



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

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## ABSTRACT

Forensic DNA laboratories rely on Y-chromosome specific short tandem repeat (Y-STR) analysis for a variety of cases where the identification of male-specific DNA is essential, with sexual assaults making up the majority of these cases.

This study focused on the comparison of three commercially available Y-STR kits and ultimately the validation of the PowerPlex® Y23 for use in forensic casework following SWGDAM and FBI validation guidelines. PowerPlex® Y (Promega Corporation), AmpFISTR® Yfiler™ (Applied Biosystems-Life Technologies) and PowerPlex® Y23 (Promega Corporation) demonstrated similar performance in precision, sensitivity, and mixture resolution. However, PowerPlex® Y23 exhibited superior discriminating power over the other amplification systems, therefore it was chosen for validation. Internal validation of PowerPlex® Y23 demonstrated the kit's ability to perform as intended by Promega Corporation for use on casework samples.

## INTRODUCTION

Y-STR analysis has been recognized as a valuable tool in forensic DNA casework for its design to target male-specific loci during the amplification process<sup>[1]</sup>. Unlike autosomal STR analysis where preferential amplification of major component female DNA can result in failure to detect minor component male DNA in admixed samples, such as those associated with sexual assault cases<sup>[2]</sup>, Y-STR analysis targets only male DNA for amplification with no amplification of female DNA<sup>[3,4]</sup>. Y-STR analysis has also proven advantageous in sexual assault cases involving multiple male assailants where autosomal STR analysis is ambiguous regarding the number of contributors to the mixture<sup>[5,6]</sup> and in cases where mixed gender DNA cannot be segregated by differential extraction<sup>[1,7]</sup>.

The intent of this research was to evaluate the performance and discrimination power of the most current, commercially available Y-STR kits and subsequently internally validate the single Y-STR system that performed optimally within the Colorado Springs Metro Crime Laboratory (Colorado Springs, CO). The eleven loci PowerPlex® Y system<sup>[8]</sup>, the sixteen loci Yfiler™ system<sup>[9]</sup>, and the twenty-three loci PowerPlex® Y23 system<sup>[10]</sup> were chosen for comparison.

## METHODS & MATERIALS

### COMPARISON

NIST Standard Reference Material 2391c and Promega Corporation Samples were used in the comparison study. All samples were quantified using Quantifiler® Duo and run on an AB 3130 Genetic Analyzer. Y-STR amplification kits were compared based on precision, sensitivity, and mixture resolution..

### VALIDATION

Genomic NIST Standard Reference Material 2391c and genomic Promega Corporation Samples, as well as non-probative buccal and blood samples previously extracted using Chelex®100 Resin were used for validation. All samples were quantified using Quantifiler® Duo and run on an AB 3130 Genetic Analyzer. Validation studies included precision, sensitivity, mixtures, stutter, analytical threshold, reproducibility, concordance, and contamination.

## RESULTS & DISCUSSION

### COMPARISON

#### Precision

- All alleles represented within the PowerPlex® Y, Yfiler™, and PowerPlex® Y23 allelic ladder, as well as each represented marker overall, exhibited an average standard deviation of less than 0.15 (Figure 1). Therefore, all three STR amplification kits are in compliance with a 95% confidence interval.

- The AB 3130 Genetic Analyzer has been found to produce precise migration with PowerPlex® Y, Yfiler™, and PowerPlex® Y23.

#### Sensitivity

- Optimal DNA target ranges:  
PowerPlex® Y = 0.25-0.5ng  
Yfiler™ and PowerPlex® Y23 = 0.5-1.0ng

- Allelic dropout was observed in all samples amplified with Yfiler™ and only 75% of samples amplified with PowerPlex® Y and PowerPlex® Y23 when DNA input concentrations were less than 0.125ng.

- For samples exhibiting drop out, an average of 91.7% of alleles were still called when using PowerPlex® Y, 79.4% when using Yfiler™, and 89.9% when using PowerPlex® Y23.

- Although PowerPlex® Y exhibited the greatest level of sensitivity, PowerPlex® Y 23 produced the greatest number of typed loci at all DNA input concentrations, by far, when compared to PowerPlex® Y and Yfiler™.

### VALIDATION

#### Precision

- The AB 3130 Genetic Analyzer has been found to produce precise migration with PowerPlex® Y23 PCR amplification system.

