Standard Operating Procedure (Sop) for Sectioning and Portioning Frozen Tissue Samples in Logistics

I. SCOPE AND PURPOSE

Frozen tissue samples are submitted to the BCR for consideration in genomic research projects. The tissue is subdivided into portions as determined by specific project protocols by the Logistics department and each is reviewed independently. Qualifying portions are submitted to a Molecular Department for extraction of DNA and RNA.

This procedure applies to all trained laboratory personnel and establishes a procedure for cutting unfixed frozen tissue for slides and creating tissue portions for molecular analyte processing.

II. PROCEDURE

A. Safety Procedures

1. Wear appropriate PPE for handling scalpel blades and when removing blades after use. Following use of a scalpel blade, dispose in sharps container.
2. Wear appropriate personal protective equipment including nitrile gloves, cut-resistant gloves under nitrile gloves, liquid impermeable lab coat, and safety glasses.
3. Use caution when working with liquid nitrogen and dry ice. Skin exposure to these materials can cause burns. Wear appropriate gloves, lab coat and face shield if needed. Work in a well-ventilated area equipped with an O_2 monitor.
4. All used materials must be disposed between samples to reduce contamination (nitrile gloves, foil, scalpel blade, petri dish, etc.)
5. Bloodborne pathogens can be present in the unfixed frozen tissue. Assume all samples are infectious. Laboratory personnel are trained annually regarding bloodborne pathogens.
6. It is possible to substitute materials and certain equipment as long as they are the equivalent of the item described above.
7. Products and disposable materials used need to be RNAse-free whenever possible and handled only with gloved hands in order to prevent contamination with skin RNAses.
8. All reagents must be made with RNAse-free materials and chemicals. Containers and tubes with samples must be kept covered when possible during the entire procedure to ensure they remain dust- and RNAse-free.
9. In the case that a reagent or disposable tool, such as foil, petri dish, or dry ice, becomes contaminated, it must be discarded.

B. Quality Control

1. **Do not let frozen tissue thaw at any time.**
2. At least two technicians must be present in order to perform this procedure. Each individual will independently quality control the sample identifier and placement of samples throughout the process. All specimens are uniquely identified and confirmed against the provided shipping manifest. Any discrepancies found must be resolved before the specimen can be sent to another area within the BCR.

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3. All sample labels are visually inspected by both individuals to ensure that the sample is being placed in an appropriately labeled vial.
4. To avoid sample contamination, change all disposable materials between specimens. Dispose of used blades in sharps container, change nitrile gloves, and replace foil and petri dish. Clean all instruments with approved cleaning solution. Use clean gauze for each sample and discard used gauze before starting a new sample.
5. Work with only one sample at a time in order to reduce risk of sample swaps.
6. If a specimen is missing, LabVantage must be updated to reflect that the specimen cannot be located.
7. Use barcode scanners to identify samples whenever possible.

C. Tissue Processing

1. Retrieve the samples from the liquid nitrogen freezer and place them in a pre-chilled 9x9 LN_2 tissue box in a filled cryocart.
2. Fill an insulated bin with dry ice.
3. Cover the dry ice with aluminum foil.
4. Before processing, sanitize the forceps and scalpel with approved cleaning solution.
5. Place a sterile 100mm Petri dish, scalpel and clean forceps on the dry ice to chill.
6. Retrieve a sample from the cryocart, placing the specimen container directly into the dry ice bin.
7. Use the shipping manifest provided by the TSS to confirm that you are processing a sample from the correct shipment. Highlight the sample on the manifest to indicate that you have taken physical custody of the tissue.
8. Take custody of the sample in LabVantage.
9. Tissue is received either snap frozen or embedded in Optimal Cutting Temperature Compound (OCT). Complete the following for each type:

a. Snap frozen tissue:

i. Remove the sample from the TSS container and place it in the chilled petri dish.

ii. Identify the area of the tissue from which a slide was cut for pathology review at the Tissue Source Site (TSS), if applicable. This is referred to as the TSS "top". The TSS "top" can be identified if the "cut" side is clearly distinguishable from the rest of the tissue, or if the TSS has placed a mark on the tissue.

