the institutional and BPC chemical inventories
B. Will have the applicable procedures downloaded from the Research Safety website. Hard copies are to be made available in addition to electronic versions
C. Will have the MSDS stored both electronically and in hard copy

V. Chemical Inventory: The Chemical Hygiene Officer, or designee(s), is required to perform an annual chemical inventory of all chemicals in the BPC and BCR and ensure proper distribution or disposal of unneeded or expired chemicals. This inventory will occur annually. This inventory is to include:

A. Notation of all laboratory chemicals
  1. The BPC chemical inventory lists all laboratory chemicals.
  2. The RINCH chemical inventory submitted to Research Safety by the BPC and BCR includes those with a 2 or greater than in any part of NFPA diamond, or a 3 or lower in any part of the GHS label, or anything meeting the requirements of a potential carcinogen, mutagen, teratogen, or reproductive toxicity
B. Manufacturers
C. General storage location(s)
D. The carcinogenic toxicity, acute toxicity, and reproductive toxicity.
  1. Store these chemicals in ventilated storage in unbreakable, chemically resistant, secondary containment.
E. Permitted volumes of flammable chemicals must be kept in a fire safety cabinet or explosion proof refrigerator. Refer to "SAF-002 Chemical Hazards and Safety" regarding flammable chemical storage.
F. Extremely toxic chemicals are to be kept in unbreakable containers in a ventilated area.
G. Order the least amount of chemical that is reasonable for current laboratory activity.

VI. Storage: A small quantity approach is used when purchasing hazardous substances to ensure both laboratory economy and safety. Chemicals are purchased by the BPC in quantities that ensure enough is on hand to maintain operations and to cover for any unforeseen circumstance. Each chemical is ordered in the smallest available quantity based upon need and rate of usage. The reasons for this approach include;

A. Unused chemicals constitute 60% of the hazardous chemical waste generated from laboratories.
B. Smaller quantities are used more quickly and reduce the chance for the decomposition of reactive compounds.
C. Problems resulting from breakage or leakage are substantially reduced.
D. Risk of accident and exposure are reduced when handling smaller containers.
  1. Procedures are in place to ensure appropriate quantities, management of inventory. BPC refer to the "LAB-038 Ordering, Receiving, and Stocking Supplies" SOP for instructions. BCR refer to "A019 Ordering, Receiving, and Stocking Supplies" SOP for instructions.

A. General considerations for storage include:

2. Both storage and working amounts of hazardous chemicals shall be kept to a minimum.
3. All chemical containers must have a legible, firmly attached label. Refer to the "SAF-014 Warning Signs and Labels" policy for details.
4. Chemicals shall be stored in containers with which they are chemically compatible.
5. Chemical reagents shall be kept in closed containers when not in use.
6. Incompatible chemicals in containers of 2.0L or 5lb capacity must be segregated. At a minimum, corrosives, flammables, combustibles, oxidizers, poisons, and water reactive chemicals should have their own designated storage in the laboratory.

VII. **Chemical Inventory – Research Institute:** In compliance with institutional guidelines, a chemical inventory is maintained on the Research Institute master chemical inventory on REX > Support Resources > Chemical Inventory. This inventory is reviewed, annually. This inventory is to include:
   - **A.** Notation of hazardous laboratory chemicals (those with a 2 or greater than in any part of NFPA diamond or a 3 or lower in any part of the GHS label) and/or are a known/suspected carcinogen, teratogen or mutagen are listed
   - **B.** Laboratory location
   - **C.** Laboratory Name: BPC Labs (includes the BPC Processing Laboratory, NCH Projects Laboratory, BCR Logistics Laboratory, and Kinnear location). BCR Labs (include BCR Histology and BCR MGL).
   - **D.** Physical state of chemical (solid, liquid, gas)
   - **E.** Volume of flammable liquids on hand

VIII. **IBCSC Hazardous Chemicals SOPs:** The IBCSC has written and made available Hazardous Chemical SOPs for the use of hazardous chemical agents in the laboratory. The BPC makes available hard copies for hazardous chemicals found in BPC laboratory areas and complies with Research Institute standards.

IX. **Material Safety Data Sheets (MSDS) and Labeling:** MSDS's must be available on site (or Central Ohio Poison Control) for each chemical listed on the inventory. All chemicals must be labeled properly.
   - **A.** Located on Research Institute's Safety page through MSDSonline, hard copies in the labs or department SharePoint pages.
   - **B.** Chemicals received will be labeled and stored with the manufacturer's original label.
   - **C.** Proper use and clean up instructions will be made available in the laboratory areas via MSDS hard copies.
   - **D.** When chemicals are placed in smaller containers that are not used immediately, these smaller containers or the racks/boxes holding these smaller containers must also carry the appropriate warnings.
   - **E.** Additional information regarding labeling can be found in "SAF-014 Warning Signs and Labels".

X. **Chemical Hygiene Training**: All employees must be instructed in the Chemical Hygiene Plan and meanings of labels, how to clean up spills, use of fire extinguishers and what the entire plan covers; this is covered by an annual Safety Presentation and attendance is documented. Each manager and designated trainers are responsible for ensuring all new employees are versed in the safe use, handling, clean up and safety equipment, etc. used with all hazardous chemicals used in this section, or that the new employee will be using; this is covered during employee onboarding and as a part of initial competency training.

XI. **Safety and Personal Protective Equipment (PPE):** The laboratory is to have all appropriate safety and PPE for the chemical hazards in the lab. Staff must also be aware of the locations and proper usage of applicable safety devices and methods. The BPC and BCR will inspect safety equipment quarterly as a part of quarterly safety walkthroughs. These include:
   - **A.** Eyewash stations
   - **B.** Appropriate fire extinguishers
   - **C.** Evacuation routes
   - **D.** Lab gowns, nitrile gloves, spill kits, safety glasses, face shields
     1. PPE must be inspected prior to each use to ensure it is in good repair. Clothing should be neat and in good repair.
   - **E.** Fume hoods, checked annually
     1. Chemicals with an NFPA rating of 4 or a GHS rating of 1 in a given category may not be used in the fume hoods in Research Building 2.

F. Flammable storage cabinet

G. Qualified personnel will inspect fire extinguishers, fire alarms, smoke detectors, fire doors, fire suppression systems, chemical fume hood, emergency showers, and biological safety cabinets at regular intervals as determined by the inspection schedule for the given piece of equipment.

XII. Chemical Waste Removal: All unused hazardous chemicals and reagents will be picked up from lab areas by Research Safety and disposed of by a certified outside contractor. Excess waste will not be accumulated as it represents a fire hazard. Refer to the "SAF-013 Biohazard and Chemical Waste Disposal" SOP.

XIII. Chemical Spills: Consult MSDS for correct procedure for spills-Refer to "SAF-002 Chemical Hazards and Safety" and "SAF-018 Biohazard and Chemical Spill Clean-up" for detailed information regarding spill identification, containment, and clean-up.

XIV. BPC and BCR Safety Representatives' Chemical Hygiene Duties: The BPC and BCR Safety Representatives have thorough knowledge of laboratory operations and the potential toxic effects of materials in use. The representatives have authority to implement and enforce the chemical hygiene plan in conjunction with BPC and BCR management.

A. Duties are as follows:

1. Work with BPC and/or BCR Managers, Hospital and Research Institute Lab Safety Committee, and employees to develop and implement appropriate chemical hygiene policies and practices
2. Certify the performance of protective equipment
3. Monitor procurement, use and disposal of chemicals used in the lab and that appropriate MSDS's are on site
4. See that appropriate audits are maintained and inspections are conducted
5. Help BPC and/or BCR Managers develop precautions and adequate facilities
6. Know the current legal requirements concerning regulated substances
7. Seek ways to improve the chemical hygiene program

B. BPC and/or BCR Managers have the overall responsibility to:

1. Ensure workers know and follow the chemical hygiene rules, protective equipment is available and in working order, and appropriate training has been provided.
2. Provide regular, formal chemical hygiene and housekeeping inspections including routine inspections of emergency equipment.
3. Know the current legal requirements concerning regulated substances.
4. Determine the required levels of protective apparel and equipment.
5. Ensure facilities and training for use of any material being ordered are adequate.

C. RINCH Responsibilities:

1. The responsibilities of designated RINCH administrative employees are defined in the RINCH Chemical Hygiene Plan.

XV. Accidents and Spills

A. Eye Contact: Promptly flush eyes with water for a prolonged period (15 minutes) and seek medical attention.

B. Ingestion: Seek medical attention. Immediately contact the Poison Control Center 1-800-222-1222 or x50436 for instructions.

C. Skin Contact: Promptly flush the affected area with water and remove any contaminated clothing. Use a safety shower when contact is extensive. Seek medical attention.

D. Clean Up: Promptly clean up spills, using appropriate personal protective equipment (PPE), equipment and proper disposal. Refer to the "SAF-013 Biohazard Materials and Chemical Disposal" SOP and the "SAF-018 Biohazard and Chemical Spill Clean-Up" SOP. Consult "SAF-002 Chemical Hazards and Safety" for further details relating to minor and major chemical spills.

E. All accidents involving spillage of hazardous substance, employee injury, or property damage must be documented via an Incident Report. BPC: Refer to SOP "ADM-009 Incident, Feedback, and Event Reporting" BCR: Refer to SOP "A013 Incidents, Feedback and Event Reporting".

XVI. First Aid Kits

A. First aid kits are made available and maintained for treatment of minor injuries or for short-term emergency treatment before getting medical assistance.

B. Kits are provided for all BPC and BCR laboratory and office areas.
C. Kits will be labeled with date received and through to the expiration date.
D. Kits are inspected quarterly and properly maintained.

XVII. Medical Care Follow-Up: All employees needing medical care following a spill should go to Employee Health Services (8:00am- 4:00pm). Their service is at no cost to the employee, without loss of pay, and at a reasonable time and place. After 4:00pm or if the injury is serious, the employee should be taken to the Emergency Department. Medical Assistance is available through 911, the Emergency Command Center (23333), or Employee Health (54135).

A. Employees will be sent for evaluations for the following reason:
1. Whenever signs and symptoms associated with a hazardous chemical develop.
2. Where exposure monitoring is over the action level for an OSHA regulated substance that has medical surveillance requirements
3. Whenever a spill, leak, or explosion results in the likelihood of a hazardous exposure, as determined by the Chemical Safety Officer, BPC management, or Institutional Chemical Safety Officer.
4. When employees are required to wear a respirator. Respirators are issued through the NCH Safety Department (x22129).

B. Where medical consultations or examinations are provided, the examining physician shall be provided with the following information:
1. The identity of the hazardous chemical(s) to which the employee may have been exposed and the MSDS's if available.
2. A description of the conditions under which the exposure occurred including quantitative exposure data, if available
3. A description of the signs and symptoms of exposure that the employee is experiencing, if any.

XVIII. Monitoring of certain hazardous chemicals: As recommended by OSHA or deemed necessary by a BPC/BCR Safety Representative or BPC/BCR Manager, monitoring of certain hazardous chemicals will be performed. .

A. Formaldehyde monitoring will take place on an as needed basis. Reasons to monitor will include, but are not limited to, an increased use of formaldehyde in the laboratory area, a decrease in the size of laboratory space, change in air flow rates or a change in the type of exhaust devices used in the laboratory. Data captured by a contracted company performing the analyses is available in hard copy and stored electronically on the BPC and BCR SharePoint sites.

XIX. References

A. N/A

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Liquid Nitrogen and Dry Ice Hazards and Safety

Procedure Number: SAF-004		
Supersedes Procedure Number: N/A		
Date Adopted: 9/17/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
	7/16/2013	Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The purpose of this policy is to emphasize the safe practices that must be employed when utilizing compressed gases, including proper handling of equipment, at all times in the Biopathology Center (BPC) and the Biospecimen Core Resource (BCR).

I. General Safety Practices

A. Liquid nitrogen

1. Due to the hazards inherent to working with liquid nitrogen, all personnel that will be handling liquid nitrogen must also be trained in procedures regarding the handling of liquid nitrogen such as EQP-001, EQP-006, etc.
2. Liquid nitrogen will vent (boil off) from the storage containers as part of normal operation (example: a 160-liter tank will vent the gas equivalent to 2 liters of liquid a day). Containers are typically of a vacuum jacketed design to minimize temperature loss. Excessive venting and/or an isolated ice build-up on the vessel walls may indicate a fault in the vessel's integrity or a problem in the process line. A leaky container should be removed from service and taken to a safe, well-ventilated area immediately**.**
3. The Material Safety Data Sheet (MSDS) or Safety Data Sheet (SDS) for Liquid nitrogen is available through The Research Institute's Safety page through MSDSonline; hard copies are in the labs, or center the BPC Sharepoint site.
   a. BPC SharePoint
   b. MSDS Online
4. Liquid nitrogen must be handled, stored and used only in containers or systems designed in accordance with applicable standards, procedures or proven safe practices.
5. All system components (piping, valves etc.) must be of the appropriate materials to withstand the extreme temperatures.
6. Pressure relief valves must be in place in systems and piping to prevent pressure build up.
7. Any system section that could be valved-off while containing liquid nitrogen must have a pressure relief valve. Pressure relief valve relief ports must be positioned to face away from personnel and any item that could be damaged by repeated exposure.

8. Transfer operations involving open cryogenic containers, such as portable liquid nitrogen containers must be done carefully, while wearing all required personal protective equipment (PPE) (See Section III below or PPE chart posted in laboratory areas for required PPE). Care must be used not to contact non-insulated pipes and system components.
   a. Transferring between open containers will be allowed only in well-ventilated areas.
   b. Do not use a funnel while transferring cryogenic liquids.
9. Use tongs or other similar devices to immerse and remove objects from liquid nitrogen.
10. Equipment changes (e.g. new models) using cryogenic materials that require process or training changes must be analyzed for risks and hazards by research safety.
11. Confined areas where liquid nitrogen is used will have an oxygen monitor. Oxygen levels must be above or equal to 19.5%.

B. Dry ice is frozen carbon dioxide. Unlike most solids, it does not melt into a liquid, but changes directly into a gas. The temperature of dry ice is around -78.5°C.

1. The MSDS or SDS for dry ice is available through Research Institute's Safety page through MSDSonline; hard copies are in the labs, and electronic copies are saved on the BPC SharePoint site.
2. Dry ice should be stored in an insulated container. The container must **not** be airtight to allow gas to escape.
3. Store in a well-ventilated area.

