



# STRATEGIES FOR INCREASING ALLELE CALLS IN FORENSIC CASEWORK USING PCR ENHANCEMENTS AND COMMERCIAL POST AMPLIFICATION CLEAN UP SYSTEMS



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## ABSTRACT

The ability to ascertain a minor component in admixtures or elucidate a profile from a degraded sample has always presented a challenge in forensic casework. Furthermore, there has been an increase in forensic DNA casework samples submitted that include degraded and trace samples which require greater analytical sensitivity. The ability to increase sensitivity with the least amount of manipulation to the sample is ideal when working with these types of samples.

Two post amplification clean up systems were evaluated: Qiagen's Minelute® PCR Purification Kit and Promega's Wizard® SV Gel and PCR Clean-Up System.

In addition one PCR enhancement reagent was evaluated: BioMatrica®'s PCRboost™. This reagent increases PCR performance, thus improving sensitivity and specificity.

In an attempt to demonstrate the diversity of the products, we examined bone samples, trace samples, serial dilutions and mock case mixtures. Using these post amplification clean up systems significant increases in detectable alleles have been noticed. Additionally, an increase in the relative fluorescent units and a lower signal to noise ratio were observed. Use of these post amplification clean up columns improves overall sensitivity and increases the probability of achieving probative results.

## GENERAL PREMISE



## MATERIALS AND METHODS

### PCR Clean Up Systems

Qiagen: Minelute® Post PCR Purification Kit

Promega: Wizard® SV Gel and PCR Clean-up System

### PCR Commercial Amplification Kits

AmpFLSTR® Profiler Plus®

AmpFLSTR® Cofiler®

AmpFLSTR® Y-File®

AmpFLSTR® Minifiler®

AmpFLSTR® Identifier®

### QPCR

ABI Prism® 7000 and 7500

### Amplification

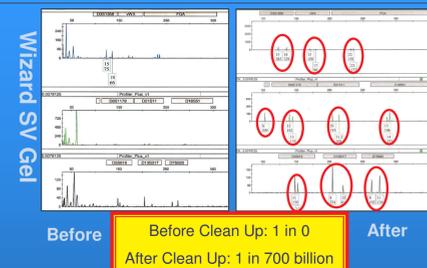
GeneAmp® PCR System 9700

### Capillary Electrophoresis

Applied Biosystems 3130xl Genetic Analyzer

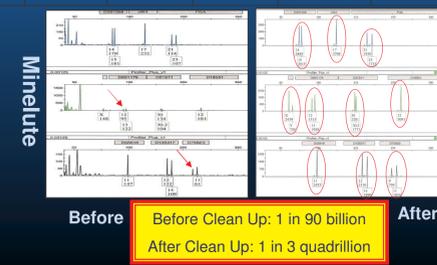
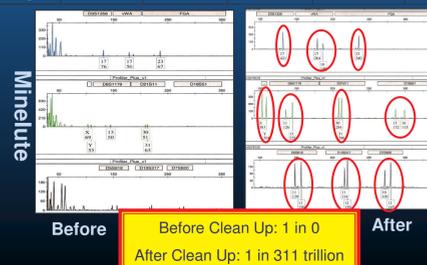
## COMPARISON DATA: FULL REACTIONS AND HALF REACTIONS

Sample	1.25 ng	0.625 ng	0.312 ng	0.156 ng	0.078 ng	0.039 ng	Sample	1.25 ng	0.625 ng	0.312 ng	0.156 ng	0.078 ng	0.039 ng
Wizard Pre Clean-Up	10	10	9(1)	7	(1)	0	Wizard Pre Clean-Up	9(1)	2(8)	0	0	0	0
Wizard Profiler Post Clean Up	10	10	10	10	7(3)	(10)	Wizard Post Half Rxn	10	10	4(6)	(2)	(1)	0
Wizard Pre Clean-Up	7	7	6(1)	2(4)	1(2)	0	Wizard Pre Clean-Up	6(1)	2(2)	0	0	0	0
Wizard Cofiler Post Clean Up	0	7	7	7	7	7	Wizard Post Half Rxn	7	5(2)	(7)	(2)	0	0
Minelute Pre Clean Up	10	10	1(5)	1(2)	(3)	0	Minelute Pre Clean-Up	10	10	8(2)	(3)	(1)	0
Minelute Profiler Post Clean Up	10	10	10	10	10	NA	Minelute Post Half Rxn	10	10	10	10	10	6(4)
Minelute Pre Clean Up	7	7	6(1)	1(4)	0	0	Minelute Pre Clean-Up	7	6(1)	3(3)	1(2)	0	0
Minelute Cofiler Post Clean Up	7	7	7	3(4)	7	3(4)	Minelute Post Half Rxn	7	7	7	6(1)	3(4)	(6)