**Note: In some cases, the TSS "top" is not identifiable. After reviewing steps II.C.10.a. and II.C.10.b. (Normal vs. Flat portioning), see step II. C.11.c. for instructions on samples that do not have an identifiable TSS "top".

iii. Weigh the sample on a clean foil square using a scale and quickly return it to the dry ice to avoid thawing.

**Note: Keep track of the TSS "top" of the tissue when transferring to and from the scale.

iv. Enter the "NCH Sample Weight" and "Processing Type" in LabVantage.

b. OCT embedded tissue:

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i. Remove the sample from the TSS container and place it in the chilled petri dish.
ii. Identify the TSS "top" of the tissue (see diagram II. C. 10 1A and 1B).
iii. Using the forceps and scalpel, carefully remove the OCT compound from the tissue.
iv. Weigh the sample on a clean foil square using the scale and quickly return it to the dry ice to avoid thawing.
**Note: Keep track of the TSS "top" of the tissue when transferring to and from the scale.
v. Enter the "NCH Sample Weight" (the weight of the entire tissue sample not including OCT, before portioning) and "Processing Type".

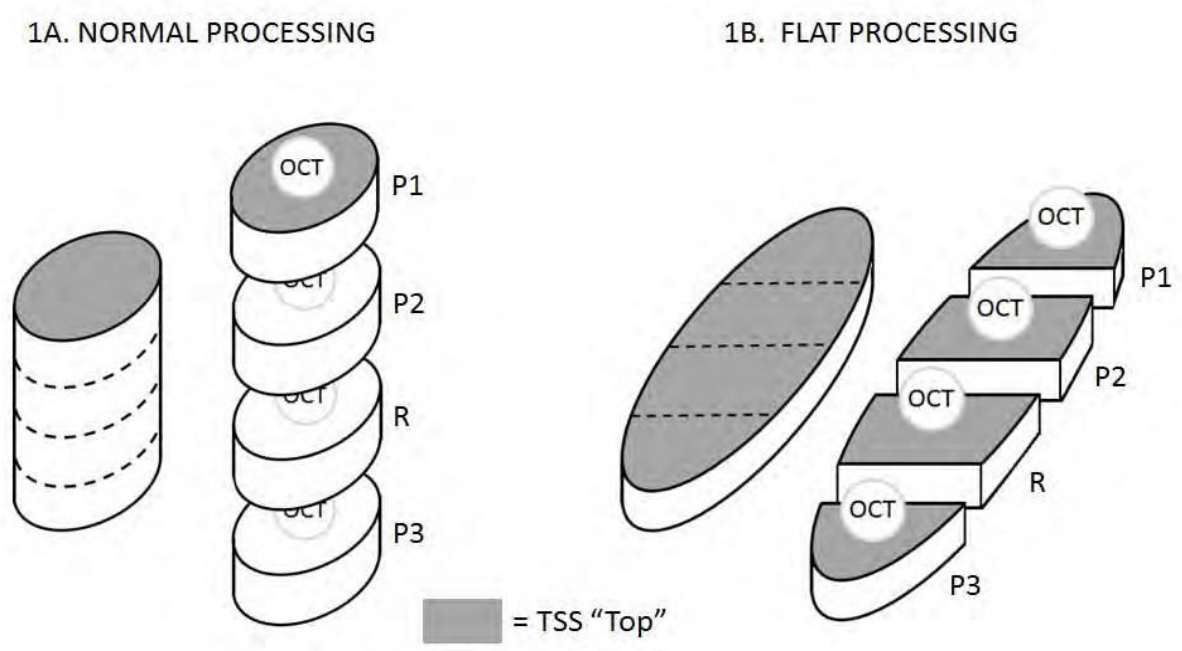
10. Using one of the following portioning methods, cut portions of tissue (note: portions must be one continuous piece of tissue)
    a. Portioning is specific to each project and thus specifications may differ from this procedure. Refer to the Working Document for the specific project for portioning details. Example: BLGSP requires only portion 1 (P1).
    b. Weight of portions is specific for each project.
        i. **Normal Portioning**: This portioning method is typically preferred. Cuts are made parallel to the TSS "top" of the tissue (see diagram 1A).
            (a) Using a scalpel, cut a portion that includes the entire TSS "top" of the tissue. This is portion one (P1). Use a scale to weigh the portion on a clean foil square and quickly return it to the dry ice to avoid thawing. Using a clean toothpick, mark portion one with a small dot of OCT on the TSS "top".
            (b) Portion two (P2) should be cut from the remaining tissue to include the area of tissue from which portion one was cut. Weigh the portion on a clean foil square using a scale and quickly return it to the dry ice to avoid thawing. Using a clean toothpick, mark portion two with a small dot of OCT on the side that portion one was cut from.
            (c) Portion three (P3) should be cut from the side of the remaining tissue opposite of where portions one and two were cut (if there is only enough tissue for three portions, portion three will be adjacent to portion two). Weigh the portion on a clean foil square using a scale and quickly return it to the dry ice to avoid thawing. Using a clean toothpick, mark portion three with a small dot of OCT on the side that was adjacent to the remaining tissue (or the side that was adjacent to portion two).
            (d) Weigh the remaining tissue on a clean foil square using a scale and quickly return it to the dry ice to avoid thawing. Using a clean toothpick, mark the remaining tissue with a small dot of OCT on the side that was adjacent to portion two.
            (e) In the event that a portion four (P4) or higher is needed (by request from the Logistics Supervisor or Virtual Microscopy (VM) department only),

