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

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Juliana Alcoforado Diniz¹, Paul Robson^{1,2,3}, Elaine Bechtel⁴

¹The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA;

²Department of Genetics and Genome Sciences, University of Connecticut School of Medicine, Farmington, CT, USA;

³Institute for Systems Genomics, University of Connecticut, Farmington, CT, USA;

⁴The Jackson Laboratory, Bar Harbor, ME, USA

Cellular Senescence Net...

KAPP-Sen TMC



Ashley M Raynock

UConn Health, UConn Center on Aging

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We use this protocol and it's working

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Abstract

To perform imaging analysis of the whole pancreas the FFPE blocks were submitted to hematoxylin and eosin stain. Initially FFPE blocks were shipped from the **Joslin Diabetes Center, University of Harvard Medical** with ice packs to **The Jackson Laboratory for Genomic Medicine (JAX)** and kept at 4°C until submission to the **JAX Histology Core** in Bar Harbor, ME.

Upon arrival the blocks were kept at 4°C to undertake an RNase Free Sectioning for Spatial Transcriptomics. 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 5µm, 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 returning 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



	A	B	C	D	E
	14	10	Ethanol, 70%	1:00	No
	15	11	Eosin	2:00	Yes
	16	12	Ethanol, 95%	0:45	Yes
	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.