

## Promega Webinars Series: Genetic Identity



IDNADEX: Improving DNA Data Exchange

### Validation Studies on DNA Mixtures Using the PowerPlex® Fusion 6C System



**INTCF-Madrid**  
**SERVICIO DE BIOLOGIA**



*Supported by a grant from the ISEC programme*



# Proposal: Validation of a single 21 STR typing system based on 6-Dye CE detection to improve DNA data Exchange locally and globally

ISEC Programme  
Targeted Call 2011  
Awarded July 2012

## Requirements to the Companies

- ✓ 21 STR covering ESS & CODIS + D2, D19 & SE33
- ✓ Concordance of primer sequences design with previous systems
- ✓ Master mix with high tolerance to PCR inhibitor for casework application

ESS STR Core Loci	CODIS STR Core Loci	Other STR Loci In Forensic Kits	Spanish Database (200.000 profiles) Year 2011	STR Markers in ESS typing kits	Proposed 21 STR Typing System
	CSF1PO		CSF1PO		CSF1PO
	TPOX		TPOX		TPOX
	D5S818		D5S818		D5S818
	D7S820		D7S820		D7S820
	D13S317		D13S317		D13S317
	D16S539		D16S539	D16S539	D16S539
FGA	FGA		FGA	FGA	FGA
TH01	TH01		TH01	TH01	TH01
VWA	VWA		VWA	VWA	VWA
D3S1358	D3S1358		D3S1358	D3S1358	D3S1358
D8S1179	D8S1179		D8S1179	D8S1179	D8S1179
D18S51	D18S51		D18S51	D18S51	D18S51
D21S11	D21S11		D21S11	D21S11	D21S11
D22S1045				D22S1045	D22S1045
D1S1656				D1S1656	D1S1656
D10S1248				D10S1248	D10S1248
D2S441				D2S441	D2S441
D12S391				D12S391	D12S391
		D2S1338	D2S1338	D2S1338	D2S1338
		D19S433	D19S433	D19S433	D19S433
		SE33		SE33	SE33
		Penta D			
		Penta E			



ELSEVIER

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

## Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://www.elsevier.com/locate/fsig)



### Improving DNA data exchange: Validation studies on a single 6 dye STR kit with 24 loci

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General characteristics of STR markers included in the Promega 6 Dye Kit (V2) and forensic biostatistical parameters in the Spanish population (N=284) Garcia et al. Forensic Sci Int Genet. 6(2) (2012)

Marker	Chromosomal Location	Basic Repeat motif	Dye Label	Allele sizes (bp)	He	DP	CE	
AMEL	X: p22.1-22.3 ; Y: p11.2	--	FL-6C™	89, 95	--	--	--	
D3S1358	3p21.31	TCTA/TCTG	FL-6C™	103-147	0.7993	0.9207	0.6930	
D1S1656	1q42.2	TAGA		161-208	0.8944	0.9626	0.7685	
D2S441	2p14	TCTA		216-252	0.7641	0.9035	0.5510	
D10S1248	10q26.3	GGAA		260-284	0.7500	0.9078	0.5179	
D13S317	13q22-31	TATC		308-358	0.7746	0.9253	0.6930	
Penta E	15q26.2	AAAGA		371-471	0.8503	0.9734	0.6954	
D16S539	16q24.1	GATA		JOE-6C™	84-132	0.7324	0.9061	0.5179
D18S51	18q21.33	AGAA		JOE-6C™	134-214	0.8732	0.9664	0.6930
D2S1338	2q35	TGCC/TTCC			224-296	0.8732	0.9629	0.6202
CSF1PO	5q33.3-34	AGAT			318-362	0.7465	0.8754	0.4550
Penta D	21q22.3	AAAGA	377-450		0.7914	0.9554	0.5833	
TH01	11p15.5	AATG/ATG	TMR-6C	72-115	0.7958	0.9179	0.5179	
vWA	12p13.31	TCTA/TCTG	TMR-6C	127-183	0.7817	0.9405	0.4253	
D21S11	21q11.2-q21	TCTA/TCTG		203-259	0.8451	0.9512	0.6930	
D7S820	7q11.21-22	GATA		269-313	0.7676	0.9397	0.4859	
D5S818	5q21-31	AGAT		321-369	0.6901	0.8658	0.3969	
TPOX	2p23-2per	AATG		393-441	0.7183	0.8397	0.3189	
D8S1179	8q24.13	TCTA/TCTG		CXR-6C™	76-124	0.8310	0.9384	0.6578
D12S391	12p13.2	AGAT/AGAC	CXR-6C™	133-185	0.8838	0.9619	0.7305	
D19S433	19q12	AAGG		193-245	0.8345	0.9178	0.6562	
SE33	6q14	Complex AAAG		270-420	0.9577	0.9790	0.8458	
D22S104	22q12.3	ATT		431-470	0.6549	0.7782	0.5510	
DYS391	Yq11.21	TCTA	TOM-6C™	86-130	--	--	--	
FGA	4q28	CTTT	TOM-6C™	143-289	0.8697	0.9624	0.7685	
DYS576	Yq	AAAG		308-356	--	--	--	
DYS570	Yq	TTTC		393-453	--	--	--	

DYS391

Promega 6C V1

D16S539

# Validation Parameters & Materials

Validation Parameters	Type & Number of Samples Analyzed
<b>Analytical Threshold</b>	20 PCR negative controls
<b>Sensitivity &amp; Stochastic Threshold</b>	3 Human genomic DNA samples of known human DNA concentration (SRM-2372A, DNA Control 2800 & 1 genomic DNA sample from an anonymous male donor) (71 samples: Quadruplicate or Triplicate analysis of 7-6 dilution series)
<b>Precision and Accuracy</b>	24 injections of the Globalfiler allelic ladder 90 human genomic DNA samples
<b>Repeatability &amp; reproducibility</b>	3 Human genomics DNA samples of known STR profile (30 samples: 5 replicates of 3 samples generated by two operators)
<b>Heterozygous balance</b>	80 Human genomic DNA samples
<b>Stutter Threshold</b>	80 Human genomic DNA samples
<b>Genotype concordance</b>	80 Human genomic DNA samples from saliva swabs of anonymous donors 10 genomic DNA samples of the DNA profiling standard SRM-2391b
<b>DNA mixtures</b>	1 male /female DNA mixture in 7 different ratios (1 ng of total DNA in triplicate) 1 male /female DNA mixture in 7 different ratios (3 ng of total DNA in triplicate) (42 samples: Triplicate analysis of 7 dilutions from 2 series) 15 DNA mixture samples from proficiency exercises
<b>Species specificity</b>	21 genomic DNA from human-associated microbial species
<b>PCR reduced reaction volume</b>	20 human genomic DNA samples of known STR profile
<b>Stability study in Case-Type Samples</b>	43 forensic DNA samples previously analyzed with different STR Kits

# Methods

## DNA Extraction



Reference samples



Forensic samples  
(Differential Lysis for Semen)

