Mammary Gland Whole Mount Protocol

- Inject animals with BrdU 2 hours before harvesting tissue (0.1cc per 10 g animal weight)
- Fix tissue for 2 hours in fresh, cold 4% paraformaldehyde (PFA) in 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 30 min Acetone 30 min Acetone 30 min Note: This step prohibits later immunohistochemical analysis by creating high background. If you want to do immunohistochemistry on these glands, skip this step.

• Rehydrate:

100% EtOH 30 min 95% EtOH 30 min

• Stain:

Hematoxylin stain:

0.13 g FeCl3·6H20
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 1/2 hours until whole gland looks purple. If you did not defat in acetone (for future immunohistochemistry), stain O/N to allow hemotoxylin to permeate through fat. Monitor stain by holding gland up to the light.

• Rinse:

Crude tap H20	rinse	(whole gland will turn blue)
Crude tap H20	overnight	
Distilled tap H20	rinse	

• Destain:

200 ml 50% EtOH + 416 ul 12N HCl for 30 min 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 1 hour
95% EtOH 1 hour
100% EtOH overnight
Complete 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:

BABB	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.
Note:	Xylenes or Histoclear can also be used for clearing glands. We have better luck
	with BABB, however.

For imaging, remove gland from tissue-cassette and press between two glass slides to flatten.

For long-term storage, transfer to methyl salicylate in glass scintiallation vials and KEEP DARK. (If exposed to light, the blue will fade to brown)

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

Representative images:



