A highly efficient method to concentrate DNA for forensic STR genotyping using DNAstable®

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Abstract

Forensic laboratories routinely use STR genotyping for identity testing of biological samples. However, forensic samples often contain low copy numbers of target DNA, making it difficult to obtain complete STR profiles. Increasing PCR amplification cycles is commonly performed to address this issue, but it can lead to stochastic effects that call into question the accuracy of the data analysis. To overcome this challenge, forensic scientists use a variety of techniques to concentrate samples in an attempt to increase the total amount of DNA available for amplification prior to the PCR step. These techniques include centrifugal filtration, sample dry-down and re-suspension, as well as precipitation with ethanol and polyethylene glycol. Such concentrating techniques have a variety of drawbacks, with the most critical being sample loss. We describe a technique for easily and effectively concentrating DNA from forensic samples using the commercially available reagent, DNAstable®. This method minimizes sample loss. We provide a case study from the Washington State Patrol Crime Laboratory, which has successfully used DNAstable to concentrate DNA for STR analysis. We show that this technique avoids many of the drawbacks associated with other DNA concentration strategies while providing accurate STR analysis results.

Methods & Materials

Preparation and Quantification of DNA Samples

DNA from eight reference swabs (Samples A - H) was extracted using a BioRobot EZ1® (Qiagen). Samples A - C and G - H were eluted in low TE (pH 8.0) buffer and samples D - F were eluted in denitized water. All samples were immediately stored in a -20°C freezer. Additionally, all extract samples were quantified using the Quantifiler® Human DNA Quantitation Kit (Life Technologies/Applied Biosystems) using a 7800 Real-Time PCR System (Life Technologies/Applied Biosystems).

Experiment 1

The purpose of the first part of this study was to examine differences in DNA recovery between Microcon® (Millipore) microfiltration and Vacufuge® (Eppendorf) vacuum centrifugation methods when DNAstable® (Biomatrica) is used to concentrate the DNA. The AmpliSTR® Yfiler® Amplification kit (Life Technologies) was used to demonstrate any differences in recovery.

Figure 1: Impact of DNAstable on DNA recovery quant values and Yfiler STR peak heights in samples in TE buffer or water.


Figure 2: Effect of various TE buffer concentrations on DNA recovery quant values and peak heights in DNA samples with or without DNAstable.

Set 1: 20 μL DNAstable in 5X TE. Set 2: 20 μL DNAstable in 10X TE. Set 3: No DNAstable in 5X TE. Set 4: No DNAstable in 10X TE. A. Average DNA recovery after dry down using Vacufuge vacuum centrifugation. B. Average Yfiler Plus STR peak heights after dry down using Vacufuge vacuum centrifugation.

Figure 3. Effect of increasing concentrations of DNAstable on peak heights using either AmpliSTR Yfiler Amplification kit or AmpliSTR IdentiFiler Plus Amplification kit. Each tube contained 0.6 ng DNA. Reference sample G & H were DNA eluted in TE buffer during purification. Set 1 was the control with 1X DNAstable in TE buffer. Set 2 contained increasing concentrations of DNAstable from 2X to 10X. Set 3 contained increasing concentrations of DNAstable from 2X to 10X, and were dried and reconstituted in water. A Peak heights determined using AmpliSTR Yfiler Amplification kit. B Peak heights determined using AmpliSTR IdentiFiler Plus Amplification kit.

Conclusions

The process of concentrating DNA to obtain more accurate and interpretable STR profiles warrants careful examination. Based on our results, the presence of DNAstable is beneficial when concentrating via vacuum centrifugation or using a microfiltration device. However, when TE approaches 10X, then a desalting method such as using a Microcon ultrafiltration device should be considered. This holds true for both AmpliSTR IdentiFiler Plus and AmpliSTR Yfiler amplification kits. With the issues that can arise from low levels of DNA, the use of optimal concentration methods needs to be carefully assessed to ensure the greatest chance of generating an accurate and robust profile.