II. Required Personal Protective Equipment (PPE)

A. Liquid nitrogen

1. A full-face shield (the eyes are the most sensitive body part to the extreme cold of the liquid and vapors) required for filling tanks and cryocarts.
2. Laboratory safety glasses required for all other use.
3. Loose-fitting thermal insulated gloves (to allow quick removal if liquid should be spilled inside). Gloves are not made to permit the hands to be immersed in a cryogenic liquid. They will only provide short-term protection from accidental contact with the liquid.
4. Impermeable, long sleeve lab coat.
5. No metal jewelry, rings, watches, etc. should be worn on hands or wrist while handling cryogenic liquids.
6. Closed-toed shoes
   a. No wide-top footwear (e.g. knee-high boots).
7. Non-cuffed long pants.

B. Dry ice

1. Safety goggles (or face shield, if preferred)
2. Loose-fitting thermal insulated gloves over nitrile gloves when bulk transferring dry ice.
3. Impermeable, long sleeve lab coat.

III. Liquid Nitrogen Special Hazards

A. Extreme Cold Hazard

1. Liquid nitrogen can rapidly freeze human tissue. This includes brief exposures that would not affect skin on the face or hands, but could damage delicate tissues such as the eyes.
2. Prolonged exposure of the skin or contact with cold surfaces or dry ice can cause frostbite. There is no initial pain but there is intense pain when frozen tissue thaws.
3. Unprotected skin can stick to metal that is cooled by liquid nitrogen and can then tear when pulled away.
4. Even non-metallic materials are dangerous to touch at low temperature.
5. Prolonged breathing of extremely cold air may damage the lungs.
6. Liquid nitrogen can cause many common materials, such as carbon steel, rubber and plastics, to become brittle or even break under stress, so only materials made for extremely cold temperatures should be used.

a. Note: supplies (e.g. cryovials) that are designed for storage in LN2 vapor may not be designed to withstand liquid LN2. If vials are suspected to have been immersed in LN2, then use extreme precaution when handling, as they may explode when handled.

B. Asphyxiation Hazard

1. Only use liquid nitrogen and dry ice in well ventilated areas.

2. Because liquid nitrogen is compressed for storage, it is constantly expanding to produce nitrogen gas. For example, one volume of liquid nitrogen vaporizes to 694 volumes of nitrogen gas at 68° F @ 1 atm.

a. Liquid nitrogen is odorless, colorless and tasteless when vaporized into the gaseous state..

b. However, extremely cold liquids and their vapors have a built-in warning property that appears whenever they are exposed to the atmosphere: the cold "boil-off" gases condense the moisture in the surrounding air, creating a highly visible fog.

c. Fog clouds do not define the vapor cloud. They define the area where vapors are still cold enough to condense the moisture in the air. The vapor cloud may extend well beyond the fog cloud.

d. Although fog clouds may be indicative of a release, they must never be used to define the leak area, which should not be entered by anyone.

e. Because liquid nitrogen vapors are undetectable to the human sensory system, never enter a suspected oxygen-deficient area without an external source of breathing air or a monitor for the atmosphere to ensure that oxygen levels are safe.

3. Dry ice sublimates, releasing carbon dioxide vapor. Carbon dioxide vapor is substantially heavier than air. In confined, poorly ventilated spaces, it can displace the air and cause lack of oxygen.

4. Evacuate the area if the oxygen sensor alarm is activated or if safety is a concern.

C. Boiling Liquid Expanding Vapor Explosion

1. Liquid nitrogen cannot be indefinitely maintained in the liquid state. If stored in a sealed container, it will continue to expand and produce enormous pressures that could rupture the container. For this reason, pressurized cryogenic containers are normally protected with multiple devices for over-pressure prevention. A pressure relief device must protect all selected equipment that may allow for the liquid to become trapped.

2. Sublimation of dry ice to carbon dioxide gas will cause any airtight container to expand or possibly explode. Dry ice storage chests have a lid, but are not airtight. Other containers for dry ice (e.g. when shipping specimens on dry ice) should never be sealed shut to allow for gas to escape.

D. Spill Evacuation

1. If a large amount of LN2 spillage occurs, leave the area immediately. Contain the area by closing the doors.

2. Contact security (722-3333), Research Safety (355-3574), and manager.

a. If located off-site call 911 immediately.

i. As soon as possible, contact a manager and research safety.

IV. Frostbite

A. For skin contact, remove any clothing that may restrict circulation to the frozen area. Do not rub frozen parts: tissue damage may result. As soon as practical, place the affected area in a warm water bath that has a temperature that does not exceed 105°F. Never use dry heat. Call a physician as soon as possible.

B. Frozen tissue is usually pain-free and appears waxy with a possible yellow color. It will become swollen, painful and prone to infection when thawed.

C. When the frozen part of the body has been thawed, cover the area with a dry sterile dressing pending medical care.

D. In case of massive exposure, remove the victims' clothing while showering him or her with warm water. Call a physician immediately. If the body temperature is depressed, the person must be warmed gradually. Shock may occur during the correction of hypothermia. Cardiac dysrhythmia may be associated with severe hypothermia.

E. If the eyes are exposed to the extreme cold of the liquid or vapors, immediately warm the frostbite area with warm water not exceeding 105°F and seek medical attention.

V. Asphyxiation

A. Signs of asphyxiation include giddiness, mental confusion, loss of judgment, loss of coordination, weakness, nausea, fainting, and up to and including death. Only a few breaths of oxygen-depleted air are required to cause a rapid drop in dissolved oxygen in the blood. Mental failure and coma follow within seconds. Symptoms or warnings are generally absent, but even if present, the loss of mental abilities, coordination and weakness may make it impossible for victims to help themselves or summon help from others. **Any employee experiencing these signs should immediately remove him- or herself from the area and go to Employee Health.**

B. Due to the potential for rapid asphyxiation in a low oxygen environment, any attempt to rescue coworkers in a confined space should be attempted only by trained personnel with SCBA (Self Contained Breathing Apparatus) or EBA (Emergency Breathing Apparatus). The risk for the "rescuer" to be overcome is real and potentially deadly.

C. Anyone suffering from a lack of oxygen should be quickly moved to an area with a normal atmosphere. If the victim is not breathing, artificial respiration should be administered immediately. Immediately contact x22233 for medical assistance. Give supplemental oxygen with respiration if oxygen is available.

VII. References

A. Nationwide Children's Laboratory Services Standard Operating Procedure, Safety-6, Cryogenic and Compressed Gas Hazards and Safety

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Electrical Hazards and Safety

Procedure Number: SAF-005		
Supersedes Procedure Number: # or N/A		
Date Adopted: 9/10/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/5/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
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Principle: Identifying and preventing electrical hazards and promoting safety is necessary for providing a safe work environment and reducing the likelihood of unnecessary damage to specimens or property at the Biopathology Center (BPC) and Biospecimen Core Resource (BCR). The purpose of this procedure is to outline electrical hazard prevention.

I. Electrical Hazards and Safety

A. Electrical equipment which is used improperly or is improperly maintained can cause serious injury, or even death. Please use the following practices:

1. Electrical devices must be properly grounded: 3-prong or double insulated.
2. Never use "cheaters" (convert 3-prong to 2-prong plugs).
3. Check cords/devices for frayed or bare wires, or other defects prior to use.
4. The use of extension cords is limited to emergency situations within Nationwide Children's Hospital see the Hospital Administrative Manual, Policy: IX-15.
5. If a piece of electrical equipment becomes abnormally hot, starts to smoke, and/or sounds abnormal, turn it off immediately, and have Biomedical Engineering, Engineering Services, or the authorized instrument technical representative inspect the equipment before it is placed back into use.
6. Never operate clinical equipment without proper training.
7. Follow the manufacturer's instructions for use and maintenance.
8. Electrical devices should be UL approved or have similar approval.
9. Never operate electrical equipment with wet hands.
10. Keep water away from electrical devices.
11. Waterless hand gel dispensers must be at least six inches from electrical outlets and switches, with that distance being measured from the center point of the dispenser to either side.
12. Never touch an electrical device and a water pipe or other ground at the same time.
13. To pull out a plug, grasp the plug firmly; do not yank the wire.
14. Do not bypass safety interlocks or other safety features
15. Multiple outlet surge protectors are to be used only for computers and their associated equipment.
16. Report any defective or malfunctioning equipment to the work area supervisor or manager.

17. Do not drape combustible material, (ex. cloth or paper) over electrical equipment.
18. All repair and maintenance of primary electrical circuits within the laboratory must be over seen by the Engineering Department.
19. Electrical outlets are tamper-proof or capped in patient care areas.
20. Electrical panels have clear access of at least 36".
21. Cords, plugs, and outlets are in good condition.

B. Special Instructions:

1. Any electrical equipment which is UL approved or sealed is not subject to this policy. This includes the following:
   a. Office equipment, such as calculators, computers, typewriters, facsimile machines, photocopiers, pencil sharpeners, intercoms, etc.
   b. Movie/slide projectors, VCRs, microfiche readers and TVs.
   c. Refrigerators, ice machines, blenders, food processors, microwaves, ovens, and mixers.
2. The following equipment has been identified as being potentially hazardous and is subject to this policy:
   a. Fans
   b. Floor buffers and automatic scrubbers
   c. Food warmers, lowerators and toasters
   d. Electric pallet jacks
   e. Electric saws, drills, and sanders
   f. Small electrical appliances
3. Electrical equipment may pose a potential hazard during intended use when any of the following criteria are present and should not be used:
   a. Historical electrical problems
   b. Continuous plugging, unplugging of equipment
   c. High voltage
   d. Use in or near water
   e. Constant movement
   f. Frayed cords
   g. Electric cord is not firmly attached
   h. Ground prong, if not equipped
   i. The housing/cabinet is damaged
   j. Not a standard design
4. All items covered by this policy will have electrical safety checks conducted, when an instrument is placed into service, has had major repair, or a problem is suspected, and documented by Biomedical Engineering, Engineering Services, or Laboratory Departmental personnel depending on the type of equipment needing inspected.
5. Grounding and polarity checks are also conducted on electrical outlets by Engineering Services when the physical integrity of the receptacle is questionable upon visual inspection or a problem is suspected.
   a. The only exceptions to the above rule are items strictly encased in plastic which renders grounding impossible.
6. New or incoming equipment undergo the same evaluation by Biomedical Engineering with electrical safety checks before being placed into service.
7. Any appliance failing to meet the specified criteria must be removed from use by the manager or designee and repaired by a qualified technician. It is not the Research Institute or Hospital responsibility to repair staff purchased small appliances.
8. An inspection label must be attached to each piece of equipment noting the date of the physical and electrical inspections and the initials of the inspectors.

C. Repair and Maintenance:

1. All repair and maintenance of electrical equipment is performed by either Engineering Services or Biomedical Engineering and conducted in accordance with standards for the control of hazardous energy.
2. All laboratory instruments and appliances are adequately grounded and checked for current leakage before initial use, after repair, following modification, or when a problem is suspected.

D. High-voltage Apparatus-Electrophoresis

1. Reasons for Caution – Electrophoresis equipment can be a major source of electrical hazard in the laboratory. The presence of high voltage and conductive fluid in the apparatus presents a potentially lethal combination. Many people are unaware of hazards associated with this apparatus; even a standard electrophoresis operating at 100 volts can deliver a lethal shock of 25 milliamps. In addition, even a slight leak in the device tank can result in a serious shock.
2. Electrophoresis Hazard Prevention
   a. Turn the power off before connecting the electrical leads
   b. Connect one lead at a time using on hand only
   c. Ensure that your hands are dry when connecting the leads
   d. Keep the apparatus away from water and water sources
   e. Turn the power off before opening the lid or reaching into the chamber
   f. Do not place other equipment or perform other procedures in close proximity to electrophoresis equipment
   g. Place electrophoresis units and their power supplies so that the on/off switch is easy to reach and the power-indicator lights are easily seen
   h. Do not disable safety devices
   i. Follow the equipment operating instructions

E. Electrical Fire:

1. In case of electrical fire, use a fire extinguisher with an “ABC” or “BC” rating. DO NOT attempt to put the fire out with water.

F. Electrical Grounding

1. Electrical equipment is either grounded or double insulated.
2. Flexible cable, electrical outlets, and plugs must be free from damage.
3. Ground fault circuit interrupters are installed in wet locations.

II. References

1. Hospital Administrative Policy IX-7 SAFETY INSPECTION OF ELECTRICAL EQUIPMENT, NONPATIENT CARE (NPC)
2. Hospital Administrative Policy IX-15 EXTENSION CORDS

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Ergonomics

Policy Number: SAF-006		
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Date Adopted: 9/10/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
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Date Approved: 9/5/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
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Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The Biopathology Center (BPC) and Biospecimen Core Resource (BCR) recognize the importance of ergonomics and comply with hospital policy when promoting and implementing ergonomically correct facilities and behaviors. See hyperlink Safety – 16.4 Ergonomics for instructions located in the BAP reference documents.

In the event of a reported work related injury (BPC: Refer to the "ADM-009 Incident Report" SOP and BCR: Refer to 'A013 Incidents and Reporting" SOP) an ergonomic evaluation will occur, if deemed necessary. The evaluation will be performed by the Nationwide Children's Safety Department. Safety will provide the BPC and BCR with recommendations for eliminating musculoskeletal disorder (MSDs) and any appropriate corrective actions based on their assessment finding.

I. References

1. N/A

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Fire Prevention and Control

Procedure Number: SAF-007		
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Principle: Fire prevention and control is necessary for ensuring the safety of all employees and the implementation of adequate precautionary measures in the Biopathology Center (BPC) and Biospecimen Core Resource (BCR). The purpose of this procedure is to give instructions on proper safety prevention and fire control.

I. Environmental Controls

A. Primary and Secondary Exits: Primary and secondary exits are provided for all laboratory and general office areas of the BPC and BCR. Exit signs designating evacuation routes are clearly posted. Staff members are trained in primary and secondary evacuation routes and procedures are in place to account for employees for which the BPC and BCR are responsible upon exiting the building.

1. See "SAF-015 Disaster and Evacuation Plan" for more details.

B. Corridors: Laboratory corridors that constitute an access to an exit are maintained clear and unobstructed at all times.

