

Promega Corporation

PowerPlex[®] Fusion 6C System

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October 8th 2014

Disclaimer

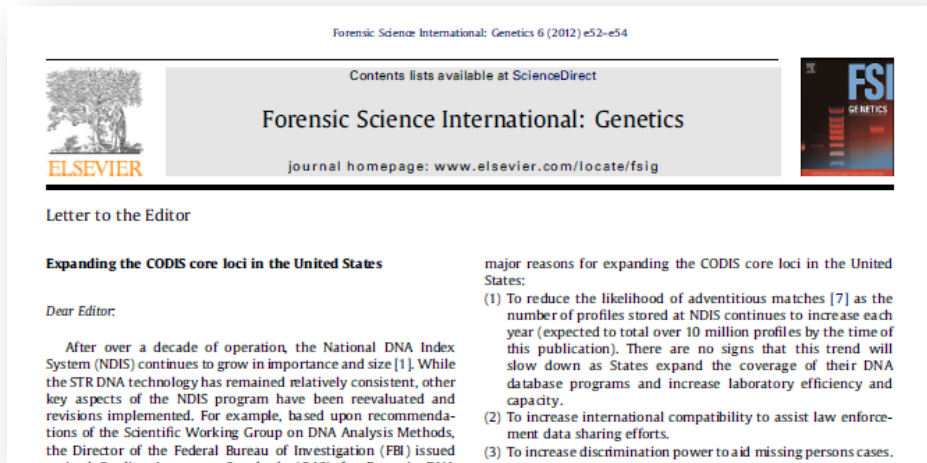
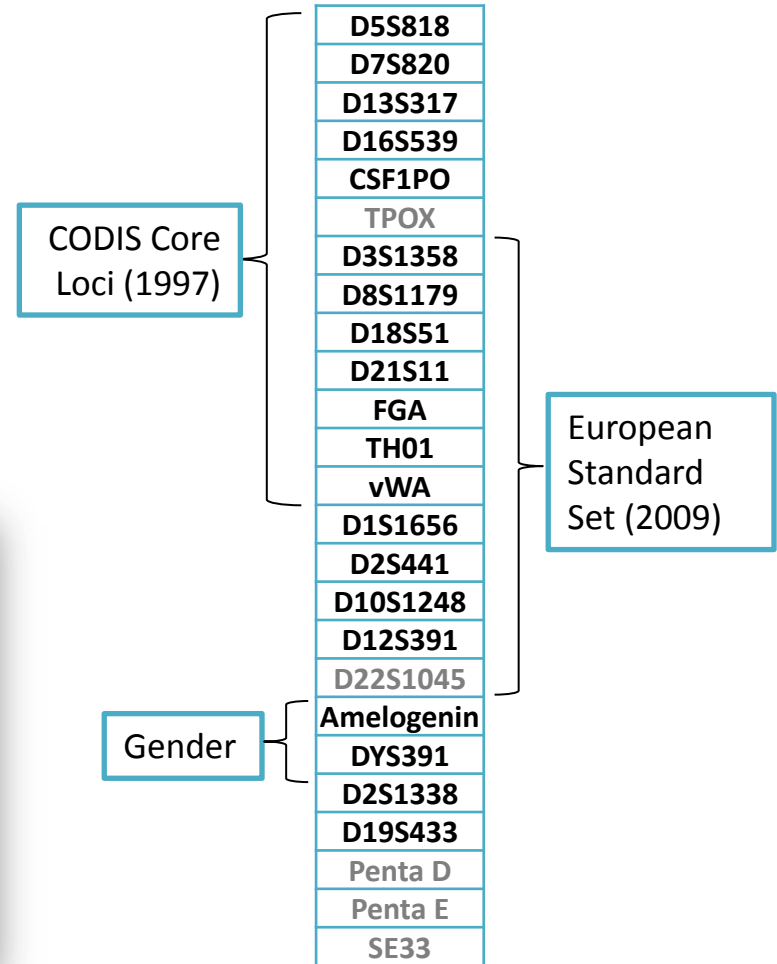
PowerPlex® Fusion 6C is still in development.

It is possible that small changes (to protocol, etc.) occur between now and product launch.

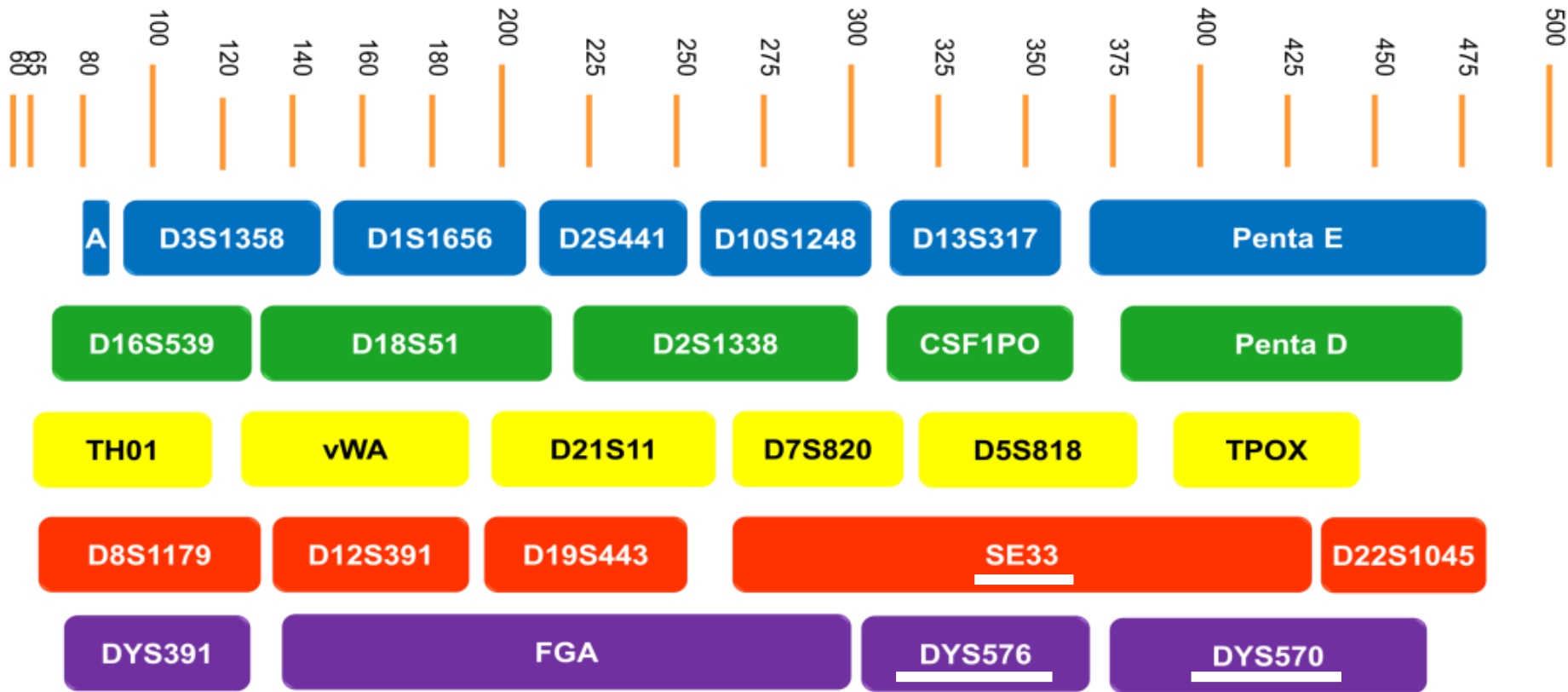
Expansion of Databases -> Expansion of STR Multiplex

CODIS core loci expansion :

- Minimize adventitious matches
- Improve international data sharing
- Increase discrimination power to aid missing persons cases



23 Autosomal STR + 3 Y-STR + Amelogenin



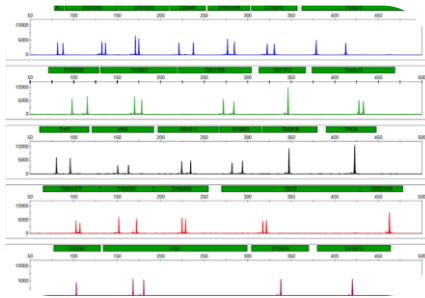
A= Amelogenin

More Loci -> Higher Power of Discrimination

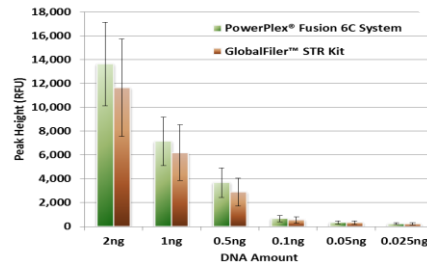
STR Kit	Number of Loci	PI (N = 1036)	How Much Better than CODIS 13?
CODIS Core 13	13	5.02×10^{-16}	-
ESS 12	12	3.04×10^{-16}	-
CODIS Core 20	20	9.35×10^{-24}	10^8
GlobalFiler™ STR Kit	23	7.73×10^{-28}	10^{12}
Investigator 24plex Kit	23	7.73×10^{-28}	10^{12}
PowerPlex® Fusion System	24	6.58×10^{-29}	10^{13}
PowerPlex® Fusion 6C System	27	1.80×10^{-32}	10^{17}

- Butler, J.M., Hill, C.R. and Coble, M.D. (2012) Variability of New STR Loci and Kits in US Population Groups. *Profiles in DNA*
- PI values do not include Amelogenin or Y-indel

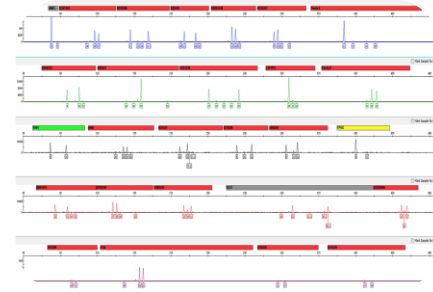
PowerPlex® Fusion 6C System Performance



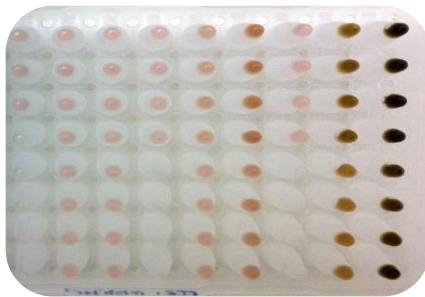
Balanced



Sensitive



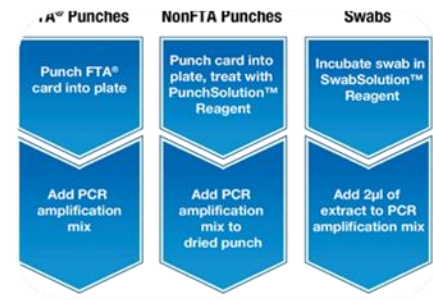
Informative in Mixtures



Robust



Fast Cycling



Direct Amp Compatible

System Components

Pre-Amplification:

Amplification Grade Water

PowerPlex® Fusion 6C 5X Master Mix

PowerPlex® Fusion 6C 5X Primer Pair Mix

2800M DNA

Post-Amplification:

WEN-ILS 500

PowerPlex® Fusion 6C Allelic Ladder Mix

Accessory Products:

