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Introduction:

This study was conducted to demonstrate the benefits of including the QIAGEN MinElute[®] PCR Purification Kit into the analysis scheme on forensic samples containing low quantities of DNA.

The QIAGEN MinElute[®] PCR Purification Kit uses a silica membrane to bind DNA fragments ranging in size from 70 bp to 4 kb. While the DNA is bound to the membrane, impurities such as unwanted primers, salts, enzymes, unincorporated nucleotides, dyes, oils, and detergents flow through the column. This causes an increase in the signal from the amplified DNA which in turn increases the peak height of the resulting data allowing previously low level samples to reach to a callable level. Removal of these impurities ensures that more DNA is injected during the electrokinetic injection on the instrumentation, thus increasing the fluorescent signal intensity.

Method:

- Two separate known human male DNA standards were prepared utilizing a standard organic extraction method in conjunction with the Millipore Microcon[®] 100 centrifugal filter device.
- Serial dilutions were prepared from DNA extracts at the following concentrations: 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078 ng.
- The samples were quantitated using the Applied Biosystems Quantifiler[®] Human Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. The results were normalized with NIST quantitation standards.
- Samples were amplified on an Applied Biosystems GeneAmp[®] PCR 9700 thermal cycler following manufacturer's specifications. Applied Biosystems' AmpflSTR[®] Profiler Plus ID[®] kit, Cofiler[®] kit, Identifier[®] kit, MiniFiler[™] kit, and the Yfiler[®] kit and Promega's PowerPlex[®] 16 system, PowerPlex[®] Y system, and the PowerPlex[®] S5 system.
- The samples were then separated and detected using an Applied Biosystems 3130xI Genetic Analyzer using manufacturer's recommendations:
 - Applied Biosystems kits: 3kv, 10 sec injections. 8.7 µl Formamide, 0.3 µl GS 500, 1 µl of sample
 - Promega kits: 3kv, 10 sec injections, 1200 sec run time. 9.5 µl Formamide, 0.5 µl ILS 600, 1 µl of sample
- Data were analyzed using GeneMapper[®] ID Software v3.2 using a threshold of 75 RFUs.
- Amplified samples from these dilutions which had data falling below the 75 RFU threshold were purified using the QIAGEN MinElute[®] PCR Purification Kit. A total of 4 washes were performed manually on each sample.
- After the post PCR clean up procedure the samples were then run on the Applied Biosystems 3130xI Genetic Analyzer under the same conditions. All purified amplification product, which ranged between 9 and 10 µl, was consumed.

¹National Forensic Science Technology Center

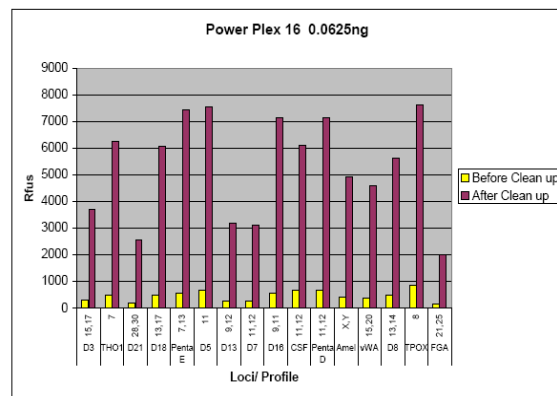
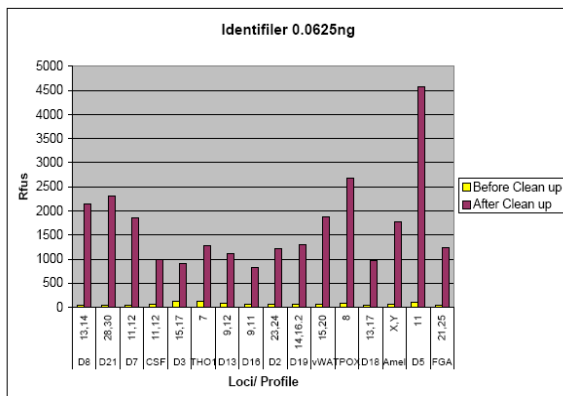
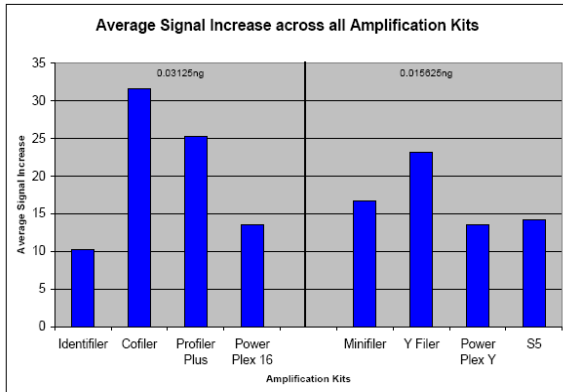
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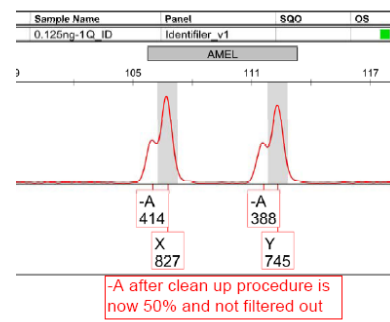
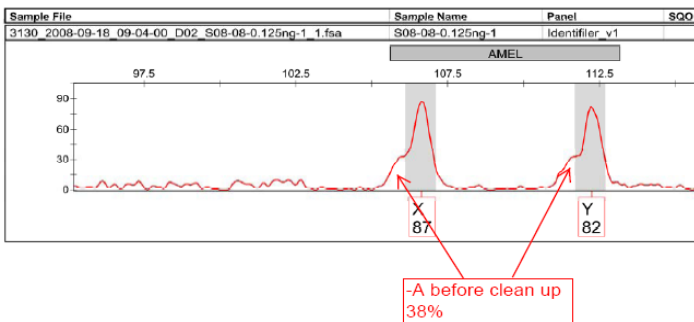
Study funded through Cooperative Agreement 2008-MU-MU-K003 with the National Institute of Justice, the research, development and evaluation agency of the U.S. Department of Justice. The NFSTC manages the Forensic Technologies Center of Excellence (FTCE) through this agreement.

Results and Discussion:

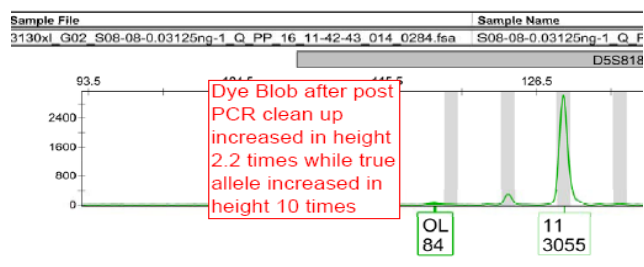
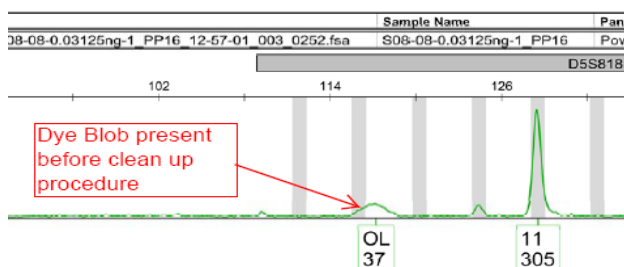
- After Post PCR clean up there was a signal increase from an original peak height of 50 RFUs to a minimum of 300 RFUs in every kit tested.



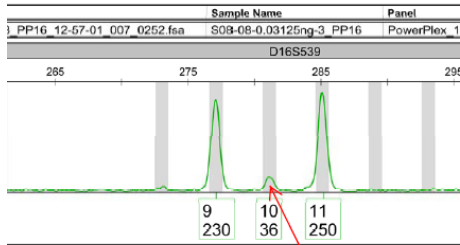
- Minus A (-A) present prior to clean up increased after clean up in a greater proportion than that of the allele. If no -A was present initially, then there was no -A after clean up.



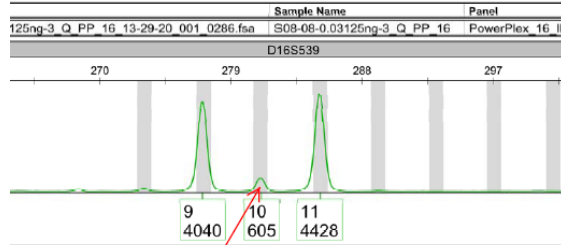
- Dye blobs did not increase in proportion to true peaks. In some instances the dye blobs were reduced in size and were even removed after clean up.



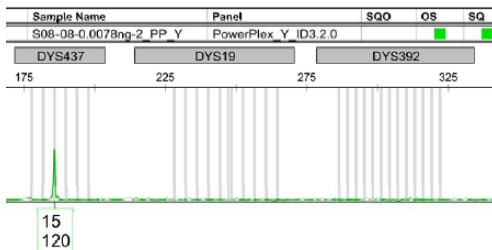
4. Any issues with the amplification of low level DNA samples such as drop in, high stutter, preferential amplification, and differential amplification were still present after clean up.



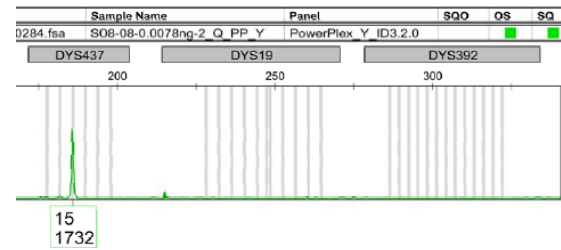
Elevated stutter before clean up



Elevated stutter still present after clean up

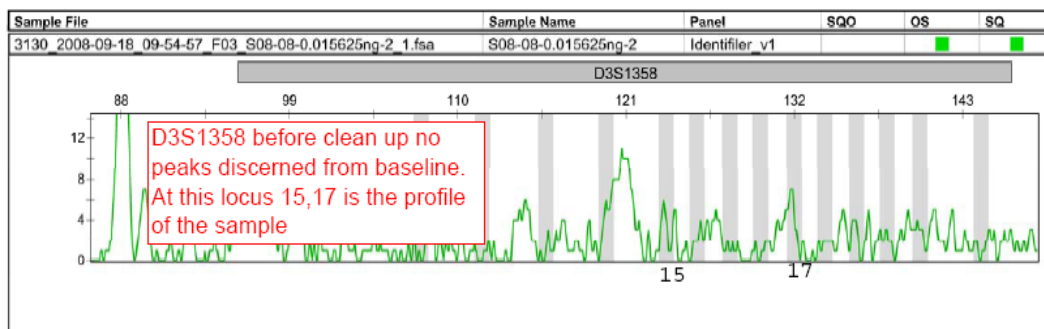


Drop out of data from Loci DYS19 and DYS392 before clean up procedure

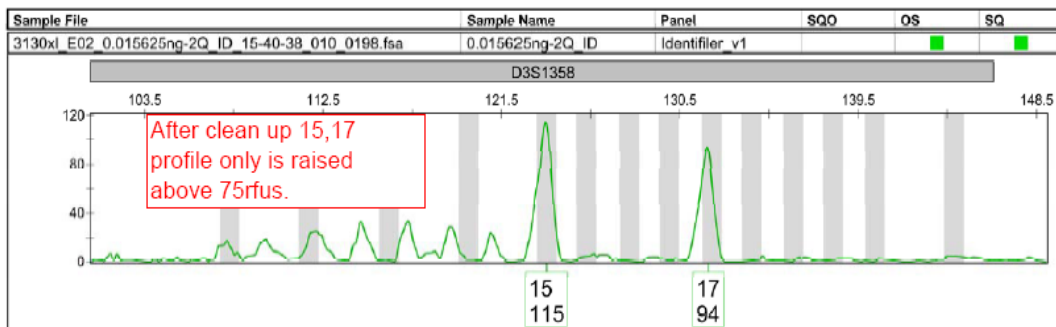


Drop out of data still occurs at the same loci after clean up procedure

In some instances peaks that were not originally discernable from the baseline were clearly identified after the purification process.

