DNA Mixture Interpretation Workshop | *Michael D. Coble, PhD*

Modified Procedures to Increase Sensitivity and the Impact on Mixture Interpretation Procedures

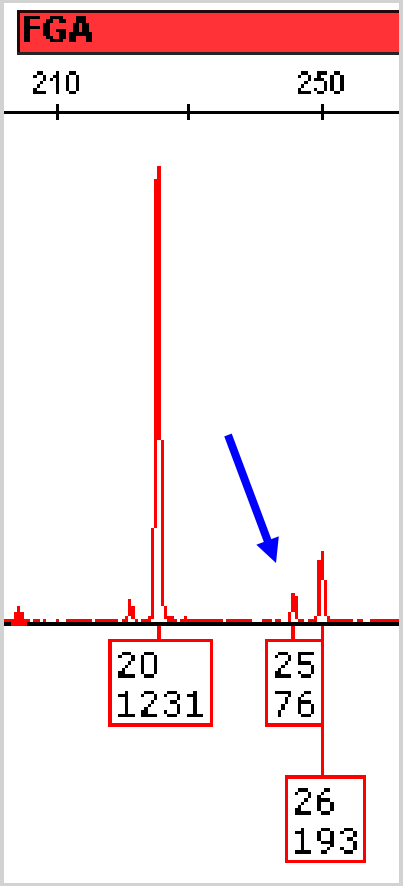
NIJ Disclaimer

- **This project was supported by NIJ Award #2008-DN-BX-K073 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice."**

DNA Mixture Interpretation

- **Mixtures from high quality DNA templates with all alleles above the stochastic threshold…**

The Reality…



Stutter ? (14.7%)

PHR = 76/193 (39.4%)

Amplification of low level (template) DNA

DNA Mixture Interpretation

- **Mixtures from low quality DNA with alleles below the stochastic threshold…**

http://thedroidguy.com/2011/03/charlie-sheen-has-some-winning-android-tiger-blood/

SWGDAM Definition of Stochastic Effects

SWGDAM Guidelines glossary:

- **Stochastic effects: the observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template**

But wait… I don't do LT-DNA testing, I always amplify 1.0 ng of DNA

Amount of DNA	~ # of cells from major component	~ # of cells from minor component
1ng	129	14
0.5ng	64	7
0.25ng	32	4
0.125ng	16	2
0.0625ng	8	1

Some Definitions of Low Template (LT) DNA

- **Working with <100-200 pg genomic DNA**
- **Considered to be data *below stochastic threshold level* where PCR amplification is not as reliable (determined by each laboratory; typically 150-250 RFUs)**
- **Having too few copies of DNA template to ensure reliable PCR amplification (allelic or full locus drop-out)**
- **Can often be the minor component of mixture samples consisting of low level DNA template amounts**

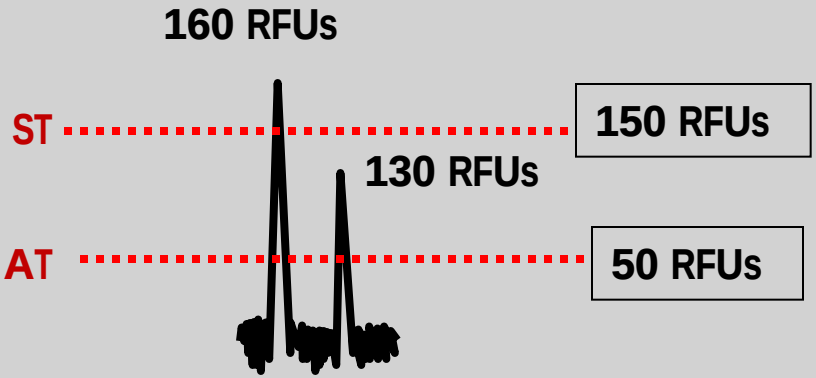
NIJ National Institute of Justice

Types of Results at Low Signal Intensity (Stochastic amplification potential)

"Straddle Data"

One allele peak above the stochastic threshold and one below

Only one allele in a pair is above the laboratory stochastic threshold



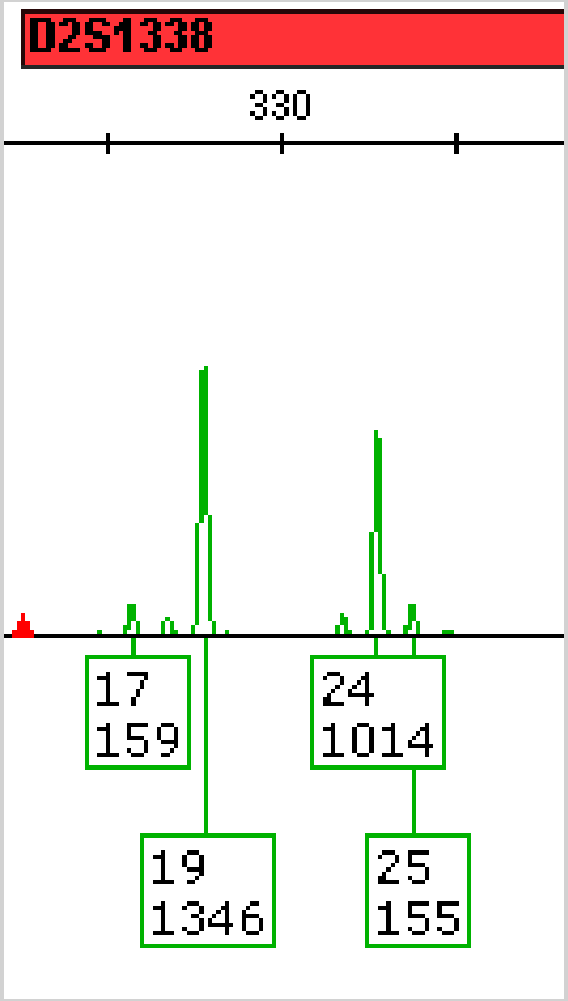
Straddle data may be caused by degradation, inhibition and LT-DNA

At low levels of input DNA, the potential for straddle data is high.