PowerPlex® 6C Matrix Standard

PunchSolution™ Kit

SwabSolution™ Kit

5X AmpSolution™ Reagent



System Compatibility

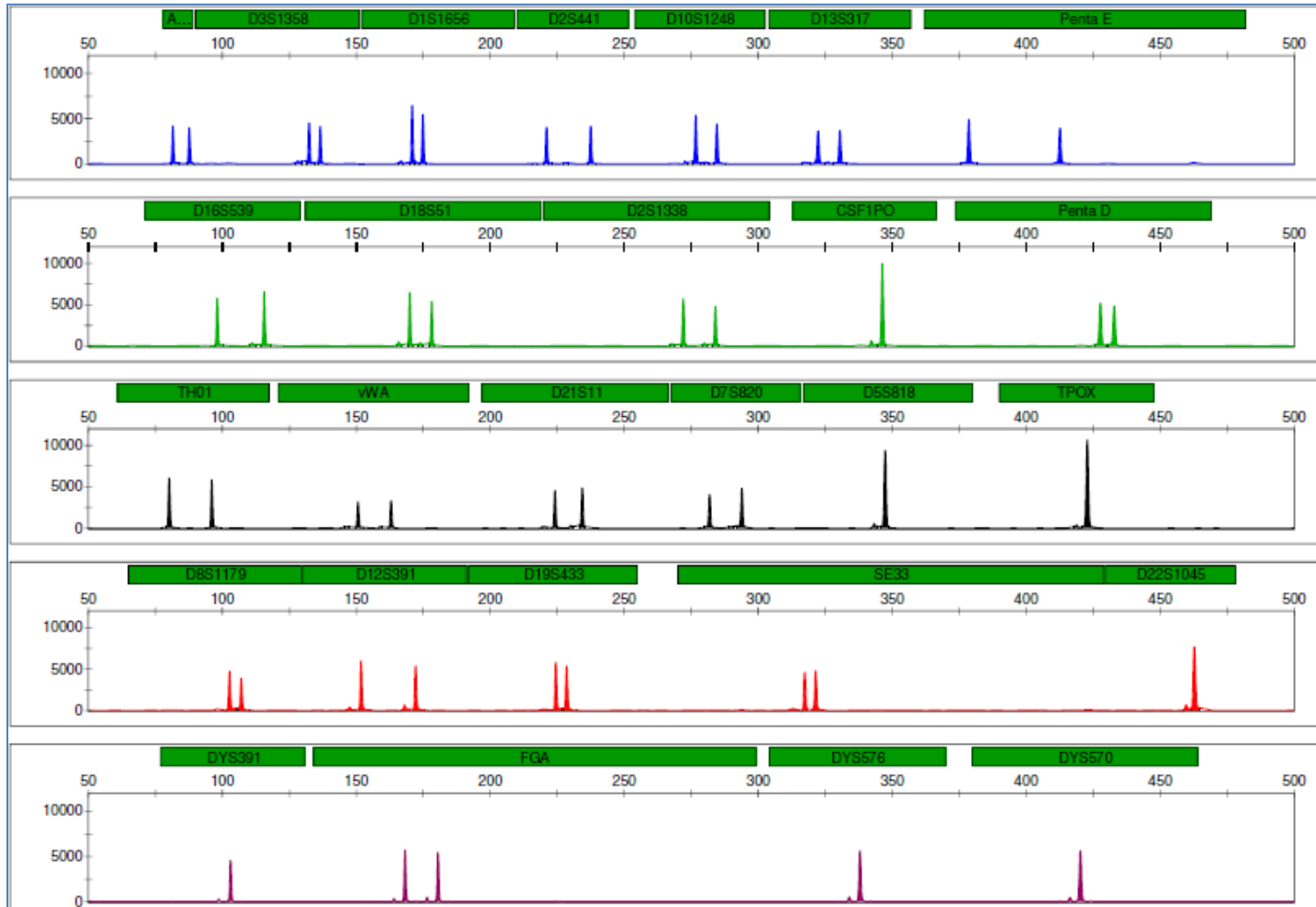
Compatibility	Fusion	Fusion 6C
Thermal cycler	GeneAmp® PCR System 9700	GeneAmp® PCR System 9700 Veriti® 96-well Thermal Cycler
CE instrument	5-dye: 31XX and 3500	6-dye: 3500 and upgraded 3130
Data collection software	<ul style="list-style-type: none"> • 3500: Data Collection v1 or v2 • 31XX: Data Collection v3.0 • 3100: Data Collection v2.0 	<ul style="list-style-type: none"> • 3500: Data Collection v1 or v2 • 3130: Data Collection v4 + 6 Dye Module License
Fragment analysis software	<ul style="list-style-type: none"> • GeneMapper® ID • GeneMapper® ID-X v1.3 (female samples will be flagged for the Y-STR) • GeneMapper® ID-X v1.4 	<ul style="list-style-type: none"> • GeneMapper® ID-X v1.3 (female samples will be flagged for the Y-STR) • GeneMapper® ID-X v1.4

GeneMapper® ID-X version 1.4: Y-Marker Check Box

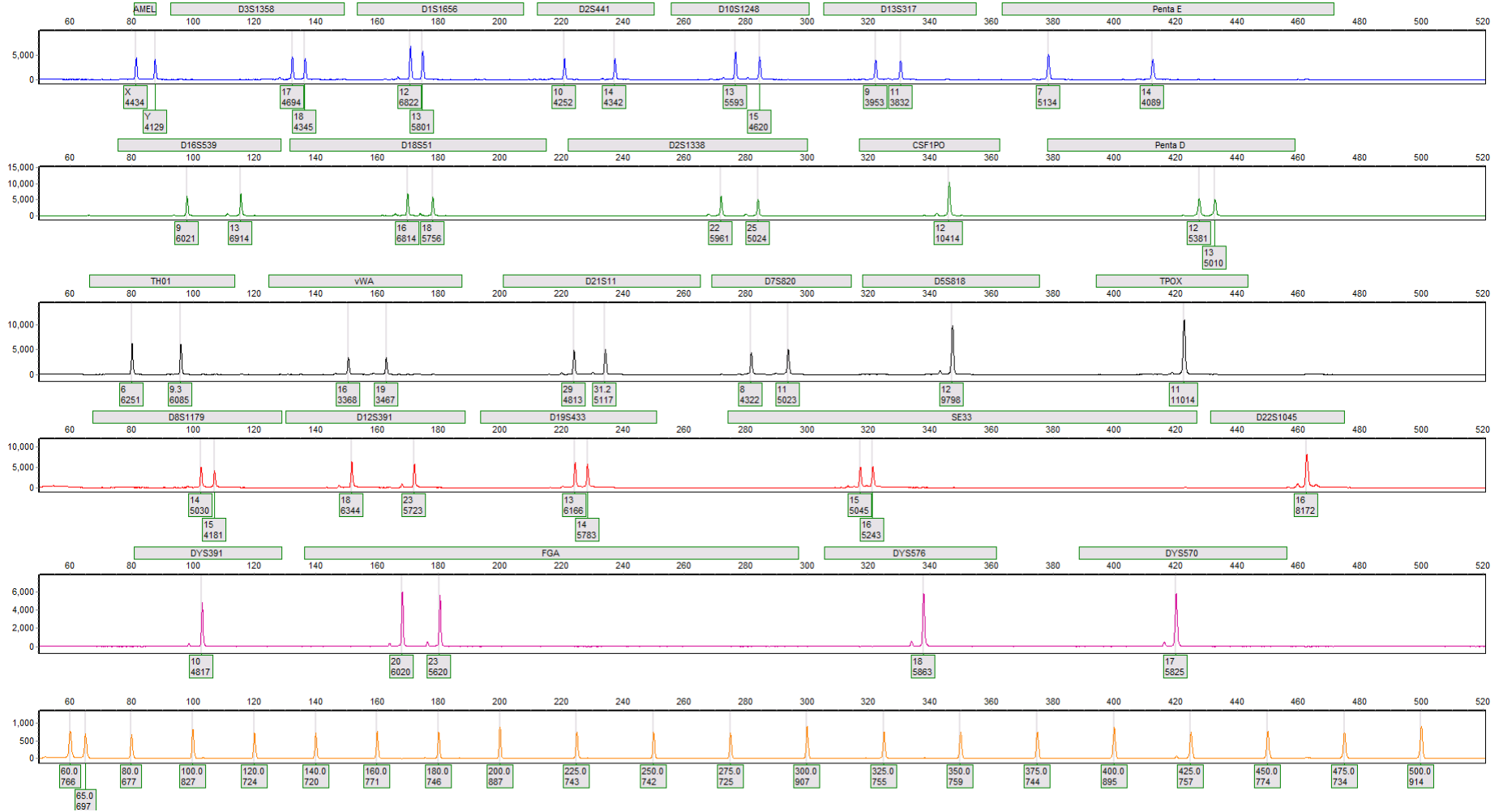
owerPlex_6dye_Bins_IDX_v2.0

Marker Name	Dye Color	Min. Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1 AMEL	Blue	83.0	92.5	X,Y	9	none	<input type="checkbox"/>	X,Y
2 D3S1358	Blue	93.0	151.5	17,18	4	none	<input type="checkbox"/>	9,10,11,12,13,14,15,16,17,18,19,20
3 D151656	Blue	153.5	208.5	12,13	4	none	<input type="checkbox"/>	9,10,11,12,13,14,14.3,15,15.3,16,16.3,17,17.3,18,18.3,19,19.3,20.3
4 D25441	Blue	211.0	251.0	10,14	4	none	<input type="checkbox"/>	8,9,10,11,11.3,12,13,14,15,16,17
5 D1051248	Blue	254.0	301.5	13,15	4	none	<input type="checkbox"/>	8,9,10,11,12,13,14,15,16,17,18,19
6 D135317	Blue	302.5	353.0	9,11	4	none	<input type="checkbox"/>	5,6,7,8,9,10,11,12,13,14,15,16,17
7 Penta E	Blue	357.0	476.0	7,14	5	none	<input type="checkbox"/>	5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
8 DY5391	Green	76.0	126.1	10	4	none	<input checked="" type="checkbox"/>	5,6,7,8,9,10,11,12,13,14,15,16
9 D18551	Green	129.1	215.5	16,18	4	none	<input type="checkbox"/>	7,8,9,10,10.2,11,12,13,13.2,14,15,16,17,18,19,20,21,22,23,24,25,26,27
10 D251338	Green	218.0	299.0	22,25	4	none	<input type="checkbox"/>	10,12,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28
11 CSF1PO	Green	309.0	363.0	12	4	none	<input type="checkbox"/>	5,6,7,8,9,10,11,12,13,14,15,16
12 Penta D	Green	370.0	461.0	12,13	5	none	<input type="checkbox"/>	2.2,3.2,5,6,7,8,9,10,11,12,13,14,15,16,17
13 TH01	Yellow	69.0	118.0	6,9,3	4	none	<input type="checkbox"/>	3,4,5,6,7,8,9,9.3,10,11,13.3
14 vWA	Yellow	122.0	192.0	16,19	4	none	<input type="checkbox"/>	10,11,12,13,14,15,16,17,18,19,20,21,22,23,24
15 D21511	Yellow	198.0	266.5	29,31.2	4	none	<input type="checkbox"/>	24,24.2,25,25.2,26,27,28,28.2,29,29.2,30,30.2,31,31.2,32,32.2,33,33.2,34,34.2,35,35.2,36,37,38
16 D75820	Yellow	267.0	316.0	8,11	4	none	<input type="checkbox"/>	5,6,7,8,9,10,11,12,13,14,15,16
17 D55818	Yellow	316.5	379.0	12	4	none	<input type="checkbox"/>	6,7,8,9,10,11,12,13,14,15,16,17,18
18 TPOX	Yellow	387.0	443.5	11	4	none	<input type="checkbox"/>	4,5,6,7,8,9,10,11,12,13,14,15,16
19 D851179	Red	72.0	131.2	14,15	4	none	<input type="checkbox"/>	7,8,9,10,11,12,13,14,15,16,17,18,19
20 D125391	Red	131.4	191.0	18,23	4	none	<input type="checkbox"/>	14,15,16,17,17.3,18,18.3,19,20,21,22,23,24,25,26,27
21 D195433	Red	191.5	256.5	13,14	4	none	<input type="checkbox"/>	5.2,6.2,8,9,10,11,12,12.2,13,13.2,14,14.2,15,15.2,16,16.2,17,17.2,18,18.2
22 SE33	Red	262.01	427.0	15,16	4	none	<input type="checkbox"/>	4.2,6.3,8,9,10,11,12,13,14,15,16,17,18,19,20,20.2,21,21.2,22,22.2,23.2,24.2,25.2,26.2,27.2,28.2,
23 D2251045	Red	428.0	479.0	16	3	none	<input type="checkbox"/>	7,8,9,10,11,12,13,14,15,16,17,18,19,20
24 D165539	Purple	75.0	135.0	9,13	4	none	<input type="checkbox"/>	4,5,6,7,8,9,10,11,12,13,14,15,16
25 FGA	Purple	136.0	297.0	20,23	4	none	<input type="checkbox"/>	14,15,16,17,18,18.2,19,19.2,20,20.2,21,21.2,22,22.2,23,23.2,24,24.2,25,25.2,26,27,28,29,30,31.2
26 DY5576	Purple	301.0	370.0	18	4	none	<input checked="" type="checkbox"/>	11,12,13,14,15,16,17,18,19,20,21,22,23
27 DY5570	Purple	380.0	464.0	17	4	none	<input checked="" type="checkbox"/>	10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25