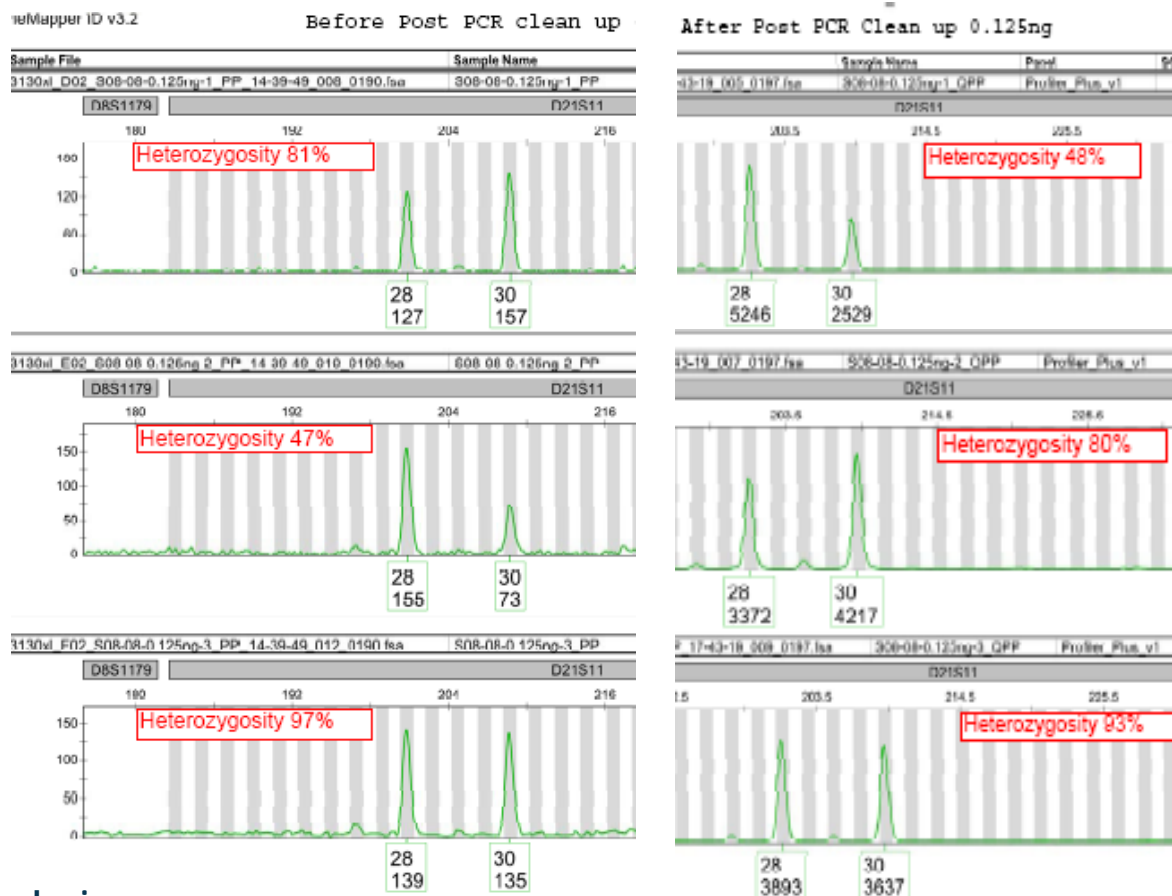


D3S1358 before clean up no peaks discerned from baseline. At this locus 15,17 is the profile of the sample



After clean up 15,17 profile only is raised above 75rfus.

5. In some instances there were fluctuations in heterozygosity when comparing samples pre and post clean up. There was no discernable trend or cause for these fluctuations.



Conclusions:

This method of post PCR clean up consistently increased the signal of samples which were previously at levels too low to be characterized at a threshold of 75 RFUs. Laboratories should perform appropriate validation studies to establish interpretation guidelines to account for the issues which occur with amplification of low level DNA samples.

Benefit to the Forensic Community:

Crime laboratories have seen an increase in the submission of requests for analysis on evidentiary items with low quantities of DNA. This study demonstrated that the QIAGEN MinElute® PCR Purification Kit consistently increased the fluorescent signal with the eight evaluated commercial STR amplification kits. This purification kit can be integrated into the laboratory process with little effort for method validation and at minimal cost. Integration of the QIAGEN MinElute® PCR Purification Kit into the DNA analysis procedure is a simple, cost effective method that can be easily implemented by a crime laboratory to increase the overall sensitivity of their DNA analysis methods.

References:

Smith, P.J., Ballantyne J. "Simplified Low-Copy-Number DNA Analysis by Post-PCR Purification." *Journal of Forensic Science*, 52(4). (2007):820-829

MinElute Handbook [Internet]. [updated 2008 Mar]. QIAGEN. [cited 2008 August 14]. Available from:

<http://www1.QIAGEN.com/Products/DNACleanup/GelPcrSiCleanupSystems/MinElutePCR PurificationKit.aspx#Tabs=t2>

The most current user and technical manuals for each kit were referenced in this study. Manuals procured from www.appliedbiosystems.com