Genomic DNA Isolation for PCR Analysis

Reagents/Equipment

Zebrafish system water
Tricaine
Small Nalgene tanks
1-L beaker
Surgical scissors
Forceps
Bench-top diaper
Eppendorf tubes (or PCR tube strips)

Method I (quick and dirty)
Quick Extract kit (Epicentre; Cat. # QE09050)

Method II (time-consuming but cleaner)
5X Passive Lysis Buffer (Promega)
Proteinase K solution (Roche)
Tris buffer-saturated phenol (Invitrogen)
Chloroform
Glycogen solution (20 mg/mL)
3M Sodium acetate, pH 5.2
70% Ethanol (aqueous)

Procedure

Anesthetization of zebrafish and tail fin clipping

1) Set up small Nalgene tanks (approximately 250 mL each) and fill them 2/3 full with system water.
2) Fill 1-L beaker with 500 mL system water containing Tricaine (80 μg/mL final concentration)
3) Place 2-5 fish to be genotyped in the Tricaine/system water solution and wait for the fish to stop moving. Quickly remove each fish with a plastic spoon and place it on a bench-top diaper soaked in system water. Be careful to 'scoop' the fish head first to avoid gill damage. Once the fish is placed onto the diaper, cut 2/3 of the tail fin with the surgical scissors and transfer it to an Eppendorf tube (or PCR tube strip). Then place the fish into a numbered isolation tank (small Nalgene tanks) and label the fin clip-containing tube with the same number.
4) Repeat as necessary until all fish are genotyped. Throughput can be increased by placing two fish per isolation tank, provided that there is a suitable means for differentiating the two fish (i.e. sex, manner in which fin is clipped, leopard strain, etc.)

Genomic DNA isolation (Method I; quick and dirty)

1) To each fin clip-containing tube, add 50 μL of the Quick Extract solution. Make sure the fin is submerged.
2) Vortex tube for 30 seconds.
3) Incubate at 68 °C for 2 minutes.
4) Vortex tube for 30 seconds.

5) Incubate at 98 °C for 2 minutes
6) Vortex tube for 30 seconds, then place tube on ice.
7) Centrifuge tube at 14,000 rpm for 2 minutes to pellet fin debris.

Genomic DNA isolation (Method II; time-consuming but cleaner)

1) To each fin clip-containing tube, add 50 μL of 1X Passive Lysis Buffer (made from the 5X solution provided in Promega Dual Luciferase kits). Make sure the fin is submerged.
2) Vortex tube for 30 seconds.
3) Incubate at 98 °C for 10 minutes.
4) Add 20 μL proteinase K solution (19 mg/mL) to each tube. Incubate at 55 °C overnight.
5) Incubate at 98 °C for 10 minutes to inactivate proteinase K.
6) Add 70 μL of Tris buffer-saturated phenol.
7) Vortex tube for 5 seconds.
8) Centrifuge tube at 14,000 rpm for 4 minutes.
9) Transfer top (aqueous) layer to new tube, being careful not to include the water/phenol interface.
10) Repeat steps 6-9 on aqueous layer.
11) Extract remaining phenol in aqueous layer with an equal volume of chloroform. Transfer genomic DNA-containing aqueous layer to new tube.
12) Add 1/10 volume 3 M sodium acetate, pH 5.2 and 1 μL glycogen (20 mg/mL) and mix by inverting tube.
13) Add 3 volumes of ethanol and mix by inverting.
14) Precipitate genomic DNA overnight at – 20 °C.
15) Centrifuge at 14,000 rpm for 15 minutes at 4 °C
16) Discard supernatant and wash pellet with cold 70% ethanol (aqueous)
17) Remove 70% ethanol and air dry.
18) Resuspend genomic DNA in MilliQ water (20 μL/fin clip).

PCR

Use 1 microliter of the genomic DNA extract per PCR reaction. Conditions will depend on the target and at least 35 cycles may be necessary for detection. DMSO may also be used as a co-solvent in the PCR reactions.