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Application Notes

Genomic DNA Purification from Cigarette Butts and Buccal Swabs Using the DNA IQ™ System

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Abstract

We describe experiments examining the ability of the DNA IQ System to isolate DNA from cigarette butts and cigarette butt paper in the presence and absence of a proteinase K digestion. In addition we describe a protocol for DNA isolation from buccal swabs in the absence of a centrifugation step. These methods simplify the pretreatment steps for these samples and demonstrate that centrifugation of the lysate following initial incubation may not be required for successful DNA isolation. Elimination of centrifugation steps make complete processing of these sample types on automated workstations possible.

Introduction

The DNA IQ System (Cat.# DC6700) employs a novel technology to easily and efficiently prepare DNA samples for short tandem repeat (STR) analysis. The unique DNA IQ Resin eliminates PCR inhibitors and contaminants frequently encountered in a wide range of forensic sample types. This resin has a defined DNA binding capacity in the presence of excess DNA and isolates a consistent amount of DNA from a given sample type. In contrast, the system exhibits high DNA binding efficiency with samples containing less than approximately 10ng of DNA. Pretreatment with proteinase K is required to ensure complete lysis of cells in the interior of tissue masses, hair, and bone. The Tissue and Hair Extraction Kit (for use with DNA IQ) (Cat.# DC6740) and Promega's custom reagents for bone extraction were designed to preprocess these difficult samples prior to DNA purification using the DNA IQ System. This article describes experiments examining two sample types: cigarette butts and buccal swabs. Several protocols were utilized with these samples to successfully isolate DNA for STR analysis using the DNA IQ System. Data presented here suggests that variations on the standard protocols can provide DNA of sufficient purity to obtain complete STR profiles.

The *DNA IQ System—Small Sample Casework Protocol* #TB296 requires a 30 minute incubation at 95°C followed by a centrifugation of the sample through a DNA IQ Spin Basket (Cat.# V1221) for DNA isolation from buccal swabs. The modified protocol described here involves a longer incubation at 95°C and eliminates the centrifugation step. DNA isolated from buccal swabs using this modified protocol was successfully amplified with the PowerPlex® 16 System.

Protocols have been developed by Promega and by external laboratories to purify DNA from cigarette butts. These protocols involve a proteinase K treatment to enhance cell lysis in order to isolate sufficient DNA for STR analysis. One goal of these experiments was to determine if sufficient DNA could be isolated without the proteinase K treatment.

Preparation of Solutions and Buffers

The DTT and Incubation Buffer were supplied with the Tissue and Hair Extraction Kit (for use with the DNA IQ System). The Lysis Buffer and the 2X Wash Buffer were supplied with the DNA IQ System. The 1M DTT, Lysis Buffer and 1X Wash Buffer were prepared as described below. Additional information can be found in the *Tissue and Hair Extraction Kit (for use with the DNA IQ System) Technical Bulletin* #TB307, which is available at: www.promega.com/tbs

Preparation of 1M DTT

Five grams of DTT was dissolved in nuclease-free water to a final volume of 32.4ml.

Preparation of Incubation Buffers

The Incubation Buffer with proteinase K and Incubation Buffer without proteinase K were prepared as described in Table 1. Buffers were mixed gently and stored on ice. The final concentration of proteinase K was 1.8mg/ml.

Table 1. Preparation of Incubation Buffer with proteinase K Solution and Incubation Buffer without proteinase K Solution.

	With Proteinase K	Without Proteinase K
Incubation Buffer	800µl	900µl
1M DTT	100µl	100µl
Stock Proteinase K Solution	100µl	0µl

Preparation of Lysis Buffer

The prepared Lysis Buffer consisted of 1µl of 1M DTT and 100µl of DNA IQ Lysis Buffer. DNA isolation from cigarette butts and buccal swabs required 200–500µl of prepared Lysis Buffer per sample.

