

Developmental Validation of the PowerPlex® Fusion System

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Abstract

The PowerPlex® Fusion System includes all the loci in Section A of the proposed expanded CODIS core loci (D.R. Hares, Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. 6 (2012) e135.). It also includes two loci from CODIS Section B (TPOX and D22S1045) and two highly polymorphic pentanucleotide loci, Penta E and Penta D. These Penta loci have been used worldwide for more than 10 years, and are included in greater than one million profiles in the CODIS database. This five color system can be used on the Applied Biosystems 3500 or 31xx series Genetic Analyzers. No upgrade is required for use on the 31xx series instruments. The PowerPlex® Fusion System has been commercially available since September 2012 and received NDIS approval in March 2013. Twelve laboratories participated in the developmental validation testing which followed the SWGDAM guidelines. Validation testing included extracted DNA, direct amplification of samples from on FTA® paper (GE Healthcare/Whatman), amplification of samples from buccal swabs pretreated with Promega's SwabSolution™ Kit, and samples from paper substrates pretreated with Promega's PunchSolution™ Kit.

System Configuration

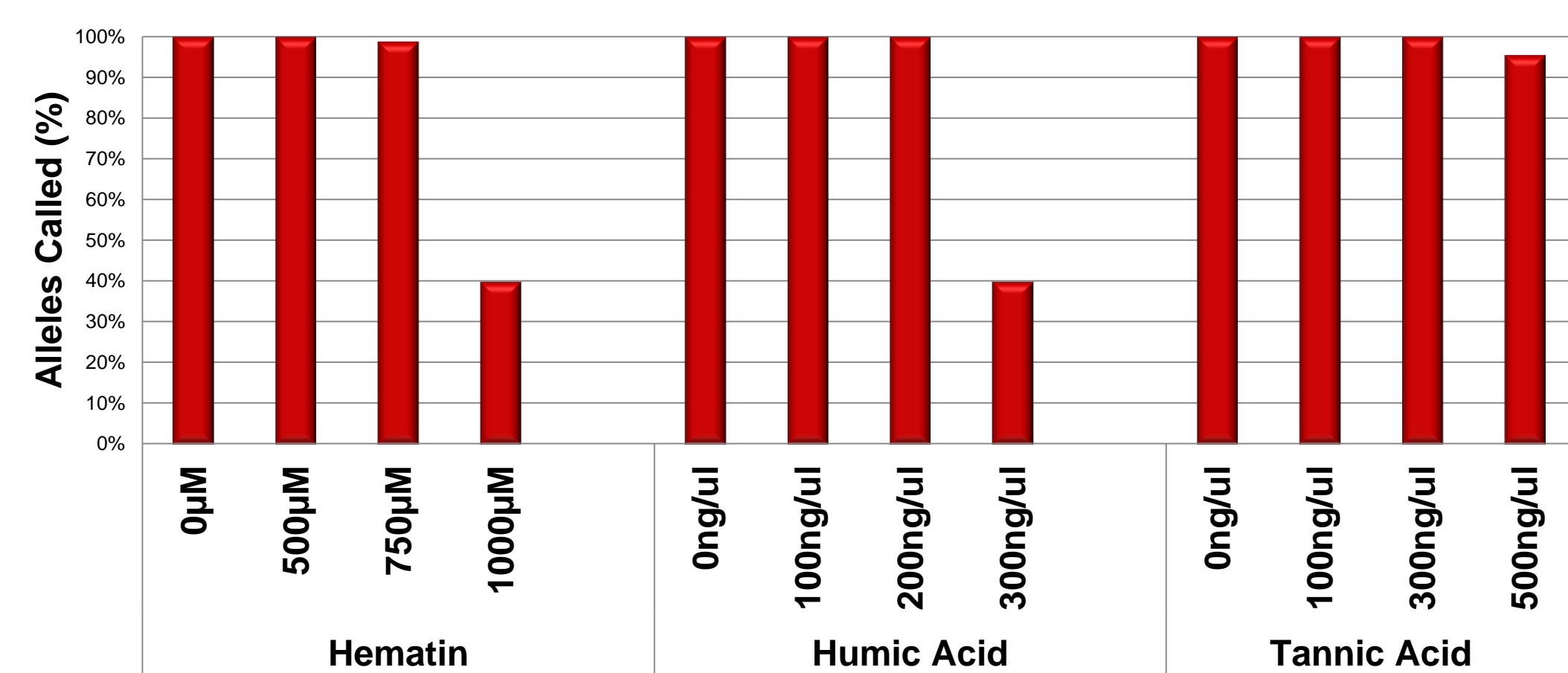


Probability of Identity for Various Sets of STR Loci

STR Typing Kits (Locus Combinations)	Total (N=1036)	African American (N=342)	U.S. Caucasian (N=361)	U.S. Hispanic (N=236)	U.S. Asian (N=97)
CODIS 13	5.02×10^{-16}	1.14×10^{-15}	2.97×10^{-15}	1.36×10^{-15}	1.71×10^{-14}
PowerPlex® 16	2.82×10^{-19}	6.09×10^{-19}	4.24×10^{-18}	1.26×10^{-18}	2.55×10^{-17}
PowerPlex® 18D	3.47×10^{-22}	5.60×10^{-22}	9.82×10^{-21}	2.54×10^{-21}	7.92×10^{-20}
GlobalFiler™	7.73×10^{-28}	3.20×10^{-27}	1.30×10^{-26}	2.27×10^{-26}	1.81×10^{-24}
PowerPlex® Fusion	6.58×10^{-29}	1.59×10^{-28}	2.35×10^{-27}	2.12×10^{-27}	1.42×10^{-25}

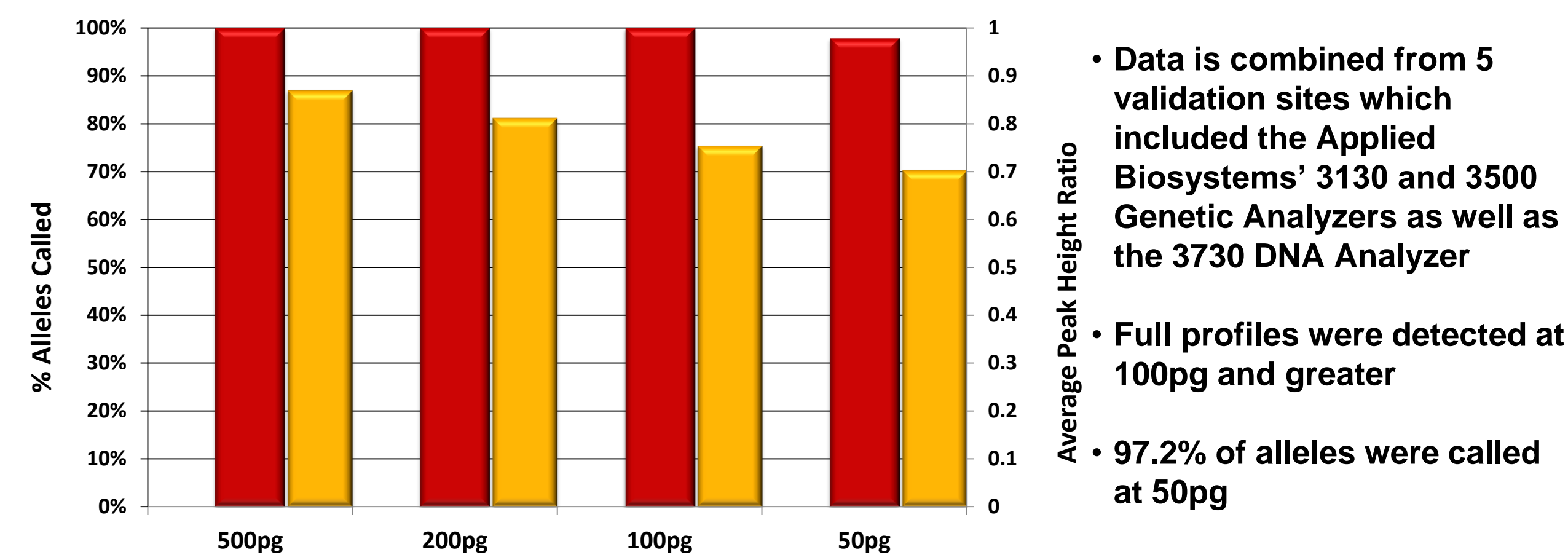
Butler, J.M., Hill, C.R. and Coble, M.D. Variability of New STR Loci and Kits in US Population Groups. [Internet] 2012. Available from: <http://www.promega.com/resources/articles/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups/>