Balanced with 1ng 2800M x 29 Cycles on a 3500



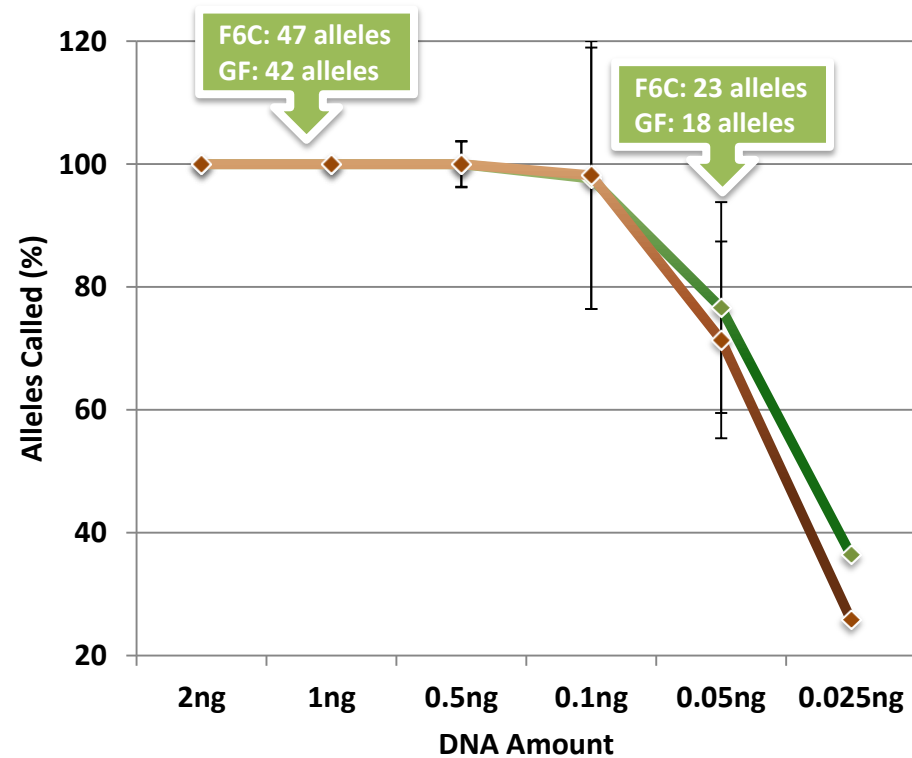
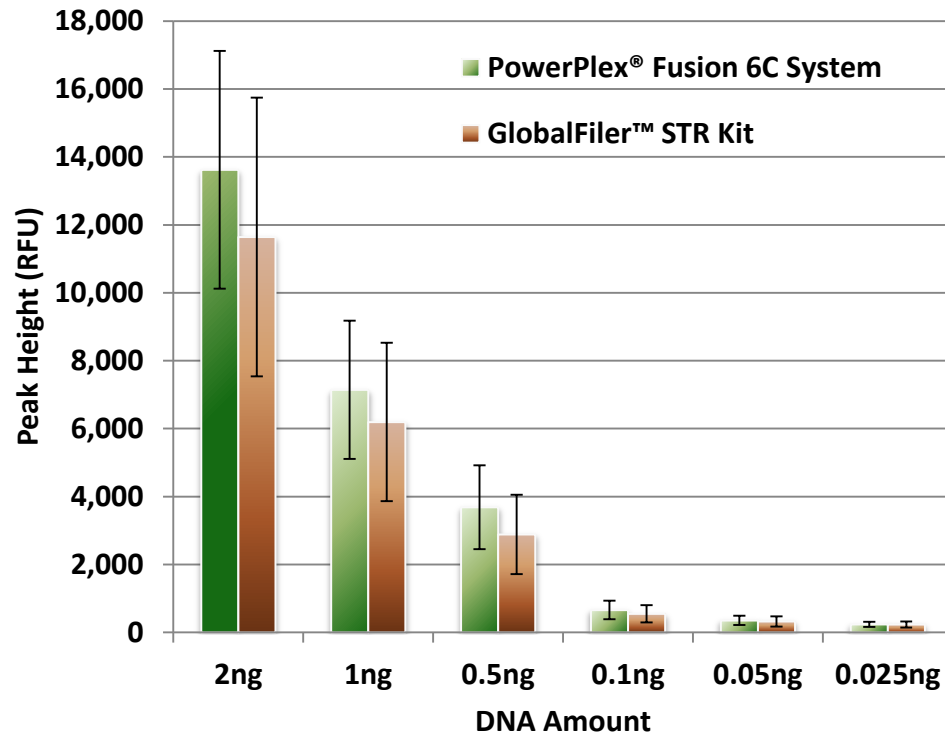
GeneMarker® HID Analysis 1ng 2800M