The issue is best avoided by re-amplifying the sample at higher input DNA

Otherwise straddle data makes locus inconclusive

"The Threshold of a Dilemma"



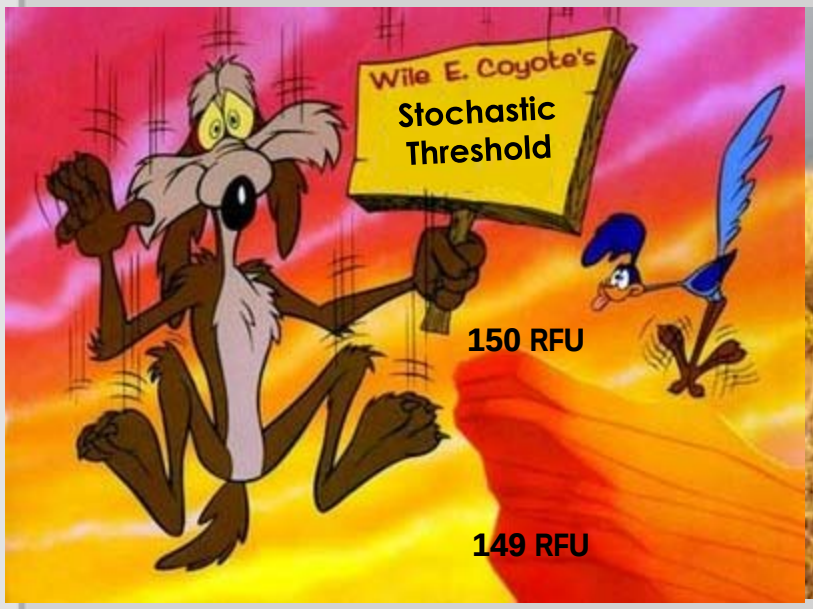
Gill and Buckleton (2010)JFS Vol 55 (1)

If ST = 150, marker is ok for stats

If ST = 175, marker is ok to use for exclusion, but **not** for RMNE (OK for LR or modified RMP)

"Falling off of a cliff"

Falling off the Cliff vs. Gradual Decline

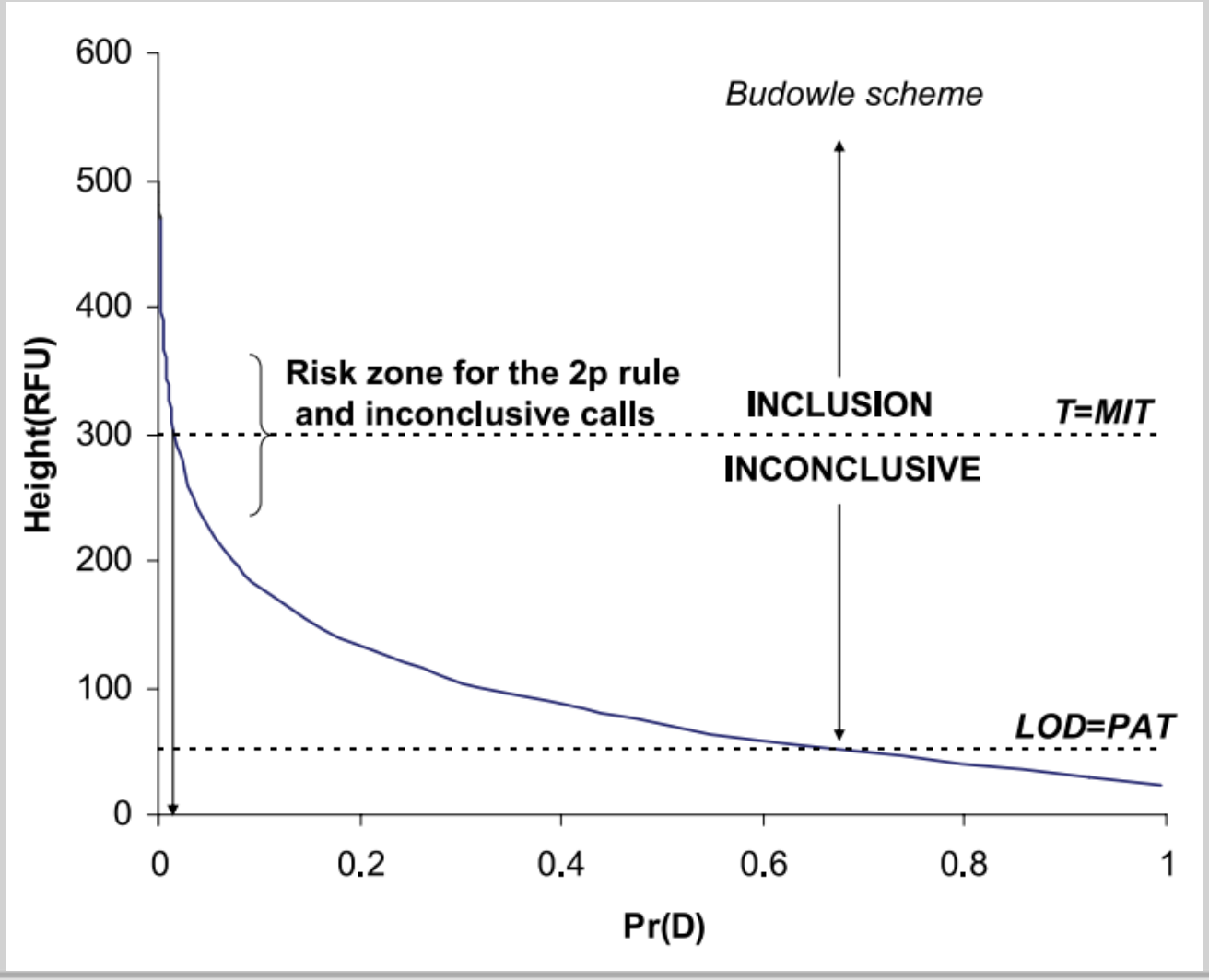


http://blog.sironaconsulting.com/.a/6a00d8341c761a53ef011168cc5ff3970c-pi

http://ultimateescapesdc.files.wordpress.com/2010/08/mountainbiking2.jpg

NIJ National Institute of Justice

Gill and Buckleton (2010)



Scientific Reasoning behind the Stochastic Threshold

- **When stochastic fluctuation is present, interpreting data becomes problematic due to the potential for:**
  - **Allele dropout**
  - **Poorly defined mixture ratios**
  - **Low template DNA**
- **Bottom line: Input levels of DNA should be sufficiently high to avoid straddle data. Mixture interpretation must be evaluated cautiously on low level data as peak intensities are highly variable.**

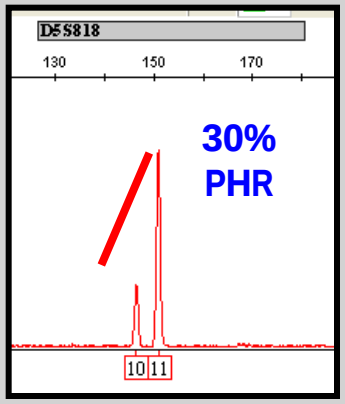
NIJ National Institute of Justice

Stochastic (Random) Effects with LT-DNA

When Combined with Higher Sensitivity Techniques

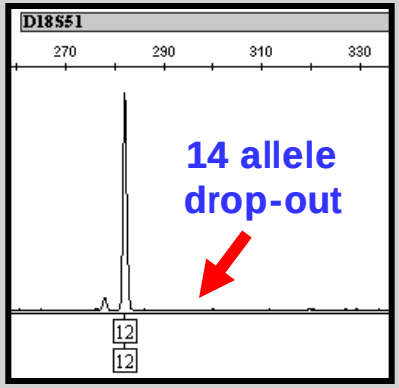
Loss of True Signal
*(**False Negative**)*

