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KAPP-Sen TMC: Tissue Section Preparation for 10x Genomics Visium CytAssist

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Cellular Senescence Net... KAPP-Sen TMC

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We use this protocol and it's working

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Abstract

Sectioning was performed by the **JAX Histology core in BH, ME.** All experiments preceding this technique were performed at 4°C and RNase-free, as described in this protocol. Tissue sections are further used for Visium Spatial Gene Expression assay performed by the **Single Cell Biology Core at The Jackson Laboratory for Genomic Medicine.**

Troubleshooting

Preparation of Workspace

1 Prepare workspace according to dx.doi.org/10.17504/protocols.io.36wgq37polk5/v1

Curl Collection and Storage

2 To verify if fixation and RNA hydrolysis during storage would lead to low yield and fragmented RNA the blocks were initially submitted to an RNA quality assay.

Blocks arrive wrapped in aluminum foil to protect from light and are stored in the dairy cooler at 4°C.

Wearing nitrile gloves cleaned with RNase Zap wipe, collect curls as follows:

2.1 Fill ice bucket with dry ice.

2.2 Put 1.5mL prelabeled Eppendorf tubes (sent by submitter with blocks) into dry ice.

2.3 Using an RNase Zap wipe, clean 5mL MacroTube (or similar) and add to dry ice bucket. This will be used to cool forceps for curl collection.

2.4 Retrieve blocks from 4°C dairy cooler and remove aluminum foil. Protect from light as much as possible during curl collection.

2.5 Using an RNase Zap cleaned blade, face into block.

2.6 Using ice tray/block prepared per Part One, step 3 above, allow block to cool and soak if needed. If using ice block, add enough di water to cover ice so blocks won't freeze to surface. Cover blocks to protect from light.

2.7 Before collecting curls, discard first two sections, then transfer curls directly into the corresponding Eppendorf tube, using cold forceps prepared per Part One, Step 4 above. Curl thickness of 10um and 2 curls per block were used. After the desired curl quantity is collected, immediately close the Eppendorf tube and seal the lid with Parafilm. Immediately return Eppendorf tube to dry ice.

Safety information

Do NOT hold bottom of tube. This will melt the curls, damaging the RNA quality.

2.8 Dip block face into molten paraffin to seal the tissue and allow to cool before rewrapping in aluminum foil and returning to 4°C dairy cooler for storage.

2.9 Repeat steps 4 through 9 with remaining blocks, using RNase Zap wipe to clean blade, forceps and brush/applicator stick between each block.

2.10 After all curls are collected, quickly transfer vials to Cryo vial box on dry ice.

2.11 Store Cryo vial box in -80°C freezer until shipping.

3 Upon arrival at The **Jackson Laboratory for Genomic Medicine,** the RNA is extracted from
curls using Qiagen's RNeasy Micro Kit (Cat. No. / ID: 74004). RNA quality is then quantified using a Tapestation High Sensitivity RNA ScreenTape (Agilent). DV200 of more than 30% is accepted by Visium CytAssist.

Section Collection

4 Wearing nitrile gloves cleaned with RNase Zap wipe, collect sections as follows:

4.1 Using a Histo-Quill Pen, hand label all slides with iLabs project number, block ID and stain if requested.

4.2 Retrieve blocks from 4°C dairy cooler and remove aluminum foil. Protect from light as much as possible during section collection.

4.3 Using an RNase Zap cleaned blade, face into block.

4.4 Using ice tray/block prepared per Part One, step 3 above, allow block to cool and soak if needed. If using ice block, add enough di water to cover ice so blocks won't freeze to surface. Cover blocks to protect from light.

Note

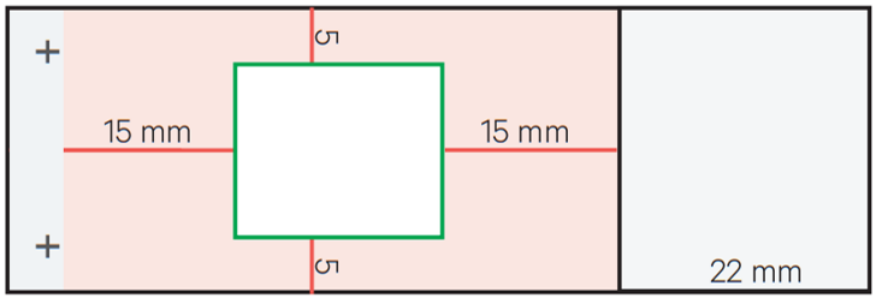
Monitor blocks carefully as they can become overhydrated very rapidly.

4.5 Using water bath, forceps and brush/applicator stick, section at 5um, discarding the first two sections before taking a ribbon. Collect sections on corresponding slides previously hand labeled in step 1.

Note

Orientation of section(s) depends on specific ST protocol for which slides will be used.

For **Visium**, orient section within the following parameters:



4.6 After sections are collected, transfer unstained slides to rack. Cover racks to protect slides from light.

4.7 Dip block face into molten paraffin to seal the tissue and allow to cool before rewrapping in aluminum foil and retuning to 4°C dairy cooler for storage.

4.8 Repeat steps 3 through 7 with remaining blocks, using RNase Zap wipe to clean blade, forceps and brush/applicator stick between each block.

Slide Drying and Storage

5 Wearing nitrile gloves cleaned with RNase Zap wipe, dry, and store slides as follows:

5.1 For Visium, dry slides at 42°C for three hours before H&E staining or transferring unstained slides to RT desiccator protected from light for storage. Unstained slides are usable for two weeks after sectioning. Store in slide box or rack labeled with iLabs project number, date and name of submitter.