Enhanced Performance from a Prototype Eight-Dye, CODIS-Focused STR System

Nicholas A Courtney‡, Karthika Divakaran‡, Brittany C Hudson, David W Nelson, Rachel Knoener, Heather Hodges, Paula Sequeira, Amanda Marquard, Bryan Gehrke, Cassidy Ames, Dawn Rabbach, Michael Lauck, and Robert S McLaren

Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

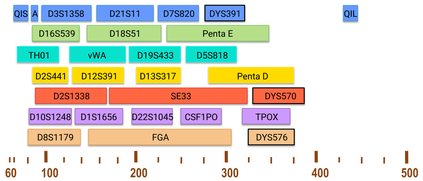
‡ denotes co-first authorship

1. Introduction and Prototype 27GY System Layout

Traditional capillary electrophoresis (CE) is widely used for forensic DNA typing due to its time- and cost-effectiveness. Eight-color STR Systems on the Spectrum CE and Spectrum Compact CE platforms enhance performance by distributing loci across more dye channels, thereby effectively reducing amplicon size. Here, we have developed a new eight-color STR System suitable for both casework and direct amplification samples that simultaneously amplifies the 20 CODIS core loci along with Penta D, Penta E and SE33 to increase discrimination and allow for wider database searching. Amelogenin and DYS391 are included for gender determination, as well as two rapidly mutating Y-STR loci (DYS570 and DYS576) and two Quality Indicators (QI). By focusing on the core CODIS loci and utilizing 8 colors, this advanced STR system can improve the success rate of generating CODIS-eligible profiles from challenging samples, such as those with degraded or low input DNA.

The Prototype 27GY System employes a novel PCR system to drastically reduce stutter artifacts across all loci, while maintaining similar workflows and sensitivity compared to traditional STR systems. The data presented here demonstrates how this drastic reduction in stutter artifacts directly translates into easier profile interpretation, simpler mixture deconvolution, and better number of contributor (NOC) determination, underscoring the unique advantages of the Prototype 27GY System.

Prototype 27GY System Layout:



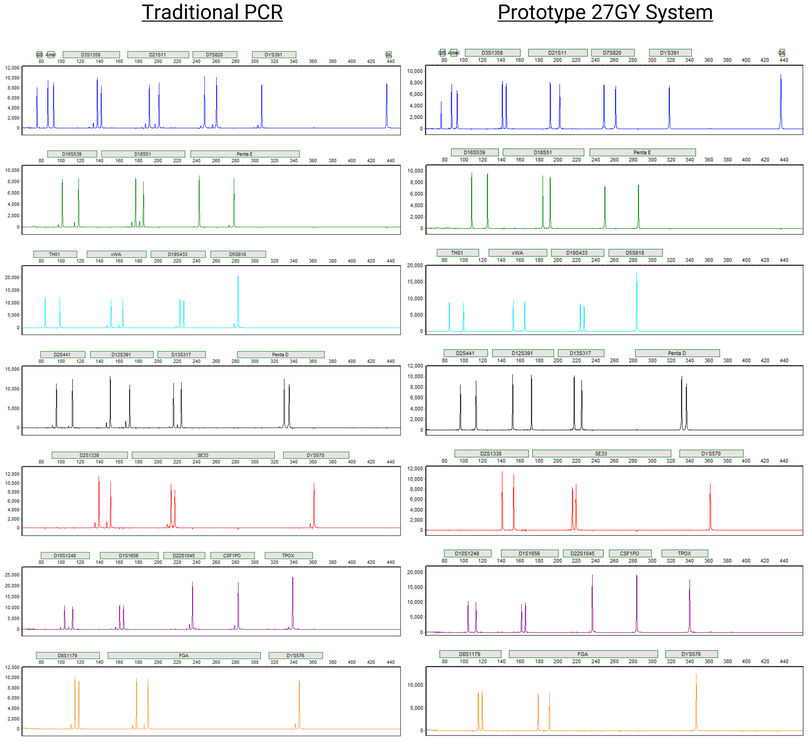
QIS = Quality Indicator Small; QIL = Quality Indicator Large

Key features:

- Minus stutter has been reduced by almost an order of magnitude across all loci.
- All 20 expanded CODIS Core Loci are under 375bp.
- Two quality indicators (QI) allow for distinction between degraded DNA and inhibited PCR reactions.

