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Long-Term Storage of Salmon Tissue Samples for DNA Extraction and Genetic Analyses

We present a protocol for field biologists to collect tissue samples for future DNA analyses. Samples must be of high quality for laboratory personnel to perform DNA-based genetic analyses. Because field studies are often in remote locations, samples must be stored without requiring specialized or electrical equipment.

Tissue Collection and Storage

Experiments in DNA stability were performed on caudal fin, adipose fin, and liver samples collected from different life stages of chinook salmon (*Oncorhynchus tshawytscha*) from the Coleman and Carson National Fish Hatcheries. Replicate 0.2–0.5 cm^3 samples of the various tissues were collected and placed in 1.5 mL microcentrifuge tubes containing either a tissue lysis–DNA stability buffer, 100% ethanol, or no solution. The buffer contained 150 mm EDTA, 50 mm Tris pH 8.0, and 2.0% n-lauroylsarcosine. Immediately after collection, replicate samples were placed under one of eight experimental conditions as follows: 1) in buffer at room temperature; 2) in buffer at 4°C; 3) in buffer at -20°C; 4) in buffer at -80°C; 5) in buffer, heated to 65°C for 30 min, and stored at room temperature; 6) in buffer, heated to 65°C for 30 min, and stored at 4°C; 7) stored dry without buffer at -80°C; and 8) in 100% ethanol without buffer and stored at -80°C. All samples were held under the indicated conditions for 3 months, with the exception of some samples held at room temperature for 1 year before DNA extraction.

Extraction Method

The DNA extraction method described here (followed by definitions) takes 2–4 h to complete, does not require toxic chemicals, and yields about 250–500 µg of DNA from 0.2–0.5 cm^3 tissue samples (Table). This yield is adequate for performing thousands of PCR-based genetic analyses.

1) Place a small sample of tissue in a 1.5-mL microfuge tube.
2) Add 500 µL of lysis buffer and mix samples.
3) Place samples at 65°C for 15 min.
4) Pulverize samples with a microfuge grinder and incubate at 65°C for 15 min.
5) Pellet debris by centrifugation at 14,000 rpm for 5 min and transfer supernatant to a clean tube.
6) Add 0.7 volumes of PEG/NaCl solution to the supernatant, mix thoroughly, and incubate at room temperature for 5–30 min.
7) Spin 5 min at 14,000 rpm, discard supernatant, and resuspend pellet in 0.2 mL TE (resuspension of DNA pellets in steps 7, 10, and 12 can be performed more quickly at 37°C) with brief (1–3 s) vortex pulses.
8) After the DNA has dissolved, add 0.5 volume of NH_4OAc; incubate on ice 5 min; and pellet protein, RNA, and fine debris by centrifugation at 14,000 rpm for 5 min.

9) Transfer supernatants to clean microcentrifuge tubesand add 0.6-1.0 volumes of IPA, mix samples gently to precipitate DNA, and incubate at room temperature for 5-30 min.
10) Pellet DNA for 5 min at 14,000 rpm, and resuspend DNA in 0.2mL TE.
11) Add NaCl to 0.1 m and 2 volumes 95% EtOH, mix gently, and ice for 5-30 min.
12) Pellet DNA for 5 min at 14,000 rpm and resuspend DNA in 0.5 mL TE buffer.

NH4OAc = Ammonium Acetate at 7.5 N, autoclaved
IPA = Isopropyl Alcohol
TE = 10 mM Tris pH 8.0, 1 mM EDTA pH 8.0, autoclaved
EtOH = Ethanol
PEG/NaCL = 20% polyethylene glycol (mw 8,000) + 2.5 M NaCl, autoclaved
NaCl = sodium chloride, autoclaved

Quantitative and Qualitative Yield of DNA

To determine the optimal conditions for storing tissue samples, DNA from the various experimental storage conditions was extracted as described above, analyzed by agarose gel electrophoresis, and viewed by fluorescence after ethidium bromide staining. High-quality salmon DNA migrates in agarose gels with an apparent size of 23-30 kilobases (kb) (Figure). As the quality of DNA decreases there is an increase in fluorescing material below 23 kb (Figure).

The yield of DNA (250-500 µg) from all tissues stored in lysis-DNA stability buffer under conditions 1-4 was equivalent (Table). Electrophoretic analysis of these DNAs (conditions 1-4) indicated that they were of high quality and about 23-30 kb in size. If the samples were pretreated at 65°C for 30 min before storage there was degradation (25%) of the DNA—probably due to the exposure of DNA to cellular nucleases that were released by heat treatment. The DNA obtained from the samples stored dry at -80°C was completely degraded. It had an average molecular weight of 0.5 kb and no DNA molecules longer than 1.0 kb. The DNA extracted from samples stored in ethanol was of high quality but the yield was only 1-5% of that from tissues stored in buffer. It seems that although ethanol does not degrade the DNA, it greatly diminishes yield. Although the adipose and caudal fin samples were stored for a year at room temperatures, the yield and quality of the DNA extracted from those tissues were indistinguishable from the liver samples that were stored for 3 months at any of the temperatures.

