Zebrafish Fin Clip

The goal of this experiment is to gain a better understanding of how zebrafish regenerate missing elements of their fins. The tail fins of zebrafish will be amputated with a dissecting scissors and allowed two weeks to recover and regenerate the lost segment of fin. Over this time period, regeneration of the caudal will be analyzed. After completion of regeneration, data may be pooled among the entire class.

1. **Hypothesis:** Before beginning this experiment, formulate a hypothesis based on what you know about regeneration in zebrafish. Your hypothesis could include information about rate of regeneration, factors that may affect the success of the fin clip, or what type of cells migrate to the site of injury first.

2. **Experimental Procedure**

- Put gloves on before starting the experiment.

- Check to make sure that all tools are within reach before starting the experiment. The Tricaine will not leave the fish anesthetized long, so the experiment will need to be done quickly to ensure the safety of the fish.

- Fill a 1L container with fish water. This will serve as the recovery tank after the amputation has been performed. Adding a small amount of antibiotic may increase the success of recovery after surgery.

- Remove a zebrafish from its tank with the small fish net and place it in a beaker of diluted Tricaine solution.

- Wait until the fish is fully anesthetized (1 or 2 minutes), then remove it from the petri dish and place it in a clean Petri dish.

- Place the petri dish with the fish under a dissecting microscope. Use the microscope to take a picture of the zebrafish, and make sure to record the zoom and position of the fish so the picture can be duplicated after amputation.

- Using your dissecting scissors and a pair of blunt forceps, lift the tail fin from the Petri dish and make a single vertical cut that is perpendicular to the rays of the fin.

- Put the fish in a recovery beaker in order to wash off the remaining Tricaine solution. If your fish does not start to move again after a few seconds, try using a transfer pipet to push water through its gills. This should help circulation in the fish and it should start moving within a few seconds.

- If you wish to keep each fish separate to follow their regeneration individually, they will each need to be kept in their own tank. Otherwise, the fish may be placed back into their original tank

3. Observing Regeneration

- Within a few hours after the tail fin has been amputated from the zebrafish, epithelial cells will migrate to the injured area and form an **apical epidermal cap**. The AEC will begin repair of the damaged area and cells in this region send information controlling the amount of new tissue necessary to repair the damaged tissue.

- A collection of undifferentiated cells called the **blastema** forms directly under the apical epidermal cap. *Note when you can distinctly see blastema formation*

- Within the next few days, the blastema will differentiate into specific cells necessary to regenerate missing parts from the fin. *What structures are you able to determine in the developing tissue?*

- Pictures should be taken using the dissecting microscope every few days for a period of 10-14 days. Lengths of regeneration can also be collected and pooled for the entire class. In this way, average regeneration data will be obtained.