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Forensic Extraction and Isolation of DNA From Hair, Tissue and Bone

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SUMMARY

The Tissue and Hair Extraction Kit (for use with DNA IQ™) provides a simple and rapid approach to processing tissue and hair samples and leads seamlessly into the DNA IQ™ System[a] protocol to purify DNA free of PCR inhibitors in either a manual or automated format. A protocol and custom reagents are also available to rapidly extract DNA from bone.

New protocols using Promega's DNA IQ™ System have recently been developed for the extraction and isolation of DNA from hair, tissue and bone samples.

INTRODUCTION

New protocols using the DNA IQ™ System have recently been developed for the extraction and isolation of DNA from hair, tissue and bone samples. The DNA IQ™ System, which uses novel paramagnetic particles to purify DNA, has a strong denaturing agent that disrupts many sample types in preparation for DNA purification. This approach has been applied to liquid blood, blood on various materials, and other samples, such as buccal and saliva swabs, and differentially extracted sperm and epithelial cells. The DNA IQ™ Lysis Buffer does not lyse cells in the interior of tissue masses, including hair and bone, so pretreatment with Proteinase K is required to ensure complete lysis. The Tissue and Hair Extraction Kit and custom reagents for DNA extraction from bone were designed to facilitate the preprocessing of these difficult samples prior to DNA purification with the DNA IQ™ System. These preprocessing steps do not significantly add to the user's "hands-on" time and still provide the extraction and isolation capabilities of the DNA IQ™ System to remove PCR inhibitors and deliver DNA for use in downstream assays. Figure 1 shows the modular approach of this system and illustrates how a few additional preprocessing steps for a diverse set of sample types flow into the standard DNA IQ™ protocol.

EXTRACTION OF DNA FROM HAIR

Hair is a common sample type discovered at crime scenes, but hair presents a problem to forensic examiners because, as hair is shed, genomic DNA undergoes programmed degradation (1). As a result, processing of nuclear DNA from hair is frequently unsuccessful. The success rate can occasionally be increased with hair follicles from shed hair if the hairs are not washed since epithelial cells are frequently attached to the follicles and contain nondegraded genomic DNA. However, contamination of genomic DNA from fingerprints on the hair shaft may be observed. The DNA IQ™ System eliminates small fragments of DNA, which act as PCR inhibitors; therefore amplification results obtained using this system may be improved over DNA extracted by standard organic methods. Figure 2 shows amplification results using the PowerPlex® 16 System[c,d,e] and genomic DNA extracted by the Tissue and Hair Extraction Kit followed by purification with the DNA IQ™ System. Figure 2A shows the balanced peak heights in an amplification of DNA isolated from a hair follicle. Figure 2B shows the amplification of DNA isolated from a second hair follicle from the same individual processed at the same time. This pattern shows loss of the larger loci typical of programmed degradation (1).

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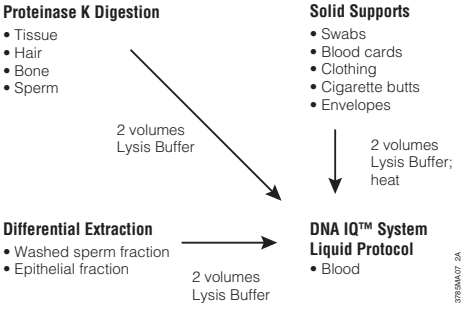

Figure 1. Flow diagram of the preprocessing and purification of DNA from a variety of sample types using the DNA IQ™ System. Different sample types are preprocessed and the DNA is purified by the standard DNA IQ™ System protocol.

Purification and sequence analysis of the hypervariable regions of mitochondrial DNA from shed hair shafts, which are generally rich in mitochondrial DNA, offer an alternative but less discriminating approach. The use of very high concentrations of Proteinase K and dithiothreitol (DTT), as described in the Tissue and Hair Extraction Kit Technical Bulletin #TB307, effectively digests hair shafts in one hour and allows for easy purification of the mitochondrial DNA using the DNA IQ™ System in either the manual or automated format. Figure 2C shows the amplification of the hypervariable region 2 of mitochondrial DNA extracted from a hair shaft using the Tissue and Hair Extraction Kit protocol followed by purification with the DNA IQ™ System.

EXTRACTION OF DNA FROM TISSUE SAMPLES

Tissue samples are fairly rare in forensic work, but they are encountered, especially in cases of arson or mass disasters. In some cases, a formalin-fixed, paraffin-embedded sample is the only available reference sample. The Tissue and Hair Extraction Kit was developed to process these difficult samples so that DNA can be easily purified with the DNA IQ™ System. Small amounts of fresh tissue, 1mg or less, give the best results and do not require mincing prior to Proteinase K digestion. Samples up to 25mg have been examined and may require mincing to help disrupt the tissue and extract the DNA.

