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UPitt TriState SenNet TMC Tissue Collection

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TriState SenNet | Cellular Senescence Net...

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We use this protocol and it's working

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Abstract

This document outlines the tissue sampling of lung and heart specimens at the TriState SenNet TMC Biospecimen Core at the University of Pittsburgh, as part of the Cellular Senescence Network Program (SenNet).

Troubleshooting

Preparation

1 Remove specimen from shipping or storage container.

2 Place specimen inside the hood over dissection table and photograph prior to sampling.

3 Select the sites from which samples will be collected, annotate and mark (if serial sampling is done)

3.1 For lung:
1. Pleura (A)
2. Broncho-vascular bundle (B)
3. Parenchyma (C)

3.2 For heart:
1. Left Ventricle (D)
2. Right Ventricle (E)
3. Left Atrium (F)
4. Right Atrium (G)

3.3 (OPTIONAL - If needed)
Wash the full-thickness core in cold PBS in a petri dish.

Flash freezing and storing

4 Cut each of the selected regions from the previous step into 9 small chunks. Chunks should be small enough to just cover the bottom of a microfuge tube (~5mm x 5mm x 5mm).

5 Dab the small chunks on a sterile gauze pad and place each one into a labeled microfuge tube.

6 Place 9 small chunks into the empty tubes to be snap frozen.
Drop tubes into liquid Nitrogen.
Leave for ~2-10 minutes.
Remove and store in -80C freezer.

Storing in formalin

7 Place one piece of the selected regions into a tubes containing 20x volume of 10% formalin (to be processed into FFPE blocks).
Store in 4C fridge.

Note: Fixation occurs at a rate of 1 mm per hour. Over-fixation causes tight cross-linking and under-fixation can compromise the tissue.

7.1 Make a notation of the time the sample went into formalin.

7.2 Based on the size of the tissue, fix the required length of time (4 to 24 hours).

7.3 After the fixation period, remove the cassette and place in 70% ETOH until scheduled processing.

Storing for fresh processing

8 Place one piece of the selected regions into a tube with cold DMEM with anti/anti.
Store in 4C fridge.

Process as soon as possible.