Modified Vic's Fix Procedure

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Required Materials:

- egg collection basket
- dilute bleach solution (1:1 bleach to deionized water)
- embryo wash (0.7% NaCl, 0.03% Triton X-100 in deionized water)
- 0.1% Triton X-100 solution in deionized water
- 1:1 PBS/Heptane mixture in a glass vial
- Vic's fixative (8 parts 37.5% formalin and 10 parts MeOH free, EM-grade 40% paraformaldehyde). Freeze at -20°C in glass vials as small aliquots.
- Petri dishes (1-1/2" diameter)
- Double stick tape
- PBTA (1x PBS, 1% Bovine Serum Albumin (BSA), 0.05% Triton X-100, 0.02% NaN_3 (Sodium Azide))
- 25-gauge needle on 5 ml syringe for hand devitellinization.

A volume of Vic's fixative equivalent to the volume of PBS <u>or</u> Heptane used in the 1:1 PBS/heptane solution is drawn up into a syringe with 20-gauge needle.

Procedure:

1) Dechorionate. The embryos are dechorionated in an egg collection basket for 2 minutes using the 50% bleach solution.

2) Rinse. After dechorionating the embryos, rinse them with embryo wash solution.

3) 0.1% Triton X-100 Treatment (Optional). The rinsed embryos are immersed in a 0.1% Triton X-100 solution to prevent them from adhering to the walls of the glass vial. Excess Triton X-100 solution is removed by blotting the bottom of the collection basket with paper towels or Kim-wipes. Be careful not to over-dry them, the point of blotting them is to just remove the excess 0.1% Triton- X-100 solution. The embryos should still be moist after blotting.

4) Heptane pre-treatment. Gently transfer the embryos to the 1:1 PBS/heptane solution. A good way to the transfer the embryos is to use a moist, finely tipped brush (such as a small paintbrush). Carefully brush the embryos from the collection basket. Since the brush is moist, the surface tension of the liquid on the brush will allow the embryos to adhere to the brush. Simply dip the embryo filled brush into the 1:1 PBS/heptane solution, which forms a bilayer with the PBS on the bottom and the heptane on the top. The embryos should form a nice MONOLAYER, they should not pileup on each other, at the interface of the two layers. Incubate the embryos for about 30-45 seconds.

5) Dispense the fixative with mild agitation. After incubating the embryos dispense the Vic's fixative from the syringe into the glass vial. The fixative should be added rapidly with gentle agitation to the embryos. A good technique to accomplish this is to very gently vortex the glass vial at a low speed (setting 1 or 2 on a standard vortexer) while

rapidly dispensing the fixative. Make sure to insert the syringe needle into the glass vial before vortexing to avoid injury. Continue the gentle vortexing for a few seconds after rapidly and completely dispensing the fixative.

6) Incubate in fixative. The embryos are allowed to fix for 15 to 30 minutes (must be determined empirically for individual antibodies) with periodic gentle agitation, but shaking vials is not necessary. Both time points give very good results for F-actin and microtubules. But, by adjusting fixation times results can be optimized. For example, a 15 minute fix is optimal for observing F-actin with a slight loss in resolution for microtubules and a 30 minute fix is optimal for microtubule observation with a slight loss in F-actin resolution. A 15 minute fix is usually fine for most antibodies.

7) Remove ALL of the fixative. After fixing, completely remove the entire bottom layer. This layer contains a solution of PBS/fixative. It is VERY IMPORTANT that as much of the PBS/fixative solution is removed as possible. A drawn out Pastuer pipette is useful in removing all of the solution with minimal loss of embryos. Any fixative residue left on the embryos prevents the embryos from adhering to the adhesive tape, which is required to remove their vitelline membranes. After removing the PBS/fixative solution, remove most of the heptane with an unmodified Pastuer pipette, leaving enough behind to cover the embryos, and replace it with fresh heptane. This also seems to help the embryos stick to the tape better, perhaps by removing some residual PBS/fixative.

8) Set up for removal of vitelline membranes. Set up a few plastic petri dishes to hold the embryos for vitelline membrane removal. To do this, apply a 3/4" strip of double stick tape inside a small plastic petri dish (35 x 10 mm).

9) Transfer the embryos to petri dishes for vitelline membrane removal. Pipette the embryos in heptane onto a 1 cm square piece of filter paper. The filter paper should be elevated on a small platform with a diameter of about 2 cm, such as a rubber stopper. Allow the heptane to evaporate, but not for too long or the embryos will dehydrate. Roughly 30 seconds should be appropriate, but the actual time varies from person to person. Transfer the embryos to the double stick tape by turning the petri dish upside down and gently pressing the double stick tape to the embryos on the 3mm paper. Using fine-tipped forceps, remove the filter paper from the petri dish and immediately cover the embryos, now stuck to the tape, with PBTA. Now remove the vitelline membrane using a 25-gauge needle mounted on a syringe.

10) Using an unmodified Pastuer pipette, transfer the devitellinized embryos to an appropriate plastic tube (e.g., 1.5 ml Eppendorf tube), allow the embryos to settle, and rinse them with fresh PBTA. Repeat this 1-2 times.

11) The embryos can now be used immediately for immunofluorescence, stored at 4°C until the next day, or taken through an alcohol dehydration series and stored longer at -20°C. For most experiments we recommend using them immediately.