C. Alarm System: An automatic smoke-detection and alarm system is provided for every area of the BPC and BCR. The alarm system is audible in all areas including the labs, walk in cooler/freezer and file room. Nothing must be placed in and around the alarm to soften the sound.

D. Public Address System: The hospital's public address system is audible in all areas of the BPC and BCR main campus. A public email may be sent to the group e-mail account that announces "Code Red", with the location of the fire as well as instructions for staff members. Safety officials may also sweep the area and inform employees to exit.

E. Fire Doors: Automatic fire doors are designed to close when the alarm is sounded. In the event of a fire, close all doors to confine the fire and smoke.

F. Fire Authority: Whenever a fire alarm is sounded and the City of Columbus Fire Department responds, the Fire Department personnel have complete authority over the situation and their instructions must be followed.

II. Hazard Evaluation

A. Periodic Reviews: BPC and BCR Safety Representatives perform a quarterly inspection of laboratory operations. The Research Institute Biosafety Department performs annual reviews. Particular attention is given to areas in which operations have changed, new chemicals have been introduced or personnel have been added since the last review.

B. Unattended Laboratory Operations: Unattended laboratory operations deserve special attention. An example of such an operation is a hot plate or pressure cooker. These and similar operations which produce heat or flame should never be left unattended.

C. Working Supplies of Flammable Liquids: Working supplies of flammable and combustible liquids are held to the minimum necessary for a safe and efficient laboratory operation. The total amount on hand is held in approved storage cabinets or in safety cans in the laboratory area.

D. Safety Cabinets and Cans: The use of approved fire-safety cabinets and safety cans may be used throughout the lab. The total design capacity is not exceeded by the contents. Containers of flammable and combustible liquids within cabinets are arranged to allow for slight expansion of the bottle or can in the event of a fire.

1. A safety can is defined as an approved container of 5 gallons or less in capacity, usually (but not always) having a spring-closing lid and spout cover and a valve arrangement that is designed to relieve internal pressure in the event of heat. Spouts are provided with flame arrestor devices.
2. Flammable and combustible liquids (flashpoint lower than 200°F/94°C) are heated only in chemical fume hoods. Hot water, steam or an electrical mantle is used; no open flame is allowed.

E. Ignition Sources: Potential sources of ignition should be minimized. The principal sources of ignition are open flames, heating elements and electrical sparks (light switches, electric motors, friction and static electricity). Where vapor levels of flammable gases are known to exist, explosion proof lights and switches are installed and electrical equipment designed for use in such atmospheres.

III. Fire Preparedness

A. Fire Extinguishers: Portable fire extinguishers are installed and maintained, including annual inspection by an outside Safety Company. This is in accordance with NFPA 10, the *Standard for Portable Fire Extinguishers*. For purposes of extinguisher placement, the BPC and BCR are defined as "ordinary hazard." Only trained personnel use portable extinguishers. Training occurs annually through the hospital CHEX program.

1. Employees are trained to use the (**PASS**) system for fire extinguisher usage:
   a. **Pull** the pin
   b. **Aim** the extinguisher at the base of the fire
   c. **Squeeze** the handles together
   d. **Sweep** the extinguisher back and forth across the surface of the burning material

B. Extinguishers may be of the following type:

1. **Class A-**Extinguishers are appropriate for solids fuels such as wood, paper and plastics. Most Class A extinguishers contain water under pressure.
2. **Class B-**Extinguishers are intended for petroleum and solvent fires. Most contain carbon dioxide or a dry chemical, such as sodium bicarbonate.
3. **Class C-**Extinguishers can be used to extinguish electrical fires. Personnel should locate and turn off power switches to a burning electrical instrument.

4. The following are the location and fire extinguisher types found in the BPC, BCR and Morphology Core:

Location	Number	Type
BPC-WA1300	1	ABC
BPC-WA1340	1	ABC
BCR-WA1100A – (two total) One at each end of the Hallway	1	ABC
BCR-WA1201 – (two total) One at each end of the Hallway	1	ABC
BCR Histology-Hallway between W241 and W243	1	ABC
BCR Molecular Genetics Laboratory between W256 and W253	1	ABC
Morphology Core-Hallway between W292 and W293	1	Halon (BC)
BPC-W344	1	ABC
Storage Room-WA0001	1	ABC
Kinnear-Office	1	ABC
Kinnear-Lab	1	ABC
Kinnear-Freezer Rooms	3	ABC
BPC Informatics- W102	1	ABC
BCR Logistics- Hallway between W230 and W233	1	ABC

5. Automatic fire-extinguishing systems are inspected and maintained annually, cared for properly and maintained in continuous service by the hospital's engineering department.
6. Fires involving piped gas systems should be extinguished by cutting off the fuel using a remote valve. If this is not possible or the flammable gas is coming from a cylinder, the room should be evacuated immediately.

C. **Fire Blankets:** Fire blankets are available for clothing fires, but personnel are trained to "stop, drop and roll" immediately after one's clothing catches fire. Fire blankets must not be used when the person is in a standing position, because the blanket can act as a chimney that delivers hot gases directly to the face and respiratory system. Fire blankets may also be used as a personal protective device when exiting an area where there is a large fire. The location of fire blankets in the BPC is as follows: **BPC**-WA1340; **Core Morphology**-W284. The BCR laboratories do not currently have fire blankets.

D. **Fire Safety Training:** A training program in fire safety is provided as part of every new employee orientation. An annual review in fire safety for every employee is required. Fire safety protocols and training is included in a computer based safety tutorial program on CHEX that every employee is required to take on an annual basis.

E. **Sounding the Alarm:** All personnel are trained in the method to sound an alarm, and how to recognize the sound of the alarm from their respective work areas. The Research Institute at Nationwide Children's Hospital has manual fire alarm boxes located at all of the exits. The BPC Kinnear Road facility also has pull stations at each exit. Employees are instructed to pull the alarm as they exit the building.

F. **Priorities:** Following are the hospital's **RACE** priorities to be followed in the event of a fire. RACE represents **Rescue, Alarm, Contain and Evacuate.**

- **Rescue** anyone in the immediate area of danger. All persons in the area should be notified immediately by calling "Code Red."

- Call 2-3333 to report the fire location and to have the **Alarm** sounded. If other staff members are evacuating the building, have them pull the manual alarm boxes located at the exit doors.
- When feasible, a portable fire extinguisher should be used to extinguish or **contain** the fire. Do not expose yourself to unnecessary danger. Close all doors leading into the area where the fire is being contained.
- All personnel should **Evacuate** the premises. If there does not appear to be an imminent danger, one person may be designated to remain in the area to explain the situation to the firefighters that respond to the emergency.

IV. References

A. N/A

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General Requirements for Personal and Lab Safety

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6/20/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The guidelines included in this procedure are supplemental to Nationwide Children's Hospital's Infection Control Manual and the Safety sections of the Administrative Manual. Nationwide Children's Hospital emphasizes employee and patient safety. The Biopathology Center (BPC) and Biospecimen Core Resource (BCR) additionally support The Research Institute Biosafety Manual. Failure to adhere to safety guidelines is reflected on annual performance review and non-compliance may lead to disciplinary action.

The BPC and BCR laboratories in which non-fixed, human samples are processed are categorized as Biosafety Level 2 (BSL-2) Containment areas as activities in these areas meet the requirements for this category. Safety SOPs are written with parameters that allow the BPC to meet the standard requirements of BSL-2 classification. Details of BSL-2 classification can be found in The Research Institute Biosafety Manual.

I. Smoking

A. All employees and contract workers are prohibited from smoking and/or using tobacco related products while at work or during their work shift. (Hospital Administrative Policy: ADMIN-V-2)

II. Food, drink, and like substances that provide potential for hand-to-mouth contact

A. Foods, drinks, and like substances (e.g., chewing gum and candy) that provide potential hand-to-mouth contact are **prohibited** in technical work areas.

B. Specimens (blood, urine, feces, sputum) containing a variety of pathogens handled daily in the technical work area and stored in laboratory refrigerators provide a potential source of contamination of food and drink. Food and drink must NOT be stored in technical refrigerators; these refrigerators are posted as such.

C. Refrigerators reserved exclusively for food storage are located in the employee lounges and/or clean areas within a department where eating and drinking are permitted.

III. Cosmetics

A. Application of cosmetics, including lip balms, in the technical work area is prohibited.

B. Use of hand creams is allowed and recommended for employees performing frequent hand washing. Lotions can prevent or minimize skin dryness and irritation due to irritant contact dermatitis associated with hand antisepsis or hand washing. The use of an approved hand lotion on a regular basis will help protect the skin and may reduce microbial shedding.

C. Only lotions approved by the Epidemiology Department may be used and can be ordered through the Research Institute Purchasing Department. Hand lotions that contain petroleum products or other oil emollients may affect the integrity of healthcare workers' gloves and should be avoided. Other lotions may interfere with the persistent effects of antimicrobial soaps or alcohol-based hand rubs.

D. Hand lotions must be dispensed from containers that are not refilled or "topped off".

E. Only Hospital approved waterless alcohol-based hand-rub can be used for decontaminating hands. When using the waterless alcohol-based hand-rub, hands must be washed every 5th time with an antimicrobial soap and water.

IV. Eye and face protection

A. Face shields, face protectors, biological safety cabinets must be used when handling toxic materials (acids and bases), bloodborne pathogens, or any materials that are likely to splash.

B. NOTE: For caustic materials, eye protection alone is not sufficient, face shields must be used.

C. These devices are stored in areas designated by the lab managers.

D. Contact lenses (especially soft lenses) will absorb solvents and vapors; therefore, they constitute a hazard during splashes or spills. Contact lenses offer no protection from splashes. They can concentrate caustic material against the cornea or prevent tears from washing a caustic substance away. **Personnel are advised not to wear contact lenses in hazardous areas of the laboratory unless protective goggles or face shields are also worn**. **If contacts are worn, manipulate contacts with clean hands and if possible, outside of the lab area.**

E. Prescription glasses are not considered to provide sufficient protection. Face shields must be worn or safety goggles must be provided and worn that fit over the prescription glasses.

F. See Personal Protective Equipment Chart posted in work areas for designated areas/activities requiring use of face masks.

V. Clothing/Personal protective equipment (PPE)

A. Clothing should be clean, neat, and in good repair.

B. All PPE is provided and maintained by the laboratory. PPE Charts are posted in all BPC and BCR Laboratories. PPE is placed in conveniently located sites within the laboratory as appropriate, and PPE use is mandated and monitored for compliance. Refer to applicable procedures and laboratory signage regarding proper PPE to wear while performing the given procedure.

C. While in the laboratory, all persons working in technical areas are to wear a long-sleeved, full-length, liquid impermeable laboratory coat that is buttoned closed. **These are provided and laundered by the Research Institute and are not to be taken home or worn outside the lab area.** Disposable gowns may be made available on an "as needed" basis.

D. Non-latex gloves are to be used when handling any specimen or potentially biohazardous substance, or when contact is made with a potentially contaminated surface.

E. Gloves are not to be worn when using equipment in the lab designated as "gloves off" or when touching any door handle throughout the lab, especially when transporting specimens between lab areas and floors. This is to prevent the contamination of the door handles.

F. In addition, chemical aprons with arm sleeves or disposable fluid-resistant gowns must be worn where there is a significant probability that potentially hazardous chemicals such as acids or bases will be splashed on the worker.

G. Personal protective clothing should be changed at appropriate intervals as per hospital policy to ensure cleanliness and it should be changed immediately if it is grossly contaminated with hazardous materials.

H. Additional protective gowns, masks, and non-latex gloves are required when collecting specimens from patients under specific infectious isolation.

I. Soiled and/or contaminated laboratory coats should be placed in the appropriate laundry chute, or bin(s), to be washed by the hospital's laundry service. Severely contaminated coats should be sealed in a plastic trash bag to prevent the spread of contamination.

NOTE: According to OSHA in 29CFR §-1910.1030, "Personal protective equipment will be considered 'appropriate' only if it does not permit blood or other potentially infectious materials to pass through to or reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use".

VI. Latex sensitivity/allergy protections

A. The Hospital strives to create a latex-free environment wherever possible.
B. The BPC and BCR are latex free; all gloves used in the BPC and BCR are non-latex.
C. During new employee orientation the Epidemiology Department is responsible for providing orientation to new employees on the use of latex products within the hospital.
D. The Epidemiology Department also conducts periodic educational sessions associated with latex sensitivity.
E. Annually, each employee completes the CHEX educational computer program on latex allergies or will be provided a presentation covering latex allergies at a staff meeting.
F. The Research Institute Purchasing Department is responsible for assuring the availability of latex-free products for employees, students, and medical staff whenever such products are available and approved by the Product Quality Cost Review Committee.
G. Any employee exhibiting symptoms of an allergic reaction to latex are to go to Employee Health Services to report the reaction.
H. Departmental Managers will work with Employee Health Services and Legal Services to identify and minimize risks associated with employee latex allergies.
I. Employee Health Services and Legal Services will monitor incidents and identify risks of staff latex sensitivity/allergic reactions through the incident reporting process.
J. Refer to the Hospital Administrative Policy IX-9 for further information on the use of latex products and the prevention of latex sensitivity/allergy.

VII. Gloves

A. Laboratories often contain hundreds if not thousands of different chemicals in their inventories. With the sheer number of different chemicals and the varying hazardous properties of these chemicals, it is impossible to find a single disposable glove that offers maximum protection to the worker for all the chemicals they use during the day. The following classification system for glove selection is designed to maximize not only the protection offered to the worker, but also minimize the impact of using more expensive gloves in the laboratory.

DISCLAIMER: Class I and II gloves are not certified for any extended exposure to harmful chemicals outside of incidental splashing. Any chemical that requires extended exposure times needs to be evaluated by Laboratory Safety (see Tier III).

