# STANDARD OPERATING PROCEDURE (SOP) FOR PREPARING FROZEN TISSUE FOR MOLECULAR ANALYSIS

## I. SCOPE AND PURPOSE

This procedure applies to all qualified Biospecimen Core Resource (BCR) laboratory personnel. The purpose of this Standard Operating Procedure is to establish a procedure for the BCR Logistics Department to prepare frozen tissue samples for molecular analysis.

## II. PROCEDURE

### A. Safety Precautions

1. Always wear personal protective equipment (PPE), including a lab coat, eye protection, cut resistant gloves, cryogenic gloves and closed toe shoes.
2. Bloodborne pathogens can be present in the unfixed frozen tissue. Assume all specimens are infectious and take universal precautions. Lab personnel are trained yearly regarding bloodborne pathogens.
3. Dry ice can cause severe burns and produces gases that are hazardous upon inhalation. All work with dry ice must be conducted in a well-ventilated room with proper PPE.
4. If a disposable item or dry ice becomes contaminated with human material such as tissue or blood, it must be discarded.

### B. MGL Transfer Logs

1. After frozen tissue samples have undergone pathology review, Virtual Microscopy (VM) will send a list of portions that have passed and may be transferred to the Molecular Genetics Laboratory (MGL) for molecular analysis. Lists are received via email in an Excel spreadsheet titled "MGL Transfer Log."
2. Print out a physical copy of the Log to be used for Quality Control purposes.
3. In LabVantage, go to "Sample Management" > "Admin Samples." Using one of the queries in the "Search By Query" column (TSS Alias, TCGA, or TCGA Patient), search for and select each case listed on the MGL Transfer Log along with the possible, associated normal control. Use copy/paste whenever possible to avoid transcription errors.
   a. Repeat this step for all cases on the Transfer Log
   b. Add all samples to a folder in LabVantage.
4. Open the folder containing all samples on the Transfer Log and select all. Click the "Enter Data" button and select "Multi-Sample List View." In the "Status" field, ensure that "Pass" or "Ready for MGL" has been selected. If any other value has been selected, contact a member of VM to clarify whether or not the case should be transferred to MGL.
5. Return to the "Admin Samples" page. Determine the freezer location (listed in the "Location" column next to each sample) of the sample(s) listed on the MGL transfer log, as well as the freezer location of the normal control for each case. **A normal control must be provided to MGL for every new case, if applicable.**

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6. All blood derived normal controls for a case are to be transferred to MGL with the first portion of tumor that has passed pathology review. If multiple bloods or DNAs exist for a case, only send the first portion
7. If there is a normal control tissue for a case or only normal tissue was submitted for the case, it should be listed on the MGL transfer log. Repeat Section II.B.4 for normal tissue to ensure that it has passed pathology review.
8. If LabVantage shows that MGL already has custody of a normal control for a case or there are no normal controls for a case, it is acceptable to send a portion without a normal control if there are no additional controls in Logistics custody.
9. Wearing all appropriate PPE, remove all tumor and normal control samples from the cryofreezer. Place tumors and normal controls for each case adjacent to one another in a 9x9 $LN_2$ box in cryocart.
10. Fill an insulated bin with dry ice. Cover dry ice with aluminum foil.
11. Before processing, sanitize forceps and scalpel handle.
12. Place a sterile 100mm petri dish, scalpel handle with new blade and clean forceps in the insulated dry ice bin to chill.
13. Pull a sample from the 9x9 LN2 box and place in the insulated dry ice bin.
14. Highlight sample on printed Transfer Log to indicate that you have begun processing the sample.
15. In LabVantage, take custody of the sample using the "Check Out" or "Force Custody" features.
16. Verify specimen label information against sample data and information in LabVantage.
17. Remove tissue sample from the cryovial and place in pre-chilled Petri dish on top of the dry ice/aluminum foil.
18. Using a scalpel and forceps, carefully remove OCT compound and any dye from the tissue specimen.
    **Note: Cover tissue with gauze while cutting to prevent accidental ejection of tissue from petri dish and processing hood.
19. If a subportion is not required proceed directly to Step 31.
    a. e.g. Burkitt Lymphoma Genome Sequencing Project (BLGSP) study samples require only one ~30 mg portion when processing.
20. If a subportion(s) is required, weigh the sample. Using the scalpel and forceps, divide the sample into as many 30mg subportion(s) as possible. It is acceptable to have one subportion that is less than 30mg.
21. In LabVantage, go to "Sample Management" > "Lab Operations Samples." Select the sample you wish to make subportion(s) from.
22. Click "Add Multi Derivative."
23. Enter the following:
    a. **Sample Template**: Portion
    b. **Number of Derivatives**: 1
    c. **Sample Type**: Primary Tumor, Metastatic or Solid Tissue Normal

