To collect *Xenopus* embryo stages:

Use a plastic transfer pippette with the tip cut off. Be gentle!

Get embryos at the right stage (use staging sheets in the frog room). If using pigmented (wild-type) embryos, and if collecting early blastomere stages, choose ones with a clear pigment difference (ventral cells dark, dorsal cells light). Albino embryos can be stained to make it easier to see what stage they are: make a stock of Nile Blue (10%) (in 50 mM sodium phosphate pH 7.8), and use it at 0.01% in 0.1X MMR to stain embryos. Doing it in 2% ficoll will make the staining the most even. The stain will survive a rinse, but alcohol washes will remove it.

Make sure you have MEMFA (MEMFA = 50 ml of 10X MEM salts, 50 ml Formaldehyde, and 400 ml DEPC water).

<u>For embryos of 2-cells to 8 cells:</u>

- Fix them: put embryos in a scint vial full of MEMFA. Let them rock or nutate, either at room temperature for 1-2 hours, or in the cold room overnight.
- Put the embryos in a petri dish full of DEPC 1X PBS, and devitellinize with forceps under the dissecting scope. Transfer them back into MEMFA as you take the membranes off (can do groups of a dozen or so).

<u>For embryos that are 1-cell stage, or stage 5 to stage 22:</u>

1. Put the embryos in a petri dish of 8 ml 0.1X MMR with an aliquot of the Proteinase-K from the frog-room freezer. Watch

them under the scope for 5-10 minutes, swirling lightly, and watch the membranes first expand away from the embryos, and then fall off.

2. Gently transfer them to MEMFA, and let them rock or nutate, either at room temperature for 1-2 hours, or in the cold room overnight.

<u>For embryos that are bent stages (stage 22 or later):</u>

1. Devitellinize manually with forceps;
2. Transfer them to MEMFA, and let them rock or nutate, either at room temperature for 1-2 hours, or in the cold room overnight.

3. Use the vacuum pump to change liquids: suck out the MEMFA, and put in DEPC 1X PBS. Leave rocking in 1X PBS for 10 minutes.

4. Then suck out half of the PBS and add half a vial of methanol from a beaker. Suck out half of that, and add half a vial more methanol. Do this like 4 times. Gently tip the tube in between to make sure embryos are not stuck to the sides, bottom, or each other. Use the stream of methanol to make sure the contents of the tube mix.

5. At the end, remove most of the liquid, and add methanol.

6. Put the tubes at -20 °C. They're good for about a month.

7. When turning off vacuum pump, make sure to break both vacuums first, by pulling out Eppendorf tubes out of corks of traps.

Recipes

10X MEM Salts:

- 1 M MOPS
- 20 mM EGTA
- 10 mM $MgSO_4$
- pH 7.4 w/ NaOH

MEMFA:

- 5 mL 10X MEM Salts
- 5 mL Formaldehyde (37%)
- 40 mL H_2O