B. Class I – Cardinal Health Esteem Stretchy Nitrile Powder-Free Exam Gloves (8810N – 8814NXX)
1. Made of nitrile material, gloves are for situations with high risk of exposure to blood or body fluids, considered a biological barrier protection glove.
2. Offers **minimum protection** against chemicals, and should not come in direct contact with harsh chemicals, change gloves immediately after any chemical splash.
3. EXEMPTION: These gloves offer excellent protection against 10% Formalin (>480 minutes contact time) and can be used in direct contact with 10% Formalin (3.7% Formaldehyde/1.5% Methanol)
4. Some examples of use: Sample Processing & Manipulation, Phlebotomy, Point of Care Testing, Culture Set-up, Basic Chemistry & Urinalysis

C. Class II – (1) Kimberly-Clark Purple Nitrile Powder-Free Exam Gloves OR (2) Cardinal Health Esteem XP Powder-Free Exam Gloves (N8850XP – N8854XP)
1. This glove type is the primary glove type for the Biopathology Center.
2. Slightly thicker nitrile material than class I, gloves are for situations with high risk of exposure to harsher chemical splashes.
3. Offers best protection against chemicals, but still should not come in direct contact with harsh chemicals, change gloves immediately after any chemical splash.
4. Need to be worn when working with the following chemicals: Methanol, Acetic Acid, Acetone, DMSO, > 50% Ethanol, > 50% Methanol, Xylene
5. NOTE: The chemicals listed above are just some of the chemicals that Class II gloves need to be worn while working with.
6. Some examples of use: Reagent Prep, Manual Slide Staining, Bench top Cleaning, Instrument Maintenance (Changing or Removing Chemicals)

D. Class III – Custom Gloves Based on Chemical and Exposure Time

1. Offers best protection against chemicals, and are able to come in direct contact with harsh chemicals, gloves are chosen on a case by case basis.
2. Contact Laboratory Safety if you have any procedures containing prolonged chemical exposure times
3. Examples of use: Working in Xylene

E. Cut Resistant Gloves – Ansell Cut Resistant Gloves (5789910-5789914)

1. To be worn underneath nitrile gloves while performing procedures that require the use of a scalpel.
2. Protects against slicing-type cuts that may result from working with scalpel blades during tissue processing.

VIII. Shoes

A. Shoes should be comfortable and rubber soled. Leather or a synthetic, fluid-impermeable material is suggested.
B. Disposable, fluid-resistant shoe covers can be worn for jobs where splashing is expected.
C. Canvas shoes (made of natural materials such as cotton) may absorb chemicals or infectious fluids and are not recommended. Some areas may prohibit the wearing of canvas shoes due to the type of testing performed in that area. Check with your manager before wearing canvas shoes.
D. Open heeled shoes are not recommended.
E. Open-toed shoes and sandals are prohibited.

IX. Hair and jewelry

A. Hair should be secured back and off the shoulders to prevent it from contact with contaminated materials or surfaces and also to prevent shedding organisms into the work area. It is also important to keep hair out of moving equipment, such as a centrifuge or microtome.
B. Do not wear jewelry that can become caught in equipment or hang into infective materials.

X. Beards and ties

A. Men with beards must observe the same precautions provided for hair. Long beards may get caught in moving equipment and all beards are a source of bacterial contamination.
B. Ties should be secured to prevent them from becoming caught in moving equipment or hanging into infective materials.

XI. Hand washing

A. For more information regarding hand hygiene, refer to Infection Control Policy IV-30 Hand Hygiene.
B. Hands should be washed frequently during the day. Wash hands, before leaving the laboratory, before and after contact with patients, before eating, and after using the restroom.
C. Hands must also be washed immediately after accidental contact with blood, body fluids, and contaminated materials.

i. When using a Hospital approved waterless alcohol-based hand-rub for decontaminating hands, hands must be washed every 5th time with an antimicrobial soap and water.

XII. Eyewash

A. Eyewash stations are located in each lab where bloodborne pathogens, acids, caustics, corrosives, and other hazardous chemicals are in use.
B. Eyewash stations must be within 100 feet and accessible within 10 seconds of hazardous chemicals or biohazardous substances.
C. Eyewash stations are approved devices attached to the cold water supply.
D. Eyewash stations are tested **weekly** to ensure proper functioning and to flush out stagnant water. (BPC: See "EQP-002 Equipment Checklists" SOP).

1. Visually inspect eyewash unit for defects
   - **i.** Flow is provided to both eyes simultaneously
   - **ii.** Nozzles or covers are present to protect from airborne contaminants
   - **iii.** Hands free flow is provided once activated
   - **ii.** Eyewash location signage is visible
2. Allow the eyewash to run for a minimum of 3 minutes to wash out any possible contaminates.
3. During the three minute flush, verify that the water temperature is tepid.

4. Clean eyepiece caps with a disinfectant such as Prospray or 10% bleach then rinse the eyepiece caps for 15 seconds and replace them on the eyewash station.

E. Document eyewash test on the appropriate form. Notify your manager of any problems that you encounter during the testing procedure.

F. Use of eyewash stations is reviewed annually with all employees in the hospital computer training module computer safety lessons (CHEX).

XIII. Emergency showers

A. Emergency showers are available in each lab and convenient to the location where caustic and corrosive chemicals are used.

B. Emergency showers must be located within 100 feet and accessible within 10 seconds of caustic and corrosive chemicals.

C. Emergency showers are tested **annually** by Engineering Services for proper function and documented in the Engineering department. The showers will also be tagged by Engineering Services with the date of testing.

XIV. Respirators and related equipment

A. There is no positive-pressure, Self-Contained Breathing Apparatus (SCBA) in the BPC and/or BCR. An Emergency Breathing Apparatus (EBA) is available for use in the event of an asphyxiation emergency outside of the Freezer Farm in Room D00630 , outside of the BCR Freezer Room near W234, and outside of WA1008 in the Research II Building first floor lobby.

B. When the situation arises for the use of SCBA, the Hospital Safety and Security Department is to be contacted immediately at ext. 23333.

C. Generally, this equipment must be worn when the nature of the respiratory hazard is unknown, when the oxygen level is below 19.5%, when the presence of the hazardous material is not detectable by the senses, when breathing the contaminant is immediately dangerous to life and health and/or during emergency response to an incident involving a hazardous substance.

D. Oxygen monitors are located in confined areas that house inert gases. Additionally, the Cytogenetics and Molecular Genetics Departments have hand-held oxygen monitoring devices. BPC alarms are configured to sound at concentrations <19.5% ambient oxygen. Staff should never enter an area that has an oxygen level lower than 19.5% or an area where the oxygen monitors are alarming.

E. **Ventilation devices** such as plastic, disposable mouth-to-mouth resuscitation bags that minimize the need for emergency mouth-to-mouth resuscitation **are not** available in the BPC and/or BCR. They are available in other areas of the institution where the need for resuscitation is predictable.

F. A **code blue** (call 22233) should be sounded and a medical response team will be summoned if such a situation arises in the BPC and/or BCR.

XV. Mouth pipetting

A. Mouth pipetting is prohibited. Pipetting aids are available for every task.

XVI. Sharp objects

A. Caution must be exercised when handling sharp objects, including needles, scalpels, and broken glass.

B. If possible, all sharp objects should be handled with mechanical devices or one-handed techniques (e.g., forceps to remove scalpel blades, and retractable Vacutainer needles).

C. **Used needles must not be bent, sheared, broken, recapped, resheathed by hand, or removed from disposable syringes.**

D. Sharp objects must be placed immediately in puncture-resistant containers and those containers should be sealed and then taped shut when being discarded properly before they are more than ¾ full or sooner if the fill line is marked less than ¾ full.

XVII. Ultraviolet (UV) Light

A. Germicidal UV light is classified as UVC light (wavelength of 180-280nm) and is the more harmful of UV rays types.

B. There are no immediate warning signs of UV overexposure. Symptoms may present in varying degrees and include sunburn or photokeratitis (welder's flash) and typically occur hours after exposure.

C. . Germicidal UV light is only used in the BCR molecular laboratory after use in the PCR hood.

XVIII. Cryostats and microtomes

A. See The Morphology Core Safety SOPs for information (including information about cryostats). The Morphology Core is CAP/CLIA accredited.
B. Creutzfeldt-Jakob agents are very rare in pediatrics and no tissues suspected of this disease are to be frozen. See "SAF-019 Precautions for Tissues Potentially Infected with Creutzfeldt-Jakob Disease".
C. Cryostats and microtomes are potentially dangerous equipment. Both use knives which may cause accidental cuts to the skin. The main difference between these two machines is that the microtome uses paraffin-embedded tissues for cutting/sectioning. A cryostat is considered a high-risk instrument because it uses frozen, unfixed tissue that can contain viable infectious agents (frozen sections). The following recommendations describe the safe use of these instruments
D. Control of Infection: The block holder and the brush should be decontaminated weekly by immersion in the suitable antiseptic solution. This is documented in the QA/QC manual found in the BCR Histology Department
E. Cryostats are decontaminated biannually when the mechanical checks are done.
F. If a suspected tissue contains mycobacteria, immediate decontamination should be done with tuberculocidal antiseptic (refer to BCR H002 "Cryostat Cleaning and Decontamination" for further details).
G. Gloves and proper protective devices should be used.

XIX. Control of Mechanical Injuries (Cryostats and microtomes)

A. Never leave knives unguarded.
B. Dispose of knives in a puncture-resistant sharps container.
C. If changing specimens without removing the finger guards, the hand wheel must be locked (see NCH Research Safety Policy 005 "Microtome and Tissue Sectioning").

XX. Good housekeeping practices

A. "Clean" and "Contaminated" Areas

i. The designation of a technical area as either "clean" or "contaminated," determines work and housekeeping practices.

B. "Clean" Areas

i. Work practices entail efforts to prevent contamination of telephones, computer keyboards, doorknobs, and other items commonly touched by both gloved and ungloved hands.
ii. Employees are required to remove gloves before touching equipment considered "clean" and when leaving the technical area.
Refer to applicable labeling, signage, and/or procedures regarding areas designated as "clean" or "gloves off" in the given laboratory.

C. "Contaminated" Areas

1. "Contaminated" surfaces must be touched with gloved hands.
i. Persons entering these areas with ungloved hands are responsible for gloving before touching "contaminated" areas/items, Refer to applicable labeling, signage, and/or procedures regarding areas designated as "contaminated" or "gloves on" in the given laboratory.

D. Under either area designation, "Clean or Contaminated", all work sites should be kept neat and uncluttered.

i. Wipe up all spills immediately following spill cleanup procedures using an appropriate cleaning agent. Wear gloves and appropriate PPE when dealing with spills and contaminated work surfaces**.**
ii. Countertops should be decontaminated at least daily (and documented – See "LAB-027 Laboratory Cleaning" SOP)—more frequently if needed—using a Nationwide Children's Hospital approved phenol disinfectant such as ProSpray® (following the phenol disinfectant directions) or freshly prepared (daily) 1:10 dilution of bleach as appropriate for the work site.

- (See *Uses of Inorganic Hypochlorite (Bleach) in Health-Care Facilities:* Uses of inorganic hypochlorite (bleach) in health-... [Clin Microbial Rev. 1997] - PubMed result)

E. Laboratory Equipment

1. Refrigerators, freezers, water baths, and centrifuges are to be cleaned and disinfected at a frequency determined by the BPC directors and/or managers of each area and when gross contamination occurs. Wear non-latex gloves, eye protection (if splashing may occur) and a lab coat during cleaning. Document cleaning and disinfecting on the equipment checklist for the given piece of equipment.

F. Transporting Specimens

1. Specimens need to be capped/closed and should be carried so that they are contained in a way to prevent the need for PPE (gloves or gowns).

G. Outer Clothing

1. Laboratory coats should be properly hung up away from heating instruments and open flames.
2. Using compressed gas cylinders or fire extinguishers as coat hooks is not appropriate. Do not obstruct the view through glass partitions in doors by hanging clothing over the glass. Store contaminated and uncontaminated personal protective garments separately.

H. Trash Disposal

1. Trash disposal is maintained on a schedule determined by Environmental Services. If a trash container is three-quarters full, Environmental Services is to be called to remove the trash, when applicable, or trash should be taken by lab staff to the appropriate receptacle for pick-up by Environmental Services.
2. **Biohazard trash must be handled in the manner outlined in "SAF-013 Biohazard and Chemical Waste Disposal."**

I. Decorations

1. Use of festive decorations should comply with hospital policy.
2. Decorations on lights, light fixtures, or instruments are prohibited.
3. Wax candles, dried arrangements, live Christmas trees, and other decorations that present potential fire hazards are also prohibited.
4. Electrical decorations are permitted if they are approved by the Hospital Safety and Security Department.

J. Personal Belongings

1. Do not store personal belongings, such as cell phones, media players, purses, coats, boots, coffee mugs, prepackaged foods or medications, in the technical work area.

K. Disposable Materials Storage

i. Do not store large amounts of disposable materials in the workplace.
ii. Hazardous liquids, such as acids or alkalis, should be stored below eye level.
iii. Store large containers near the floor.
iv. Optimal storage conditions of at least an 18-inch clearance from the bottom of ceiling-mounted sprinkler heads must be maintained.
    i. Where shelving runs along a wall, storage is allowed to go up to the ceiling as long as nothing is placed with 18"
v. Do not store any materials in hallways or near exits where they may be an obstacle to emergency personnel or a quick exit of the area.
vi. Maintain at least 36" walkways.
vii. Disposable materials should not be stored on the floor.

L. Exit Routes

i. Exits and aisles must not be obstructed in any way.
ii. No trash, supplies, equipment, or furniture should be permitted in exit routes or aisles.
iii. Exit doors must not be obstructed, bolted, or blocked in any way.
iv. Fire doors must not be obstructed in any way that prevents automatic closing in case of fire.
v. **NOTE:** Do not cover or block access to fire extinguishers, fire alarm boxes, safety showers, or exits at any time, for any reason. There should be adequate clearance around fire extinguishers, fire alarm boxes, and safety showers.

XXI. Glassware

A. When handling glassware, the following practices should be employed:

1. Discard broken or chipped glassware in either sharps container, or appropriate labeled cardboard box. All contaminated glassware must be disposed of in red bagged glass boxes.
2. Do not remove stoppers on glass tubing by force. Those that are stuck should be cut off.
3. Decontaminate glassware that has been exposed to infectious agents before washing.
4. Dispose of broken pieces in a specially marked, separate, puncture-resistant container.
5. Handle hot glass with heat-retardant gloves.
6. Handle broken glassware only with mechanical devices.

XXII. Use of centrifuge

A. Aerosols

1. Centrifugation procedures must minimize the possibility of aerosolization.

B. Operation

i. Do not operate any centrifuge unless the covers are closed.
ii. Keep hair, clothing, or dangling items out of the way.

