Paraffin Embedding Protocol

Day 1

Materials:
1X PBS
Ethanol (30%, 50%, 60%, 70%, diluted with ddH2O)
Glass vials with screw on lids
Orbital rocker

Procedure:
1.) If tissues are in sucrose, do several washes back into 1X PBS.
- 3 washes x 30'
2.) Dehydrate tissue using the following washes:
30% EtOH 1 hour RT Rocking
50% EtOH 1 hour RT Rocking
60% EtOH 1 hour RT Rocking
70% EtOH 1 hour RT Rocking
70% EtOH overnight RT Rocking
Notes:
- Should do longer 1X PBS washes if time allows.
- Use at least 200 proof ethanol.
- Do ethanol washes quickly. Do not leave tissues exposed to air.

Days 2-3

Materials:
Ethanol (85%, 95%, diluted with ddH2O, 100%,)
Xylene
Orbital rocker
Paraffin
Warm Paraffin bead tray

Procedure:
1.) Use tissues from the overnight wash in 70% ethanol made on Day 1.
Do the following steps:
85% ethanol 1 hour RT Rocking
95% ethanol 1 hour RT Rocking
100% ethanol 30 ' RT Rocking
100% ethanol 1 hour RT Rocking
1:1 Xylene:EtOH 1 hour RT Rocking
100% Xylene 30' RT Rocking
100% Xylene 1 hour RT Rocking
1:1 Xylene:Paraffin 1 hour 60ºC-65ºC in bead trays (small flask) on paraffin oven.
100% Paraffin 1 hour 60ºC-65ºC in bead trays on paraffin oven.
100% Paraffin 3 hour 60ºC-65ºC in bead trays on paraffin oven.
100% Paraffin 3 hour 60ºC-65ºC in bead trays on paraffin oven.
100% Paraffin 3 hour 60ºC-65ºC in bead trays on paraffin oven.
100% Paraffin overnights 60ºC-65ºC in bead trays on paraffin oven.

Day 4

Materials:
Vacuum oven (We don't have this machine. But if you can use this, it is best for your results)
Metal heat block (for transport of vials)
Paraffin molds and embedding rings

Procedure:
1) Fill clear paraffin mold with paraffin. Rest on heated surface.
2) Take tissues out from paraffin container (flask) and quickly put tissues in the paraffin molds on center. Arrange tissue in correct orientation in warm paraffin.
3) Add a little bit of warm paraffin to top of clear mold. Swirl around, then quickly press embedding ring onto the clear mold. Fill the rest of the mold to the top of the embedding ring.

4) Move mold to unheated surface and let paraffin harden slightly while maintaining correct position of tissues.

5) Let harden on benchtop. Samples will keep in the mold at RT overnight.

Paraffin Sectioning Protocol

Day 5 – Sectioning

Materials:
Microtome
Warm water tray
Paintbrush
Glass slides (Superfrost Plus)
Embedded sample

Procedure:
1) Heat water in water tray. Turn light on while using the water tray.
- Let water heat for at least 1 hour before needed.
2) Clean blade and around cutting surface with a kimwipe. Let dry completely.
3) Cut off excess wax from embedded sample.
- Cut off excess wax from embedding ring. Can also trim around sample if desired.
4) Lock microtome blade in place and make sure blade guards are closed. Lock microtome handle and clamp embedded sample onto microtome.
5) Adjust sample position so it sits straight and even right above the blade. Lock into place.
6) Unlock and quickly turn handle until sample starts cutting a little. Try to capture the first full section using wooden probe to encourage future sections to stick to each other and create a ribbon of paraffin sections.
- Use 5-10 micron (Using 5micron for best staining result) thickness for sections.
7) Gently place cut sections into warm water tray using tweezers and metal probe.
8) Use metal probe to gently maneuver the sections onto a glass slide.
9) Let slide dry upright in drying rack until most of the moisture is gone.
10) Transfer slides to adjust platform to finish drying. Leave on platform overnight on air.
11) Store in plastic slide carrier/slide box until ready to stain.

Hematoxylin and Eosin (H&E) Staining

(Modified From Baylor College of Medicine)

Protocol for H&E staining:

•Place slides containing paraffin sections in a slide holder (glass or metal)

•Deparaffinize and rehydrate sections:

1) 3 x 3´ Xylene *(blot excess xylene before going into ethanol)*
2) 3 x 3´ 100% ethanol
3) 1 x 3´ 95% ethanol
4) 1 x 5´ PBS

•While sections are in PBS, skim surface of hematoxalin with a Kimwipe to remove oxidized particles. Blot excess water from slide holder before going into hematoxalin.

•Hematoxalin staining:

5) 1 x 3´ Hematoxalin
6) Rinse PBS
7) Dip 4-5x (fast) Acid ethanol *(to destain)*
8) Dip 4-5x (fast) Ammonia water (0.3%)

•Eosin staining and dehydration:

9) 1 x 3-5´ Eosin *(up to 5 minutes for an older batch of eosin)*
10) 3 x 3´ 95% ethanol
11) 3 x 3´ 100% ethanol (*blot excess ethanol before going into xylene)*
12) 3 x 3´ Xylene
13) Coverslip slides using Permount (xylene based).
14) Place a drop of Permount on the slide using a glass rod, taking care to leave no bubbles.
15) Angle the coverslip and let fall gently onto the slide. Allow the Permount to spread beneath the coverslip, covering all the tissue.
16) Dry overnight in the hood.

Reagents for H&E staining:

- **Acid Ethanol:** 0.5 ml concentrated HCl + 50 ml 70% ethanol
- **Hematoxylin**
- **Eosin**
- **Permount:**