The Power to Solve... from sample to analysis

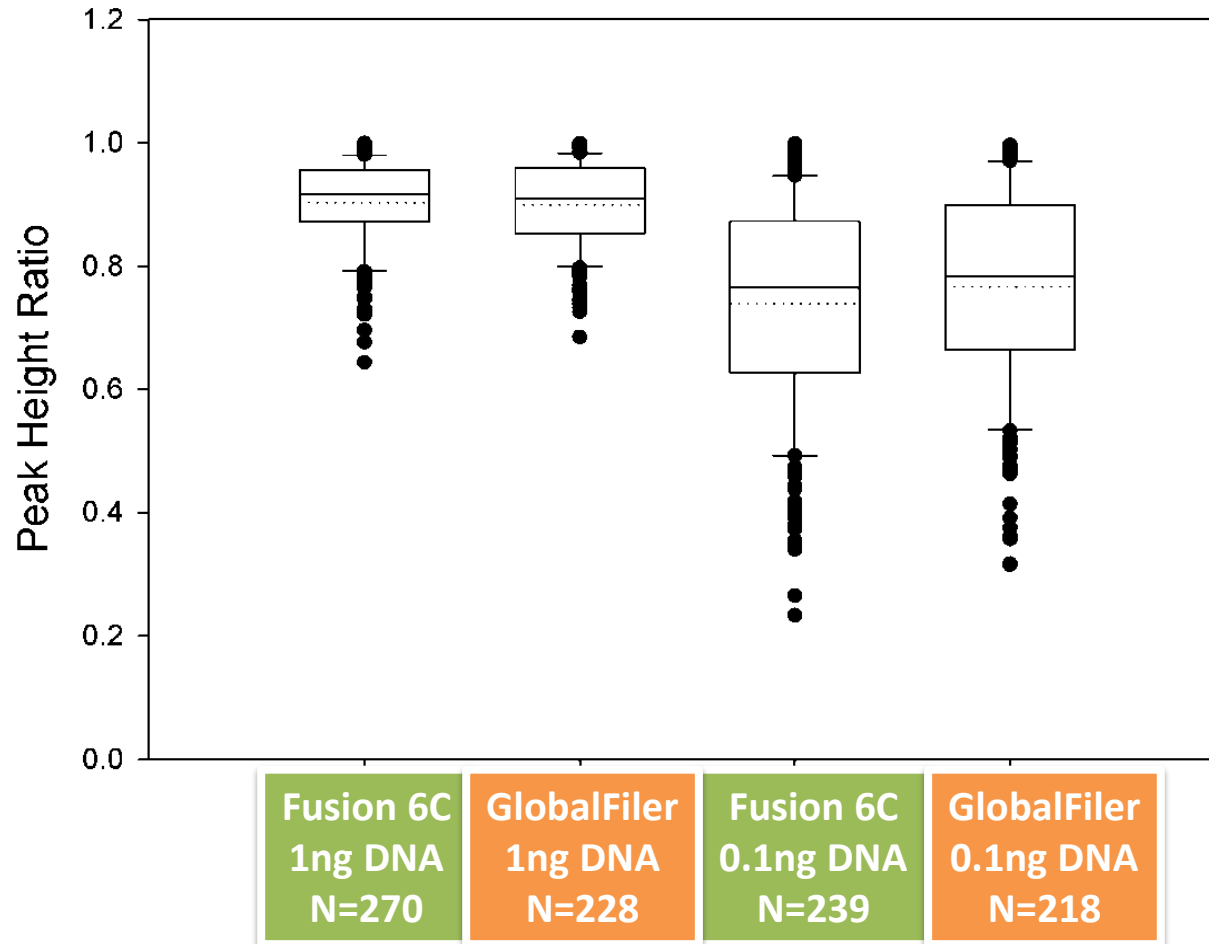
Promega Corporation

Sensitivity: Peak Height and Alleles Called

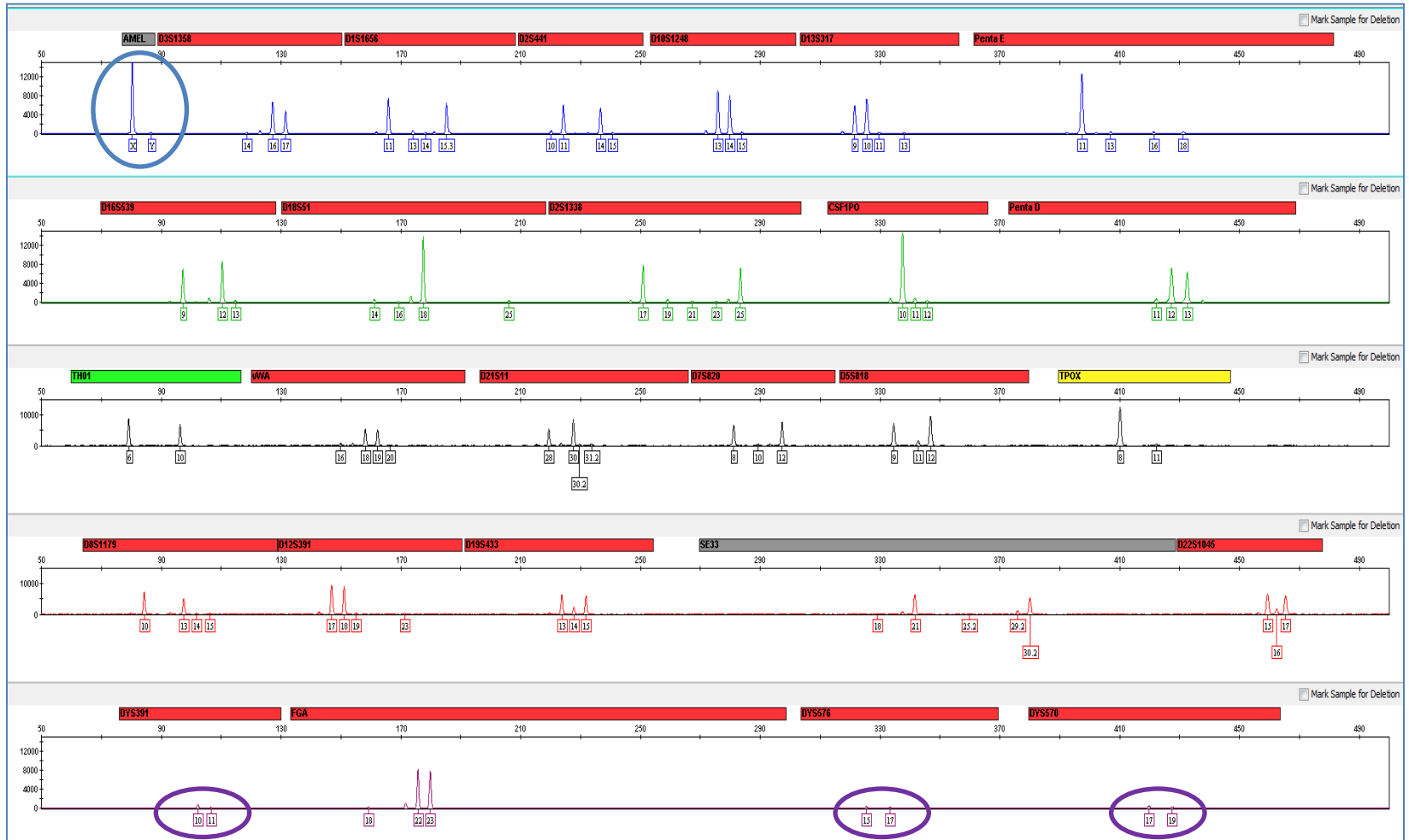


- 29 cycles
- 3500 (1.2kV, 15 second injection)
- 175RFU threshold

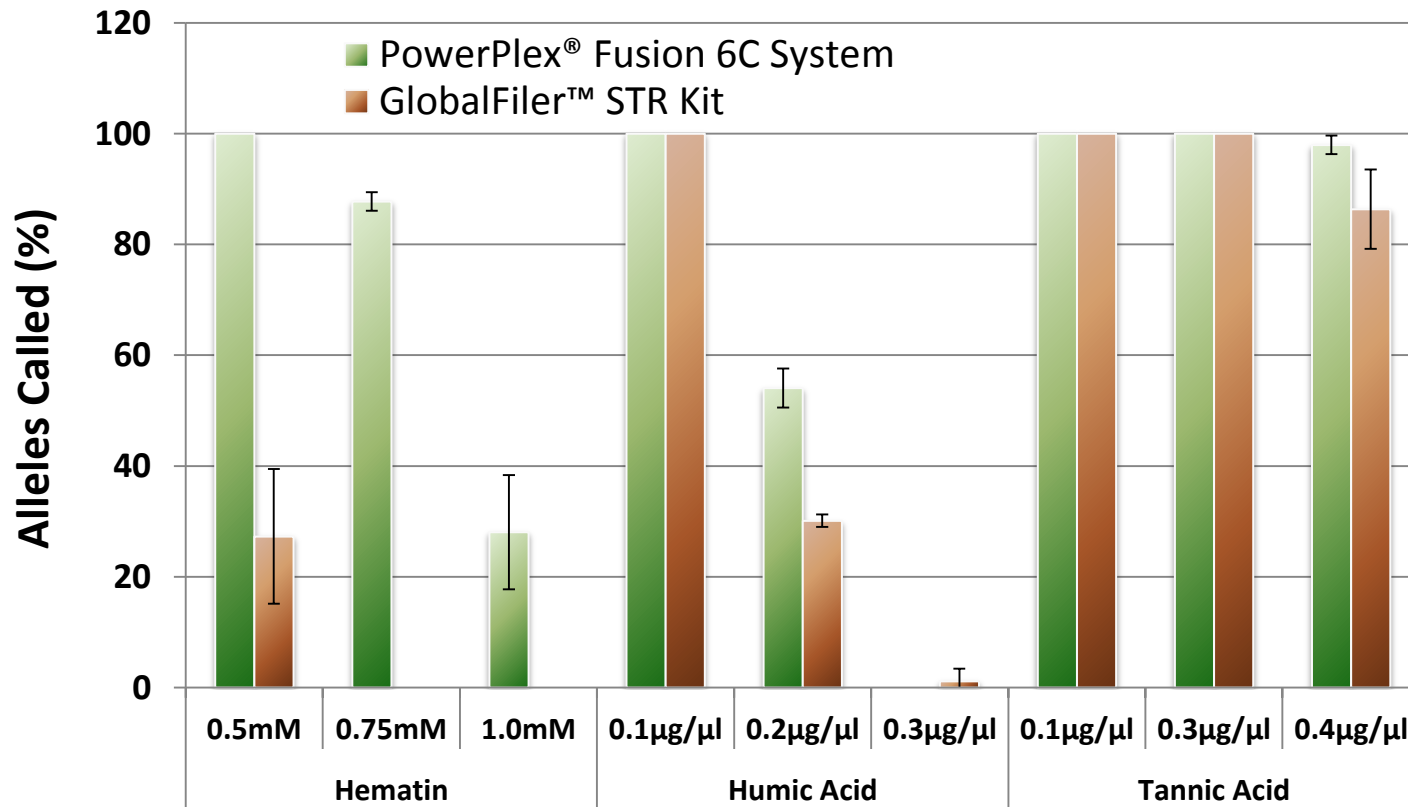
Sensitivity: Peak Height Ratio



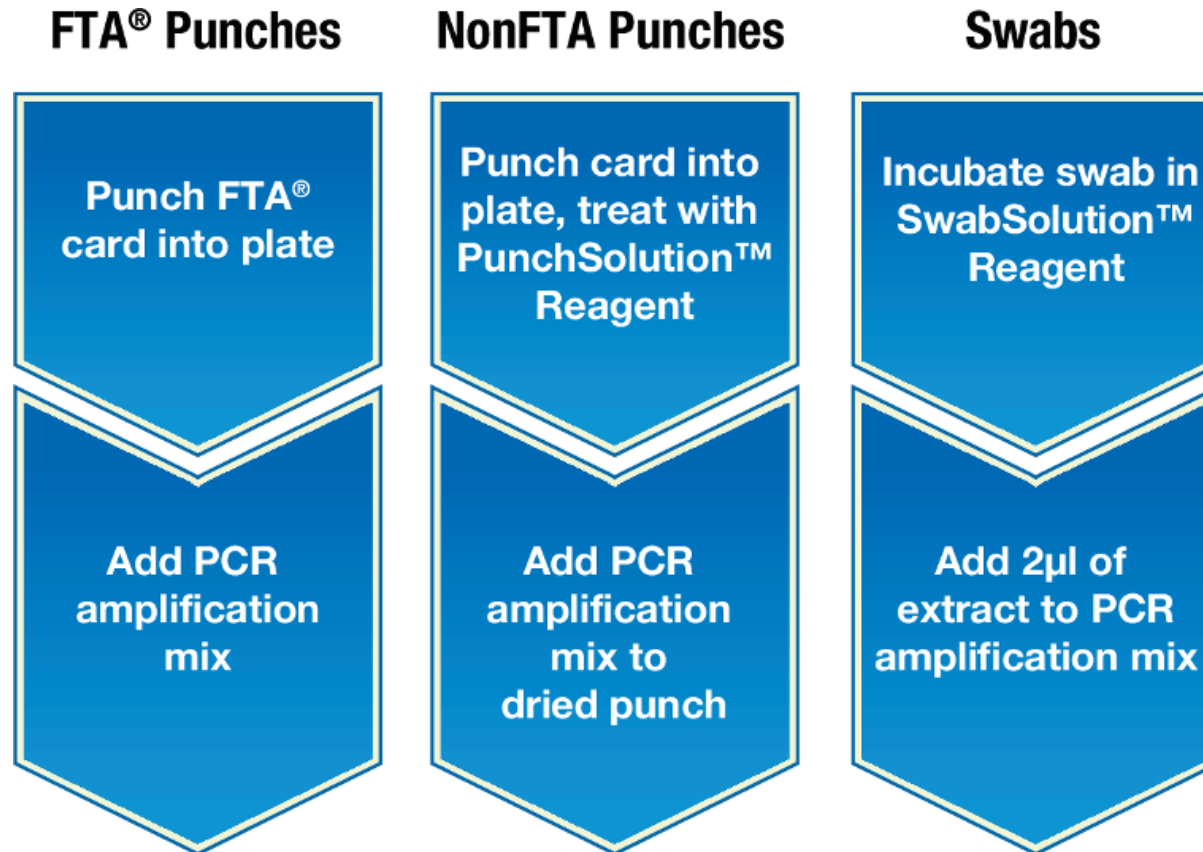
1 : 1 : 20 (Male 1 : Male 2 : Female) Mixture



