Paraffin Embedding Protocol

Day 1

Materials:

1X PBS

Ethanol (30%, 50%, 60%, 70%, diluted with ddH_2O)

Glass vials with screw on lids

Orbital rocker

Procedure:

1.) If embryos 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
50%EtOH	1 hour	RT	Rocking
60% 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 embryos exposed to air.

Paraffin Embedding Protocol

Days 2- 4

Materials:

Ethanol (85%, 95%, diluted with ddH_2O, 100%,)

Citrisolv/Citrisolv Hybrid

Orbital rocker

Paraffin

Warm Paraffin bead tray

Procedure:

1.) Use embryos 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 Citrisolv:EtOH	1 hour	RT	Rocking
100% Citrisolv	30’	RT	Rocking
100% Citrisolv	1 hour	RT	Rocking
1:1 Citrisolv:Paraffin	1 hour	60°C – 65°C	in bead trays
100% Paraffin	1 hour	60°C – 65°C	in bead trays
100% Paraffin	3 nights	60°C – 65°C	in bead trays

Notes:

- Can use Histoclear or Xylene instead of Citrisolv if needed.

As of 3/2/11

Paraffin Embedding Protocol

Day 5

Materials:

Vacuum oven

Metal heat block (for transport of vials)

Paraffin molds and embedding rings

Microscope (fits onto top of paraffin dispenser)

Sharp-tipped tweezers

Small plastic lid (for containing working embryos on paraffin dispenser)

Bench pad (to protect clothes)

Procedure:

1.) Turn vacuum oven on at least 1 hr. before needed. Make sure heat block for transport of vial is in the oven.

2.) Take vial out of bead container in paraffin dispenser. Pour out paraffin in waste container. Quickly fill container with fresh paraffin. If doing more than one vial, put vials in bead containers until ready for transport.

3.) Put vial in heat block for transport to oven. Loosen lid.

4.) Put vial into oven (leave in heat block). Close door and latch completely.

5.) Tighten vent (black knob on top right side of oven). Turn vacuum on (make sure vacuum port is open by loosening black knob on top left side of oven). Wait until pressure reaches 15 Hg, then tighten vacuum port knob to close it off.

6.) Leave vial in oven for 1 hr.

- During this step, label paraffin embedding rings and set up in room with the paraffin dispenser.

7.) Remove heat block and vial from oven and transfer vials to bead trays in paraffin dispenser.

8.) Pour embryo and paraffin out into small lid resting on heated surface of paraffin dispenser.

9.) Fill clear paraffin mold with paraffin. Rest on heated surface.

10.) Arrange embryo in correct orientation in warm paraffin (Use microscope if needed).

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

12.) 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.

13.) Let harden on benchtop. Samples will keep in the mold at RT until ready to do sectioning.

Paraffin Sectioning Protocol

Day 6 – Sectioning

Materials:

Microtome
Tweezers
Paintbrush
Citrisolv
Warm drying platform
Wooden probe
Warm water tray
Metal Probe
Glass slides (Superfrost Plus)
Slide drying rack
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 and a little bit of Citrisolv. 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 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 warm drying platform to finish drying. Leave on platform for at least 1 hour.

11.) Store in plastic slide carrier until ready to stain.

Paraffin Staining Protocol

Day 7 – Staining

Materials:

100% Citrisolv (3 staining dishes)
100% ethanol, 200 proof (2 staining dishes)
90% ethanol (1 staining dish)
70% ethanol (1 staining dish)
50% ethanol (1 staining dish)
ddH_2O (1 staining dish)
Nuclear Fast Red (NFR) (1 staining dish)
Slide rack

Procedure:

1.) Load slide rack with slides.

2.) Do the following series of dewaxing, dehydrating, staining, and cleaning steps:

Reagent	Soak Time (minutes)
100% Citrisolv	10
100% Citrisolv	10
100% Citrisolv	10
100% ethanol	5
100% ethanol	5
90% ethanol	3
70% ethanol	3
50% ethanol	3
ddH_2O	3
ddH_2O	3
ddH_2O	3
Diluted NFR	1 very quick dip
ddH_2O	5
ddH_2O	5

- Empty staining dish after each ddH_2O wash and refill with fresh ddH_2O.

- Pour NFR into staining dish right before using. Pour back into opaque container immediately after staining slides.

3.) Gently blot slides in slide rack on paper towels to remove excess water.

4.) Remove slides from rack and lay on paper towel. Let dry for rest of day or overnight.

Paraffin Staining Protocol

Day 8 – Adding Coverslip

Materials:

Aquamount
24 x 50 mm coverslips
22 x 50 mm coverslips
22 x 60 mm coverslips
Kimwipes

Procedure:

1.) Make sure glass slide is completely dry. On a paper towel, lay down coverslip, dot coverslip with Aquamount, slowly put glass slide (section side down) on top of coverslip, and gently push down on slide to squeeze out bubbles and excess Aquamount.

- Another method is to put Aquamount in small dots directly on slide (section side up), then slowly add coverslip and lightly presses around sections.

2.) Let slides dry ~1 hr.

3.) Visualize while Aquamount is still slightly wet for best results.

- You can clean excess Aquamount off of slides using water and kimwipes if needed.
- Aquamount tends to shrink excessively when dry.