Buccal swabbing and extraction of high quality sunfish (*Lepomis*) DNA for use in PCR analysis

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BioTechniques 38:188-192 (February 2005)

The acquisition of high quality DNA for use in phylogenetic and molecular genetic population studies is a primary concern for evolutionary and genetic researchers. While such DNA is easily obtained from study organisms, it often requires the sacrifice of the subjects in question. Such destructive or lethal sampling has the potential to seriously impact the genetic makeup of populations under investigation and should be avoided whenever possible.

Many nondestructive DNA sampling methods have been developed and are used with a variety of taxa in applications ranging from genetic stock assessment to molecular forensics. Toe clips have been used as sources of DNA for population genetic studies of the Great Plains toad, *Bufo cognatus* (1). DNA suitable for PCR amplification and analysis of microsatellites in honey bees (*Apis melliera*) has been obtained from wing clips (2). The molecular phylogeny of the family Chinchillidae has been investigated using DNA from hair, blood, feces, and ear tissue (3). In fish, sources of DNA available for nonlethal sampling include: fin clips, scales, barbels, muscle, blood, and sperm (4,5). DNA suitable for microsatellite analysis and genotyping has even been obtained from chimpanzee (*Pan troglodytes*) feces (6).

A standard method of collecting DNA with minimal invasiveness from humans involves buccal swabbing to dislodge epithelial cells from which the DNA can then be extracted (7,8). Among the advantages of this method are rapidity and simplicity (9). These characteristics make buccal swabbing adaptable to a wide variety of situations and particularly amenable to large sample sizes.

We have developed a field sampling method for obtaining high quality DNA from sunfish (*Lepomis*) that employs a variation on the buccal swab method and results in the collection of DNA suitable for PCR amplification and polymorphic analysis. The ease of this method—coupled with its scalability to include large sample sizes, its ambient temperature of field storage and preservation, and its simplicity of sample transport—should make it applicable to field-oriented population and conservation genetic studies involving a wide range of fish.

Bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) sunfish were caught in situ using standard angling equipment and bait of mealworms. The sunfish originated from Lake Wapalanne in Northwestern New Jersey. Once caught, the fish were held temporarily (from 20 min to 1.5 h) in buckets of lake water.

When approximately 20 fish were captured, buccal smears were taken from each fish by sterilely swabbing their mouths using the wooden ends of sterile cotton-tipped applicators (Moore Medical Corp, New Britain, CT, USA). Cheek cells from the applicator ends were fixed and preserved on site by resuspension into 100 µL of 100% ethanol in 1.5-mL microcentrifuge tubes. After taking buccal smears, fish were returned to their lake habitat. In the laboratory, the fixed tissue samples were stored at 4°C for 24–96 h before extraction.

For DNA extraction, the ethanol fixative was first dried from the tissue samples for 10–20 min on a Thermo Savant SpeedVac® vacuum dryer (GMI, Albertville, MN, USA) set at the lowest drying temperature. Tissue samples were then resuspended in 50 µL of TE and RNase (10 mM Tris-Cl, 1 mM EDTA, pH 8.0, 1 U RNase per 50-µL aliquot). Tissues were lysed by a 5-min incubation at 95°C, then cooled on ice for an additional 5-min incubation, and then centrifuged briefly to collect water that condensed on the side of the microcentrifuge tube. The DNA concentration averaged 0.5–1 ng/µL based on electrophoretic analysis and comparison to known molecular weight standards. DNA was stored frozen at -20°C until later PCR amplification.

To test the quality of the extracted sunfish DNA, PCR was performed employing amplification primers for detecting microsatellite polymorphisms (10). The PCR amplification conditions principally followed the directions of Vander Zwan et al. (11). Microsatellites were amplified in 20-µL reactions containing 1–1.5 ng fish DNA, 10% ThermoPol buffer (New England Biolabs, Beverly, MA, USA), 5 pmol of each primer, 200 µM dNTPs (New England Biolabs), and 1.0 U *Taq* DNA polymerase. All amplification was performed in a Mastercycler® gradient thermal cycler (Eppendorf AG, Hamburg, Germany). The PCR products were subjected to electrophoresis on a 1.5% agarose gel in 1× sodium borate buffer (12). The products on the agarose gels were stained with ethidium bromide and imaged using the Gel Documentation System (Ultralum, Claremont, CA, USA) and Scion computer software (Scion, Frederick, MD, USA).

