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Cross Consortium Benchmarking Project - UMN Tissue Resecting and Processing Protocol V.1

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Cellular Senescence Net...

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the tissue processing from surgical procurement of the organ to histological processing into FFPE and OCT Frozen blocks.

Surgical Procurement Protocol provided by surgical fellow Abraham Matar
Lung and skin resecting and processing protocol provided by Mythili Dileepan
Histology protocols provided by UMN BLS Histology Core - Colleen Forster and Adam Lewis

Guidelines

Ethics Statement:
This protocol requires prior approval by the users' local Institutional Animal Care and Use Committee (IACUC), Institutional Review Board (IRB) or equivalent ethics committee(s), depending on the source of the tissue.

Surgical Procurement

1 **Lung Sample:**
1. Prior to excision the main bronchus is stapled with a TA Stapler. Similarly, the pulmonary
artery and vein are stapled with a TA stapler, and the right lung is removed from the pleural
cavity and stored in a sterile basin filled with ice.
2. The right upper lung lobe is flushed with 500 ml of cold sterile saline
3. A piece of the upper lung lobe is excised with dimensions of 8 × 6 × 0.5 cm^3.
4. The lateral and superior (cephalic) borders are marked with a marking pen.
5. The samples are then passed off to the researcher.

2 **Skin Sample:**
1. Abdominal skin from the right lower flank is excised with dimensions of 4 × 8 × 0.5 cm^3.
2. The lateral and superior (cephalic) borders are marked with a marking pen.
3. The samples are then passed off to the researcher.

Lung Tissue Resecting

3 **In OR:**
1. Receives an 8 × 6 × 0.5 cm^3 lung piece from a surgeon. The lung piece is cut into two pieces with
dimensions of 4 × 6 × 0.5 cm^3. The far-left (lateral) piece is called Wedge A, while the other piece, closer to the medial side, is called Wedge B.
2. Both wedges are then cut in half again, resulting in pieces with dimensions of 4 × 3 × 0.5 cm^3. The lateral-cephalic corner of all four blocks is marked with red paint.
3. The upper portions are placed directly into 10% neutral buffered
formalin (NBF) at room temperature, while the lower portions are placed in RPMI 1640 Medium with HEPES on ice, appropriately labeled in their respective containers.
4. Samples brought to gross room.

4 **In Gross Room:**

FFPE Samples -
1. Place 10% NBF wedges on shaker for 2 hours at room temperature
2. After 2 hours, the pieces 10%NBF will be excised according to the diagram and marked with paint according to the color system depicted in the accompanying diagram to maintain the orientation of each piece in relation to the organ's anatomical position.
3. Once tissue is cut to size and color marked, place into appropriately labeled 15-ml tubes containing 10% NBF.
4. Incubate at 4°C for 48 hours.

5. After 48 hours, wash the pieces in distilled water and place in 70% ethanol made with diethylpyrocarbonate (DEPC) water.

Fresh Frozen Samples -

1. Prepare isopentane bath by adding isopentane to a metal container sitting in dry ice for at least 30 minutes prior to use.
1. Fresh tissue sections will be excised according to specifications and marked with paint according to the color system depicted in the accompanying diagram to maintain the orientation of each piece in relation to the organ's anatomical position.
2. Place marked samples in cold isopentane for 4-5 minutes.
3. Once frozen, tissue sections are placed into labeled cryovials and stored at -80°C.

