β-galactosidase histochemistrical analysis

Whole mount X-gal staining and histology

1. Dissect embryos in PBS
2. Fix embryos with 4% parafomaldehyde/PBS at 4°C (E7.5/5min; E8.5/10min; E9.5/20min; E10.5 to later/30min)
3. Wash with lacZ rinse 3 X 20 min at 4C°
4. Stain with X-gal staining solution O/N at 37°C.

Solution:

lacZ rinse (for 500 ml)
100 mM sodium phorsphate (pH7.0-7.5) (I use PBS routinely)
1 ml 1 M $MgCl_2$ (final 2 mM)
1 ml 10% NP-40 (0.02%)
0.01% sodium deoxycholate (optional for embryos <E10.5)

X-gal staining solution (for 10 ml)
04 ml 25 mg/ml X-gal in dimethylformamide (1mg/ml final)
0.1 ml 0.5 M (1.6 g in 10 ml) potassium ferricyanide (5 mM) (Sigma P-8131, FW 329.2)
0.1 ml 0.5 M (2.1 g in 10 ml) potassium ferrocyanide (5 mM) (Sigma P-9387, FW 422.4)
in lacZ rinse solution

Sectioning of X-gal stained embryos

1. Postfix embryos in 4% PFA/PBS at RT for 1 hr.
2.

X-gal staining frozen sections

After fixation, placentas were washed with PBS for 5 minute three times and then passed through graded sucrose solution from 10%, 20% and 30% in PBS for 8-12 hours each step at 4C. Placentas were embeded in O.C.T. compound (Sakur Finetek Inc.) on a block of dry ice. After freezing, samples were stored at –80°C until they were processed for sectioning. Sections were cut at 14 um thick with a Reichert-Jung cryostat (2800

frigocut) at temperature of –20°C and mounted on Superfrost/plus microscope slides (Fisher Scientific). Sections were allowed to dry at RT up to one hour and rinsed with PBS for 5 minute three times, then stained with X-gal staining solution (the same as that for whole mount staining) at 37°C overnight. After staining, sections were washed with PBS three times for 5 minute and postfixed with 4% paraformaldehyde for 2 minute and counterstained with hematoxyline and Eosin Y.