Fish room manual for visiting scientists

Ahituv Lab

Purpose

This document is intended to give a brief overview of the Ahituv lab Zebrafish facility. Visitors working with zebrafish should known and understand these procedures before working with fish.

Zebrafish as a model

Small size, ease of care, external fertilization - development and rapid maturation have all contributed to the usefulness of zebrafish as a model for genomic research. The lab has two strains of fish; AB “wild type” and the “Casper”. The Casper strain is bred to be transparent throughout its life. This makes it easier to see where GFP is expressed.

Visitor responsibilities

All visitors working with zebrafish are responsible for the fish they are using. Return the fish to the system on the rack where they were found and feed them with a few pellets of Ziegler adult zebrafish diet if they are not back by noon. **All fish must in the system by 3:30PM**. Please put a “more food” tag on the tank when you return fish to the system after breeding, this will be removed the next morning. Breeding tanks are to be cleaned and dried after each use. Use the white scrub pad for all plastic parts. The green scrub pads will scratch the tanks. Be sure to get the corners. All tanks, nets, strainers, and plates are to be cleaned and rinsed with R/O water. Place clean and rinsed tanks, lids and screens on the top rack of rolling cart to dry. When dry, check again for cleanliness and put onto storage shelves. Keep room and work areas (benches, sink area, and injection stations) clean, wipe up spills, refill sump, and restock supplies.

Embryo injections

A general overview of the injection protocol (though we have made some minor changes) is Fisher, S., et al., *Evaluating the biological relevance of putative enhancers using Tol2 transposon-mediated transgenesis in zebrafish.* Nature Protocols, 2006. **1**(3): p. 1297-1305.

The duration of a zebrafish injection experiment, at minimum, is a three day process.

Day 1: the night before the injection, set up mating pairs between 5 PM – 6 PM. (30 min)

Day 2: injection day, prepare injection mix and inject embryos between 8:30 AM – 12 PM.

4 – 6 hours post injection, screen for healthy developing embryos anywhere between "High stage" to "75% Epiboly" stage depending on how long the embryos have been developing (refer to zebrafish development chart). Move all healthy embryos into a new dish with new Egg Water. (30 min)

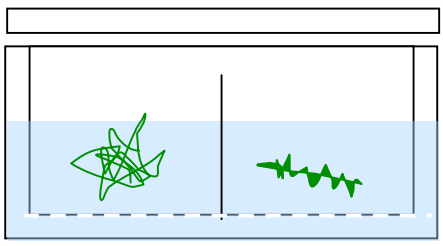
Day 3: 24 hours post injection, screen for healthy embryos, move all healthy embryos into a new dish with new Egg Water and check for gene expression (if applicable)

Day N: Embryos must be checked at least once a day. Move all healthy embryos into a new dish with new Egg Water. Determine when you expect to see enhancer expression and we can make special arrangements if you want to keep the embryos more than 48 hours post-injection or over the weekend.

You will have to determine the time to test for GFP expression with enhancers that you inject. A good resource for understanding zebrafish embryonic development and determining time points to test for enhancer activity in a given tissue is Kimmel, C., et al., *Stages of Embryonic Development of the Zebrafish.* Dev. Dyn., 1995. **203**: p. 253-30.

Setting up breeding pairs

All fish belong to Ahituv lab members, so please use only the fish that are you are specifically told to use. Dates of breeding are listed on tank labels and fish should not breed more than once per week at max. Please contact the training lab member to reserve an injection date and station. Sign-ups are allowed no more than 10 days in advance. **Due to limited resources, only one injection date per week is permitted.**



Set up a mating tank about 1/3-1/2 full of system water and place the inner tank with divider inside. Putting a piece of plastic plants in each side of the tank may help, as zebrafish prefer vegetated areas for spawning, but this is optional. Put male and female fish on opposite sides of the divider and cover (they jump!). Set tank on the breeding shelves overnight.

In the morning, remove the divider. Fish should mate within 20-30 minutes*, but some begin to lay eggs immediately.

To collect eggs first move the parents to a new tank, remove the inner tank, and pour the water with eggs through a tea strainer. Rinse the eggs with Egg Water** to clean them and put them in a petri dish of Egg Water.

Often, one male and one female are enough, but sometimes we put multiple males and multiple females in breeding tanks.

*Observing the pair for a few minutes can help you predict if they are going to lay eggs. Normal mating behavior involves the male chasing the female around the tank and swimming right next to her. I have also observed that some of the AB males tend to look pink when they are chasing females. If the female is chasing the male away or the fish are ignoring each other completely, it's unlikely that the pair will lay eggs. If this behavior continues for >30 minutes, it might be wise to put another male in the tank.
**Egg water is the blue solution in a tank below the system water (salt water) reservoir. Concentrated stock is below the brine shrimp table and the egg water recipe is on page 10.5 of The Zebrafish Book.

Supplies (needles, injection mold, and injection solution) needed for injections are provided by Ahituv lab unless you are injecting on a routine basis. If it is the case, please make arrangements with us to prepare your own supplies.

Injection Mix:
1uL 125ng/uL Endotoxin-free plasmid DNA (your construct)
1uL 175ng/uL TOL2 RNA
2 uL Sterile water
1uL 2%Phenol red

Prepare the injection mix on ice. Prepare a needle and load 1-2uL of injection mix (this is more than enough for a day of injections and allows the rest to be held aside in case there is a problem with the first needle)

Set up injection station

1. Turn compressed air tank valve counterclockwise to open the valve.
2. Turn pressure gauge valve clockwise to 40 PSI.
3. Switch on power for PicoSpritzer unit.
4. Turn pressure valve on PicoSpritzer unit clockwise to 20 PSI – 40 PSI.
5. Set duration time between 30 milliseconds – 200 milliseconds.

(Note: the pressure and duration time may need to be adjusted to deliver the DNA properly.)

Shut off injection station

1. Remove injection needle from pipette holder.
2. Turn compressed air tank valve clockwise to close the valve.
3. Turn pressure gauge valve counterclockwise.
4. Turn pressure valve on PicoSpritzer unit counterclockwise.
5. Hit foot pedal to release the air in the system. All air gauge dials should read 0.
6. Switch off power for PicoSpritzer unit.

Fish room setup

The fish room has two racks, with a combined capacity of 1776 breeding fish. The capacity can be greater since tank densities can be increased for fish not actively breeding. Tank capacity for breeding fish is 8 fish/1.5L tank and 16 fish/3.0L tank.

System water is made from R/O (reverse osmosis) water to which various salts and other chemicals have been added to approximate conditions of the zebrafish's natural habitat. Water quality is maintained with a combination of mechanical, biological, and chemical filtration. The system also includes sterilization by UV light. Each rack has a self contained filtration system. The system is designed to decrease cross contamination between individual tanks. Cross contamination can still occur by splashing, nets, re-tanking fish, etc. Proper tank hygiene is also important. Dirty tanks can spread disease to all tanks in a rack in a short period of time. Water flows from the tank into a horizontal trough which in turn deposits the water over a 150 micron filter pad into the main sump.

The main sump contains media which acts a a substrate for nitrifying bacteria. Nitrification of fish waste is performed by two different species of bacteria. *Nitrosomona* spp. oxidizes the ammonia produced by the fish into nitrite. *Nitrobacter* spp. in turn converts the nitrite into nitrates. Bacteria capable of reducing nitrate do exist but they are not suited to a aquarium environment, therefore, nitrate is removed by 10% daily water changes.

Additional mechanical filtration is achieved through 50 micron canister filters. There are also canisters that hold activated charcoal for chemical filtration. The charcoal also clarifies the water before it passes through the UV sterilization modules.

