Trekker and Seeker Preserving and Sectioning Tissue Protocol-At-A-Glance

I. Introduction

Proper handling and preservation of tissue is essential for maintaining tissue RNA quality prior to the **Trekker™ Single-Cell Spatial Mapping Kit** (Cat. Nos. SK017 & SK020) and **Seeker™ Spatial Transcriptomics Kit** (Cat. Nos. SK003 & SK004) workflows. This document describes guidelines for harvesting and preserving fresh murine brain and cryostat sectioning for the two workflows. Optimal conditions for other tissue types may differ from these guidelines; please use protocols and conditions ideal for your tissue type.

II. Required Materials (Not Provided)

- Dry Ice
- Isopentane (Thermo Fisher, Cat. No. O3551-4)
- Liquid Nitrogen
- Plastic tissue molds (Electron Microscopy Sciences, SKU 70180-AS)
- Optimal Cutting Temperature (OCT) Compound (Leica, Product ID 14020108926)
- 1X PBS
- Kimwipes or paper towels
- Styrofoam container -or- liquid nitrogen dewar
- Gloves for handling dry ice and liquid nitrogen
- Metal bowl for isopentane bath
- Tweezer or forceps
- Spoon or spatula
- Supports for plastic molds while sitting in isopentane bath

III. Protocol

A. Preparation before harvesting

1. Option 1: Isopentane Bath

1. Place dry ice or liquid nitrogen in a safe container and create a flat surface.
2. Depending on the size of your tissue and the plastic mold you are using, place an appropriate amount of isopentane in a metal container and place it on the flat surface.
3. Fill the area around the isopentane container with dry ice or liquid nitrogen. Ensure the dry ice or liquid nitrogen outside of the metal isopentane container is level with the amount of isopentane.
4. Place two supports on the edge of the isopentane bath so that the plastic mold can rest on the supports while in the isopentane bath without exposing the tissue and the OCT to isopentane.
5. Prepare and label a plastic mold for each tissue for embedding.
6. Place a bubble free layer of OCT compound in the bottom of each mold and leave at room temperature.
7. Prepare a cold bath of 1X PBS for washing tissue after dissection.
8. (**Optional**): Depending on your tissue of interest, consider anesthetizing and whole animal perfusion with ice cold HEPES-Sucrose Cutting Solution. (Refer to https://www.protocols.io/view/fresh-frozen-mouse-brain-preparation-for-single-nu-j8nlkedb1l5r/v1)

2. Option 2: Dry Ice only

1. Place dry ice in a styrofoam container and create a flat surface.
2. Prepare and label a plastic mold for each tissue for embedding.
3. Place a bubble free layer of OCT compound in the bottom of each mold and leave at room temperature.
4. Prepare a cold bath of 1X PBS for washing tissue after dissection.

B. Sample collection and handling

1. Clean freshly harvested and trimmed tissue in a bath of cold PBS to wash off any blood, hair and loose tissue.
2. Place the tissue on Kimwipes and gently blot dry.
3. Immediately place freshly harvested tissue into prepared plastic molds with OCT compound at the bottom.
4. Carefully cover the fresh tissue with bubble-free OCT compound.
5. Once the tissue is fully embedded in OCT compound, do one of the following depending on which protocol is being followed (from Section A).
   a. **Option 1**

      Rest the plastic mold on the supports. Ensure the mold is partially submerged in the isopentane but the OCT compound and tissue do not come in contact with it.

   b. **Option 2**

      Rest the plastic mold on the flat dry ice surface.
6. Let freeze for a few minutes depending on the size and volume of your molds.
7. Once the OCT compound and embedded tissue are completely frozen, place the mold in a plastic bag and store the block in the –80°C freezer for at least one day before further processing.

C. Sectioning tissue

NOTE: Optimum cutting temperature and acclamation time in the following steps will depend on your tissue type and block size.

1. After at least one day in the –80°C freezer, remove the block from the mold and trim the excess OCT compound from the block.
2. Mount the block onto a cold chuck from a cryostat set at –18°C by adding a layer of OCT compound to the chuck and pressing the block in the desired orientation into the fresh OCT compound.
3. Place the chuck with the mounted block into the cryostat with the tissue resting on the chuck for freezing and complete mounting.
4. Leave the block to acclimate to the cryostat for at least 20 min.
5. Once the chuck is frozen to the block, secure the chuck in place for sectioning.
6. Proceed to Section V.B "Tissue Sectioning" in the Seeker or Trekker kit user manual you are using.

Appendix. Troubleshooting Guide

Problem	Possible Explanation	Solution
Tissue is tearing and sections poorly.	Poor fixation of tissue may benefit from perfusion before harvest	Try an additional perfusion step to further preserve tissue: https://www.protocols.io/view/fresh-frozen-mouse-brain-preparation-for-single-nu-j8nlkedb1l5r/v1?step=1
	Tissue may be too cold or too warm.	Adjust sectioning temperature.

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