Extraction of genomic DNA from zebrafish embryos for PCR

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Reference: http://zfin.org/zf_info/zfbook/chapt9/9.3.html

1. Add 50 µl of extraction buffer per embryo.

2. Incubate at 50 °C for 2 - 3 hr or O/N. Tap the tube occasionally during the incubation.

3. Incubate at 95 °C for 10 min to heat-inactivate Proteinase K.

4. Spin at 12,000 rpm for 10 min at 4 °C.

5. Use 2 – 3 µl to set up PCR reaction in a total volume of 10 µl.

Optionally, you can use ethanol precipitation to get more pure DNA.

6. Add 100 µl of EtOH, mix and place on ice for 20-30 min.

7. Centrifuge at full speed at 4 °C for 10 min, remove supernatant and add 200 µl of 70% EtOH.

8. Spin again for 2 min, remove liquid and air-dry pellet.

9. Resuspend the DNA in 20 µl of 10 mM Tris (pH 8.0).

10. Proceed with PCR reaction. Usually 1/4 to 1/2 (5-10 µl) of the DNA isolated from one embryo is sufficient.

Extraction buffer

	Stock	Final	Total 50 ml
Tris HCl (pH 8.0)	1 M	10 mM	0.5 ml
EDTA	125 mM	2 mM	0.8 ml
SDS	10%	0.5%	2.5 ml
Proteinase K*	20 mg/ml	200 μg/ml	0.5 ml

* Add just before use.

	Stock	Final	Total 50 ml
Tris HCl (pH 8.0)	1 M	10 mM	0.5 ml
NaCl		200 mM	
EDTA	125 mM	10 mM	4 ml
SDS	10%	0.5%	2.5 ml
Proteinase K*	20 mg/ml	200 μg/ml	0.5 ml