STANDARD OPERATING PROCEDURE (SOP) FOR PREPARATION OF TISSUE SAMPLES FOR PROTEOME CHARACTERIZATION CENTERS (PCCs)

I. SCOPE AND PURPOSE

Frozen tumor samples that qualify for Biospecimen Core Resource (BCR) projects and shipped to Genome Characterization Centers (GCC) and Genome Sequencing Centers (GSC) may also have a sub portion shipped to a Proteome Characterization Center (PCC) for proteomic analysis, if available and requested.

This procedure applies to all qualified BCR laboratory personnel and establishes a procedure to prepare and ship qualified frozen tumor samples for proteomic analysis.

II. PROCEDURE

A. Safety Procedures

1. Always wear appropriate personal protective equipment (PPE).
2. Lab personnel must take annual bloodborne pathogens training. Always use universal precautions as bloodborne pathogens can be present in unfixed frozen tissue.
3. Dry ice is an asphyxiant and can cause severe burns. All work with dry ice should be conducted in a room with oxygen sensors or with sufficient airflow to prevent asphyxiation.

B. Quality Control

1. Sample quality control will occur in teams of two, and each individual will independently perform quality control by confirming sample identifiers.
2. All sample labels are visually inspected by both individuals to verify that the sample is being placed in an appropriately labeled vial.
3. Use dry ice to ensure the specimen stays as cold as possible throughout the entire procedure.
4. Discard any dry ice or other disposable items that become contaminated with tissue.

C. Preparing samples for PCC centers

1. A list of cases that have qualified for a specified research project and are ready to be shipped to a PCC will be generated by Project Management and given to the Logistics Department. Lists are typically generated in groups by cancer type.
2. Contact the Program Director or Logistics Supervisor for details regarding how subportions should be created for each PCC. Weight and origin of tissue, vial type, pathology metrics, etc. may vary with each PCC.
3. Using LabVantage and the details provided in II. C. 1, the Logistics Supervisor (or designee) will determine which portions from each case will be used for proteomics.
4. To identify the piece of tissue that will be shipped for proteomic analysis, use the following rules:
    a) Use the next most adjacent piece of tissue to the sub portion that is being shipped for molecular analysis. E.g., If portion 1 was shipped to a GCC/GSC use portion 2 for proteomics.

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   b) Do not send if the only remaining tissue is from the same portion that was used for the GCC/GSC analyte.
   c) Confirm that the selected tissue meets pathology metrics:
      i. At least 1% tumor nuclei
      ii. Less than 100% necrosis
5. Save the generated worksheet of portions to be processed as an Excel document and distribute to the Logistics team for processing.

D. Preparing Pooled Samples for Shipment

1. Go to LabVantage > "Sample management" > "Lab Operations Samples". Select the subportions to be pooled and click "Pool Sample".
2. Enter the following:
   a. Select Template: portion
   b. Copies: 1
   c. Container Type: cryovial
   d. Custodial Domain: CO-Logistics
   e. Selection Unit: mg
   f. Quantity needed: indicate how much tissue from each sub portion will be pooled
3. Click "Save".
4. LabVantage will redirect to the "Child Confirmation Samples" page.
   a. Select the sub portion and click "Confirm".
   b. Each sub portion depleted from the pooling procedure will need to be manually disposed of as consumed in Admin Samples.
   c. The newly pooled sub portion will have the same barcode number as one of the subportions used to create it.
5. Return to the "Lab Operations Samples" page. Find and select the child portion created.
6. Click "Print Label". Enter the following:
   a. Label Method: Logistics – Proteomics
   b. Printer: Select the desired printer
   c. Copies: 1
   d. Click "OK".
7. Retrieve the printed label and apply to vial type specified by each PCC.

E. Preparing Non-Pooled Samples for Shipment

1. Use provided identifier to find the sample to be processed in LabVantage.
2. Take custody, or Check Out, the sample.
3. On the "Lab Operations Samples" page, create a sub portion derivative.
4. Find and select the child portion created.
5. Click "Print Label". Enter the following:
   a. Label Method: Logistics – Proteomics
   b. Printer: Select the desired printer
   c. Copies: 1

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d. Click "OK".

