Preparation Of Genomic DNA

(Source: A. Fritz from Zebrafish Book 5th Edition)

Large sample number, very quick and dirty, adequate for PCR

This protocol is most suitable for samples consisting of 1-20, diploid, 2-3 day old embryos. Embryos need not be removed from their chorions. DNA prepared with this procedure is only good for PCR analysis, but is unsuitable for digests and Southern blots. Because PCR does not require high-molecular-weight DNA, samples can be vortexed and frozen.

1. Transfer embryo(s) into microfuge tube and remove excess liquid with drawn-out Pasteur pipette.
2. Add extraction buffer, 50µl or 10µl per embryo whichever is larger, and incubate at 50-56°C for 2-3 hours (longer is OK). Vortex occasionally.

**PCR Extraction buffer:**

10 mM Tris pH 8, 2 mM EDTA, 0.2% Triton X-100, 200 µg/ml Proteinase K

3. Boil samples in water bath for 5-10 minutes to inactivate Proteinase K.
4. Spin in microfuge for 1 minute, store at -20°C.
5. Use 10-15 µl to set up PCR reaction in a total volume of 50 µl, proceed with your favorite PCR protocol.

Large sample number, still quick but less dirty

This protocol produces cleaner preparations and probably somewhat higher yields per embryo. DNA prepared with this method from single haploid embryos is satisfactory for multiple-primer (multiplex) PCR reactions. The protocol is provided for the preparation of DNA from single haploid embryos, but, of course, can be adapted for more embryos.

1. Remove the embryo from the chorion. Sperm tends to stick to the chorion, and sperm DNA can be amplified in a PCR reaction; it is therefore essential to remove the chorion, especially if you expect the genotype of the haploid embryo to differ (i.e. mutant) from that of the UV-treated sperm (usually wild type).
2. Transfer embryo into microfuge tube, remove liquid with Pasteur pipette, rinse by adding some dH2O, and remove as much liquid as possible with drawn-out Pasteur pipette.
3. Add 50 µl extraction buffer and incubate at 50°C for about 3 hours. Mix occasionally.

**DNA Extraction buffer:**

10 mM Tris pH 8.2, 10 mM EDTA, 200 mM NaCl, 0.5% SDS, and 200 µg/ml proteinase K

4. Add 100 µl EtOH, mix, and place on ice for 20-30 minutes.
5. Centrifuge in microfuge for 10 minutes, remove supernatant and add 200 µl 70% EtOH. Spin again for 2 minutes, remove liquid and dry pellet.
6. Resuspend the DNA in 20 µl TE, store at -20°C.
7. Proceed with PCR reaction. Usually 1/4 to 1/2 (5-10 µl) of the DNA preparation from one embryo is sufficient.

Isolation of high-molecular-weight genomic DNA

This protocol is essentially that of Sambrook et al. (i.e. Maniatis), described on page 9.16 ff. of Vol. 2. References, modifications, and alternatives can be found there. Genomic DNA prepared by this method can be used for all purposes, including construction of libraries. Care should be taken to carry out all mixing and resuspension steps gently; do not vortex. Store DNA at 4°C, do not freeze.

a. Place embryos, tissue or adult fish into suitable container, remove all liquid and quick-freeze in liquid nitrogen. If desired, specimen can be stored at -80°C.

b. Prepare extraction buffer. About 10 ml per 1 g of material or 10 volumes per volume of ground fish (see #3, below) is appropriate. Have extraction buffer ready in 50 ml plastic tube or a small beaker.

**Genomic DNA extraction buffer:**

10 mM Tris pH 8, 100 mM EDTA pH 8, 0.5% SDS, 200 g/ml Proteinase K

3. Grind material into a powder using a high speed blender or mortar and pestle, keeping it frozen in liquid nitrogen. When finished, allow the liquid nitrogen to evaporate.

4. Slowly add powder to extraction buffer, allowing it to spread and wet on the surface of the buffer. Then shake to submerge material.
5. Incubate at 50°C for at least 3 hours (up to overnight) with occasional gentle swirling.
6. Cool solution to room temperature and extract two times with one volume of equilibrated phenol. MIX GENTLY until an emulsion has formed; separate phases by centrifugation at 3000-5000 x g for 10 minutes. Remove aqueous phase carefully using a wide-bore pipette.
7. Extract a third time with phenol:chloroform:isoamyl alcohol (25:24:1), centrifuge, then transfer aqueous phase into fresh tube and add NaCl to a final concentration of 200 mM. Overlay with 2 volumes of ethanol by slowly letting it run down the side of the tube. Swirl the tube gently until the solution is thoroughly mixed. The DNA will precipitate immediately and should be easily visible.

8. Over a Bunsen burner, seal the end of a Pasteur pipette and melt it into a U-shape. Remove the precipitated DNA using the Pasteur pipette and transfer it into a tube containing 70% ethanol. Let DNA stand in 70% ethanol for about 5 minutes and gently move it around from time to time using the Pasteur pipette.

9. Remove the DNA from the 70% ethanol with the Pasteur pipette, let excess liquid drip off, and place the Pasteur pipette with DNA sticking to it inverted into a microfuge rack. Let the DNA air dry for 5 minutes.

NOTE: If the DNA precipitate becomes fragmented in the 70% ethanol solution and fails to stick to the pipette, centrifuge the tube for 5 minutes at 5000 x g to pellet the DNA. Then remove as much ethanol as possible. Let the DNA air dry until the last visible traces of ethanol have evaporated.

10. Resuspend the DNA in an appropriate volume (5-10 ml per gram of starting material) of 10 mM Tris pH 8, 5 mM EDTA and 100 µg/ml (DNAse free) RNAse A. Genomic DNA is hard to resuspend, this may take several hours. Place DNA solution at 37°C and gently mix and swirl from time to time.

11. Extract the DNA solution once with phenol:chloroform:isoamyl alcohol (as above) and transfer aqueous phase to fresh tube.

12. Add 0.1 volume of 7.5 M ammonium chloride and overlay with two volumes of ethanol. Precipitate DNA by slowly inverting tube until solution is thoroughly mixed. Repeat steps 8 and 9. Rinse twice in 70% ethanol.

13. Resuspend DNA in TE, usually 1-2 ml per g of starting material is good. Resuspending can be facilitated by warming the DNA solution to 50°C.

14. Determine the concentration by measuring OD at 260 nm in a spectrophotometer. 1 OD unit is 50µg/ml.