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

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[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711;
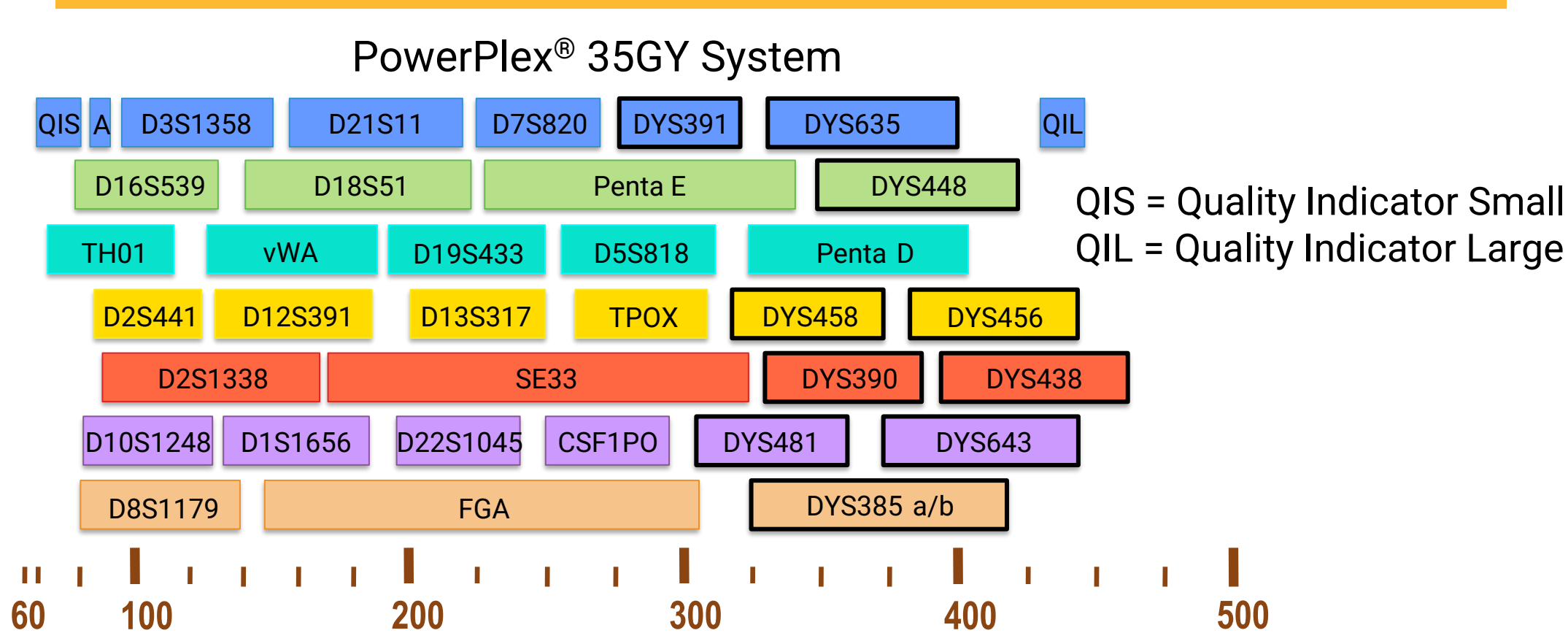
[2]National Institute of Standards and Technology, Applied Genetics Group, 100 Bureau Dr, Gaithersburg, MD 20899
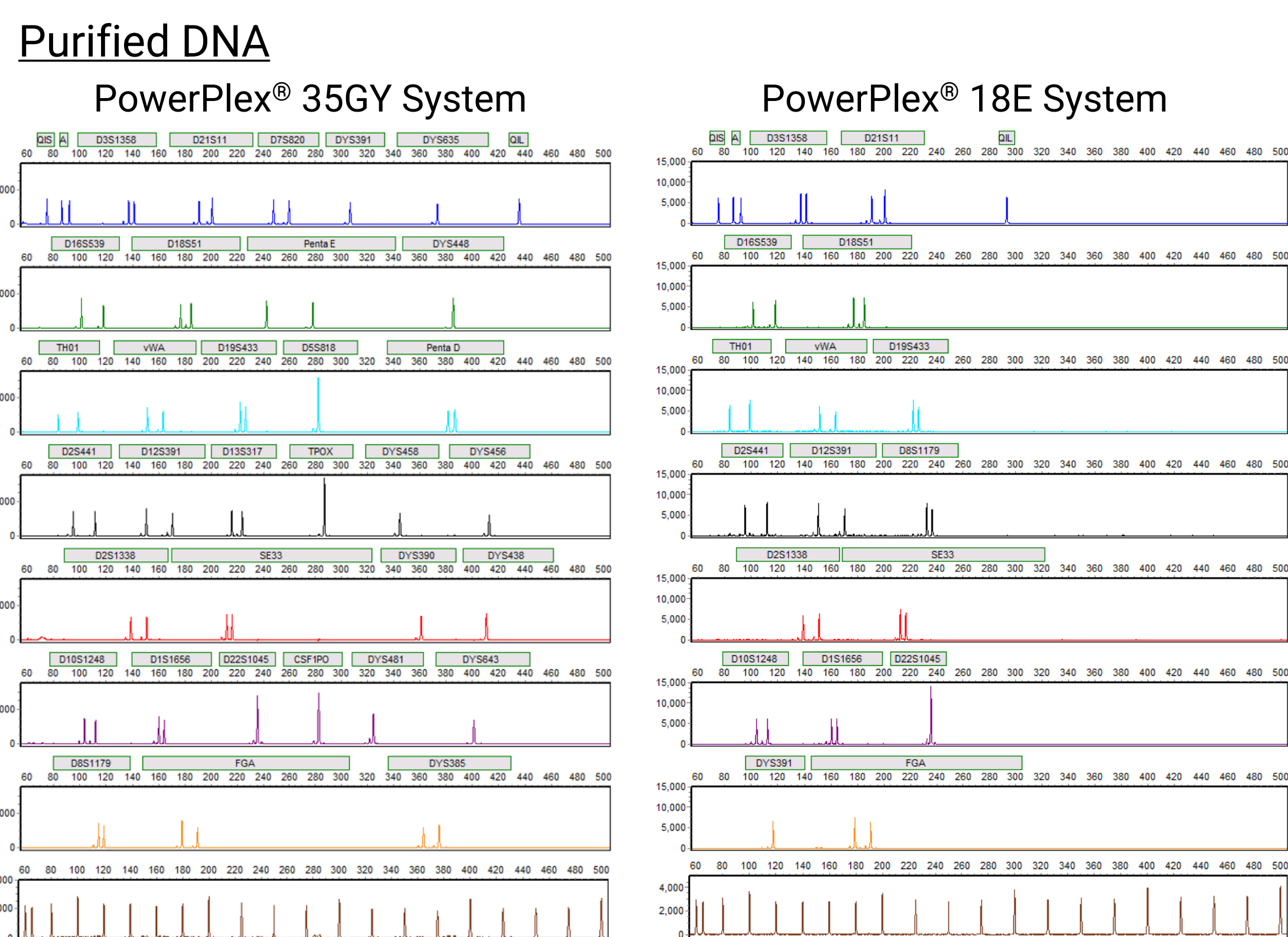
## 1. Introduction

