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. $MgCl_2$
9. EtOH
10. TE
11. Tween 20
12. NaOAC
13. Gelatine
14. NP40
15. Proteinase K
16. ddH_2O
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 ddH_2O.
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 ddH_2O (*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 µl R primer (20 µM)

1 µl 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 µl pcr to check the yield.

Recipes

1. 1x RAPD buffer

   1.55 ml 150 mM $MgCl_2$

   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 H_2O and autoclave at 121 °C for 20 min. Store at 4 °C.

2. $RAPD^+$ (100ml)

   30 µl dATP

   30 µl dCTP

   30 µl dGTP

   30 µl dTTP (each 100 mM)

   150 µl 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 µg/ml Proteinase K

Store at -20 °C.

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