X-GAL Staining of Embryos

To obtain homozygous mutant embryos for PCR analysis. (Make sure your stock is balanced with a lacZ balancer chromosome; e.g. wg-lacZ, Ubx-lacZ, ftz-lacZ.)

1) Collect germ band retracted embryos (i.e. older than 8 hours at 25°C). Overnight collections also work, but pick out germ band retracted (i.e. older) embryos.

2) Fix the embryos in one of two ways:
   a. with MeOH/Heptane (i.e. just pop the embryos with MeOH)
   b. with 1% glutaraldehyde/PBS 1:1 with heptane for 15 minutes

3) Devitellinze (pop) the embryos with MeOH.

4) Wash 3 X with 1 ml of MeOH (just add the MeOH and let embryos sink).

5) Wash 3 X 1 min with 1 ml PBT.

6) Rock embryos on nutator in 1 ml of PBT for 6 hours (or until the end of the day).

7) Wash once with 1 ml staining solution, and then rock O/N in 1 ml staining solution in the dark (i.e. wrap the tubes in foil).

All steps at room temperature

X-GAL staining solution for 2 ml:
1780 ul PBT
150 ul 2% X-GAL in DMF
70 ul Ferri-ferro cyanide
2 ul 1M $MgCl_2$

Ferri-ferro cyanide:
0.1M K ferricyanide
0.1M K ferrocyanide

Keep at 4°C in the dark (we have some in the refridgerator).

8) After staining wash 3 X with PBT

9) Hand pick the white embryos (5-10) with a small pipet man, and put in 0.5 ml tube.

10) Remove all the PBT, replace with 20 ul squish solution, and mash with rounded pipet tip.

Squish solution:
10 mM Tris 8.0
1 mM EDTA
25 mM NaCl
200 ug/ml Proteinase K

11) Heat the mashed embryos at 37°C for 15 min

12) Inactive the proteinase K by heating at 85°C for 15 min

13) Spin down junk. Use 1-5 ul for PCR