Heterozygote Peak Imbalance



Identifiler, 30 pg DNA, 31 cycles

Allelic **Drop-out**



Identifiler, 30 pg DNA, 31 cycles

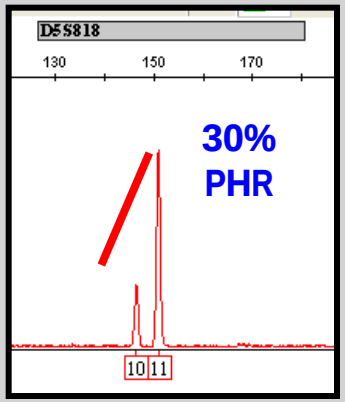
NIJ National Institute of Justice

Stochastic (Random) Effects with LT-DNA

When Combined with Higher Sensitivity Techniques

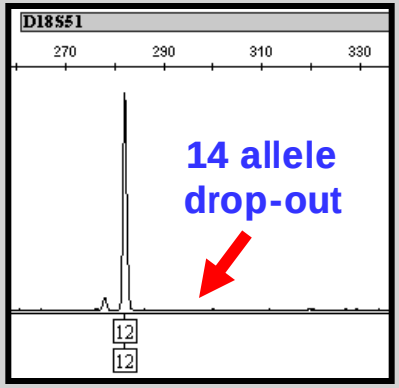
Loss of True Signal (False Negative)

Heterozygote Peak Imbalance



Identifiler, 30 pg
DNA, 31 cycles

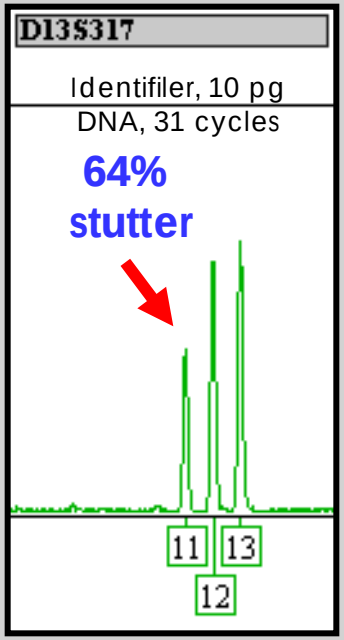
Allelic **Drop-out**



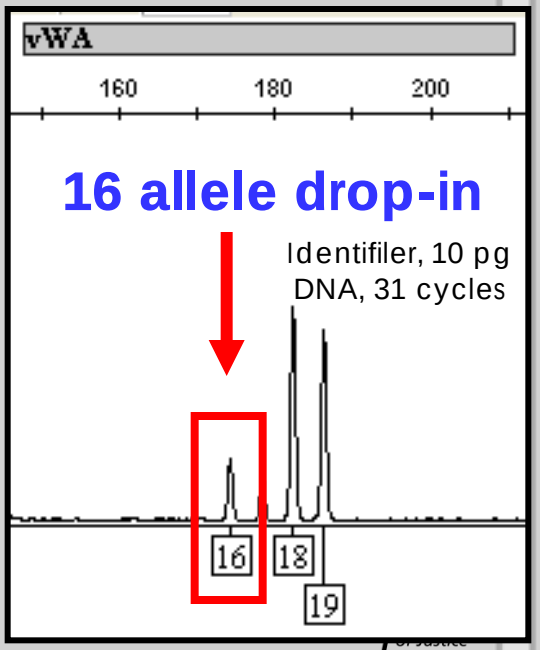
Identifiler, 30 pg
DNA, 31 cycles

Gain of False Signal (False Positive)

Higher Stutter



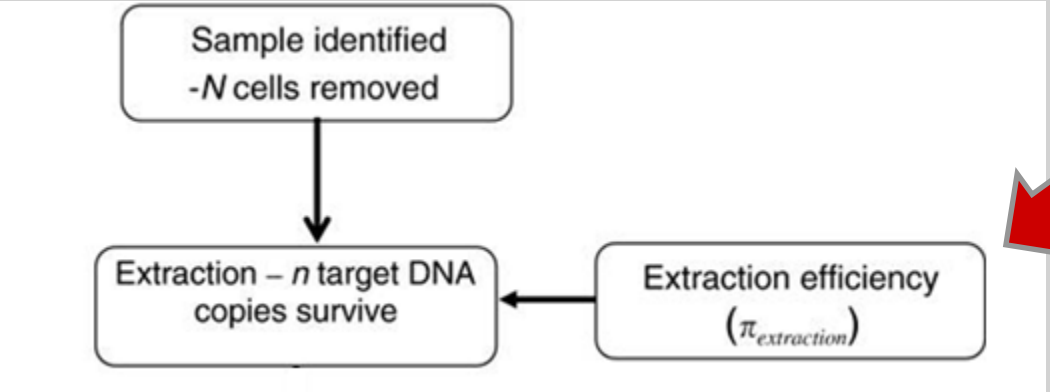
Allelic Drop-in

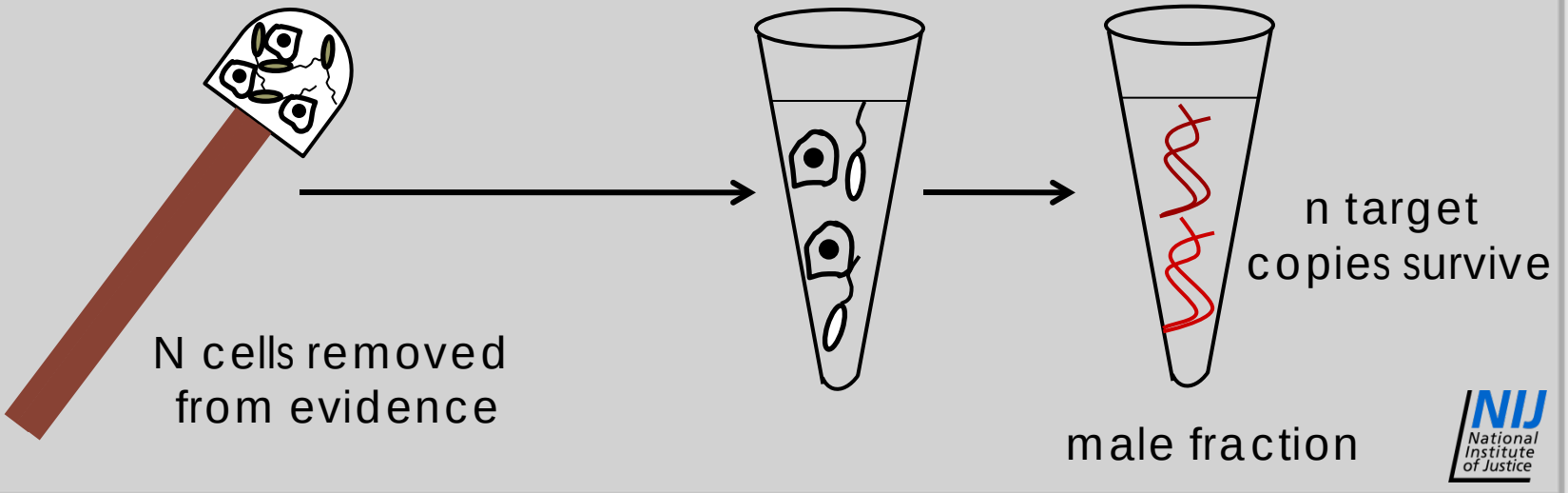


Targets to improve DNA quantity and Increase Sensitivity

Gill, Curran, and Elliot (2005) NAR

A graphical simulation model of the entire DNA process associated with the analysis of short tandem repeat loci