C. Contamination

i. Avoid centrifugation of uncovered tubes of specimens (blood, urine, or sputum) or flammable liquids.
ii. Centrifugation creates a vacuum and causes liquids to become volatile (e.g., contaminated items become aerosols, flammable liquids become bombs).

D. Infectious Agents

i. All cultures or specimens likely to contain agents that are infectious by aerosol inhalation must be centrifuged within sealed centrifuge tubes in tightly covered trunnion cups or rotors. For example, this pertains to the processing of specimens for the culture of acid fast bacilli (AFB).

E. Cleaning

i. Centrifuges should be routinely cleaned and disinfected with a Nationwide Children's Hospital approved phenol disinfectant. Document cleaning and disinfecting on the appropriate form.

F. Balance of Tubes

i. When operating a centrifuge, maintain proper tube balance.
ii. Imbalance of the rotor causes vibration. Even when the imbalance is not great enough to trigger the imbalance detector, tubes break more frequently and wear on the centrifuge is increased.

XXIII. Fume hoods and biological safety cabinets

A. Fume hoods are available and must be used whenever volatile, flammable or other similar compounds or noxious/toxic materials are used; see "SAF-003 Chemical Hygiene Plan" for more information.

B. Similarly, biological safety cabinets (HEPA filtered, laminar flow hoods) must be used whenever working with clinical materials for microbiological, virological, and serological infectious disease tests and other assays; See related safety procedure "SAF-010 Laboratory Exposure Plan" for more details.

C. Employees are instructed on proper use of these hoods.

D. All hoods are **inspected annually** to ensure proper performance, and inspection certificates or stickers are displayed prominently.

XXIV. Temperature monitoring

A. An appropriate thermometric standard device of known accuracy (guaranteed by the manufacturer) must be present on all temperature-controlled instruments and environments and should be checked daily. Equipment failures, particularly with refrigerators or freezers containing reagents and specimens could affect the quality of specimens

1. Items such as water baths and heat blocks used for procedures need only be checked on days in use of patient testing.

B. Rees Freezer Monitoring Alarm system monitors all temperature controlled storage units, 24 hours a day, 7 days a week, and 365 days a year. See applicable freezer monitoring and alarm SOPs for the given laboratory area for further details, including but not limited to daily temperature checks using a back-up method, contingency monitoring plans, and system functionality verification checks. Contact a manager if uncertain where to find the given information for the laboratory area.

C. Temperature controlled equipment and temperature monitoring systems are validated annual by BioMedical Engineering or applicable Vender (ex. Rees Scientific).

D. If a piece of temperature sensitive equipment needs to be taken out of service, document all details in Q-Pulse using the type of activity to capture date and details of when the equipment is taken out of service. Reference SOP BPC-ITS-023 "Q-Pulse –Assets Module" for further details.

XXV. Transport of Specimens

A. Blood, other body fluids, secretions/exudates from **all** patients are considered infective.

B. All specimens of blood and body fluids are placed in a well-constructed container with a secure lid to prevent leakage during transport.

1. All specimens should be transported at appropriate temperature. Refer to the "LAB-047 Specimen Storage" SOP.

C. Care should be taken when collecting each specimen to avoid contaminating the outside of the container or the laboratory form accompanying the specimen.

D. An outer leak proof container (approved zip-lock bag is used (i.e., bagging) and biohazard insignia labels appear on all specimens).

i. NOTE: A labeling exemption exists in section (d)(2)(xiii)(A) of the Bloodborne Pathogen Standard **that exempts facilities that handle all specimens with standard precautions, provided the containers are recognizable as containing specimens, to transport specimens within the facility utilizing containers that do not display a biohazard insignia or red-color coding. This exemption applies only while the specimens remain within the facility. All employees who will have contact with the specimens must be trained to handle all specimens with standard precautions. If the specimens leave the facility (e.g., during transport, shipment, or disposal), a label or red color-coding is required.** OSHA FAQ

E. The shipment of dangerous goods and infectious substances is governed by regulations issued by the Department of Transportation (DOT) in 49 CFR 171-180 and the U.S. Postal Service (USPS) in 39 CFR 111. The International Civil Aviation Organization Technical Instructions (ICAO TI) govern all dangerous goods shipments by air. These technical instructions are published annually in the International Air Transport Association (IATA) *Dangerous Goods Regulations (DGR)* manual. The IATA DGR contains all requirements of the ICAO TI plus additional, more restrictive regulations than ICAO TI. These regulations address how shippers of dangerous goods and infectious substances must properly pack, label, and declare shipments to ensure that the public and transportation workers are protected from exposure to any agent that may be contained in a package. These regulations apply to anyone who handles, offers transport, transports dangerous goods and/or causes dangerous goods to be transported.

F. All laboratory staff package and ship infectious material in accordance with applicable federal, state and local regulations.

G. Training must include an overview of the DOT and IATA regulations as well as detailed training applicable to the trainee's specific job functions (e.g., preparing labels and shipper's declarations). IATA certification takes place every 2 years.

XXVI. Cell phones

A. Cell phone usage in not allowed in contaminated areas of the laboratory. If you wish to use your cell phone as a personal listening device, refer to "XXVII", below.

B. Cell phone usage is not permitted in patient care areas.

XXVII. Personal listening devices

1. Personal listening devices are allowed to be used in both clean and contaminated areas of the laboratory. If they are used in contaminated areas, they must be stored completely underneath the lab coat and are not to be touched with gloves and/or contaminated hands.

2. Devices are not permitted to be stored in lab coat pockets or on any surface in contaminated areas.

3. Headphones are also to be stored underneath lab coats and are not to be touched with gloved and/or contaminated hands. It is recommended that a set of headphones/ear buds be designated for lab use only to minimize the potential for blood borne pathogen exposure.

4. Personal listening devices are not allowed to be used in patient care areas.

5. It is recommended that these items are decontaminated before using in a clean area using appropriate disinfectant wipes.

6. Headphones are to be worn only in one ear. This applies to all BPC and BCR areas, including both laboratory and administrative spaces.

XXVIII. Receipt of (Potentially) Radioactive Biospecimens:

A. The BPC and BCR do not work with radioactive materials and are not supposed to receive any potentially radioactive biospecimens.

B. Should a contact from a submitting institution ask about submission of post-treatment biospecimens from a patient that has undergone treatment involving radioactive chemicals (ex. MIBG scans – patients receive Iodine-123 as a part of this process), the requestor will be instructed to wait before collecting and sending the specimen. The wait time should be based on the type of radiation exposure and should be a sufficient amount of time to render the specimen non-radioactive.

C. Should a biospecimen or shipping container labeled as radioactive be received:

1. STOP – Do not open the package or touch the biospecimen.
2. Contact Research Safety at x52776.
    i. Research Safety team members will create a temporary, secured area to open the package and contain any radiation within that area. They will be the ones to open the packaging utilizing specialized PPE.
3. Contact the Clinical Research Associate (CRA) who submitted the biospecimen and request written permission to destroy the biospecimens.

XXIX. References

1. 49 CFR 171-180
2. 39 CFR 111
3. Uses of inorganic hypochlorite (bleach) in health-... [Clin Microbial Rev. 1997] - PubMed result
4. Nationwide Children's Hospital's Infection Control Manual and the Safety sections of the Administrative Manual
5. OSHA 29CFR §-1910.1030

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Hazardous Waste Disposal

Policy Number: SAF-009		
Supersedes Procedure Number: BPC Safety Manual		
Date Adopted: 9/18/2012	**Prepared By:** Lauren Noyes, BPC Regulatory Coordinator	
	Reviewed By: Teresa Tabler, Business Analyst	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
6/20/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: This policy outlines the Biopathology Center's (BPC) and Biospecimen Core Resource's (BCR) actions as they relate to the proper disposal of hazardous wastes.

I. **Hazardous Waste Disposal:** The BPC, BCR and Research Institute at Nationwide Children's Hospital (RINCH) have a responsibility to the community to ensure that appropriate hazardous waste handling policies are developed and rigorously followed. Local, state, and federal authorities strictly regulate the disposal of chemical and microbiological wastes and effluents into the environment. Individual variations from jurisdiction to jurisdiction require that the BPC, BCR and RINCH carefully review the regulations applicable to its locale. Part of this review should include a cataloging or inventory by RINCH of waste generated and the time that hazardous waste is stored on-site before disposal. Depending on the amount of waste generated, special storage permits may be required. Special attention should also be given to the packaging and manifesting requirements of the facility that will provide ultimate disposal services for the hazardous waste.

II. By EPA regulations, the laboratory retains the responsibility for all chemical hazardous waste regardless of disposal agency methods. The laboratory should develop specific waste handling and disposal procedures to reduce the potential risk to personnel that handle hazardous waste in the laboratory, and those involved in disposing of the waste (See "SAF-013 Biohazard and Chemical Waste Disposal" procedure for step-wise instructions). These procedures may call for segregation of wastes by specific type, labeling of waste containers to identify specific hazards, and training.

III. Specific labels and/or signage should include the hazards and the person to contact in case of a spill during the disposal process. Various waste-disposal methods include incineration, dilution and disposal into the sewer systems, use of landfills or disposal sites, recycling and chemical or biological degradation in disinfection or autoclaving. Neutralization or chemical deactivation is also a disposal method where one chemical added to another chemical can deactivate its potential hazard. All of these methods have their place within the BPC and BCR waste disposal system and the laboratory should develop a specific policy for disposal of each individual hazardous waste stream. Where possible, the laboratory should attempt to substitute less hazardous materials for use in procedures. Of particular interest is the handling of patient fluids in the laboratory. Current recommendations suggest that blood and blood products should be considered a hazardous waste and they should be treated before disposal to eliminate any viral or bacterial

hazard. Those materials normally excreted by patients can be disposed of into the sewer system. Refer to the "SAF-013 Biohazard and Chemical Waste Disposal" SOP.

IV. Reduction of hazardous waste: The Research Institute at Nationwide Children's Hospital will take steps to continually reduce waste. Refer to the Research Institute Chemical Hygiene Plan for details. The BPC and BCR will participate in initiatives set forth by the Research Institute and comply with all mandated action items.

V. In brief:

A. All potentially infectious waste is handled as RED-BAGGED trash and disposed of as per hospital regulations. If in doubt as to what should be considered red bagged-trash, consult the Research Biosafety Officer or the Research Facility Manager.

B. Hazardous chemicals are disposed of through the regularly scheduled waste disposal days coordinated by Safety and Security through the Research Institute pick up site (Research Wexner 1 W124). If in doubt as to what is considered a hazardous chemical, consult the Research Biosafety Officer or the Research Facility Manager.

VI. References

1. BPC Safety Manual

Biopathology Center
The Research Institute at
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700 Children's Drive, WA1300
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Laboratory Exposure Control Plan

Policy Number: SAF-010		
Supersedes Procedure Number: BPC Safety Manual		
Date Adopted: 9/18/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
	7/16/2013	Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The Laboratory Exposure Plan was enacted protect all laboratory employees from occupational exposure to blood-borne pathogens including, but not limited to, HBV, HCV, and HIV, and to ensure the employer provides a safe working environment for every employee in the Biopathology Center (BPC) and Biospecimen Core Resource (BCR). This plan is designed to maintain compliance with all governing bodies, meet hospital regulations, and provide instructions for reducing the likelihood of exposure to biological substances that may contain harmful pathogens and increasing the safety of the workplace for all employees.

I. **Laboratory Exposure Control Plan:** This plan is designed to meet Occupational Safety and Health Administration (OSHA) and hospital regulations for workplace standards to reduce the likelihood of transmission of blood-borne pathogens. Refer to "Nationwide Children's Hospital Exposure Control Plan for OSHA's Bloodborne Pathogen Standard". It is designed for use as a supplement to the hospitals Infection Control Manual and Exposure Control Plan and in addition to BPC and BCR specific plans.
   - A. **Employee Rights and Responsibilities:** Every employee must adhere to the policies stated in the Hospital Exposure Plan, BPC/BCR Laboratory Exposure Plan and each individual section plan. As an employee you have the right to a safe workplace and the freedom to file complaints without fear of punishment. You also have the responsibility to learn and adhere to all the provisions of exposure plans.
   - B. **Personnel Risk for Exposure**: Personnel risk is defined as one of the following;
     1. High Risk: Central receiving staff, processing staff, distribution staff and managers when performing technical duties in the lab.
     2. Moderate Risk: Clerks, IT staff, commercial representatives (sales or repair), biomedical engineering and environmental service personnel.
     3. Low Risk: Customer Service Representatives, Administrative staff.
   - C. **General Methods for Compliance:** The following guidelines will be followed in order for the BPC and BCR to be compliant.
     1. Universal Precautions will be followed, Personal Protective Equipment (PPE) will be used and made readily available (impermeable gowns, gloves, shields and safety glasses).

2. Engineering controls will be provided and maintained, i.e. eyewash stations, ventilation, hand washing facilities.
3. Every employee will follow proper work practice controls, i.e. hand washing, proper sharps handling and disposal, proper biohazard labeling, decontamination of equipment and workbench surfaces.
4. Hepatitis B vaccination or declination, including documentation.
5. Post exposure incident evaluation and follow up.
6. Employee training sessions and annual hospital in-service/Blood-borne Standard training.
7. Enforcement: Each manager will be required to enforce documented policies. Incident reports should be used and employees reminded that continued violation of safety rules will lead to disciplinary actions. Employee annual evaluations will include adherence to safety regulations.

D. General Procedures within the BPC and BCR:

1. All lab coats for staff will be the impermeable type regardless of task assignment. Hand washing is mandatory after removing PPE and/or before leaving any laboratory area, except a clean area. All gloves will be of non-latex construction.
2. Handling of body fluid (including urine) designated as having a possibility of blood contamination when there is risk of a splash requires impermeable lab coat, safety glasses, and gloves.

