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624.1_URMC_HTC_Cryosectioning Non-Inflated Fresh-Frozen Embedded Lung for Nuclear Isolation or Multi-plexed In Situ Hybridization

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Gloria S Pryhuber[1], Cory Poole[2]

[1]University of Rochester Medical Center; [2]University of Rochester

Human BioMolecular Atl... LungMap2 Consortium 2 more workspaces

Gloria S Pryhuber
University of Rochester Medical Center

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We use this protocol and it's working

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Abstract

This protocol describes steps to prepare non-fixed, fresh frozen tissue that has been embedded in OCT, CMC or other embedding material.
The protocol has been used in preparation for DARTFISH and MERFISH assays, as well as for representative serial section H&E stains of the tissue blocks used in these assays.

Guidelines

Care is taken to reduce RNA degradation including preventing introduction of RNases.
The optimal cutting temperature of the cryostat will depend on the tissue and the embedding material.

Materials

·RNaseZAP – Sigma, R2020-250ML
·SuperFrost Plus – FisherBrand, 22-037-246
·Accu-Edge High Profile Blades – VWR, 25608-961
·P200 Pipette with tips
·15 mL conical tube with about 10 mL ultrapure water
·VectaBond treated Coverglass – Provided by outside lab

Troubleshooting

Safety warnings

Microtome blades are sharp and unfixed tissue has the ability to more easily pass along various pathogens.
Wear a lab coat, gloves, and any required PPE while preparing these samples

Before start

Label the slides or containers for the cryosections to be made as needed.

Tissue Processing – Cutting Fresh Frozen Lung Sections for FISH

1 Prepare the SuperFrost Plus slides by placing them into a staining rack.

2 Rinse the slides with ultrapure water and spray them with RNaseZAP, allowing them to sit for a few minutes.

3 Rinse the slides with more ultrapure water and, while keeping them in the slide rack, dry them on the slide warmer for about 20 minutes. Keep preparing the cryostat during this time.

4 Spray the inside of the cryostat with RNaseZAP and after 3 minutes wipe down the inside with 100% ethanol making sure the chamber is dry.

5 Place a new blade into the chamber and allow to cool for 5 minutes. During this time, place the tissue block to be sectioned onto the chuck, or aside in the cryostat chamber if cutting more than one, and allow them to equilibrate to the cryostat chamber temperature

6 Place the sample (example, fresh frozen embedded in OCT, stored at -80) to be cut on the chuck and trim until a full section can be cut.

7 Cut sections at the desired thickness, generally 10 micron, and place the section onto a room temperature treated coverglass.

8 Pipette 50 µL of water from the 15 mL conical onto the correctly labeled slide for the section.

9 Place the section with the TISSUE SIDE UP onto the slide. Place into the cryostat to allow the water to freeze, adhering the section to the slide.

10 Place the slide into a slidebox on dry ice or in the chamber until finished.

11 SWITCH BLADES BETWEEN DONORS to prevent contamination of nuclei/cell material between donors.

12 Repeat until finished, keeping slides frozen throughout. Place slides in slide box equilibrated to temperature in the cryostat chamber.

13 Slides may be stored (briefly) at -80 but it is thought to be better to use or, if needed, ship on dry ice, within 24-48 hours of sectioning

14 Analysis of RNA quality of the tissue being sectioned can be achieved by isolating RNA from 2 – 20 µm tissue sections and testing for RIN and DV200.

Tissue Processing - Cutting Frozen Tissue Curls for Single Nuclei Isolation

15 Label cryovials appropriate for the tissue section curls to be cut

16 Spray the inside of the cryostat with RNaseZAP and after 3 minutes wipe down the inside with 100% ethanol making sure the chamber is dry.

17 Place a new blade into the chamber and allow to cool for 5 minutes. During this time, place the tissue block to be sectioned onto the chuck, or aside in the cryostat chamber if cutting more than one, and allow them to equilibrate to the cryostat chamber temperature

18 Place the sample (example, fresh frozen embedded in OCT, stored at -80) to be cut on the chuck and trim until a full section can be cut.

19 Cut the tissue sections for sn isolation at the desired thickness, generally 40 micron, allowing the tissue to curl. Gently recover tissue curl into a labelled cryovial. May place up to ~ 10 curls into a 1.5 ml cryovial. The thickness and number of sections collected per tissue block will depend on tissue type, density and desired yield.

20 Approximately every 10 thick section, cut 1 or 2 full face, serial 10 micron sections on SuperFrost Plus slides to stain with H&E to demonstrate morphology of the tissue being collected for sn isolation.

21 SWITCH BLADES BETWEEN DONORS to prevent contamination of nuclei/cell material between donors.

22 Cryovials containing cryosectioned tissue curls may be shipped on dry ice

23 Analysis of RNA quality of the tissue being sectioned can be achieved by isolating RNA from 2 – 20 µm tissue sections or isolated nuclei and testing for RIN and DV200.