#### Sensitivity

- A target DNA input range of 0.5-1.0ng is acceptable for use with the AB 3130 Genetic Analyzer

- Full profiles can be obtained from as little as 0.125ng template DNA

- Partial profiles can be generated from as little as 0.0312ng template DNA

#### Mixtures

##### Male/Female

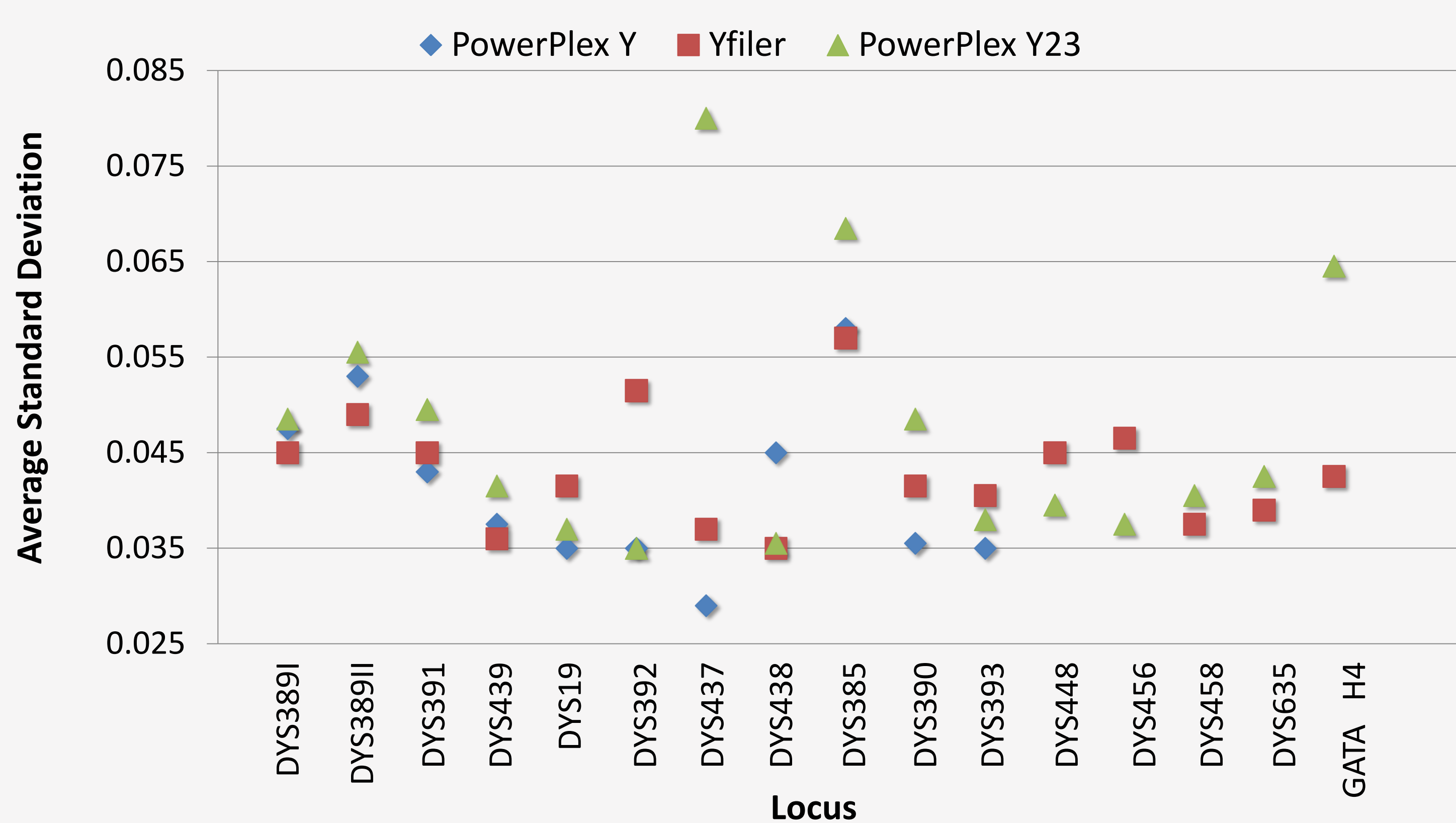
- Full profiles containing only male donor alleles were obtained.

##### Male/Male

Table 2. PowerPlex® Y23 Male/Male Mixture Results for Each Ratio Tested

	1:1	1:2/2:1	1:5/5:1	1:9/9:1	1:19/19:1
Full Profile For Both Males	Yes	Yes	Yes	No	No
Major/Minor Discernible	No	No	Yes	Yes	Yes
Minor Donor Drop-Out	No	No	No	Yes	Yes

Figure 1. Precision as a Function of Average Standard Deviation per Shared Locus



#### Mixtures

##### Male/Female

- Full profiles containing only alleles from the male donor were obtained for PowerPlex® Y, Yfiler™, and PowerPlex® Y23.