Robust against Inhibitors



Multiple Sample Types for Direct Amplification

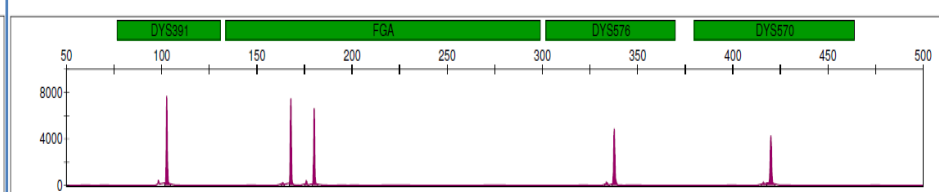
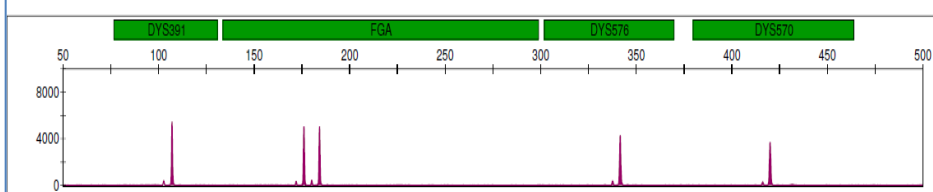
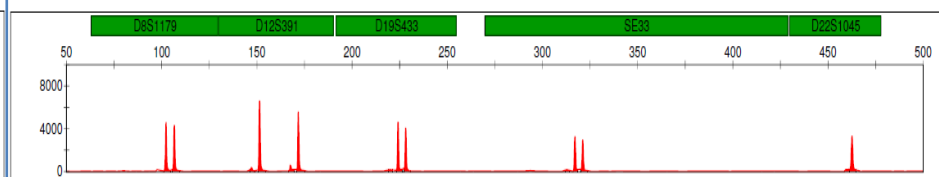
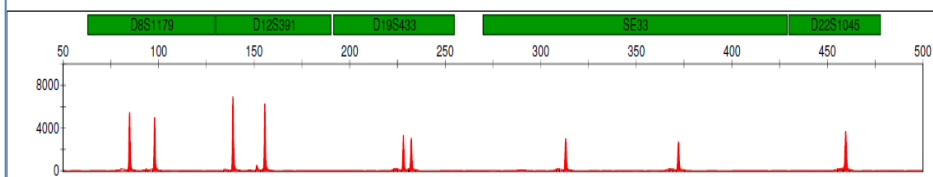
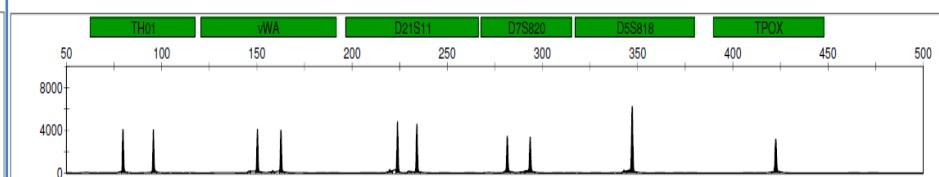
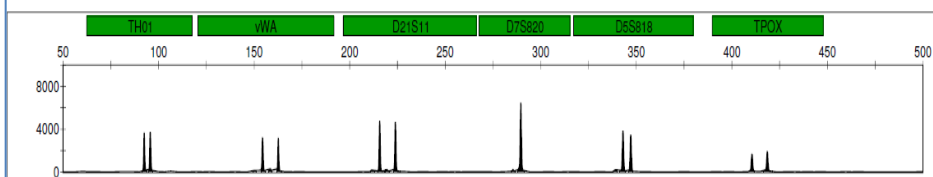
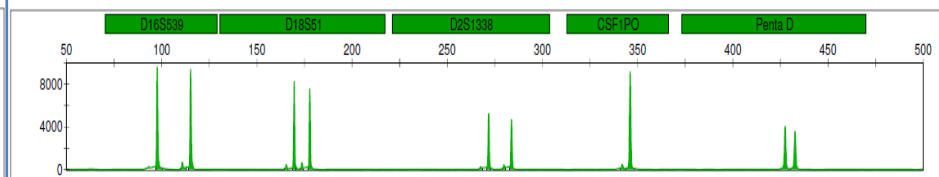
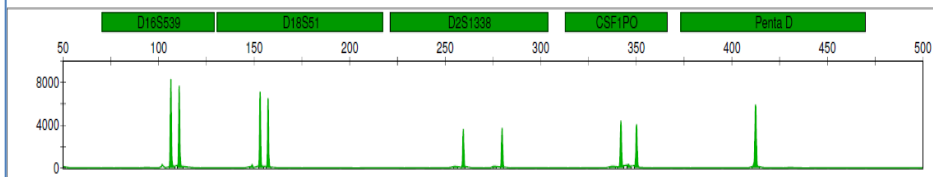
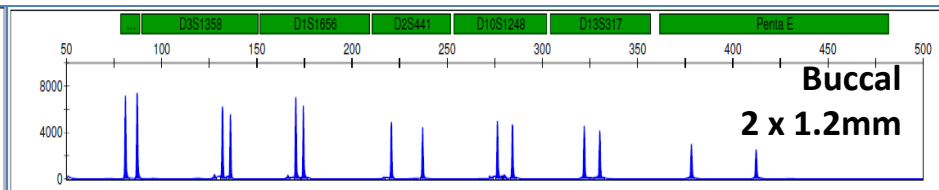
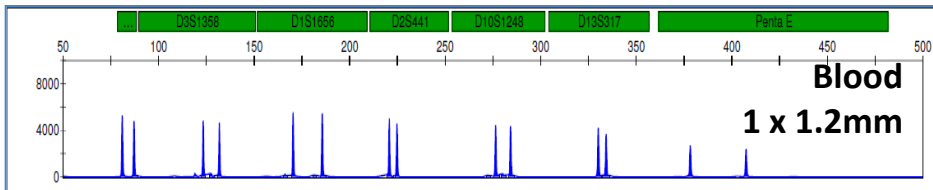


Sample Compatibility

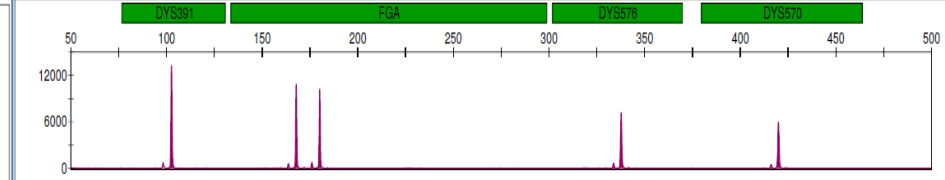
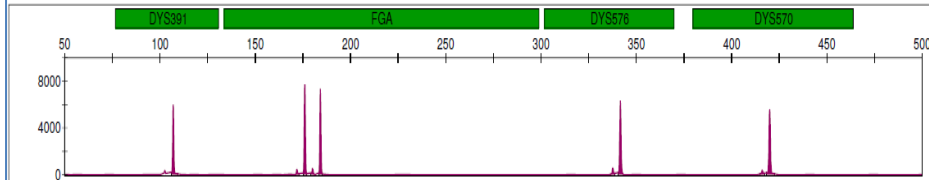
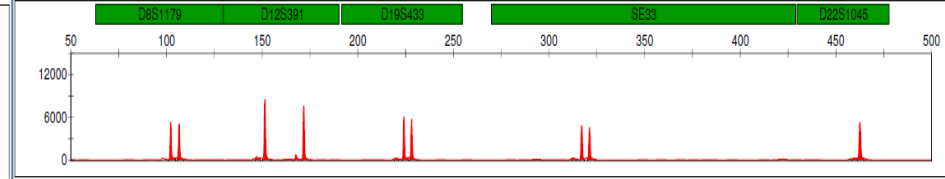
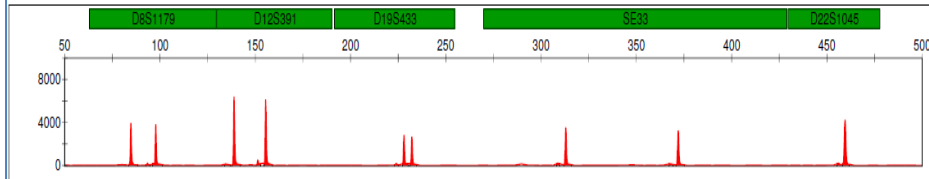
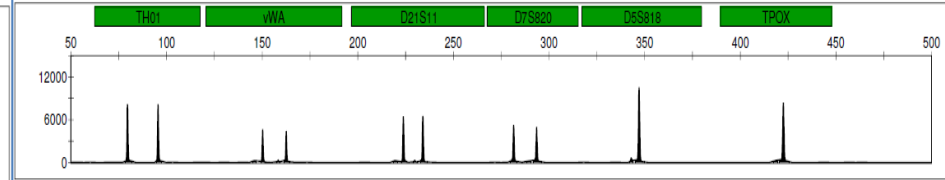
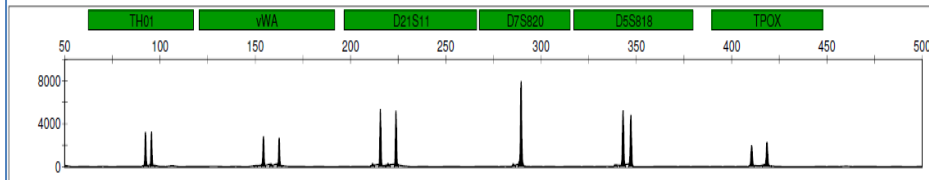
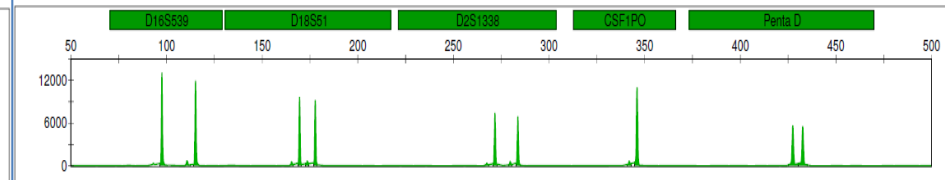
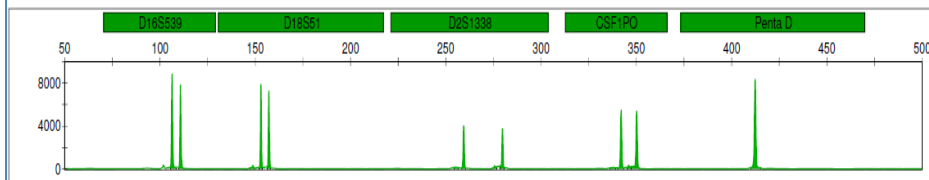
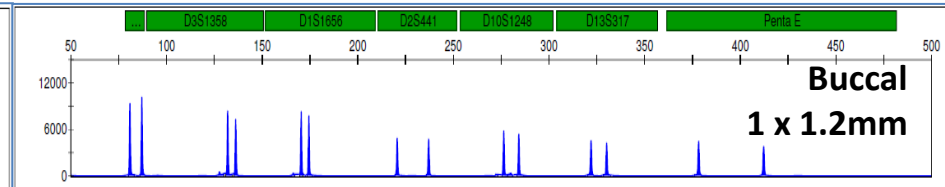
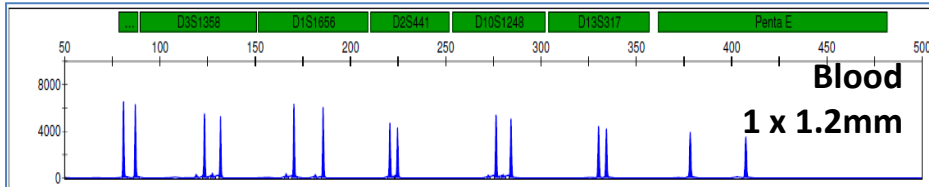
Sample Type	Reaction Volume	Number of Cycles	Final Extension	PunchSolution™	SwabSolution™	AmpSolution™	Amplification Time
Purified DNA	25µL	29	5:00	-	-	-	61 minutes
Blood FTA® (1X1.2mm)	25µL	26*	10:00	-	-	-	60 minutes
Blood FTA® (1X1.2mm)	12.5µL	25*	10:00	-	-	Yes	58 minutes
Buccal FTA® (2X1.2mm)	25µL	26*	10:00	-	-	-	60 minutes
Buccal FTA® (2X1.2mm)	12.5µL	25*	10:00	-	-	Yes	58 minutes
Blood nonFTA (1X1.2mm)	25µL	26*	10:00	Yes	-	-	60 minutes
Blood nonFTA (1X1.2mm)	12.5µL	24*	10:00	Yes	-	Yes	56 minutes
Buccal nonFTA (1X1.2mm)	25µL	25*	10:00	Yes	-	-	58 minutes
Buccal nonFTA (1X1.2mm)	12.5µL	24*	10:00	Yes	-	Yes	56 minutes
Buccal Swab Extracts	25µL	26*	10:00	-	Yes	-	60 minutes
Buccal Swab Extracts	12.5µL	25*	10:00	-	Yes	-	58 minutes

*Conditions optimized at Promega, labs will need to perform internal validation based on desired sample types.

