Whole mount immunocytochemistry

(Fuchs lab)

1. Obtain embryos from uterus (I presume you or someone in the lab is doing this).
2. Depending on the antibodies to be used, fix the embryos in cold methanol for 10 min or 2.0% paraformaldehyde for 30 min.
3. After fix, wash them in PBS + 1.0% BSA. Remember, if you place them in cold methanol for fix, when you place them into PBS for the wash they will float and move on top of the PBS rather fast until they rehydrate (about 1 min) You may need to sink them with your forceps.
4. If fixed in PFA, you must permeabilize the cell membranes using PBS+0.5% Triton X-100 + 2.0% PFA for 30 min.
5. Order the M.O.M. kit from Vector Labs. Follow directions in the MOM kit for making the IgG block and protein block.
6. Place the embryos in drops (50-100microliter) of IgG block. Leave them for at least 1 hr at r.t. Can go overnight at 4 degrees as long as you keep them from dehydrating. THIS STEP is mandatory if your primary antibody is a mouse monoclonal.
7. Wash 3X in PBS + 0.5% triton + 1%BSA 15 min each.
8. Place embryos into MOM protein block for 30 min.
9. Dilute antibodies in fresh protein block.
10. Place embryos in drops of protein block containing abs. Can incubate 1hr at r.t. or go overnight at 4 degrees.
11. Wash 3X PBS+BSA+0.5%Triton
12. Dilute 2ndary abs into protein block.
13. Place embryos into drops of 2ndary abs. 1 hr r.t. (don't go too long in 2ndaries)
14. Wash embryos as in step 11.
15. Mount embryos

a. To do this you need to be a little careful. You have to make 4 posts of wax which your coverslip will rest on. You can't place the coverslip directly onto the embryo on your slide. If you do, you will smash the embryo. I make a 13:1 dilution of paraffin:petroleum gelly (vaseline). I melt it together in a beaker over a flame and then pour it into a 10ml syringe. I use an 18 or 20 gauge needle to make the 4
posts, one at each corner of the cover slip.

b. I place the embryo onto a superfrost + slide with as little of wash buffer as possible. I then surround the embryo with antifade and place the coverslip onto the slide.

c. SLOWLY and CAREFULLY push the coverslip down always watching with a dissecting scope when you start pushing down on the embryo. Once you touch the embryo, stop pushing. Seal the coverslip with nail polish.

You are ready to look at them.