Preparation of 1X Wash Buffer

The 1X Wash Buffer was prepared by adding ethanol and isopropyl alcohol to the 2X Wash Buffer as shown in Table 2. The buffer was inverted to mix and stored at room temperature. The bottle was closed tightly to avoid evaporation.

Table 2. Preparation of 1X Wash Buffer.

	DNA IQ™ System (Cat.# DC6700,400 samples)	DNA IQ™ System (Cat.# DC6701,100 samples)
95-100% Ethanol	35ml	15ml
100% Isopropyl alcohol	35ml	15ml

DNA Purification from Cigarette Butt Papers and Filters

Cigarettes that were analyzed had been smoked by a male and a female individual and stored for 2 days in an airtight bag. A 5mm section was removed from the butt end of each cigarette. The paper and filter were separated, and each was cut into small pieces and placed in a 1.5ml microcentrifuge tube. Each sample received 200µl of freshly prepared Incubation Buffer with proteinase K or Incubation Buffer without proteinase K and was incubated at 56°C for 30 or 120 minutes, as indicated in Table 3. The entire contents of each tube was transferred to a DNA IQ™ Spin Basket (Cat.# V1221) seated in a clean 1.5ml tube. Samples were centrifuged at room temperature in a microcentrifuge for 2 minutes at maximum speed. Spin baskets were removed and discarded. Each tube received 400µl of prepared Lysis Buffer and 7µl of resuspended DNA IQ Resin. Samples were vortexed for 3 seconds at high speed and incubated at room temperature for 5 minutes. After brief vortex mixing at high speed, the tubes were placed in the MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5342). Separation occurred immediately, and the solution was removed and discarded without disturbing the pellet. For the first wash step, 100µl of prepared Lysis Buffer was added to each sample. The tubes were vortexed briefly at high speed and placed in the magnetic stand. The Lysis Buffer was removed and discarded without disturbing the pellet. A second wash was performed by adding 100µl of 1X Wash Buffer to each tube. Tubes were removed from the magnetic stand, vortexed for 2 seconds at high speed and returned to the magnetic stand. The 1X Wash Buffer was carefully removed and discarded. The wash steps were repeated twice more with 100µl of 1X Wash Buffer per wash for a total of four washes. During the final wash step, all of the 1X Wash Buffer was carefully removed, and the tubes were allowed to air-dry with the lids open in the magnetic stand for 5 minutes. Drying the DNA IQ Resin for more than 20 minutes can significantly reduce yield.

To elute the DNA, 25µl of DNA IQ Elution Buffer was added to each sample. Tubes were vortexed for 2 seconds at high speed and incubated at 65°C for 5 minutes. After a brief vortex mixing, the tubes were replaced on the stand to capture the DNA IQ Resin. The liquid phase, which now contains the DNA, was immediately transferred to a fresh tube. The DNA is stable at 4°C for short-term storage or at –20°C or –70°C for extended periods.

DNA Purification from Buccal Swabs

The heads of freshly prepared buccal swabs were removed and placed in 1.5ml tubes. Each tube received 250µl of prepared Lysis Buffer or enough to cover the swab. The tubes were closed, vortexed for 30 seconds and incubated for 2 hours at 95°C. Following incubation, the tubes were vortexed for an additional 30 seconds, and the swabs were removed. The DNA IQ Resin was vortexed for 10 seconds at high speed to completely resuspend the resin, and 7µl of resin was added to each tube. This DNA solution/resin mix was vortexed briefly and incubated at room temperature for 5 minutes. The tubes were briefly vortexed at high speed and placed in the magnetic stand. Separation occurred instantly. All liquid was removed and discarded without disturbing the resin. For the first wash, 100µl of prepared Lysis Buffer was added to each sample. The tubes were removed from the stand and vortexed for 2 seconds at high speed. The tube was replaced in the stand, and the Lysis Buffer was removed and discarded. Three additional washes were performed with 100µl of 1X Wash Buffer, as described above for the cigarette butt protocol. DNA was eluted in 50µl of DNA IQ Elution Buffer.