TECHNICAL NOTE

David Sweet,[1] D.M.D, Ph.D.; Miguel Lorente,[2] M.D., Ph.D.; José A. Lorente,[2] M.D., Ph.D.; Aurora Valenzuela,[2] M.D., Ph.D., B.D.S.; and Enrique Villanueva,[2] M.D., Ph.D.

An Improved Method to Recover Saliva from Human Skin: The Double Swab Technique

REFERENCE: Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: The double swab technique. J Forensic Sci 1997;42(2): 320–322.

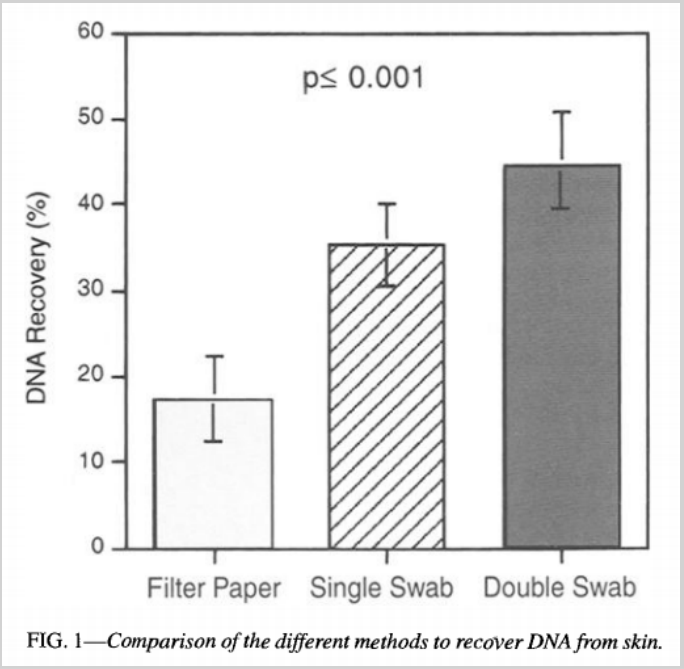
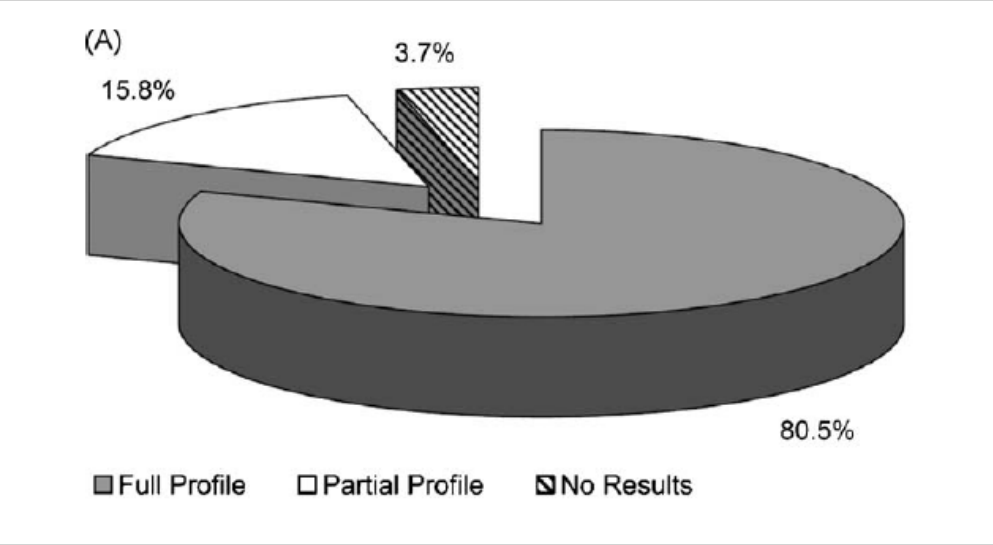


FIG. 1—*Comparison of the different methods to recover DNA from skin.*

NIJ National Institute of Justice

Extraction Efficiency

- **Defined using several different methods**
  - **Full vs. Partial STR Profiles**



M. Stangegaard et al. "Automated extraction of DNA from reference samples from various types of biological materials on the Qiagen BioRobot EZ1 Workstation." Forensic Science International: Genetics Supplement Series 2 (2009) 69–70

Extraction Efficiency

- **Defined using several different methods**
  - **Number of loci successfully genotyped**
  - **Pass/Fail System**

E. Milne et al. "Buccal DNA Collection: Comparison of Buccal Swabs with FTA Cards." Cancer Epidemiol Biomarkers Prev 2006;15(4). April 2006

No. loci genotyped successfully	*n* (%)	
	FTA card	Buccal swab
4	101 (82.8)	59 (48.4)
3	20 (16.4)	40 (32.8)
2	1 (0.8)	11 (9.0)
0 or 1	0 (0)	12 (9.8)

Table 1. Success of genotyping from FTA card and buccal swabs at three loci in 122 subjects

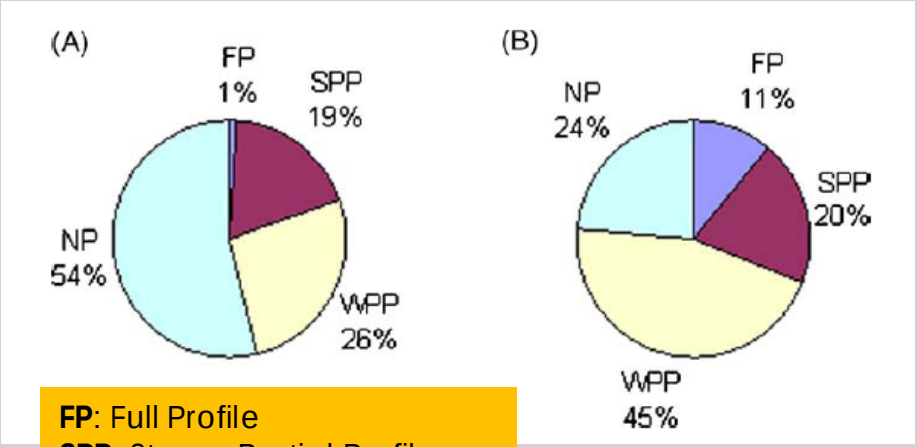
Locus	Buccal swabs	FTA cards, *n* (overall %)		
		Pass	Fail	Total
MTHFR 677C>T (RFLP)	Pass	95 (77.9)	2 (1.6)	97 (79.5)
	Fail	25 (20.5)	0 (0)	25 (20.5)
	Total	120 (98.4)	2 (1.6)	122 (100)
MTHFR 677C>T (real-time PCR)	Pass	109 (89.3)	0 (0)	109 (89.3)
	Fail	13 (10.6)	0 (0)	13 (10.6)
	Total	122 (100)	0 (0)	122 (100)
ACE *I/D*	Pass	65 (53.3)	11 (9.0)	76 (62.3)
	Fail	37 (30.3)	9 (7.4)	46 (37.7)
	Total	102 (83.6)	20 (16.4)	122 (100)
XPD 1012G>A	Pass	107 (87.7)	0 (0)	107 (87.7)
	Fail	15 (12.3)	0 (0)	15 (12.3)
	Total	122 (100)	0 (0)	122 (100)