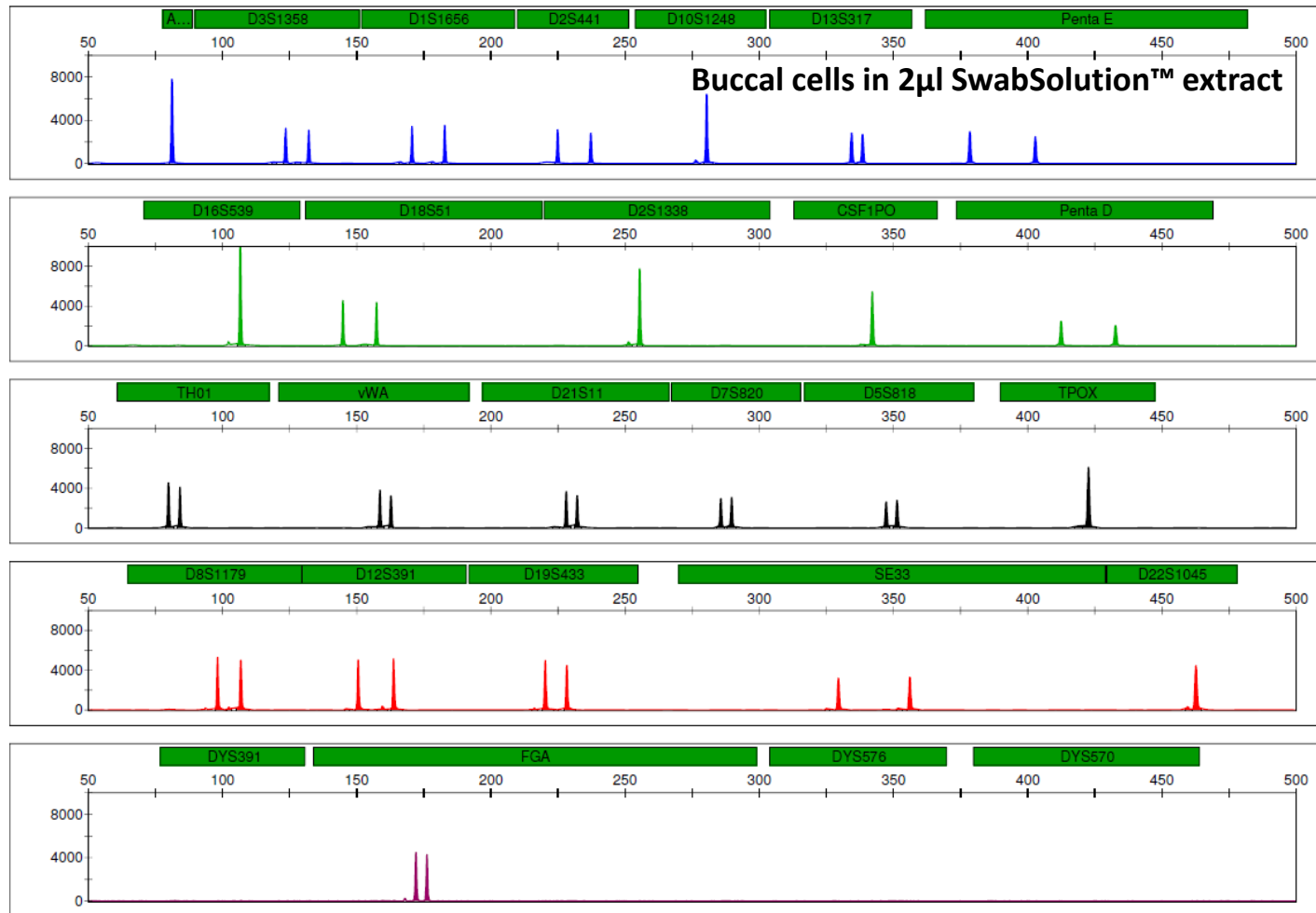
FTA[®] Punches, 12.5 μ l Reaction Volume, 25 cycles



NonFTA Punches, 12.5 μ l Reaction Volume, 24 cycles



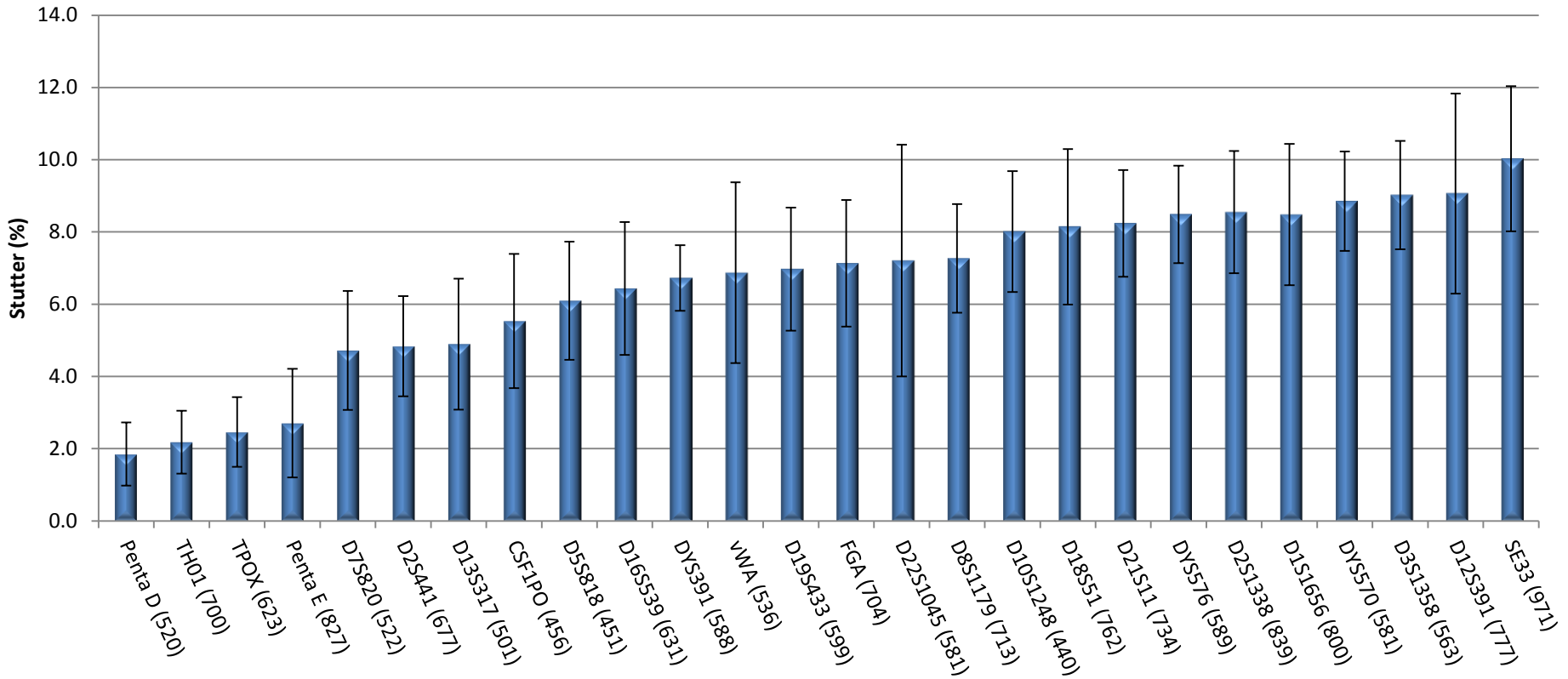
Buccal Swab, 12.5 μ l Reaction Volume, 25 cycles



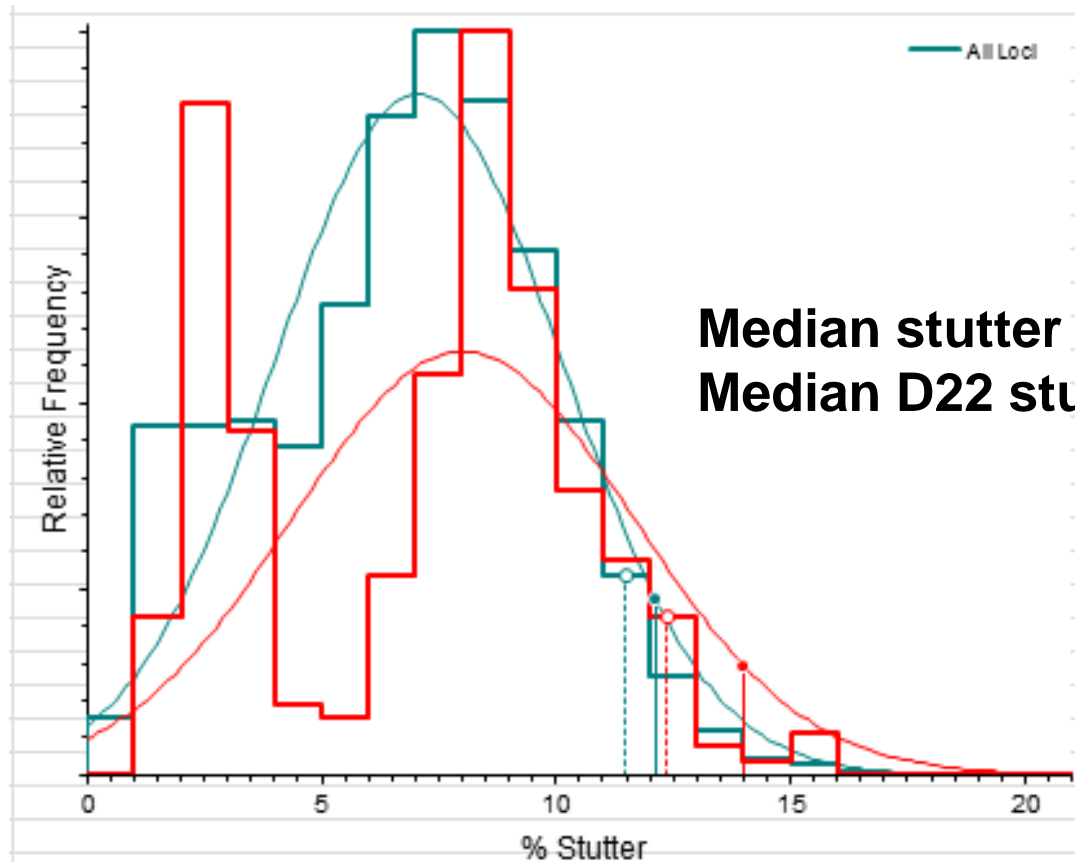
Concordance Summary

- Collaboration with NIST
 - Data from 658 unrelated samples
 - NIST US population set (652 samples)
 - SRM 2391c (6 samples)
- Results
 - PowerPlex® Fusion 6C is fully concordant with NIST SRM 2391c certified values
 - Out of 33,558 alleles compared, 2 discordant calls were observed
 - 0.006% discordance
 - 99.994% concordance between PowerPlex® Fusion 6C and NIST final data set

Stutter Values (From NIST Population Samples)

