Preparing dechorionated eggs

(Removal of the outer layer of the egg by trypsin treatment)

To remove the follicular cells (the chorion) from the eggs, which improves microscopic observation yet does not perturb development, trypsin should be added to the eggs.

1. Put an aliquot of 1ml 1% Trypsin solution (in filtered TAPS-buffered (5 mM, pH 8.2)) seawater (prepared in advance and stored at -20 °C) to defrost. Be sure to use a form of trypsin that is not inactivated by calcium - which is 10 mM in sea water.
The most common problem for poor dechorionation is that the pH drifts. If dechorionation is poor try adding more TAPS pH buffer during the dechorionation reaction.

2. Add 1ml 1% trypsin solution to the 4.5 ml eggs and agitate on an orbital shaker for 1hr 30 min. at 20°C (or until approx. 10% of the eggs begin to lose their outer layer) to dechorionate the eggs.

4. Once approx. 10% of the eggs begin to dechorionate gently pipette approx. 10 times without making air bubbles to dechorionate the remaining eggs.

5. Wash eggs to remove trypsin, chorionated eggs and dead eggs. Gently swirl in a circular motion to collect dechorionated eggs and remove the excess sea water. Add fresh TAPS filtered sea water and repeat 4 or 5 times or until most of the dead or chorionated eggs are removed leaving only dechorionated eggs.

6. Transfer the dechorionated eggs to a larger a fresh Petri dish which has been GF-coated and store at 16°C. (The dechorionated eggs should be smooth in appearance. If the eggs have been left too long in the trypsin solution they will form cytasters (the eggs take on a golf ball texture). Eggs like these should be discarded.)