Cancer Proteomics Tumor Analysis Consortium
Prospective Biospecimen Collection Protocol
Breast Cancer
v1.9

Overview

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) sponsored by the NCI Office of Cancer Clinical Proteomics Research is a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust, quantitative, proteomic technologies and workflows. The overarching goal of CPTAC is to improve our ability to diagnose, treat and prevent cancer. To achieve this goal in a scientifically rigorous manner, the NCI launched CPTAC to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provide this data with accompanying assays and protocols to the public.

CPTAC consists of a network of Proteome Characterizations Centers (PCC) and a Data Coordinating Center (DCC) serving as a hub and central repository for CPTAC data. CPTAC will be expanded to include 1) a network of Tissue Source Sites (TSS) to obtain clinical specimens for proteomic and genomic analysis, 2) a Biospecimen Core Resource (BCR) to serve as a repository for tissue and associated, de-identified clinical data submitted to the program, and 3) a Genomic Characterization Center (GCC) dedicated to the genomic analysis of CPTAC specimens.

Purpose

The purpose of the three options in this protocol are to establish minimum procurement parameters for ductal and lobular breast cancer stage IIA – IIIC specimens to be submitted to the CPTAC for proteomic and genomic analysis. Tissue will be obtained from newly diagnosed, untreated patients undergoing definitive surgery for breast cancer or by needle core biopsy at the time of placement of a vascular access device prior to neoadjuvant therapy for breast cancer.

The protocol builds on CPTAC experience with human tissues obtained from The Cancer Genome Atlas (TCGA) program and specifically aims for:

- Minimized specimen processing and ischemia time with the ischemia time recorded.
- Sufficient total material from each patient divided into multiple, homogeneous samples suitable for independent processing for proteomic and genomic analysis.
- Histological assessment and quality assurance by the BCR (through frozen sectioning) of all tissue specimens utilized for each analytical platform.

- Improved determination of weights of individual samples for improved estimates of protein yield.

Scope

The three options described in this protocol apply to any breast cancer patient biospecimens submitted by a Leidos Biomedical Research, Inc. subcontractor to the CPTAC BCR.

Requirements

Patient Inclusion Criteria

- Newly diagnosed patients with invasive ductal or lobular breast cancer, Stage IIa-IIIC, undergoing either definitive surgical tumor resection for breast cancer OR placement of a vascular access device as a prelude to neoadjuvant therapy for breast cancer. The inclusion criteria include patients with more than one newly observed and independent breast masses.

Patient Exclusion Criteria

- Prior history of other malignancies within the past 12 months other than treated basal cell carcinoma of the skin or surgery-only treated DCIS of the ipsilateral or contralateral breast (as long as no tamoxifen was administered).
- Other malignancies at the time of surgery.
- Any prior systemic chemotherapy, endocrine therapy or biological therapy for any cancer.
- Prior history of radiation therapy involving the breast such as mantle field radiation for Hodgkins Disease, radiotherapy for lung cancer, etc.
- Patients who are found to have a diagnosis other than invasive breast cancer as a result of the surgery.

Regulatory (before procurement)

- IRB approval received and documented with the CPTAC BCR.
- MTA/DUA agreement received and documented with the CPTAC BCR.

Tissue Procurement and Shipping

- Signed patient consent (maintained at the tissue source site, copy to CPTAC BCR not required).
- Cancer tissue per protocol.
- Normal tissue per protocol.
- Blood per protocol.
- Shipping Manifest completed and accompanying tissue shipment.
- CPTAC Tissue Submission Form (contains details regarding procurement along with minimal patient information) completed and electronically submitted within 1-2 working days after tissue procurement Secure access to the electronic clinical data management system with the CRF to be provided by the CPTAC BCR.

- Adherence to BCR shipping instructions (the BCR will provide the shipping cryoport and cover the cost of shipping).

