X-GAL STAINING OF MOUSE EMBRYOS

REAGENTS REQUIRED

25% glutaraldehyde
400mM potassium ferricyanide in ddH2O [K3(Fe(CN)6)], stored at -20 in the dark
400mM potassium ferrocyanide in ddH2O [K4(Fe(CN)6)], stored at -20 in the dark
20mg/ml X-Gal in DMSO or DMF, stored at -20 in the dark
100mM EGTA, pH 8.0
500mM MgCl2
500mM Phosphate Buffer, pH 7.4
10% Nonidet P-40 (NP-40)
1% Sodium deoxycholate (Na-deoxy.)
1M tris-HCl, pH 7.3
Phosphate Buffered Saline

WORKING SOLUTIONS TO MAKE AND STORE AT RT

β-Gal FIX: 0.1M PO4, 5mM EGTA, 2mM MgCl2
- 100mL 0.5M PO4 buffer
- 25mL 100mM EGTA
- 2mL 0.5M MgCl2
- 373mL ddH2O

β-Gal WASH: 0.1M PO4, 2mM MgCl2, 0.01% Na-deoxy., 0.02% NP-40
- 100mL 0.5M PO4 buffer
- 2mL 0.5M MgCl2
- 1mL 10% NP-40
- 5mL Na-deoxy.
- 392mL ddH2O

β-Gal STAIN: 0.1M PO_4, 2mM $MgCl_2$, 20mM tris-HCl pH 7.3, 0.01% Na-deoxy, 0.02% NP-40 (+5mM K3(Fe(CN)6), 5mM K4(Fe(CN)6),on 1mg/ml X-gal)
- 100mL 0.5M PO4
- 2mL 0.5M MgCl2
- 10mL 1M tris-HCl, pH 7.3
- 5mL 1% Na-deoxy.
- 1mL 10% NP-40
- 344.5mL ddH2O

Make up solutions minus last three components which will be added immediately before use
- 6.25mL 400mM K3(Fe(CN)6)
- 6.25mL 400mM K4(Fe(CN)6)
- 25mL 20mg/ml X-gal

M. Lamarche, 2011

Protocol:

1 Dissect out embryos into a dish of cold PBS

2 Wash embryos once in cold PBS on ice (~5 mins without rocking)

3 Fix the embryos

Add 400uL glutaraldehyde to 50mL cold FIX solution. Aliquot 1.2mL cold fixative into 2mL round bottomed microtubes containing one embryo each.
Incubate on ice for:

E6 to E8 – 10 to 15 min
E9 to E11 – 15 to 20 min
E12 to E13.5 – 20 to 30 min
** for E13.5, use 25 min

4 Wash fixed embryos.

Transfer embryos to pre-labelled 12 well dish. Incubate 3x 5min in 2mL of β-gal WASH solution at room temperature with rocking.

5 Stain embryos

Pre-warm stain working solution to 37°C. Just before adding stain to wells, add the appropriate volume of K3(Fe(CN)6), K3(Fe(CN)6), and X-gal.
Add 2mL stain per well.

Stain for 1 hour to overnight (or longer) at 30-37°C. Avoid incubators with added CO2.
** Tris-HCl is only required in stain solution if staining overnight
** If incubating for longer than a few hours, be sure to wrap plate in parafilm
**Both specific staining and background staining increase with increased incubation time.
** When looking for activity in melanocytes, embryos are stained at 37°C for 48 hours

6 Transfer embryos to a fresh 12 well dish and wash stained embryos 3x in PBS at RT with rocking for (2 minutes for first wash, 5 minutes for 2nd and 3rd wash)

Pictures can be taken at this point or fixed and photographed later

7 Fix stain with 4% formaldehyde in PBS at RT for at least 1 hour (with rocking)

Embryros can be stored in this solution

8 Wash embryos in PBS (2x 5 minutes at room temperature with rocking) and photograph

9 Clearing

Incubate embryos in 50% glycerol, 50% PBS, then
80% glycerol, 20% PBS
** incubate in glycerol:PBS mixture until embryo has gone from floating on top of solution to being submerged and laying on bottom of tube or well
**can store in the last glycerol:PBS solution

10 Genotyping embryos

Cut off a small portion of the bottom of the embryo (ex. Tail + 1 leg) and digest using proteinase K.
** Digestion is more successful after embryos have been cleared vs. when sitting in formaldehyde fix solution