Tissue embedding in paraffin for sectioning

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Reference: Abler LL, Keil KP, Mehta V, Joshi PS, Schmitz CT and **Vezina CM** (2011). A High Resolution Molecular Atlas of the Fetal Mouse Lower Urogenital Tract. ***Dev Dyn*** 240:2364-2377. PMC3177421.

All steps should be conducted with gentile agitation on an orbital shaker, unless otherwise instructed.

Day 1

1. Tissue fixation
   - Place tissues in at least 6 volumes of 4% paraformaldehyde (US biologicals #10043-1LT) and incubate overnight at 4°C.
2. Wash 5 min at 25°C in Phosphate Buffered Saline (PBS). Repeat 3 times.

3. **Optional: Tissue embedding in agar prior to embedding in paraffin.**
   - If you wish to embed multiple tissue pieces in the same paraffin block, then prepare a solution of 1% Bacto Agar (w/v, BD #214010) in PBS.
     - Heat solution in microwave for 15 seconds or until boiling.
     - Remove and swirl solution. Repeat heating/swirling until agar has dissolved.
     - Cool agar and maintain at 65°C. Add a plastic Pasteur pipette to agar bottle so that it is pre-warmed.
   - Remove tissues from PBS and place on OptiPlus™ Positive-Charged Barrier Slides (3 x 1/3 test areas, Biogenix # XT014-SL). Slides can be re-used multiple times if cleaned with deionized water between each use and stored in a dust-free container.
   - Use Kimwipe to blot excess moisture from tissues.
   - Add pre-warmed agar solution to tissues. Quickly align tissues in a row and ensure they are properly oriented and devoid of air bubbles.
   - Incubate slide at 4°C for 5 min (no agitation—this step is to solidify agar) and return bottle containing agar to 65°C incubator.
   - Remove slide from 4°C. Use a razorblade to trim agar into a rectangular strip. If tissues aren't oriented as desired, use forceps to remove them from agar and re-embed them.
   - Multiple agar strips can be arranged together in rows and additional agar can be added to the rows to glue them into a multi-tissue block. Use forceps to sweep out air bubbles that accumulate between the strips.
   - The 1% agar solution can be stored at 25°C for up to 6 months.

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4. Dehydrate tissues
   - Place each tissue or agar block into a Shandon Tissue Cassette (Fisher #B1003500GR).
   - ***IMPORTANT*** Use large volumes (>100mL) of each alcohol solution, long incubation times, and gentle agitation to ensure complete dehydration.
   - 15 min at 25°C in 25% ETOH (Pharmco-Aaper #111000-200E200G)/ 75% PBS.
   - 15 min at 25°C in 50% ETOH / 50%PBS.
   - 20 min at 25°C in 75%ETOH/25%H_20.
   - Quick wash in 100% ETOH.
   - 30 min at 25°C in 100% ETOH (cover vessel with plastic wrap).
   - Overnight at 4°C in 100% ETOH (cover vessel with plastic wrap).

Place Paraplast chips (Fisher# 23-021-399) in a 250 mL glass beaker and place beaker in 65°C oven for melting. You will need this on Day 2.

Day 2

1. Final Dehydration / Paraplast Embedding
   - Incubate 20 min at 25°C in 100% ETOH (cover vessel with plastic wrap).
   - Incubate 45 min at 25°C in 100% XYLENE (cover vessel with aluminum foil).
   - Drop Shandon cassette into paraffin. Tap cassette with forceps to remove air bubbles. Incubate at least 3 hours to overnight (depending on tissue size) min at 65°C in 100% Paraplast (Fisher# 23-021-399). During this time, the paraffin mix should be swirled every 15 minutes.
   - Warm aluminum molds (ted pella #27521) and glass pasteur pipettes with bottoms broken off and fire polished) to 65°C.
   - When aluminum molds have warmed, place a small amount of paraplast in each mold and keep at 65°C.
   - Remove one cassette from paraplast, remove tissue or agar block and place it into aluminum mold. Place Shandon cover on mold. Keep at 65°C.
   - Repeat above step for all tissue pieces.
   - Remove one tissue-containing mold from incubator. Place on table. Add paraplast dropwise until the shandon cover is full. Incubate at 25°C overnight (if you are in a hurry to section, the samples need to incubate at 25°C for at least 15 min, they can then be transferred to 4°C for 15 min, and then to -20°C for 15 min.)
   - Incubate tissue blocks at -20°C for 15 min.
   - Carefully squeeze aluminum mold to separate it from paraplast. Use razorblade to trim excess paraplast from top and sides of block.
   - Ensure block is properly labeled and store at -20°C until ready for sectioning.
   - Remaining paraplast can be stored long term at 65°C in a covered container.

2. Sectioning
   - Obtain slides (Superfrost Plus, Fisherbrand #22034979).

- Obtain microtome blades (Accuedge low profile #4689).
- Warm flotation water bath to 53°C.
- Engage safety break on right of microtome.
- Ensure that microtome (Leica Model # HM315) blade angle is set to 5°.
- Ensure that microtome thickness is set to 5 microns.
- Place microtome blade in holder.
- Place tissue block in holder.
- Disengage microtome brake. Use manual dial on right of microtome to move the block out of the cutting plane.
- Use microtome right crank to move block up or down to make it even with the blade.
- Use left manual dial to advance block forward until it approaches the blade.
- Orient the face of the block by disengaging the lock on the left of the block holder, and using the thumbscrews to adjust the x- and y- axes of the block until it is parallel with the blade.
- Engage the block holder lock.
- Use microtome right crank to advance the block forward and begin sectioning.
- Once the sections contain tissue, place one of every ten sections on a slide. View slide under scope to determine whether you've reached the correct tissue region. Continue with this process, stopping every 10 section to microscopically verify tissue region, until the appropriate region is reached.
- Cut a ribbon of two sections, remove them from the ribbon with a razor blade, and float them on the 53°C water bath for 5 minutes.
- Place sections on slide and place slide into a slide box.
- When sectioning is complete, store slides at 25°C and allow at least 24h for them to dry before processing sections.