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.

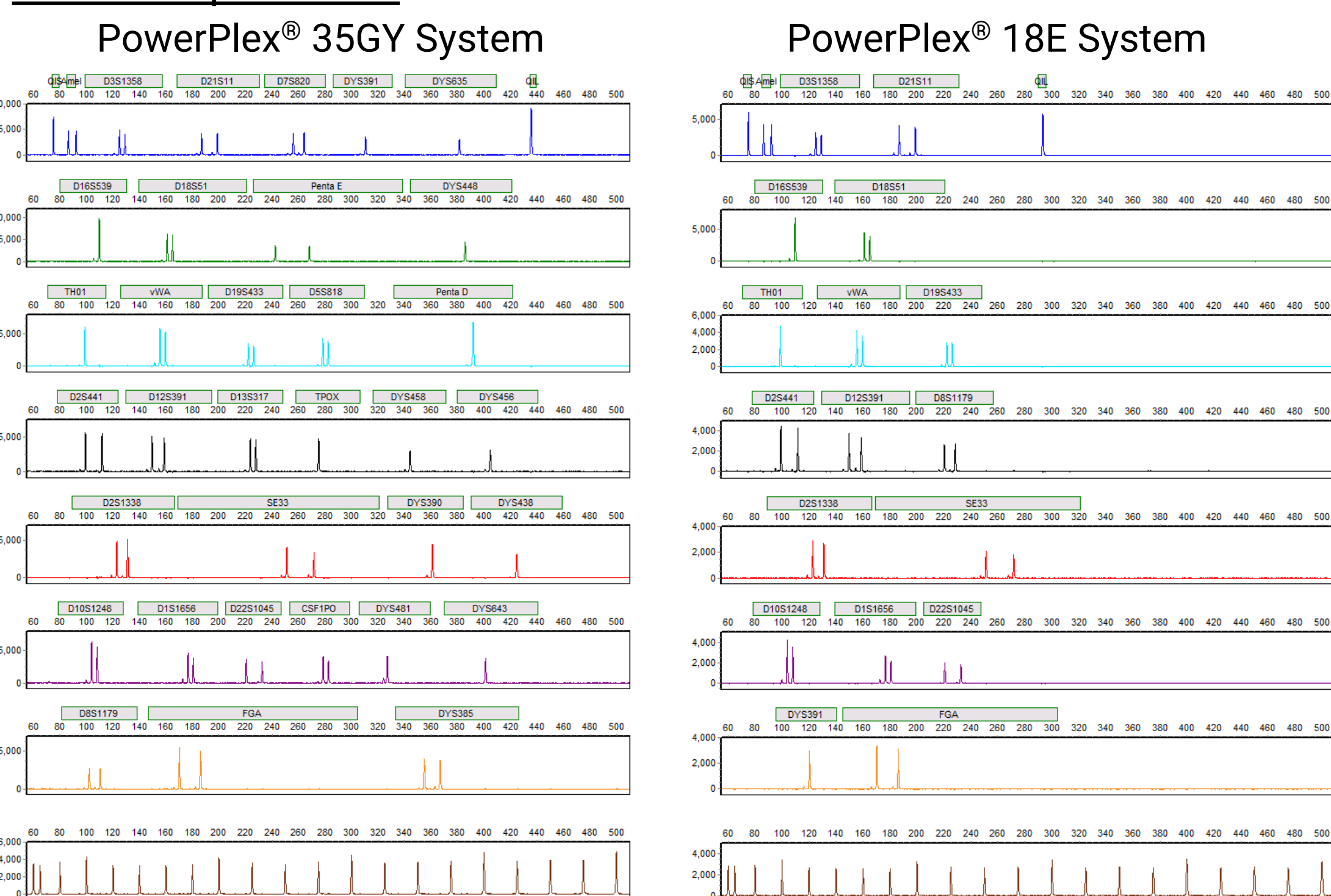## 2. PowerPlex® 35GY and 18E System Layouts