cut the portion from the remaining tissue to include area marked with OCT. Remove the OCT dot, weigh the portion on a clean foil square using a scale and quickly return it to the dry ice to avoid thawing. Using a clean toothpick, mark portion four with a small dot of OCT on the side that was previously marked with OCT. Follow this method for any subsequent portions needed until the remaining tissue is depleted.

ii. **Flat portioning**:

(a) This portioning method is typically only be used when "normal" portioning is not possible (for tissue that is irregular or flat). In flat portioning, cuts are made perpendicular to the TSS "top". Multiple or all portions contain a part of the TSS "top" (see diagram 1B).

(b) Portions one (P1) and two (P2) should be cut adjacent to one another. Portion three (P3) should be cut from the opposite side of the remaining tissue (or adjacent to P2 if there is only enough tissue for three portions). Weigh each portion on a clean foil square on a scale and quickly return to the dry ice to avoid thawing. Using a clean toothpick, mark the TSS "top" on each portion with OCT (NOT the side that is adjacent to the previous portion as is done in "normal" portioning).



c. Processing Method Subtypes: All portions derived from the same piece of parent tissue must be cut using the same processing method and assigned the same "Processing Required" in LabVantage (all "Normal" or all "Flat"). However, tissue with an unidentifiable TSS "top" and differences between the way portions are marked with OCT can be accounted for by choosing one of the following processing method subtypes (see diagrams 1C and 1D):