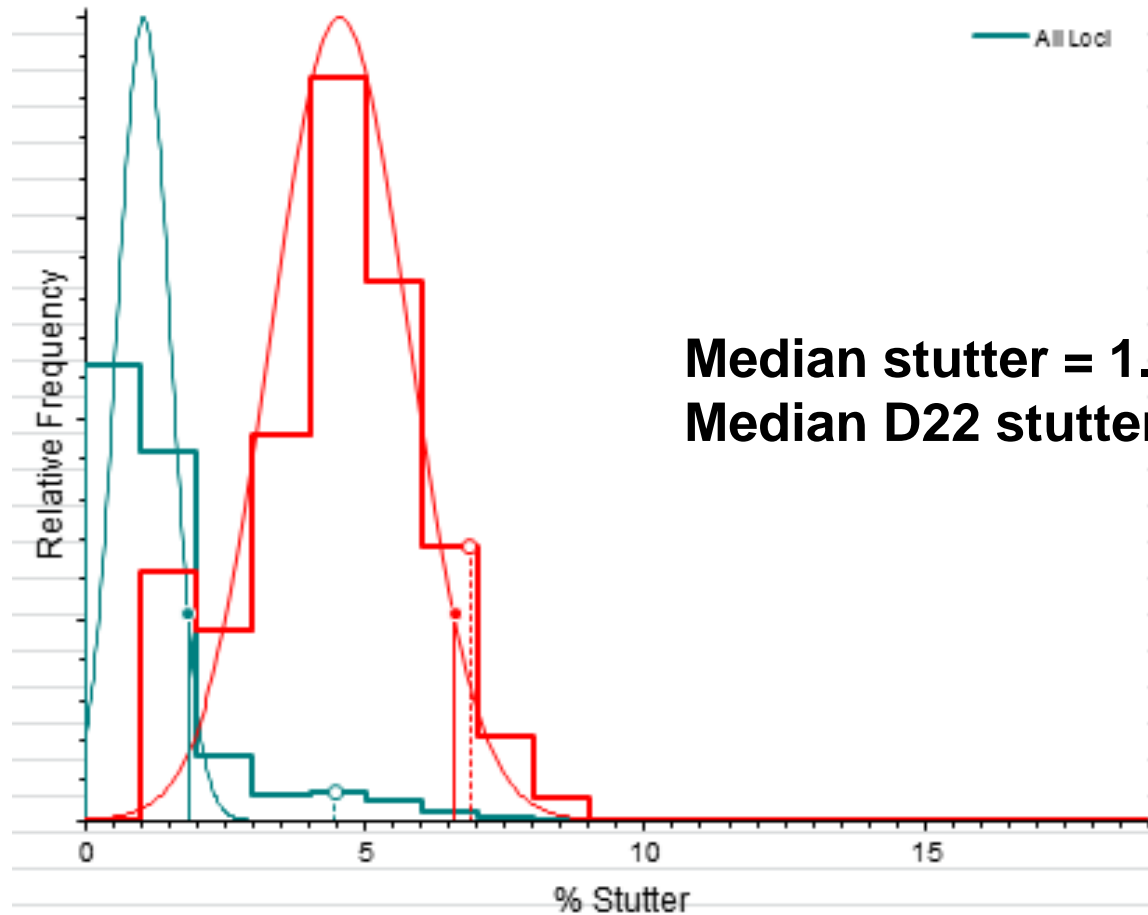


Stutter: NIST Summary



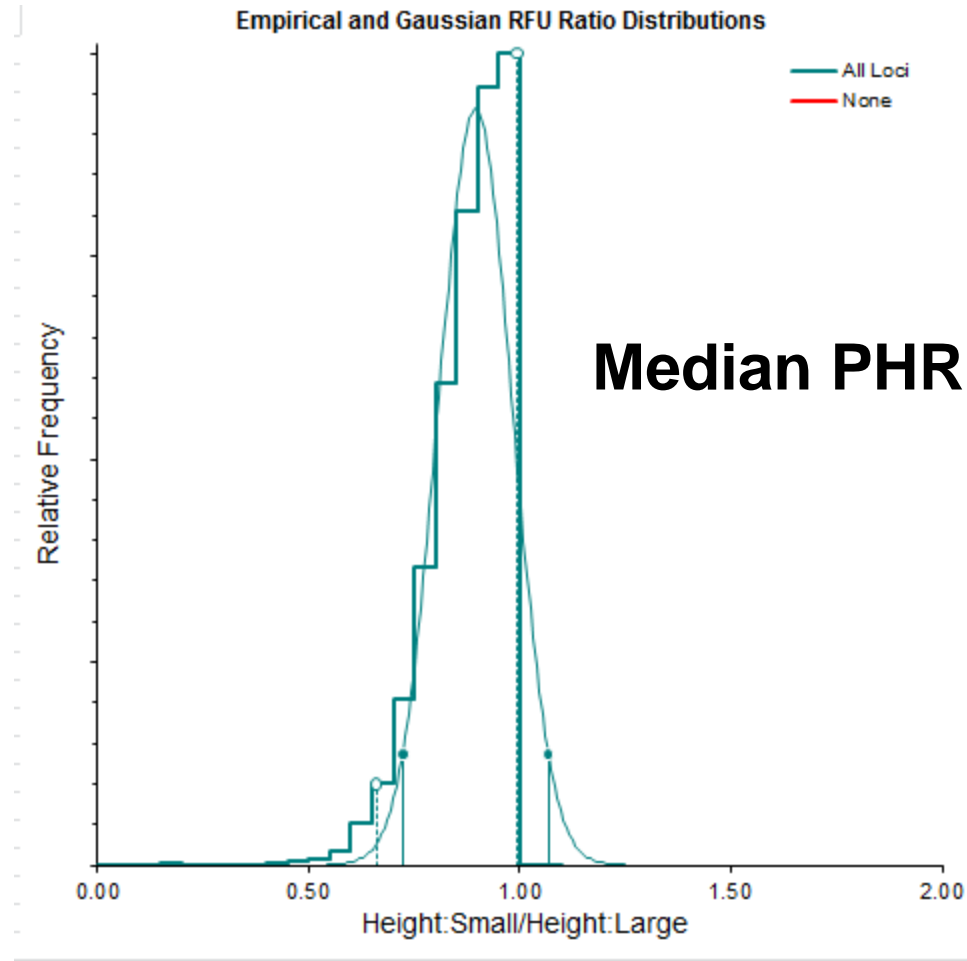
Median stutter = 7.06%
Median D22 stutter (in red) = 8.03%

Plus Stutter: NIST Summary



Median stutter = 1.04%
Median D22 stutter (in red) = 4.57%

NIST Peak Height Ratio



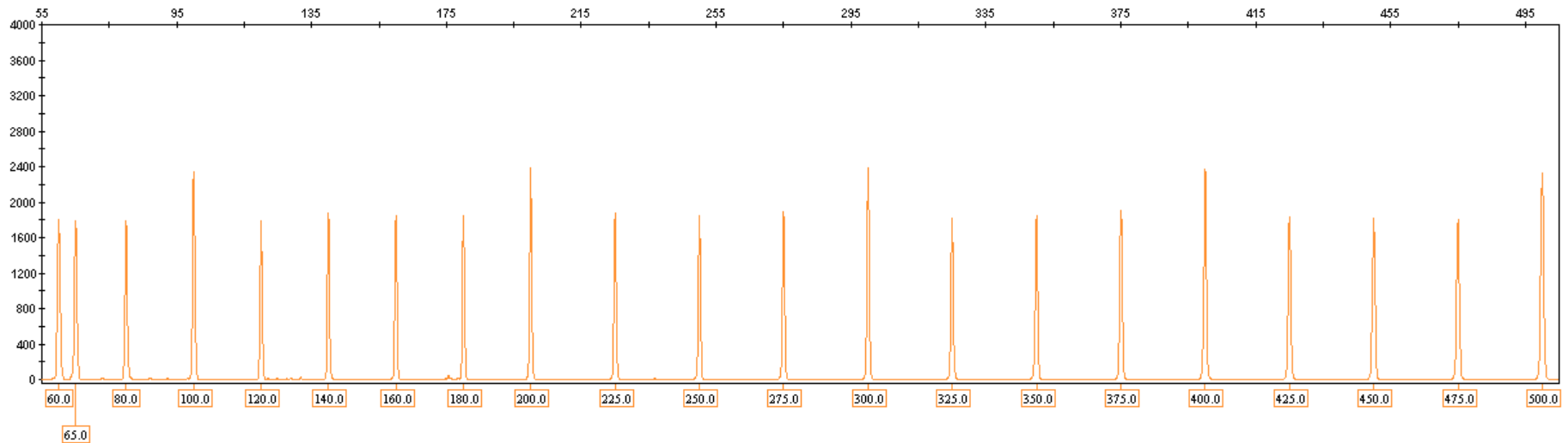
PowerPlex® Fusion 6C System

Kit Components and Sizes

Component	50 reaction kit DC2705	200 reaction kit DC2720
PowerPlex® Fusion 6C 5X Primer Pair Mix	1 x 250µl	4 x 250µl
PowerPlex® Fusion 6C 5X Master Mix	1 x 250µl	4 x 250µl
PowerPlex® Fusion 6C Allelic Ladder Mix	1 x 25µl	4 x 25µl
WEN Internal Lane Standard 500	1 x 200µl	2 x 200µl
2800M Control DNA	1 x 25µl	1 x 25µl
Water, Amplification Grade	1 x 1,250µl	5 x 1,250µl

Component	PowerPlex® 6C Matrix Standards DG4900
6C Matrix Mix	1 x 150µl
6C Matrix Dilution Buffer	5 x 200µl

WEN ILS 500



Same fragments, new orange dye

PowerPlex® 6C Matrix Standards: All Fragments in One Tube

Setup a Dye Set

* Dye Set Name: PromegaJ6
* Chemistry: Matrix Standard
* Dye Set Template: []

Arrange Dyes

Dye Selection	Reduced Selection	Calibration Peak Order
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	6
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	4
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	3
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	5

Parameters
The parameters will be used for instruments configured with 36cm capillary array and polymer POP4

Matrix Condition Number Upper Limit: 8.0
Locate Start Point: * After Scan 800 * Before Scan 5000
* Limit Scans To: 2750
Sensitivity: 0.4
* Minimum Quality Score: 0.95

Notes
After scan changed to 800

Close

Export Spectral Calibration Results | E-Signature | View Spectral Calibration Report | Print

Calibration Run | History View

Calibration Information

Dye Set	Chemistry Standard	Calibration Date	Capillary Array Serial Number	Is Signed
Promega J6	Matrix Standard	25-Sep-2014 07:33:51 AM	L31480210	No
Promega J6	Matrix Standard	23-Sep-2014 01:43:51 PM	L31480210	No
Promega J6	Matrix Standard	23-Sep-2014 01:03:44 PM	L31480210	No

Capillary Run Data

Capillary	1	2	3	4	5	6	7	8
Run 1	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Run 2	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Run 3	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Overall	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed

Legend: Passed Failed Borrowed Not Calibrated

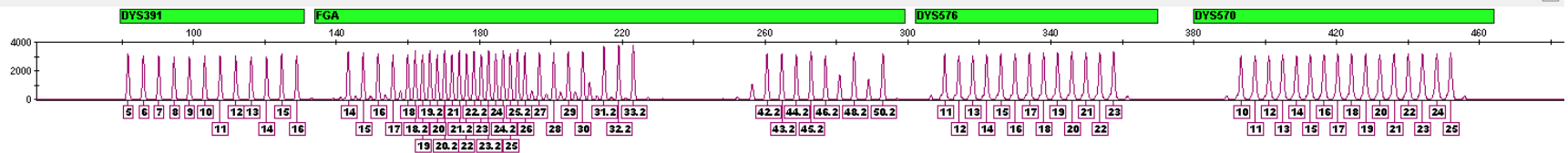
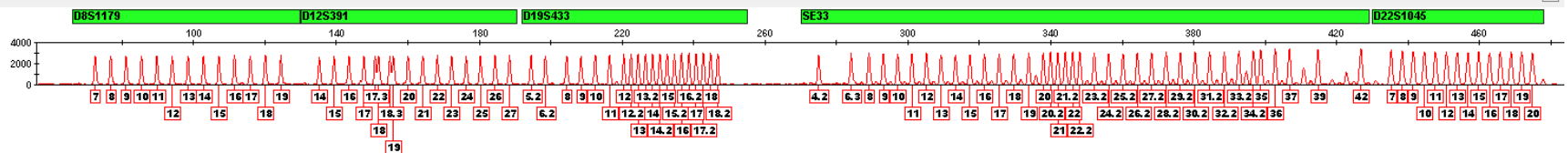
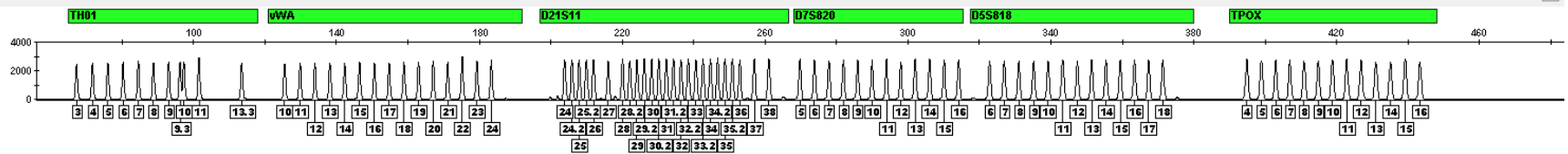
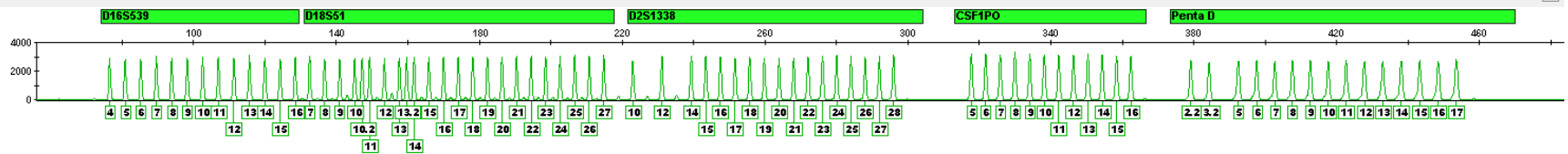
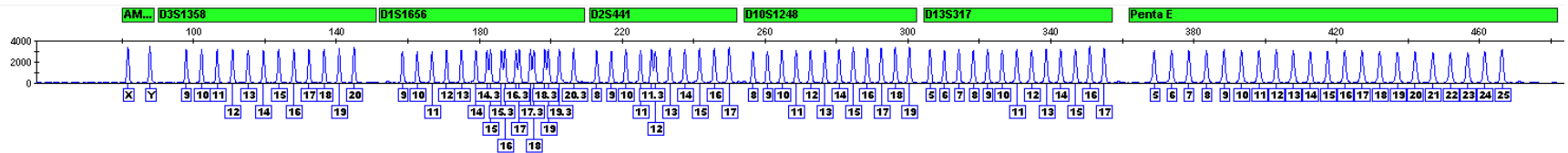
Capillary 4 - Run 1: Quality Value: 0.997857 Condition #: 5.388942 Status: Passed Message: qp:0.998 cc:5.389

Intensity vs Scan Number

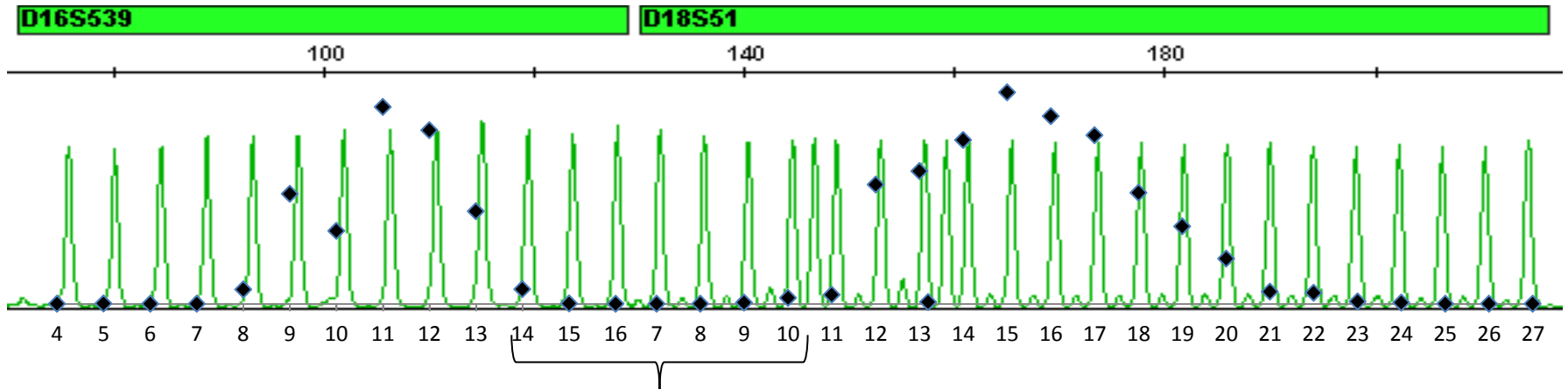
Calibrated Data

Intensity vs Scan Number

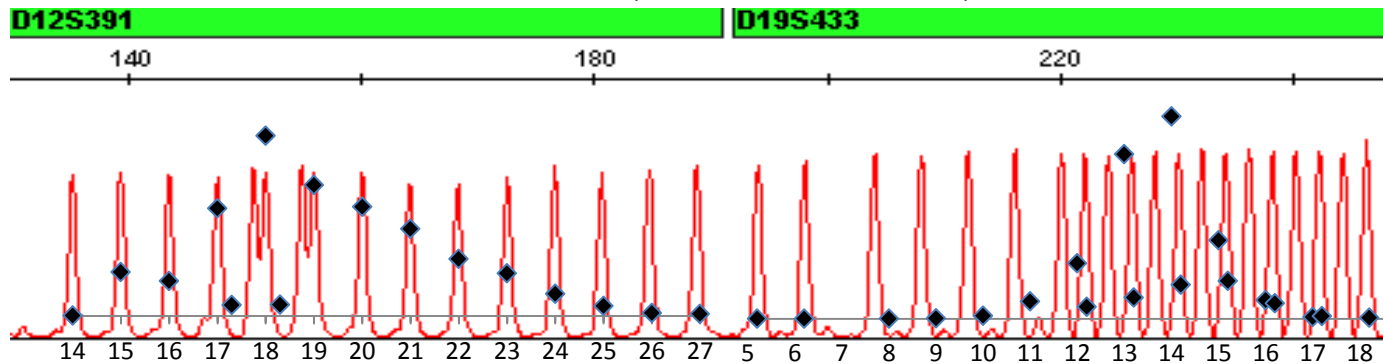
434 Allelic Ladder Fragments



Wider Panel Ranges Reduce OL and OMR Calls

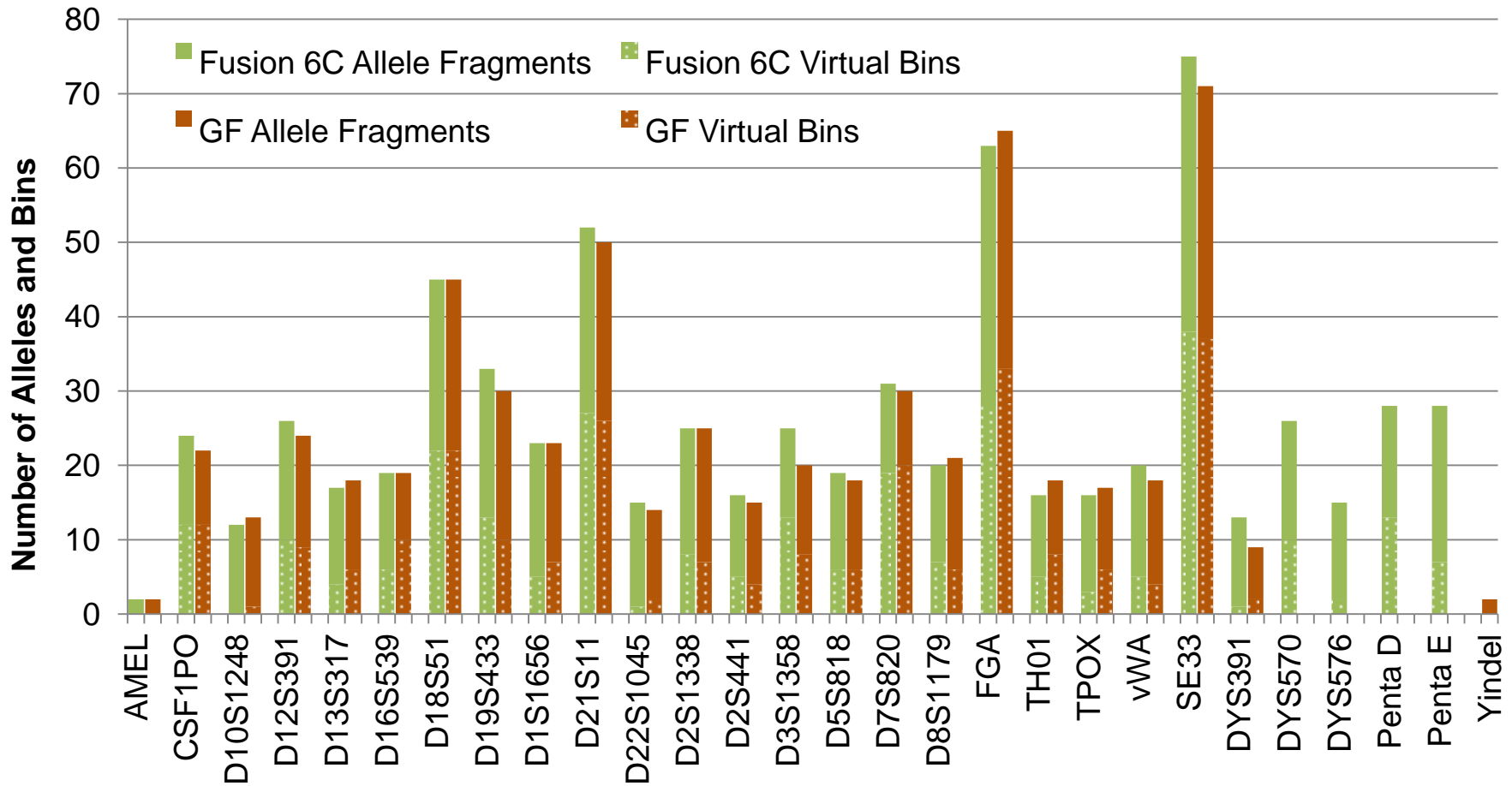


Frequencies of alleles at the edge of panel ranges are very low, but there when you need them



Enhanced Allelic Ladder

More Accurate Allele Calls



Is PowerPlex® Fusion 6C System for You?



Summary

- 6-dye STR amplification system designed to meet new CODIS guidelines
- Contains 27 loci including SE33 and 3 Y-STR loci
- Improved mixture interpretation
- One kit for both casework and database use
- Short PCR cycling time (around 1 hour)

- Launch date: Early 2015
- Seeking collaborators for developmental validation effort

Acknowledgments

Research & Development

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More Information Available

Search "Fusion 6C" on Promega.com...

The screenshot shows the Promega website interface. At the top, there is a navigation bar with the Promega logo, a search bar, and links for 'Items: 0', 'Catalog', 'Quick Order', 'My Orders', 'Find My Gene™', 'Register', and 'Log In'. Below the navigation bar, there are tabs for 'Products + Services', 'Resources', and 'Support'. The main content area features a large green banner with the text 'PERFORMANCE PACKED. More Loci. More Choices. More Information.' and an image of a PCR tube. To the right of the banner, there are sections for 'Try In Your Lab', 'Ordering Information', and social media sharing options (Facebook Share and Twitter Tweet). Below these, there are sections for 'RELATED PRODUCTS' and 'RELATED RESOURCES'. The bottom of the page has a blue background with the text 'The Power to Solve... from sample to analysis' and 'Promega Corporation'.

PowerPlex® Fusion Systems

PERFORMANCE
PACKED.
More Loci. More Choices.
More Information.

Designed to meet CODIS and European standards, the PowerPlex® Fusion Systems enable laboratories to:

- Achieve the most inter-database compatibility and highest discrimination of any autosomal STR kit.

RELATED PRODUCTS

SwabSolution™ Kit, PunchSolution™ Kit and 5X AmpSolution™ Reagent

RELATED RESOURCES

The Power to Solve... from sample to analysis

Promega Corporation