Internal Validation of Promega® PowerPlex® Y23 Amplification Kit for Use in Forensic Casework

Jordan L. Clarke, [1] B.S.*; Jody West, [2] B.S.; Kristin Meyer, [2] M.F.S.; Pamela Staton, [1] Ph.D.

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

2 North Carolina State Crime Laboratory, 121 East Tryon Road, Raleigh, NC 27603

Abstract

The North Carolina State Crime Laboratory Forensic Biology section does not currently use a Y-STR kit in casework processing. An internal validation was performed on the PowerPlex® Y23 PCR Amplification kit in accordance with the Scientific Working Group for DNA Analysis Methods (SWGDAM) validation guidelines, and the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (September 2011 revision). Sensitivity results demonstrated that the Y23 system could consistently generate full profiles at concentrations of 0.03125ng, and full male profiles were also observed in several samples at concentrations as low as 0.0156ng. Male/female mixture study results indicated that full male profiles could be consistently obtained at ratios as extreme as 1:16,000, illustrating the specificity of the Y23 for male DNA amplification. Additionally, a study was performed to explore the viability of Y23 PCR product over a period of several weeks. These studies identified the most efficient and appropriate operating procedures for the PowerPlex® Y23 amplification kit that meets the laboratory's needs and requirements.

Introduction

Y-STR systems can be an effective tool in distinguishing between males of different paternal lineage. Generating male profiles can be useful in identifying missing persons and human remains, distinguishing male contributors in complex autosomal DNA mixtures, and potentially excluding male contributors in samples containing minor male components. Y-STRs are implemented in the Combined DNA Index System (CODIS) in conjunction with traditional STRs to provide more information on missing person cases and unidentified remains at the NDIS level**.** Y-STRs are also beneficial in sexual assault evidence where the female contributor overwhelms the male contributor, or when differential extractions cannot effectively separate the male and female contributors. The increase in number of loci allows for greater discrimination among males with no paternal relation while the two rapidly mutating loci, DYS570 and DYS576, allow for possible discrimination among paternally related males. Paternally related males can be used to identify unidentified remains, missing persons, and/or if the actual suspect standard is unavailable, but one can be obtained from a brother, father, or other paternal relative.

Materials and Methods

Instrumentation and Chemistries

- Qiagen® EZ1 Advanced XL & DNA Investigator Kit
- Qiagen® QIAgility™
- Qiagen® Investigator® Quantifiler® Duo Kit
- Applied Biosystems™ 7500 Real-Time PCR Instrument
- **Promega® PowerPlex® Y23 System Amplification Kit**
- Applied Biosystems™ 9700 Thermal Cycler
- Applied Biosystems™ 3500*xl* Genetic Analyzer
- Applied Biosystems™ Genemapper™ ID-X software v 1.4

Validation Studies Performed:

- Analytical Threshold and Precision Studies
- Sensitivity and Stochastic Studies
- Non-probative/Mock Study and NIST Concordance
- Mixture Studies
- Contamination and Reproducibility Studies
- Robustness of Amplification Product and 2800M Stability

Analytical Threshold Study

The analysis threshold was set to 1 Relative Fluorescent Unit (RFU) and all known artifacts and alleles were removed for data analysis. Calculations were performed by dye color for the minimum RFU, maximum RFU, average RFU, and standard deviation.

Sensitivity and Stochastic Study

Three unrelated male samples were used to prepare DNA concentrations from 0.5 ng to 15 pg.

Seven known male samples were selected based on being the most genetically distinct from each other across all loci, specifically at the DYS385 locus and dilutions were prepared for each at 31 pg and 15 pg. Each dilution for both studies was amplified in triplicate using the total target DNA input amount.