NIJ National Institute of Justice

Typical Definition of Extraction Efficiency

- **The number of observed full STR profiles**

- **Divided into three categories:**
  1. **Full Profile**
  2. **Partial Profile**
  3. **No Profile**



FP: Full Profile
SPP: Strong Partial Profile
WPP: Weak Partial Profile
NP: No Profile

K.M. Horsman-Hall et al. "Development of STR profiles from firearms and fired cartridge cases." Forensic Science International: Genetics 3 (2009) 242–250

NIJ National Institute of Justice

Extraction Efficiency Results in the Literature

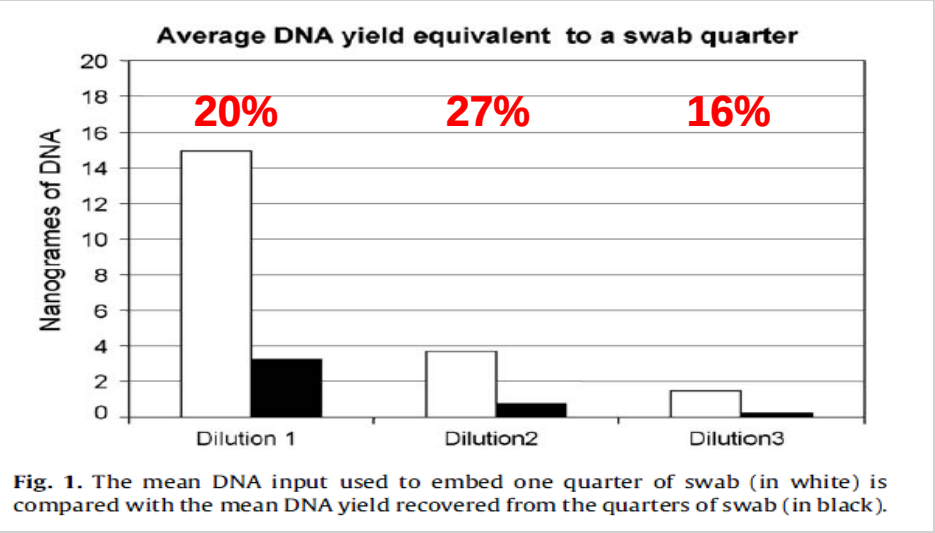


Fig. 1. The mean DNA input used to embed one quarter of swab (in white) is compared with the mean DNA yield recovered from the quarters of swab (in black).

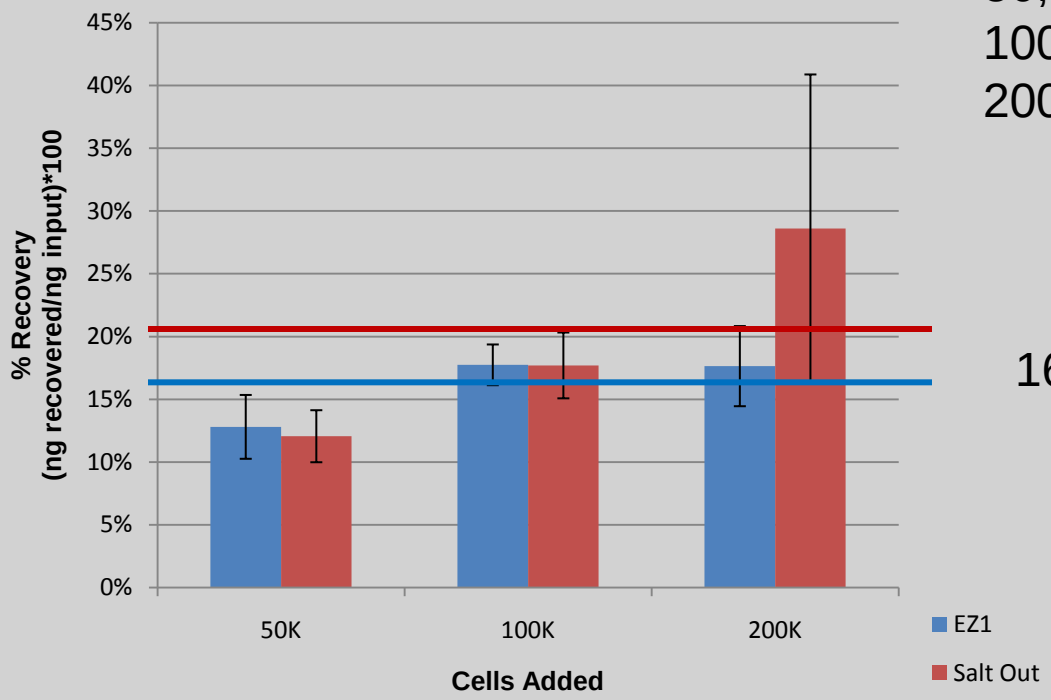
A. Colussi et al. "**Efficiency of DNA IQ System in recovering semen from cotton swab.**" Forensic Science International: Genetics Supplement Series 2 (2009) 87-88.

Liquid Blood Dilutions	Volume of Liquid Blood Extracted (µL)	BioRobot® EZ1, DNA (ng)	BioRobot® EZ1 with cRNA, DNA (ng)	Organic Extraction, DNA (ng)
1:10	0.1	8.025	10.000	7.900
1:50	0.02	0.213	2.250	1.840
1:250	0.004	0.050	0.260	0.263
1:1250	0.0008	0.000	0.040	0.038
1:2500	0.0004	0.000	0.013	0.000
		33%		**33%**

R. Kishore et al. "**Optimization of DNA Extraction from Low-Yield and Degraded Samples Using the BioRobot EZ1 ad BioRobot M48.**" J Forensic Sci, September 2006, Vol. 51, No 5.