E. Manager's Responsibilities:

1. Provide PPE, label storage areas and always make supplies readily available.
2. BPC: Ensure employees are trained and maintain applicable documentation using the appropriate competency forms (See "PER-004 Standard Operating Procedure Training") and CHEX tool. Confirm at the time of the employee's annual performance review that the annual CHEX requirement has been completed and keep a copy of the CHEX completion certificate and any competency forms in the personnel file.
3. BCR: Ensure employees are trained and maintain applicable documentation using the *BCR New Employee General Orientation and Safety Checklist* and CHEX tool. The checklist documents administrative and sectional training for bloodborne pathogen and exposure control safety.
4. Ensure that proper work practice controls are followed in every area. Remember that these controls depend on the behavior of the employee.
5. Specify computer terminals and telephones as to their degree of hazard and appropriate precautions to be taken.
6. Specify any atypical circumstances in your section that may require protection.

II. References

A. Nationwide Children's Hospital Exposure Control Plan for OSHA's Bloodborne Pathogen Standard

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Microbiological Hazards and Safety

Policy Number: SAF-011		
Supersedes Procedure Number: BPC Safety Manual		
Date Adopted: 9/17/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
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Principle: The purpose of this document is to highlight policies and procedures implemented by the Biopathology Center (BPC) and Biospecimen Core Resource (BCR) in relationship to identifying potential microbiological hazards, implementing and utilizing safe practices, and education on such hazards and safety.

I. **Introduction:** For practical purposes, microbiological hazards in the laboratory may be divided into three categories:
   - **A.** Those specimens and procedures that expose the employee to acquiring viral hepatitis (specifically hepatitis B and C) and acquired immunodeficiency syndrome (AIDS). Such hazards are ubiquitous throughout the laboratory.
   - **B.** Those pathogens that are most commonly examined for within the microbiologic laboratory. Such hazards include exposure to bacteria, mycobacteria and fungi.
   - **C.** Those specimens and procedures involving the highly virulent agents, such as viral hemorrhagic fevers and rickettsia.
   - **D.** Because medical history and examination cannot reliably identify all patients infected with pathogens, Universal Precautions must be exercised when the contacting or handling of solid tissues or fluids of any person is anticipated. These precautions presume that all such materials are likely to transmit blood-borne pathogens. The Centers for Disease Control and Prevention (CDC)-recommended guidelines for the protection of the laboratory worker appear in the Morbidity and Mortality Weekly Report (MMWR), Volume 36, August 1987 and MMWR update, Volume 37, No.24, June 1988.
   - **E.** In the event that an employee cuts/or is exposed to a biospecimen the submitting institution will be contacted and information will be requested on whether the patient has an infectious disease. In some situations this data may not be available. Any information obtained will be forwarded to Employee Health.

II. **Routes of Infection:** Infections may occur by the following routes:
   - **A.** Aerosols of infectious solutions can be formed when removing the stoppers or plugs from sample tubes, when dropping solutions onto hard surfaces, by centrifuging unstoppered tubes and by heating liquids too rapidly.

B. Ingestion of microbiological organisms due to the failure of hand washing thoroughly before eating or drinking can result in hand-to-mouth spread of infection.

C. Direct inoculation from an accidental needle stick is an obvious route of infection. Broken glass or animal bites can also produce direct inoculation. Small scratches or paper cuts on the fingers and broken cuticles can be easily contaminated by specimens.

D. Certain organisms, including the hepatitis B and C (HBV, HBC) and human immunodeficiency virus (HIV), can enter the human body through direct contact with mucous membranes, e.g. ocular conjunctivae. Hands must be thoroughly washed before leaving the laboratory areas, rubbing the eyes, changing contact lenses or applying cosmetics.

E. Mosquitoes, ticks, fleas and other ectoparasites are potential sources of infection via bites, especially if an animal colony is in the building.

III. Blood-borne Pathogens Potential Exposure**:** Potential exposure to blood-borne pathogens is the most common microbiologic hazard facing laboratory workers. Refer to "SAF-010 Laboratory Exposure Plan" SOP and the hospital's "IC-VII-1 Exposure Control Plan for OSHA Bloodborne Pathogen Standard". In this regard, Occupational Safety and Health Administration (OSHA) has enacted the Blood-borne Pathogens Standard (BPS), codified as 29CFR§1910.1030 (57 FR 29206, July 1, 1992). Its purpose is "to reduce occupational exposure to HBV, HCV, HIV and other blood-borne pathogens." The BPS requires each laboratory to develop an Exposure Control Plan that includes the following elements:

A. Purpose of the plan

B. General program management (responsible persons, availability to employee, annual review and update)

C. Exposure risk determinations

D. Methods of compliance (engineering and work-practice controls, PPE and housekeeping)

E. Hepatitis B vaccination program

F. Post exposure evaluation and follow-up, involvement of a health care professional and the professional's written opinion and medical record keeping.

G. Labels and signs

H. Training methods and record keeping (topics)

I. Copy of BPS (29CFR§1910.1030 (57 FR 29206, July 1, 1992)

IV. General Comments: All BPC and BCR employees must follow the practice of "universal precautions." In addition, the laboratory area should identify potential exposure situations for each job category employee. Once a potential source of exposure is identified, engineering and work-practice controls should be implemented to lessen or remove the potential exposure. The laboratory provides appropriate PPE, such as gloves, laboratory coats, face shields, masks and safety glasses. Employees are instructed when and how to use this equipment.

V. Universal Precautions

A. Blood and other body fluids from all patients are considered infectious. All specimens of blood and body fluids are placed in a well-constructed container with a secure lid to prevent leakage during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container or the laboratory form accompanying the specimen.

B. All persons processing blood and body fluid specimens (e.g. removing tops from vacuum tubes) should wear gloves, lab coats and safety glasses. Facial barrier protection or Plexiglas shields should be used if splashes or sprays of blood or body fluids can occur.

C. Mechanical pipetting devices must be used for manipulating all liquids in the laboratory. Mouth pipetting is strictly prohibited.

D. To prevent injuries, all sharp objects should be used with caution. Needles must not be recapped, purposely bent, broken by hand, removed from a disposable syringe or otherwise manipulated. After they are used, all sharps are to be placed in a clearly labeled, puncture resistant container for transport to disposal sites. Sharps containers are placed close to the work site. To prevent overfilling and resultant accidental skin punctures, sharps containers are removed when they are 2/3 full. Do not overfill. It is the responsibility of all laboratory personnel to replace full sharps containers.

E. Laboratory work surfaces should be decontaminated with appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed**.** Disinfect surfaces according to "LAB-027 Laboratory Cleaning" SOP and hospital's policy "IC-VII-1 Exposure Control Plan for OSHA Bloodborne Pathogen Standard".

F. Contaminated materials used in laboratory tests should be decontaminated before reprocessing, or placed in bags and disposed of in accordance with institutional policies for disposal of infective waste (red-bagged trash). Refer to "SAF-013 Biohazard and Chemical Waste Disposal" SOP.

G. Scientific equipment that has been contaminated with blood or other body fluids should be decontaminated and cleaned before repaired in the laboratory or transported to the manufacturer. Equipment that cannot be completely decontaminated must be labeled with the biohazard insignia.

H. Hands and other skin surfaces must be washed immediately and thoroughly if contact with blood or other body fluids occurs. Hands must be washed immediately after gloves are removed and after completing laboratory activities. All PPE should be removed before leaving the laboratory.

I. Gloves must be worn when it can be reasonably anticipated that the healthcare worker will have contact with blood, or other potentially infectious materials, mucous membranes, non-intact skin or when handling contaminated items or surfaces. Gloves should be replaced as soon as feasible if they become torn, punctured or lose their ability to function as a barrier. Disposable gloves should not be washed or decontaminated for re-use because this will compromise the impermeability of the material. All gloves used in the BPC and BCR are non-latex**.**

J. Work areas should be covered with absorbent paper when appropriate. Aerosols must be prevented during pipetting, mixing, shaking, vortexing and centrifuging operations.

VI. Ebola:

A. Biological materials with known Ebola contamination will be handled by the BPC per guidance from the Centers for Disease Control, Institutional policies and the College of American Pathologists in addition to the standard implementation of universal precautions.

VII. Hepatitis B (HBV), Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV) Exposure Follow Up:

A. The hospital offers hepatitis B vaccination at no cost to all employees. An employee may decline vaccination, but must sign an appropriate form expressing this declination, which will be kept on file with Employee Health Services. Refer to the hospital policy "IC-VII-1 Exposure Control Plan for OSHA Bloodborne Pathogen Standard".

B. The hospital has a policy for follow-up of any incident of exposure to blood-borne pathogens. See the hospital Infection Control Manual. Follow-up includes accident investigation, employee treatment and medical consultation and corrective actions to prevent similar incidents. Refer to the hospital policy "IC-IV-20 Employee Exposures to Patients' Blood or Body Fluids".

VIII. Tuberculosis (TB) Exposure and Infection Control Plan: The hospital mandates that only those staff having direct patient contact or those working in patient care areas including volunteers will need to have an annual TB skin test employees are tested for TB annually. BPC and BCR Employees are exempt from annual TB skin tests unless otherwise notified based upon the hospital's requirement criteria. Refer to the hospital's policy "IC-IX-1 Tuberculosis Exposure Control Plan".

A. Employees undergo annual mandatory education (CHEX) regarding TB including dangers, symptoms, exposure control, and general information such that employees have adequate knowledge and can safely protect themselves and others.

B. The BPC employees utilize Universal Precautions to prevent exposure to aerosols, including the utilization of face shields, tissue processing hoods, biological safety cabinet sashes, fume hood doors, and centrifuge bucket lids (when applicable) when there is potential for TB infected aerosols. These protections methods are in addition to the Personal Protective Equipment required when handling specimens, or when it can be reasonably anticipated that contact with potentially infectious substances, including nitrile gloves, impermeable laboratory coat, and eyewear protection.

1. Centrifuge bucket lids are utilized when bronchial alveolar lavage, sputum, or tracheal aspirate samples are being centrifuged to reduce the potential for contact with harmful aerosols.

IX. Labels and Signs: To clearly identify items containing or contaminated by biohazardous materials, a comprehensive warning label program is in place. Labels exhibiting the universal biohazard insignia (see

below) are placed on containers of regulated waste, refrigerators containing blood or other potentially infectious materials and sharps disposal containers. The containers for storage, transport or shipping are labeled or color-coded (red-bagged) and closed before being stored, transported or shipped. The BPC, BCR and the hospital uses universal precautions in the handling of all specimens.

THE UNIVERSAL BIOHAZARD INSIGNIA

X. References

1. N/A

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Biohazard and Chemical Waste Disposal

Procedure Number: SAF-013		
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Date Adopted: 9/17/2012	**Prepared By:** Mary McNulty, Research Assistant; Molly Myers, Research Associate	
	Reviewed By: Erin Pumplun, BPC Program Manager	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
10/30/2012		Nilsa C. Ramirez M.D., BPC Director (signature on file)
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Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The purpose of this procedure is to provide instructions for the proper disposal of biohazardous waste in the Biopathology Center (BPC) and Biospecimen Core Resource (BCR). The BPC and BCR have the responsibility to ensure that appropriate hazardous waste handling policies are rigorously followed and compliant. Compliance with this procedure will reduce potential risk to personnel that handle hazardous waste in the laboratory and those involved in disposing of the waste.

I. Equipment

- **A.** Red biohazard trash bin
- **B.** Gray biohazard trash bin
- **C.** Red biohazard trash bags
- **D.** Sharps container
- **E.** 2000 mL Erlenmeyer Flask
- **F.** Fume Hood
- **G.** "Dirty" Sink

II. Materials

- **A.** Bleach
- **B.** Water
- **C.** Alcohol
- **D.** Neutralizer
- **E.** Packaging Tape

III. Warnings/Precautions

A. Biohazard: a biological agent/substance that can pose potential risk, harm, or danger to living organisms if exposure occurs.

B. Employ universal precaution throughout all steps of this process. Refer to "SAF-011 Microbiological Hazards and Safety" for a definition.

C. Wear Personal Protective Equipment (PPE), including gloves, lab coats, and eye protection while handling of biohazard and/or chemical waste materials.

D. Biohazard waste can be harmful to the body. Report any biohazard exposures to BPC and BCR Management and Employee Health Services immediately.

E. Disposal of biohazard waste must be compliant with local, state, and federal regulations. Local, state, and federal authorities strictly regulate the disposal of biohazard waste and effluents into the environment. Individual variations from jurisdiction to jurisdiction require laboratory review of the regulations applicable to its locale (includes cataloging/inventory of the waste generated and the time hazardous waste is stored on-site prior to disposal). Special storage permits may be required depending on the amount of waste generated. Special attention should also be given to the packaging and manifesting requirements of the facility that will provide ultimate disposal services for the hazardous waste. By EPA regulations, the laboratory retains the responsibility for the chemical hazardous waste regardless of the disposal agency methods. The Research Institute Facilities Manager and the Research Institute Biosafety Officer can provide documentation of proper handling and disposal of chemical and biohazardous waste, upon request.

F. Where possible, the laboratory should attempt to substitute less hazardous materials for use in procedures.

G. Always dispose of biohazard waste materials according to the specific criteria listed in the NCH CHEX training, CITI Special Laboratory Hazards, and CITI Basic Biosafety Training. Refer to the "PER-002 Training Certifications" SOP for instructions to complete this training.

H. It is highly recommended to treat patient fluids (such as blood/bone marrow and derivatives) prior to disposal to eliminate viral/bacterial hazard.

I. Hands must be washed frequently when handling potentially hazardous materials. See "SAF-004 General Requirements for Personal and Lab Safety" for details.

J. MSDS can be found on Research Institute Safety Page through MSDSonline. Copies of MSDS may be on each department's SharePoint and/or hard copies are kept in each lab.

IV. Quality Control

A. Quality control will be completed on this procedure through annual updates of the procedure manual or earlier if deemed necessary due to changes in techniques.

B. The laboratory trainer will evaluate the employee's ability to perform this procedure.

C. Specimens and specimen containers undergo visual quality assurance during all steps of the handling process to ensure there are no characteristics outside normal expectations. Examples include change in specimen color, change in specimen consistency, and/or broken storage container.

V. Procedure

A. Handle all potentially infectious/hazardous waste as red-bagged trash and dispose according to Nationwide Children's Hospital policies.

1. Refer to the Research Institute's policy regarding biohazard waste disposal: Research-(Safety)-001-01.
2. Contact the Research Facilities Manager and the Research Institute Biosafety Officer if you have any questions regarding NCH biohazard waste policies and procedures.