Inhibitor Tolerance



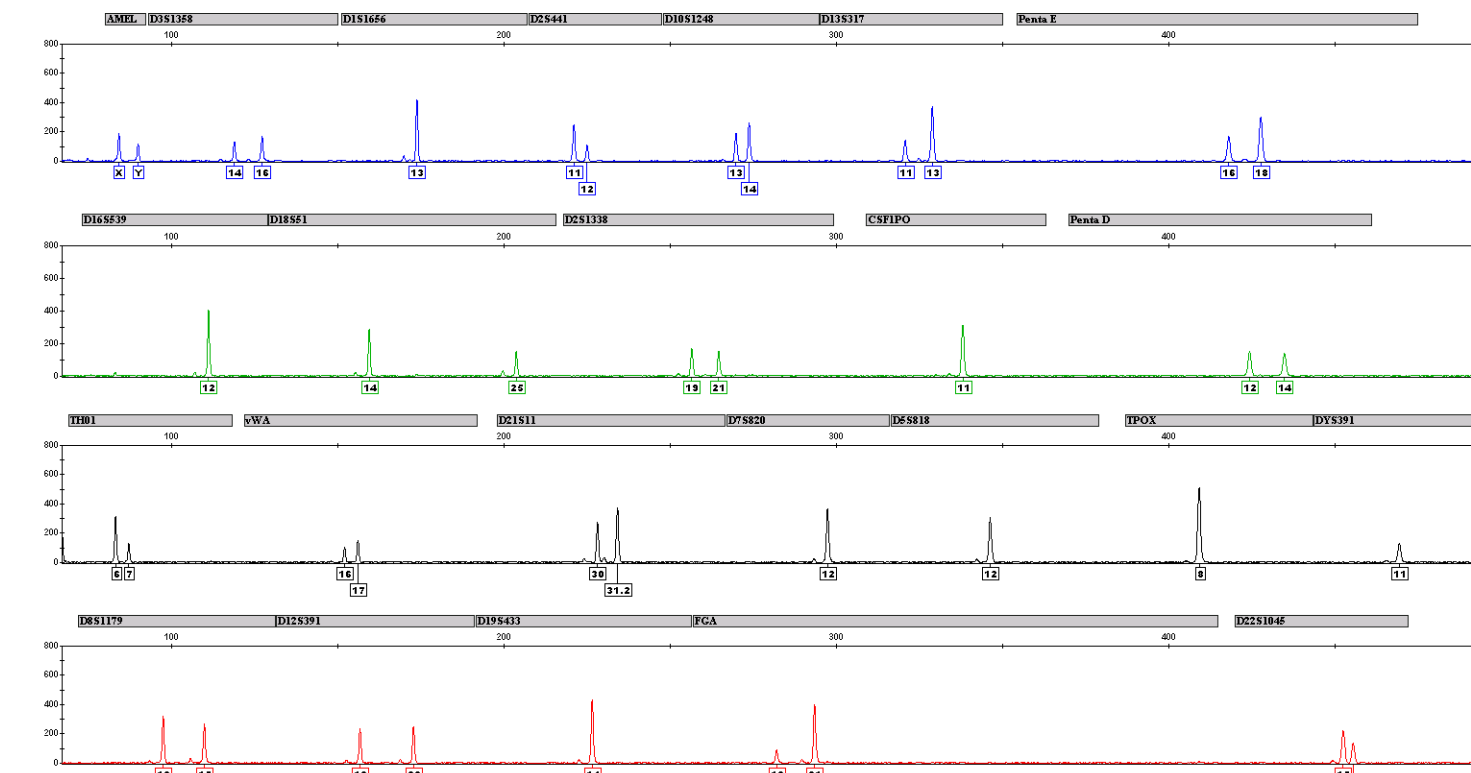
500pg of DNA (n=3) was amplified in the presence of the inhibitors