This extraction protocol has been used to obtain DNA from bacteria, fungi, plants, nematodes, and whiteflies. In all of these systems, the DNA was of sufficient quantity and quality to perform restriction enzyme and PCR analyses.

Conclusions

We present a simple protocol to store small salmon tissue samples for future DNA extraction. We developed a DNA extraction protocol to rapidly obtain sufficient amounts of DNA for 500-1,000 PCR-based genetic analyses. The yield of DNA is sufficient to permit nonlethal collection of samples from fin tissues. The protocol can be performed with standard laboratory equipment and chemicals. Optimal storage conditions require placing tissues in buffer at any temperature with no pretreatment at 65°C. The yield of DNA from samples stored under these conditions was qualitatively and quantitatively equivalent. DNA prepared by this protocol has been successfully used as template for PCR-based genetic analyses of several salmon populations (to be described elswhere). We have used this procedure to extract high-quality DNA from several species of marine fishes, insects, nematodes, fungi, bacteria, and plants. In all of these species, the yield of DNA was adequate for performing thousands of PCR analyses.

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Table. Effect of storage conditions on DNA yield and size.

Storage conditions[a]	DNA yield[b]	DNA size[c]
1) In buffer at room temperature (RT)	0.5-1.0	23-30 kb
2) In buffer at 4°C	0.5-1.0	23-30 kb
3) In buffer at -20°C	0.5-1.0	23-30 kb
4) In buffer at -80°C	0.5-1.0	23-30 kb
5) In buffer, heated to 65°C for 30 min and stored at RT	0.5-1.0	0.1-30 kb[d]
6) In buffer, heated to 65°C for 30 min and stored at 4°C	0.5-1.0	1.0-30 kb[e]
7) Stored dry without buffer at -80°C	–	0.5-1.0 kb
8) In 100% ethanol without buffer and stored at -80°C	0.025-0.050	23-30 kb

[a]Six replicate samples were collected and stored as indicated.
[b]Quantitated as µg/µL by electrophoresis with quantitation standards.
[c]Determined by migration in agarose gels compared to DNA size standards.
[d]The majority of DNA was 23-30 kb, however there was some DNA degradation down to 0.1 kb.
[e]The majority of DNA was 23-30 kb, however there was some DNA degradation down to 1.0 kb.

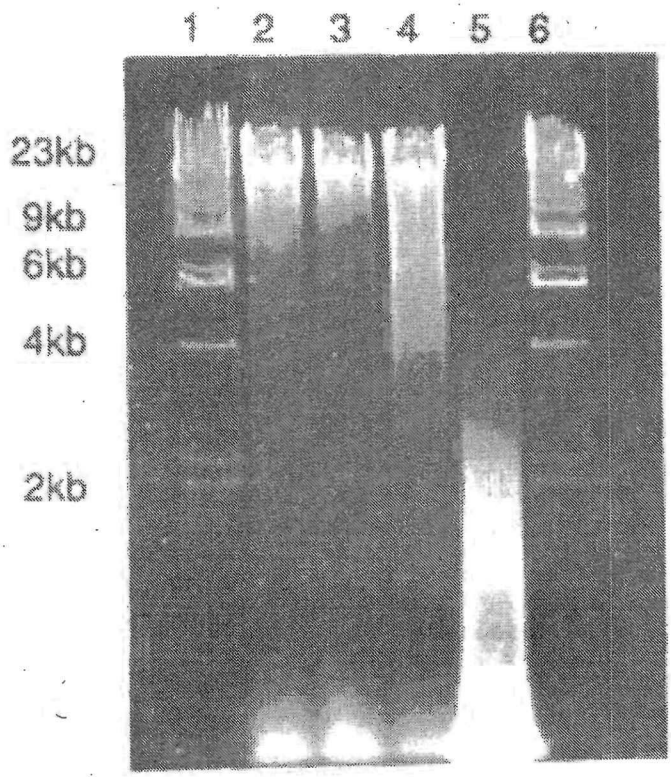

Figure. Electrophoretic analysis of DNA extracted from liver (lane 2), caudal fin (lane 3), and adipose fin (lane 4) tissues stored in the tissue lysis-DNA stability buffer, and from liver tissue stored dry without buffer for 3 months at -80°C (lane 5). Lanes 1 and 6 contain DNA size markers with sizes indicated on the left.