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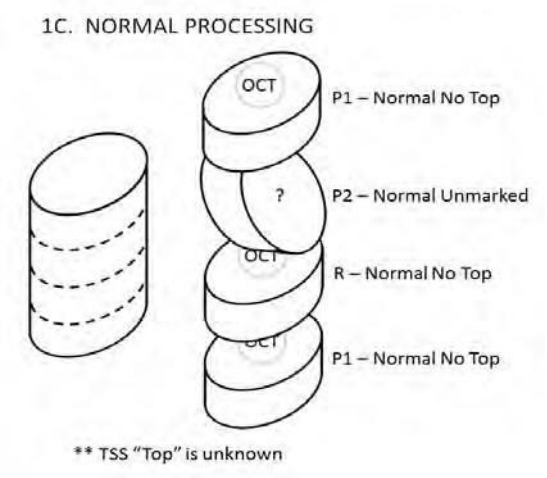
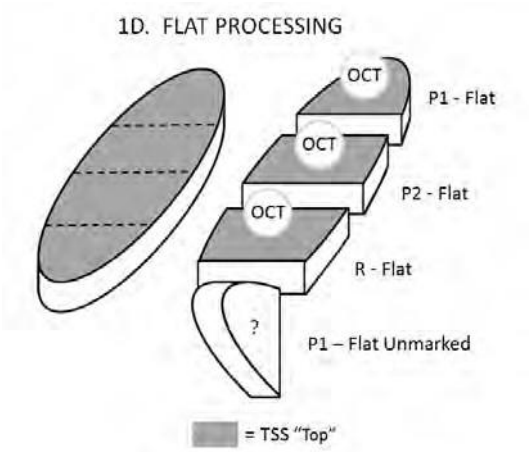
i. **Normal Unmarked**: typically used when a case has been portioned using the "Normal" portioning method, but the Logistics technician has lost the orientation of an individual portion. The portion should NOT be marked with OCT. This processing method subtype can also be used when the TSS "top" of a case is unknown and there is only enough tissue to make one portion.
ii. **Flat Unmarked**: typically used when a case has been portioned using the "Flat" portioning method, but the Logistics technician has lost the orientation of an individual portion. The portion should NOT be marked with OCT.
iii. **Normal No Top**: typically used when the TSS "top" of the parent tissue is unknown. Portions are cut in a fashion similar to the "Normal" processing method, however the OCT marking on P1 does NOT indicate the TSS "top" of the tissue (the side of the tissue that P1 is cut from is arbitrarily chosen).
iv. **Flat No Top**: typically used when the TSS "top" of the parent tissue is unknown and the "Normal No Top" processing method cannot used (due to tissue that is irregular or flat). Portions are cut in a fashion similar to the "Flat" processing method, however the OCT marking on all portions does NOT indicate the TSS "top" of the tissue (one side of the parent piece of tissue is arbitrarily chosen and marked with OCT on all portions).





**Note: Marking portions with OCT may also be completed after all portions for a case have been cut and weighed (ensure the TSS "top" of is kept track of during cutting and weighing).

11. In LabVantage, complete the following:
    a. Enter the "NCH Sample Weight" (the weight of the entire tissue sample not including OCT, before portioning) and "Processing Type".
    b. Create the portions and enter the portion weights.
    c. Enter the "Processing Required" for all portions and any remaining tissue.

d. Print "Logistics" and "Processing" labels for all portions and any remaining tissue.

12. Place portion labels on cryovials with the following cap colors and place the cryovials in the dry ice bin to chill:
    a. Portion one labels – Blue
    b. Portion two labels – Green
    c. Portion three labels – Red
    d. Portion four and above – Yellow
    e. Remaining tissue – White
13. Place each portion in the corresponding cryovial labeled in the previous step.
14. Place all portions and the empty TSS container adjacent to one another in a pre-chilled 9x9 LN_2 tissue box in the cryocart.
15. Discard the foil, petri dish, blade, nitrile gloves, and any other materials used to process the case in the appropriate biohazard and/or sharps container.
16. Repeat steps II. C. 3 through II. C. 13 for each desired case/tissue type.
17. Once all cases have been portioned, create a CDT in LabVantage of all P1s and P2s to Histology.
    **Note: The P3s that were created will be used in the event that both P1 and P2 fail pathology review for a case. P3s and remaining tissue will be stored in the Logistics LN_2 freezer for future use if needed. An "Internal Transfer Log" will be emailed to the Logistics department from VM containing any additional portions that need to be transferred to Histology.
    ** Note: Portions sent to Histology and banked may vary based on specific project protocols. Confirm work flow with Logistics Supervisor.
18. Print the CDT report using the PackageTransferCDT Report found on the NCH BCR SharePoint> Reports > Transfers.
19. Two technicians should perform quality control on all portions created to ensure the following:
    a. All portions have been labeled with the same TSS ID as the empty TSS container they were placed adjacent to in the 9x9 LN_2 box.
    b. All P1s and P2s are placed in a box together to be transferred to Histology. The portion identifiers and "Processing Required" should read exactly as is listed on the CDT report, and the portions should be placed in the box in the same order as they are listed on the CDT report.
20. P3s and remaining tissue specimens should be stored in the Logistics LN_2 freezer and have freezer locations recorded in LabVantage utilizing barcode scanners.