Sensitivity



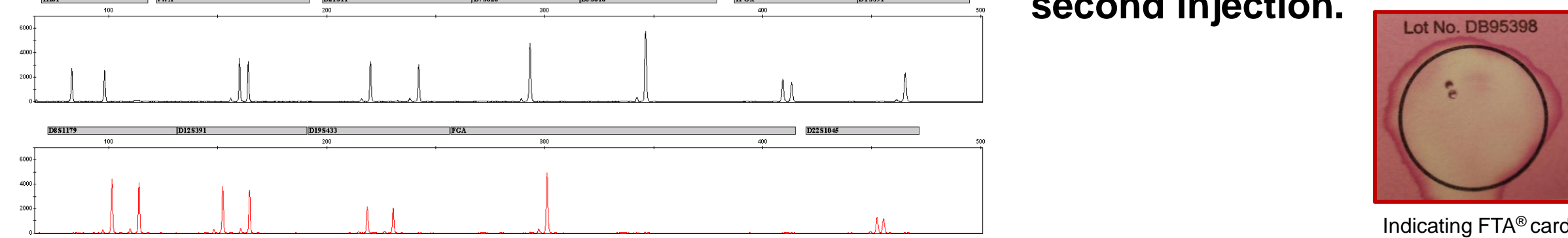
- Data is combined from 5 validation sites which included the Applied Biosystems' 3130 and 3500 Genetic Analyzers as well as the 3730 DNA Analyzer
- Full profiles were detected at 100pg and greater
- 97.2% of alleles were called at 50pg

Amplification (30 cycles) of a 50pg extracted DNA followed by electrophoresis on an Applied Biosystems' 3130x/ Genetic Analyzer using a 3kV, 5 second injection.

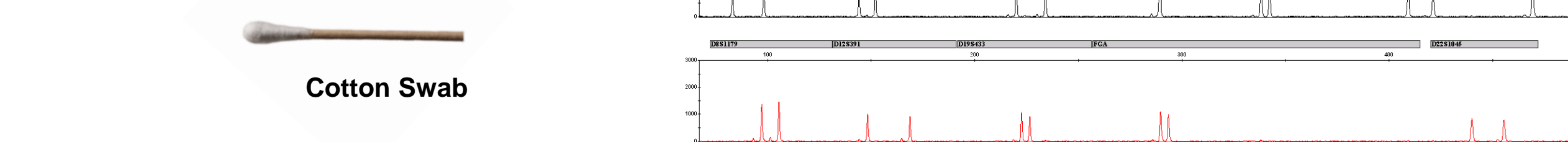


Direct Amplification

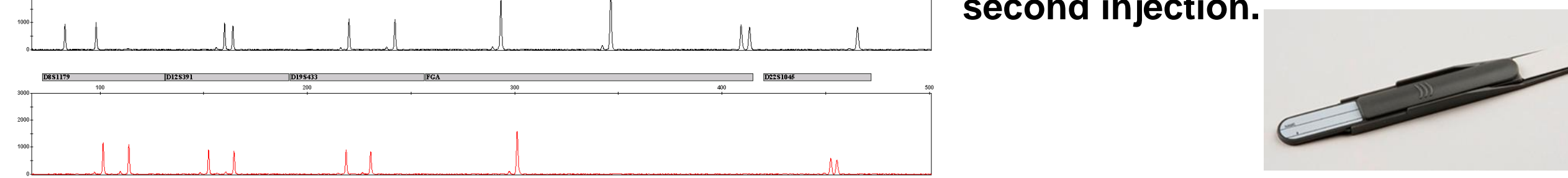
Amplification (27 cycles) of a 1.2mm punch of a buccal sample on FTA® Paper followed by electrophoresis on an Applied Biosystems' 3130x/ Genetic Analyzer using a 3kV, 5 second injection.