Severely compromised samples, such as those exposed to elevated temperatures, present additional difficulties. Much of the DNA may be degraded and protein may be altered to the point of being resistant to Proteinase K digestion. In these cases, the best approach is to mince the sample to expose as much tissue as possible and to use larger amounts of tissue. Also, the digestion time should be increased as the additional protein in large samples will compete for DNA binding sites on the DNA IQ™ Resin and reduce DNA recovery.

Because the DNA IQ™ System isolates a consistent but limited amount of DNA, samples that contain very large amounts of microbial DNA will be less suitable, as the microbial DNA will compete with the small amount of human DNA for binding to the DNA IQ™ Resin. The use of three times the normal amount of DNA IQ™ Resin may reduce this effect. Figure 3A shows the profile of a 1mg tissue sample extracted with the Tissue and

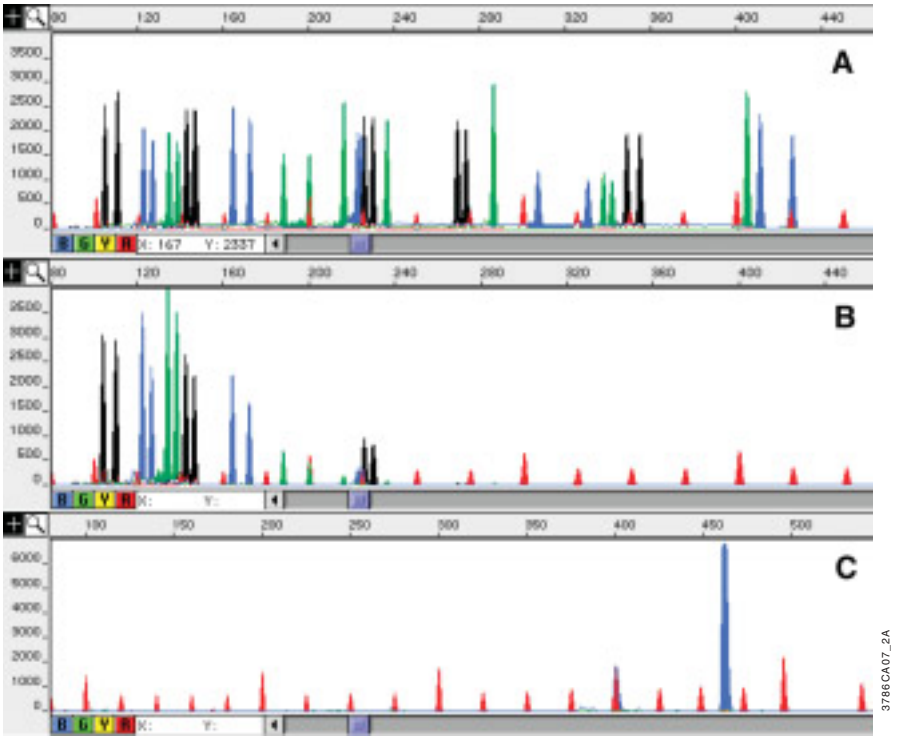

Figure 2. Analysis of DNA purified from hair follicles and hair shafts. Single hair follicles and shafts were preprocessed using the Tissue and Hair Extraction Kit (for use with DNA IQ™) and then DNA was purified using the DNA IQ™ System. DNA from follicles from the same individual was amplified with the PowerPlex® 16 System and analyzed on an ABI PRISM® 310 Genetic Analyzer. Panel A shows balanced peaks while Panel B shows loss of larger alleles, indicating the follicle was undergoing programmed DNA degradation. Panel C, DNA from a hair follicle was amplified with a fluorescently labeled primer set specific for the hypervariable region 2 of mitochondrial DNA and analyzed on the ABI PRISM® 310 Genetic Analyzer.