QIS = Quality Indicator Small
QIL = Quality Indicator Large

*All primers pairs in PowerPlex® 18E shared with PowerPlex® 35GY, except DYS391 and D8S1179

## 3. Amplification

### Purified DNA



PowerPlex® 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® HID Software for Spectrum CE Systems.
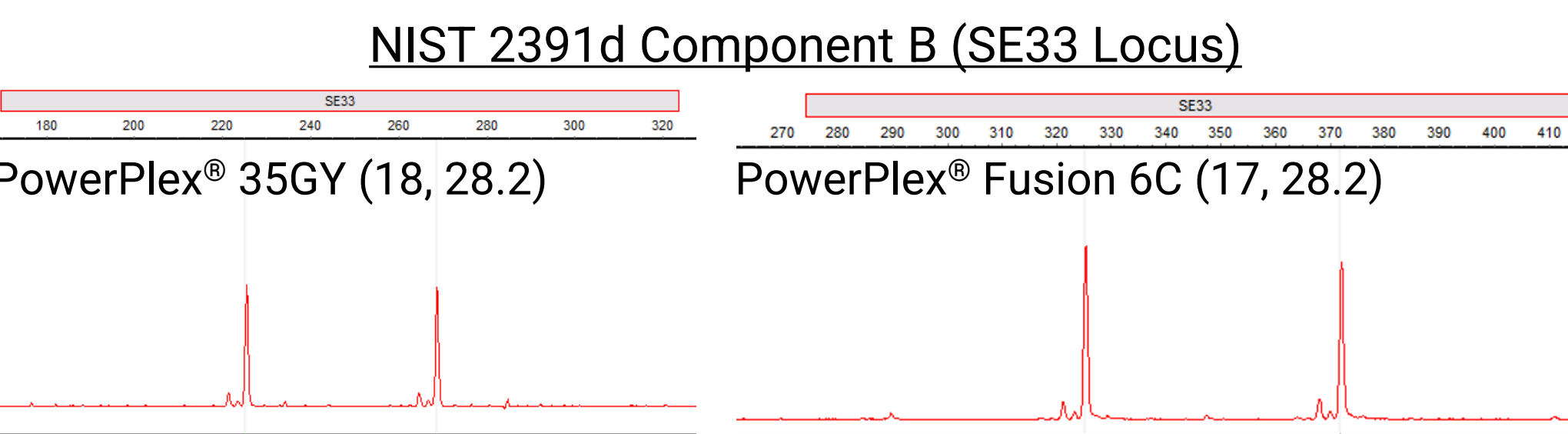
### Direct Amplification



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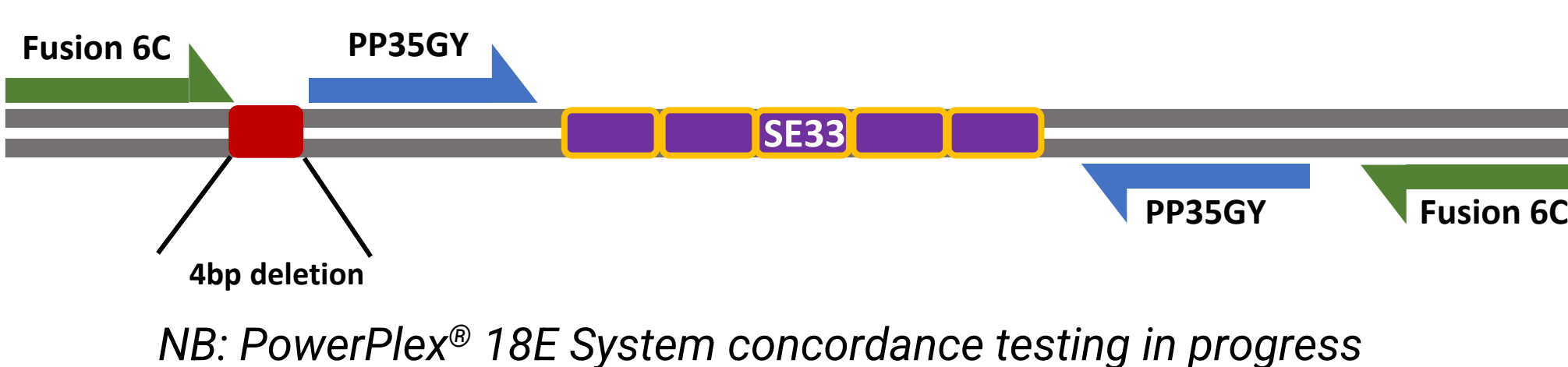
## 4. Concordance