## DNA Quantification



Real time PCR Quantifiler Duo Kit  
Targets: RPPH1 , SRY & IPC

## PCR Amplification



1 X 96°C for 60 s  
30 X 96°C for 5 s, 60-62°C  
for 60 s  
1X 60°C for 5 min

## CE Detection ABI3500



Sample Injection: 15 s at  
1.2kv  
CE: 1550 s at 13 kv

## Data Analysis

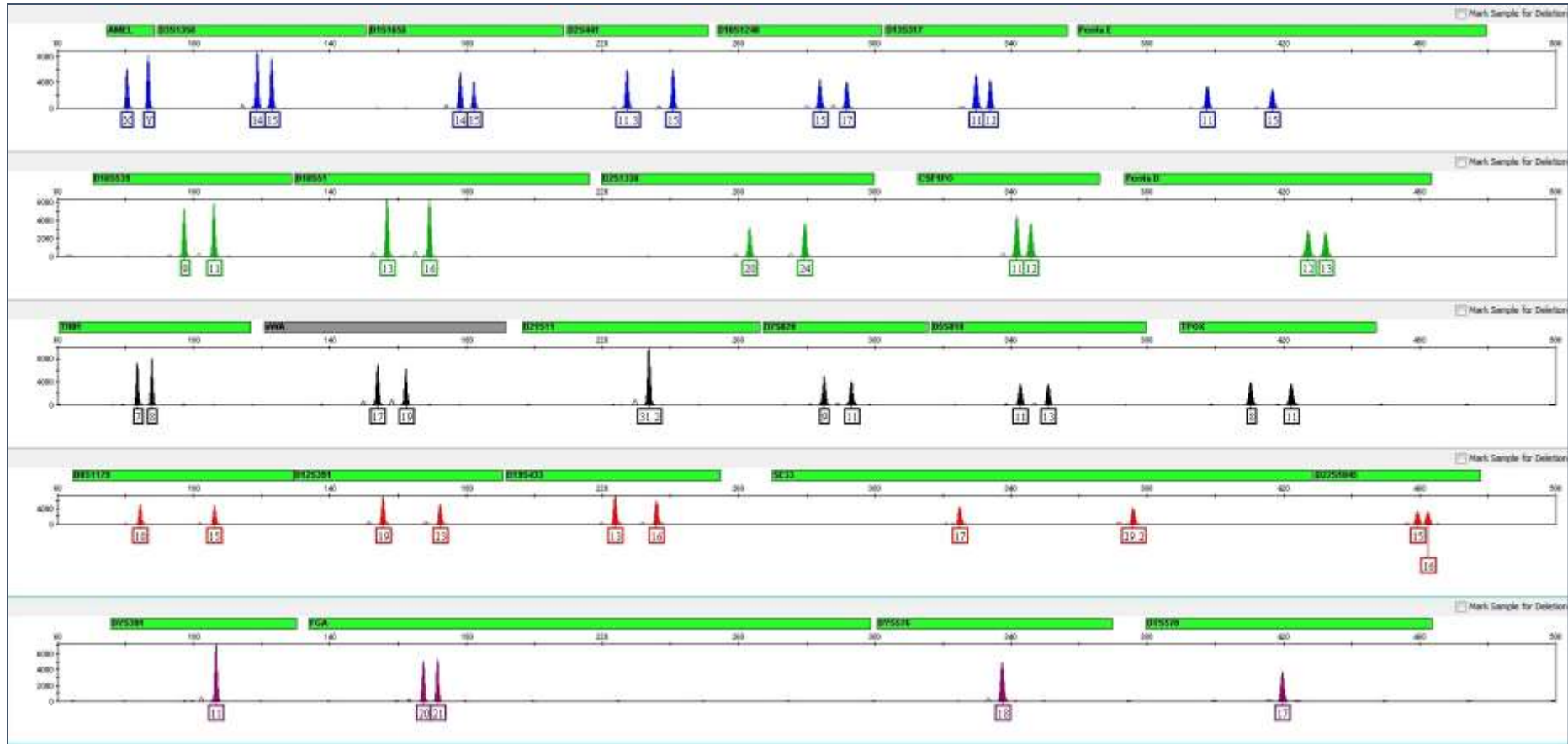


GM-IDX  
Promega 6 Dye Manual  
Peak Amplitude 100 – 200  
RFU



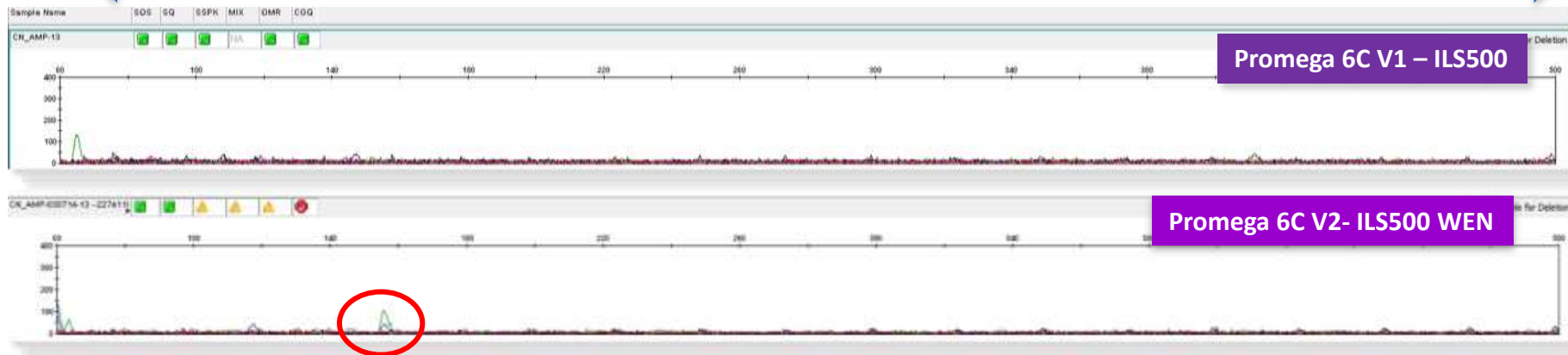
Management of samples, analysis  
and results  
Comparison & Concordance studies

# Promega 6 Dye STR profile from a reference saliva swab (DNA input: 500 pg; PCR Reaction volume :12.5 ul)



# Analytical Threshold : 20 PCR negative controls

Range of analysis: 65-500 bp; Peak Detector: 1 RFU



## Promega 6C V1

	Blue	Green	Yellow	Red	Purple
Average RFU	12,00	13,84	12,58	13,11	8,36
Standard deviation	4,55	4,23	5,59	4,07	3,25
Max. RFU	56	33	44	36	38
Min. RFU	3	3	2	2	2
Average RFU + 3 Standard deviation	25,65	26,54	29,33	25,32	18,10
Average RFU + 10 Standard deviation	57,50	56,18	68,43	53,80	40,82
2 X (Ymax - Ymin)	106	60	84	68	72

AT: 70 RFU

AT: 100 RFU

## Promega 6C V2

	Blue	Green	Yellow	Red	Purple
Average RFU	5,31	11,39	5,87	6,93	7,11
Standard deviation	2,94	4,52	6,14	2,38	6,04
Max. RFU	47	104	59	42	47
Min. RFU	1	2	1	1	1
Average RFU + 3 Standard deviation	14,12	24,95	24,29	14,09	25,24
Average RFU + 10 Standard deviation	34,67	56,57	67,28	30,77	67,52
2 X (Ymax - Ymin)	92	204	116	82	92

