Bob's Embryo BrdU-labeling protocol

Labeling:

1. Collect, dechorionate and wash embryos in Nitex basket as usual

2. Air dry embryos on the net for 3-5 minutes depending on the humidity. Wipe liquid away from bottom of screen and inside of basket with a kimwipe. Set the basket upside down on the bench top. Use a dissecting scope to check dryness. Embryos have a dull sheen as opposed to being really shiny when they are properly dry (this isn't always obvious). Be careful not to overdry, or they won't devitellinize after fixation.

3. Permeablization: Put the basket in a weigh boat and add octane to cover the embryos. Swirl the embryos to the middle of the basket, and then bounce the basket to spread the embryos in a monolayer on the net. Incubate 3 minutes.

4. Transfer to Schneider's + BrdU. This is the trickiest part. Carefully remove the basket from the octane and blot from the bottom, first by setting the basket on a paper towel, and then by wiping with a kimwipe. Do this quickly. IMMEDIATELY check the embryos under the dissecting scope. Watch the octane evaporating. When it is almost gone, or just gone (this can take only a few seconds), put the basket in a new weigh boat and add Schneider's + 1 mg/ml BrdU BY POURING ON TOP. If you pour outside the basket and let the media run up from underneath then the embryos will float which is not good. If you've done it correctly, the embyros will stay stuck on the basket (don't worry if a few or even some float). Incubate for 15 min or for however long you want to do the pulse. Be careful not to overdry the embryos when you remove them from the octane. If they wrinkle a little bit they are OK, if they shrivel up, then they are too dry and they won't devittelinize.

5. Fixation: Remove the basket from the Scheider's 1 min before the end of your pulse, and place in a watch glass with heptane. Squirt the embryos off the bottom of the net with heptane. They should come off fairly easily. If they are cemented on there, then they are too dry. Transfer them to an eppendorf or some other vessel to do the fixation. This can be tricky because the embryos will stick to the sides of the pipette tip sometimes. Fix as you normally do. I use 37% formaldehyde for 5 min. Less intense fixations also work fine.

6. Remove fix, add MeOH and shake to devittelinize. Take only the ones that sink. If none of them sink, then the embryos got too dry at some step.

7. Wash 3 X in MeOH, 3 X in EtOH, and store at -20°C

Detection:

1. Wash 1 X in MeOH. Remove MeOH and add 2M HCl with 0.1% Tween. Incubate 40 min. IMPORTANT: make sure acid is really 2M or it won't work.

2. Wash 2 X 2 min in 0.1 M Na Borate

3. Wash 3 X 3-5 min in PBT

4. 1° antibody for 1 hour at RT. 1:20-1:100 Becton Dickinson anti BrdU mouse monoclonal (dilution depends on the lot). No blocking should be necessary, but this depends on the lot again.

5. Wash 3 X 10 min in PBT

6. 2° antibody for 1 hour. Whatever you want. I use 1:500 Goat anti mouse Rhodamine from Jackson. You can also do histochemistry. DAB staining with HRP secondary works great if you get no background.

7. Wash 3 X 10 min in PBT and mount or stain.