Mixture Studies

- Mixture Set A – Constant female and varying male concentrations
- Mixture Set B – Constant male and increasing female concentrations
- Mixture Set C – Varying male:male mixtures at 0.5 ng input DNA
- Mixture Set D – Male:male mixtures from mixture set C at 31 pg and 15 pg input DNA

Non-probative/Mock Study

- 1 Adjudicated Case
- 3 Proficiency Tests
- 3 Differential
- 3 Aspermic Post-coital
- 3 Semen and 3 Saliva Dilutions

Results and Discussion

Analytical Threshold and Precision Studies

An analytical threshold was determined to be 150 RFU and the precision study demonstrated that the PowerPlex® Y23 system and instrument can resolve alleles that differ by a single base pair size.

Sensitivity and Stochastic Study

Table 1. Sensitivity Studies; Percentage of Complete Profiles for Each Dilution

Input Target	% Complete Profiles
0.5 ng	100%
0.25 ng	100%
0.125 ng	100%
0.0625 ng	100%
0.03125 ng	98%
0.0156 ng	76%

For stochastic studies, there was minimal dropout at DYS385 for both target DNA amounts. However, based on the results obtained, the stochastic threshold would be within the range of 150 RFU to 625 RFU.

Non-probative/ Mock and NIST Concordance

The results obtained from the non-probative studies were not as complete as expected due to lack of profiles obtained and not great quality samples.

The NIST Components that were required all resulted in the correct profiles.

Mixture Studies

Full male profiles at all ratios were obtained without interference from the overwhelming female DNA for both mixture study A and B. This demonstrates that large amounts of female DNA as high as 1:16,000 ratio will not inhibit or interfere with the PowerPlex® Y23 amplification system.

Mixture study C results showed that the peak heights were representative of major and minor contributors for all ratios. However, the peak height ratios were not completely representative of the input ratios as the ratio difference increased (Table 2).

Table 2. Actual Peak Height Ratios versus the Expected PHR of Mixture C per Allele (at one loci)

Marker	Ratio (A:B)	Allele	Contributor A	Contributor B	Actual Ratio	Expected Ratio
DYS389 I	1:19	12		4783.333	0.050	0.050
		13	241.000			
	1:9	12		4159.667	0.103	0.110
		13	428.333			
	1:3	12		4202.333	0.371	0.330
		13	1557.000			
	1:1	12		3214.667	1.203	1.000
		13	3866.000			
	3:1	12		1485.333	2.487	3.000
		13	3693.667			
	9:1	12		535.333	6.769	9.000
		13	3623.667			
	19:1	12		545.333	8.482	19.000
		13	4625.333			

Mixture study D results exhibited the phenomena referred to as "flip flopping"; which refers to instances where the expected major contributor's alleles is actually smaller than the allele of the expected minor contributor (Figure 1).

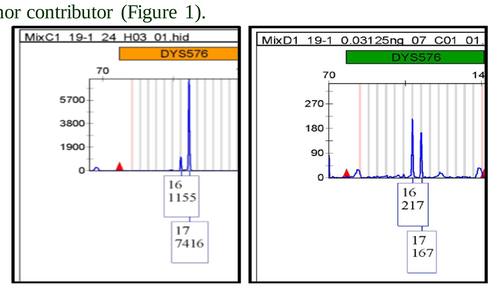

Figure 1. Occurrence of "Flip Flopping" from Optimal DNA Input Amount (left) and Low DNA Template Amount of 31pg (right).

Contamination and Reproducibility

The reproducibility of the PowerPlex® Y23 Amplification kit was demonstrated through the repeated amplification of the known standards as well as the 2800M positive amplification control. Contamination was not observed in any of the positive or negative controls used throughout the validation to demonstrate no sample to sample contamination.

Robustness of Amplification Product and 2800M Stability

In both the amplification product (Figure 2) and 2800M stability (Table 3) studies, all DNA profiles obtained contained the expected results up to eight weeks post-amplification (initial preparation).