D. SPECIAL PROCESSING RULES

1. Breast Normal Tissue

a. Identify if there are any breast normal tissues included in incoming shipment.
b. All breast normal tissue is portioned and sent to Histology.
c. Breast normal tissue is typically processed into 100 mg portions with no limit on the number of portions (i.e. approval is not needed if these exceed 3 portions).

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Note: Portion size and criteria may change depending on project specific guidelines.

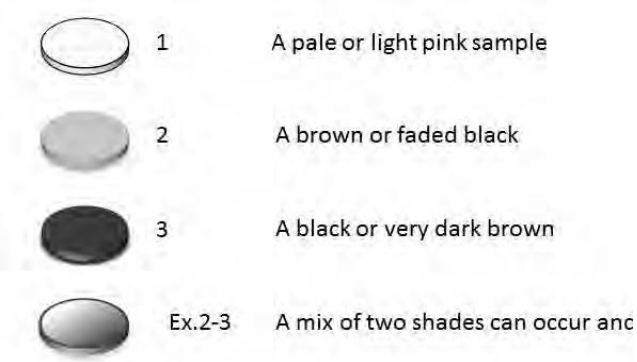
d. Notify Histology if portions will require a top and bottom slide when samples are transferred using a CDT.
e. If the normal tissue portion passes pathology review, the entire 100 mg portion is transferred as a single subportion to MGL.

2. Sarcoma

a. Identify if there are any sarcoma tissues included in incoming shipment.
b. Sarcoma tissues will be portioned using normal protocol and transferred to Histology.
c. Any Sarcoma normal tissue that passed pathology review will be sub-portioned into 30 mg portions for Molecular Processing and **ALL** sub-portions for passing portion need to be transferred to MGL.
d. Sarcoma portioning rules are subject to change based on MGL yield data reports.

3. Melanoma

a. Identify if there are any Melanoma (includes Uveal Melanoma) tissues in the incoming shipment. If applicable, remove any OCT from around the tissue.
b. Using judgment, make a determination on how dark the tissue is.
    i. A light sample is considered a 1.
    ii. A brown or faded black is considered a 2.
    iii. A black or very dark brown is considered a 3.
    iv. A mix of two shades can occur and is labeled as both.



c. Print an identifying label for the tissue. Select sample in LV > Select Print Label > Label Method (Logistics Label), Printer (Select appropriate Brady label printer), Copies (1). > Select OK.
d. Place the label beside the Melanoma tissue. Making sure to capture both the tissue and label, take a picture of the sample.
e. Continue processing the sample.

E. Archive the image

1. Attach the camera to the computer using the camera cord.
2. Find the picture in the camera files and move the picture onto your computer desktop.

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3. Go to http://rex/BPC/TCGA-BCR and select Sample Images > Melanoma > Select (Year Shipment Received) > Upload > Upload Picture > Browse
4. Find and Select the picture that was uploaded for your sample.
5. Name the picture with the TSS ID, TCGA #, and Tissue identification #. An example would be 12-AAAA-01A.
6. Save the picture.

F. Macrodissection

1. A specimen is annotated per SOP C041, "Coordinating and Tracking Macrodissection and/or Portioning Cases through the BCR Pipeline". This annotation will go to the Logistics Supervisor, or designee, through the Naughty List Annotation Report.
2. The Logistics Supervisor, or designee, sends an email to the Logistics team with the specimen identifier and location.
3. Obtain the specimen from storage.
4. Cut additional portions of the sample as instructed by the Logistics Supervisor (i.e. portions 4-6, 7-9) and CDT to Histology.

III. REFERENCES

A. BCR-SOP C041, "Coordinating and Tracking Macrodissection and/or Portioning Cases Through the BCR Pipeline"

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

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version 2, Effective Date **8/25/2015**

1. Wrote that materials used need to be RNAse-free whenever possible
2. Included language to refer to Working Document for specific project portioning details regarding OCT embedded tissue
3. Breast Normal Tissue is typically processed into 100 mg unless dictated by project guidelines

B. Version 1, Effective Date **08/26/2014** - New
This SOP replaces the Logistics Section of SOP LH003, "Sectioning and Portioning TCGA Frozen Tissue Samples"

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Signatures

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

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