Cryoprotection and Processing of Embryonic Tissue for β-gal Labeling/*in situ* Hybridization

For anesthetizing adult mice:

- use Avertin (ref. – Manipulating the mouse embryo, 2nd Ed.; p. 416)

- stock solution: 10g of 2,2,2 – tribromoethyl alcohol (T4,840-2)
  10 ml of tert-amyl alcohol (24,048-6)
  (both from Aldrich)
  mix in 50ml falcon tube
  cover in foil & store at 4°C

- working solution: prepare 2.5% solution in PBS (250λ of stock + 9.75ml PBS).
  Stir vigorously until dissolved

- dose: 0.016mg/g body weight (typical adult ≈ 20-25g; use ~ 400λ mouse).

Dissection:

• Dissect embryos in PBS over ice – remove yolk sac to genotype (E8.5-10.5) or use small (2mm) piece of tail (E11.5-early postnatal stages) (place in 200γ tubes; add 50λ tail digest yolk sac or 100λ to tail and incubate o/n at 60°C; heat inactivate at 100°C for 10 min).

• Fix embryos (up to E14.5) or brains (E14.5-P0) with 4% paraformaldehyde (PFA) for 20 minutes at 4°C. Cryoprotect as described below.
Note: For adults perfuse intracardially with saline, then PFA. Fix in PFA o/n and cryoprotect.

•For whole mount X-gal labeling on embryos skip the embedding and go directly to the X-gal wash buffer step described below; for sectioning proceed with cryoprotection.

Cryoprotection:

• Rinse in PBS

• 15% sucrose for 1 hour
• 30% sucrose until submerged (o.n)

} Begin genotyping when applicable

• Embed embryos with genotype of interest in OCT

Freezing:

• Freeze using the 2-methyl-butane/acetone method. This has been adapted as follows from: CRYOTECHNIQUES FOR LIGHT MICROSCOPY (Mark Donovan and Henry Preston).
Liquid Nitrogen - Isopentane Method (-150°C)
REAGENTS REQUIRED
•Isopentane (equivalent to 2-methyl butane)
• Liquid nitrogen
• OCT embedding compound
METHOD
1 Place 50ml of isopentane in a pyrex or polypropylene beaker.
2 Immerse the beaker in a dewar or styrofoam container of liquid nitrogen.
3 Stir the isopentane until opalescent (about 2-3 minutes) and temperature reaches -150°C.
4 Place OCT compound (or similar) in a cryomould and orientate the specimen within it.

5 Immerse the specimen into the cooled isopentane until frozen (usually takes 2-3 minutes).
6 Place the frozen block into the cryostat for sectioning or store at -20°C until required. The specimen will require warming to its optimal cutting temperature before

• Section @10-12μm with CT = -20°C; OT = -18°C
mount on Fisher Biotech ProbeOn Plus slides (No. 15-188-52) as these accommodate 50ml solution aliquots in glass coplin jars

X-gal reaction:

• Fix sections with PFA for 5min in coplin jar

• Wash with X-gal washing buffer 2X, 10min each

1ml of 1M $MgCl_2$ [2mM]
500λ of Igepal Ca-30 [0.1%]
0.25g deoxycholate [0.05%]
500ml PBS

• Incubate in Xgal rxn buffer o/n at 37°C

0.106g potassium ferrocyanide
0.082g potassium ferricyanide
48ml of Xgal wash buffer
2ml of X-gal substrate stock solution (25mg/ml) [1mg/ml]
50ml total volume

(100x stock solution of potassium ferrocyanide/potassium ferricyanide in watee can be prepared in H20 and stored at -20°C)

For section *in situ* hybridization on embryonic tissue freeze as described above and follow the section *in situ* protocol available on the Joyner website under the protocols heading.