National Forensic Science Technology Center
7881 114th Avenue North, Largo, FL 33773
Phone (727) 549-6067 Fax (727) 549-6070
www.NFSTC.org

Forensic Technology Testing & Evaluation Report Form

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Project Information

Title: Effects of using the QIAGEN MinElute® PCR Purification Kit on Eight Commercially Available STR Kits
Evaluation Type: Post-PCR Purification
Stakeholder (s): NFSTC
Start Date: August 2008
End Date: January 2009

Manufacturer Information:

Manufacturers: Applied Biosystems, Promega and QIAGEN Inc.

Abstract:

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

The study was designed to evaluate the similarities and differences in capillary electrophoresis signal detection when using the QIAGEN MinElute® PCR Purification Kit on amplified DNA obtained from commonly used short tandem repeat (STR) commercial amplification kits. The STR amplification methods used in this study were the Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, Minifiler™ kit and Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system and PowerPlex® S5 system.

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. Removal of these impurities ensures that more DNA is injected during the electrokinetic injection on the instrumentation, thus increasing the fluorescent signal intensity.

All single source samples were extracted using a standard organic extraction method and quantitated using the Applied Biosystems Quantifiler® Human DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. 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 and amplified with each STR multiplex following the manufacturer's specifications using an Applied Biosystems GeneAmp® PCR 9700 thermal cycler. A portion of the amplified DNA from these dilutions was purified using the QIAGEN MinElute® PCR Purification Kit. Both purified and non-purified samples from each of the dilutions were separated and detected using the Applied Biosystems 3130*xl* Genetic Analyzer. The data were analyzed using GeneMapper® ID Software v3.2 using a threshold of 75 RFU. The fluorescent signals from both purified

and non-purified samples were compared for all eight STR multiplexes to assess the change in fluorescent signal, stutter ratio, heterozygosity and baseline noise.

Preliminary results of low-level samples have shown increased signal levels after cleanup using the QIAGEN MinElute® PCR Purification Kit. This has consistently been shown during preliminary trials using Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, and Identifiler® kit. Results for the dilution series using all of the above listed amplification kits will be presented in the poster.

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.

Keywords: QIAGEN, QIAGEN MinElute® PCR Purification Kit, low-level DNA, polymerase chain reaction, STR, forensic

Product Specifications

Product Name: QIAGEN MinElute® PCR Purification Kit, Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® Kit, Identifiler® kit, Minifiler™ kit, and Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system and PowerPlex® S5 system

Storage Conditions: Refrigerator/Freezer

Number of reactions:

- Applied Biosystems' AmpfℓSTR® Profiler Plus® kit – 100 reactions
- Applied Biosystems' AmpfℓSTR® Cofiler Plus® kit – 100 reactions
- Applied Biosystems' AmpfℓSTR® Identifiler® kit–200 reactions
- Applied Biosystems' AmpfℓSTR® Minifiler™ kit – 100 reactions
- Applied Biosystems' AmpfℓSTR® Yfiler® kit – 100 reactions
- Promega PowerPlex® 16 system – 100 or 400 reactions
- Promega PowerPlex® Y system – 50 or 200 reactions
- Promega PowerPlex® S5 system – 100 or 400 reactions

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Evaluation

Objective(s):

To evaluate the effects of using post-PCR purification to boost signal of low-level DNA samples using eight commercially available STR amplification kits commonly used in U.S. forensic laboratories.

Reagents and consumables:

- Buccal swabs from 2 donors
- Phenol:chloroform:Isoamyl alcohol (25:24:1)
- DTT (Dithiothreitol)
- Proteinase K (10 ng/ul)
- Applied Biosystems Human DNA Quantifiler Kit
- Applied Biosystems Human Male Y Quantifiler Kit
- TE Buffer
- 16 capillary array
- POP4
- Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® Kit, Identifiler® kit, Minifiler™kit, and the Yfiler® and Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system
- 10x Genetic Analyzer Buffer
- Dye Set G5 Spectral/ matrix standards
- Dye Set F Spectral/ matrix standards
- Internal Lane Sixe Standards
- Formamide
- QIAGEN MinElute® PCR Purification Kit

Equipment:

- AB 7500 RT PCR
- AB 9700 Thermal cycler
- AB 3130*xl* Genetic Analyzer

Experimental Design:

1. Collect DNA from two individuals
2. Perform organic extraction in conjunction with Millipore Microcon 100
3. Quantitate using Quantifiler Human and Quantifiler Y Kits on the 7500. Normalize the results with NIST Quantitation standards.
4. Calculate, combine and dilute samples to generate a large volume of at least 1 ml to obtain the following target concentrations for amplification
    - Single source dilutions:
        i. 1.0 ng

ii. 0.50 ng
iii. 0.25 ng
iv. 0.125 ng
v. 0.0625 ng
vi. 0.03125 ng
vii. 0.015625 ng
viii. 0.0078 ng

5. Quantitate after dilutions using Quantifiler Human Kit on the 7500 and normalize with the NIST standards

6. Amplify the appropriate amount of each sample with each of the following kits: Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, Minifiler™ kit and Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system and PowerPlex® S5 system using manufacturer's recommended amplification conditions

- Profiler Plus/Cofiler (50ul reaction) cycling parameters 95°C hold for 11 mins; 30 cycles 94°C for 1 min, 60°C for 1 min and 72°C for 1 min; and a 60°C final extension for 45 mins.
- Identifiler (25 ul reaction) cycling parameters: 95°C hold 11 mins; 30 cycles 94°C for 1 min, 60°C for 1 min and 72°C for 1 min; and a 60°C final extension for 45 mins.
- Minifiler (25 ul) reaction cycling parameters: 95°C hold (11 min); 30 cycles 94°C (20 sec), 59°C (2 min)and 72°C (1 min); and a 60°C (45min) final extension
- YFiler (25 ul reaction) cycling parameters: 95°C hold(11 min); 30 cycles 94°C (1 min), 61°C (1 min) and 72°C (1 min); and a 60°C (80 min) final extension.
- PowerPlex 16 (25 ul reaction) cycling parameters: 95°C hold (11 min); 96°C hold (1 min); 10 cycles ramp 100% to 94°C (30 sec), ramp 29% to 60°C (30 sec) and ramp 23% to 70°C (45 sec); 22 cycles ramp 100% to 90°C (30 sec), ramp 29% to 60°C (30 sec) and ramp 23% to 70°C (45 sec); and a 60°C (30 min) final extension.
- PowerPlex Y 25 ul reaction cycling parameters: 95°C hold (11 min); 96°C hold (1 min); 10 cycles ramp 100% to 94°C (30 sec), ramp 29% to 60°C (30 sec) and ramp 23% to 70°C (45 sec); 22 cycles ramp 100% to 90°C (30 sec), ramp 29% to 58°C (30 sec) and ramp 23% to 70°C (45 sec); and a 60°C (30 min) final
- PowerPlex S5 (25 ul reaction) cycling parameters: 96°C hold (2 min); 30 cycles 94°C (30 sec), 60°C (2 min) and 72°C (90 sec); and a 60°C (45 min) final extension

7. Prepare and run samples on 3130*xl* using manufacturer's recommended running conditions on half of the prepared amplified product
8. Applied Biosystems kits: 3 kv, 10 sec injections 8.7 ul Formamide, 0.3 ul GS 500, 1 ul samples.
9. Promega kits: 2 kv, 10 sec, 1200 sec run time 9.5 ul Formamide, 0.5 ul ILS 600 1 ul sample
10. Analyze data using Genemapper ID v 3.2. Determine for each kit the limit of sensitivity. This will be defined as the point at which loci drop below 100 RFUs.
11. Samples that have loci dropping below 100 RFUs will then be cleaned up using the QIAGEN MinElute® PCR Purification Kit and run on the 3130*xl* using the same run conditions as before. The only difference being that the entire cleaned up sample is added to run plate – this ranged from 9 to 10 ul of sample.

12. Analyze data using the Genemapper ID v3.2 and compare data before cleanup with a focus on changes in fluorescent signal, heterozygosity and amplification artifacts.

Findings

Strengths

1. Across all kits there was a signal increase from 50 RFUs to 300 RFUs and higher. In the majority of cases the signal increase was larger.
2. Dye blobs did not increase in signal in the same proportion as the true allele peaks did. Dye blobs had a smaller signal increase, were decreased or were completely removed after post-PCR clean up.
3. Any effects of low-level sample amplification were the same before and after the purification step. The use of post-PCR cleanup did not introduce any deleterious effects to the low-level samples that were not there before.
4. The addition of the post-PCR cleanup step did not introduce any contamination or allelic drop in.

Opportunities for Improvement

1. –A before purification increased more than the signal increase of the main allele peaks after purification.
2. Heterozygosity in some kits fluctuated after purification. There was no clear trend; in some instances heterozygosity increased; in some it decreased; and in some cases, it remained the same. This may be a consequence of electrokinetic injection.

Recommendations

1. Each laboratory should conduct their own validation to determine the quantity at which the purification yields the best results since instrument sensitivity will also affect the end results.
2. The purification step can be easily implemented and takes minimal time. However, it cannot be done across all samples since samples with high quantities will give off-scale results.
3. Laboratories should also take into account that low-level samples that have peaks mixed in with baseline will be raised to callable levels.
4. The issues surrounding the amplification of low-level DNA samples (allelic dropout, locus imbalance and stochastic effects, etc.) are still a concern when interpreting data after using the post-PCR cleanup method. Validations studies should be conducted so that laboratories establish appropriate data interpretation guidelines.