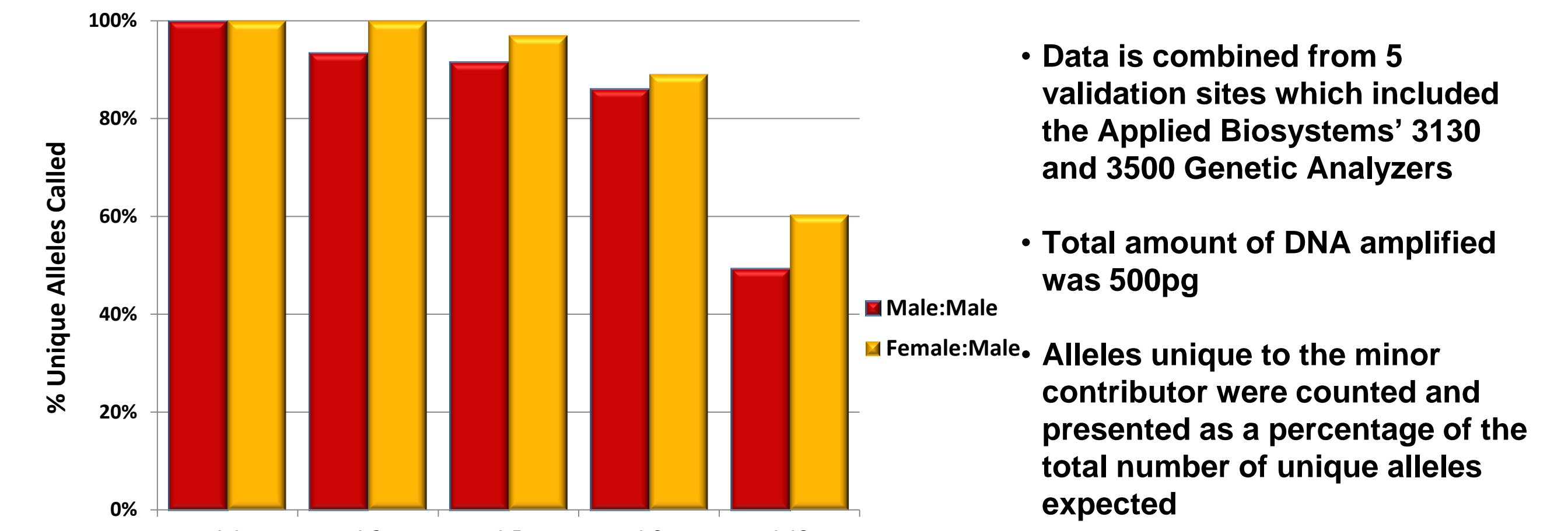
Amplification (27 cycles) of 2µl of a swab lysate pretreated with SwabSolution™ followed by electrophoresis on an Applied Biosystems' 3130x/ using a 3kV, 5 second injection.



Amplification (27 cycles) of a 1.2mm punch of a buccal sample on a Bode Buccal Collector pretreated with PunchSolution™ followed by electrophoresis on an Applied Biosystems' 3130x/ using a 3kV, 5 second injection.

