FIXATION & LACZ STAINING OF MOUSE EMBRYOS (BLACK LAB)

1. Euthanize female mouse

2. Remove uterus to a petri dish of PBS-A

3. Remove embryos with yolk sac

4. Remove all membranes (**including the amnion**); save yolk sac in tail buffer for Southern, if necessary

5. Rinse embryos in PBS-A to remove all blood, etc.

6. Fix embryos in FIX SOLUTION at 4°C in 24- or 6-well plates (5 ml fix in a 6 well; 1 ml fix for 24 well)

8.5-9.5 dpc	30 min.
10.5-11.5 dpc	60 min.
12.5 dpc	1.5 hours
13.5 dpc	2 hours
14.5 dpc	2.5 hours
15.5 dpc	3 hours
16.5-17.5 dpc	4 hours

   For 15.5 dpc and younger, no skinning is required; for 15.5 and older, skinning is required

7. Rinse quickly with PBS-A; then incubate in PBS-A for 30 min.

8. Put embryos in STAINING SOLUTION overnight in the dark (wrap in foil) at RT
   For 13.5-15.5 dpc embryos, the 'bros should be permeablized with detergent during staining
   make staining solution FRESH

9. Rinse quickly with PBS-A; then incubate in PBS-A for 30 min.

10. FIX again overnight in 4% formaldehyde (salmon colored--see below)

11. Embryos can be stored indefinitely in 4% formaldehyde or in PBS-A at 4°C

SOME EMBRYOS MAY NEED TO BE **CLEARED FOR WHOLE MOUNT PHOTOGRAPHY**
(especially true for 12.5 and older; skinned embryos CANNOT be cleared)

1. following fixation, rinse embryos in PBS-A

2. DEHYDRATE embryos in ethanol
   put embryos in GLASS scintillation vials
   add 10 ml 50% ethanol; incubate for 30 minutes
   add 10 ml 70% ethanol; incubate for 30 minutes
   add 10 ml 100% ethanol; incubate for 30 minutes
   add 10 ml 100% ethanol; incubate OVERNIGHT (can be stored at -20°C indefinitely)

3. Next day, add 10 ml 100% ethanol; incubate for 30 minutes

4. Add 10 ml 1:1 benzyl benzoate: benzyl alcohol; WATCH CAREFULLY, embryos will clear quickly

5. To photograph put in glass petri dishes in FRESH 1:1 benzyl benzoate: benzyl alcohol
   be sure that embryos are completely submerged for photography

ALL WORK FOR CLEARING MUST BE IN **GLASS** AND MUST BE **ANHYDROUS**
(plastic will melt and water will cause a white precipitate that cannot be removed)

SOLUTIONS

10% formaldehyde from paraformaldehyde
mix 50 ml dH_2O with 10 g paraformaldehyde
heat and stir on a hot plate (solution will remain cloudy)
add ~100 µl of 1.0 N NaOH which will make the solution go clear
filter the solution through a 0.8 micron filter (0.45 micron filter can also work)
add 10 ml 10X PBS-A
QS to 100 ml with dH_2O
aliquot and store at -20°C

FIX SOLUTION	for 10 ml
2% formaldehyde	2 ml of 10% stock
0.2% glutaraldehyde	80 µl 25% stock (Sigma EM grade)
1X PBS-A	1 ml of 10X stock
	6.92 ml of dH_2O

add 5 µl of phenol red (solution should be SALMON colored--light pink)
if necessary, adjust pH by adding 10% HCl until proper color (a few microliters)

STAIN SOLUTION (10 ml)

0.5 ml	100 mM pottasium ferrocyanide (store stock in 1 ml aliquots at -20°C)
0.5 ml	100 mM pottasium ferricyanide (store stock in 1 ml aliquots at -20°C)
20 µl	1.0 M $MgCl_2$
100 µl	Xgal in DMF (100 mg/ml) (store stock at -20°C)
1.0 ml	10X PBS-A
7.9 ml	dH_2O

To permeablize--	add NP40 to a final conc of 0.02%	20 µl of 10% NP40
	add sodium deoxycholate at 0.01%	20 µl of 5% sodium deoxycholate
	add Tris pH=7.4 to a conc of 20mM	200 µl of 1.0 M Tris pH=7.4

10X STOCK OF PBS-A (500 ml)

NaCl	40.0 g
KCl	1.0 g
KH_2PO_4	1.0 g
Na_2HPO_4	5.75 g

dissolve into a final volume of 500 ml dH_2O and autoclave