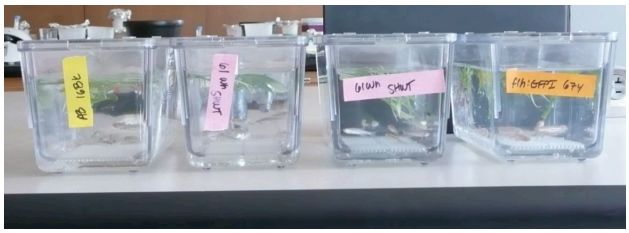
Zebrafish fertilization and embryo isolation

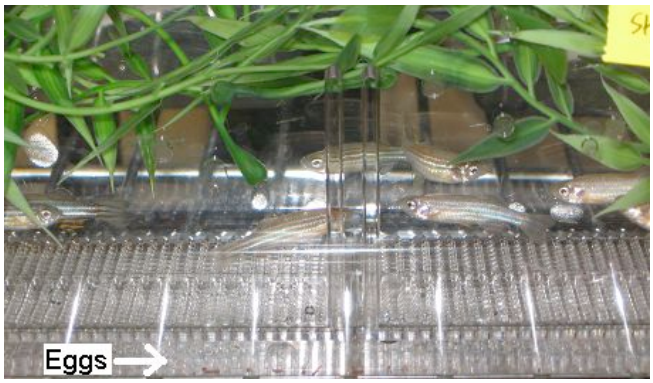
The following protocol outlines the basics on how to get embryos for use in experiments. Make sure to identify the kinds of zebrafish that will be used in the experiment (mutant, wild-type, transgenic, etc).

1. Obtain the desired females and males and put them together into a mating tank (top compartment). To identify the sex of the zebrafish, keep in mind that females tend to be larger with a white, round belly and a silver-blue streak whereas males are somewhat yellowish, have a flat belly, and tend to be more active.

2. Label the tank according to your lab's specifications and place the label horizontally. A typical label should contain: 1) the genotype of the fish, 2) the name or the location of the fish's home tank 3) the date. The labels are essential since they are there to help identify which tanks have eggs to avoid confusion with other tanks. Also, the labels can help identify what type of zebrafish is in there. Leave the tank in the dark overnight and check in the next morning.



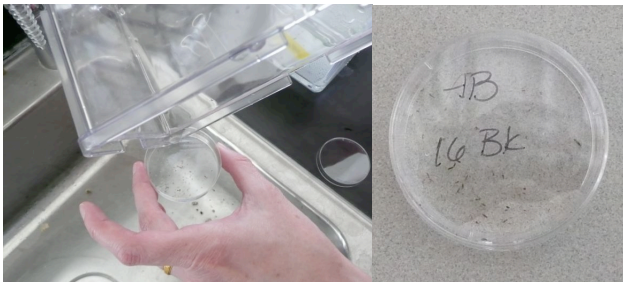
3. Zebrafish typically lay eggs within the first two hours after the lights come on. Look for eggs in the lower compartment, lift the tank and look from underneath if necessary. If there are eggs, turn the label vertically to indicate the presence of eggs.



4. Obtain the lower part of the new fertilization tank and fill it with water (first picture below). Carefully, move the upper compartment of the fertilization tank (together with the adult fish) to the new tank or put the fish back in their home tank. Always make sure to move the label with the adult fish-most strains of adult zebrafish look exactly the same. The fish will be moving and splashing, be quick during this procedure for the fish not to dry out and suffocate.

5. Drain most the water out of the initial tank filled with eggs (let the eggs settle to the bottom if they are not there already). Take the tank to a sink and gently and slowly pour the water out through one corner, making sure not to lose any eggs. Pour until all the remaining eggs and water cover one corner. Make sure to have a Petri-dish nearby.

6. Pour the remaining eggs with fish water into a Petri-dish and label it.



7. After the eggs are collected, they need to be sorted. Sorting entails moving the good, fertile embryos/eggs into a new dish away from the infertile eggs and any debris that is in the dish (scales, waste products of the fish, etc.). Fertile embryos will always look beautiful and symmetrical (picture to the left below). In fertile embryos will often just stay stuck at the one cell stage, and then later they will look like big dark masses inside a chorion (picture to the right below).