Genotyping for Single Zebrafish (Fin Clip) or Zebrafish Embryo

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[Abstract] Zebrafish is increasingly used as a genetic model organism in biomedical studies. This protocol provides a detailed procedure about the identification of the genotype of an adult zebrafish or a zebrafish embryo.

Materials and Reagents

1. Tricane (Sigma-Aldrich, catalog number: 886-86-2)
2. dNTPS (QIAGEN, catalog number: 201900)
3. Proteinase K (Roche Diagnostics, catalog number: 03115836001)
4. MEOH
5. DNA polymerase
6. $MgCl_2$
7. Tris
8. KCl
9. $MgCl_2$
10. Gelatine
11. BSA
12. NP40
13. Tween 20
14. PCR lysis buffer (see Recipes)
15. RAPD+ (see Recipes)
16. 1x RAPD buffer (see Recipes)
17. 2x RAPD+ buffer (see Recipes)
18. Tricane solution (see Recipes)

Equipment

1. Standard tabletop centrifuges
2. Incubator (55 °C and 65 °C)
3. PCR thermal cycler

4. 96-well PCR plate
5. Razors
6. Plastic plates
7. Plastic beaker
8. Forceps
9. Spoon

Procedure

A. Fin clip:

1. Bring clean plastic plates, razors, Tricane, a plastic beaker, forceps and a spoon to fish room.
2. Prepare a 96-well plate, with 100-200 µl of MEOH in each well.
3. Add 20 ml Tricane to the beaker and add clean water to dilute the tricane (1:15 dilution).
4. Put the fish into the tricane solution and let the fish sleep. Move the fish to a clean plate and cut a small piece of the tail fin.
5. Quickly put the fish back to the single box and put the tail into one well of the PCR plate with MEOH in it.
6. Label both the single box and the well.
7. Make sure the tail is in the MEOH (the fish can be kept in single boxes for up to 5 days). *Note: A single embryo can be kept in MEOH directly.*

B. Genomic DNA extraction:

1. Remove as much MEOH as possible.
2. Incubate at 55 °C for 5 min to evaporate all MEOH.
3. Add 100 µl lysis buffer per well (200 µl lysis buffer for 72 hpf embryo).
4. Incubate at 55 °C O/N and heat inactivate at 95 °C for 10 min.
5. Centrifuge at 4 °C at 1,000 rpm for 2-10 min.
6. Store DNA at -20 °C or for long time at -80 °C.

C. PCR after genomic DNA extraction

1. PCR reaction set up

X µl	RAPD+ (make up to 20 µl)
1-2 µl	DNA
1 µl	Forward primer (20 µM)
1 µl	Reverse primer (20 µM)
1 µl	Taq Polymerase

For multiple PCRs, make a master mix (keep mixture and the plate on ice).

2. PCR program

94	1 min
94	30 sec
54	2 min
73	1 min
	go to ii. 5x
94	30 sec
55	30 sec
73	1 min
	go to vi. 35x
4	hold
	end

Use H_2O as the negative control to make sure the buffer is clean.

3. Run 2-5 μl PCR reaction to check the DNA yield.
4. Digest DNA fragment

Cut DNA in 30 μl reaction:

DNA	2-5 μl (dependent on the yield)
Enzyme*	0.5 μl
10x buffer	3 μl

Cut for about 10 h.

* Enzyme can be selected by the dCAPs program online:

http://helix.wustl.edu/dcaps/dcaps.html

May get incomplete digestion.

5. Run gel and examine the PCR results.

Recipes

1. Tricane solution

400 mg Tricane powder in 97.9 ml ddH_2O and use 2.1 ml Tris (pH 9) to adjust pH to 7.

2. 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.

3. RAPD+ (100 ml)

   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.

4. 2x RAPD+ might work better for PCR than 1x RAPD+

   100 ml

   3.1 ml 150 mM $MgCl_2$

   3 ml 1 M Tris (pH 8.3)

   15 ml 1 M KCl

   3 ml 0.1% Gelatine

   Add 75.9 ml H_2O and autoclave at 121 °C for 20 min and add 2x nucleotides.

5. 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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