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

Yale Murine TMC - Frozen Tissue Sectioning Protocol for Spatial Transcriptomics (DBiTSeq) and Immunofluorescence

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ABSTRACT

Protocol for frozen tissue sectioning for use in DBiTSeq and immunofluorescence staining

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88925

Tissue Embedding

1 Embed tissue according to this protocol: dx.doi.org/10.17504/protocols.io.kqdg3x8keg25/v1

Cryosectioning

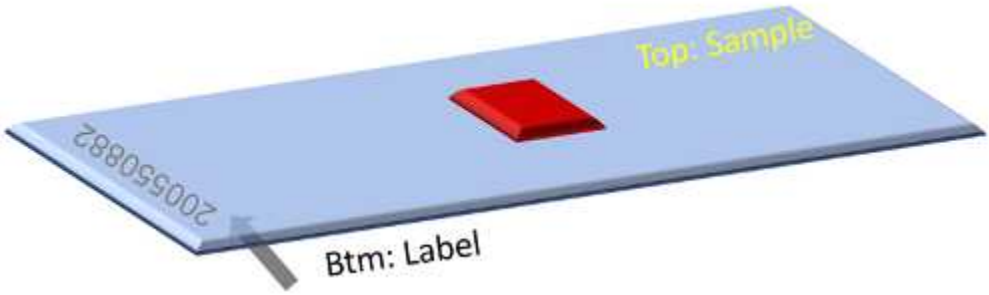
2 Spray the workplace and tools (blades, tweezers, brushes, etc) with RNAseZap to decontaminate the environment. Spray 100% ethanol onto the cryostat to remove any residue of RNAseZap or reagent residue. Set the cryostat to -20°C. Prepare dry ice in a foam box. Place the empty clean slide box in the dry ice.

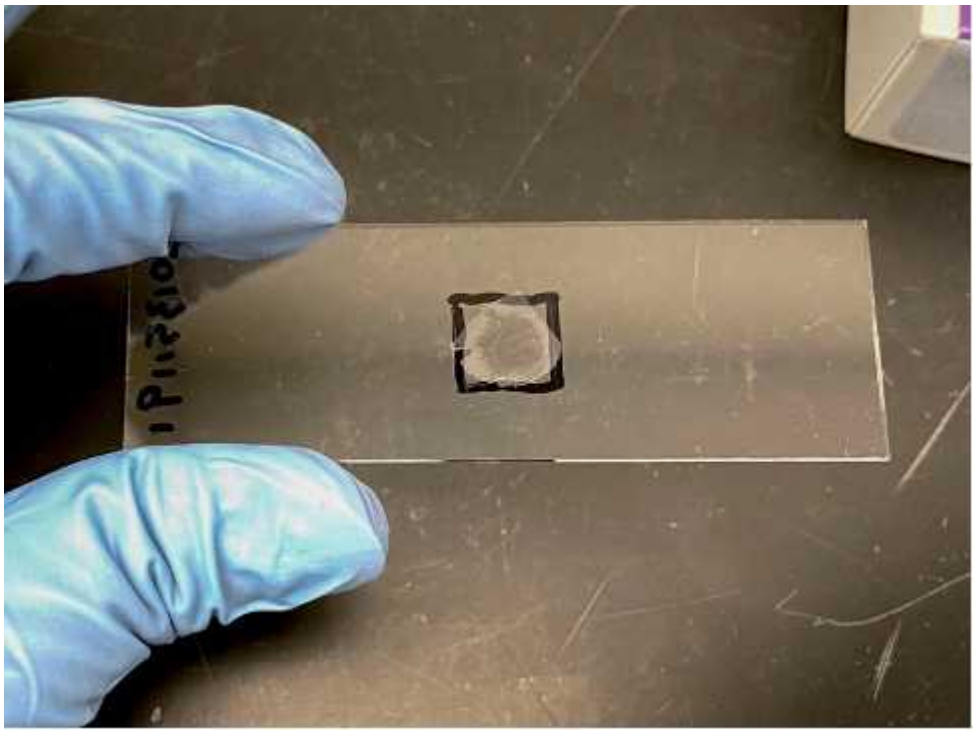
3 Remove tissue blocks from the -80°C freezer/dry ice and allow them to warm in cyrostat to sectioning temperature (-20°C) for 20 minutes. Locate the region of interest in the tissue block.

4 Trim the block if needed. Collect the defect tissue slides on a regular glass slide to test for RIN number. Usually, 3-4 sections of a 1 cm x 1 cm tissue on a glass slide is good for a RIN test.

5 Adjust cyrostat thickness to 7-10 µm. For spatial transcriptomes, 7-10 µm is the best thickness, but generally 8-13 µm works as well.

6 One section per slide needs to be put onto the center of the Ultra clean poly-L-lysine glass slide (see picture below). The glass slides are at room temperature. Label the bottom side of the slide for sample name with a marker. Make sure the top side of the slide is free of dust or any labeling. Use Dustoff gas to blow off the surface.





7 Once the tissue slice is picked up onto the glass slide, allow 30 seconds to 1 min at room temperature before transferring to a slide box in dry ice.

8 Save the slide box in a sealed plastic bag at -80° C before use. The bag helps prevent water condensation on the slides.

9 For slides used for H&E, thaw them at room temperature for 10 min, then dry them with compressed air for at least 5 min. Make sure no moisture is observed on the slide. Dip the slides in 4% paraformaldehyde at room temperature for 15 min (alternative: 30 min in pre-chilled methanol at -20° C). Wash with deionized water 3 times. Air blow dry the slides or leave them at

room temperature overnight before doing H&E staining.