Patient Data

- CPTAC Baseline Case Report Form containing the patient's history and status at surgery along with diagnostic information completed and electronically submitted. Secure access to the electronic clinical data management system with the CRF to be provided by the CPTAC BCR.
- Pathology Report (de-identified, following the guidelines from to the 7th Edition 2013 AJCC) including ER, PgR, and HER2 status submitted within 5 days after overall (pathology + molecular) qualification. This may be in the form of a scanned document submitted electronically.
- At least one representative image of a hematoxylin/eosin-stained slide from a formalin-fixed piece of the carcinoma in SVS, JPG, or TIFF format. FFPE H&E diagnostic slides/images representative of the diagnosis in the pathology report should be submitted within 5 days after overall (pathology + molecular) qualification. Slides will be returned.
- CPTAC One-Year Case Report Form with updated history and status one year after the date of CPTAC tissue procurement. Secure access to the electronic clinical data management system with the CRF to be provided by the CPTAC BCR.

Tumor Specimen Inclusion Criteria

- Two or more tumor tissue core biopsies or surgical resection segments that together total more than 200 mg and on average are composed of greater than 60% tumor cell nuclei and less than 20% tumor necrosis, as judged by frozen tissue section review of each core or tumor segment.
- Thirty minutes or less total ischemia time.

Tissue Procurement Procedures

<u>Option 1 – Tumor Excision at Lumpectomy/Mastectomy</u>

In this approach the tumor in a patient who has received no neoadjuvant therapy is removed as per standard of care for a surgical resection procedure. The pathologist and/or surgeon must quickly determine what proportion of the tumor is required for clinical diagnostics and whether and what remaining tumor tissue can be sampled and frozen for research purposes. When in doubt, the surgical resection specimen must always be properly preserved for clinical assessment.

Tumor Tissue Procurement

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue embedding and freezing. Label each cryomold with an appropriate ID and "T" to indicate tumor tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Excise the tumor with the goal of minimizing time of ischemia to the tissue. Record the time the tumor was excised.

- Divide the tumor tissue which is procured for research purposes and not needed for clinical management into segments that are no larger than 1 cm x 1 cm x 0.5 cm. Depending upon the size of the tumor, 2-4 such tumor tissue segments may be procured, embedded, and frozen.

- Gently place each tissue segment in the well of an "Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx). The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6). Place the tissue flat in the cryomold, with its largest two dimensions sitting parallel to the bottom of the mold. Avoid placing the tissue in the mold in any other orientation (Fig. 7). Each tissue segment should be placed in a different cryomold.

- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the CryoCooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

- Record time when segments are frozen. No more than **30 minutes** should have elapsed from the time of excision to freezing.
- Store at vapor phase LN2 temperature until shipping.

Normal Tissue

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue embedding and freezing. Label each cryomold with an appropriate ID and "N" to indicate normal tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Divide the non-malignant tissue which is procured for research purposes and not needed for clinical management into segments that are no larger than 1 cm x 1 cm x 0.5 cm. Depending upon the size of the normal breast tissue, 2-4 such tissue segments may be procured, embedded, and frozen. Sample non-malignant tissue as far from the tumor site as possible.

- Gently place each segment in the well of an "Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx). The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6). Place the tissue flat in the cryomold, with its largest two dimensions sitting parallel to the bottom of the mold. Avoid placing the tissue in the mold in any other orientation (Fig. 7). Each segment shall be placed in a different cryomold.

- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

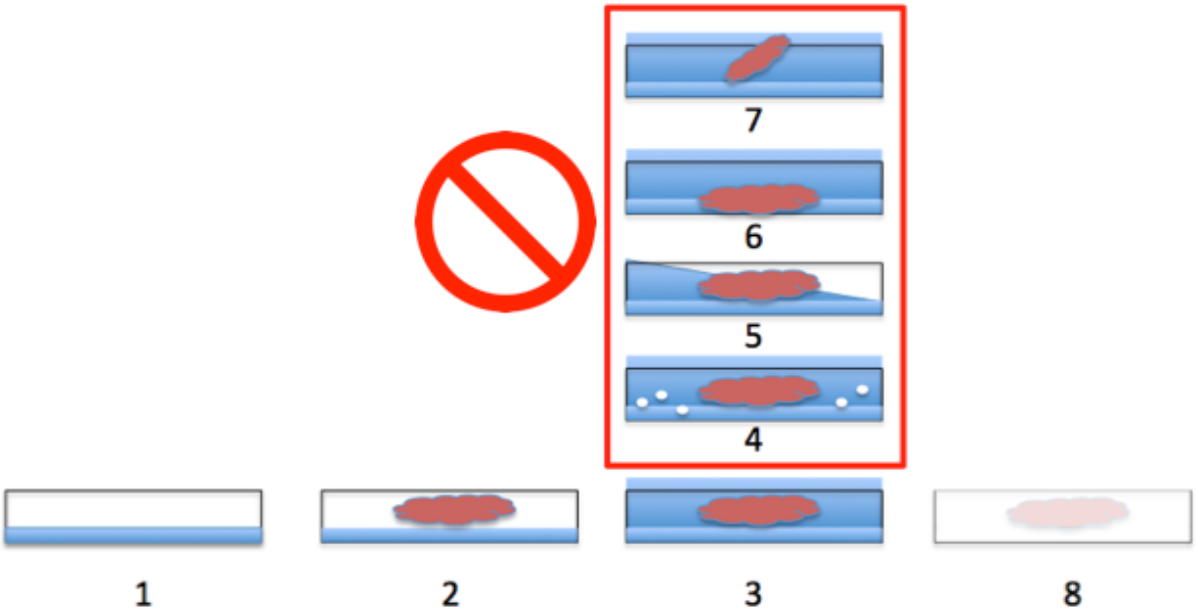
- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the CryoCooler. Lay the mold flat in the vapor phase; do not

tilt the mold and ensure that OCT is evenly covering the entirety of the tissue face (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

- Record time when segments are frozen. No more than **30 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping



Figures 1-8. Steps for embedding and freezing fresh tissue in OCT compound. See protocol. Figures 1, 2, 3, and 8- proper steps for embedding. Figures 4-7- improper methods for embedding- (4) air bubbles in OCT compound; (5) OCT not evenly covering tissue; (6) tissue sitting against floor of cryomold; (7) tissue not oriented flat in the mold.

Option 2 - Large-Bore Percutaneous Tumor Coring Before Lumpectomy/Mastectomy

In this approach the tumor in a patient who has received no neoadjuvant therapy is sampled by needle core biopsy *in situ* prior to being removed as per standard of care for a surgical resection procedure. It is the clinical judgment of the surgeon to determine whether the tumor is of sufficient size to perform a needle core biopsy prior to surgical excision and pathological evaluation without compromising the diagnostic integrity of the specimen. When in doubt, the surgical resection specimen must always be properly preserved for clinical assessment.

Tumor Tissue Cores

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue core embedding and freezing. Label each cryomold with an appropriate ID and "T" to indicate tumor tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

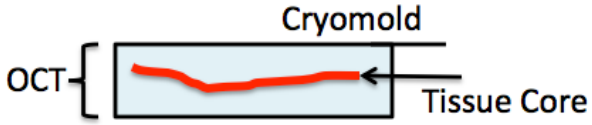
- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Obtain the large-bore (~10 ga.) needle core specimens to be submitted to the CPTAC BCR from the tumor (minimum of 2 attempts, 3 or more preferred).

- Record the time each core is obtained.

- Ensure that the core is devoid of excess fluid or blood, but do not attempt to 'blot' the tissue core with gauze.

- Gently place each core longitudinally (see below) into the cryomold, ensuring that it is not touching the bottom of the mold. The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6).



- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the CryoCooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue face (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

- Record time when segments are frozen. No more than **30 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping.

Normal Tissue Cores from the Same Patient

- Identify normal appearing breast tissue as far from the tumor as possible.

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue core embedding and freezing. Label each cryomold with an appropriate ID and "N" to indicate normal tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

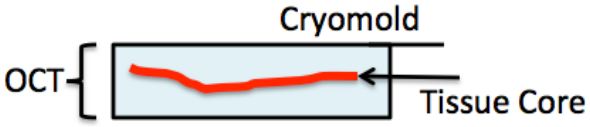
- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Record the time each core is obtained.

