

May 26, 2023

U54 SCENT Normal Lung Surgical Tissue Collection Procedure

DOI

dx.doi.org/10.17504/protocols.io.n92ldpozxl5b/v1



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Protocol Citation: Carolyn Glass, andrew.nixon, Matthew Hartwig, Nancy McGreal, SSCRS, Mary Jordan* 2023. U54 SCENT Normal Lung Surgical Tissue Collection Procedure. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.n92ldpozxl5b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: May 25, 2023

Last Modified: May 26, 2023

Protocol Integer ID: 82483

Keywords: u54 scent normal lung surgical tissue collection procedure, collection of lung tissue, lung tissue, surgical resection at duke university hospital, department of pathology, precision pathology center, tissue, duke university hospital, surgical resection

Funders Acknowledgements:

NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director
Grant ID: U54AG075936

Abstract

This document outlines the required criteria for inclusion and collection of lung tissue obtained by surgical resection at Duke University Hospital through the Biorepository and Precision Pathology Center (BPRC) in the Department of Pathology.



Materials

Personal Protective Equipment:

- Lab coat
- Gloves

Equipment:

- Leica Autostainer XL
- Microscope

Materials:

- Bleach
- Xylene
- Alcohol, 100%
- Alcohol, 95%
- Alcohol, 80%
- Hematoxylin - Pureview Hematoxylin 660+
- Eosin - Pureview Eosin 615
- Clearing Solution - Pureview Focus-AQ
- Bluing - Pureview PH Blue
- Chemical waste container
- Wypall pads
- Slide staining rack
- Pre-cut H&E control slide

Troubleshooting

Inclusion and Exclusion Criteria

1 Inclusion Criteria:

- Any age
- All sexes
- Grossly normal lung obtainable through surgical pathology

Exclusion Criteria:

- No recent treatment with chemotherapy (recent <1 year)
- No recent treatment with radiation (recent <1 year)
- No history of COVID
- No recent colds (<1 year),etc
- If smoker or past smoker, check emphysematous changes in CT

Notes:

- Check recent CT scans to determine if there is evidence of acute or chronic on-going disease

After Collection:

- For quality assurance, H&Es of all grossly normal lung specimens obtained will be generated by the BRPC and reviewed by pathologist to ensure microscopic normal lung.

Collection Protocol - Adult Tissue

2

U54Clinical Research Coordinator will screen for lung resection cases where normal tissue may be available. Refer to inclusion and exclusion criteria for surgical resection for U54.

3

U54Clinical Research Coordinator will approach patients for consent and enroll patients in BRPC study at Duke when consent occurs.

4

Upon day of collection, U54Clinical Research Coordinator will be notified of tissue availability from Surgical Pathology

5

Receive 1 fragment of normal lung tissue (total size collected will be 3×3×3 cm; can take less if not available) from the resected site.

6

Process the fragment into the following samples. Make note of the weight and dimensions of each sample. Create a QC for each sample collected:



- a. FFPE (3×3×0.5 cm)
 - b. Snap frozen: split in half
 - i. ½ snap freeze in cryovial
 - ii. ½ will be cut into “rice-size piece”
 1. Wash tissue with PBS to remove the blood and epithelial lining fluid.
 2. Using blade, mince tissue into approximately 20 small pieces (1-2mm).
 3. Evenly distribute the rice sized pieces into 3 cryopreserved tubes with 1mL freezing media each.
 4. Cells should be frozen slowly at 1°C/min. Put tubes in the freezing container (e.g., Mr. Frosty) with isopropyl alcohol and place them overnight in a -80°C freezer.
 5. Next day, transfer tubes to liquid nitrogen
 - c. If tissue remaining: 3 additional cryovials of snap frozen tissue cut into rice sized pieces and OCT.
- 7 Route FFPE samples to Research Histology Lab to:
- a. Create an H&E from each FFPE sample and route to the pathologist for review.
 - b. After review, create 2 tissue scrolls from the tissue blocks and coordinate RNA and DNA extraction.
 - c. Once RNA and DNA extraction is complete, the frozen LN2 samples will be shipped to lab for scRNAseq

Collection Protocol - Adult Blood

- 8 U54Clinical Research Coordinator will coordinate to have blood tubes dropped off prior to the procedure, if patients consented to BRPC optional blood component(s). An email will be sent to the Substrate Services Core Research Support regarding blood facilitation the day before surgery.
- 9 Drop off: (label each tube with MRN and BRPC ID)
 - a. 2 × 10mL EDTA tubes
 - b. 3 × 8.5mL ACD tubes
- 10 Make a note to prioritize EDTA tubes over ACD tubes if not all can be filled.
- 11 Once tubes are available, notify the Substrate Services Core Research Support of blood for pickup via email and provide transport form with the available blood tubes. They will complete further processing.

Collection Protocol - Pediatric Tissue

- 12 U54 Clinical Research Coordinator will screen for lung resection cases where normal tissue may be available. Refer to inclusion and exclusion criteria for surgical resection for U54.
- 13 U54 Clinical Research Coordinator or BRPC team member will approach patient's in-person for consent and enroll patients in BRPC study at Duke when consent occurs.
- 14 Upon day of collection, U54 Clinical Research Coordinator will be notified of tissue availability from Surgical Pathology.
- 15 Receive 1 fragment of normal lung tissue (total size collected will be 3×3×3 cm; can take less if not available) from the resected site.
- 16 Process the fragment into the following samples. Make note of the weight and dimensions of each sample. Create a QC for each sample collected:
 - a. FFPE (3×3×0.5 cm)
 - b. Snap frozen: split in half
 - i. ½ snap freeze in cryovial as normal
 - ii. ½ will be cut into "rice-size piece"
 1. Wash tissue with PBS to remove the blood and epithelial lining fluid.
 2. Using blade, mince tissue into approximately 20 small pieces (1-2mm).
 3. Evenly distribute the rice sized pieces into 3 cryopreserved tubes with 1mL freezing media each.
 4. Cells should be frozen slowly at 1°C/min. Put tubes in the freezing container (e.g., Mr. Frosty) with isopropyl alcohol and place them overnight in a -80°C freezer.
 5. Next day, transfer tubes to liquid nitrogen.
 - c. If tissue remaining: 3 additional cryovials of snap frozen tissue cut into rice sized pieces and OCT.
- 17 Route FFPE samples to Research Histology Lab to:
 - a. Create an H&E from each FFPE sample and route to the pathologist for review.
 - b. After review, create 2 tissue scrolls from the tissue blocks and coordinate RNA and DNA extraction.
 - c. Once RNA and DNA extraction is complete, the frozen LN2 samples will be shipped to lab for scRNAseq.



Collection Protocol - Pediatric Blood

- 18 U54 Clinical Research Coordinator will coordinate to have blood tubes dropped off prior to the procedure, if patients consented to BRPC optional blood component(s). An email will be sent to the Substrate Services Core Research Support regarding blood facilitation the day before surgery.
- 19 Drop off: (label each tube with MRN and BRPC ID)
 - a. 2 × 4mL EDTA tubes
- 20 Once tubes are available, notify the Substrate Services Core Research Support of blood for pickup via email and provide transport form with the available blood tubes. They will complete further processing.