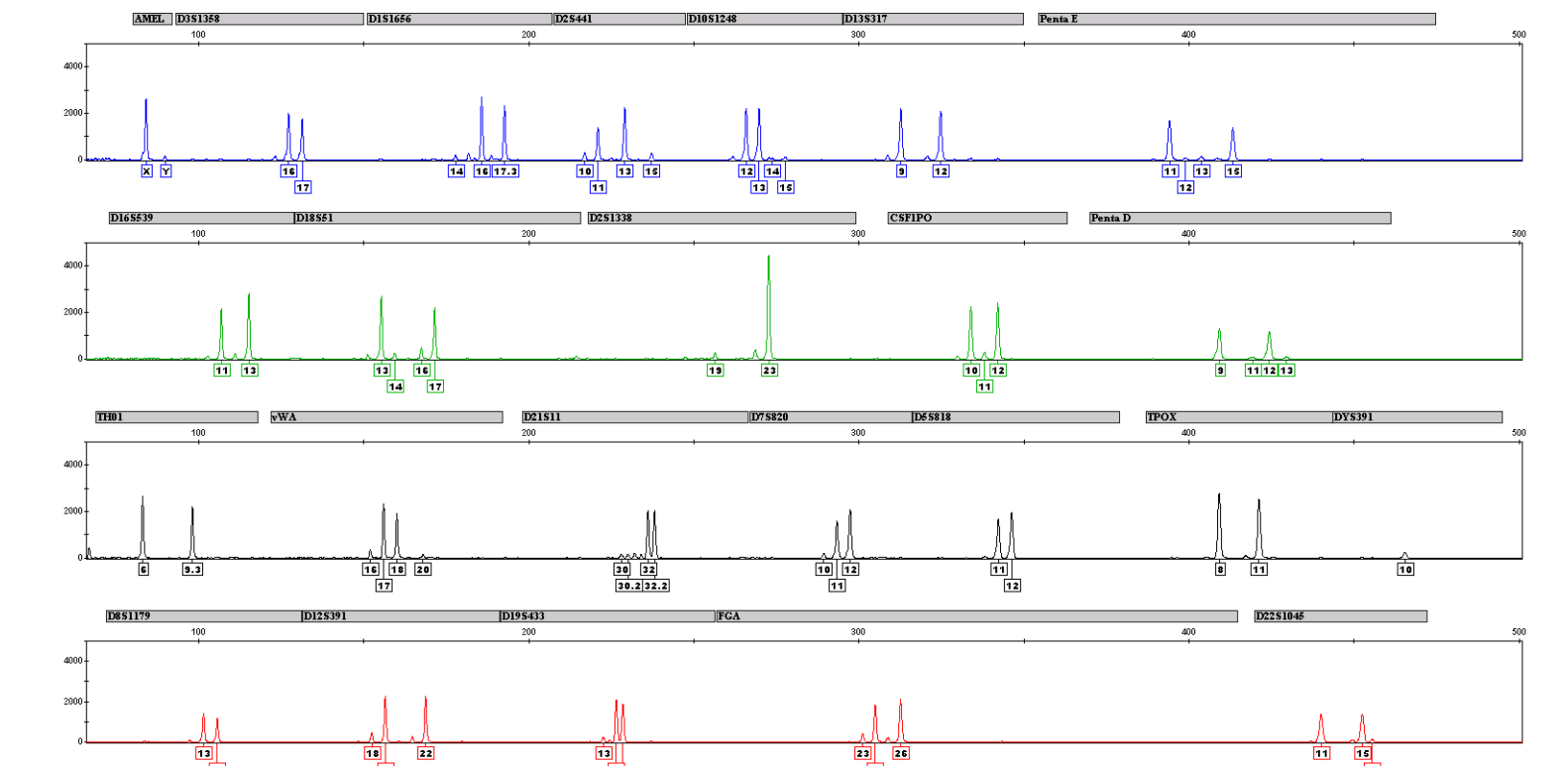


Mixtures



- Data is combined from 5 validation sites which included the Applied Biosystems' 3130 and 3500 Genetic Analyzers
- Total amount of DNA amplified was 500pg
- Alleles unique to the minor contributor were counted and presented as a percentage of the total number of unique alleles expected

Amplification (30 cycles) of a 1:9 mixture (500pg) Male:Female DNA followed by electrophoresis on an Applied Biosystems' 3130x/ Genetic Analyzer using a 3kV, 5 second injection.



Summary

- The PowerPlex® Fusion System is a rapid (1.5 hour thermal cycling), robust and sensitive system that meets the requirements of the new CODIS Standard.
- The system is based upon the same proven five color chemistry used in several of the systems Promega introduced in the last two years. This allows for use of the kits on existing configurations of the Applied Biosystems 3100/3130 and 3500 series Genetic Analyzers.
- The PowerPlex® Fusion System is a one kit solution for both extracted DNA and direct amplification. The PowerPlex® Fusion System is optimized for extracted DNA, but allows for direct amplification from a variety of substrates.

Acknowledgements

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Contributors	Laboratory
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