AT: 70 RFU

AT: 200 RFU

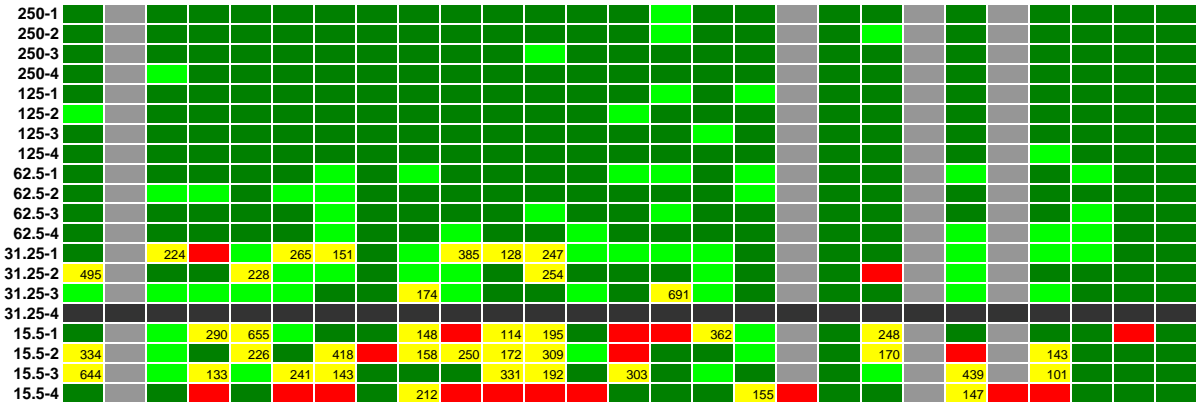


# Sensitivity study & stochastic threshold (Promega 6C V1)

Heat maps summarizing the sensitivity results of the last five DNA serial dilutions (from 250 to 15.25 pg)

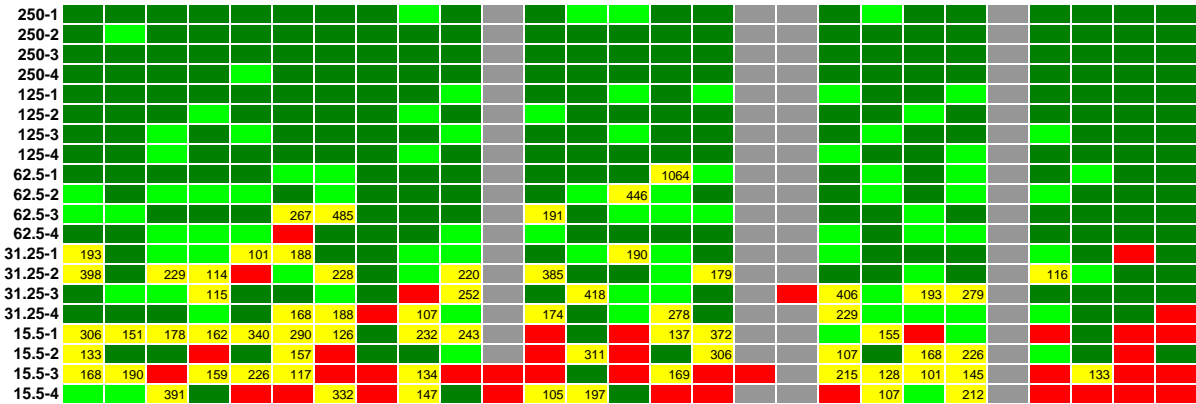
D  
D D 1 D P D P D D D D D D D D D D D D  
3 1 D 0 1 e D D 2 C e D D D 8 1 1 2 1 D D  
S S 2 S 3 n Y 1 S S n 2 7 5 S 2 9 S 6 Y Y  
A 1 1 S 1 S t S 8 1 F t T 1 S S T 1 S S S 1 S S S  
M 3 6 4 2 3 a 3 S 3 1 a H v S 8 8 P 1 3 4 E 0 5 F 5 5  
E 5 5 4 4 1 9 5 3 P 0 W 1 2 1 O 7 9 3 3 4 3 G 7 7  
L 8 6 1 8 7 E 1 1 8 O D 1 A 1 0 8 X 9 1 3 3 5 9 A 6 0

2372A



DNA Input (pg)	Observed Dropout
250	0 %
125	0 %
62	0 %
31	17 %
15	47 %

2800M



DNA Input (pg)	Observed Dropout
250	0 %
125	0 %
62	8 %
31	20 %
15	61 %

## Stochastic threshold

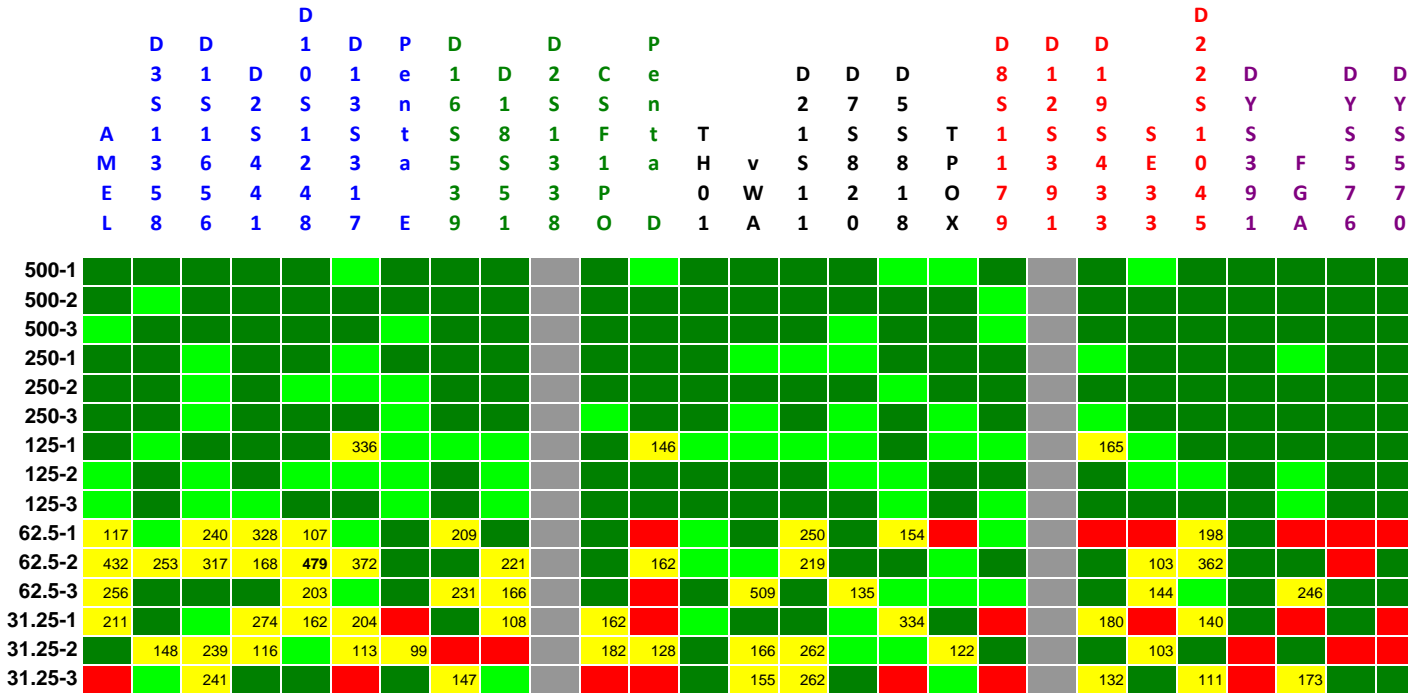
RFU value of the highest surviving false homozygous peak when allele drop-out is observed at heterozygous markers:

1064 RFU for D21S11 at 62.5 pg of DNA input

# Sensitivity study & stochastic threshold (Promega 6C V2)

Heat maps summarizing the sensitivity results of five DNA serial dilutions (from 500 to 31 pg)

## GENOMIC DNA (DNA01)



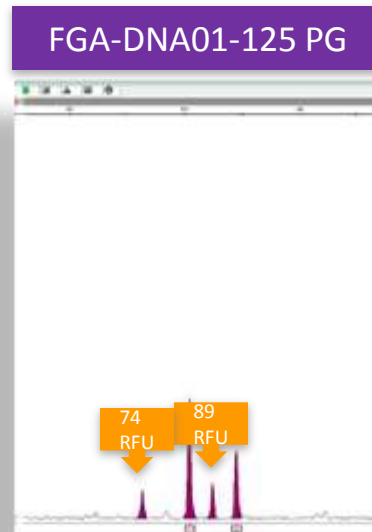
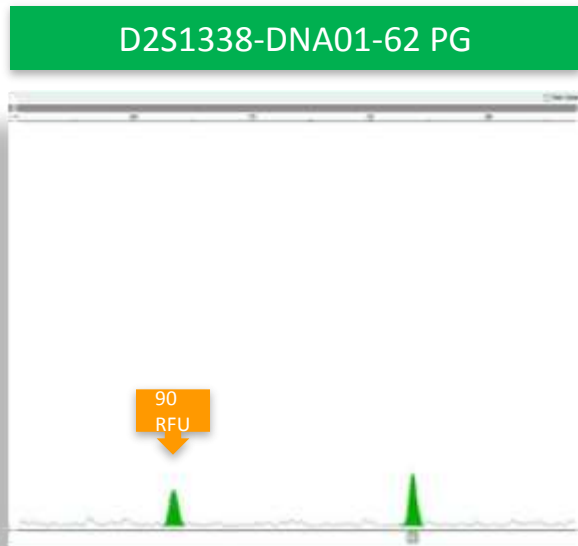
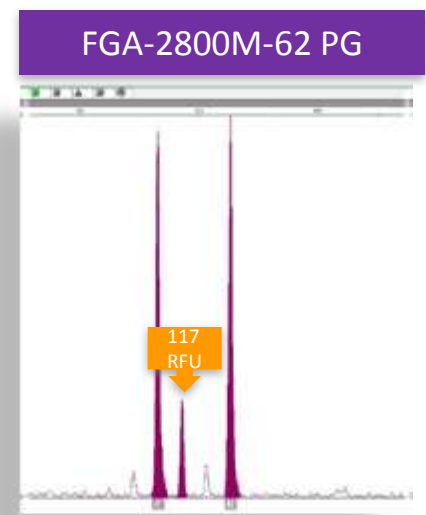
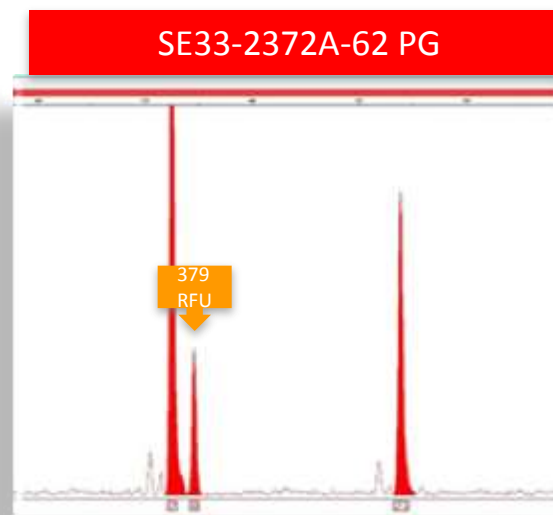
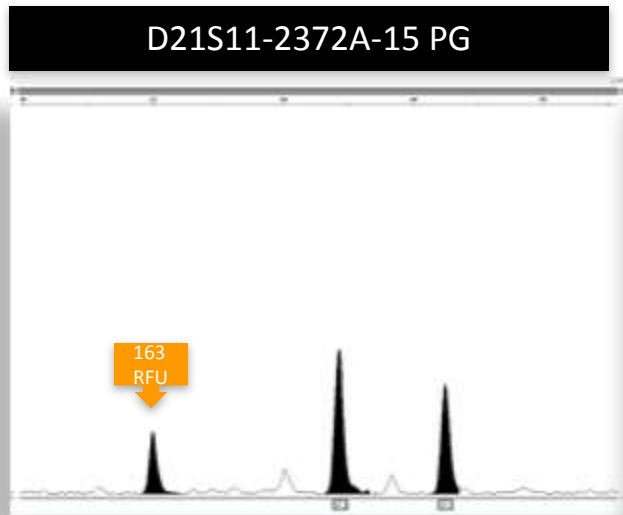
DNA Input (pg)	Observed Dropout
500	0 %
250	0 %
125	4 %
62	45 %
31	56 %

## Stochastic threshold

RFU value of the highest surviving false homozygous peak when allele drop-out is observed at heterozygous markers:

479 RFU for D10S1248 at 62.5 pg of DNA input

# Sensitivity study: Incidence of Drop-in at Low DNA input

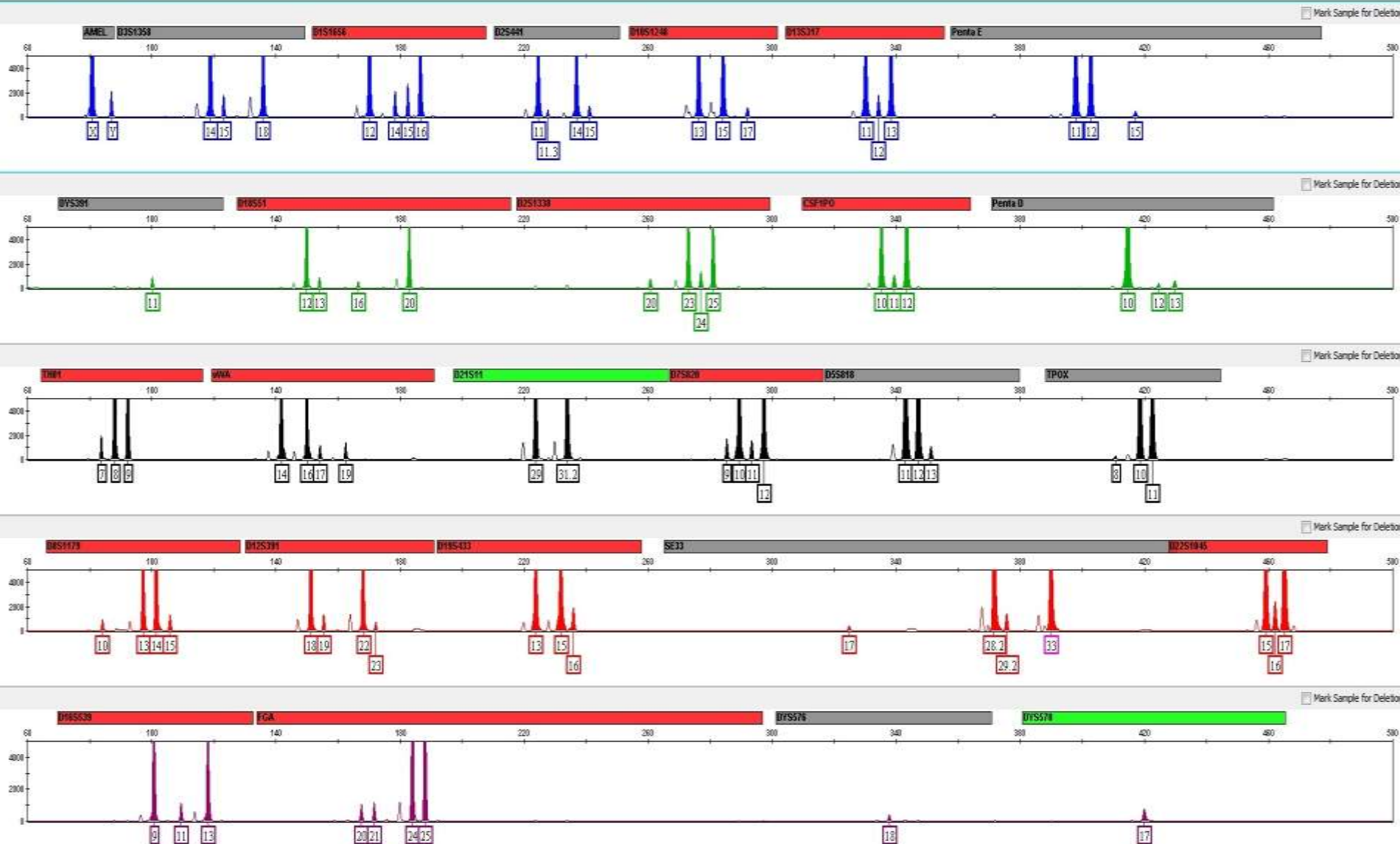


Detected Incidence of Drop-in for the last four dilution series over a threshold of 100 RFU (1,012 Determinations)

0.3 %

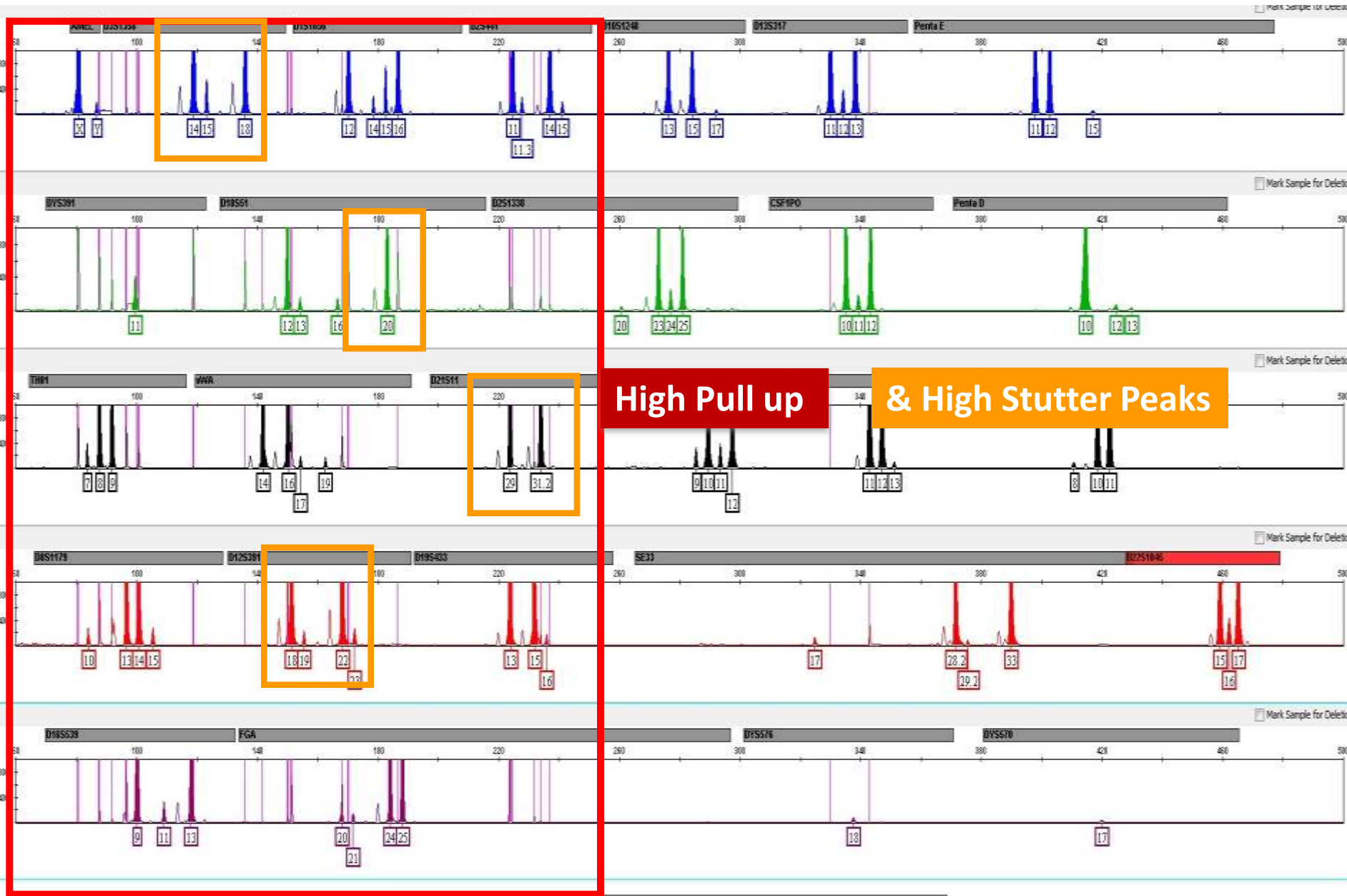


# Full profile of the minor male contributor: Series of 1 ng of total DNA at 8:1 mixture ratio



DNA input of the minor male contributor: 111 pg

# Full profile of the minor male contributor: Series of 3 ng of total DNA at 15:1 mixture ratio



DNA input of the minor male contributor: 186 pg

# Body fluid mixture samples from GHEP & GEDNAP proficiency exercises

Two Contributors

DNA SAMPLES	BODY FLUIDS & CONTRIBUTORS		[DNA] ng/ul
DNAMIX01-L1	♀	♂	Total: 5.7 Male: 0.8
DNAMIX01-L2	♀	♂	Total: 0.7 Male: 0.4
DNAMIX01-L3	♀	♂	Total: 0.7 Male: 0.6
DNAMIX02-L1	♂	♂	Total: 13 Male: 15
DNAMIX03	♀	♂	Total: 4 Male: 2
DNAMIX04	♀	♂	Total: 13 Male: 8
DNAMIX05	♀	♂	Total: 9 Male: 3
DNAMIX06	♀	♂	Total: 1.7 Male: 1
DNAMIX07	♀	♂	Total: 3.3 Male: 1.8
DNAMIX08	♀	♂	Total: 1.9 Male: 1.8
DNAMIX09	♀	♂	Total: 1.7 Male: 1.3
DNAMIX10	♀	♀	Total: 3 Male: 0

Three Contributors

DNA SAMPLES	BODY FLUIDS & CONTRIBUTORS			[DNA] ng/ul
DNAMIX11	♀	♂	♂	Total: 7 Male: 3
DNAMIX12	♀	♂	♂	Total: 1.6 Male: 1.7
DNAMIX13	♀	♀	♂	Total: 3.5 Male: 1.7
DNAMIX14	♀	♀	♂	Total: 1.7 Male: 0.8

