Zebrfish Embryo DNA Preparation

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[Abstract] This protocol explains how to extract DNA from a single zebrafish embryo. It does not require the use of expensive kits.

Materials and Reagents

1. Proteinase K (Roche Diagnostics, catalog number: 03115836001)  
2. 1 M Tris (pH 8.3)  
3. NaCl  
4. KCl  
5. CaCl₂·2H₂O  
6. MgSO₄·7H₂O  
7. Sterile water  
8. 10% Tween 20 (EMD Biosciences, catalog number: 655207)  
9. 10% NP40 (Merck KGaA, catalog number: 492018)  
10. Embryo lysis buffer (see Recipes)  
11. 1x PCR buffer (see Recipes)  
12. E3 (see Recipes)

Equipment

1. PCR Thermal cycler  
2. Centrifuges  
3. Incubator  
4. 96-well plate

Procedure

A. Freezing single live embryos  
   1. Wash dechorionated embryos 3x with E3.  
   2. Place a single embryo into a well of a 96-well plate and remove all excess buffer.
(Store dry at -20 °C if needed).

**B. DNA preparation:**

1. Add 50 μl lysis buffer to single (live or in situ'd) embryos.
2. Incubate at 98 °C for 10 min to lyse cells. Spin down.
3. Add 5 μl Proteinase K (10 mg/ml stock) to single embryos.
4. Incubate at 55 °C for at least 2 h (longer the incubation, cleaner the DNA).
5. Incubate at 98 °C to heat kill Proteinase K.
6. Vortex thoroughly and spin down debris.
7. Use 2 μl of single embryo DNA per PCR reaction.

**Recipes**

1. **1x PCR buffer**
   For 50 ml
   - 10 mM Tris-HCl (500 μl of 1 M Tris) (pH 8.3)
   - 50 mM KCl (2.5 ml of 1 M KCl)
   - 47 ml sterile water
   (Can be stored at RT for several months)

2. **Embryo lysis buffer (1x PCR buffer with tween 20 and NP40)**
   For 10 ml of lysis buffer
   - 9.4 ml 1x PCR buffer
   - 300 μl NP40 (10% stock) ***Make fresh
   - 300 μl tween 20 (10% stock) each time***

3. **E3**
   - 60x E3 stock (2 L)
   - NaCl 34.4 g
   - KCl 1.52 g
   - CaCl₂·2H₂O 5.8 g
   - MgSO₄·7H₂O 9.8 g
   Add distilled water up to 2,000 ml.
   Store at RT.
   To dilute to 1x for rearing zebrafish, use 160 ml of stock and fill to 10 L with ddH₂O
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