1. PURPOSE

1.1. The purpose of this procedure is to outline the process for generating the images necessary to evaluate biobanked TCRB samples for use in sequencing studies.

2. SCOPE

2.1 This procedure is applicable to all individuals who perform a function related to the generation and imaging of histology specimens associated with TCRB BioBank material.

3. DEFINITIONS

3.1 IMAGING: Series of procedures that are performed in order to digitally capture microscopic images.

3.2 TCRB: Texas Cancer Research BioBank.

3.3 TCRB Pathologist: Pathologist representing TCRB's interests at each biobanking sub-site and regional center (currently in Houston, Dallas and Lubbock).

3.4 H&E: Hematoxylin and Eosin

3.5 FFPE: Formalin-Fixed Paraffin-Embedded

3.6 Acquire: TCRB's proprietary and secure database

3.6 Pathology Sampling Site: The locaton at which tumor and normal tissue is selected and processed for biobanking and histology.

3.6 TCRB Sub Site: A pathology sampling site that is overseen and coordinated by one of the TCRB regional biobanks. These sites typically procure specimens but do not engage in long-term biobanking or imaging.

3.6 TCRB Regional BioBank: A site that (in addition to its own pathology sampling) identifies, establishes and coordinates pathology sampling at affiliated TCRB sub-sites. The Regional BioBank receives all research biobank and histology samples from all affiliated sampling sites (including its own) and provides BioBanking and Research Histology Imaging services.

3.7 W-G: Wright Geimsa; hematology stain used on smears, used herein primarily on smears of cell concentrates from pleural or ascities fluids.

3.8 OCT: Optimal Cutting Temperature Embedding Media (Tissue-Tek) for cryopreseration and cryosectioning of tissues.

3.9 Tier 1 and Tier 2 Imaging: Initial semi-quantitative (Tier 1) and secondary fully quantitative (Tier 2) imaging protocols for banked specimens.

4. RESPONSIBILITY

4.1 The pathologist (or designated equivalent) representing TCRB at the TCRB Regional BioBank is responsible for:

4.1.1 Overseeing the outlined tissue procurement process (SOP-100) to ensure that TCRB BioBank tissues are represented by appropriate formalin-fixed or W-G material.

4.1.2 Overseeing the submission of TCRB formalin-fixed material for paraffin embedding and generation of evaluable H&E slides.

4.1.3 Appropriately imaging the H&E or W-G slides, scoring them for required TCRB elements and reporting this information to TCRB, via Acquire, in coordination with the TCRB Regional BioBank.

4.1.4 Comparing the tumor/normal histology with the written pathology report on the specimen and confirming similar histology.

4.1.5 Assisting TBRB Regional BioBank personnel in their response to requests from TCRB to send selected BioBank specimens and H&E or W-G slides to the appropriate TCRB facilities for further processing/imaging.

4.2 The Pathology Sampling Site at which tumor and normal tissue procurement occurs is responsible for:

4.2.1 Ensuring that the appropriate regulatory approvals are in place to allow for collection of any patient samples to be used for research purposes.

4.2.2 Receiving and documenting the required IRB-approved, valid consent from the patient to allow for collection of tumor and normal tissue samples for the TCRB BioBank [note that this may occur after the actual sampling but must occur in accordance with the local IRB-approved TCRB protocol document].

4.2.3 If the Pathology Sampling Site is a sub-site of one of the TCRB Regional BioBanks (currently at Dallas, Houston and Lubbock), notifying the TCRB Regional BioBank that research material has been procured, stabilized and made available for transfer.

4.3 The TCRB Regional BioBank is responsible for:

4.3.1 Training individuals and making arrangements for the collection and transport of tissue samples to the TCRB BioBank from Pathology Sampling Sites.

4.3.2 Ensuring that the appropriate Materials Transfer Agreements and regulatory approvals are in place locally to allow for the transfer of tissue samples, images

and slides to the Human Genome Sequencing Center's Nucleic Acids Core or other approved site.

4.3.3 Working with TCRB Sub Sites to ensure that the appropriate Materials Transfer Agreements are in place to allow for transfer of tissue samples to the TCRB Regional BioBank.

4.3.4 Deidentifying and alphanumerically encoding the patient specimens collected (tumor and normal tissue). This deidentification and encoding can occur through the use of sterile containers with barcodes and labels. It should follow the standard operating procedures of the TCRB Regional Biobank.

