

Sectioning of Paraformaldehyde-fixed Mouse Urogenital Sinus (UGS) Tissues with a Vibrating Microtome

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At least one day prior to starting

- 1. Prepare agarose.
 - A. Prepare a 4% low-melt agarose solution in PBS (2g agarose in 50mL PBS).
 - B. Microwave briefly to get agarose into solution, cool to 62°C to prepare for embedding.
 - C. Agarose solution may be stored at 25°C, then melted and reused for up to 6 – 8 weeks
- 2. Prepare polystyrene ring mold.
 - A. Remove membrane from 12mm diameter Millicell culture plate well insert and retain polystyrene ring to use as an agarose mold.
 - B. Soak rings overnight in RNase inhibitor solution.
- 3. Prepare vibrating microtome blades by stabilizing with stiffer microtome blades.
 - A. Collect used microtome blades and cut in half with metal snips.
 - B. Break Wilkinson double edge blade in half so separated into two blades.
 - C. Use superglue to attach one Wilkinson razor blade to cut microtome blade.
 - D. Use glue sparingly to avoid getting glue on cutting edge of razor blade.
 - E. Leave 3 – 4mm gap between edge of microtome blade and cutting edge of razor blade.

Prepare tissue samples (fresh UGS+bladder tissue samples are fixed overnight at 4°C in 4% paraformaldehyde and dehydrated through a series of MeOH / PBSTw washes and stored in 100% MeOH at –20°C)

- 1. Remove dehydrated tissue from –20°C storage.

- **2.** Rehydrate tissues through series of MeOH / PBSTw washes at 25°C with gentle agitation.
 - **A.** Wash tissues 1 x 10 min with 75% MeOH / 25% PBSTw.
 - **B.** Wash tissues 1 x 10 min with 50% MeOH / 50% PBSTw.
 - **C.** Wash tissues 1 x 10 min with 25% MeOH / 75% PBSTw.
 - **D.** Wash tissues 2 x 10 min with 100% PBSTw.

Embed tissue in agarose

- **1.** Transfer one UGS+bladder into petri dish containing PBSTw and use forceps to remove loose tissue from UGS. Use spring scissors to remove and discard approximately two-thirds of bladder.
- **2.** Prepare tissue and mold for embedding.
 - **A.** Place the polystyrene ring mold, flat surface down, on a 25°C plain glass microscope slide.
 - **B.** Remove agarose solution from 62°C oven and add drop-wise until mold is filled.
 - **C.** Incubate melted agarose solution in mold at 25°C.
- **3.** Embed tissue
 - **A.** Remove UGS tissue from PBSTw and blot dry on absorbent wipe (note: it is important to dry tissue as completely as possible so that agarose directly contacts the tissue).
 - **B.** After agarose has incubated in the mold for 1 min 50 sec, transfer UGS tissue from absorbent wipe to mold and gently poke tissue into agarose solution with forceps.
 - **C.** For sagittal sections, orient UGS tissue on side, parallel to slide & suspended in middle of agar solution.
 - **D.** After UGS tissue is oriented, place mold in refrigerator to set agarose.
 - **E. Note:** adjust agarose cooling time based on how first tissue sample sinks in agarose. The goal is to suspend the tissue in the middle of the agarose solution before it solidifies. If tissue sample rapidly sinks to bottom, increase agarose solution cooling time by 10 sec increments; if sample does not sink, decrease cooling time.
 - **F. Note:** if UGS tissue is oriented in a grossly inappropriate plane for sectioning, carefully peel sample out of solidified agarose plug & re-embed; for very minor adjustments in tissue orientation, adjust/bevel the flat bottom of the agarose plug with a razor blade.

Vibrating microtome sectioning

- **1. Prep vibrating microtome.**
 - **A.** Set blade angle to 35° and tighten set-screw.
 - **B.** Fill deluxe specimen bath with 1X PBS.
 - **C.** Pack wet ice around specimen bath.
- **2. Mount tissue sample.**
 - **A.** Remove solidified agarose plug from mold and blot bottom of plug surface dry with absorbent wipe.
 - **B.** If necessary for orientation, cut round border of plug on one side to create a flat edge, then cut a small bevel in that edge.
 - **C.** Place drop of superglue onto vibrating microtome specimen mounting disk.
 - **D.** Press agarose plug, flat surface down, into glue & allow glue to dry.
 - **E.** Slide specimen mounting disk into deluxe specimen bath, taking care not to cut plug on blade.
 - **F.** Tighten mounting screw to hold specimen disk in place.
- **3. Section.**
 - **A.** Use high speed to advance blade into agarose plug by 100mm to approach tissue sample.
 - **B.** Once near tissue sample, adjust microtome settings to speed = 2, amplitude = 4, cut thickness = 50mm.
 - **C.** Use blunt forceps to collect each tissue section as it comes off of blade.
 - **D.** Transfer tissue sections into individual wells of 24-well culture plate containing 0.5mL PBSTw / well and chill plate on wet ice.
 - **E.** Examine tissue sections under microscope, discard those that will not be useful and photograph remaining tissue sections for your records.
 - **F.** Store sections at 4°C in PBSTw for use on following day.

Solutions

- 4% agarose: 2g low-melt agarose in 50mL PBS
- PBSTw: 1X PBS + 0.1% Tween-20, add 1mL of 0.2M sodium azide per 1 mL PBSTw to prevent contaminating growth, sterile filter to remove insoluble material/contaminants
- 0.2M sodium azide: Dissolve 1.3g sodium azide in 100mL double-distilled H₂O, pH to 7.6 (Note: quite sensitive to pH change so need very little NaOH to adjust)

Abbreviations

- MeOH = methanol
- PBS = phosphate-buffered saline
- PBSTw = 1X PBS + 0.1% Tween-20
- PFA = paraformaldehyde

- UGS = urogenital sinus

Equipment, Reagents, Supplies

- Eliminase RNase remover, cat # 0435532, Fisher
- MeOH, cat # A411-4, Fisher
- PBS, w/out Ca & Mg, MP Biomedicals powdered media, cat # ICN1760420, Fisher
- SeaPlaque low-melt agarose, cat # 50101, Lonza
- Tween-20, cat # BP337-100, Fisher
- Deluxe specimen bath for vibrating microtome, cat# 39053745, Leica Microsystems
- Glass slides (plain), cat # 12-549, Fisher
- Loctite 404 quick set instant adhesive, general purpose, cat # 46551
- Millipore Millicell culture plate insert, 0.4m, 12mm diameter, cat # PICM01250, Fisher
- Vibrating microtome, Model VT1000A, Leica Microsystems
- Wilkinson Sword Classic double edge razor blades