A Method Utilizing Buccal Swabbing for Collection and Extraction of High-quality, Newt (Notophthalmus) DNA for Use in Phylogenetic Analyses

by

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Abstract

The acquisition of high-quality DNA for use in phylogenetic and molecular population genetic studies is a primary concern for evolutionary and genetic researchers. While such DNA is easily obtained, it often requires the sacrifice of the subjects in question. Many non-destructive DNA sampling methods have been developed and are used with a variety of taxa in applications ranging from genetic stock assessment to molecular forensics. We have developed a field sampling method for obtaining high-quality DNA from newts (Notophthalmus) which employs a variation on the buccal swab method and results in the collection of DNA suitable for PCR amplification and polymorphism analysis. The ease and benefits of this method should make it applicable to field-oriented population and conservation genetic studies involving a wide range of amphibians.

Introduction

The acquisition of high-quality DNA for use in phylogenetic and molecular population genetic studies is a primary concern for genetic and evolutionary researchers. While such DNA is easily obtained from studied 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 non-destructive 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. DNA suitable for PCR amplification and analysis of microsatellites in honey bees (Apis mellifera) has been obtained from wing clips. The molecular phylogeny of the family Chinchillidae has been investigated using DNA from hair, blood, feces, and ear tissue. In fish, sources of DNA available for non-lethal sampling include: fin clips, scales, barbels, muscle, blood and sperm DNA suitable for microsatellite analysis and genotyping has even been obtained from chimpanzee (Pan troglodytes) feces and sperm whale (Physeter macrocephalus) teeth and scrimshaw.

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. Among the advantages of this method are rapidity and simplicity. 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 newts \textit{(Notophthalmus)} which employs a variation on the buccal swab method and results in the collection of DNA suitable for PCR amplification and polymorphism analysis. The benefits of this method include its scalability to include large sample sizes, its ambient temperature of field storage and preservation, and its simplicity of sample transport. The ease of our method should make it readily applicable to field-oriented population and conservation genetic studies involving a wide range of amphibians.

**Materials and Methods**

Red-spotted newts \textit{(Notophthalmus viridescens)} were caught in situ using standard seining methods. The newts originated from Lake Wapalanne in Northwestern New Jersey on the grounds of the New Jersey School of Conservation. Once caught, the newts were held temporarily (20 min - 1.5 hr) in shallow buckets of lake water.

When \~40 newts were captured, buccal smears were taken from each newt by steriley swabbing their mouths using the wooden ends of sterile cotton-tipped applicators (Moore Medical Corp, New Britain, Connecticut). Cheek cells from the applicator ends were fixed and preserved on site by re-suspension into 100 \(\mu\)L of 100% ethanol in 1.5 mL microfuge tubes. After taking buccal smears, newts were returned to their lake habitat. In the laboratory, the fixed tissue samples were stored at 4\(^\circ\)C for 24-96 hours before extraction.

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

To test the quality of the extracted DNA, PCR was performed employing amplification primers for detecting microsatellite polymorphisms in red-spotted newts\(^1\). The PCR amplification conditions principally followed the directions of Vander Zwan et al.\(^2\). Microsatellites were amplified in 20 \(\mu\)L reactions containing: 1-1.5 ng newt DNA, 10% ThermoPol buffer (New England Biolabs, Inc., Beverly, Massachusetts), 5 pmoles of each primer, 200 \(\mu\)M dNTPs (New England Biolabs Inc., Beverly, Massachusetts), and 1.0 unit Taq polymerase. All amplification was performed in a Mastercycler Gradient Thermocycler (Eppendorf Inc., Germany). The PCR products were subjected to electrophoresis on a 2\% agarose gel in 1X sodium borate buffer\(^3\). The products in the agarose gels were stained with ethidium bromide and imaged using an Ultralum gel documentation system (Ultralum, Inc., Claremont, California) and Scion Image computer software (Scion, Inc., Frederick, Maryland).

**Results and Discussion**

The quality of the isolated newt 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 newt (Figure 1). Microsatellite regions have been successfully amplified at all available newt loci (Nvi2, Nvi7, Nvi11, Nvi14, Nvi18, Nvi19, and Nvi24), although data from only the Nvi14 locus \textit{(Forward primer: 5' AAGGTCATCTAAACAAAGAGT 3', Reverse primer: 5' ACAGCATGGGCACAGTAT}
3') is presented here (Figure 1). Moreover, we have amplified larger polymorphic microsatellite alleles of 300 basepairs using the marker Nvi14 (data not shown). High-resolution agarose gel electrophoretic analyses using comparison to known concentrations of HiLo molecular weight marker (Minnesota Molecular, Madison, Minnesota) were employed to determine the size ranges of unamplified genomic DNA. We found molecular weights ranged from 1000 to 7000 basepairs.

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

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References