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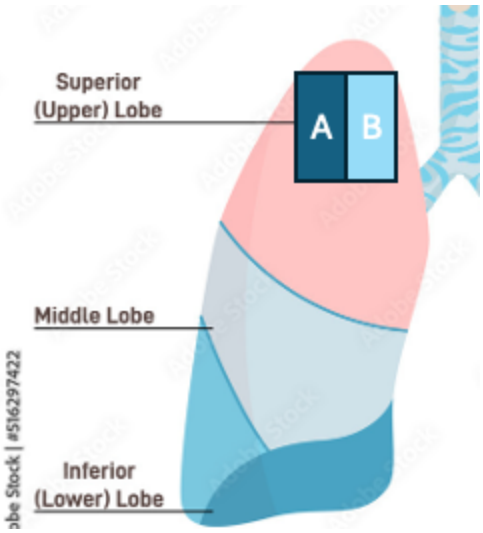
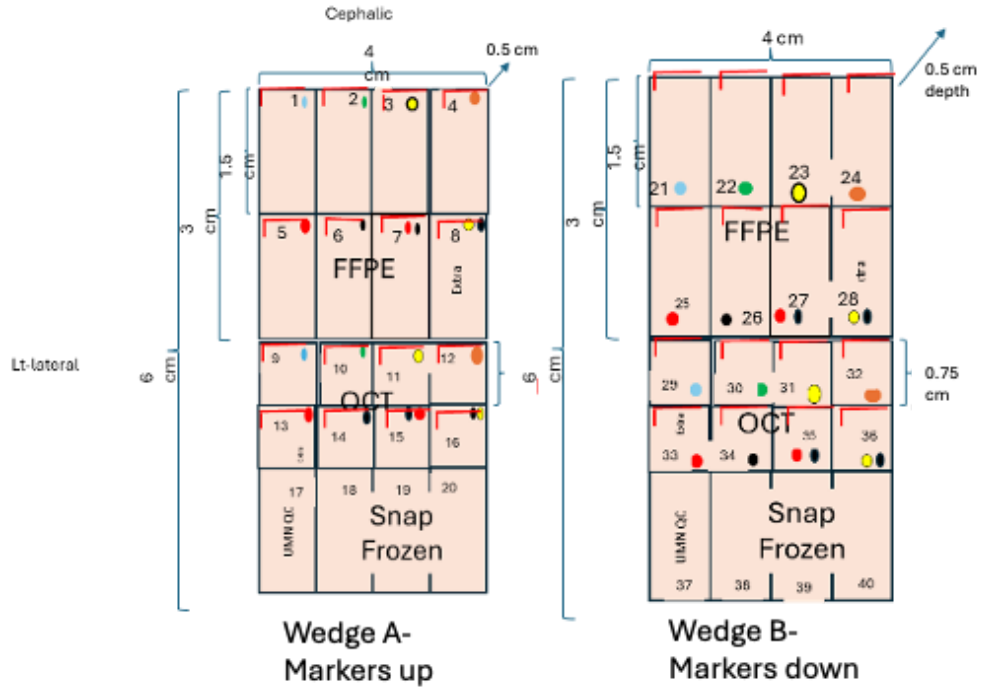


Lung Tissue Block Location

6 Sample Location Diagram



Lung Sample Location Diagram

Skin Tissue Resecting

7 **FFPE Samples -**

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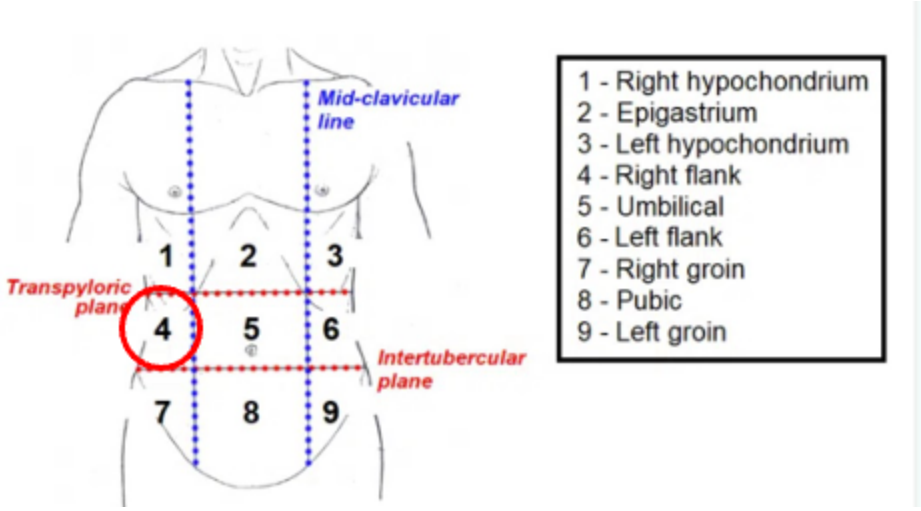