Extracted Cell Line Efficiency

Swabbed 100 µL of a solution containing human epithelial cells in a Teflon tube (n=12 per quantity)

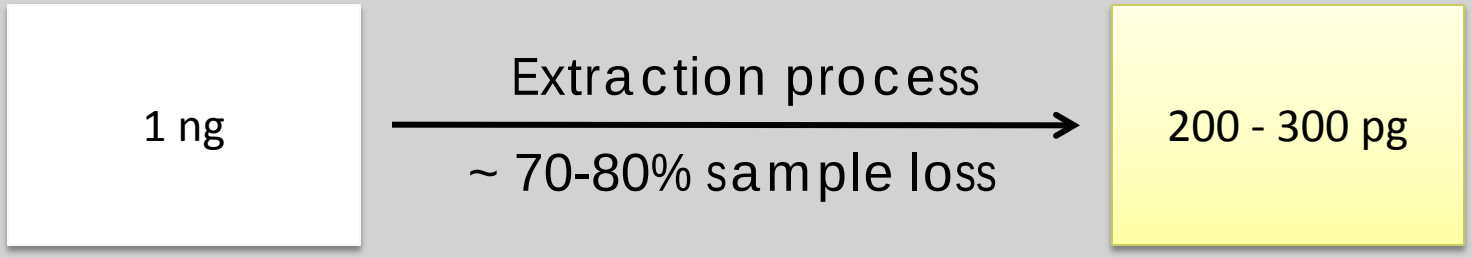


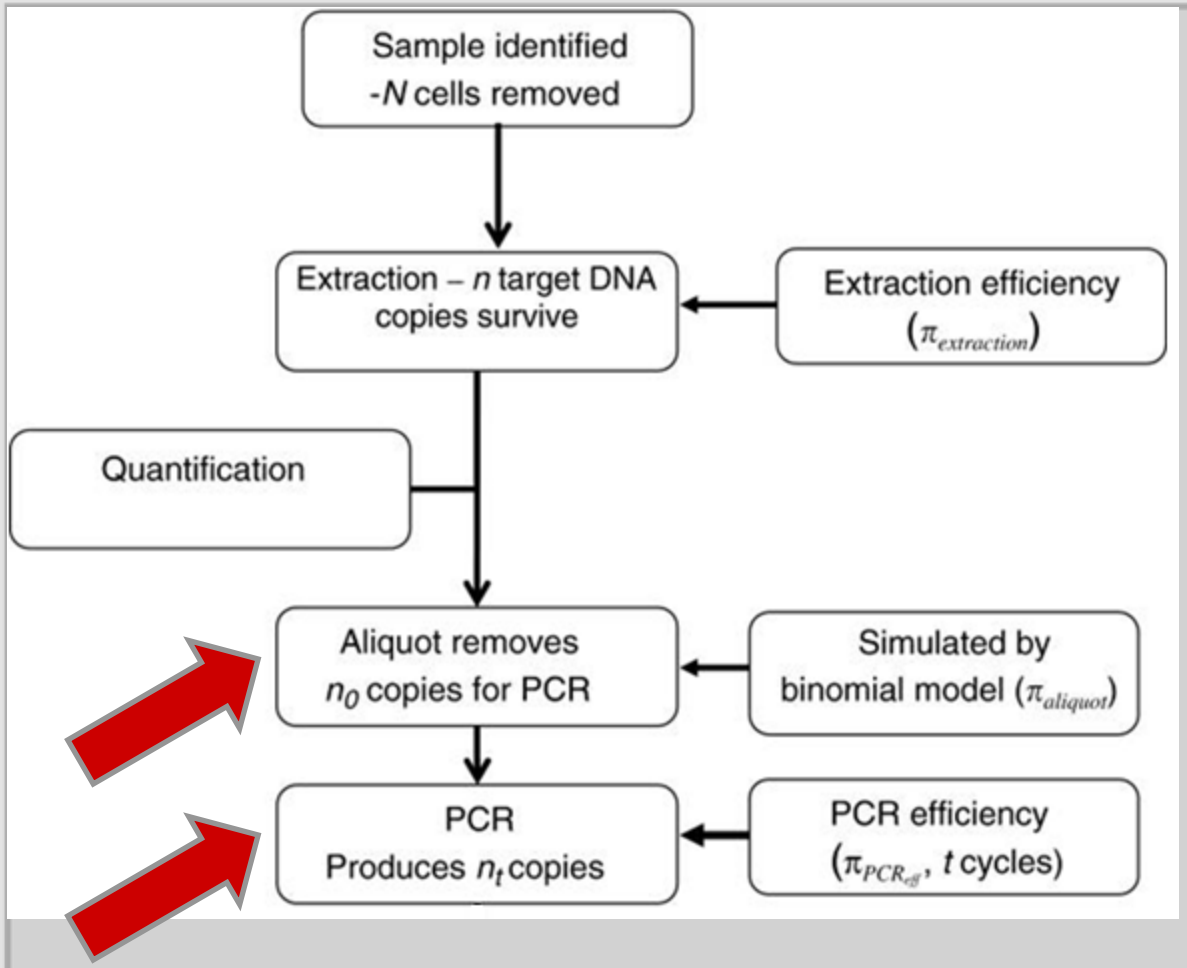
50,000 cells (300 ng)
100,000 cells (600 ng)
200,000 cells (1200 ng)

16-21% avg. recovery

Why Does This Matter?

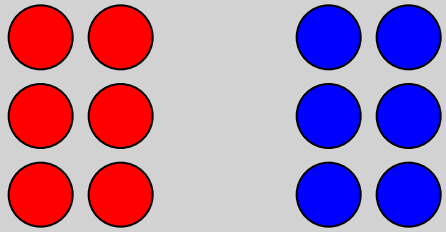
- **Low extraction efficiency could lower sample quantity into the Low Template DNA (LT-DNA) range**