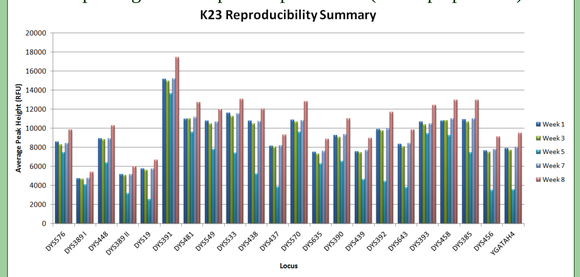

Figure 2. Average RFU Values at Each Loci for Weeks 1, 3, 5, 7, and 8.

Table 3. Average Peak Heights across all Loci for each 2800M Dilution

Date Prepared	Average Peak Height (RFU)
5.26.2015	3519.565
6.10.2015	7068.826
6.18.2015	7963.333
7.1.2015	7957.58
7.8.2015	9670.29
7.14.2015	7397.319
7.22.2015	10046.04

It is recommended that more studies should be performed testing the 2800M dilutions for longer periods of time to see how long a 2800M dilution may be stored and used for casework purposes.

Conclusions

This internal validation demonstrates the potential benefit of implementing the PowerPlex® Y23 kit in other forensic casework laboratories and will assist the North Carolina State Crime Laboratory's Forensic Biology section in evaluating the addition of Y-STR analysis in the processing of sexual assault evidence.

- Analytical Threshold set to 150 RFU
- Stochastic Threshold within 150 – 600 RFU
- Sensitivity of kit to obtain full profiles as low as 31pg and 15 pg

Future Studies

- More sensitivity and stochastic studies with lower DNA template amount and larger sample population
- More Non-probative/Mock samples to represent actual casework
- Complete the stutter study
- Test instrument parameters other than 24 second injection and 30 cycles for amplification
- Validate casework AB® 3500xL Genetic Analyzer

References

Applied Biosystems. Applied Biosystems 3500/3500xL Genetic Analyzer User Guide. Rev 06/2010.

Buscher, A., Zastrow-Arkens, S., Kauraka, D., Hinton, N., Culhane, S., and Degroot, G. "Internal Validation and Implementation of the PowerPlex® Y23 System. *Wisconsin State Crime Laboratory Bureau*.

Federal Bureau of Investigation. "Quality Assurance Standards for Forensic DNA Testing Laboratories." July, 2009.

Federal Bureau of Investigation: Laboratory Services. "Frequently Asked Questions (FAQs) on the CODIS Program and the National DNA Index System,"

North Carolina State Crime Laboratory ISO Procedures. Forensic Biology: DNA Casework Procedures. Raleigh, NC.

Kline, Margaret C., Butts, Erica L.R., Hill, Carolyn R., Coble, Michael D., Duewer, David L., and Butler, John M. "The New Standard Reference Material® 2391c: PCR-based DNA Profiling Standard". *U.S. National Institute of Standards and Technology*.

Promega® PowerPlex® Y23 System Technical Manual, March 2015

Scientific Working Group of DNA Analysis Methods (SWGDAM). Quality Assurance Standards for Forensic DNA Testing Laboratories. September, 2011.

Scientific Working Group on DNA Analysis Methods. "SWGDAM Interpretation Guideline for Y-Chromosome STR Typing by Forensic DNA Laboratories." 9 January 2014.

Vermeulen M, Wollstein A, van der Gaag K *et al. (2009)* Improving global and regional resolution of male lineage differentiation by simple single-copy Y-chromosomal short tandem repeat polymorphisms. *Forensic Science International: Genetics*, **3**, 205–213.

Acknowledgements

The author thanks the North Carolina State Crime Laboratory Forensic Biology/DNA section for the opportunity to perform the internal validation. The author thanks Jody West for his assistance and supervision throughout the project. The validation benefitted from the constant input and guidance from Kristin Meyer, and valued assistance from Tim Baize and Amanda Overman. The author thanks Jody West, Kristin Meyer, and Dr. Pamela Staton for their input and review of the final validation project.