

Genotyping Transgenic Zebrafish Using Genomic DNA Extracted from Clutch of Embryos

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[Abstract] Transgenic zebrafish can serve as very useful genetic tools to study a variety of biological processes. Identification of the right transgene founder and the subsequent transgenic animals are always tedious and time consuming. This protocol provides a relatively rapid and easy method to identify the founder parent using a clutch of embryos.

Materials and Reagents

1. Zebrafish embryos
2. MeOH
3. Phenol
4. Chloroform
5. Isoamyl alcohol (IAA)
6. NaCl
7. KCl
8. MgCl₂
9. EtOH
10. TE
11. Tween 20
12. NaOAC
13. Gelatine
14. NP40
15. Proteinase K
16. ddH₂O
17. Phenol: chloroform: isoamyl alcohol (25:24:1)
18. Primers (custom ordered from IDT)
19. 1x RAPD buffer (see Recipes)
20. RAPD⁺ (see Recipes)
21. PCR lysis buffer (see Recipes)

Equipment

1. PCR thermal cycler
2. Incubator
3. Glass pipette

Procedure

A. Collect embryos

Groups of 25-40, 1 transgenic embryo in clutch of 40 embryos should be detected, fix in MeOH, store at -20 °C.

1. Remove MeOH and dry embryos in 55 °C incubator.
2. Add 10 µl/embryo of lysis buffer (w/ Rnase & Dnase).
3. Incubate at 37 °C, overnight.
4. Extract with 1 volume (vol.) of phenol: chloroform: isoamyl alcohol (25:24:1); vortex 15 sec and spin for 1 min and then extract top aqueous layer.
5. Repeat phenol: chloroform: isoamyl alcohol extraction.
6. Extract with 1 vol. of chloroform: IAA (24:1).
7. Add NaCl to 0.3 M (*Do not use NaOAc as it will precipitate DNA into a slurry!).
8. Add 2 vol. of cold EtOH (should see DNA precipitate into cloud).
9. Prepare glass pipette with hook at the end.
10. Remove DNA by stirring glass pipette into Eppendorf.
11. Carefully wash or remove pellet from pipette with 70% EtOH and into new eppendorf; dry pellet.
12. Dissolve DNA into 100 µl of TE or ddH₂O.
13. Add 10 µl NaOAc and 220 µl EtOH.
14. Spin for 15 min at max speed at 4 °C.
15. Wash pellet with 70% EtOH and spin at max speed at RT for 5 min.
16. Dry pellet and resuspend in ddH₂O (*i.e.* 50 µl).

B. Transgene PCR detection

1. To detect transgene, using primers that span the promoter and cDNA sequence or primers that span the Tag sequence such as Myc, GFP if used in the transgene.
2. PCR set up

X µl	RAPD + (make up to 20 µl)
2 µl	DNA
1 µl	F primer (20 µM)

- 1 μ l R primer (20 μ M)
 1 μ l Tag polymerase
3. PCR program
- 94 °C 1 min
 94 °C 30 sec
 54 °C 2 min
 73 °C 1 min
 go to ii. 5x
 94 °C 30 sec
 55 °C 30 sec
 73 °C 1 min
 go to vi. 35x
 4 °C hold
 end
- Run 2-5 μ l pcr to check the yield.

Recipes

1. 1x RAPD buffer
- 1.55 ml 150 mM MgCl₂
 1.5 ml 1 M Tris (pH 8.3)
 7.5 ml 1 M KCl
 1.5 ml 0.1% Gelatine (heat gelatin to dissolve completely)
 12.05 ml
- Add 88 ml H₂O and autoclave at 121 °C for 20 min. Store at 4 °C.
2. RAPD⁺ (100ml)
- 30 μ l dATP
 30 μ l dCTP
 30 μ l dGTP
 30 μ l dTTP (each 100 mM)
 150 μ l BSA (20 mg/ml)
- Aliquot and store at -80 °C.
3. PCR lysis buffer
- 1x RAPD buffer
- 0.3 % Tween 20
 0.3% NP40
 100 μ g/ml Proteinase K

Store at -20 °C.

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