

Mammary Gland Whole Mount Protocol

- Optional: Inject animals with BrdU 2 hours before harvesting tissue
- Fix tissue for 2 hours in fresh, cold 4% paraformaldehyde (PFA) on ice.
- Rinse tissue in 70% ethanol until ready to stain (up to 2 weeks).
- All the following steps are conducted by submerging tissue-cassettes containing glands into the solution. Thus, many glands can be processed in one jar at once.

- Defat:
 - Acetone 3 x 30 min
 - Note: This step prohibits later immunohistochemical analysis by creating high background. If you want to try to do immunohistochemistry on these glands, skip this step (or use the carmine alum protocol instead).

- Rehydrate:
 - 100% EtOH 1 x 30 min
 - 95% EtOH 1 x 30 min

- Stain:
 - Hematoxylin stain:**
 - 0.13 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
 - 13.5 ml distilled water
 - Dissolve, and add 1.74 mL stock (10%) Harris hematoxylin *
 - Add 200 ml 95% ethanol
 - Adjust pH to 1.25 with concentrated (12N) HCl (pH is critical)
 - Make stain fresh each time, and check that stain turns blue by putting a tiny amount in a weigh boat and running under *crude tap water*. Stain should turn bright light blue. If not, throw out and try again.

 - Place glands in hematoxylin stain for 1.5 hours until whole gland looks purple. If you did not defat in acetone (for future immunohistochemistry), stain O/N to allow hematoxylin to permeate through fat. Monitor stain by holding gland up to the light.

- Rinse in Crude tap H_2O until water is clear

- Destain:
 - Acid ethanol 2 x 30 min
 - (200 ml 50% EtOH + 416 ul 12N HCl)
 - Monitor destaining by holding gland up to the light. You should be able to clearly see blue epithelium with a fairly clear background.

- Dehydrate:
 - 70% EtOH 2 x 30 min
 - 95% EtOH 2 x 30 min
 - 100% EtOH 2 x 30 minComplete dehydration of gland is critical for complete clearing. Incomplete dehydration will result in brown background in the fat. Use fresh 95% and 100% EtOH and don't take shortcuts.
- Clear:
 - Xylene 2 x 1 hour (or can go overnight)
- An alternate method of clearing the glands is to use BABB for 1-2 hours
 - BA = benzylalcohol
 - BB = benzylbenzoate
 - Mix these chemicals 1:2 (BA:BB)
 - Note: leaving glands in BABB for extended time results in the lightening of the hematoxylin stain.
- For imaging, remove gland from tissue-cassette and press between two glass slides to flatten. Secure with mini binder clips and view under a dissecting microscope.
- For long-term storage, transfer to methyl salicylate in glass scintillation vials and KEEP DARK. (If exposed to light, the blue will fade to brown)

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- STOCK HEMATOXYLIN (10%):
 - Harris hematoxylin (Fisher, powder)
- Add 10g of powder to 100 ml of 95% ethanol, leave overnight stirring and it will go into solution. Keep covered in foil. Let sit at least 3 weeks to 1 month for best stain.

Representative images:

