

Standard Operating Procedure for Zebrafish Breeding Colony Maintenance

Purpose

The animal facility staff maintains numerous strains of fish that require adherence to strict breeding procedures. These procedures ensure the availability of adequate experimental animals, proper cross breeding of strains and identification of mutant strains.

Holding and Breeding Tank Procedure

1. Breeding tanks are set up at the beginning of each week. Additional mating may be requested by the investigators throughout the week.
2. Fish are typically bred every day Monday through Thursday.
3. Breeding tanks are designed with a barrier to allow the eggs to be separated from the adults to prevent cannibalism. These tanks can have a screen bottom insert, a slotted plastic insert, or a sloped insert that is inserted into a tank.
4. Use a 3 liter tank for pair, group, or timed mating.
5. Animals selected for breeding in stock group tanks will be removed and placed into a holding tank.
6. Holding and breeding tanks can be in several locations depending on the investigator.
7. Generate a bar code label for each new breeder holding tank.

Feeding and Transferring Fish Breeders

1. To transfer fish from the holding tank to a breeding tank, carefully net the fish and place them into the selected breeding tank.
2. Breeding fish are fed twice a day in the morning and afternoon.
3. The afternoon feeding should be completed by 2:15 PM to allow breeders to eat before being transferred to breeding tanks at approximately 3:00 PM.
4. The breeding tank will always be to the right of the holding tank.
5. Breeding is completed overnight and eggs will drop through the barrier.
6. Transfer the breeding fish to the holding tank in the morning being careful to avoid netted fish jumping out of the net.
7. Feed fish once they are transferred into the holding tanks in the morning.

Egg Production

1. Check for eggs in all breeding tanks Tuesday through Friday.
2. Some breeders are located in the light boxes. Egg collection will depend on the light cycle at the time.
3. If eggs are present write the date on the breeding tank.
4. Leave the breeding tanks containing eggs next to the holding tanks.
5. If eggs are NOT present, dump the water into the system trough, rinse the breeding tank and replace it in the same location and refill with system water.
6. Each lab is responsible for collecting eggs from the breeding tanks.
7. FLSC staff will be responsible for collection of stock eggs produced.
8. Breeder tanks are disassembled and sanitized every two weeks or as needed.

Egg collection

1. The supplies needed for egg collection include: 3 inch petri dishes, pipette, egg water (Methylene Blue Solution).
2. Stock egg collection is completed by FLSC staff by 11:00 AM.
3. Lab egg collection is at the discretion of each lab.
4. FLSC staff will contact labs if eggs are not collected by 3:00 PM.
5. Pour tank of fish eggs into a strainer over the sink, rinse eggs with RO water.
6. Fill as many 3 inch petri dishes ½ full of egg water according to the number of eggs collected.
7. Turn the strainer upside down over the egg water petri dish, rinse eggs into the dish with RO squeeze bottle.
8. Distribute eggs using a pipette placing 50 eggs per petri dish.

9. Label dishes with the family #, breeder's ID (♀♂), type of crossing (in-cross or out-cross) and the date collected.
10. Place petri dishes in the incubator according to the date collected. Each level of the incubator is labeled Tuesday, Wednesday, Thursday or Friday for experimental eggs.
11. All stock fish eggs are placed at the bottom of the incubator.
12. Petri dishes may be stacked up to 5 high to save space.
13. Eggs are in the incubator for 4 days.
14. Each lab will screen eggs and shift their petri dishes as needed in the incubator.
15. Stock eggs will be screened at least once during the 4 day incubation period.
16. At day 4, fry that have hatched are moved to a small tank or beaker in 513 or 516 counter.
17. Fill each beaker or tank with system water.
18. Typically all albino and transgenic fish are placed in a water bath in 513 or on the counter in 516 depending on investigator room location.

Preparation of Egg Water (Methylene Blue Solution)

1. Stock solution is prepared by investigator labs.
 - a. 25 ml salt stock solution, 5 ml HCL concentrate, 10 gm Sodium bicarbonate brought to 1 liter with RO water.
 - b. Salt stock solution is made by adding 120 g Instant Ocean® to 1 liter RO water.
2. Add 200 ml stock solution to 5 liters RO water.
3. Then add 20 microliters of Methylene Blue stain and mix.
4. Apply date sticker to Egg Water carboy indicating expiration 3 months from date of mixing.