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KAPP-Sen TMC: Tissue Section Preparation and H&E Staining

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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

Before performing further spatial transcriptomic analysis, FFPE blocks were submitted to hematoxylin and eosin stain. All these procedures were performed by **JAX Histology Core** in **Bar Harbor, ME** where blocks were always kept at 4C. For the following protocol gloves cleaned with RNase Zap wipes must be worn during preparation for and completion of this protocol and whenever handling FFPE blocks intended for spatial transcriptomics. If gloves contact a surface not previously cleaned with RNase Zap during this protocol, clean gloves again.

Preparation of the Workspace

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

Section Collection

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> **Note**
>
> Before beginning section collection, check project instructions for curl request. If curls are also requested, they will need to be collected immediately prior to section collection.

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 sections as follows:

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

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

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

2.4 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.

2.5 Using water bath, forceps and brush/applicator stick prepared per Part One, steps 2 and 4, section at 5um, discarding the first two sections before taking a ribbon. Collect sections on corresponding slides previously hand labeled in step 1.

2.6 After sections are collected, transfer unstained slides to rack prepared per Part One, step 5, and slides for H&E to Leica staining rack. Cover racks to protect slides from light.

2.7 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.

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

Routine H&E Protocol: Leica AutoStainer XL

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	A	B	C	D	E
	Step	Station	Reagent	Time, min:sec	Exact
	1	1	Xylene	3:00	No
	2	2	Xylene	3:00	No
	3	3	Xylene	3:00	No
	4	4	Ethanol, 100%	2:00	No
	5	5	Ethanol, 100%	1:00	No
	6	6	Ethanol, 95%	1:00	No
	7	7	Ethanol, 70%	1:00	No
	8	Wash 2	Tap Water	1:00	No
	9	9	Mayer's Hematoxylin	4:00	Yes
	10	Wash 4	Tap Water	2:00	No
	11	8	Ammonium Hydroxide Water, 0.1%	1:00	No
	12	Wash 5	Tap Water	1:00	No
	13	Wash 3	Tap Water	2:00	No
	14	10	Ethanol, 70%	1:00	No
	15	11	Eosin	2:00	Yes
	16	12	Ethanol, 95%	0:45	Yes

	A	B	C	D	E
	17	13	Ethanol, 95%	0:30	Yes
	18	14	Ethanol, 100%	2:00	No
	19	15	Ethanol, 100%	2:00	No
	20	16	Ethanol, 100%	1:00	No
	21	17	Xylene	2:00	No
	22	18	Xylene	2:00	No

Slide Scan Submission

4 Slides will then be scanned by the **JAX Light Microscopy Core in Bar Harbor, ME** using Nanozoomer (Hamamatsu) HT2.0. Images will be acquired with brightfield scans at 40X.

Images can be accessed by NDP software or submitted to further imaging analysis at The Jackson Laboratory for Genomic Medicine, Farmington, CT. These images are also used to determine the Region of Interest for further Spatial Transcriptomics assays.