Dechorionating 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

Dumont #5 forceps (2)
2 mg/mL Pronase in E3 medium (Pronase Solution made from solid powder (Roche 165921))
Fire-polished wide-bore Pasteur pipet

Procedure

When raised in E3 medium at 28.5 °C, zebrafish embryos develop normally outside of their chorions. Chorions can be removed easily using two forceps (it is critical that the tips are sharp and that their ends can touch). Using one forcep to hold the chorion, make a tear in the chorion with the other forcep. The chorion is then held in a region opposite that of the tear, and the embryo is gently pushed through the opening by passing the chorion and embryo between the tips of the other forcep. Embryos younger than 15 hpf are particularly fragile and great care must be taken not to damage them.

Alternatively, embryos can be enzymatically dechorionated in bulk using the following procedure:

1) Incubate the embryos in the Pronase Solution at room temperature for a brief time (typically 1-10 minutes, depending on the embryonic stage). Constantly check the condition of the chorions by gently depressing them with a poker (made by attaching a loop of 6X flyfishing tippet to a capillary tube) and looking at them under a stereomicroscope. When the chorions no longer return to a spherical shape, the Pronase treatment is complete.
2) Thoroughly wash the embryos (3 or 4 times) in E3 medium to remove the Pronase solution. Using the fire-polished Pasteur pipet, pipet the embryos back and forth in the E3 medium. The Pronase-treated chorions should break open, releasing the embryos.
3) Dechorionated embryos should be handled using fire-polished Pasteur pipets to minimize damage.