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d. **Prep Type**: Portion
e. **Quantity**: Weight of portion in mg (if a portion is less than 30mg, ensure this portion is the last portion to be entered on the list)
f. **Unit**: mg

24. Repeat section II.B.23 for all portions to be created (click "Add" to add another portion field).
    a. Check the "Mark Depleted" Box
    b. Click "Save"
25. LabVantage will redirect to the "Child Confirmation Samples" page. Select all subportions, place into a folder, and click "Confirm." Return to the "Lab Operations Samples" page.
26. Select folder with the subportion(s). Click "Print Label." Enter the following:
    a. **Label Method**: Logistics – Label
    b. **Printer**: Select the printer you wish to print to
    c. **Copies**: 1
    d. Click "OK."
27. Retrieve labels from printer. Label Eppendorf tube(s) and place on dry ice bin to chill.
    **Note: Vials should be fully chilled before used to store samples.
28. Place subportion(s) into chilled Eppendorf tubes.
    a. If more than one sub portion has been created, ensure any subportion weighing less than 30mg is placed in the Eppendorf tube labeled with the last subportion number.
29. Place the subportions, along with the original portion vial, adjacent to each other in a 9x9 LN2 box in the cryocart.
30. Once all cases on the MGL Transfer Log (along with any corresponding normal control samples) have been processed, create a Custodial Domain Transfer (CDT) of the first subportion of each portion processed (Ex: -01A-11, -01A-21, -11A-11 etc.)
31. If subportions were not required (e.g. BLGSP Study), create a Custodial Domain Transfer (CDT) with all portions (along with any corresponding normal controls) being sent to MGL.
32. In the Packages and CDTs Tab in LV. Select your created CDT and EDIT it to represent the format listed below.
    a. Project Name, MGL Log Date (if Applicable), Date being sent to MGL, TSS ID's present in CDT.
33. Go to the BCR TCGA SharePoint site. In the "BCR Reports" page, expand "Report Category: Transfers." Click "PackageTransferCDT." In the "Report Viewer – CDTPackageTransfer" page, select the CDT number created in section II.B.23 in the "CDT" dropdown menu. Click "Apply."
34. Once the CDT Report has been generated, click "Actions." Click "Print." Select the desired printer and click "OK."

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35. With one technician reading aloud all identifiers on the samples in the 9X9 LN2 box, and another technician listening and comparing to the CDT report and printed Transfer Log, confirm the following:
    a. All subportions are labeled with the same case and tissue type as the parent portion they are adjacent to in the 9x9 LN2 box.
    b. All subportions match what is listed on the CDT report (only the first subportion from each portion processed should be listed on the CDT report. Place these subportions along with corresponding blood derived normal in a separate 9x9 LN2 box to be delivered to MGL as the samples are read).
    c. All cases being transferred to MGL for the first time are accompanied by a normal control when applicable.
    d. All blood derived normal samples are labeled correctly (the TSS Alias on the original TSS label matches the TSS Alias on the label printed from LabVantage).
36. Any remaining subportions that were created but not shipped in the CDT to MGL (-01A-12, -01A-13, etc.) should be stored in a liquid nitrogen freezer and banked in a locations in LabVantage.
37. Any case not requiring subportions compare the sample with the MGL Log and CDT to MGL.
38. Bury 9x9 LN2 box in dry ice in an insulated box, and deliver along with CDT report to MGL for processing.
    **Note: Do not deliver samples to MGL after 4pm.
39. Send an email to the BCR MGL and Logistics Departments, giving notification that MGL Transfer Log has been processed and delivered.