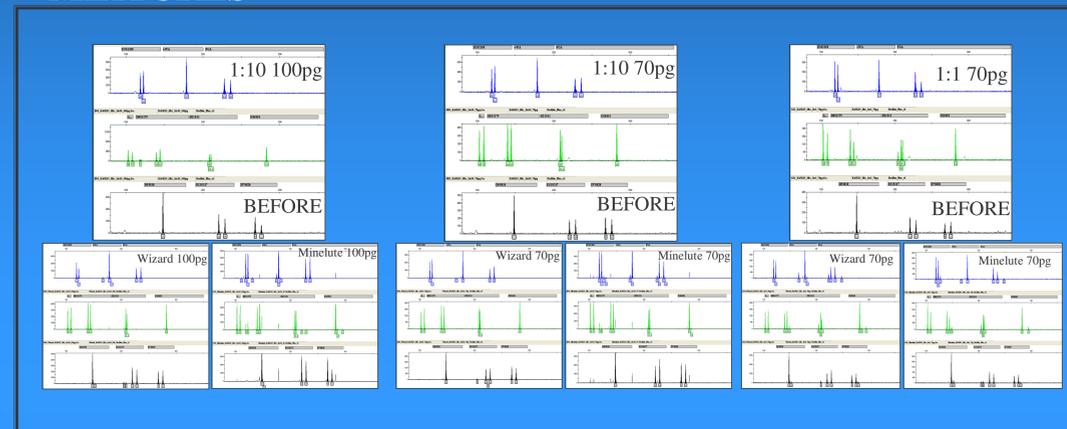


Sample	1.25 ng	0.625 ng	0.312 ng	0.156 ng	0.078 ng	0.039 ng
Wizard Pre Clean-Up	410-850	212-400	97-272	50-134	70	0
Minelute Pre Clean-Up	172-705	133-445	51-152	50-121	50-70	0
Wizard Post Clean-Up	2400-4200	1850-2900	651-1600	118-634	61-385	61-136
Minelute Post Clean-Up	3800-8600	902-3200	428-1435	60-464	166-725	82-360

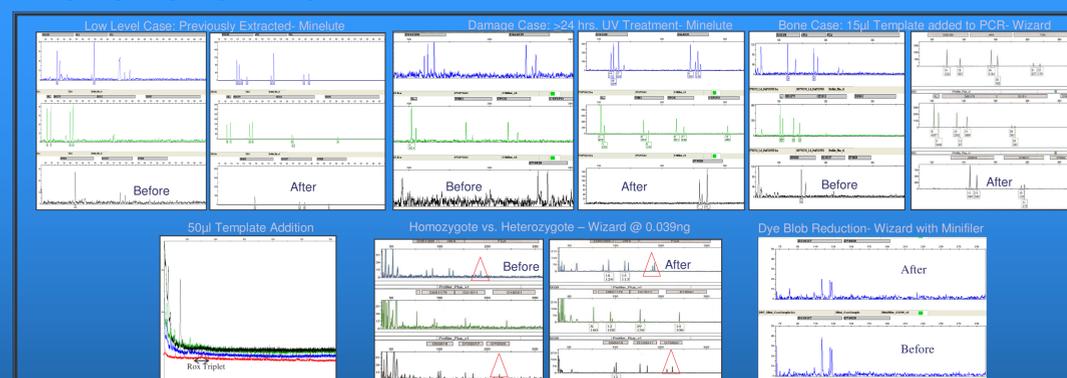
Sample	1.25 ng	0.625 ng	0.312 ng	0.156 ng	0.078 ng	0.039 ng
Wizard Pre Clean-Up	70-400	62-108	0	0	0	0
Wizard Post Clean Up	276-1300	108-371	50-134	82-91	51	0
Minelute Pre Clean Up	300-800	115-400	61-200	61-83	52	0
Minelute Post Clean Up	3300-7500	2300-7300	708-3000	175-1500	116-500	79-587



## MIXTURES



## CASE STUDIES



## CONCLUSIONS

- Quick and easy way to increase number of loci 'called'
- Better sensitivity than other methods
- Use with difficult casework
- Only uses amplified product
- Cleaner baseline
- Use of a half reaction

## REFERENCES

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[http://www.promega.com/applications/pcr/featuresandbenefits/Wizard\\_SV\\_Gel\\_PCR\\_Clean-Up\\_System.htm](http://www.promega.com/applications/pcr/featuresandbenefits/Wizard_SV_Gel_PCR_Clean-Up_System.htm)

<http://www.qiagen.com/Products/DnaCleanup/DnaCleanupSystems/Minelute/PCR/PurificationKit.aspx>

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