Additional Study[1]: A comparison of the average fold increase in peak heights by varying the number of washes, volumes and performing the process with the QIAcube® robot

Introduction:

As a continuation to the MinElute® study certain components of the process which were kept constant initially were now changed to see how they would affect the average fold increase of low level samples. For this study only the AmpfℓSTR® Identifiler® kit was used with a concentration of 0.0625ng/µl and 0.125ng/µl to minimize stochastic effects associated with low level amplifications. All samples were run in triplicate

Experimental Design:

Varying the number of manual washes:
0.0625ng/µl and 0.125ng/µl concentrations were processed with the MinElute® kit varying the number of washes starting from one wash up to four washes. These concentrations were also processed on the QIAcube®. Samples were also done with no washes where all of the amplified product was added to the Genetic Analyzer.

Varying volumes:
Samples with a concentration of 0.125ng/µl were processed using the MinElute® kit on the QIAcube®. Volumes of amplified product put through the process and volumes of cleaned up product put onto 3130XL Genetic Analyzer were varied.

- Volumes of 5µl and 10µl were put through the cleanup process and all of the resulting cleaned up product was placed on the Genetic Analyzer.
- All of the amplified product was put through the cleanup process and only volumes of 1µl, 5µl and 10µl were added to the Genetic Analyzer.

Mixtures: Two mixtures were run through the cleanup process a 1:15 and 1:20 mixture.
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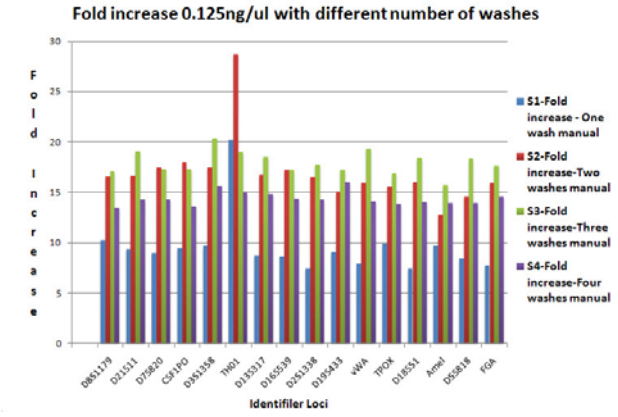
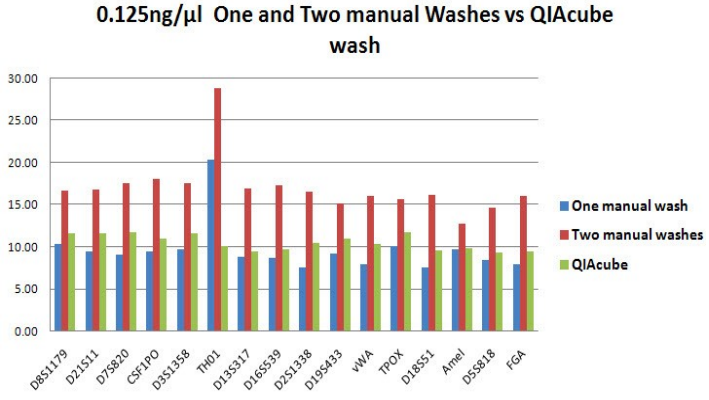
Results:

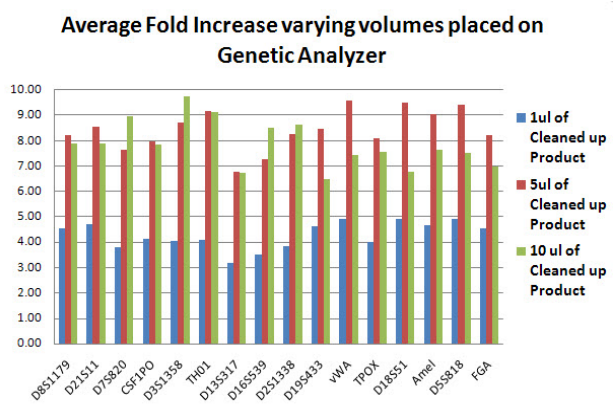


The fold increase is related to the number of washes performed. Three washes gave the best average fold increase. However based on time and reagent use two washes would be more efficient since the fold increase is not much lower than with three.

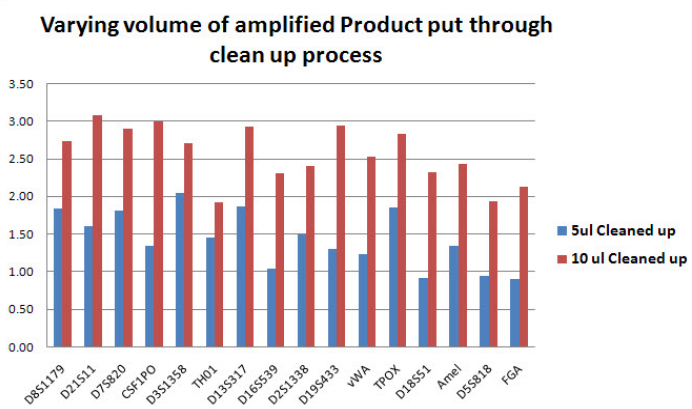


The QIAcube® which only does one wash performed better than one manual wash but not as good as two manual washes. It is worth noting that when this was done with a starting concentration of 0.0625ng/µl the average fold increase stayed consistent with 0.125ng/µl result using the QIAcube® but fluctuated with the manual technique.

[1]Original Study: The Benefit of Using the QIAGEN MinElute® PCR Purification Kit for Post PCR Cleanup on Low Level DNA Samples, 2008 Authors: *O'Brien, Robert I, BS[1*]; Sutherland, Carrie B, BS[1] ; Figarelli, Debra A, BS[1]; Ring, Joan G, MS [1], and Grates, Kirk M, BA[1]*



The average fold increase does vary with the volume of cleaned up product added to the Genetic analyzer. The QIAcube® elutes a final volume of approximately 15µl. By adding less volume to Genetic Analyzer there can be product left over for future testing.



By reducing the volume of amplified product put through the cleanup process this greatly reduces the average fold increase. The fold increase was not as much for samples in which 5µl of amplified product was cleaned up. However these samples had a more consistent percentage heterozygosity than before as shown in the table below.

	Heterozygosity %					
Locus	**Set 1-PreQ**	**Set 1-PostQ**	**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

Other Results:

For one experiment all 25µl of amplified product was run on the Genetic Analyzer without the clean up process. The result was a decrease in signal on the instrument and sizing of the data did not occur because the peak heights of the size standard were lowered below the calling threshold. For the 1:15 and 1:20 mixtures there was an increase in both the major and minor as a result the minor was now at a callable level. However the major was increased so much it went offscale which resulted in artifacts such as pull up being called. In some cases the pull up was higher than the minor which would cause problems in interpretation.