Zebrafish 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. $CaCl_2 \cdot 2H_2O$
6. $MgSO_4 \cdot 7H_2O$
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 severalmonths)
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
$CaCl_2.2H_2O$	5.8 g
$MgSO_4.7H_2O$	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 ddH_2O

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