- ✓ Allele Concordance with other kits
- ✓ MNC
- ✓ MNM



<http://www.ghep-isfg.org>



<http://www.gednap.de>

SALIVA

SEMEN

BLOOD

# Allele Concordance with other Kits (Intra & Inter-lab comparison)

Two Contributors

DNA SAMPLES	BODY FLUIDS & CONTRIBUTORS		Allele Concordance (Globalfiler, Identifiler, NGMSE, ESX17,...)
DNAMIX01-L1	♀	♂	OK Partial DNA profile from the minor male contributor at a 7:1 DNA ratio
DNAMIX01-L2	♀	♂	OK
DNAMIX01-L3	♀	♂	OK
DNAMIX02	♂	♂	OK
DNAMIX03	♀	♂	OK Non-concordance at SE33 among different Kits (primer designs)
DNAMIX04	♀	♂	OK
DNAMIX05	♀	♂	OK Allele 28 at D12S391 (Out of ladder)
DNAMIX06	♀	♂	OK
DNAMIX07	♀	♂	OK
DNAMIX08	♀	♂	OK
DNAMIX09	♀	♂	OK
DNAMIX10	♀	♀	OK



# Allele Concordance with other Kits (Intra & Inter-lab comparisons)

Three Contributors

DNA SAMPLES	BODY FLUIDS & CONTRIBUTORS			Allele Concordance (Globalfiler, Identifiler , NGMSE, ESX17,...)
DNAMIX11	♀	♂	♂	OK CE Resolution of Microvariants at D12S391
DNAMIX12	♀	♂	♂	OK CE Resolution of Microvariants at D12S391
DNAMIX13	♀	♀	♂	OK
DNAMIX14	♀	♀	♂	OK

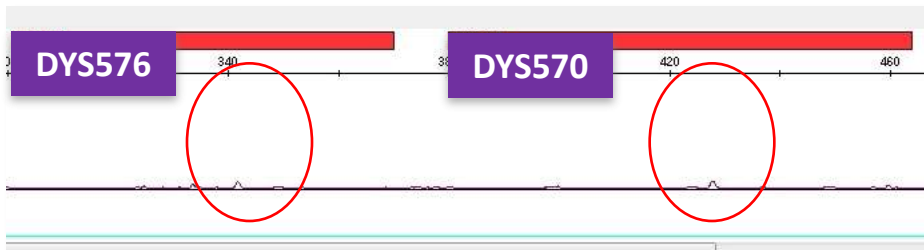
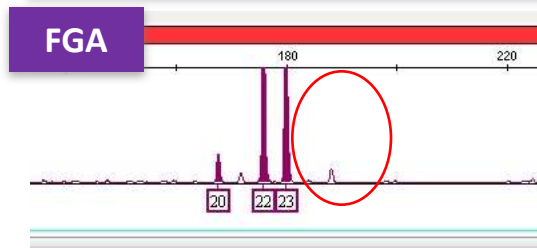
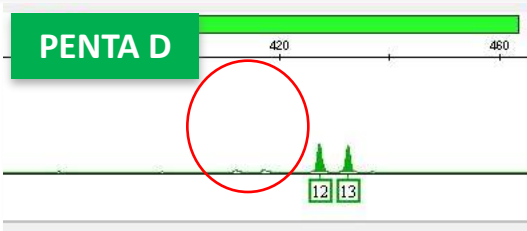
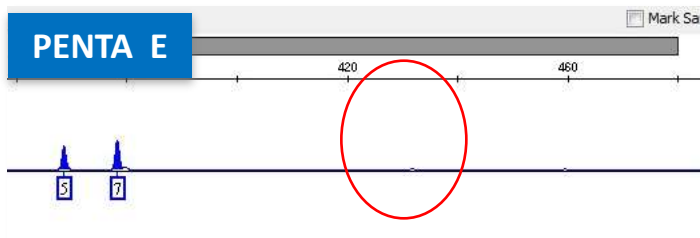
# Partial DNA profile from the minor male contributor

(Female:male mixture ratio 7:1)

DNAMIX01-L1



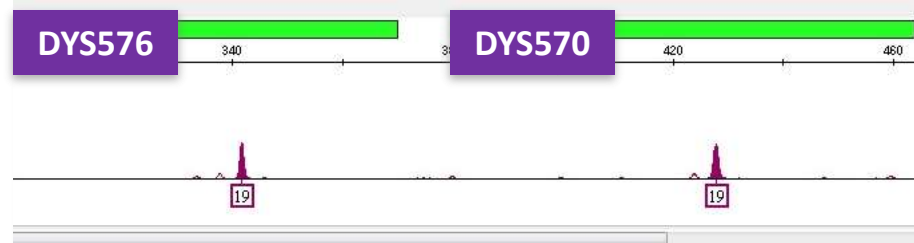
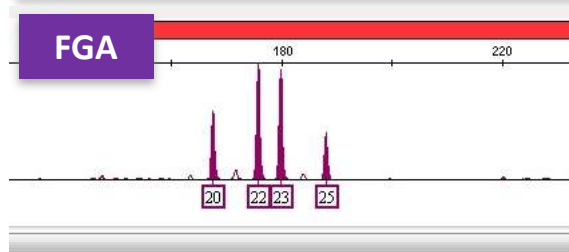
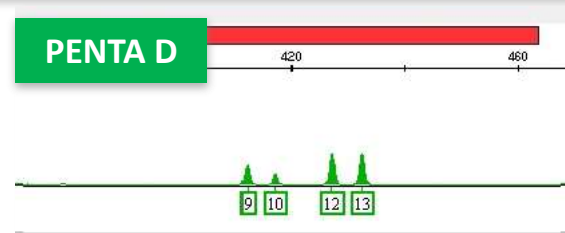
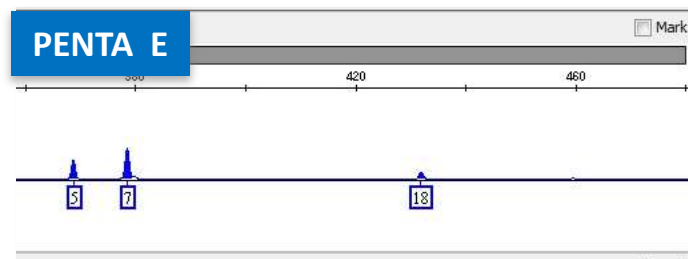
Total: 5.7  
Male: 0.8



DNAMIX01-L2



Total: 0.7  
Male: 0.4



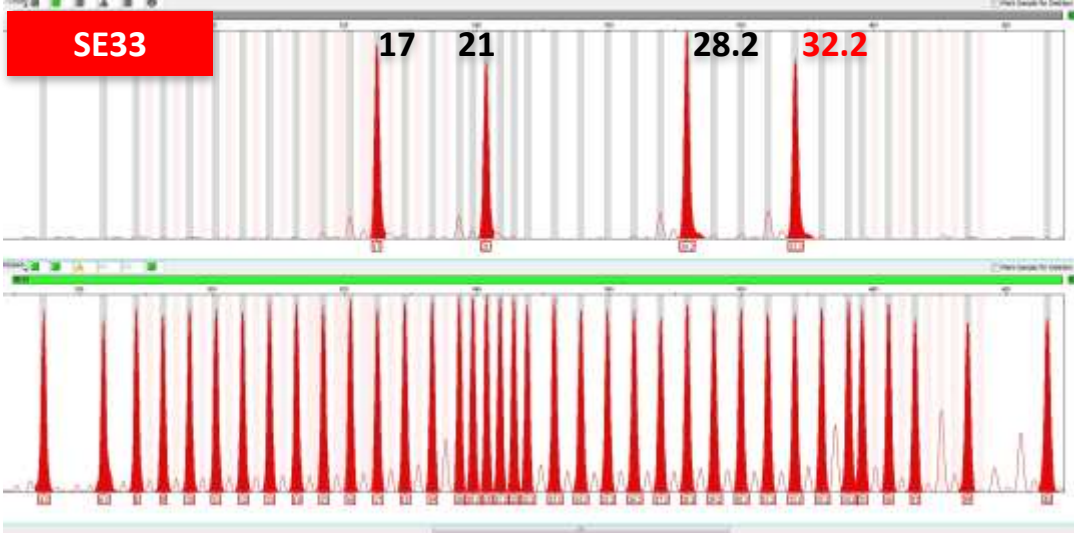
# Non-concordance at SE33 among different Kits (primer designs)