6. Retrieve the printed label and apply to vial type specified by each PCC.

F. Processing all PCC samples (pooled and non-pooled)

1. Pull all samples from the appropriate freezers and place in a 9×9 liquid nitrogen (LN_2) tissue box. Place the tissue box in a filled cryocart.
2. Remove a sample to be processed from the LN_2 tissue box and place in an insulated bin filled with dry ice for processing.
3. Compare all identifiers on the sample label to the Excel list of samples generated by Logistics Supervisor in II. C. 4. to ensure the correct sample is being processed.
4. Cut a sub portion (per SOP L020, "Sectioning and Portioning of TCGA Frozen Tissue Samples") from the sample according to the weight determined in step II. C. 2. **Note: Weigh tissue and return to dry ice as quickly as possible to avoid thawing.
5. If depleting a case, mark the top of the parent portion vial with a black marker to indicate that it has been depleted and can be disposed of after quality control (QC) is performed.
6. Some PCC portion standards may require the combining (pooling) of subportions to meet the minimum weight required by the PCC. Samples that require pooling should be indicated on the worksheet generated in step II. C. 3.
7. Place vial(s) on dry ice to chill.
8. Place tissue in appropriately labeled vials (if using the Precellys® Ceramic Beads Kit (1.4 mm) provided by the University of Texas MD Anderson Cancer Center, ensure tissue is placed on top of beads and vial is completely chilled).
9. Place remaining tissue (if any) back into the parent vial from which it came. Update the remaining weight of the parent sample.
10. Place the parent portion and sub portion adjacent to one another in a new 9×9 LN_2 tissue box in the cryocart.
11. Sanitize the forceps scalpel handle and dispose of any used foil, gauze and scalpel blades and replace with new materials.
12. Repeat steps II. D. 1-7 and II. E. 1-5 for each sample listed in worksheet generated in II. C. 4.
13. When all cases on the list have been processed, compare all subportions to the adjacent parent portions in the 9x9 LN_2 box to ensure:
    a. The correct label was printed for each case.
    b. The correct portions have been pulled and processed.
14. Store the parent samples in the cryofreezer and update their locations in LabVantage.
15. In LabVantage, go to "Sample Management" > "Lab Operations Samples". Search for and select all subportions created for proteome characterization. Add the subportions to a new folder by selecting the sub portion, selecting "…to a new folder" in the "Search By Folder" column and clicking "ADD".
16. Take custody of all subportions.

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17. Contact the Molecular Genetics Lab (MGL) to obtain a new Matrix Plate ID(s) for the box(s) containing the samples to be shipped for proteome characterization.
18. Label a new 9×9 LN_2 tissue box(s) with the Matrix Plate ID(s) on all sides.
19. In LabVantage, go to "Storage" > "Boxes".
    a. Click "+Add". Enter the following information:
        i. Status : Empty
        ii. Description: Matrix Plate ID obtained from MGL - Cancer Type - Proteomics (Ex: A17N Rectal Proteomics).
        iii. Study Id: Cancer Type
        iv. Type: Sorted
        v. Active: Yes
        vi. Storage Unit Type: Box 9×9
        vii. Custodial Domain: CO – Logistics
        viii. Matrix Plate ID: Matrix Plate ID obtained from MGL
    b. Click "Save".
    c. Go to "Storage" > "File Samples".
    d. Click the look up icon next to "Select Target Storage Unit".
    e. Click "Sorted Box".
20. In the pop up window, use one of the queries in the "Search by a Query" column to search for the box(s) created in II. E. 13. (the "MyBoxes" query will yield any boxes in the current user's custody). Click a box in which to file samples.
21. Check the "Scan Alias" box and select "TCGA" from the drop down list.
22. With a barcode scanner, scan samples into appropriate location in the box and place the physical samples into the box labeled with the correct Matrix Plate ID. Click "Save".
23. Go to "Storage" > "Boxes". Using one of the queries in the "Search By Query" column, search for and select the box(s) containing the subportions for proteome characterization.
    a) Select the Box and click "Edit"
    b) In the "Ship Id (Center)" drop down; select the center to which the box will be shipped. E.g., Center20:GCC-20-MD Anderson-Proteomics
24. Go to the BCR SharePoint site. In the "Reports" page, expand "Transfers and Shipping".
25. Click "shippingforms". Enter the following information:
    a) Plate: Matrix Plate ID (E.g., A17N)
    b) Shipping Date
    ** Note: Data must be generated overnight in the warehouse after the Box is assigned to Ship Id (Center) before Shipping Form can be generated.
26. Hit the "Enter" Key. Once the report has been generated, click "Actions". Click "Export" > "Excel".
27. Save a copy of this report in the designated folder on SharePoint.

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28. To perform quality control (QC) on the outgoing box(s), two Logistics technicians will compare each sample in the box(s) to the Shipping Information Proteomics form to ensure that the form properly reflects the contents of the box and that the correct samples are being shipped.
29. Prepare box(s), Shipping Information Proteomics form(s) for shipping.
    a) MD Anderson samples will be shipped on dry ice.
    b) All other PCC projects will be shipped using a cryoport unless otherwise determined by BCR Management.
30. In LabVantage, create a package and place the box(s) in the package.
31. Email the FedEx tracking number to the Program Director and Logistics Supervisor and verify that PCC has been notified of the shipment.
32. Upon approval by the Logistics Supervisor and/or Program Director, ship the Fed Ex package to the appropriate PCC and ship the virtual package created in Lab Vantage.

III. REFERENCES

A. BCR SOP L020, "Sectioning and Portioning of Frozen Tissue Samples"
B. BCR SOP S009, "Bloodborne Pathogen and Exposure Control Plan"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version 2, Effective Date **08/26/2014**
1. New format used
2. Minor word and grammatical changes made throughout
3. Removed the Equipment and Materials section
4. Removed reference to retired BCR SOP LH003, " Sectioning and Portioning TCGA Frozen Tissue Samples"
5. Updated references to Sapphire to LabVantage to reflect the current name for the BCR Laboratory Information Management System
6. Updated to reflect new process, including
    a) Added rules to identify tissue that needs to be shipped for proteomic analysis
    b) Clarified sample preparation of pooled samples versus non-pooled samples

B. Version 1, Effective Date 7/10/12 – New

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Signatures

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

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