Forensic Technology Testing & Evaluation Report

Forensic Technologies Center of Excellence (FTCoE)

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Final Report Date: 11/11/09

Project Title:	**Projected Start Date:**
Comparing the Effectiveness of Varying the Amount of Manual Buffer Washes to Use of the QIAcube™ When Performing the QIAGEN MinElute® Post-PCR Clean Up	**6/8/2009**
Evaluation Type: (Instrument, kit, procedure, product-to-product comparison study, etc.)	**Projected End Date:**
Instrument and Procedure	**6/30/2009**
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Evaluation Overview

Evaluation Summary:

This project is a continuation of a recently completed study using the QIAGEN MinElute Post-PCR cleanup to purify and concentrate amplified DNA on eight of the most common commercially available STR kits.

In that study, the evaluation team used four washes, the maximum number of washes recommended by the manufacturer. In this study, the samples were put through the post-PCR cleanup process and the number of washes was varied from one to four to determine the ideal number of washes that would yield the maximum result.

The sample was also run on the QIAcube, a fully automated purification system, to determine how effective this robotic equipment is in performing the wash procedure. In addition, a subset of samples did not undergo the cleanup procedure and all of the amplified product was placed on the 3130xl Genetic Analyzer. This provided data to confirm whether use of the MinElute system is necessary to increase signal. The results were then compared to determine which method produces the highest boost in signal and which method is the most efficient. In addition, the volume of sample that was cleaned up and added to the Genetic Analyzer after clean up was varied since some lab systems are not allowed to consume all of their amplified product.

The comparison was performed using only one Identifiler® STR kit at a concentration which produced peak heights in the 50 RFU range and showed no stochastic effects due to low-level DNA amplification.

Experimental Design

Procedural outline for evaluation:

1. The QIAcube was checked for contamination: Since contamination is a concern when dealing with amplified DNA, the QIAcube was first evaluated to ensure it did not introduce any contamination. The following steps were performed to check for contamination:

    1.1. Samples with DNA were run on the QIAcube, then blanks were processed in the QIAcube.

    1.2. The blanks were analyzed on the 3130xl Genetic Analyzer to evaluate if any residual DNA was transferred from the previous samples. This indicated whether contamination could

occur from one run to the next.

1.3. The QIAcube was then used to process alternating samples that did and did not contain DNA. The samples without DNA were then analyzed on the 3130xl. This step indicated whether any DNA was transferred from one sample to the other during a run.

2. Once it was determined the QIAcube exhibited no sign of carry-over contamination, the following evaluation commenced:

2.1. A concentration was determined which yielded a profile that exhibited peak heights in the 50 RFU range or showed no stochastic effects.

2.2. Triplicate sets of this sample were analyzed on the 3130xl to determine the baseline.

2.3. Each triplicate set was processed using the MinElute cleanup procedure.

2.4. The 1st set underwent 1 wash during the MinElute cleanup.

2.5. The 2nd set underwent 2 washes during the MinElute cleanup.

2.6. The 3rd set underwent 3 washes during the MinElute cleanup.

2.7. The 4th set underwent 4 washes during the MinElute cleanup.

2.8. The 5th set underwent the MinElute clean up using the QIAcube.

2.9. The 6th set did not undergo any washes; all of the amplified DNA was placed on the instrument with no cleanup.

2.10. Sets of samples were also processed on the QIAcube and the volumes were adjusted. The volumes of amplified product being added to the cleanup system were varied and the volume of cleaned up product being added to the Genetic Analyzer was varied.

3. 5ul and 10ul of amplified product was added to the MinElute clean up. The samples were processed in triplicate and all of the resulting clean up product was added to the Genetic Analyzer. In addition, the total volume of amplified product was added and volumes of 1ul, 5ul and 10ul were added to the Genetic analyzer. This was also processed in triplicate.

4. To determine if the minor peak could be brought up to a level where a full profile could be achieved, two samples containing a 1:15 mixture and a 1:20 mixture were put through the QIAcube in triplicate.

Notes: All triplicate set samples were heated and snap-cooled before being run on the Applied Biosystems™ 3130xl Genetic Analyzer, and evaluated using Genemapper® ID to determine how much of

a peak height increase occurred from the baseline samples run on the instrument before clean up, and the samples run with no washes, different number of washes, the robot, and varying volumes of samples cleaned up and volumes added to the Genetic Analyzer. The results obtained were used to determine which method produced the highest increase in signal as well as which method was the most efficient, balancing increase in signal with time taken to complete the procedure.

Product(s) Specifications:

Brief description of Product(s)/Technology/Procedure being evaluated:

<table>
<tr><th>Product Name(s)</th><th>Model Number:</th><th>Serial/Lot Number:</th><th>Dimensions:</th></tr>
<tr><td rowspan="3">QIAcube – The QIAcube is a robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits. The unit is designed to process up to 12 samples per run.</td><td></td><td>5818</td><td>25.6in-W,
24.4in-D,
22.4in-H</td></tr>
<tr><td>Cost:</td><td>Weight:</td><td>Power Req.:</td></tr>
<tr><td></td><td>155.4lb</td><td>100-120V AC</td></tr>
<tr><td>Storage Conditions</td><td colspan="3">Room Temperature</td></tr>
<tr><td>Operational Conditions:</td><td colspan="3">Room Temperature</td></tr>
<tr><td>Associated costs:
(consumables, maintenance, etc.)</td><td colspan="3">$5,760.00</td></tr>
</table>

Photo/Image of Product(s):

Evaluation
Instrumentation

Instrument Setup Performed by*:*
____ Manufacturer
____ Manufacturer and Evaluator(s)
_X___ Evaluator(s) Only

Instrument Setup Comments:

The instrument was relatively easy to set up; however, protocol sheets had to be requested from the Manufacturer for final positioning of reagents.

