## The Power of 8 Colors: Next Generation CE STR Analysis

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#### 1. Introduction

The ability to detect 8 colors with the new Spectrum CE System allows for the development of 8-color STR systems with more loci as smaller amplicons for better performance with degraded and inhibited samples. The 8-color PowerPlex® 35GY System combines the CODIS 20 core loci along with Penta D, Penta E, SE33 and ten Y STR markers for familial searching and analysis of sexual assault evidence. The 8-color PowerPlex® 18E System combines all of the ENFSI recommended loci. Amelogenin and DYS391 are included for sex determination in both systems. The use of 8 colors enables 15 autosomal STR loci to be present as amplicons under 250bp in both 8-color systems. Two quality indicators (QIS and QIL) are included that aid in determining if poor quality profiles are due to degradation, inhibition, or absence of human DNA.

#### 4. Concordance

PowerPlex<sup>®</sup> 35GY typing of 661 NIST U.S. population set samples and 5 samples in the NIST SRM 2391d PCR-Based DNA Profiling Standard demonstrated a 99.97% concordance rate. Out of 38,999 alleles compared, 10 discordant allele calls were observed.

- One null allele in DYS635
- One discordant allele in D7S820
- One discordant allele in CSF1PO
- Seven discordant alleles in SE33, including Component B of SRM 2391d – caused by a TTTT (6/7) or TTG deletion (1/7)

#### 7. Ancient Bone Sample



#### 2. PowerPlex<sup>®</sup> 35GY and 18E System Layouts



300

# $\frac{\text{NIST 2391d Component B (SE33 Locus)}}{PowerPlex^{\textcircled{B}} 35GY (18, 28.2)} \xrightarrow{\underline{523}} 200 300 320} PowerPlex^{\textcircled{B}} Fusion 6C (17, 28.2)$

The TTTT deletion results in one full repeat difference between PowerPlex<sup>®</sup> 35GY and other STR chemistries. However, PowerPlex<sup>®</sup> 35GY is concordant with sequencing data and with respect to the actual number of repeats present.



NB: PowerPlex<sup>®</sup> 18E System concordance testing in progress

#### 5. Performance with Inhibitors

#### PowerPlex<sup>®</sup> 35GY System (60 to 500bp)



DNA from an ~800 years old petrous bone sample was analyzed. Quantification of human DNA (ng/µL) performed with PowerQuant<sup>®</sup> System

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0.09905	0.0009	0.03235	3.06	110.06				

PowerPlex<sup>®</sup> 35GY and 18E profiles of 1ng ancient DNA amplified for 29 cycles in 25µL reactions. Quality Indicator peaks confirmed absence of inhibition. Profiles were obtained at 21 STR loci and Amelogenin with the PowerPlex<sup>®</sup> 35GY system, and at 16 STR loci and Amelogenin with the PowerPlex<sup>®</sup> 18E system.

#### 8. Differential Extraction Samples

PowerPlex<sup>®</sup> 35GY System (60 to 500bp)



#### 3. Amplification

200



PowerPlex<sup>®</sup> 35GY and 18E profiles of 1ng 2800M Control DNA amplified for 29 cycles in 25µL reactions. Electrophoresis performed on the Spectrum CE System. Data analyzed using GeneMarker<sup>®</sup> HID Software for Spectrum CE Systems.

#### **Direct Amplification**

PowerPlex<sup>®</sup> 35GY System

PowerPlex<sup>®</sup> 18E System

500

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#### PowerPlex<sup>®</sup> 18E System (60 to 350bp)



PowerPlex<sup>®</sup> 35GY and 18E profiles of 1ng 2800M Control DNA amplified for 29 cycles in 25µL reactions with increasing hematin concentrations. Electrophoresis performed on the Spectrum CE System. Data analyzed using GeneMarker<sup>®</sup> HID Software for Spectrum CE Systems.

#### 6. Quality Indicator Performance

Designed for amplification of purified DNA and direct amplification
Debugt amplification with inhibitory

The PowerPlex<sup>®</sup> 35GY and 18E Systems are 8-color STR

#### PowerPlex<sup>®</sup> 18E System (60 to 350bp)



PowerPlex<sup>®</sup> 35GY and 18E profiles of total DNA present in a mock sexual assault sample (0.25µL semen spotted on buccal swab) and the resulting sperm fraction following differential extraction using the Prototype Maxwell DE System. Blue dye channel shown.

#### 9. Conclusions

chemistries



PowerPlex<sup>®</sup> 35GY and 18E profiles of 1.2mm buccal FTA<sup>®</sup> punches amplified for 25 cycles in 12.5µL reactions. Electrophoresis performed on the Spectrum CE System. Data analyzed using GeneMarker<sup>®</sup> HID Software for Spectrum CE Systems.



Quality Indicator peaks (QIS and QIL) provide data interpretation assistance.

- A. Balanced STR profile with balanced QIS and QIL peaks successful amplification
- B. No STR peaks with balanced QIS and QIL peaks no template DNA amplified in sample
- C. Sloping STR profile with balanced QIS and QIL peaks degraded DNA in sample

D. Sloping STR profile with low or missing QIL peak – inhibited sample

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Funding - This work was in part supported by the NIST Special Programs Office: Forensic Genetics.

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- Robust amplification with inhibitors
- Includes two Quality Indicators (QIS and QIL) to help with data interpretation

### Concordance testing showed 99.97% concordance with the NIST data set

- Only 10 discordant alleles called from 661 individuals tested
- Most discordant alleles observed in SE33. PowerPlex<sup>®</sup> 35GY System concordant with SE33 sequencing results

#### **Offers improvements over previous STR chemistries**

- Amplification of an ~800-year-old bone sample resulted in a significant amount of genetic information because of the increase in the number of loci below 250bp
- The SE33 locus in PowerPlex<sup>®</sup> 35GY and 18E Systems is the smallest of any current STR chemistry on the market

#### **Acknowledgements:**

We thank Dr. Meradeth Snow at the University of Montana for providing the DNA extract of the petrous bone sample described in Section 7 and Jessica Seifarth for performing the PowerQuant<sup>®</sup> analysis. We also thank Nick Courtney for performing the differential extraction described in Section 8.

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PS445 | November 2022