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Embryology Tool-Kit

Rationale and Background:
Materials and equipment that are useful for many aspects of embryology, but are particularly useful for use with embryos and larvae of marine invertebrates. Many of the following techniques are adapted from the MBL at Woods Hole Embryology Course.

Protocols:

- **Braking Mouth Pipette**

Extremely useful for transferring embryos in small volumes of liquid.

Materials:
Latex rubber tubing (or silicone)
Blue p1000 pipette tips (transfer pipette holder)
Mouth piece
UltraFine gel loading pipette tip (brake) – traditionally, glass pipettes are used, but plastic pipettes have the advantage of flexibility.
Transfer pipette

Directions:
Cut a length of tubing to slightly longer than your arm, enough that you will be able to comfortably hold the transfer pipette with the mouthpiece in place.
Attach a mouthpiece to one end.
Make a cut in the tubing about 1/3 of the way down from the mouthpiece; not so close to the mouthpiece that the brake will become blocked with saliva, or so near to the transfer pipette that it could become clogged with fluid.
Cut the thickest part of the brake and insert into the tubing. The direction of the brake should not matter.
Insert the fine end of the p1000 tip into the end of the tubing. The large end of the pipette tip fits the end of a transfer pipette made from a pulled Pasteur pipette.
Before use, check that there are no leaks in the tubing and seal all joints with parafilm. When you blow air out of the pipette, you should be able to produce bubbles with enough force, and suck up water (and embryos) with great control and precision, using your tongue to stop the flow of water.

- **Gelatin Dishes (Henry and Martindale, 2011; MBL Woods Hole Embryology Course)**

Gelatin can be used to prevent charged embryos from sticking to transfer pipettes, operating needles and plastic culture dishes.

Materials:
<u>5X Gelatin Stock solution</u>
(Can be stored at room temperature and should last for several months)
0.25g (0.5%) Knox unflavoured gelatin – dissolve before adding formalin, may need to heat gently

(Kraft Foods Inc. – found in the baking aisle of the grocery store)
250 ul Formalin (0.19% formaldehyde)
50 ml dH2O

Directions:
For coating dishes and transfer pipettes, dilute to 1X working strength with dH2O before use. Add several mls to a small petri dish (35mm), swirl to coat the dish, and pour into the next dish. A thin gelatin coating will remain in the first dish. Let dishes dry completely, propped upside down overnight. Mark a stack of gelatin dishes with a stripe on the side of the dish. Rinse 3X with dH_2O before use.

- Wet Mounts of Live Invertebrate Embryos and Larvae

For observing embryos and larvae under a compound microscope.

Materials:
Slides
Coverslips
Non-Toxic Modelling Clay (such as Van Aken Plastina)
Rain-X (available at hardware stores)

Directions:
Place a small amount of Rain-X on a KimWipe.
Wipe one side of a clean slide with Rain-X. Allow to dry.
Polish slide to clean film left behind by Rain-X. Your slide should now form a nice bead when liquid is added. Do the same with a coverslip.
Place a drop of liquid (filtered seawater) containing your embryo of interest on the slide.
In order to not crush your embryo, you can use clay "feet" on the corners of your coverslips. Draw the corner of a clean, Rain-X treated coverslip across the modelling clay. It will deposit a small amount.
Carefully place coverslip over sample, using forceps if needed.
You should now be able to roll your specimen under the coverslip, to reach the appropriate orientation for imaging or observations.