**C. MGL Tissue Requests**

1. If the Molecular Genetics Lab (MGL) processes a case and it is determined that more tissue is required for molecular analysis, an email will be sent to the Logistics Department containing what is needed. Emails are typically titled "MGL Tissue Request." If it is unclear what type of sample is needed (tumor, normal or blood), contact MGL for clarification.
2. Upon receipt of Tissue Request email, the Logistics Supervisor, or designee, reviews all cases per sections II.C.5 through 7. All data are compiled into an Excel spreadsheet accompanied by the action that needs to be taken for each sample. Actions to be taken are as follows:
    **a. Need Sub-portions cut**
    **b. Send Sub-portion to MGL**
    **c. Special Request**
    **d. Send to Histo**
    **e. No Tissue Remaining**
    **f. Blood to MGL**
    **g. DNA to MGL**
3. This spreadsheet is sent to all original email recipients and saved on G: Drive > BCR > BCR-Logistics > MGL Tissue Requests.
4. Tissue Requests are saved according to date tissue was requested.

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5. In LabVantage, go to "Sample Management" > "Lab Operations Samples." Search for each case listed in the MGL Tissue Request.
   a. If a subportion still remains in Logistics custody for the case and sample type, select the lowest subportion number available to transfer to MGL (Ex: if -01A-12 and -01A-13 are available, select -01A-12).
   b. If no subportions remain for the case and sample type, check to see if there are any portions remaining for the sample type (Ex: -01A-02, -01A-03). If portions are available, select each portion and click "Enter Data". If a portion has a value of "Ready for MGL", "Pass" or "Pass-Not Used" in the "Enter Value" field, it may be subportioned for MGL. If a portion has a value of "Not Used", it must first be transferred to Histology to have a slide cut for pathology review. If a portion has a value of "In Progress", it is currently undergoing pathology review and is not ready to be transferred to MGL. If a portion has any other value, it may not be transferred to MGL.
6. Determine the freezer location of the samples (listed in the "Location" column next to each sample) to be transferred to MGL.
   **Note: In most cases, samples listed on an MGL Tissue Request do not need to be accompanied by a normal control, as MGL has already previously received tumor and normal controls for these cases. However, for each case it should still be verified in LabVantage that no blood derived normal controls remain in Logistics' custody.
7. List all samples to be transferred to MGL, any samples that need to go to Histology, and any cases that do not have any more passing tissue available using the action words listed in section II.C.2.
8. Print out a physical copy of the Tissue Request to be used for Quality Control purposes
9. Follow section II.B.4 through39 in the "MGL Transfer Log" section above to process any portions pulled for the MGL tissue request, as well as to prepare and create a CDT for subportion(s) to be transferred to MGL.

**D. Quality Control**

1. All sample labels are visually inspected and verbally confirmed by two trained individuals to ensure that the sample is being placed in an appropriately labeled vial.
2. Ensure the specimen stays frozen throughout the entire procedure.
3. When performing electronically, IDs must be copied and pasted to avoid transcription errors
4. Use barcode scanners to identify samples whenever possible.
5. Use copy/paste whenever possible to avoid transcription errors.

## III. REFERENCES - None

## IV. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version 2, Effective Date 12/31/2014
   1. New template used

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2. Minor word changes and clarifications made throughout
3. Major changes made throughout, including:
   a. Title changed from "Molecular Genetics Lab (MGL) Transfer Logs" for clarification
   b. Equipment and materials section and all references to Prospray were removed
   c. Safety Precautions section was updated
   d. Quality Control section was updated to include use of barcode scanners and copy/paste to reduce errors
   e. Requirement to print out log was added
   f. Use of new spreadsheet system for tissue requests was added

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

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## Signatures

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

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