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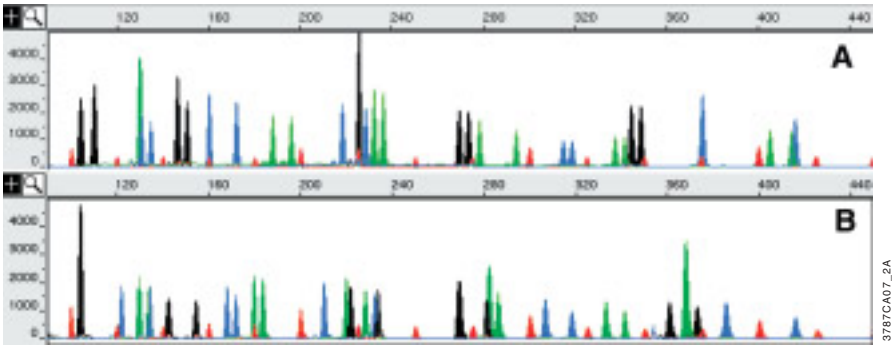

Figure 3. Analysis of DNA purified from tissue samples. One milligram of fresh tissue (Panel A) or a 10µm thin section of formalin-fixed, paraffin-embedded tissue (Panel B) was preprocessed using the Tissue and Hair Extraction Kit (for use with DNA IQ™) and then DNA was purified using the DNA IQ™ System. DNA was then amplified with the PowerPlex® 16 System and analyzed on an ABI PRISM® 310 Genetic Analyzer.

Hair Extraction Kit, followed by DNA purification with the DNA IQ™ System and amplification with the PowerPlex® 16 System.

Protocols for automated DNA isolation from tissue have been developed for Beckman Coulter's Biomek® 2000 Laboratory Automation Workstation and have been successfully used in forensic laboratories.

Formalin-fixed, paraffin-embedded tissue samples pose additional problems, as the DNA and protein are frequently cross-linked. Thin sections of this sample type can be easily processed and do not need to be deparaffinized when performing an overnight Proteinase K digestion. The addition of two volumes of DNA IQ™ Lysis Buffer will then dissolve the tissue sample. Amplification of DNA purified from formalin-fixed, paraffin-embedded tissue is frequently successful, but success is somewhat dependent on the fixation time. Doubling the extension time for each amplification cycle may help to increase inter-allelic balance if severe imbalance is initially observed. Figure 3B shows the genetic profile of DNA purified from a 10µm section of formalin-fixed, paraffin-embedded tissue.

EXTRACTION OF DNA FROM BONE

Although not a common sample type in most laboratories, bone is encountered frequently enough to warrant a standard protocol. Promega scientists have collaborated with an external laboratory to provide a protocol and custom reagents that allow for rapid purification of DNA from bone using a Proteinase K digestion in a decalcifying buffer, followed by a slight modification of the DNA IQ™ System protocol. The Incubation Buffer/Proteinase K Solution provided in the Tissue and Hair Extraction Kit will not efficiently extract DNA from bone samples.

The bone protocol has been tested on mass grave remains under various soil conditions, which can profoundly affect DNA quality. As with other sample types, the DNA IQ™ Resin removes very small DNA fragments, which are a cause of PCR inhibition, leading to improved amplification results. Figure 4 shows the genetic profile of DNA isolated from bone found at a mass grave site. Our custom bone reagents and the DNA IQ™ System were used for DNA isolation, and amplification was performed using the PowerPlex® 16 System.

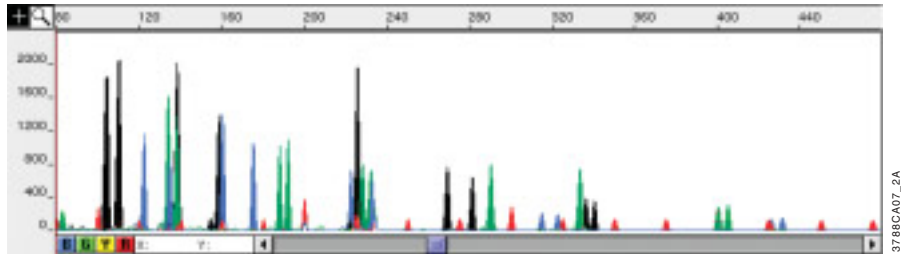

Figure 4. Analysis of DNA purified from bone. A bone fragment from a mass grave site was pulverized, preprocessed with the custom bone extraction reagents and DNA was purified with the modified DNA IQ™ System protocol. DNA was then amplified with the PowerPlex® 16 System and analyzed on an ABI PRISM® 310 Genetic Analyzer.

CONCLUSION

The Tissue and Hair Extraction Kit provides a procedure for dissolving tissue masses in either individual tubes or 96-well plates. To process bone samples, the related custom bone extraction reagents are required.

For additional information about available protocols, visit **www.promega.com/profiles/**

REFERENCES

1. Linch, C.A. *et al.* (1998) Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. *J. Forensic Sci.* **43**, 305–14.