High-performance DNA from forensic samples using the EZ1 DNA Tissue Kit together with the EZ1 DNA Forensic Card protocols

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Introduction

Forensic science demands the efficient purification of small amounts of DNA from a wide range of low-volume and trace samples. Reproducible isolation of pure DNA is critical for successful downstream analyses. Sample throughput demands for forensic science continue to increase because of the introduction of DNA databanks and the expanding volume of casework samples. Automating time-consuming and labor-intensive sample prep can increase both reproducibility and throughput.

Materials and methods

Samples were mixed with 190 µl Buffer G2 and 10 µl Proteinase K. For absorbent samples, distilled water was added to a final sample volume of 200 µl. The samples were incubated at 56°C for 15 minutes with mixing. After incubation, any solid material was removed from the tubes using sterile forceps. Samples were processed using the BioRobot® EZ1 workstation, the EZ1 DNA Tissue Kit, and the Trace Sample Protocol encoded on the EZ1 DNA Forensic Card. Purified DNA was eluted in 100 µl water and quantified by absorbance (A_{260}) corrected for background (A_{320}).

Dried blood samples (4 paper discs, each of 3.25 mm diameter) were processed using the Dried Blood Protocol.

Results

The quantitative amplification and detection of small amounts of DNA is invaluable for case work analyses, especially of mixed samples. DNA purified from dried blood samples performed well in sensitive real-time PCR (Figure 1).

Multiplex PCR of STR loci allows for high discrimination typing of specimens and has become an invaluable tool in forensic analyses. STR results (Figure 2) show clean and easily interpretable quantitative STR profiles obtained using DNA from hair, demonstrating the high quality of DNA obtained using the BioRobot EZ1 system.

Table 1. Yields of DNA From a Range of Samples

Sample type	DNA yield (µg)
Buccal swabs	0.7–1.2
Cigarette butts	0.1–0.6
Dried blood on textile	0.2–0.7
Dried blood on paper	0.04–0.2
Hair	0.1–0.2

Reliable Real-Time Quantitative PCR

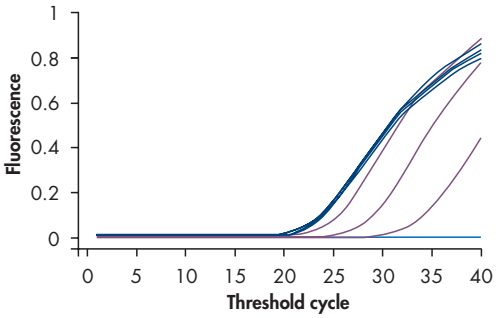
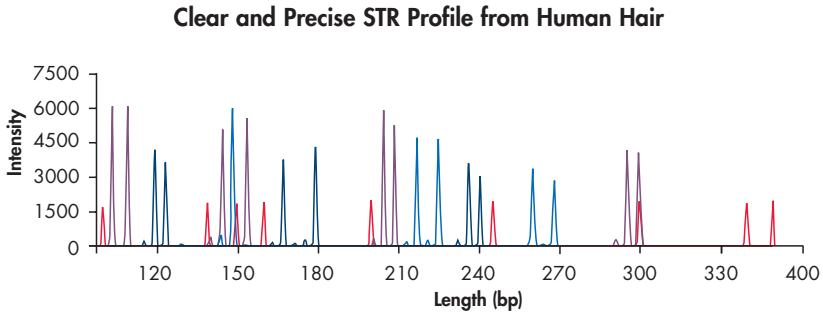


Figure 1 Reproducible real-time PCR using DNA from dried blood. Purified DNA (5 µl) was used in each of 6 duplicate 25 µl real time PCR of the human β-actin gene. Four disks contain approximately 5–15 µl blood. **Dark blue**: dried blood samples. **Violet**: β-actin control DNA (1, 0.1, and 0.01 ng). **Light blue**: negative control.

Figure 2 STR profile of a 3-day old human hair sample at 9 different loci. DNA was purified from hair root sections (2 cm) using the BioRobot EZ1 workstation with the EZ1 DNA Tissue Kit and the Trace Sample protocol. Purified DNA (15 ng) was used in a 25 µl PCR of nine STRs. The DNA samples were analyzed on the ABI PRISM® 310 Genetic Analyzer after co-amplification (28 cycles) of 9 short tandem repeat (STR) sequences plus the Amelogenin gene, using the AmpFLSTR® Profiler Plus® Kit. **Dark blue**: D3S1358/vWA/FGA; **Violet**: D8S1179/D21S11/D18S51 plus the amelogenin gene; **Light blue**: D5S818/D13S317/D7S820.



Conclusions

Clean and easily interpretable STR profiles and PCR amplifications were obtained for all analyses. Consistent high-performance in sensitive forensic applications illustrates the high-purity, integrity, and optimal concentration of DNA purified using the EZ1 DNA Tissue Kit and the BioRobot EZ1 workstation.

- DNA purified from a single human hair provided highly informative STR profiles
- DNA purified from dried blood performed consistently well in quantitative real-time PCR

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Tissue Kit (48)	48 Reagent Cartridges (Tissue), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2 ml), 50 Elution Tubes (1.5 ml), Buffer G2, Proteinase K	953034
EZ1 DNA Forensic Card	Pre-programmed card for BioRobot EZ1 Forensic Protocols	9015864
QuantiTect Probe PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR MasterMix, 2 x 2.0 ml RNase-free water	204343
BioRobot EZ1	Robotic workstation for automated purification of nucleic acids using EZ1 kits, installation, 1-year warranty on parts and labor	900705

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