The quality of the isolated bluegill DNA is high enough to allow PCR amplification of simple sequence-length polymorphisms without further purification. There is some background visible along with the polymorphic DNA bands, but the bands themselves are clearly visible for each individual fish (Figure 1). Microsatellite regions have been successfully amplified at several bluegill loci (Lma20, Lma21, Lma87, Lma102, Lma120, and Lma124), although data from only the Lma20 locus (forward primer, 5′-GGCACTAATCTAATTGTAGCC-3′; reverse primer, 5′-TTGTGTGTCTGC-ATTGGAATC-3′) is presented here (Figure 1). Moreover, we have

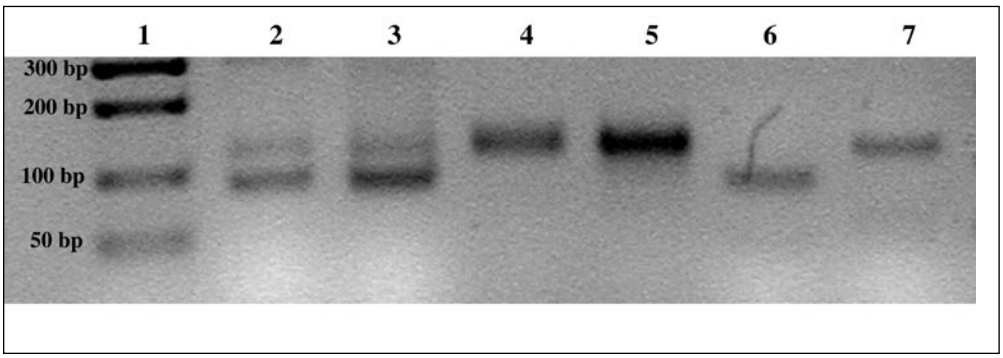

Figure 1. PCR products of bluegill DNA (from Lake Wapalanne), using Lma20 primers to amplify microsatellite regions. Lane 1, molecular weight markers (HiLo marker), size indicated in basepairs. Lanes 2–7, bluegill Lma20 microsatellite polymorphisms. Heterozygotes and homozygotes for the Lma20 marker are clearly delineated in the individual fish. Agarose gel (1.5%) stained with ethidium bromide. The image was inverted to a negative by Scion computer software.

amplified larger polymorphic microsatellite sequences of 300 bp using the marker Lma120 (data not shown). High-resolution agarose gel electrophoretic analysis using comparisons to known concentrations of the HiLo marker (Minnesota Molecular, Madison, MN, USA) were employed to determine the size ranges of unamplified genomic DNA. We found molecular weights ranged from 1000 to 7000 bp.

To our knowledge, this is the first application of buccal swabbing in fish for purposes of DNA extraction. Variations on this collection method have been commonly used among mammals for many years, but it is possible differences in the epithelial tissues of fish made researchers less apt to consider employing this method for sample acquisition. Now that this method has been demonstrated to work efficiently in sunfish, we hope that it will be attempted in both field and laboratory work for many fresh and saltwater fish.

ACKNOWLEDGMENTS

Foremost, we thank the New Jersey School of Conservation for the use of their facilities. We also offer our gratitude for the angling assistance of Scott Kight, Stephanie Smith, Andrew Mickens, Nick Reed, Chris Sikes-Keilp, Aimee Luers, and Joe Satran. Finally, we wish to thank Lisa Campanella for her editorial help with this manuscript. This work was supported in part by a Sokol grant for undergraduate research and a Weston Scholars grant.

COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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Received 20 July 2004; accepted 6 October 2004.

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