Extraction of genomic DNA from zebrafish embryos for Southern Blotting

Written by Li-Hua Li on 11-9-2009

Reference: Kawakami et al., PNAS 97:11403; http://zfin.org/zf_info/zfbook/chapt9/9.3.html

1. Place 30–60 embyos in a microfuge tube with 10 volumes of DNA extraction buffer (e.g., 200 µl of extraction buffer for 60 embryos).

2. Incubate at 50 °C for 3 hr or O/N. Tap* the tube occasionally to mix the reaction, or extraction yield would be low.

3. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1).

4. Seal the top of the tube with parafilm, invert the tube gently 3–5 x and rock the tube at Speed 2 for 20 min at RT.

5. Remove the parafilm and spin at 12 K RPM for 5 min.

6. Transfer# the upper aqueous phase to a fresh microfuge tube.

7. Add the same volume of extraction buffer (less proteinase K) used in Step 1 to the organic phase, invert the tube 10 x gently and spin at 12 K RPM for 5 min.

8. Pool the aqueous phase.

9. Add an equal volume of chloroform to the pooled aqueous phase, invert the tube 10x gently and spin at 12 K RPM for 5 min.

10. Transfer the upper aqueous phase to a fresh microfuge tube and add sodium acetate (pH 5.2) to 0.3 M.

11. Add an equal volume of ice-cold isopropanol and invert the tube 10x gently. You may see thread-like genomic DNA (gDNA).

12. Spin at 12k RPM at 4 °C for 15 min and discard the supernatant.

13. Add 1 ml of ice-cold 70% ethanol to the pellet, invert 10x and spin at 12 K RPM at 4 °C for 5 min.

14. Remove the 70% ethanol completely and air-dry the pellet at RT for 20 min.

15. Add 100 µl of 10 mM Tris (pH 8.0) to the dried pellet and rock the tube at Speed 2 gently overnight at 4 °C. Tap the tube occasionally to help DNA dissolve completely.

16. Measure DNA concentration. Typically, the yield from 60 embryos is 500-600 ng/µl of genomic DNA.

17. Run 5 µl of gDNA on 1% agarose gel to see if there is any degradation of DNA.

[*] As gDNA is fragile, mechanical forces easily shear gDNA. Therefore, do not vortex the tube containing gDNA throughout the procedure.

[#] When transferring solution with genomic DNA, use large-bore pipet tips.

DNA Extraction Buffer

	Stock	Final	Total 50 ml
Tris HCl (pH 8.0)	1 M	10 mM	0.5 ml
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

[&] Add just before use. Don't try to heat-inactivate proteinase K since exposure of DNA to high temperature winds up degradation of DNA.