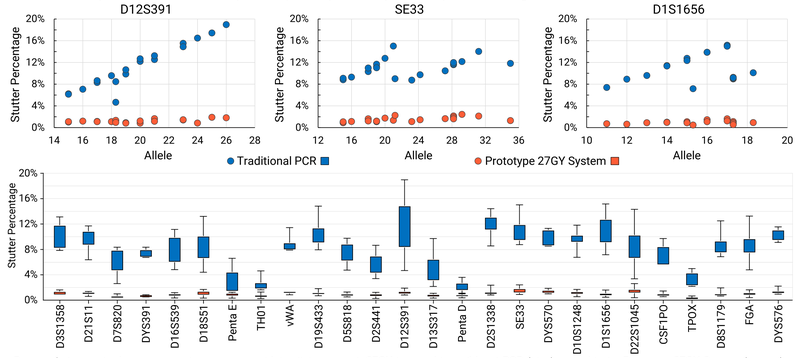
2. Amplification of Purified DNA

- 1ng of 2800M control DNA was amplified using either traditional PCR or the Prototype 27GY System.
- The Prototype 27GY System uses similar primer sequences, reaction setups, and cycling parameters as the Traditional PCR approach. Both systems were amplified in 25µl reaction volumes using 29 cycles.
- Amplification products were injected on a Spectrum Compact CE and analyzed using GeneMarker® HID Software for Spectrum CE Systems using standard injection conditions.



3. Minus Stutter Quantification by Locus and Allele

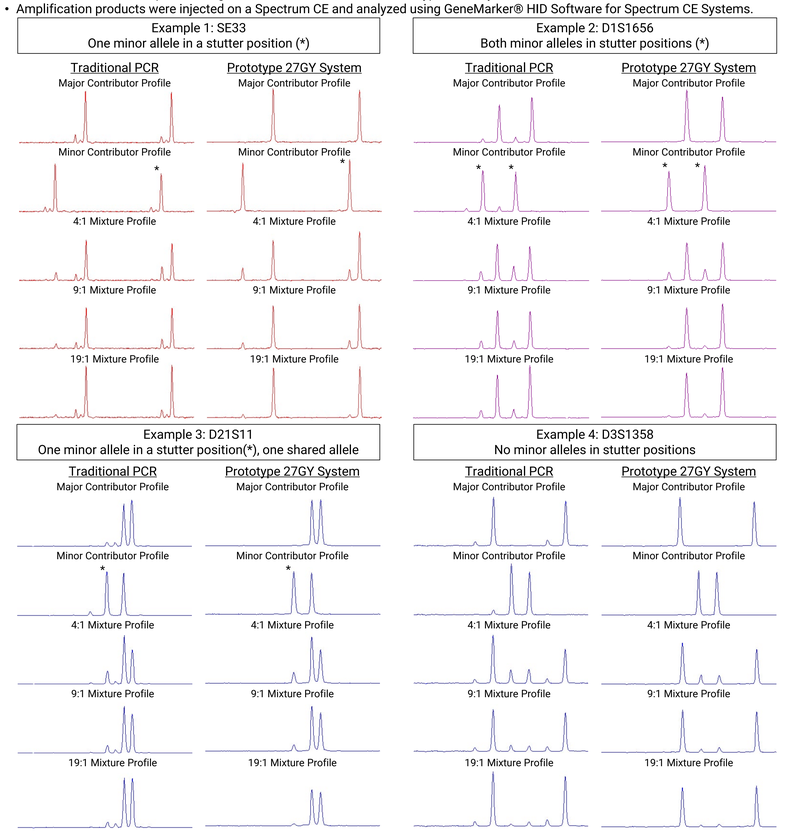
- DNA samples from 13 individuals were amplified using either traditional PCR or the Prototype 27GY System.
- Amplification products were injected on a Spectrum CE and analyzed using GeneMarker® HID Software for Spectrum CE Systems.
- Minus stutter peaks (i.e., minus one repeat unit) were quantified per locus, per allele using a 20RFU threshold.



Range of observed minus stutter values across multiple alleles at each 27GY locus with traditional PCR (blue) and with the Prototype 27GY System (orange). Boxes depict inter-quartile range (25th-75th percentile). Whiskers depict the minimum and maximum stutter values.