4.3.5 Logging in the receipt of the tissue sample(s) in the TCRB site Clinical Sample Log and Acquire.

4.3.6 Confirming that proper documentation of consent is available for each patient tissue sample collected.

4.3.6 Submitting the formalin-fixed tissue or OCT-embedded frozen (if needed) samples to pathology for FFPE and generation of H&E slides to be reviewed and imaged by the TCRB Pathologist.

4.3.7 Verifying that the tissue detailed in the written requisition matches the tissue received by the BioBank and imaged by the TCRB Pathologist.

5. REFERENCES AND APPLICABLE DOCUMENTS

5.1 TCRB BioBank SOP-100 Procurement and Shipment of Tumor, Tissue, and Blood for TCRB

5.2 Attachment #1: TCRB Pathology Collection Form

5.3 TCRB Acquire: https://TCRBAcquire.research.bcm.edu/

6. MATERIALS AND METHODS

A. MATERIALS PRODUCED DURING TISSUE COLLECTION

1.1 Pathology Material
The tissue produced for imaging during sample procurement is described in the Collection SOP (SOP-100) and consists of FFPE tissue from the ends and/or intervening segments of banked tumor and normal tissue. If this material was procured at a TCRB Regional BioBank sub-site, it will be transferred to the TCRB Regional BioBank according to the protocols and procedures in place locally.

1.2 Informed Consent
Informed consent for tissue collection will be obtained from each study participant by the surgical team, either at the time of consent for operative intervention or at some time afterwards according to local IRB-approved protocol. The consent explicitly includes permission for DNA, RNA, and protein extraction, genomic sequencing, release of de-identified clinical information, post-

treatment re-contact, and release of de-identified genomic and clinical information to scientific databases. The informed consent must be received at the TCRB Regional BioBank prior to any activities beyond specimen banking.

1.3 Specimen Coding

At the time of enrollment, procurement or at some point prior to tissue being entered into the TCRB Regional BioBank, patient/samples will be assigned an identification number that must be unique for the TCRB Regional BioBank. This identifier will be associated with all tissue samples collected, as well as all data elements acquired. In the event that the sample qualifies for TCRB, it may subsequently be assigned a different number generated by Aquire/TCRB. The original number may be retained at the TCRB Regional BioBank for local biobanking purposes or it may be changed to the TCRB number, depending on local and TCRB preference.

1.4 Pathology Report

The final pathology report (including any/all addendums) on the specimen from which the banked tumor and normal tissues were procured will be received, coded to remove patient identifiers according to local biobanking protocol, and made a part of the associated clinical data for the banked material.

B. TISSUE TARGETS FOR PROCESSING AND IMAGING

There are three main types of tissues being collected from each patient: Blood, normal tissue and tumor. Blood is being collected by the PaxGene tube method and normal tissue and tumor tissue are being collected for several different types of analytical processes and storage in the TCRB Regional BioBanking Centers for later use by TCRB in the event that they qualify. Tissue sections for FFPE and permanent histology are collected from the tumor and normal tissues as described in SOP-100. These are stored in 10% Neutral Buffered Formalin at the TCRB Regional BioBank, as part of the case, until processing for FFPE section generation, H&E staining and imaging.

C. TISSUE PROCESSING AND IMAGING

H&E Slide Generation

Required are those materials routinely used in the processing, paraffin embedding, sectioning and H&E staining of formalin-fixed or frozen tissues in the histology laboratory of the TCRB Regional BioBank Pathology Department.

Imaging

There are two levels of TCRB imaging:

- Tier 1: Qualitative (initial determination of specimen adequacy for TCRB)
- Tier 2: Quantitative (detailed multi-parametric imaging to more fully characterize each tumor, make images available for wide distribution and allow for quantitative investigation of virtually any captured image data, potentially to correlate with the results of sequencing studies)

Required for Tier 1 imaging are those materials routinely used by Pathology at the TCRB Regional BioBank for imaging of slide-mounted tissue sections, typically a digital camera and associated computer

with software for image processing and storage. Tier 1 imaging is performed on all FFPE sections / W-G smears associated with a banked sample.

