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Tissue Slice Preparation for Visium Analysis

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Stephen Fisher[1], Marielena Grijalva[1], Rong Guo[1], Sarah A Johnston[1], Hieu Nguyen[1], John Renz[2], Jean G Rosario[1], Steven Rudich[2], Brian Gregory[1], Junhyong Kim[1], Kate O'Neill[1]

[1]University of Pennsylvania; [2]Gift of Life Donor Program

Stephen Fisher
University of Pennsylvania

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of tissue slices for processing with the 10X Genomics Visium imaging system. Ensuring that the tissue and specific region of tissue of interest is mounted within the fiducial frame of the Visium slide is the most critical step of this protocol. Establishing a clear workflow and line of communication between the individual mounting the tissue and the subject matter expert/pathology is critical to avoiding incorrect mounting on costly Visium slides.

Materials

- OCT embedded tissue block

- BioAnalyzer High Sensitivity Chip **Agilent Technologies** **Catalog #**5067-4626
- Illumina Library Quantitation Complete kit (Universal) **Kapa Biosystems** **Catalog #**KK4824
- Visium Spatial Gene Expression Slide & Reagents Kit 16 samples **10x Genomics** **Catalog #**1000184
- 10X Visium slide **10x Genomics** **Catalog #**1000185
- Microscope slide 75mm x 25mm x 1mm **VWR International (Avantor)** **Catalog #**16004-368
- 0.2mL PCR Tube Strips, 8-Tube Strip; 120/Pk. **Thermo Fisher** **Catalog #**E0030124286
- DNA LoBind 1.5mL microcentrifuge tubes **Fisher Scientific** **Catalog #**13-698-791
- MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL **Thermo Fisher** **Catalog #**4346907
- QuantStudio™ 3 Real-Time PCR System, 96-well, 0.2 mL, laptop **Thermo Fisher** **Catalog #**A28567

Troubleshooting

Before start

Prior to preparing tissue for 10X Genomics Visium analysis, the permeability of the tissue needs to be assessed using a 10X Genomics Visium Spatial Tissue Optimization Slide & Reagent Kit (10X Genomics; PN-1000193). This process is detailed in the **10X Genomics document CG000238**. The tissue-specific permeabilization optimization values are used in Step 8.2.

1 Use a cryostate to acquire a tissue slice of approximately 10µm from the OCT tissue block.

2 Mount on a standard slide and perform H&E staining for quality control and the selection of the region of interest (Kruse et al., 2021).

Tip: When mounting the slice, take particular care to note the orientation of the slice with respect to the tissue block and any orientation marks on the tissue block. This will be particularly important for step 4 below, when a second slice is positioned on a 10X Visium slide based on a pathologist review.

3 Request pathologist review of H&E brightfield image for quality and mark the region of interest within the tissue, as the 10X Visium imaging is confined to a 6.5mm x 6.5mm region.

4 Acquire second tissue slice from OCT tissue block and mount on a 10X Visium slide such that the region of interest, identified by the pathologist, is within the fiducial frame of the Visium slide.

Tip: You need to be extra careful here to align the slice using the morphology cues and orientation markings on the tissue block, as noted during step 2 above.

5 Acquire third tissue slice from OCT tissue block and mount on the same Visium slide in a second frame, such that the region of interest is within the fiducial frame of the Visium slide.

6 Process the Visium slide with **H&E Staining for 10X Genomics Visium Imaging**.

6.1 **Quality control**: If the region of interest wasn't properly aligned to the slide fiducial frame, you can reset the slide, destroying the existing slice in the process. A slide can only be reset once and will likely result in a decreased number of unique transcripts detected (see **10X Genomics Document CG000332**).

7 For later 3D anatomical positioning, track the numbering of each slice taken from the OCT tissue block.

8 Process the Visium slide according to the 10X Genomics "**Visium Spatial Gene Expression Reagent Kits User Guide**".

Step-specific processing notes are included below.

8.1 **Step 1.1b**: When applying the gasket to encase the slide in step 1.1b, care must be taken to make sure the gasket properly creates the leakproof wells.

8.2 **Step 1.1e**: Step 1.1e is the permeabilization step and the time required is dependent on the tissue specific permeability optimization previously run (see **10X Genomics document CG000238**). The permeabilization times used for our tissues are listed below.
- Ovary tissue: 24 minutes
- Fallopian Tube tissue: 30 minutes
- Cervix tissue: 30 minutes
- Uterine tissue: 30 minutes

8.3 **Step 3.4**: During step 3.4, consider diluting the aliquot 1:3 as we often found that the concentration was outside of the range for accurate quantification using an Agilent Bioanalyzer high sensitivity DNA assay. If diluting, then also adjust the cDNA total yield calculations accordingly.

8.4 **Quality control**: The peak of the Agilent Bioanalyzer trace at step 4.7 should roughly be gaussian centered around 480. If the peak is to narrow or asymmetric, then size selection was not performed correctly and we recommend rebuilding the library from the cDNA step 3.4.

8.5 **Tip**: An Illumina MiSeq run can prove helpful to rebalance the libraries, if needed, and confirm the libraries are properly binding to the flow cell. MiSeq results do not have sufficient sequencing depth for downstream 10X protocol analyses.