

Rapid Sampling and Identification of Mitochondrial and Genomic DNA

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PCR-ready mitochondrial and genomic DNA is effectively extracted using the QuickExtract™ DNA Extraction Solution 1.0 from a diverse range of sources, including human teeth, hair and buccal cells.

Introduction

Analyses of genomic (gDNA) or mitochondrial DNA (mtDNA) provide powerful methods for identifying the specific origin of a human or animal sample. Since maternally inherited mtDNA is present at hundreds to thousands of copies per cell and is not subject to recombination, it provides a valuable tool for forensic scientists, molecular anthropologists, and researchers studying metabolic and other human disorders. PCR amplification and sequencing of two hypervariable segments of the non-coding control region of mtDNA is a reliable method of forensic identification, extinct species comparison, or ancient population analysis.¹⁻² Examinations of both coding and non-coding regions of mtDNA have provided insights into a number of mitochondrial mutations involved in aging and human disease.³⁻⁴

Since potential samples for mtDNA extraction range dramatically in abundance and quality, a simple, efficient DNA extraction method is required. Here, we demonstrate that high quality mtDNA and gDNA are readily extracted from human tooth, hair or buccal (cheek swab) samples using a simple 3 to 8 minute protocol with EPICENTRE Biotechnologies' QuickExtract™ DNA Extraction Solution 1.0 or BuccalAmp™ DNA Extraction Kit, which also includes the QuickExtract Solution 1.0. Following the DNA extraction, the hypervariable region 1 (HV1) of the human mitochondrial control region (commonly used for mtDNA typing) was amplified using the FailSafe™ PCR System (EPICENTRE). The same samples were separately examined by multiplex FailSafe PCR for the presence of dystrophin genomic DNA sequences. The results demonstrate the utility of the method for generating high quality DNA for analysis of either mitochondrial or genomic DNA.

Methods

DNA extraction

DNA was extracted from a freshly pulled human hair and root, a human tooth (6 months post-shedding), and human buccal cells obtained using Catch-All™ Sample Collection Swabs. The tooth and hair samples

were placed in the QuickExtract Solution, vortexed, heated to 65°C for 6 minutes, then 98°C for 2 minutes.⁵ Samples were vortexed and then cooled on ice. The rapid protocol used to extract DNA from the buccal cell samples consisted of simply rotating a Catch-All Swab with the sample in the QuickExtract Solution and then heating the solution at 65°C for 1 minute and 98°C for 2 minutes.⁶

FailSafe PCR amplification

PCR was performed using 2 to 5 µl of each 500 µl DNA sample in a 25 µl reaction containing the FailSafe PCR Enzyme Mix, FailSafe PCR 2X PreMix D, and primers to the HV1 of mtDNA (10 pmoles of each). The primers used were: HV1.5: 5'- CAC CTG TAG TAC ATA AAA ACC CAA CCC; and HV1.3: 5'- AAG GTT GAT TGC TGT ACT TGC TT, which amplify a 78 bp region of the D-loop (of the non-coding control region) of human mtDNA. The DNA was denatured at 95°C for 2 minutes, and then cycled for 30 cycles, each consisting of: 95°C for 20 seconds; 60°C for 30 seconds; and 72°C for 30 seconds. PCR reactions were analyzed by agarose gel electrophoresis.

PCR amplifications were also performed using 9 primer sets to various regions of the dystrophin gene.⁷ The 18 primers were designed to work well for multiplex PCR (the simultaneous amplification of different targets) in order to identify large deletions in the gene. Two to 5 µl of each DNA sample was used in a 25 µl reaction containing the FailSafe PCR Enzyme Mix, FailSafe PCR 2X PreMix C, and 2 pmoles of the 18 different dystrophin primers. The DNA was denatured at 95°C for 2 minutes, and then cycled for 30 cycles, each consisting of: 94°C for 20 seconds; 58°C for 30 seconds; and 72°C for 60 seconds. Reactions were analyzed by agarose gel electrophoresis.

Results

DNA was successfully extracted from a human tooth (shed 6 months earlier and stored at room temperature), a freshly pulled human hair, and human buccal cells collected with a Catch-All Collection Swab. The extracted DNA samples were stored at -20°C for 2 years and then amplified by FailSafe PCR using primers to the HV1 of the human mitochondrial control gene, which is commonly amplified and sequenced for identification of human remains and for

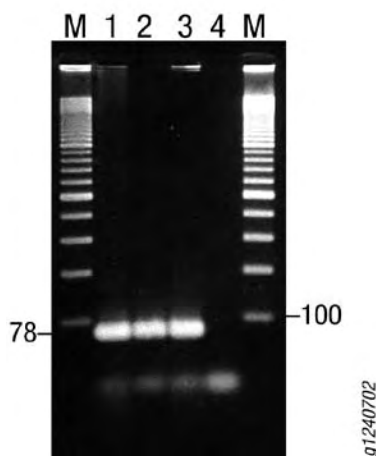


FIG 1. PCR amplification of QuickExtract™ mitochondrial DNA with primers to HV1 results in abundant PCR products. Lane M, 100 bp ladder; Lane 1, tooth DNA amplicon; Lane 2, hair shaft DNA amplicon; Lane 3, buccal cell DNA amplicon; Lane 4, no DNA template control.

