University Animal Care Committee Standard Operating Procedure		
Document No: 15.5	**Subject:** Bleaching of Zebrafish Embryos	
Date Issued: May 5, 2016	**Revision:** 1	**Page No:** 1

Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the correct procedure for bleaching zebrafish embryos.

1) **Introduction and Definitions**: Every new line to be introduced to the fish facility must be treated with bleach solution to reduce transfer of potential pathogens. Only bleached embryos can go into the system of the main fish facility. Bleaching is best done between 24-30 hpf (hours post fertilization). The bleach treatment makes the chorion harder and the embryos will not be able to hatch on their own; therefore, they must be manually dechorionated with pronase. Check with the facility that has shipped the embryos as they may have already done the bleaching. If the bleaching has not been done, proceed as described below.

2) Materials:

- Breeding tank insert (Techniplast)
- Commercial bleach (sodium hypochlorite 3-6%)
- Reverse osmosis (RO) water
- Zebrafish embryos (24-30hpf)
- 2 sterile or autoclaved containers that large enough to hold ~1L of RO water
- Pipette
- Small glass beakers (4)
- Timer
- Pronase (20 mg/ml, Sigma P-5147, or similar)
- Petri dish
- 70% ethanol

3) Procedures:

***Preparation of Bleach Solution*:**

- Add 1L of RO water to a sterile or autoclaved container.
- Add 0.7ml of commercial bleach to water and swirl gently until well mixed.

Bleaching of Zebrafish Embryos:

- Clean the bleaching area with 70% ethanol.
- Transfer zebrafish embryos from shipping container to small glass beaker with pipette. Add bleach solution directly to glass beaker, ensuring that embryos are submerged completely. Keep embryos submerged in bleach solution for exactly 5 minutes.
- Transfer embryos with pipette to a second beaker containing only RO water. Keep embryos in RO water for exactly 5 minutes.
- Transfer embryos with pipette to first beaker, containing bleaching solution. Keep embryos submerged in bleach solution for exactly 5 minutes.
- Transfer embryos with pipette to a third beaker containing only RO water. Keep embryos in fresh RO water for exactly 5 minutes.
- Transfer embryos with pipette to a fourth beaker containing only RO water. Keep embryos in fresh RO water for exactly 5 minutes.

- Transfer embryos to clean petri dishes containing RO water at a density of 50 embryos per 100mm petri dish.

Pronase Dechorionation of Zebrafish Embryos:

1. Transfer up to 50 zebrafish embryos from the fourth RO beaker to a petri dish with a pipette.
2. Add 1 ml of pronase. Keep zebrafish embryos submerged for exactly 3 minutes.
3. Pour off pronase containing RO water and refill with fresh RO water. Repeat this step for a total of 3 washouts.
4. Refill dish with RO water. Some embryos will have hatched immediately, others may require up to 24-hours to hatch or can be hatched by gently swirling or pipetting up and down.

References:

1) Nicole Compo, DVM
2) Zebrafish Husbandry Course 5th International, Leading Advances in Zebrafish Husbandry, September 27-30, 2016, Bugaggiate (VA), Italy

Revised: October 22/2020