4. Two-Person Mixture Samples

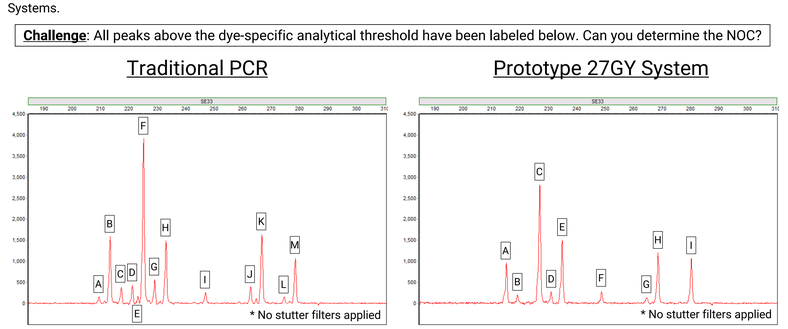
- Mock 2-person samples were created by mixing a designated major and minor contributor's DNA at the indicated ratios.
- These mixtures were amplified using either traditional PCR or the Prototype 27GY System.
- Amplification products were injected on a Spectrum CE and analyzed using GeneMarker® HID Software for Spectrum CE Systems.



5. Number of Contributors (NOC) Determination

- A mock forensic sample was created by mixing multiple known contributors' DNA at varying ratios.
- This mixture was then amplified using either traditional PCR or the Prototype 27GY System using 1ng total DNA per reaction.
- Amplification products were injected on a Spectrum Compact CE and analyzed using GeneMarker® HID Software for Spectrum CE Systems.

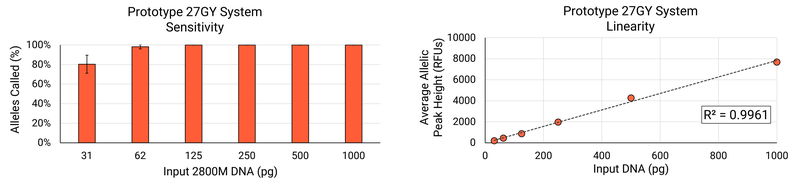
Challenge: All peaks above the dye-specific analytical threshold have been labeled below. Can you determine the NOC?



Answer Key: This is a 5-person mixture. Listed in order of abundance, individual donor genotypes in the Prototype 27GY System electropherogram are C,H (40%), C,E (30%), A,I (20%), B,G (5%), and D,F (5%). This corresponds to (F,K), (F,H), (B,M), (C,J), and (G,I) in the traditional PCR electropherogram.

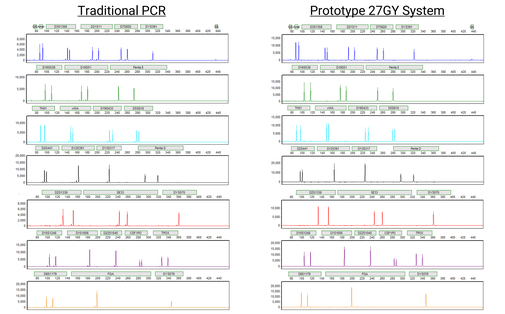
6. Sensitivity and Direct Amplification

- A sensitivity series was performed by amplifying a serial dilution of 2800M control DNA with the Prototype 27GY System (n=8 per dilution, 29 cycles of PCR).
- Amplification products were injected on a Spectrum CE and analyzed using GeneMarker® HID Software for Spectrum CE Systems.



Direct Amplification:

- A buccal swab was processed in 1mL SwabSolution™ using standard protocols.
- Direct amplification of the buccal swab sample was performed in 12.5µl reaction volumes using the following parameters.
  - Traditional PCR: 2µl swab extract and 25 cycles of PCR.
  - Prototype 27GY System: 2µl swab extract and 26 cycles of PCR.
- Amplification products were injected on a Spectrum CE and analyzed using GeneMarker® HID Software for Spectrum CE Systems.



7. Conclusions

The Prototype 27GY System has drastically reduced stutter artifacts, resulting in

- Easier profile interpretations.
- Simpler mixture deconvolutions.
- Better determination of the number of contributor (NOC).

The Prototype 27GY System utilizes eight colors to reduce amplicon sizing.

- All CODIS core loci are < 375 bp for better performance with challenging samples such as degraded DNA.

The Prototype 27GY System utilizes a similar workflow as traditional STR chemistries.

- Designed for amplification of purified DNA and direct amplification, with similar sensitivity compared to traditional STR systems.

Corresponding author: nick.courtney@promega.com