

Bleaching Zebrafish Eggs to Sterilize

This protocol should be used to bleach eggs in order to sterilize them whenever embryos are transferred from the quarantine room to the main fish room. Bleaching only sterilizes the surface of the eggs. It cannot sterilize within the chorion.

1. Collect eggs and raise to 4-30 hours post fertilization (hpf) before bleaching.
2. Using a dissecting scope, transfer the healthy eggs to a clean Petri dish being extra careful to not transfer any unhealthy eggs or detritus. Only perfectly transparent eggs should be transferred. If there is any cloudiness, fuzziness, or inclusions (e.g. spores) in the eggs then do not transfer them.
3. Prepare bleach solution by adding 0.1ml of 5.25% sodium hypochlorite (Fisher, Cat.# Fisher:SS290-1, kept at 4°C) to 170ml clean Egg water.
4. Prepare bleaching apparatus by getting a 6 well plate, and filling 3 of the wells with bleach solution, and the other 3 with egg water.
5. To bleach eggs, put 30-40 eggs at a time into a 100 μ m nylon cell strainer (Falcon, Ref#352360). Let eggs remain in bleach solution for 2 minutes. Wash eggs by dipping the tea strainer into one of the unused egg water wells for 2 minutes.
6. Repeat 2X. By the end of this step you will have used all 6 wells.
7. Rinse 1 more time in egg water in some other container.
8. Transfer to new Petri dishes and discard any imperfect embryos.
9. Dechorionate embryos between 48 and 72hpf. Bleaching toughens the chorion and prevents natural hatching so the embryos must be dechorionated manually.
10. Before placing the larvae on the system at day 5 for rearing, visually examine all larvae to ensure they are healthy. If there is any sign of death and disease then sac the whole Petri dish.