B. Biohazard disposal at the Kinnear Road location:

1. Dispose all non-fluid patient specimens (tissue) in the red biohazard bins located in the laboratory.

2. Dispose all non-sharp materials that come in contact with any biohazard materials/patient specimens (such as packaging materials, foil, gauze, petri-dishes, gloves, etc.) in the red biohazard bins located in the laboratory. Bins must be double-bagged.
3. Dispose all sharps materials that come in contact with any biohazard materials/patient specimens (such as pipette tips and blades) in the red sharps containers located at the laboratory stations.
   a. Do not fill sharps containers more than ¾ full or above the fill line.
   b. Make sure the lid on the sharps container is secure.
   c. Close the sliding portion on the lid until it snaps shut.
   d. Place packing tape over the lid on the sharps containers to ensure the lid is properly secured.
4. Biohazard waste is picked up the first Tuesday of the month.
   a. An employee of Stericycle who has arrived to pick up the biohazard trash calls the lab from the dock.
   b. Red-bag trash and sharps containers are combined into one large red bag.
   c. The combined items are placed in a large gray biohazard bin.
   d. Complete the Regulated Medical Waste sticker (UN 3291) and place on the side of the gray bin.
   e. Transport the filled gray bin to the dock and exchange for an empty gray bin.
5. Red bags are double-bagged and replaced monthly in the red bag trash cans

C. Biohazard disposal at Nationwide Children's Hospital (NCH) WA1340/WA1342 location:

1. Dispose all non-fluid patient specimens (tissue) in a jar containing formalin in the fume hood labeled "Remnant Tissue to be Discarded".
   i. Once per month, use forceps to remove fixed tissue and discard in the biohazard trash.
   ii. Dispose of the used formalin per steps V.E.3.
2. Dispose all non-sharp materials that come in contact with any biohazard materials/patient specimens (such as packaging materials, foil, gauze, petri-dishes, gloves, etc.) in the red biohazard bins located in the BPC laboratory. Bins must be double-bagged (done by Environmental Services).
3. Dispose all sharps materials that come in contact with any biohazard materials/patient specimens (such as pipette tips and blades) in the red sharps containers located at the laboratory stations
   a. Fill sharps containers no more than 3/4 full, or to the container's fill line (if one is available).
   b. Make sure the lid on the sharps container is secure.
   c. Close the sliding portion on the lid until it snaps shut.
   d. Place packing tape over the lid on the sharps containers to ensure the lid is properly secure.
   e. Place the sharps container on top of the large biohazard bin in the BPC laboratory, WA1340, for Environmental Services pickup.
4. NCH Environmental Services picks up biohazard trash up to twice a week.
   a. Do not overfill biohazard red bins.
   b. Contact/page environmental services staff member to change the biohazard trash if needed to prevent overfilling.
5. Dispose patient fluids accordingly:
   a. Dispose any fluids normally excreted by a patient (ex. sweat, urine) directly in the sink (into the sewer system) and flush with water.
   b. Dispose any formalin liquid in contact with patient specimens in the formalin waste container located in the biological fume hood.
      i. Label the container as described is step V.E.3.a., below.
      ii. Formalin will be disposed of via the Hazardous Waste procedure established by Research Safety. Refer to steps V.E.3, below.
   c. Dispose any patient liquids not normally excreted by a patient (such as blood/bone marrow and their derivatives, supernatant from aspirate or lavage processing) in an Erlenmeyer flask containing the recommended amount of bleach to create a 10% bleach solution.
      i. Determine the maximum amount of solution that can fit into the flask.
      ii. Calculate 10% of that amount.

- Ex. 10% of a 2000mL flask would be 200mL
- Ex. 10% of a 500mL flask would be 50mL

iii. Add the calculated amount of bleach to the flask and place in the fume hood designated for the laboratory for which you are in.

iv. Immediately label the flask "Biological waste in 10% bleach" and indicate the date the container was prepared.

- Ex. "Biological waste+200mL Bleach" for a 2000mL flask
- Ex. "Biological waste+50mL Bleach" for a 500mL flask

v. Pour the biological waste into the labeled Erlenmeyer flask.

- The flask may be filled up to the last marking.

vi. Allow the patient liquid specimens to sit in the bleach/water solution for a minimum of 24 hours.

vii. Dispose the solution down a designated "dirty" laboratory sink and flush with water.

D. Biohazard trash disposal at Nationwide Children's Hospital (NCH) W344, W233/W234, W256/W253, and W292/W293 locations:

1. Dispose all non-fluid patient specimens (tissue) in the biohazard trash to be autoclaved. Refer to the SOP, "SAF-020 Autoclaving Biohazard Trash".

2. Dispose all non-sharp materials that come in contact with any biohazard materials/patient specimens (such as packaging materials, foil, gauze, petri-dishes, gloves, etc.) in the red biohazard bins located in the laboratory.

3. Dispose all sharps materials that come in contact with any biohazard materials/patient specimens (such as pipette tips and blades) in the red sharps containers located at the laboratory stations.

a. Fill sharps containers no more than 3/4 full, or to the container's fill line (if one is available).

b. Make sure the lid on the sharps container is secure.

c. Close the sliding portion on the lid until it snaps shut.

d. Place packing tape over the lid on the sharps containers to ensure the lid is properly secured.

e. Place the sharps container on top of the large biohazard bin the BPC laboratory, WA1340, for Environmental Services pickup.

4. When biohazard bags are 3/4 full, they must be loosely tied off with autoclaving tape and autoclaved.

a. Refer to "SAF-020 Autoclaving Biohazard Trash" for further instruction.

5. Dispose patient fluids accordingly:

a. Dispose any fluids normally excreted by a patient (ex. sweat, urine) directly in the sink (into the sewer system) and flush with water.

b. Dispose any patient liquids not normally excreted by a patient (such as blood/bone marrow and their derivatives) in an Erlenmeyer flask containing the recommended amount of bleach to create a 10% bleach solution. Refer to steps listed under V.C.5.c.

E. Proper Disposal of Hazardous Reagents/Chemicals:

a. Refer to Material Safety Data Sheets (MSDS) for information on proper disposal of the desired reagent.

i. If the method of disposal is unclear, refer to other sections of the MSDS that describe the risks associated with this reagent to determine the best route of disposal.

ii. If still uncertain, contact the manufacturer of the reagent for their recommended disposal method(s).

iii. An MSDS sheet can also be requested at this time if one was not previously available. Please document any conversation with the manufacturer for future reference.

b. Contact the Research Biosafety Officer with any extraneous questions.

2. Disposal in "dirty" sink:

a. "Dirty" Sinks are located in the fume hoods. If an additional "dirty" sink is available outside of the fume hoods, it is labeled as such.

- **b.** Reagents able to be disposed in this manner include reagents of low environmental and health risks, as well as diluted acids.
- **c.** Examples of low-risk reagents include, L-glutamine, Pen-strep, and 0.9% NaCl (injection, USP)
- **d.** Wear appropriate Personal Protective Equipment (PPE).
- **e.** Pour liquid reagents down dirty sink, located in the fume hood, with copious amounts of water.
- **f.** If plastic bottle is recyclable, rinse the bottle, dispose of the cap, and place the bottle in the appropriate recycle bin.
- **g.** If plastic bottle is not recyclable, thoroughly rinse and discard.

3. Disposal of Hazardous reagents (General):

- **a.** Properly label the container of chemical waste to be disposed of with:
  - **i.** "Hazardous Waste"
  - **ii.** The Principal Investigator's name
  - **iii.** Room Number
  - **iv.** Date pick-up was requested
- **b.** Notify the Research Biosafety Officer that waste is ready for pick-up by completing the form at http://rex/ResearchSafety. Once the webpage is open, select the link, "Hazardous Chemical Waste Pick Up Request".
- **c.** Pick-up will occur every other Tuesday. Ensure chemical waste for pick-up is properly stored in the meantime (ex. flammable waste should be stored in a flammables cabinet).

VI. References

A. N/A

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Warning Signs and Labels

Procedure Number: SAF-014		
Supersedes Procedure Number: BPC Safety Manual		
Date Adopted: 9/18/2012	**Prepared By:** Lauren Noyes, Biospecimen Core Coordinator	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
4/29/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: Warning signs and labels provide a uniform policy for indicating the presence of certain hazards within the Biopathology Center (BPC) and Biospecimen Core Resource (BCR); they should be used constantly.

I. **Area Designation and Posting:** Laboratory area designations vary depending on risk as per Material Safety Data Sheets (MSDS) protocols and are determined on the basis of the relative risk associated with the hazards present. The importance of microbiological, chemical, and radiation hazards is considered in assessing relative risk present in a given area. Generally, risk designations are permanent unless the work activity or location changes.

   The BPC and BCR laboratories in which human samples are handled are designated as Biosafety Level 2 (BSL-2) laboratory spaces (refer to The Research Institute Biosafety Manual). As such, a sign that includes the biohazard insignia, the laboratory's biosafety level, "Human Source Material", laboratory contacts, emergency telephone number, and required procedures for entering and exiting the laboratory must be posted on BSL-2 laboratory doors.

II. **Hazardous Chemical Labels:** Containers of hazardous chemicals are labeled, tagged, or marked with the following information:
   - **A.** The identity or contents of the container, the chemical name or scientific designation of any element, chemical compound or mixture of elements and/or compounds.
   - **B.** Appropriate hazard warnings, including any words, pictures, symbols or combinations that convey the health and/or physical hazards of the container's contents. Hazard warnings are specific as to the effect of the chemical and specific target organs involved.
   - **C.** Refer to the "SAF-003 Chemical Hygiene" SOP.

III. **Storage Container Labels:** Laboratories are also required to ensure that containers of hazardous chemicals in use or storage are labeled with the identity or contents of the container. Appropriate hazard warnings are required for manufacturers; regular inventories can reveal containers purchased before manufacturers were required to use adequate precautionary labeling.

IV. **Secondary Container Labels:** Any secondary container into which hazardous chemicals are transferred from labeled containers must be labeled with the chemical identity of the contents and any precautionary handling hazards, including specific effects of the chemical and target organs affected. The only permissible exceptions to this requirement are containers intended for immediate use only by a person who does the transfer; in this case, the container should remain in the custody of the person who does the transfer.
   A. Aliquots of chemicals used for specimen processing, as listed in this procedure, must each be labeled with the following information
      1. Chemical Name
      2. Date Aliquoted
      3. Expiration Date
      4. Lot Number
      5. Aliquot volume
      6. NFPA rating and/or GHS rating – this label may be adhered to the container in which the aliquots from a given chemical are held.

V. **Existing Labels:** Existing labels on containers carrying hazardous chemicals should not be removed unless the container is immediately marked with the required re-labeling information.

VI. **Label Requirements:** When required, containers are also labeled with date of receipt, date of preparation and/or date opened and date of expiration. BPC: Refer to the "LAB-038 Receiving, Stocking, and Stocking Supplies" SOP. BCR: Refer to "A019 Ordering, Receiving, and Stocking Supplies."

VII. **Flammable Liquid Sign:** Refrigerators and freezers approved for storage of flammable liquids are identified as such with a sign or label. Other refrigerators are labeled as "not for use for storage of flammable liquids" and "not for storage of food and drink".

VIII. **General Information:** General information signs are posted as needed.

IX. **References**
   A. N/A

Biopathology Center
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Disaster and Evacuation Plan

Procedure Number: SAF-015		
Supersedes Procedure Number: N/A		
Date Adopted: 9/17/2012	**Prepared By:** Teresa Tabler, Senior Research Associate	
	Reviewed By: Lauren Noyes, Biospecimen Core Coordinator	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
7/22/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
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Principle: The purpose of this procedure is to outline the Biopathology Center's (BPC)/Biospecimen Core Resource's (BCR) response plan to emergencies determined by Hospital and/or Research Administration.

I. Policy:

A. The hospital-wide emergency preparedness (EP) plan is located in the office area (WA1300) of the BPC, in the office area (WA1100A) in the BCR, in the office area of 1305 Kinnear Road, Suite 140, and on the Nationwide Children's Hospital Intranet.

B. BPC and BCR disaster call trees and personnel call lists shall be maintained and updated as personnel changes occur. The BPC call tree is provided to each employee and is available on the Emergency call tree located on BPC SharePoint > Biospecimen Core > BPC Documents-Forms > Safety > Safety Contact Information. The BCR call tree is available on the BCR SharePoint > Project and QMS > Safety. Call trees are also located in the Emergency Preparedness binders for each area (BPC, BCR, Kinnear). An emergency call list, along with a work unit phone list, will also be kept on a bulletin board or wall near the doors of each main office area (WA1100A, WA1201 and WA1300).

C. Emergencies will be announced over the public address system, through the Children's Hospital Alert System (CHAS), and via e-mail. Drills will be noted as such. For specific instructions for each of the disasters below, refer to the hospital wide EP Manual.

1. Disasters may also be categorized by NCH using following codes:

- **a.** Code Adam = infant/child abduction
- **b.** Code Black = bomb threat
- **c.** Code Gray = severe weather
- **d.** Code Red = fire
- **e.** Code Orange = hazardous material spill/release
- **f.** Code Blue = medical emergency – pediatric
- **g.** Code Yellow = disaster
  - Disaster plan will be activated
- **h.** Code Violet = Violent or combative person

i. Code Silver = Person with a weapon or hostage situation

II. Responsibilities:

A. It is the responsibility of all BPC and BCR employees to be familiar with this policy and the included special instructions. It is the responsibility of BPC and BCR management to enforce this policy and ensure employees are adequately trained.

III. Special Instructions:

A. Communicating emergencies/disasters:

1. An e-mail (if computers are functioning) will be sent with the nature of the emergency and any other pertinent information. This is the main line of communication as it reaches people in the quickest timeframe.

2. The BPC Director and BCR Principal Investigator will activate the emergency call tree when deemed necessary and relay all needed information.

a. The emergency call tree will be updated with the departure of current staff and/or hiring of new staff.

B. Evacuation:

1. Evacuations should be performed horizontally, first, to the closest point of safety.

a. Emergency lighting is adequate for a safe evacuation.

2. If your area needs to evacuate, meet in the staff parking garage (N-Garage). Always follow direction from fire/safety personnel during an evacuation or any emergency situation.

i. Enter the garage through the person door (general badge access is granted) or through the car garage doors.

- The car garage doors will be opened by security in the event an evacuation is required.

ii. Travel down the down-ramp and meet at the blue light where the ramp dead-ends into the chain-link fence.

a. The Kinnear Facility will evacuate to the front of the SciTech building by the SciTech sign.