DNAMIX03

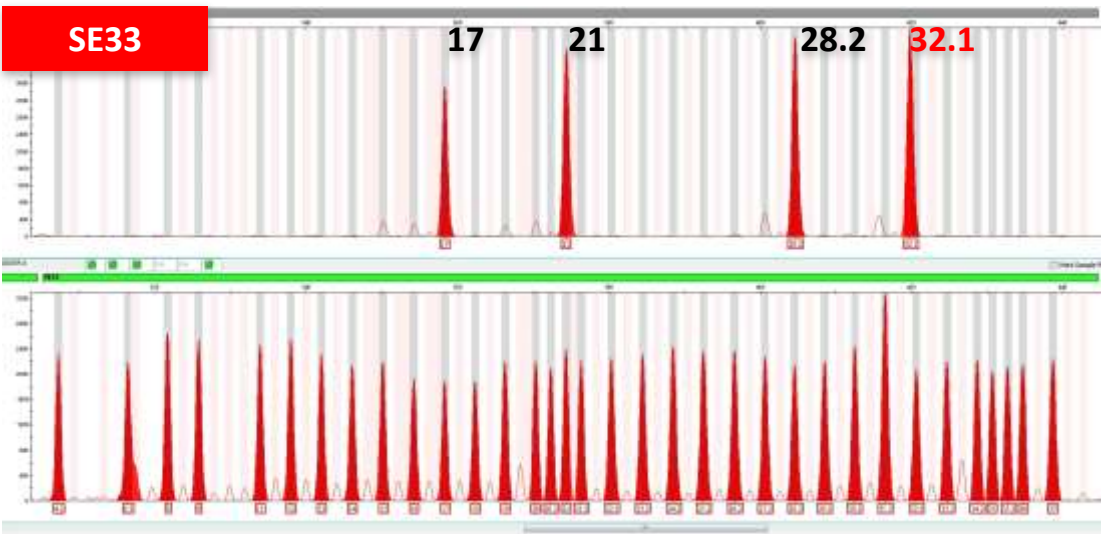


Total: 4  
Male: 2

Promega 6C V2



Globalfiler



# Allele 28 at D12S391 (Out of ladder)

DNAMIX05



Total: 9  
Male: 3

Promega 6C V2



Globalfiler



# CE Resolution of Microvariants at D12S391

DNAMIX11



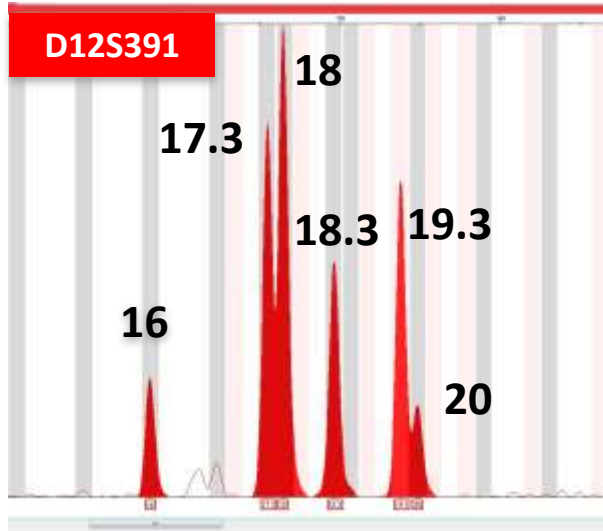
Total: 7  
Male: 3

DNAMIX12

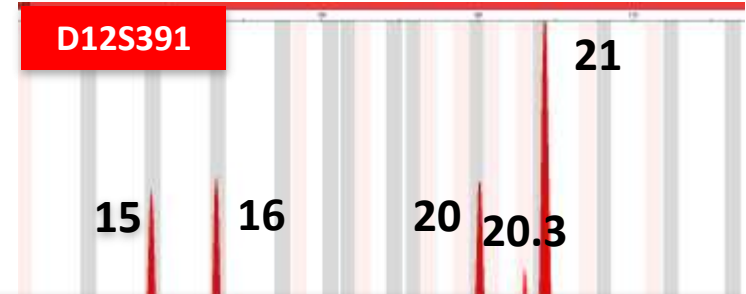


Total: 1.6  
Male: 1.7

Promega 6C V2

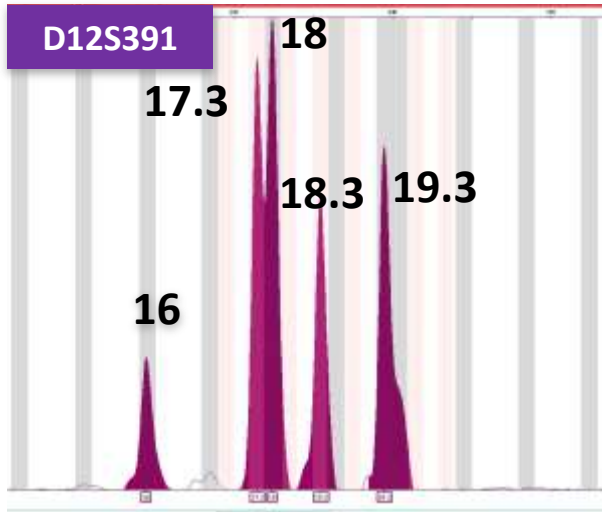


D12S391

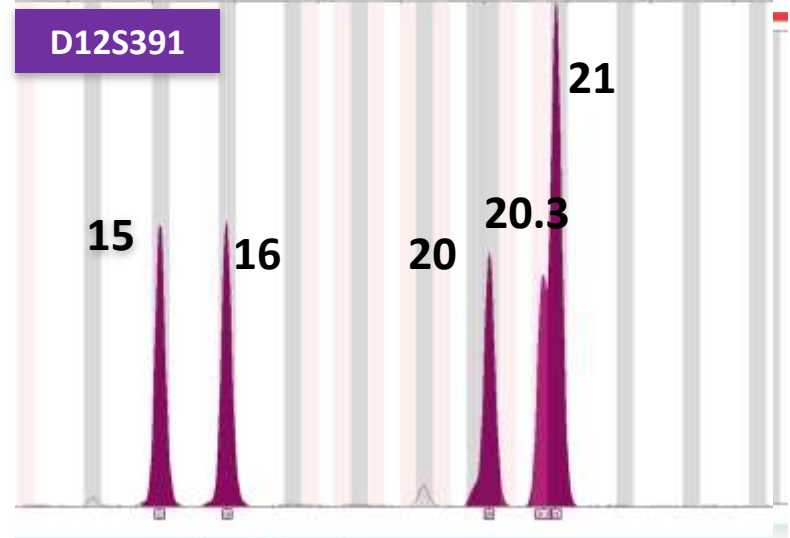


Automatic allele calling of 20.3  
By GMIDX reanalysis with:  
**No Smoothing**

Globalfiler



D12S391



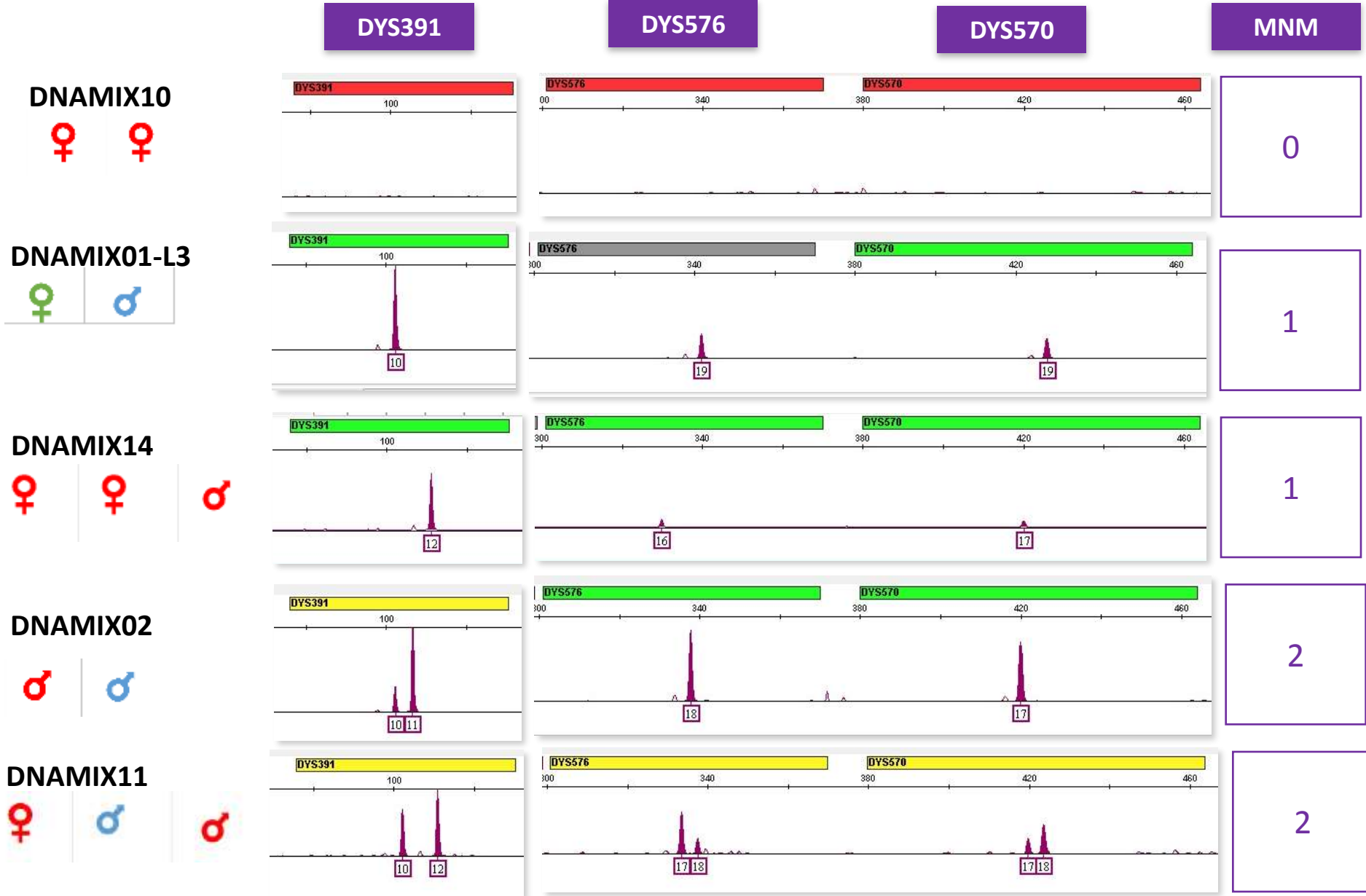
# Minimum Number of Contributors

DNA SAMPLES	BODY FLUIDS & CONTRIBUTORS		Minimum Number of Contributors			
			Total ≠ of Alleles	≠ STR with 3 alleles	≠ STR with 4 alleles	MNC
DNAMIX01-L2	♀	♂	68	12	5	2
DNAMIX01-L3	♀	♂	68	12	5	2
DNAMIX02	♂	♂	71	14	6	2
DNAMIX03	♀	♂	72	10	8	2
DNAMIX04	♀	♂	71	14	5	2
DNAMIX05	♀	♂	72	9	8	2
DNAMIX06	♀	♂	67	9	6	2
DNAMIX07	♀	♂	64	16	1	2
DNAMIX08	♀	♂	68	11	6	2
DNAMIX09	♀	♂	71	5	10	2
DNAMIX10	♀	♀	77	7	12	2

# Minimum Number of Contributors

DNA SAMPLES	BODY FLUIDS & CONTRIBUTORS			Minimum Number of Contributors			
				Total ≠ of Alleles	≠ STR with 5 alleles	≠ STR with 6 alleles	MNC
DNAMIX11	♀	♂	♂	105	8	3	3
DNAMIX12	♀	♂	♂	90	3	2	3
DNAMIX13	♀	♀	♂	77	2	0	3
DNAMIX14	♀	♀	♂	90	5	1	3

# Minimum Number of Males ?





## Concluding Remarks

- ✓ The data reported demonstrated the Promega 6C PCR amplification system followed by CE detection in a 3500 genetic analyzer generates high quality, reproducible, precise, accurate, and sensitive profiling STR data, even from sub-nanogram amounts of genomic DNA template.
