

Zebrafish Breeding for Genetic Crossing
IACUC Standard Procedure
Effective Date: April 2022

Objectives:

Fish are bred in order to generate embryos for experiments and for maintenance of the breeding stock. Transgenic fish are bred in order to generate animals carrying transgenes for labeling or genetic manipulation and genetic crosses are performed to produce and/or identify particular genotypes and phenotypes. Key points are to maintain good water quality, reduce stress when possible and restore the fish to normal feeding and a flowing water system as soon as possible.

Description of procedure:

It is recommended to cross adult fish at a ratio of 1 male to 1 female or 2 males to 3 females for group mating fish in off-system breeding tanks overnight at a density of 5 or less fish per liter of water. Fish may be separated by sex using a divider. The fish will be maintained in a crossing tank consisting of an upper (breeding) and lower (embryo collection) chamber separated by plastic mesh to allow the eggs to fall where breeding adults do not have access. Fish will commonly consume their eggs if they are accessible. Some facilities have bulk breeding tanks for mass mating and in this case, the individual protocol for each apparatus applies.

Embryos from successful crosses will be collected the following day. Changing water in the morning is strongly advised for group mating and recommended for paired mating. After crossing, adult fish will be returned to system tanks as soon as possible. All fish in crossing tanks are transferred to fresh system water no longer than 20 hours after setup. Laboratory personnel unable to return fish to racks or transfer fresh system water within 20 hours off-system should immediately contact the facility manager for assistance. Fish that are undergoing identification by offspring analysis (pair crossing) are occasionally housed off-system during phenotyping of the offspring. Pair-crosses are maintained in off-system tanks for a maximum of 3 days with daily fresh water changes. Off-system tanks are labeled with setup date and housed in a designated section in order to allow monitoring by facility staff. Fish will be allowed a minimum 1 week recovery period between productive crosses, with 2 week recovery periods being ideal. **Adverse Effects** Crossing as described should have no adverse effects but the expected phenotype of the cross should be taken into account **and potential adverse effects described in the IACUC protocol.**