LacZ Staining on Cryosections

1. Fix the samples in 0.2% Glutaraldehyde solution with 2mM $MgCl_2$ at 4°C O/N.

0.2% Glutaraldehyde solution	
25% Glutaraldehyde – stock	400 µl
1M $MgCl_2$ (prepare in H_2O)	100 µl
1x PBS	50 ml

2. Decalcify the samples in 10% EDTA with 2mM $MgCl_2$ at 4°C.

   Note: The decalcification time is dependent on tissue type and sample stage (e.g. ~ 1 week for E16.5 embryonic heads).
3. Wash the samples in 1XPBS containing 2mM $MgCl_2$ at 4°C O/N.
4. Dehydrate the samples in sucrose solution.
   a) Dehydrate the samples in 15% sucrose with 2mM $MgCl_2$ in at RT until the samples sink down.
   b) Dehydrate the samples in 30% sucrose/50% OCT with 2mM $MgCl_2$ at 4°C O/N.
   c) Dehydrate the samples in OCT with 2mM $MgCl_2$ at 4°C O/N.
   d) Embed the samples in OCT on dry ice and store the samples at -80°C.
5. Section the samples into 8µm thick slices and air-dry the slides at RT or 37°C.
6. Rinse the slides in PBS with 2mM $MgCl_2$.
7. Wash the slides in PBS with 2mM $MgCl_2$ for 10 minutes.
8. Stain the samples in LacZ staining solution.
   a) Prepare LacZ staining solution and protect from light.
   b) Stain the slides in LacZ staining solution at 37°C in a jar wrapped in aluminium foil.
   c) Check the development of signal under the microscope.

d) After staining, the LacZ staining solution can be filtered and kept at 4°C to be reused.

LacZ Staining Solution (****LIGHT SENSITIVE****)	
1M $MgCl_2$	1 ml
1% NaDOC (Sodium deoxycholate)	5 ml
1% NP-40 (Nonidet P-40)	2.5 ml
50 mM K Ferri (Potassium ferricyanide), $K_3Fe(CN)_6$	50 ml
50 mM K Ferro (Potassium ferrocyanide), $K_4Fe(CN)_6 \cdot 3H_2O$	50 ml
1M Tris (pH 7.3)	10 ml
40 mg/ml X-gal	12.5 ml
1x PBS	

Note: Store freshly made staining solution in 50ml aliquots (wrapped in aluminium foil) at -20°C.

9. Wash the slides twice in PBS at RT for 5 minutes each.
10. Fix the slides in PFA or Formalin for 30 minutes.
11. Rinse the slides in dH_2O.
12. Counterstain the sections for 3 minutes in Nuclear Fast Red.
12. Wash the slides twice with dH_2O.
13. Wash the slides in 70% Ethanol for 2 minutes.
14. Stain the sections in Eosin for 1 minute 15 seconds.
15. Wash the slides in 95% Ethanol.
16. Wash the slides twice in 100% Ethanol for 2 minutes each.
17. Wash the slides twice in Xylene for 2 minutes each.
18. Mount the slides.