

Ordering Information

Product	Contents	Cat. no.
MinElute PCR Purification Kit (50)	50 MinElute spin columns, buffers, collection tubes (2 ml)	28004
MinElute PCR Purification Kit (250)	250 MinElute spin columns, buffers, collection tubes (2 ml)	28006
QIAcube (110 V)	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	9001292*
QIAcube (230 V)	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	9001293†

* For use in North America.

† For use in rest of world.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Discover more about MinElute PCR purification and other QIAGEN products that aid forensic casework analysis at www.qiagen.com/goto/forensics.

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Enhancing forensic analysis of trace DNA using the MinElute[®] PCR Purification Kit*

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The QIAGEN[®] MinElute PCR Purification Kit improves forensic casework analysis of trace DNA samples, which often result in poor-quality profiles. This post-PCR process concentrates DNA and removes unwanted PCR components, thus increasing amplicon uptake during electrokinetic injection.

Introduction

Improving sensitivity in trace DNA casework applications by post-PCR purification is becoming increasingly important. Success rates using standard forensic methods to obtain intelligible profiles from low-level DNA samples range around only 30–50%. Efforts to enhance sample processing usually focus on the preanalytical or pre-PCR steps of the analysis, yet there can be tremendous value in post-PCR method optimization. A number of techniques have been described for post-PCR purification including filtration, enzyme-mediated digests, increased cycle number amplification, and increased electrokinetic injection times. These methods, however, can often lead to allele dropout and increased background, which make interpretation of results difficult. Post-PCR purification using the MinElute PCR Purification Kit is an alternative means to analyze low amounts of DNA. Requiring approximately 15 minutes, this is a quick and simple procedure that is easily incorporated into existing protocols.

This study demonstrates that post-PCR purification using the MinElute PCR Purification Kit reduces background, improves allele detection, boosts analysis sensitivity, and minimizes artifacts.

Materials and methods

DNA was isolated from 2 previously typed blood samples on cotton cloth and FTA[®] paper. The resulting isolates were serially diluted to generate DNA input concentrations as low as 5 pg. Fifteen single tandem repeat (STR) loci and the sexing locus Amelogenin were amplified in a standard ►

* Data excerpted from Smith, P.J. and Ballentyne, J. (2007) Simplified low-copy-number DNA analysis by post-PCR purification. *J. Forensic Sci.* **52**, 1556. Published with permission from Wiley-Blackwell.

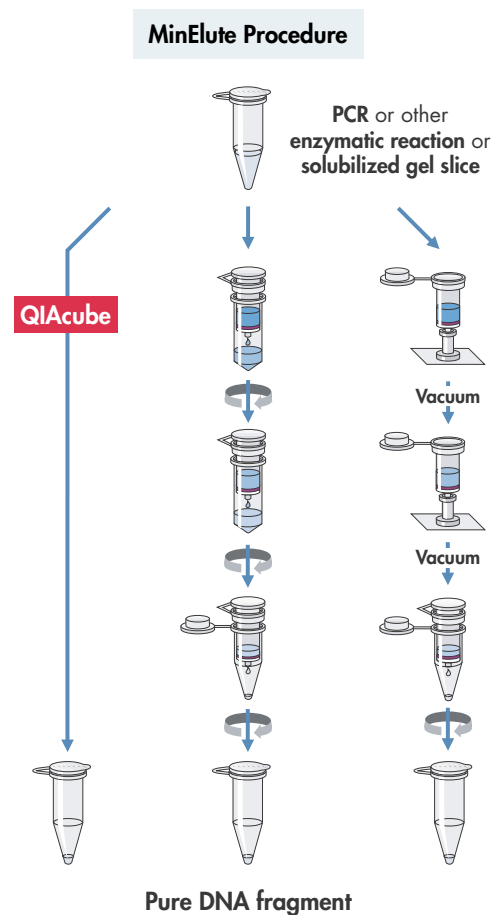


Figure 1. The MinElute procedure. Post-PCR purification of the amplified product using the MinElute PCR Purification Kit can be performed in approximately 15 minutes using either a microcentrifuge or a vacuum manifold. Alternatively, the procedure can be automated on the QIAcube[®].



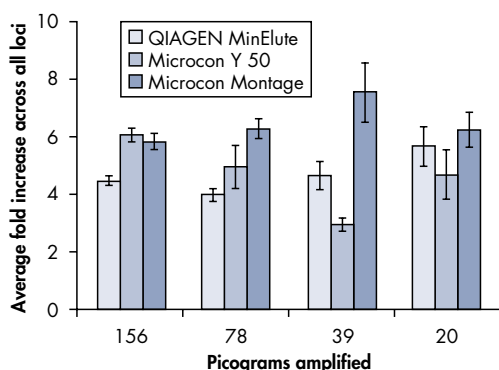


Figure 2. Compared efficacy of different post-PCR purification methods. Increased allelic signal intensity of two filtration-based methods (Microcon-50 and Microcon Montage PCR[®]) compared to that achieved with the QIAGEN MinElute PCR Purification Kit. Although Microcon Montage PCR yielded greater signal enhancement, both filtration-based methods produced off-ladder calls and other artifacts, which were absent in the profiles generated after purification with the MinElute PCR Purification Kit.



Figure 3. QIAcube. The QIAcube enables full automation of QIAGEN spin-column kits, making sample processing with the MinElute PCR Purification Kit fast and efficient.

28-cycle PCR and the products were then purified using the MinElute PCR Purification Kit (Figure 1), 2 filtration-based methods, and an enzyme-mediated digest method (1). The purified product from each method and unpurified samples were separated and detected by electrophoresis, and evaluated via a comparison of profile integrity and relative fluorescent signal intensity. Samples purified with the MinElute PCR Purification Kit were studied in more detail to determine threshold input of template DNA and to optimize electrokinetic injection volume.

Results

Allelic signal intensity was enhanced by the MinElute PCR Purification Kit and both filtration-based methods, with average increase compared to unpurified samples ranging between 3- and 8-fold (Figure 2). The enzyme-based method resulted in poor-quality data, including extraneous peaks and decreased relative fluorescence intensity. Nonspecific products resulting in off-ladder allele calls were present in most sample replicates purified by filtration-based methods. These artifacts were absent from samples processed with the MinElute PCR Purification Kit and the resulting DNA profiles exhibited significantly reduced background and clear allele signals (Figure 4). Subsequent experiments with the MinElute PCR Purification Kit yielded full DNA profiles from samples as small as 78 pg and partial profiles from as little as 5 pg DNA (Table 1). Injecting the entire purified product can significantly improve profile integrity (Table 1) and boost allelic signal intensity up to 19 times, though this enhancement must be balanced with slightly increased stutter and spurious peaks that result from increased injection volume.

Table 1. Alleles detected after purification with the MinElute PCR Purification Kit

PCR product	156 pg	78 pg	39 pg	20 pg	10 pg	5 pg
1.5 µl unpurified	30	15–25	5–9	0–1	0	0
1.5 µl purified	30	30	27–28	9–19	5–13	0–5
Entire purified product	N/D	30	30	30	22–28	12–27

Discussion

Ease of use, high eluate purity, and significant improvement of signal intensity make the MinElute PCR Purification Kit highly suited for enhancing forensic analysis of trace DNA samples. This simple method is easily incorporated into existing protocols and a highly efficient workflow can be achieved via automation on the QIAcube (Figure 3). MinElute columns have a uniquely designed silica membrane that binds DNA while impurities, such as unincorporated nucleotides (dNTPs), salts, and primers, are washed away. DNA that is ready to use can then be eluted in as little as 10 µl elution buffer. The removal of these unwanted amplification components favors

amplicon input during electrokinetic injection and this leads to increased fluorescent signal intensity and reduced background. The data presented confirm that post-PCR processing with the MinElute PCR Purification Kit is superior to enzyme-mediated digests and filtration-based purification methods, as the latter produce artifacts that make interpretation of resulting DNA profiles difficult. Subsequent studies in this and another lab have demonstrated that standard 28-cycle PCR combined with post-PCR processing with the MinElute PCR Purification Kit significantly improves profiles from extended interval DNA samples in analysis of sexual assault cases (2) and achieves equal or better results than increased cycle number procedures such as the “low-copy-number” method (3).

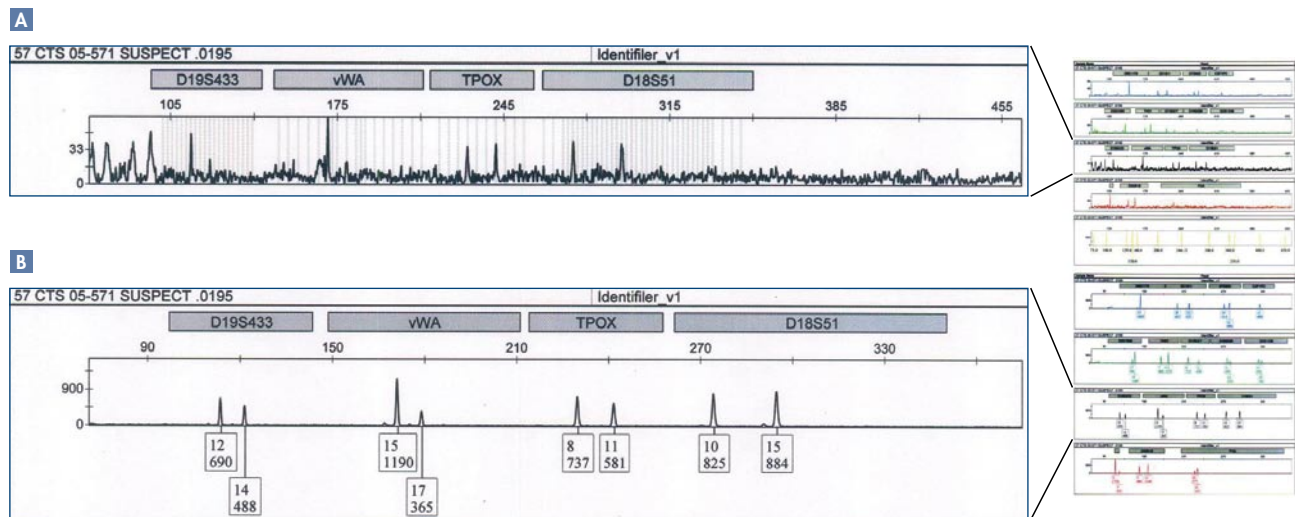


Figure 4. Enhanced sensitivity in STR analysis. STR analysis was carried out using **A** 20 pg DNA without post-PCR purification and **B** 20 pg DNA purified after PCR using the MinElute PCR Purification Kit. (Data kindly provided by Pamela Smith, Texas Department of Public Safety, Corpus Christi, TX, USA.)

Conclusions

- Post-PCR processing using the MinElute PCR Purification Kit is a rapid and straightforward method for enhancing detection sensitivity in the analysis of trace DNA.
- Enhanced STR signal and reduced signal-to-noise ratio is achieved by concentrating the sample and effectively removing PCR components that interfere with downstream analysis.
- DNA profiles produced by standard 28-cycle PCR coupled with purification with the MinElute PCR Purification Kit exhibit strong, easily interpreted allele signals without artifacts.
- Use of the MinElute PCR Purification Kit, especially in conjunction with the QIAcube, standardizes sample processing, thereby improving consistency of results and reliability of profiles.

References

1. QIAGEN (2007) *User-developed protocol: Forensic post-PCR purification protocol using the MinElute® PCR Purification Kit.*
2. Mayntz-Press, K.A., Sims, L.M., Hall, A., and Ballantyne, J. (2008) Y-STR profiling in extended interval (≥ 3 days) postcoital cervicovaginal samples. *J. Forensic Sci.* **53**, 342.
3. Forster, L., Thomson, J., and Kutranov, S. (2008) Direct comparison of post-28-cycle PCR purification and modified capillary electrophoresis methods with the 34-cycle “low copy number” (LCN) method for analysis of trace forensic DNA samples. *Forensic Sci. Int. Genet.* **2**, 318.