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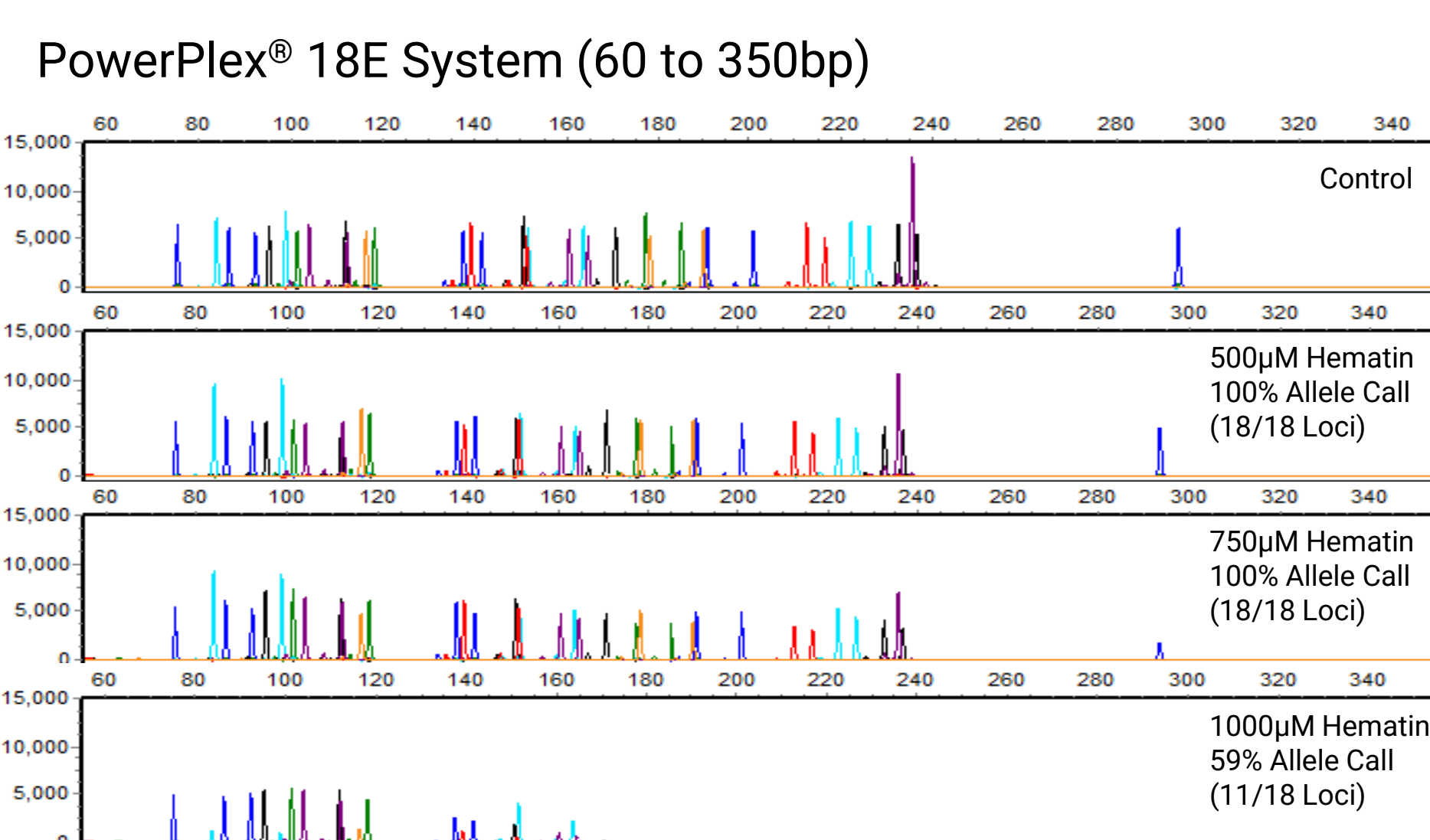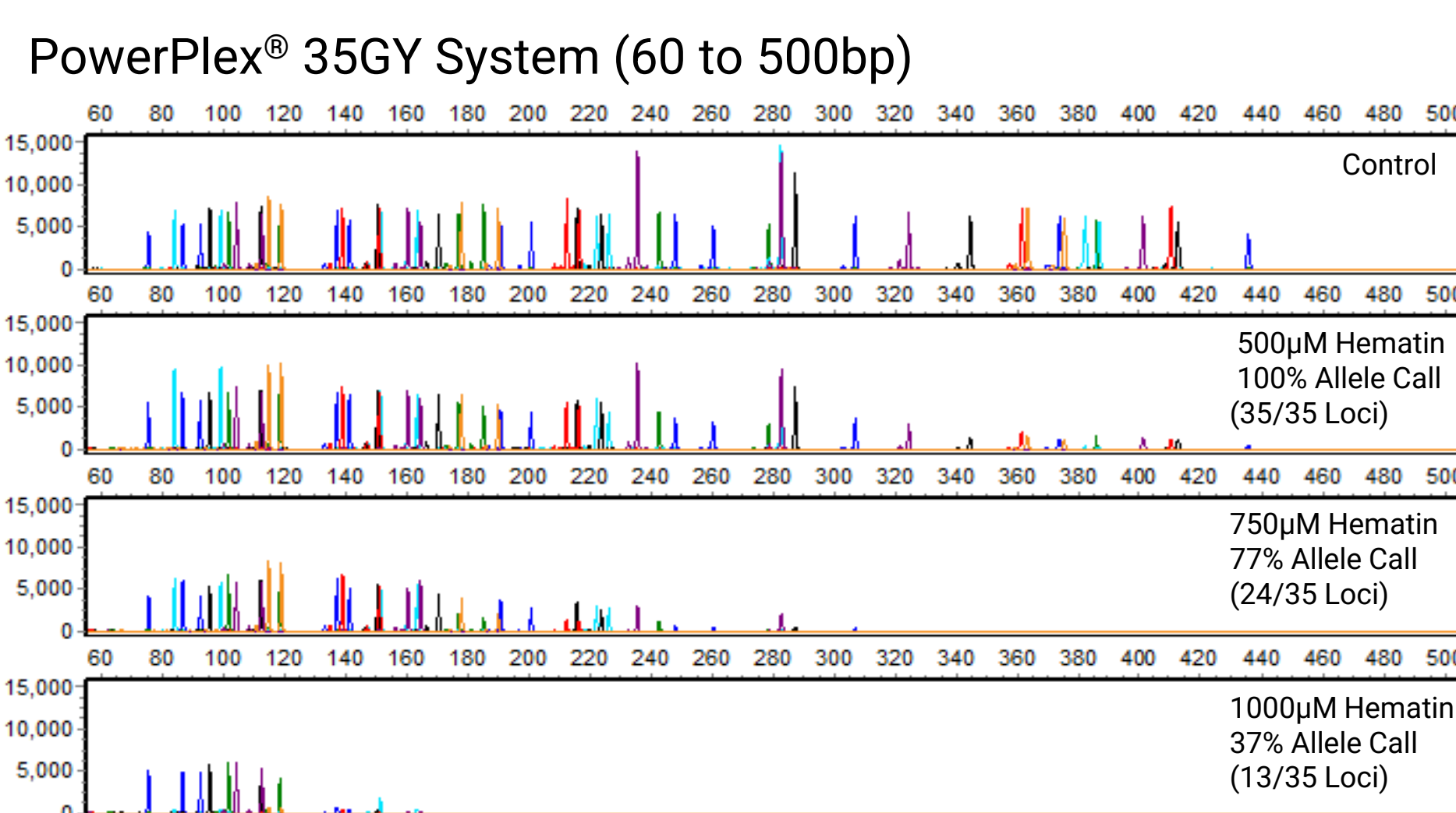
- One null allele in DYS635
- One discordant allele in D7S820
- One discordant allele in CSF1PO
- Seven discordant alleles in SE33, including Component B of SRM 2391d – caused by a TTTT (6/7) or TTG deletion (1/7)

