Bleaching Zebrafish Embryos

Reagents and Supplies

E3 medium

	quantities for 5 L of 60X stock
5 mM NaCl	86 g
0.17 mM KCl	3.8 g
0.33 mM $CaCl_2$	14.5 g $CaCl_2 \cdot 2H_2O$
0.33 mM $MgSO_4$	24.5 g $MgSO_4 \cdot 7H_2O$
0.00001% (w/v) Methylene Blue	to be added to 1X solution

70% Ethanol
Tap water
0.004% NaOCl in E3 medium (Bleaching Solution made from 10-13% NaOCl stock (Aldrich 425044))
30 mg/mL Pronase in E3 medium (Pronase Solution made from solid powder (Roche 165921); can be stored at –20 °C)
Deep 90-mm Petri dishes
Tea strainer

Procedure

Embryos should be bleached between 10 and 28 hpf. After 28 hpf, the chorion is partially degraded and the sodium hypochlorite solution can damage the embryo. Since bleaching interferes with hatching, Pronase is added to facilitate this process.

1) Sterile working area with 70% ethanol.
2) Step up five washing solutions in the following order: Bleaching Solution, Tap water, Bleaching Solution, Tap water, Tap water.
3) Transfer embryos to a clean tea strainer and incubate them in each bath (5 minutes/each). Be sure that embryos are submerged in each treatment.
4) Wash the embryos into a new Petri dish with 50 mL E3 medium (no more than 75 embryos/dish)
5) Add 10 μL Pronase Solution to each Petri dish and incubate at 28.5 °C.
6) After 24 h make sure that all embryos have hatched and dechorionate with clean forceps if necessary.