Level of Operator Knowledge as Set by Manufacturer *(check one)***:**
____ Non-Scientist
__X__ Technician
____ Scientist

Standards, Controls and Samples Used in Evaluation:

Male DNA extracted from Sperm cells, Positive Control- 9947A, TE Buffer.

Post-Evaluation Findings

Strengths/Results:

Results:
Procedure #1: The entire amplified product was cleaned up and concentrated with MinElute and all of it was placed on the Genetic Analyzer.

Manual —varying number of washes— Concentration of 0.125ng/ µl

Number of Washes	Average Fold Increase
1	9.5
2	16.9
3	17.9

4	14.3
No washes (all amplified product added to Genetic Analyzer)	No data produced; even size standard was suppressed.

Manual —varying number of washes— Concentration 0.0625ng/ µl

Number of Washes	Average Fold Increase
1	7.3
2	11.5
3	13.9
4	13
No washes (all amplified product added to Genetic Analyzer)	No data produced; even size standard was suppressed.

QIAcube – performs 1 wash

Concentration	Average Fold Increase
0.125ng/ µl	10.4
0.0625ng/ µl	9.7

Conclusions:

Based on time and use of reagents, two manual washes produced the best results. The QIAcube, however, produced the most consistent results from one concentration to another. This is probably because the pipetting is more consistent utilizing the robot versus manual processing.

For the remainder of the study, all samples were processed using the QIAcube to eliminate variability with pipetting. A concentration 0.125ng/ µl was used because there was minimal or no stochastic effects.

Procedure #2: All of the amplified product was cleaned up and concentrated with MinElute and different volumes were added to the Genetic Analyzer

Volume added to Genetic Analyzer	Average Fold Increase
1 µl	4.2
5 µl	8.4
10 µl	7.8

Conclusion:

The volume added to the Genetic Analyzer affected the fold increase. However, based on these results, there is not much difference between adding 5 µl or 10 µl; to conserve product for possible retesting, 5 µl would be the preferred volume to add.

Procedure #3: Different volumes of the amplified product are cleaned up and concentrated using the MinElute and all resulting product is placed on the Genetic Analyzer.

Volume of product put through MinElute	Fold Increase
5 µl	1.4
10 µl	2.5

Conclusion:

These increases were minimal. 5 µl at some loci did not show an increase; there was actually a decrease. However, before the cleanup process there were 13 peaks with heights below 100 RFUs. After cleanup there were only 2 peaks with heights below 100 RFUs. Upon examination of heterozygosity it was seen that after cleanup there was more consistency in the heterozygosity than before.

The following table displays heterozygosity results before and after cleanup on a triplicate set where only 5µl of amplified product was put through the MinElute cleanup.

	Heterozygosity %					
Locus	Set 1–Pre Q	Set 1–Post Q	Set 2– PreQ	Set 2– Post Q	Set 3– Pre Q	Set 3–Post Q
D8	75	75	74	76	97	75
D21	83	83	58	80	51	83
D7	61	58	61	60	87	60
CSF	83	82	77	81	63	80
D3	76	78	56	78	69	78
D13	97	98	75	96	61	94
D16	82	90	82	94	45	93
D2	92	85	68	87	54	87
D19	67	69	76	64	64	64
vWA	79	88	91	82	73	82
D18	49	49	96	45	75	45
Amel	94	95	98	96	76	100
FGA	72	92	79	91	55	86

When only cleaning up 5µl of sample, on average there was an increase; however, at some loci there was not an increase in peak heights (FGA and D18 had some lower peak heights after cleanup).

Locus	Peak Heights	
	Pre Q	**Post Q**
D18	162,79	211,104
	185,177	374,169*
	205,153	268,122*
FGA	85,61	75*,69
	96,122	143,130
	111,61	121,104

*Peak heights after clean up.

Strengths

- The MinElute system always provides some amount of signal increase regardless of what factors are changed. To implement this into the laboratory system it would be up to the agency to decide what factors they would adjust depending on their needs and their current policies (e.g., procedures for retaining amplified product for future testing, etc.).
- Manual MinElute is easy to perform and is very quick even if more than one wash is performed
- The QIAcube is just as easy to operate and presents some advantages:
    - It is automated so the analyst is freed up to conduct other tasks
    - It does not cause contamination between samples or from one run to the next
    - It can process less than twelve samples as long as tubes are balanced inside the robot
    - It performs a more consistent pipetting so the fold increase is close to the same every time
    - It has built-in self-checks which ensure set up is done correctly
    - It is very easy to operate

Areas for Improvement:

Two manual washes gave a better increase in peak heights than the QIAcube wash. However, the increase was not consistent when using different starting concentrations of DNA. If the QIAcube could be programmed to perform 2 washes instead of one, it might produce a better, more consistent signal increase.

Limitations of Technology:

The QIAcube and the manual post-PCR cleanup process are limited by the starting amount of amplified

DNA available. If the DNA is degraded or too low to provide a proper amplification, the peak heights will be increased, but they will reflect the same stochastic effects as the original data before clean up.

In mixture samples that have a major-minor situation, where the major is high (e.g., 1000 RFUs or higher), increasing the entire sample causes the major peaks to be offscale. This in turn causes the baseline to be raised which causes artifacts such as “pull up” to occur so frequently that even though the minor is pulled above the calling threshold, it is not decipherable from artifacts that are now called because they too are above the threshold.

Training Requirements:

Minimal training is required to perform manual cleanups or to use the QIAcube. Both processes are relatively easy to perform.

Health and Safety Issues:

QIAcube uses ethanol, a flammable liquid.

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