- ✓ The study demonstrated that the combination of autosomal STRs and Y-STRs in the PowerPlex® 6-dye system maximizes the information obtained from DNA mixtures regarding both the minimum number of total contributors and the minimum number of specific male contributors.
- ✓ The Promega 6C system provides equivalent overall performance to previous forensic STR PCR kits, but with enhanced discrimination power for a better match efficiency that would reduce the chance of adventitious matches during DNA data exchange among national DNA databases

## Concluding Remarks

Multiplex STR System	Probability of Identity	Probability of Paternity Exclusion
<b>NGMSElect</b>	$2.5 \times 10^{-21}$	99.9999998%
<b>Globalfiler</b>	$3.1 \times 10^{-26}$	99.99999995%
<b>Promega 6C System</b>	$3.7 \times 10^{-29}$	99.999999994%

(Spanish population. [Garcia et al. Forensic Sci Int Genet. 6\(2\) \(2012\)](#))

✓ The use of Promega 6C System would be also very beneficial to improve discrimination power of DNA analysis not only in criminal DNA databases, but also in many other forensic applications of autosomal STR profiling including missing person identification, disaster victim identification, mass grave investigations and kinship analysis.

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Douglas Storts & The Promega Team  
Kit Design & Production



Oscar Garcia  
Biostatistical Calculation with the Spanish Population Data



## PROJECT TEAM

Pablo Martín  
Lourdes Fernández de Simón  
Gracia Luque  
M<sup>a</sup> José Farfán  
Antonio Alonso



INTCF-Madrid  
SERVICIO DE  
BIOLOGIA



BIOLOGY DEPARTMENT STAFF



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