Required for Tier 2 imaging is a system capable of high-resolution quantitative image analysis. This system is currently provided by the TCRB Regional BioBank at BCM in Houston. Tier 2 imaging is reserved for those cases that meet TCRB qualification standards and are selected for transfer to BCM for further processing and potential sequence analysis.

D. IMAGING PROCEDURES (TIER 1 AND TIER 2)

Tier 1 (Qualitative) Imaging **The TCRB Regional BioBank Pathologist (The Pathologist) will:**
1. Receive notification from the BioBank that a potential TCRB sample has been procured, coded and banked appropriately, including receipt of the informed consent document and final pathology report.
2. Review the tissue procurement notes to determine if there were any variances in specimen procurement protocol that could affect imaging considerations (i.e. only one FFPE section taken from a small fragment of banked tissue; representative sections taken from homogenized tissue or cell preparations such as cell blocks or smears). This may be done locally or via Acquire in the event that this information has been transferred to Acquire. a. If this is done via Acquire, The Pathologist will mark the appropriate indicator box/button to record his/her review of these notes and provide any comments.
3. Confirm and review the valid informed consent document and final pathology report on the original specimen. This may be done locally or via Acquire in the event that this information has been transferred to Acquire. a. If this is done via Acquire, The Pathologist will mark the appropriate indicator box/button to record his/her review of these notes and provide any comments. b. If there is a problem with either the consent document (e.g. it is not signed) or final pathology report (e.g. the diagnosis does not match the histology as reviewed below from the sampled material), The Pathologist will mark the appropriate indicator box/button in Acquire to indicate that the case cannot proceed further and will provide appropriate comments. i. This action will prompt Acquire to notify the appropriate TCRB oversight entity for awareness and potential resolution of the problem. ii. The Pathologist will note this locally and return all materials to the TCRB Regional BioBank for possible local resolution of the issue according to individual TCRB Regional BioBank procedures and protocols.
4. Submit the formalin-fixed or frozen tissue sections for routine embedding, sectioning and H&E-slide generation as a research specimen, maintaining the coded identification number and specimen identification block numbers (e.g. A2-3) as the identifying numbers on the appropriate cassettes. a. Routine local parameters for fixation, embedding, tissue section thickness and H&E staining will be used unless a TCRB-specific protocol for pathology processing is in place or becomes in place. b. In some instances, in which material in addition to that frozen during intraoperative consultations was not available to TCRB, the original frozen section slides (2 are typically produced, at separate tissue levels) may be the only material available for any further imaging, in which case the following protocol will be followed as closely as possible.
5. Receive and retain 2 consecutive H&E-stained sections (1 each on 2 glass slides) per tissue section. These are part of the research materials and not the original pathology specimen materials. a. These are to be transferred back to the TCRB Regional BioBank to reside with the other materials in the case as the conclusion of The Pathologist's review and imaging.
6. Receive and retain all FFPE blocks from which the slides were cut. These are part of the research materials and not the original pathology specimen materials. a. These are to be transferred back to the TCRB Regional BioBank to reside with the other materials in the case as the conclusion of The Pathologist's review and imaging.

7. Perform digital imaging and image capture using routine image settings and protocols.
    a. Imaging should include any low, medium and high power fields necessary to document criteria evaluated below.
    b. Images should be stored in a loss-less format such as TIFF.
    c. A set of all images on permanent media (e.g. CD or DVD) is to be transferred back to the TCRB Regional BioBank to reside with the other materials in the case as the conclusion of The Pathologist's review and imaging.
8. Rate the following histologic criteria semi-quantitatively for each H&E section (based on TCGA requirements which may be updated in the future) of both tumor and normal tissue:
    a. % Tumor Nuclei
    b. % Necrosis/Hemorrhage
    c. % Stroma/Normal Tissue
9. For each W-G smear, only the percent Tumor cytology and non-tumor cytology are reported.
   Note: An OCT block can be used for percent necrosis, if needed.
10. Report the above 3 parameters for each tissue section in the appropriate place in either the Acquire pathology portion of the case or in Attachment #1: TCRB Pathology Collection Form until such as it can be transferred to Acquire.
    a. The Acquire average for each of these parameters on the banked tissue will be based on the ratings of the two sections taken from the ends of each banked tissue specimen (i.e. A1-2 and A2-3 will be averaged to determine the parameters for banked tissue section A2).
    b. A local record of these ratings is to be transferred back to the TCRB Regional BioBank to reside with the other materials in the case as the conclusion of The Pathologist's review and imaging.
    c. Once all parameters have been reported for all histologic sections, The Pathologist will report to TCRB that a case has been banked and evaluated and will transmit the specimen and imaging data on the case.