- Obtain the large-bore (~10 ga.) samples to be submitted to the CPTAC BCR from the normal breast tissue (minimum of 2 attempts, 3 or more preferred).

- Ensure that the core is devoid of excess fluid or blood, but do not attempt to 'blot' the tissue core with gauze.

- Gently place each core longitudinally into the cryomold (see below), ensuring that it is not touching the bottom of the mold. The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6).



- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the cryocooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue face (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

- Record time when segments are frozen. No more than **30 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping.

Option 3 - Large-Bore Percutaneous Coring During Port Placement

In this approach the tumor in a patient who has received no neoadjuvant therapy is sampled by needle core biopsy at the time of port placement. It is the clinical judgment of the surgeon to determine whether the tumor is of sufficient size to perform an accurate needle core biopsy without impacting the clinical management of the patient. Tissue procurement procedures are identical to those described in *Option 2*.

Tumor Tissue Cores

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue core embedding and freezing. Label each cryomold with an appropriate ID and "T" to indicate tumor tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

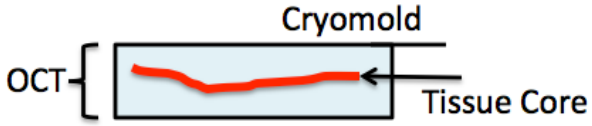
- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Obtain the large-bore (~10 ga.) needle core specimens to be submitted to the CPTAC BCR from the tumor (minimum of 2 attempts, 3 or more preferred).

- Record the time each core is obtained.

- Ensure that the core is devoid of excess fluid or blood, but do not attempt to 'blot' the tissue core with gauze.

- Gently place each core longitudinally (see below) into the cryomold, ensuring that it is not touching the bottom of the mold. The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6).



- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the CryoCooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue face (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

- Record time when segments are frozen. No more than **30 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping.

Normal Tissue Cores from the Same Patient

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue core embedding and freezing. Label each cryomold with an appropriate ID and "N" to indicate normal tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

- Identify normal appearing breast tissue as far from the tumor as possible.

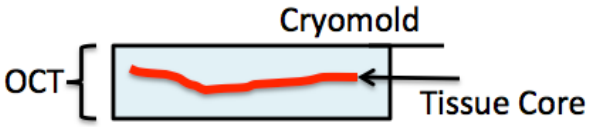
- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Obtain the large-bore (~10 ga.) samples to be submitted to the CPTAC BCR from the normal breast tissue (minimum of 2 attempts, 3 or more preferred).

- Record the time each core is obtained.

- Ensure that the core is devoid of excess fluid or blood, but do not attempt to 'blot' the tissue core with gauze.

- Gently place each core longitudinally into the cryomold (see below), ensuring that it is not touching the bottom of the mold. The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6).



- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the cryocooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue face (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

- Record time when segments are frozen. No more than **30 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping.

Blood Collection

- Peripheral venous blood **MUST** be collected prior to administration of anesthesia.
- Obtain 10 ml of peripheral whole blood collected by standard venous phlebotomy. The blood should be collected in the KEDTA (lavender top) vacutainer tube provided with the biospecimen procurement kit.

- Whole blood specimens should be processed and frozen as per CPTAC blood processing protocol within 1 hour of collection.

References:

Wilson, R and Kavin, S, Comparison of Large-Core Vacuum-Assisted Biopsy and Excision Systems *In* Brun del Re, R. (Ed.) Minimally Invasive Breast Biopsies, Springer, pp. 23-41, 2010.

Tebbit CL, Zhai J, Untch BR, Ellis MJ, Dressman HK, Bentley RC, Baker JA, Marcom PK, Nevins JR, Marks JR, Olson JA Jr. Novel tumor sampling strategies to enable microarray gene expression signatures in breast cancer: a study to determine feasibility and reproducibility in the context of clinical care. Breast Cancer Res Treat. 2009 Dec;118(3):635-43. 2009 Feb 18. PubMed PMID: 19224362; PubMed Central PMCID: PMC3786337.