UAB Zebrafish Research Facility Guidelines: Bleaching of Zebrafish Embryos

This protocol describes how to bleach zebrafish embryos at 24 to 30 hours post fertilization to remove pathogens. This process will not eliminate intracellular organisms like Pseudoloma (Microsporidia).

Equipment:

6 glass dishes (Fisher 13-675-F)
200 ml graduated cylinder, plastic or glass, for measuring solutions
Pipetter and tips for dispensing 240 ul volume
Tea strainer, for holding embryos
Timer

Needed solutions:

The base for the solutions is filter sterilized fish water (system water), which we will call FSFW.

1. 400 mls of .001% sodium hypochlorite (bleach) solution, which we will call BS (see below)
2. 200 mls of 0.05% sodium thiosulfate solution, which we will call TS (see below)
3. 600 mls FSFW

BS: Solution is 0.001% sodium hypochlorite. This is made by mixing 0.24 mls (240 ul) of standard household bleach (6.15% hypochlorite, original unscented) with 400 mls of FSFW.
TS: Sodium thiosulfate solution is 0.05%. Mix 0.1 g of sodium thiosulfate into 200 mls FSFW.

Procedure:

Prepare dishes. Each should hold a little under 200 mls.
Dish 1: should contain FSFW
Dish 2: should contain BS
Dish 3: should contain FSFW
Dish 4 should contain BS
Dish 5 should contain TS
Dish 6 should contain FSFW
Add up to 100 embryos to the tea strainer. "Bad" embryos must be removed before bleaching.
Place strainer into dish 1 to rinse embryos in FSFW for 1 minute. Agitate gently periodically.
Move strainer to dish 2 for 5 minutes. Agitate gently periodically to be sure bleach contacts all surfaces of each embryo.
Move strainer to dish 3 for 3 minutes. Agitate gently periodically.
Move strainer to dish 4 for 3 minutes. Agitate gently periodically.
Move strainer to dish 5 for 3 minutes. Agitate gently periodically.
Move the strainer to dish 6 for 5 minutes. Agitate gently periodically.
Tap the strainer on the top edge of an open Petri plate (100 mm) to transfer the bleached embryos into the plate for incubation. The embryos can alternatively be rinsed from the strainer into the Petri plate with embryo media or FSFW.

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