Protease Dissociation

(Source: D. Frost and D. Sepich from Zebrafish Book 5th Edition)

1. Rinse embryos in sterile, calcium-free Ringer's solution, 15 minutes.
2. Transfer the embryos to a dish containing Custom ATV solution (Irvine Scientific) or 0.25% trypsin, 1 mM EDTA, pH 8.0 in sterile PBS. Incubate at 28.5°C and monitor the dissociation with a microscope. Intermittently triturate with a sterile, narrow-bore Pasteur pipette and continue until you see mostly single cells.
3. Add $CaCl_2$ to 1-2 mM and fetal calf serum to 5-10% to stop the reaction.
4. Centrifuge at 100-300 x g for 3 minutes.
5. Discard the supernatant and resuspend the cells in L-15 (Sigma) supplemented with 0.3 mg/ml glutamine, 50 U/ml penicillin, 0.05 mg/ml streptomycin, and 0.8 mM $CaCl_2$.
6. Repeat step 4 and resuspend in supplemented L-15 (as in step 5) with 10% embryo extract and 3% fetal calf serum to make a final concentration of 15 embryos per ml.
7. Plate cells on plastic or coated glass and incubate at 28.5°C without additional atmospheric CO_2.