Tier 2 (Quantitative) Imaging
The TCRB Tier 2 Imaging Pathologist will:

1. Receive 1 of the 2 glass slides from each section rated in the Tier 1 protocol above.
    a. This will occur as part of the events subsequent to selection of a TCRB case for further analysis. This occurs when TCRB determines that, on Tier 1 reported data, a case exists that suits its current sequencing schedule. At that time, TCRB requests that the Regional BioBank shall send the requested banked tissue (frozen and possibly RNAlater-frozen) to the Nucleic Acids Core Facility for qualitative and quantitative analyses and that the associated H&E / W-G slides shall be sent to the TCRB Regional BioBank Tier 2 Imaging Pathologist (currently Michael Ittmann MD, PhD, at BCM) for Tier 2 imaging.
2. Direct the scanning and quantitative imaging of each slide on an appropriately-equipped imaging platform
    a. Currently Nuance (scanning) and InForm (analysis).
3. Perform and review automated image analysis of TCRB qualifying parameters, genertating a quantitative rating for each.
    a. % Tumor Nuclei
    b. % Necrosis/Hemorrhage (using FS of OCT material if necessary)
    c. % Stroma/Normal Tissue (% non-tumor nuclei in W-G smears)

4. Perform any additional image analyses that may be indicated in the future as part of the Tier 2 protocol.
5. Store the quantitative scanned slide data on appropriate permanent media if indicated by TCRB
    a. As the glass slide will be retained, rescanning is always possible and permanent storage of scanned data may be deemed impractical/cost-prohibitive.
6. Report the results of Tier 2 imaging in the appropriate place in Acquire or in Attachment #1: TCRB Pathology Collection Form until such time as it can be entered into Acquire.
7. Indicate in Acquire or in Attachment #1 that Tier 2 pathology imaging is complete
    a. This will generate a prompt to the Tier 1 pathologist that these results are available, serving as QC/QA review of the Tier 1 estimates.
    b. Together with the results of the DNA/RNA analysis, this will generate the data necessary to qualify a sample for TCRB sequencing. In the event that nucleic acid indices and Tier 2 imaging exceed TCRB minimal thresholds, the Sequencing Core Facility will be prompted that a TCRB-qualified case is ready for sequencing or other relevant analyses.

E. DOCUMENT REVISION HISTORY

Date	Version #	Change Summary
02/02/2012	01	Original

Attachment #1: TCRB Pathology Collection Form

***This collection form is limited to the collecting of specimens for the Texas Cancer Research Bio-bank. Data collected on this form is to be entered into the TCRB Acquire database. ***

TCRB Pathology Collection Form

Tumor Specimen and Aliquot Pathology

Please record the pathology of the Tumor Specimen and the individual aliquots if available. The pathology of the individual aliquots is used to determine the best aliquot(s) of the specimen for RNA/DNA extraction as quality varies from location within the tumor.

1. Specimen Barcode: **Specimen Label:**	**Percent Tumor Nuclei:**	**Percent Tumor Necrosis:**	**Percent Stroma:**
2. Specimen Barcode: **Specimen Label:**	**Percent Tumor Nuclei:**	**Percent Tumor Necrosis:**	**Percent Stroma:**
3. Specimen Barcode: **Specimen Label:**	**Percent Tumor Nuclei:**	**Percent Tumor Necrosis:**	**Percent Stroma:**
4. Specimen Barcode: **Specimen Label:**	**Percent Tumor Nuclei:**	**Percent Tumor Necrosis:**	**Percent Stroma:**
5. Specimen Barcode: **Specimen Label:**	**Percent Tumor Nuclei:**	**Percent Tumor Necrosis:**	**Percent Stroma:**

Tumor or Normal Specimen Barcode and Label Fields: Sticking a printed copy of the respective specimen label or barcode on the form is an accurate method to complete these fields. The label may also be hand written, but a copy of the printed label is preferred to eliminate transfer errors.