### NIST 2391d Component B (SE33 Locus)



PowerPlex® 35GY (18, 28.2)   PowerPlex® Fusion 6C (17, 28.2)

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



NB: PowerPlex® 18E System concordance testing in progress

## 5. Performance with Inhibitors

### PowerPlex® 35GY System (60 to 500bp)



Control
500µM Hematin 100% Allele Call (35/35 Loci)
750µM Hematin 77% Allele Call (24/35 Loci)
1000µM Hematin 37% Allele Call (13/35 Loci)

### PowerPlex® 18E System (60 to 350bp)



Control
500µM Hematin 100% Allele Call (18/18 Loci)
750µM Hematin 100% Allele Call (18/18 Loci)
1000µM Hematin 59% Allele Call (11/18 Loci)

PowerPlex® 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® HID Software for Spectrum CE Systems.

## 6. Quality Indicator Performance
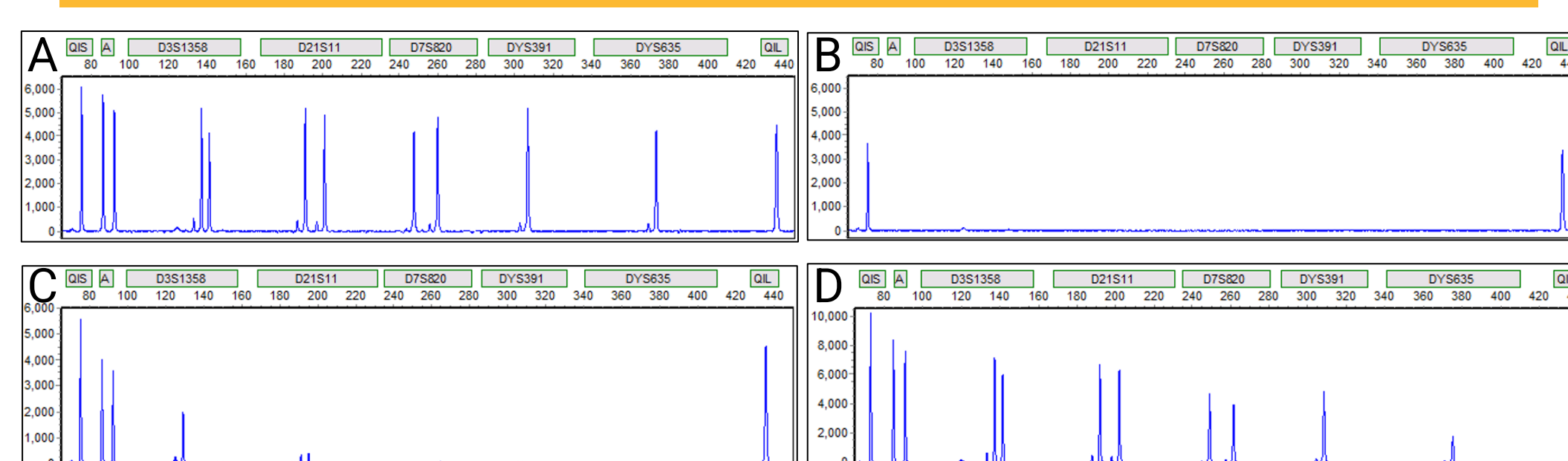


Quality Indicator peaks (QIS and QIL) provide data interpretation assistance.

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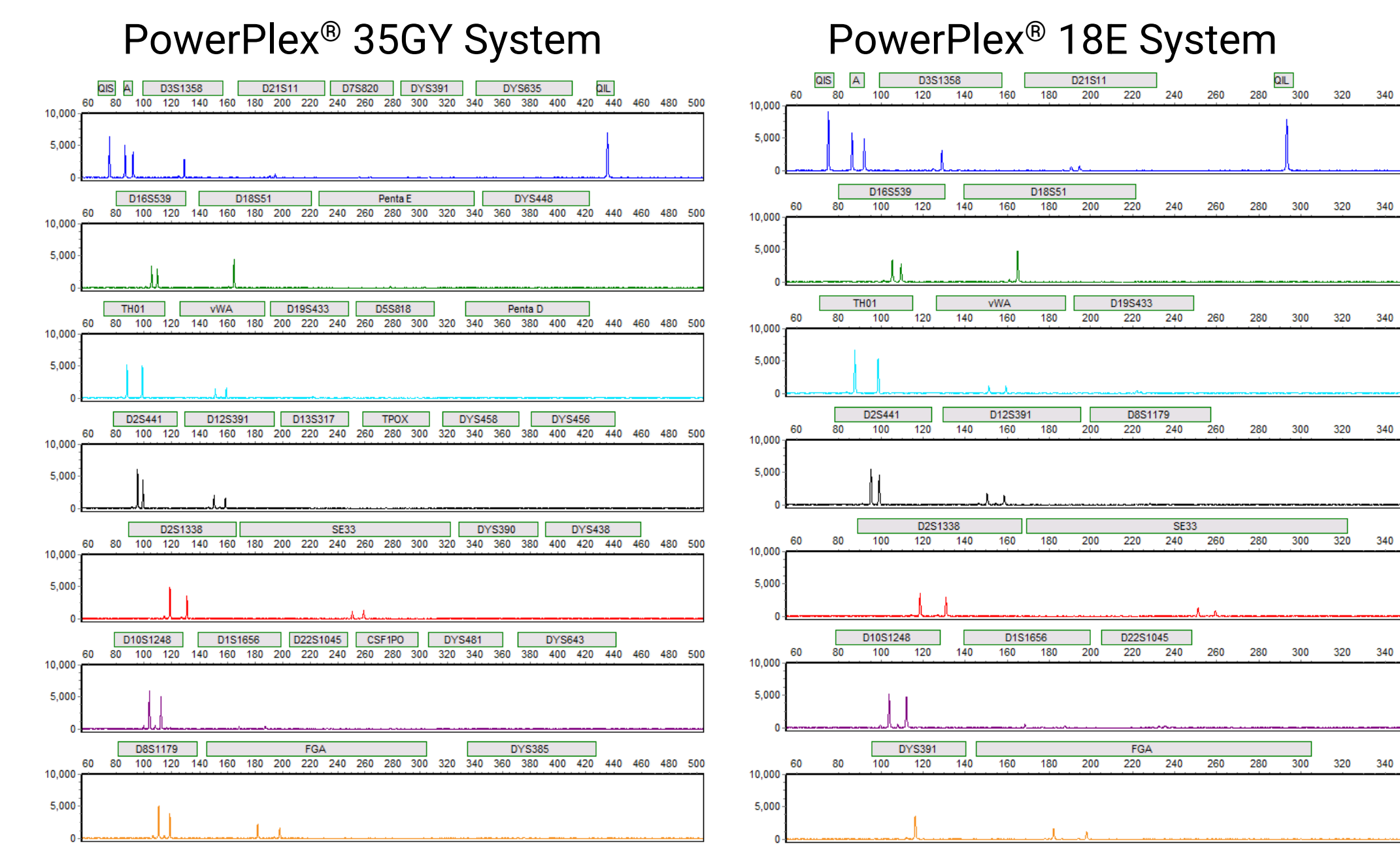D. Sloping STR profile with low or missing QIL peak – inhibited sample