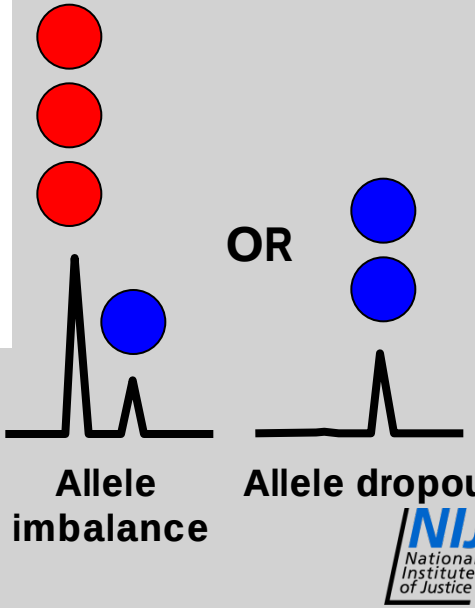


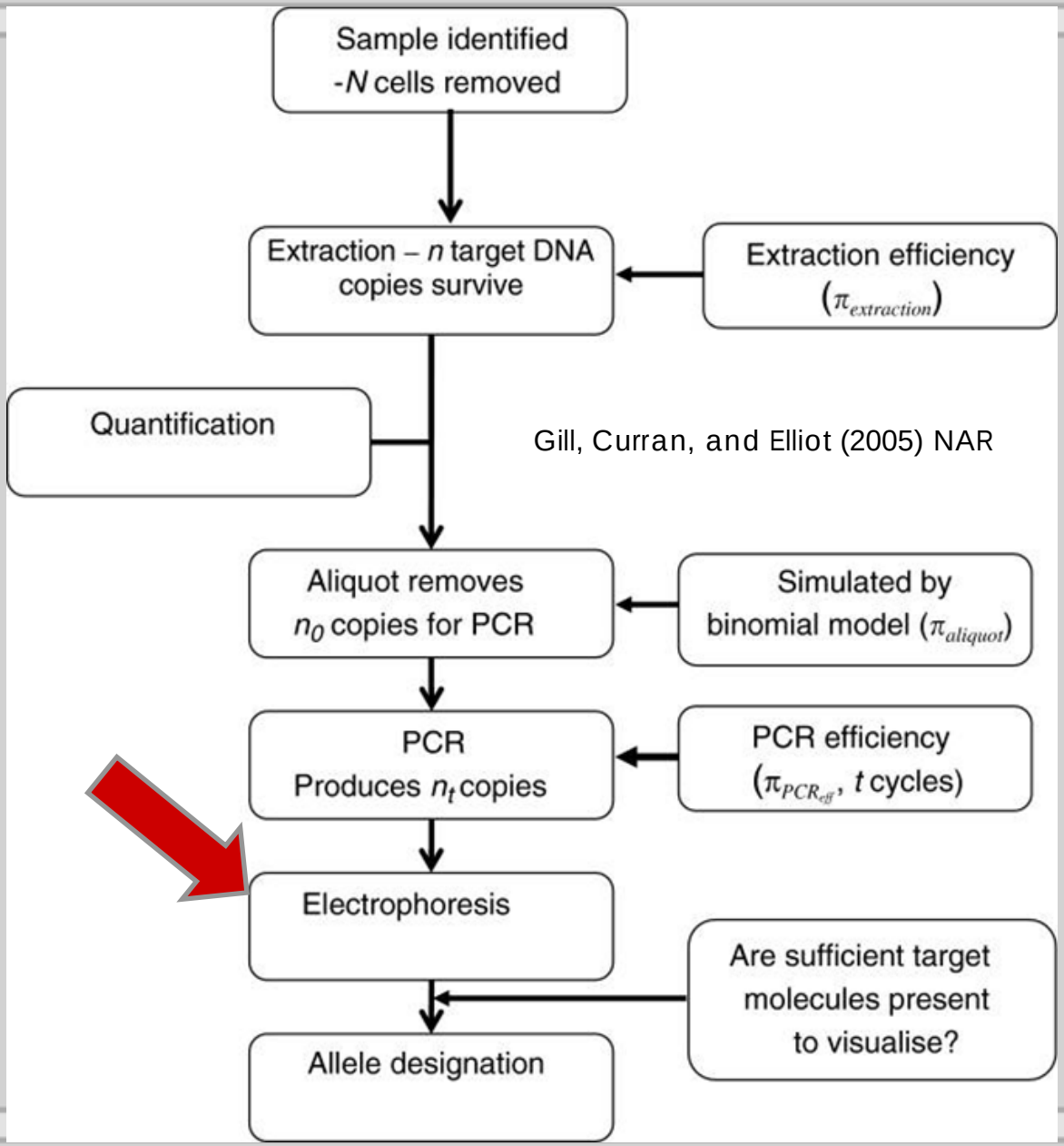
Gill, Curran, and Elliot (2005) NAR

True amount



What might be sampled by the PCR reaction…




Sample identified
-N cells removed
Extraction – n target DNA copies survive
Extraction efficiency ($\pi_{extraction}$)
Quantification
Gill, Curran, and Elliot (2005) NAR
Aliquot removes n_0 copies for PCR
Simulated by binomial model ($\pi_{aliquot}$)
PCR
Produces n_t copies
PCR efficiency ($\pi_{PCR_{eff}}$, t cycles)
Electrophoresis
Are sufficient target molecules present to visualise?
Allele designation

Modified Procedures to Increase Sensitivity

- **See Budowle *et al.* (2009) *Croatian Medical Journal* 50: 207-217 for a number of examples.**

Modified Procedures to Increase Sensitivity

- **Reduced PCR volume**
  - **Advantages: Concentrates PCR product**
  - **Disadvantages: Can concentrate PCR inhibitors, can increase pipetting errors with lower volumes, decreases the volume of template added to the reaction**

  - **Gaines *et al*. (2002) *J. Forensic Sci*. 47(6):1224-1237**
  - **Leclair *et al*. (2003) *J. Forensic Sci*. 48: 1001-1013**

Reduced Volume PCR

- **Possibility of lower volume PCR to effectively concentrate the amount of DNA in contact with the PCR reagents**
  - **Gaines *et al*. (2002) *J. Forensic Sci*. 47(6):1224-1237**
  - **Leclair *et al*. (2003) *J. Forensic Sci*. 48: 1001-1013**

- **Can samples be concentrated or can extraction volume be reduced?**

NIJ National Institute of Justice

Leclair et al. (2003) JFS 48:1001-1013

40 uL PCR

Panel A:
2ng / 40μL
(condition #4)

Panel B:
1ng / 20μL
(condition #5)

Panel C:
0.500ng / 10μL
(condition #6)

Panel D:
0.250ng / 5μL
(condition #7)

Panel E:
0.500ng / 40μL
(condition #12)

Panel F:
0.500ng / 20μL
(condition #9)

Panel G:
0.500ng / 10μL
(condition #6)

5 uL PCR

Panel H:
0.500ng / 5μL
(condition #3)