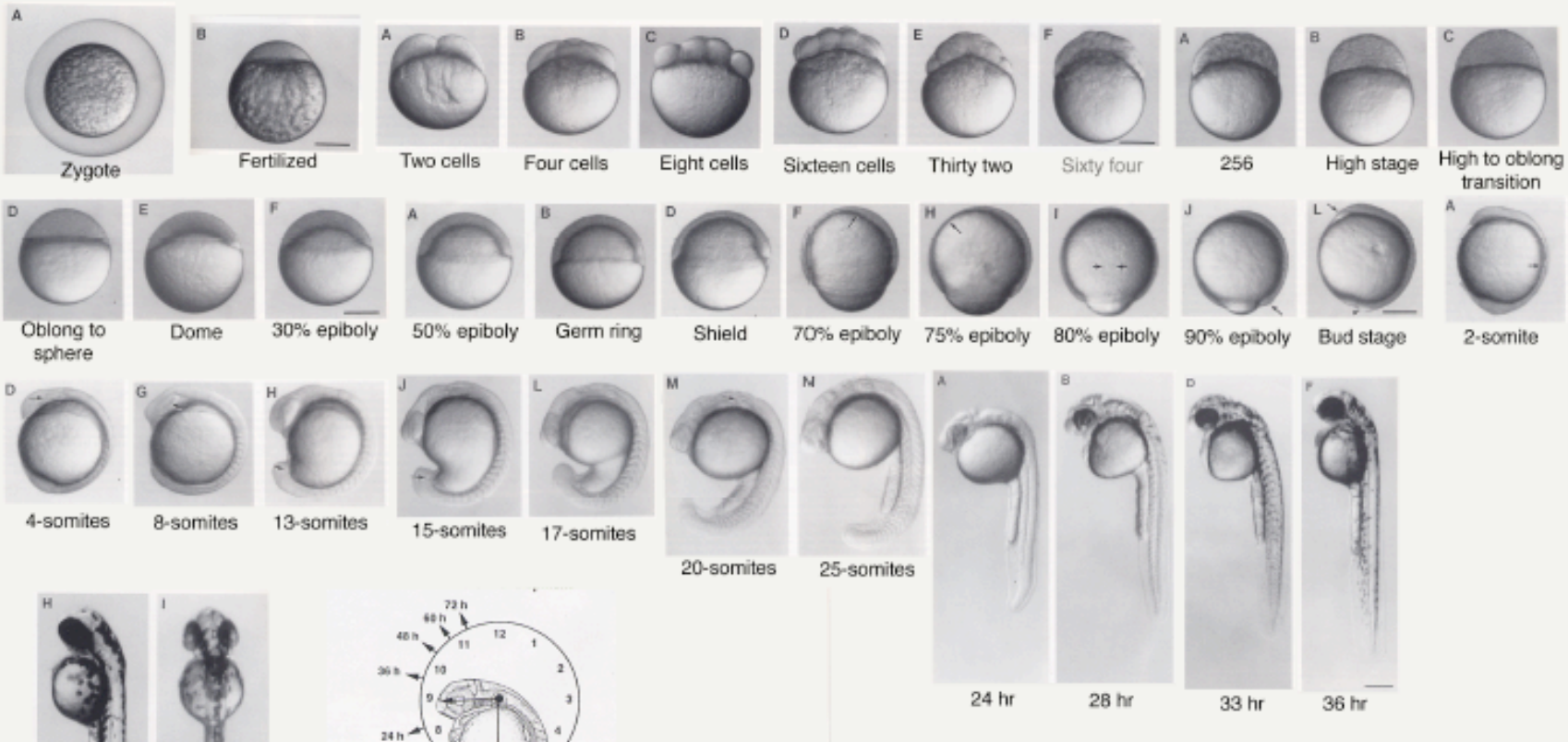
After sterilization, the water is pumped through manifolds into the individual tanks. The water flow to the tanks is controlled by valves at each tank. The water adjustments should be made at these valves and not at the manifold.

The pH and temperature are monitored and adjusted automatically. The room itself is kept at ~25° C, with in-sump heaters as backup. The pH is maintained at 7.2 – 7.6 by injection of Sodium bicarbonate. You should not need to make any adjustments to these settings. If you have any questions or notice any unusual conditions, for example, the room seems colder than normal, contact a lab member.

On weekdays the fish are fed three times daily. In the morning and evening the fish are fed live brine shrimp nauplii. In the afternoon the fish are fed a dry prepared pellet food (Ziegler Adult Zebrafish Diet).

The fish room lights are on a 14 hour on, 10 hour off cycle. A timer turns the light on at 8 AM and off at 10 PM. **No work is to be done in the fish room during the lights off cycle.**

Zebrafish Development



From: Kimmel et al. Stages of embryonic development of the zebrafish
Dev. Dyn. 203:253-310, 1995