## 7. Ancient Bone Sample


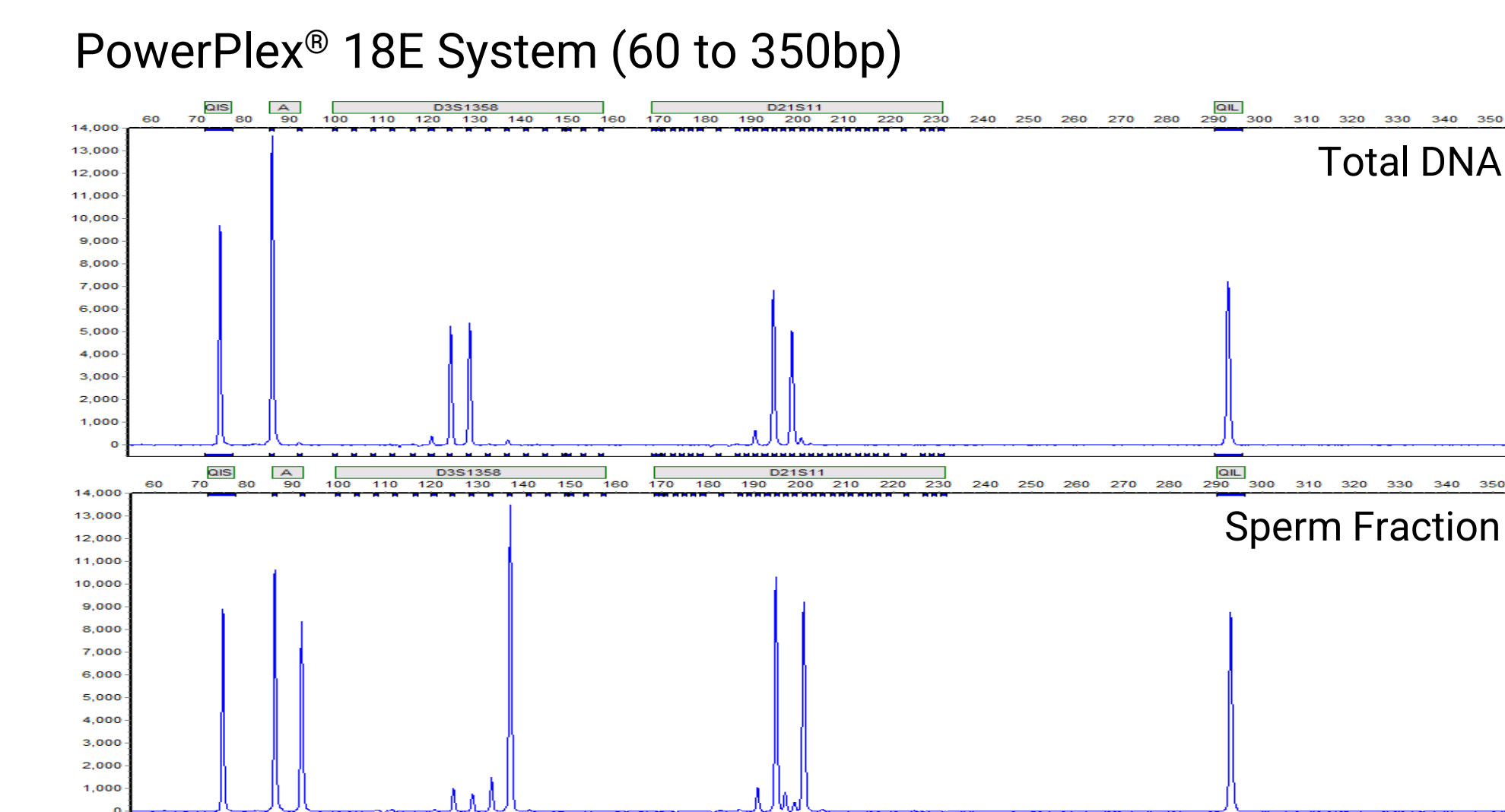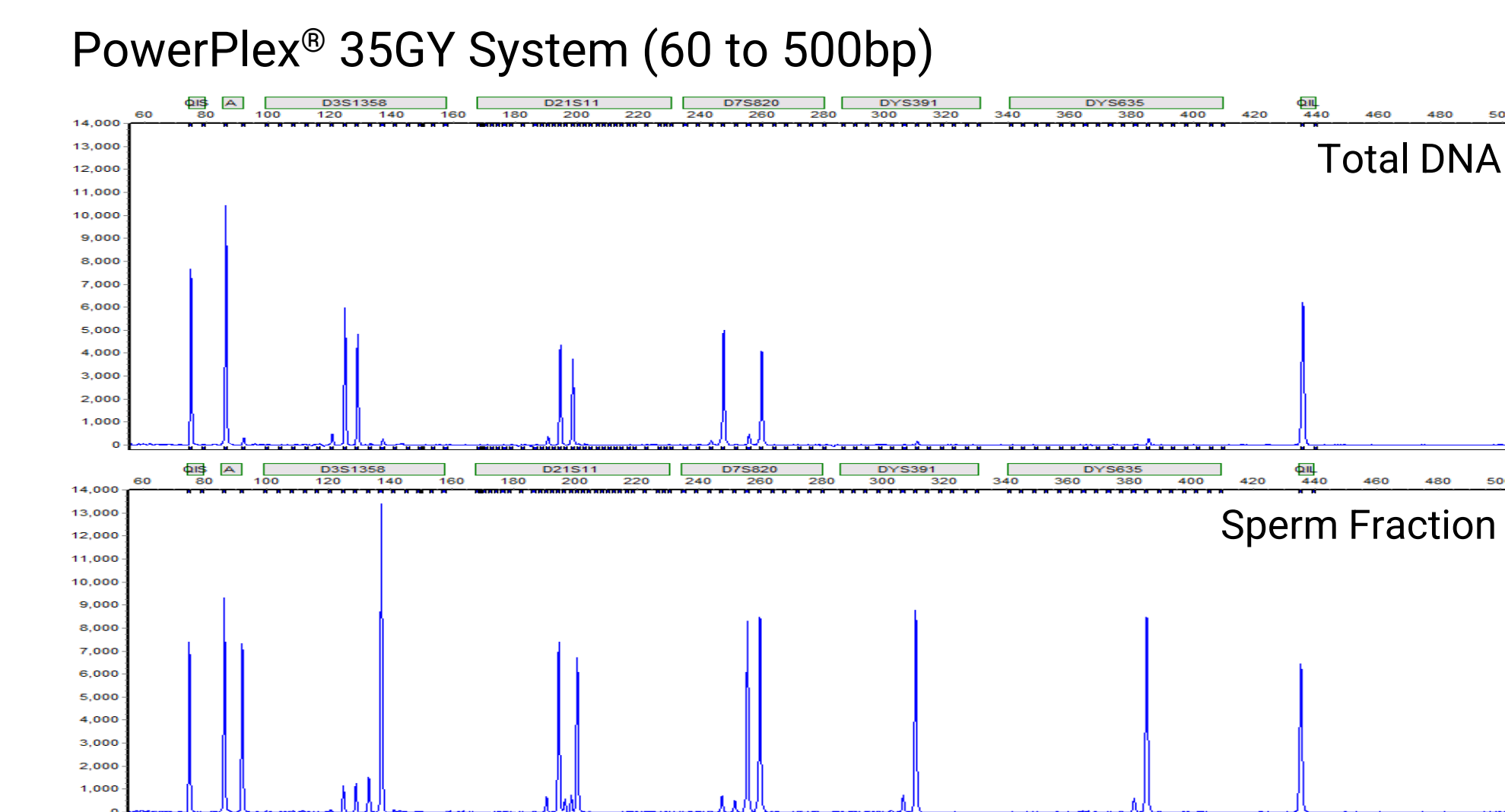
PowerPlex® 35GY System   PowerPlex® 18E System

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

| [Auto] | [Deg] | [Y] | [Auto]/[Y] | [Auto]/[Deg] |
|--------|-------|-----|-----------|--------------|
| 0.09905 | 0.0009 | 0.03235 | 3.06 | 110.06 |

PowerPlex® 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® 35GY system, and at 16 STR loci and Amelogenin with the PowerPlex® 18E system.

## 8. Differential Extraction Samples

### PowerPlex® 35GY System (60 to 500bp)



Total DNA
Sperm Fraction

### PowerPlex® 18E System (60 to 350bp)



Total DNA
Sperm Fraction

PowerPlex® 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

**The PowerPlex® 35GY and 18E Systems are 8-color STR chemistries**
- Designed for amplification of purified DNA and direct amplification
- 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® 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® 35GY and 18E Systems is the smallest of any current STR chemistry on the market