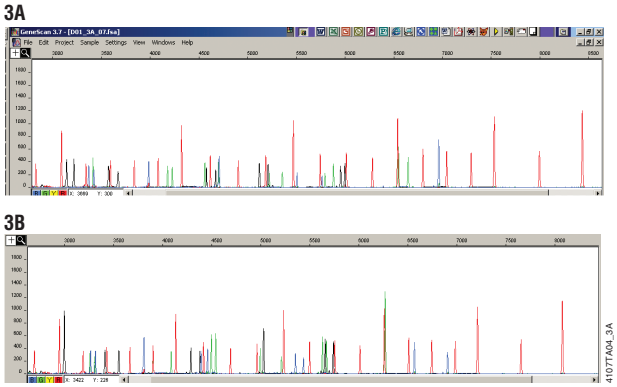

Figure 1. Analysis of DNA purified from cigarette paper with proteinase K treatment. DNA was isolated from cigarette papers that were incubated for 120 minutes in Incubation Buffer with proteinase K. The eluted DNA (1µl) was amplified with the PowerPlex® 16 System without prior quantification, and the amplified fragments were analyzed with an ABI PRISM® 3100 Genetic Analyzer. **Panel A.** Amplification results of DNA from sample 3A. **Panel B.** Amplification results of DNA from sample 3B.

Table 3. Amplification Results for DNA Samples Purified from Buccal Swabs and Cigarette Papers and Filters Using the DNA IQ™ System.

Sample Number	Sample Type	Gender	Incubation Time	Proteinase K Treatment	Centrifugation	Amplification Results
1A	Cigarette Paper	Female	120 minutes	No	Yes	Full profile
1B	Cigarette Paper	Male	120 minutes	No	Yes	Full profile
2A	Cigarette Filter	Female	120 minutes	Yes	Yes	Partial profile
2B	Cigarette Filter	Male	120 minutes	Yes	Yes	No profile
3A	Cigarette Paper	Female	120 minutes	Yes	Yes	Full profile
3B	Cigarette Paper	Male	120 minutes	Yes	Yes	Full profile
4A	Buccal Swab	Female	120 minutes	No	No	Full profile
4B	Buccal Swab	Male	120 minutes	No	No	Full profile
5A	Cigarette Paper	Female	30 minutes	No	Yes	Full profile
5B	Cigarette Paper	Male	30 minutes	No	Yes	Full profile

Analysis of Purified DNA Using the PowerPlex®16 System

DNA quantitation was not performed. To assess DNA quality, 1µl of DNA sample was added to each PowerPlex 16 reaction. Amplification was performed with the GeneAmp® PCR System 9600 Thermal Cycler, and amplified fragments were detected with the ABI PRISM® 3100 Genetic Analyzer and analyzed with the GeneScan® collection software, as described in the *PowerPlex 16 System Technical Manual* #TMD012.

Results with DNA from Cigarette Papers and Filters

Full STR profiles were obtained in all 6 samples from cigarette paper (see Table 3). Representative data are shown in Figure 1. Amplification of DNA isolated from the two cigarette filters resulted in a partial STR profile or no STR profile. The partial profile for sample 2A showed good amplification of the smaller alleles with loss of the larger alleles (data not shown). This can be an indication of too much template DNA in the amplification reaction. If that is the case, eluting the DNA in 50–100µl, as recommended in the *DNA IQ System—Databases Protocol* #TB297 and the *DNA IQ System—Small Sample Casework Protocol* #TB296, would be more appropriate. The absence of peaks with sample 2B suggests contamination or the absence of DNA in the cigarette filter. Formation of a gel upon heating cigarette filters has been reported by other laboratories, therefore it is possible that, after the heating step, the cigarette filter formed a gel that affected DNA extraction or amplification. Figure 2 shows STR profiles from samples 5A and 5B, which were incubated for 30 minutes in Incubation Buffer without proteinase K. DNA quality is highly dependent upon the quality of the original sample. Even though these samples were not stored under ideal conditions, it was surprising to obtain full profiles from samples that were incubated in Incubation Buffer without proteinase K for only 30 minutes. This illustrates the ability of the DNA IQ Lysis Buffer to extract sufficient amounts of DNA for amplification from cigarette paper.

