β-gal immunohistochemistry (Black Lab 2005)

- The primary antibody we use is from ICN (now MP). It is rabbit anti-β-galactosidase, the catalog number is 55976 and the web page is: http://www2.mpbio.com/servlet/ItemDisplay?i=12363&catnum=55976.

For all of the following protocols, we use a dilution between 1:300 and 1:1000 of primary antibody. The antibody is diluted directly before use into 3% normal goat serum in 1x PBS.

- The secondary antibody we use varies depending on what type of picture we require. We have successfully used a number of anti-rabbit HRP conjugated secondary antibodies from Sigma. Also, we regularly use a fluorescent green anti-rabbit secondary antibody from Molecular Probes called Alexa Fluor 488 (catalog #A-11034). Both are usually used at a dilution of 1:300 diluted directly before use into 3% normal goat serum in 1x PBS.

- For transgenic lines that express high levels of β-galactosidase we embed the embryo in paraffin wax, section, and perform the IHC on the sections (best results within 2 months of cutting). This is by far the best way since the histology is usually better and the sections are easier to cut and last longer.

- For lines that express lower levels of β-gal, including the GT-Rosa line (ROSA26R), we have been unable to detect any signal after paraffin embedding, so instead we use frozen sections.

Protocols are on the following pages.

1. β-gal IHC with paraffin sectioned slides

-Fix embryos in 4% paraformaldehyde overnight, then store in 1xPBS or 100% methanol until use.

-Dehydrate embryos through a series of ethanol washes into 100% ethanol, clear with three washes in xylene (until embryo is translucent, 10-80 mins depending on age) and then infiltrate with paraffin wax at 65°C for 2-3 hours at pressure. Allow to set at room temp as a wax block.

- Cut sections 5-6 microns thick. These can be cut in advance but we have the best luck when the sections are less than 2 months old. Store at room temperature in dry conditions

- Dewax and hydrate the sections through a series of three xylene washes of 1 min each, followed by two washes in 100% ethanol, then thru 95%, 80%, 70% ethanol and into 1XPBS. Leave in the 1xPBS for around 5 mins.
- If you are planning to use a peroxidase conjugated secondary antibody, incubate the sections in 1.5% hydrogen peroxidase (in PBS) for 20 mins. Rinse in PBS for another 5 mins
- Block sections in 3% normal goat serum for 30 minutes.
- Carefully drain away goat serum.
- For each slide, add 100-200µl primary antibody (diluted 1 in 1000 in 3% normal goat serum) and cover carefully with coverslip
- Incubate at RT for 1-3 hours OR at 4°C overnight. To prevent the slides drying out, do this in humid conditions (we use a tuperware box with wet tissues, sealed with saran wrap).
- Rinse in 1XPBS for 1 hour. Coverslips should fall off naturally, do not force. Change PBS 3 times or so.
- For each slide, add 100-200µl diluted secondary antibody and cover with coverslip
- Incubate at RT for 1 hour in humid conditions. If using a fluorescent antibody, incubate in the dark
- Again rinse in 1xPBS for 30-60 minutes.
- If using fluorescent antibody, mount using Slow-Fade media. If using HRP conjugated secondary, develop image using a peroxidase substrate kit. We use either DAB or Vector SG kits from Vector labs, catalog numbers SK-4100 and SK-4700.

2: β-gal IHC with frozen sections

- Fix embryos in 4% paraformaldehyde overnight, then store in 1xPBS.
- Place embryos into 10% sucrose solution and incubate 3-15 hours with motion at 4°C. Repeat with 20% and 30% sucrose solutions.
- Bring to RT. Remove embryo from sucrose solution and place in small plastic mold. Immerse in O.C.T. compound (Tissue-Tek #4583), place on dry ice and allow to freeze at -20°C for 2 hours-overnight.
- Cut sections 10-20 microns thick on cryostat. These can be cut in advance but should be frozen after cutting and used pretty soon.
- Place slides in the 1xPBS for around 5 mins.
- Fix slides for 10 mins in 4% PFA
- Rinse 5 mins in PBS

(-If you are planning to use a peroxidase conjugated secondary antibody, incubate the sections in 1.5% hydrogen peroxidase (in PBS) for 20 mins. Rinse in PBS for another 5 mins.)- Block sections in 3% normal goat serum for 30 minutes.
- Carefully drain away goat serum.
- For each slide, add 100-200µl primary antibody (diluted 1 in 1000 in 3% normal goat serum) and cover carefully with coverslip
- Incubate at RT for 1-3 hours OR at 4°C overnight. To prevent the slides drying out, do this in humid conditions (we use a tuperware box with wet tissues, sealed with saran wrap).
- Rinse in 1XPBS for 1 hour. Coverslips should fall off naturally, do not force. Change PBS 3 times or so.
- For each slide, add 100-200µl diluted secondary antibody and cover with coverslip
- Incubate at RT for 1 hour in humid conditions. If using a fluorescent antibody, incubate in the dark
- Again rinse in 1xPBS for 30-60 minutes.
- If using fluorescent antibody, mount using Slow-Fade media. If using HRP conjugated secondary, develop image using a peroxidase substrate kit. We use either DAB or Vector SG kits from Vector labs, catalog numbers SK-4100 and SK-4700.