Skin Tissue Block Location

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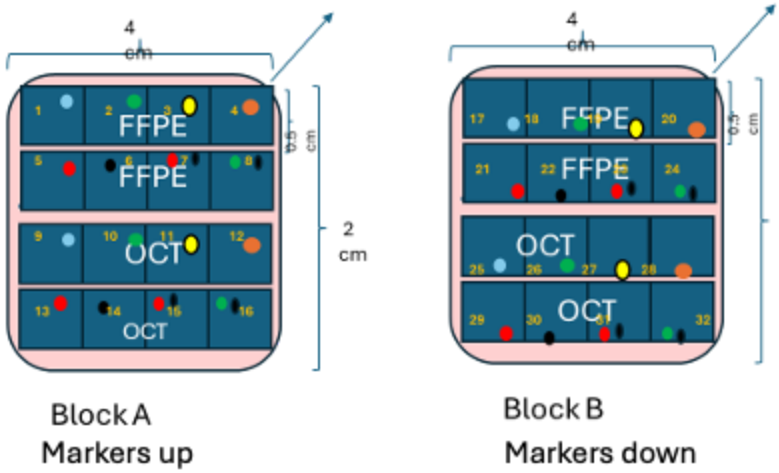

Skin Sample Location Diagram

FFPE Blocking

10 dx.doi.org/10.17504/protocols.io.yxmvme3d9g3p/v1

OCT Blocking

11 **Tissue Blocking Protocol for OCT Embedding**

Purpose
This protocol describes the procedure for embedding an already frozen tissue sample into Optimal Cutting Temperature (OCT) compound. The process includes orienting the tissue, embedding it in OCT, curing the compound, and labeling the block, all done inside a cryostat at -20°C for subsequent sectioning.

Scope
This protocol applies to tissue samples that have already been frozen and are now ready for blocking in OCT compound for subsequent sectioning and imaging.

Materials and Reagents
Embedding Medium: Optimal Cutting Temperature (OCT) compound (commercially available).
Embedding Molds: Metal or plastic molds for embedding and orienting tissue.
Cryostat: For embedding, maintaining frozen tissue, and curing OCT at -20°C.
Specimen Labels: To identify the tissue block (Sharpie marker).

Preparation
Step 1.1: Ensure the tissue is fully frozen before beginning the embedding process. The tissue should have been frozen and maintained at a temperature of -20°C or lower.

Embedding Process
Step 2.1: Place a small amount of OCT compound into a metal or plastic embedding mold.
Step 2.2: Inside the cryostat at -20°C, orient the already frozen tissue within the mold, ensuring the cutting surface (the surface that will be sectioned) is facing up. Proper orientation is essential for obtaining high-quality sections.
Step 2.3: Carefully fill the mold with additional OCT compound to completely encase the tissue, ensuring there are no air bubbles trapped in the medium.

Freezing and Curing
Step 3.1: Once the tissue is properly oriented and fully encased in OCT, allow the mold with the tissue and OCT to remain inside the cryostat at -20°C. Allow the OCT compound to freeze and cure completely. This process should take approximately 30 minutes to 1 hour.
Step 3.2: Verify that the OCT has fully solidified and that the block is firm and stable before proceeding to the next step.

Labeling

Step 4.1: Once the OCT block is fully solidified and cured, carefully remove the tissue block from the mold.

Step 4.2: Using a Sharpie marker, label the tissue block with the specimen ID to ensure proper identification during sectioning and imaging.

Acknowledgements

LifeSource - **https://www.life-source.org/**