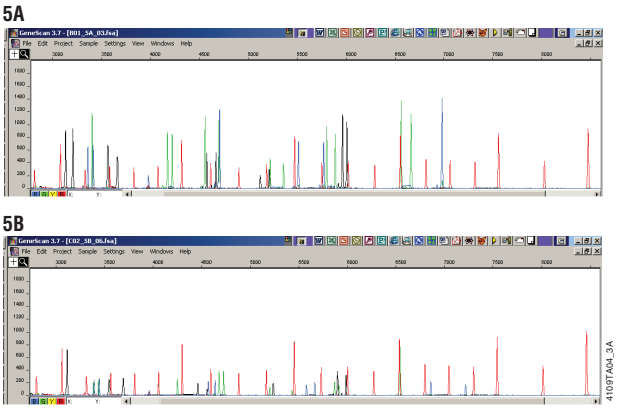

Figure 2. Analysis of DNA purified from cigarette paper without proteinase K treatment. DNA was isolated from cigarette papers that were incubated for 30 minutes in Incubation Buffer without proteinase K. The eluted DNA (1µl) was amplified with the PowerPlex 16 System without prior quantification, and the amplified fragments were analyzed with an ABI PRISM® 3100 Genetic Analyzer. **Panel A.** Amplification results of DNA from sample 5A. **Panel B.** Amplification results of DNA from sample 5B.

Results with DNA from Buccal Swabs

Both fresh buccal swabs provided full profiles with peak heights averaging 3,000RFU and excellent sister-allele balance (Figure 3). The elimination of centrifugations and DNA quantitation makes this protocol an attractive method for obtaining DNA from buccal swabs for the purpose of amplification. From an automation standpoint, this allows protocols that are entirely "walkaway".

Figure 3. Analysis of DNA purified from buccal swabs without centrifugation. DNA was isolated from buccal swabs that were incubated for 120 minutes in Incubation Buffer without proteinase K. The eluted DNA (1µl) was amplified with the PowerPlex® 16 System without prior quantification, and amplified fragments were analyzed with an ABI PRISM® 3100 Genetic Analyzer. **Panel A.** Amplification results of DNA from sample 4A. **Panel B.** Amplification results of DNA from sample 4B.

Summary

The DNA IQ™ System isolates high-quality DNA that does not require quantitation, even from samples that are commonly regarded as less reliable, such as cigarette butt paper. Amplification of this DNA using the PowerPlex 16 System gives full profiles. The standard protocol for DNA isolation from buccal swabs using the DNA IQ System involves a 30 minute incubation followed by centrifugation. By increasing the incubation time in Incubation Buffer without proteinase K to 120 minutes and including a vortex mixing before and after incubation, the DNA IQ System was capable of isolating sufficient DNA for amplification without centrifugation steps and without the use of DNA IQ™ Spin Baskets. This makes the protocol more amenable to an automated format.

DNA isolated from cigarette paper was more successful in amplification than DNA from the cigarette filter. The partial profile from sample 2A suggests that DNA isolation and amplification from cigarette filter is possible with further optimization.

Full profiles can be obtained from these samples with relative ease and without the need for quantitation when using the DNA IQ System with or without proteinase K treatment. However we recommend that a proteinase K treatment always be included when isolating DNA from cigarette paper and filter, as DNA quality and quantity may vary considerably and will depend on the age and quality of the starting material. For recommendations for the pretreatment of other sample types, see the *DNA IQ—Small Sample Casework Protocol* #TB296.

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