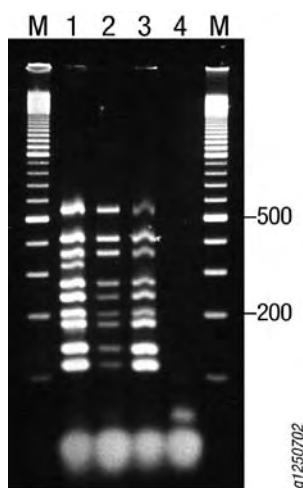


FIG 2. Nuclear genomic DNA, prepared by QuickExtract™ DNA Extraction Solution and amplified by multiplex FailSafe™ PCR with primers to the dystrophin gene, produces abundant PCR products from tooth, hair, and buccal cells. Lane M, 100 bp ladder; Lane 1, tooth DNA amplicons; Lane 2, hair DNA amplicons; Lane 3, buccal cell DNA amplicons; Lane 4, no DNA template, negative control.

comparative DNA studies. All 3 types of tissue treated with QuickExtract Solution produced the expected 78 bp amplicon (FIG 1).

Primers to the dystrophin gene were used to examine the genomic DNA present in the extracted samples. Nine primer sets, which amplify different dystrophin exons, have been used to detect genomic deletions present in muscular dystrophy patients.⁷ DNA extracted from all 3 sources was subjected to multiplex PCR analysis with these primers and all 9 amplicons were readily detected (FIG 2).

Conclusion

PCR-ready mitochondrial and genomic DNA is effectively extracted using the QuickExtract™ DNA Extraction Solution 1.0 from a diverse range of sources, including human teeth, hair and buccal cells. The extraction procedure consists of simply heating the sample in the QuickExtract Solution for less than 10 minutes, after which the sample is ready for even multiplex FailSafe™ PCR analysis. Only minimal sample handling is required, and enough DNA is obtained for over 100 PCR amplifications. The simplicity of the method

makes it highly amenable to automated sample handling.

References

1. Willerslev, E. *et al.*, (2005) *Proc. Biol. Sci.* **272**(1558), 3.
2. Budowle, B. *et al.*, (2003) *Annu. Rev. Genomics Hum. Genet.* **4**,119.
3. Chomyn, A. and Attardi, G. (2003) *Biochem. Biophys. Res. Commun.* **304**(3),519.
4. Schapira, A.H. (2006) *Lancet* **368**(9529), 70.
5. Jarvis, B.W. (2004) *EPICENTRE Forum* **11**(6), 18.
6. Jarvis, B.W. (2004) *EPICENTRE Forum* **11**(4), 4.
7. Beggs, A.H. *et al.*, (1990) *Hum. Genet.* **86**(1), 45.

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QEC091H 100 Swabs
Swabs are individually packaged in sterile, hard-pack plastic cylinders.

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FailSafe™ PCR PreMix Selection Kit

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Note: Each PreMix volume has been modified to match the Enzyme Mix volume.

PCR Analysis of Fingernail DNA Using QuickExtract™ DNA Extraction Solution

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In addition to blood and hair, which are tissues commonly used to obtain DNA for PCR analysis, fingernails or toenails are other potential sources of human DNA.¹ Nails have the advantage of ease of transport and storage for forensic work. DNA from nails can also be used for the diagnosis of superficial mycotic infections.² In the realm of veterinary care, toenail clippings are beneficial for canine genetic studies, yielding five-fold more DNA for PCR than canine buccal cells.³

DNA is extracted simply by heating the nail samples in the QuickExtract™ DNA Extraction Solution at 65°C for 15, 30 or 45 minutes and then prepared for PCR by heating at 98°C for 2 minutes (FIG 1). QuickExtract Solution also gives high yields of PCR-ready DNA from most other types of samples, including hair follicles, quill-end cells of feathers, tissue culture cells, or mouse tail snips, using similar protocols.

QuickExtract provides an easy, reliable method for extracting DNA for diagnostic and forensic applications.

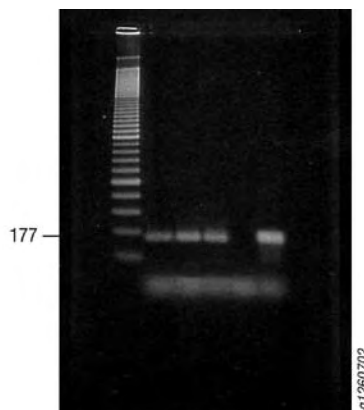


FIG 1. End-point PCR amplification of DNA extracted from fingernails using QuickExtract™. Lane 1, 100 bp ladder; Lane 2, 15 min extraction; Lane 3, 30 min extraction; Lane 4, 45 min extraction; Lane 5, cycled without template, negative control; Lane 6, buccal cell positive control. The reactions used EPICENTRE's FailSafe™ PCR System with PreMix E and human cyclophilin A primers. The primer sequences were, 5' primer: 5'-CAT ACG GGT CCT GGC ATC TTG and 3' primer: 5'-GCC ATT CCT GGA CCC AAA GC. Cycling conditions were: 94°C (2 min) and 26 cycles of 94°C (30 sec), 55°C (30 sec), 72°C (30 sec). The amplicon produced was 177 bp.

Acknowledgment

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References

1. Kaneshige, T. *et al.* (1992) *Nucl. Acids Res.* **20**(20), 5489.
2. Kardjeva, V. *et al.* (2006) *J. Clin. Microbiol.* **44**(4), 1419.
3. Oberbauer, A.M. *et al.* (2003) *Vet Res Commun.* **27**(1), 27.

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