Benchmarks

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Protein Salting-Out Method Applied to Genomic DNA Isolation from Fish Whole Blood

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Isolation of genomic DNA is a primary molecular biology technique that has been increasingly applied in the research fields of aquaculture, fisheries and fish biology. There are two major DNA isolation procedures, the organic extraction method and the protein salting-out method. Both methods differ in the manner in which partially hydrolyzed polypeptides were separated and precipitated following cell lysis and proteinase K treatment. The organic extraction method is based on the use of organic solvents such as phenol and chloroform. The protein salting-out method is based on the use of a saturated salt solution such as sodium chloride. The organic extraction method is toxic and difficult, and DNA product is sometimes degraded upon usage and storage because of contaminants. In contrast, the protein salting-out method is fast, easy, safe and inexpensive, and the quality of the DNA product is high. Most fish biologists commonly use the method of phenol/chloroform extraction for genomic DNA isolation. The

protein salting-out method has been proven useful in other species and can be an alternative approach for isolating DNA from fish.

We developed a protein salting-out method based on Miller et al. (3) and several protocols collected from different laboratories for isolating genomic DNA from sea bass blood cells. Fish erythrocytes are nucleated and comprise more than 98% of the cellular components of the blood (1), so a small blood sample can yield large amounts of DNA. In the protocol, the lysis step of fish nucleated blood cells was divided into two successive steps with two types of buffers differing in the sodium dodecyl sulfate (SDS) component, which can facilitate the cell lysates to reach homogeneity upon mixing and optimize the number of cells being lysed. Isolated DNA was suitable for polymerase chain reaction (PCR) and Southern blotting. The procedures were rapid and straightforward for isolation of DNA from a large number of fish.

The blood was drawn from the caudal vein of sea bass with syringes containing 0.1 M EDTA at a concentration of 20 μ L/1 mL of blood. Part of the blood was used for the DNA extraction, while the remaining blood was preserved in 95% ethanol at a proportion of 1:3. Forty microliters of freshly drawn blood or the blood cell pellets centrifuged from 160 μ L ethanol-preserved blood were resuspended in a 15-mL polypropylene tube containing 3 mL of resuspension buffer (0.1 M Tris-HCl, 0.01 M NaCl, 0.1 M EDTA, pH

8.0). The cells were immediately lysed by adding 3 mL of cell lysis buffer (0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, 1% SDS, pH 8.0) and gently inverting the tube to mix thoroughly. The lysate was then ready for DNA isolation or for storage at room temperature for several months. To proceed with the DNA extraction, the cell lysates were treated with proteinase K (150 μ g/mL) at 55°C for 2 h or at 37°C overnight in cell lysis buffer. Next, 2 mL of 6 M NaCl were added to reach a final concentration of 1.5 M, and the solution was vortex-mixed vigorously for 15 s, chilled on ice for 5–10 min, followed by centrifugation at 1200 $\times$ g for 30 min to precipitate partially hydrolyzed polypeptides. If the supernatant did not look clear, it was transferred to a new tube and centrifuged again for another 15 min. Approximately 6 mL of clear supernatant containing genomic DNA were transferred to another 15-mL polypropylene tube containing an equal volume of isopropanol. The tubes were inverted gently several times, causing long strands of high-molecular-weight DNA to appear, and the DNA was pelleted by centrifugation at 1200 $\times$ g for 15 min. The DNA pellet was then washed in 70% ethanol and moderately dried. The dried DNA pellet was resuspended in 500 μ L of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) overnight and stored at 4°C. DNA was quantified using a spectrophotometer.

Using the described technique, DNA can be isolated very efficiently from a small amount of whole blood.

Figure 1. Southern hybridization of one unknown DNA clone on the salt-extracted and *Hind*III-digested genomic DNA from fish blood cells. Male sea bass genomic DNA was subtracted against female genomic DNA, and then one of the subtracted DNA clones was used to probe *Hind*III-digested male DNA (lanes 1–6), intersex DNA (lane 7) and female DNA (lanes 8–13).

Depending on the number of blood cells, the yield of genomic DNA from 40 μ L of fresh whole blood ranged from 125 to 250 μ g or at times as much as 500 μ g. The quality of DNA isolated from sea bass blood was high. The optical density (OD)₂₆₀/OD₂₈₀ ratio was found to be between 1.8 and 2.0, and 5 μ g of uncut genomic DNA after an intensified short running using a 0.8% agarose gel showed the intact DNA of approximately 50 kbp without shearing (data not shown).

Five micrograms of salt-extracted DNA cleaved with restriction enzymes *Bam*HI, *Hind*III or *Sau*3AI and electrophoresed on a 0.8% agarose gel for 5 h showed complete digestion (data not shown), and the resulting gels were used in Southern hybridization for restriction fragment-length polymorphism (RFLP) analysis (Figure 1). The isolated genomic DNA was also found suitable for use in PCR. Using a version of the subtractive hybridization technique [representational difference analysis (RDA) (2)], salt-extracted DNA cleaved with *Bam*HI, *Hind*III or *Sau*3AI was ligated to oligonucleotide adaptors and amplified by PCR for preparation of tester and driver DNA. Subsequently, different products after the first, second and third DNA subtraction steps were amplified by PCR (data not shown).

The DNA extraction protocol described has been used in more than 100 fish blood samples for fulfilling our research needs. According to individual needs, the procedures can be scaled up 3-fold for blood samples and reagents and can be processed in 50-mL polypropylene tubes or scaled down to 4 μ L of blood samples and one tenth of the reagents and processed in microcentrifuge tubes.

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