3. Total building evacuation is only performed at the direction of the BPC and BCR Management, Columbus Fire Department Scene Commander, and/or NCH security.

a. Carefully follow any instructions provided by the aforementioned individuals.

4. Immediately notify all affected areas of the need to evacuate.

5. Take the EP Manual during evacuation.

a. This should include a list of all chemicals stored within the area and a list of all personnel's phone numbers. It is located in the office area of the BPC (WA1300), office area of the BCR (WA1100A) and the office area of 1305 Kinnear Road, Suite 140.

b. Take personal effects only if time permits.

c. Evacuation routes are posted in all lab and clerical areas. They are also located on BPC SharePoint > Biospecimen Core > BPC Documents – Forms > Safety and BCR SharePoint > Project and QMS > Safety

d. For all evacuations, employees are to assist those with apparent or known disabilities to safety. See section III.C for further details.

6. Each area (lab or clerical area) will have a designated "responsible person" (may be manager or designee) that accounts for all personnel on duty and reports back to BPC and BCR management.

a. All staff will be notified of the responsible person for each area.

C. Evacuations of persons with disabilities:

1. Persons using crutches/canes/walkers:

a. Have the individual sit on a sturdy chair, preferably with arms, and follow the procedure for non-ambulatory persons, below.

2. Non-Ambulatory persons (wheelchairs):
    a. Evacuation may not be needed or advisable. Most stairwells are designed to provide temporary protection from fire or other danger. An able-bodied volunteer should stay with the non-ambulatory person in the platform area of the stair well while a second person notifies emergency personnel of the exact location of the wheelchair user.
        i. If immediate evacuation is necessary, consider the following:
            - Wheelchairs have movable parts and may not be designed to withstand stress or lifting.
            - You may need to remove the batteries attached to life-support equipment.
            - In a life-threatening emergency, it may be necessary to remove the person from their wheelchair.
            - Wheelchairs should be used to descend stairwells, if at all possible.
            - Check evacuation routes for obstructions before assisting the person to the exit.
            - Delegate other volunteers to bring the wheelchair
                1. Reunite the person with wheelchair as soon as it is safe to retrieve.
            - Always consult with the non-ambulatory person on the best way to assist them.
3. Visually-impaired persons
    a. Act as a sighted guide, offer elbow, and escort them to safety. As you walk describe where you are and communicate all obstacles. Inform the person of the location once a safe place is reached.
4. Hearing impaired persons
    a. Write a note describing the emergency and nearest evacuation route.
    b. Turn the light switch off and on to gain attention. Indicate with gestures what is happening and what to do.

D. Visitors
1. All visitors will be required to sign in with the Research receptionist (in the first floor lobby area).
    a. Upon sign-in, visitors will have access to the evacuation route of the BPC and BCR and meeting location.
2. Visitors will be accounted for if evacuation is needed.

E. Specimens
1. During the event of a disaster (internal or external), the rescue of specimens to prevent loss will be completed as possible (with personnel safety at the forefront of all decisions).
    a. The discretion and order in which specimens are rescued will be decided by the BPC Director, depending on the type and effects of the emergency situation as it is presented.
    b. Any rescue of specimens will follow approval/direction from emergency personnel as well as NCH and Research Institute Management.
2. Research Institute Safety Personnel will employ the NCH Risk Assessment Team (RAT) to the BPC and BCR if necessary.
    a. The RAT will help determine the scope of response needed.
        i. Any outside assistance deemed necessary will be determined and gathered. This could include any of the following (not meant to be a complete list):
            - Sources to help move specimens
            - Contacting other facilities to house specimens temporarily
            - Refrigeration/Freezer supplies

F. Code Silver
1. Announced for any area in research/near a BPC or BCR space

a. If possible, employees should barricade themselves into rooms that can lock and that have doors without windows.
   i. BPC
      - WA1301
      - WA1302
      - WA1310
      - WA1320
      - WA1341
   ii. NCH Projects
      - W345
      - W351
   iii. Kinnear Road
      - Conference Room
   iv. BPC Informatics
      - W103A
   v. BCR
      - WA1101
      - WA1102
      - WA1103
      - WA1104
      - WA1105
      - WA1106
      - WA1107
      - WA1108
   vi. BCR Logistics
      - W234
   vii. Morphology Core
      - W279A
      - W285

b. If possible, move heavy/large items (bookcases, desks) in front of the locked door.
c. Silence all cell phones.
d. Close blinds to outside windows.
e. Get onto the floor and stay low.
f. Remain calm and quiet.
g. Fight back against intruder as a last resort.
   i. Throw heavy items
   ii. Take any action necessary to protect yourself

G. Tornado
1. Will be announced as a Code Gray – Tornado.
2. The Code Gray – Tornado portion of the NCH Emergency Preparedness Manual must be followed.
3. All Employees should take shelter in a secure area indoors away from any glass.
   a. All BCP employees in Wexner and Wexner II and BCR employees on upper floors of Wexner II should go to the back hallway on the first floor between Wexner and Wexner II.
   b. BCR Employees in WA1100A and WA1201 should remain in the office area with all doors closed.
   c. Employees at the Kinnear Road facility should go to the conference room and close the door.

IV. References

1. ADMIN-DIS-1.6 Laboratory Emergency Preparedness

2. Nationwide Children's Emergency Preparedness Manual

Biopathology Center
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Electrical Power Outage

Procedure Number: SAF-016		
Supersedes Procedure Number: N/A		
Date Adopted: 9/10/2012	**Prepared By:** Teresa Tabler, Senior Research Associate	
	Reviewed By: Teresa Tabler, Senior Research Associate	
Date Approved: 9/5/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
	7/16/2013	Nilsa C. Ramirez M.D., BPC Director (signature on file)
Electronic signature captured in Q-Pulse (effective 11/18/2013)		

Principle: The Research Institute at Nationwide Children's Hospital will maintain an emergency power back-up system in the event of a power outage.

I. Scope and Purpose:

A. To provide an adequate level of emergency power to support refrigerators, freezers, incubators, etc. to ensure preservation of specimens.

II. Safety Precautions:

A. In the event of a generator failure, the Hospital Engineering Services Department will bring a rental generator on a flatbed. The primary fuel oil/diesel vendor is Beem's BP, and Marble Cliff Oil is the backup provider.

III. Quality Control:

A. Ohio Cat performs quarterly preventive maintenance on all generators and a yearly load test.

1. Ohio Cat provides documentation to NCH Engineering Services Department and is on file in the Hospital Engineering Shop office. Ohio Cat also keeps a record of their preventive maintenance and load testing.

B. NCH Engineering Services Department performs a generator test on all generators on the main campus on the 3rd Wednesday of every month.

IV. Policy

A. NCH Hospital Engineering Services Department maintains a current procedure for handling power outages.

1. See Hospital Administrative Policy IX-1 Electrical Power Outage.

B. Emergency power outlets are identified (red outlets) in Research II (RBII). All power to the Wexner building (RBI) is backed-up by emergency generator. All BPC and BCR temperature-controlled storage equipment have an emergency power supply.

C. Unnecessary power-consuming devices will not be used during any power outage.

D. If necessary, the Disaster Plan will be activated when an outage is in effect. Refer to Emergency Preparedness Manual, "Utility Failure Plan" for additional information.

V. References

A. Hospital Administrative Policy IX-1 Electrical Power Outage
B. NCH Emergency Preparedness Manual

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Biohazard and Chemical Spill Clean-up

Procedure Number: SAF-018		
Supersedes Procedure Number: N/A		
Date Adopted: 9/18/2012	**Prepared By:** Laci Bloomfield, Research Associate	
	Reviewed By: Teresa Tabler, Business Analyst	
Date Approved: 9/17/2012	**Approved By:** Nilsa C. Ramirez M.D., BPC Director (signature on file)	
Date Revised:	**Date Reviewed:**	**Signature:**
6/20/2013		Nilsa C. Ramirez M.D., BPC Director (signature on file)
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Principle: The purpose of this procedure is to provide instructions on how to safely clean up and properly dispose of biological/chemical spills using spill kits in the Biopathology Center (BPC) and Biospecimen Core Resource (BCR). All spills resulting in overt exposure, injury/illness of personnel, or breach of containment must be immediately reported to BPC and BCR management and Research Safety.

I. Equipment

- **A.** Biological Spills
  - **1.** Red Biohazard Bin
- **B.** Universal Spills and Formaldehyde spills
  - **1.** Phone

II. Materials

- **A.** Kits
  - **1.** Biological Spill Kit
  - **2.** Universal Spill Kit
  - **3.** Formaldehyde Spill Kit
- **B.** PPE
  - **1.** Lab Coat
  - **2.** Gloves
  - **3.** Safety Glasses

III. Warnings/Precautions

- **A.** Failure to comply may result in the spread of hazardous materials and/or biological substances.
- **B.** Failure to report a spill that results in overt exposure, injury/illness of personnel, or breach of containment may result in corrective action.
- **C.** Failure to use the proper personal protective equipment PPE may result in injury or illness.
- **D.** For a chemical spill:
  - **1.** Consult the chemical's Material Safety Data Sheet (MSDS) to confirm any specific or special instructions related to clean-up.

a. MSDS are made available in the laboratory area and on the Research Institute Safety webpage through MSDSonline.

2. A major chemical spill is defined as more than one gallon of a hazardous material or biological substance, or a substance that has high toxicity or poses a fire or life threat. Should a major spill occur, evacuate the laboratory and contact both hospital security and the Research Institute Biosafety Officer. If employee exposure also occurs (ex. splashed/soaked clothes) contact Employee Health, as well.
   a. Minor spills may be addressed with the applicable spill kit. If the employee(s) is uncomfortable or is experiencing any health compromises, evacuate and contact Hospital Security and the Research Institute Biosafety Officer.
3. Complete an incident report. BPC: See "ADM-009 Incident Report" SOP for instructions. BCR: See "A013 Incidents and Reporting" SOP for instructions.

E. For a biological spill :
1. Use universal precautions. See "SAF-011 Microbiological Safety and Hazards"
2. If employee exposure occurs (ex. splashed/soaked clothes) seek Employee Health.
3. Complete an incident report. BPC: See "ADM-009 Incident Report" SOP for instructions. BCR: See "A013 Incidents and Reporting" SOP for instructions.

IV. Quality Control

A. Quality control will be completed on this procedure through annual updates of the procedure manual or earlier if deemed necessary due to changes in techniques and/or best practices.
B. The laboratory trainer will evaluate the employee's ability to perform this procedure.

V. Procedure

A. Biological/Chemical Spill Clean-up:
1. Put on lab coat, safety glasses and gloves.
2. Open the Biological spills kit.
   a. Spill Kit storage locations are:
      i. BPC Processing Lab: WA1340 on labeled blue floor rack near the fume hood
      ii. Biospecimen Core/Internal Projects Lab: W344 under the sink labeled as "clean"
      iii. Kinnear Location: On the countertop next to the laboratory sink
      iv. BCR Logistics: W233 under the sink.
      v. BCR MGL: W251 and W244 on the window shelves.
      vi. BCR Histology: W240 spill kit is under the sink on the north side.
3. Put on disposable gown, gloves, face mask, and shoe covers over PPE donned in step V.A.1.
4. Obtain the packet of absorbent granules from the biohazard spill kit.
5. Cover the entire spill with absorbent granules.
6. After a semi-solid forms, use the scraper to scoop the material into a red biohazard bag.
7. Wipe up the last remnants of the spill with the kit's absorbent towels and dispose of in a red biohazard bag.
8. Thoroughly wipe down the contaminated area with a germicidal cloth.
9. When area is cleaned, dispose of all materials used to clean up the spill in a red biohazard bag.
10. Tightly close the bag and dispose of in the red biohazard trash bin.
11. Remove disposable gown, shoe covers, gloves, face mask and dispose in the red biohazard bin.
12. Remove lab coat and place in the hamper.
13. Wipe hands with germicidal cloth.

B. Universal Spill Clean-up:
1. Put on lab coat, safety glasses, and gloves.
2. Identify the spilled liquid and verify that it is compatible with the spill clean-up material.
   a. Ex. Spilled liquid cannot contain hydrofluoric acid (HF)
      i. Consult the MSDS if unsure.

ii. Contact Hospital security and the Research Institute Biosafety Officer for assistance if the chemical is incompatible.

3. Open the spill kit and put on the provided rubber boots, rubber gloves, and the goggles.
4. Surround the spill with the white "socks" provided to keep spill from spreading and entering the floor drains.
5. Make sure the area is clear of other people to prevent falls.
6. Cover the spill with sorbent pads and/or Super Sorbent Particulate to reduce vapors and to soak up the spill.
7. Remove saturated sorbent pads and/or media and place into the yellow disposal bag.
8. Label the yellow bag with the name of the liquid spilled.
9. Re-cover the spill area with the Super Sorbent and work into the floor with a stiff broom.
10. Sweep up the safety sorbent and place into the yellow disposal bag.
11. Repeat steps 9 and 10 until the floor is completely dry.
12. Remove PPE and place into the yellow disposal bag.
13. Contact the NCH Research Biosafety Office for proper disposal of the spill.

C. Formaldehyde (formalin) Spill Clean-up:

1. Put on lab coat, safety glasses, and gloves.
2. Identify the amount of formalin spilled.
    a.) If less than 100mL, proceed to step 3.
    b.) If greater than 100mL, or uncomfortable, evacuate the lab and contact NCH Security.
3. Open the formaldehyde spill kit.
4. Put on the gown, gloves, goggles, and respirator.
5. Sprinkle FSC-1 granules evenly over the spill. The fluid should begin to solidify. Add more granules if necessary.
6. Use the disposable scoop and scraper to remove the solidified spill and place in the white disposal bag.
7. Clean the surface with a hard surface wipe or disinfectant.
8. Wipe up any remaining residue with the white wiper pads provided and place into the white disposal bag.
9. Remove respirator, gown, goggles, and gloves and place in the white disposal bag.
10. Seal bag with twist tie and contact the NCH Research Biosafety Office for instructions regarding proper disposal.

VI. References

1. Bodily Fluid Pickup At-A-Glance Guide
2. Spill Response Kit Usage Procedure
3. Formaldehyde Clean-up Kit