##### Male/Male

- Full genetic profiles for both male donors were produced at a mixture ratio of 1:1 for all Y-STR amplification kits.

- A major and minor contributor were discernible at mixture ratios of 1:9/9:1 and 1:19/19:1 for all Y-STR amplification kits. However, minor donor allele drop-out was present at these ratios for all kits (Table 1).

Table 1: Average percentage of minor alleles called in male/male mixture profiles generated from a 1:9/9:1 and 1:19/19:1 mixture ratio.

	PowerPlex® Y	Yfiler™	PowerPlex® Y23
1:9/9:1	58.33%	82.86%	78.26%
1:19/19:1	33.33%	50.00%	50.00%

#### Stutter

- The majority of loci exhibited stutter ratio values comparable to those reported by Promega Corporation, except for DYS389II, DYS549, DYS390, DYS385, DYS456, and GATTA H4 which exhibited notably higher stutter ratio values.

- The CSPD Metro Crime Laboratory will use the highest stutter ratio observed per locus (validation calculated vs. manufacturer determined).

#### Analytical Threshold

- The analytical threshold value to be implemented by the CSPD Metro Crime Lab is 70 RFU, and is based on the Example in SWGDAM Guidelines - Negatives calculated value of 68 FRU<sup>[11]</sup>.

#### Reproducibility

- PowerPlex® Y23 was capable of generating identical Y-STR profiles for multiple amplifications of the same sample, and therefore demonstrates reproducibility.

#### Concordance

- Data generated using PowerPlex® Y23 was 100% concordant with data generated using Yfiler™ for all shared loci.

#### Contamination

- PowerPlex® Y23 System has appropriately demonstrated the lack of detectable cross-contamination between wells.

## CONCLUSIONS

### COMPARISON

The number one priority of forensic DNA testing is to produce genetic profiles that exhibit high powers of discrimination so that greater accuracy of exclusions and inclusions can be achieved. PowerPlex® Y23 consistently produces a high number of allele calls at a wide range of DNA input concentrations, as well as a high number of minor contributor allele calls in male/male mixtures. Therefore, PowerPlex® Y23 was chosen for validation.

### VALIDATION

Internal validation of PowerPlex® Y23 has verified the amplification system's ability to perform as intended by Promega Corporation.

## REFERENCES

- Butler JM. Fundamentals of Forensic DNA Typing. Academic Press, San Diego, 2009.
- Cerri N, et al. Mixed stains from sexual assault cases: autosomal or Y-chromosome short tandem repeats?. Croatian Medical Journal, 2003; 44: 289-292.
- Daniels DL, Hall AM, Ballantyne J. SWGDAM Developmental Validation of a 19-locus Y-STR System for Forensic Casework. Journal of Forensic Science, 2004;49(4): 1–16.
- Krenke BE, et al. Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex. Forensic Science International, 2005; 148(1): 1-14.
- Hanson E, Ballantyne J. A Highly Discriminating 21 Locus Y-STR "Megaplex" System Designed to Augment the Minimal Haplotype Loci for Forensic Casework, 2004; 49(1): 1-12.
- Prinz M, Sansone M. Y Chromosome-specific Short Tandem Repeats in Forensic Casework, 2001; 42(3): 288-291.
- Shewale JG, Sikka, SC, Schneida E, Sinha S. DNA Profiling of Azoospermic Semen Samples from Vasectomized Males by Using Y-PLEX™ 6 Amplification Kit. Journal of Forensic Science, 2003; 48(1): 1-3.
- Promega Corporation. Technical Manual: PowerPlex® Y System Instructions for Use of Product DC6760 and DC6761. Promega Corporation, 2012.
- Applied Biosystems-Life Technologies. User's Manual: AmpFISTR® Yfiler™ PCR Amplification Kit. Life Technologies Corporation, 2012.
- Promega Corporation. Technical Manual: PowerPlex® Y23 System Instructions for Use of Product DC2305 and DC2320. Promega Corporation, 2012.
- 21<sup>st</sup> International Symposium on Human Identification. Mixture Interpretation Workshop: Principles, Protocols, and Practice. Analytical Thresholds: Determination of Minimum Distinguishable Signals. Presented by Catherine M Grgickac. October 11, 2010. San Antonio Texas. Accessed Online September 2012. <http://www.cstl.nist.gov/div831/strbase/mixture/3%20-%20Analytical%20Threshold.pdf>

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