Validation of PowerPlex® Y-23 Amplification Kit for West Virginia State Police Biochemistry Department

Clarinda K Ellison, BS[1]; Melissa Runyan, MS[2]; Angela Gill, MS[2]; Jennifer Hayden, MSFS[1]; Pamela Staton, PhD[1]

[1]Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701

[2]West Virginia State Police, 725 Jefferson Road South Charleston, WV 25309

Abstract

An internal validation of Promega's PowerPlex® Y23 amplification kit was performed for the West Virginia State Police Biochemistry Department, which concluded the kit performed as expected. An internal validation is mandatory for any new kit before it can be introduced into case work by Standard 8 of the FBI Quality Assurance Standards. The purpose is to ensure that the product not only meets the need of the laboratory but will also produce reliable, reproducible, and robust results. To accomplish this, 5 studies for the internal validation plus 2 additional ones were performed:

- Sensitivity
- Reproducibility
- Precision
- Mixture
- Known and Non-Probative
- Concordance
- Contamination
- Thermal Cycler Cycle Number*
- Water control*

* Denotes the two extra studies

Introduction

Y-STR testing is a valuable tool available for use in DNA casework. Y-STR like autosomal STR testing uses short tandem repeat units found within the human genome, but the difference is that Y-STRs are only found on the male Y-chromosome. This unique feature makes them useful for obtaining the male profile in a mixture containing a high concentration of female DNA and a low concentration of male DNA.

Materials

Instrumentation and Reagents

- Biorobot EZ1 with EZ1 DNA investigator kit, Sarkosyl, DTT, and Proteinase K
- Applied Biosystem 7500 Real-time PCR system and Quantifiler® Duo
- GeneAmp® PCR System 9700 and Powerplex® Y23 amplification kit
- 3130xL Genetic Analyzer with Hi-Di™ formamide, Powerplex® Y23 ladder, ILS 500Y23® , and Buffer
- Genemapper® ID software v3.2

Method

Cycle number

- 3 serial dilutions from three different sources
- Each ran at 29, 30, and 31 cycles

Water

- Same dilution sets used in cycle number study
- Distilled water vs. amplification grade water

Sensitivity

- Data from cycle number and water study

Reproducibility & Precision

- SERI positive control amplified 5 times

Mixture

- 2 male mixtures
- 3 male mixtures
- Male and female mixture

Concordance and non-probative

- Premade samples tested against Powerplex® Y

Contamination

- All negative controls were generated from other tests

Results

Cycle Number

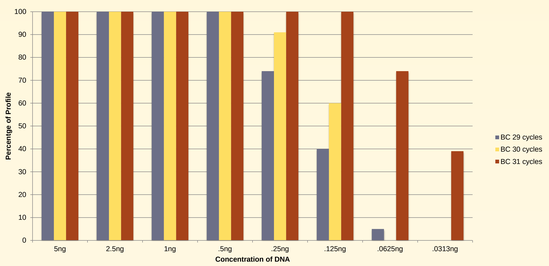

Figure 1. Percentage of the profile obtained for the serial dilution of sample BC ran at 29, 30, and 31 cycles. The injection time was 5 seconds at 3kV on the 3130xL genetic analyzer.

Sensitivity

Concentration (ng)	28000M (RFUs)	BC (RFUs)	FI (RFUs)
5	5597.87	3874	4455.45
2.5	4470.9	2419.18	2417.78
1	3386.13	1004.25	986.26
0.5	1008.71	489.53	410.18
0.25	431.43	241.89	212.19
0.125	212.09	157.98	153.89
0.0625	146.76	112.47	142.25
0.0313	128.23	117.5	194.75

Table 1. The average peak heights of all the dye channels measured in RFUs. The samples were amplified at 30 cycles and placed on the genetic analyzer for 5 second injection at 3 kV.

Results Continued

Precision

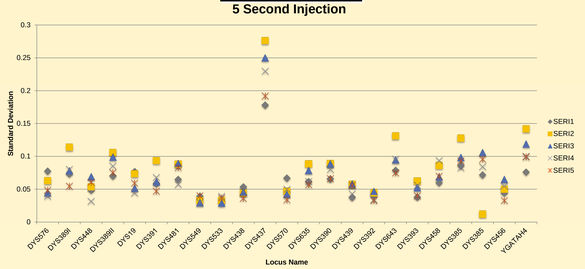

Figure 2. Standard deviation of fragment size of the five Seri samples injected with each of the capillaries at a 5 second injection time. Consistently there is poor migration of the locus DYS437.

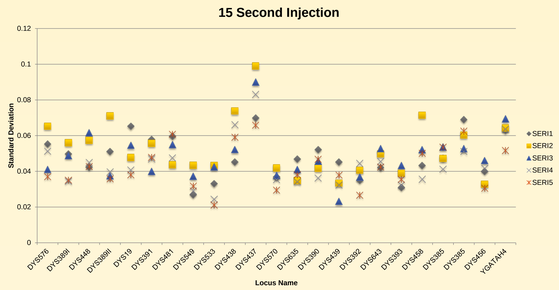

Figure3. Standard deviation of fragment size of the five Seri samples injected with each of the capillaries at a 15 second injection time. Consistently there is poor migration of the locus DYS437.

Mixture

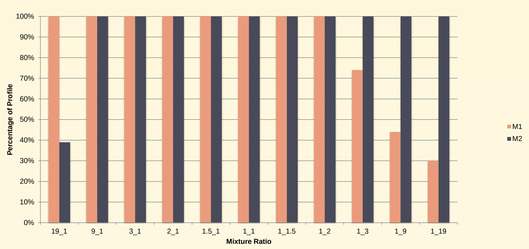

Figure 4. Percentage of each male profile observed at each mixture ratio injected for 5 seconds at 3kV

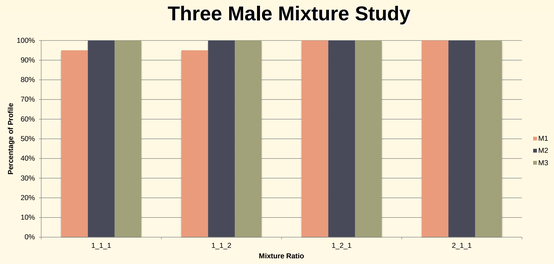

Figure 5. Percentage of each male profile observed at each mixture ratio injected for 5 seconds at 3kV.

Results Continued

Male and Female Mixture Study

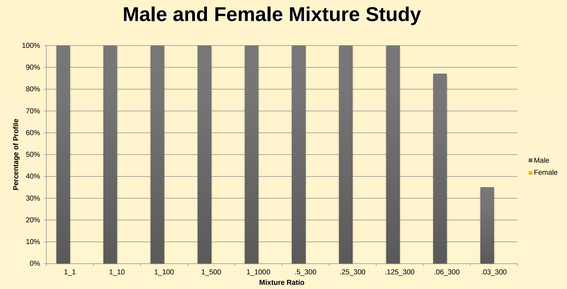

Figure 6. Percentage of the profile that was seen of male DNA at each mixture ratio injected for 5 seconds at 3kV.

Conclusion

Cycle Number Test

- 30 cycles allowed the most consistent amplification

Water

- No difference were observed between distilled water and amplification grade water

Sensitivity

- Target range found to be 0.5 ng

Reproducibility & Precision

- The kit produces reproducible results
- Standard deviation fell within acceptable range

Concordance

- Powerplex® Y23 was concordant with Powerplex® Y

Contamination

- No contamination was observed

Future Work

- Different substrates
- Degraded and inhibited samples

Acknowledgements

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