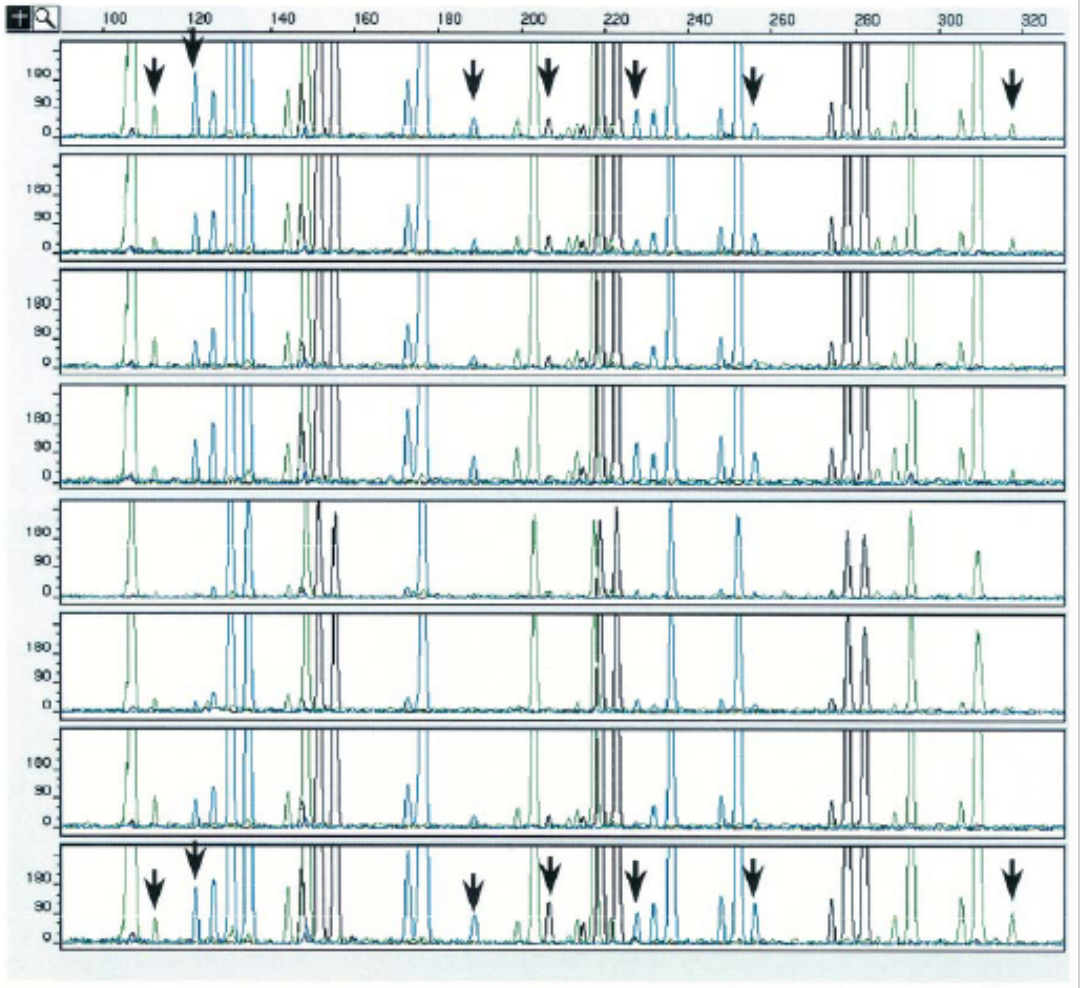


FIG. 5—*Effects of a reduction of PCR reaction volume and DNA template concentration on amplification of a casework sample with a minor profile representing 2% of the total mixture.*

NIJ National Institute of Justice

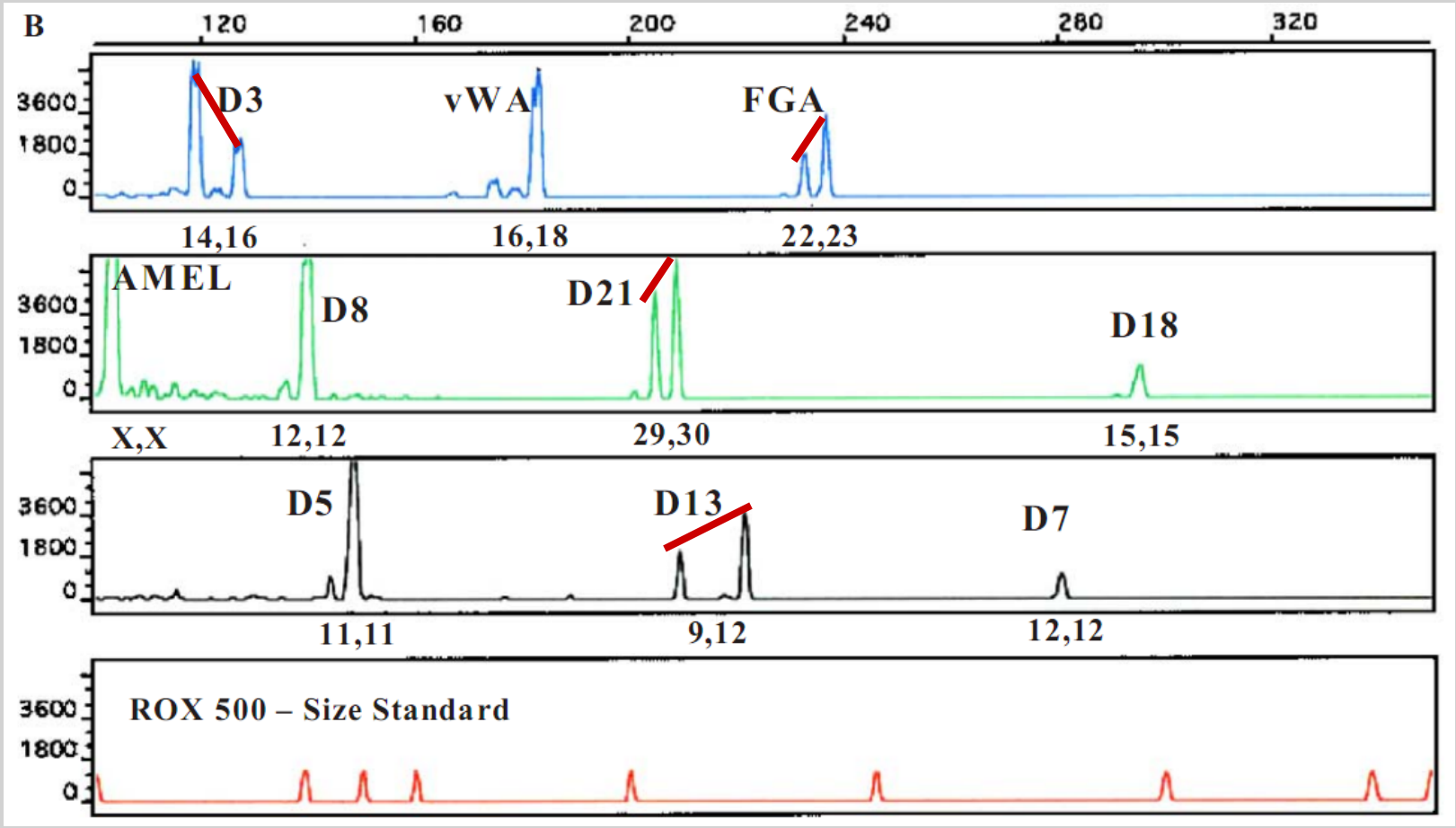
Modified Procedures to Increase Sensitivity

- **Whole Genome Amplification**
  - **Advantages: Increase the quantity of template prior to PCR**
  - **Disadvantages: Amplifies *all* DNA (bacterial), can preferentially amplify targets (affect PHRs)**

  - **Hanson and Ballantyne (2005) *Analytical Biochemistry* 346: 246-257**

Whole genome amplification strategy for forensic genetic analysis using single or few cell equivalents of genomic DNA

Hanson and Ballantyne (2005)



5 pg vaginal epithelial cells

Modified Procedures to Increase Sensitivity

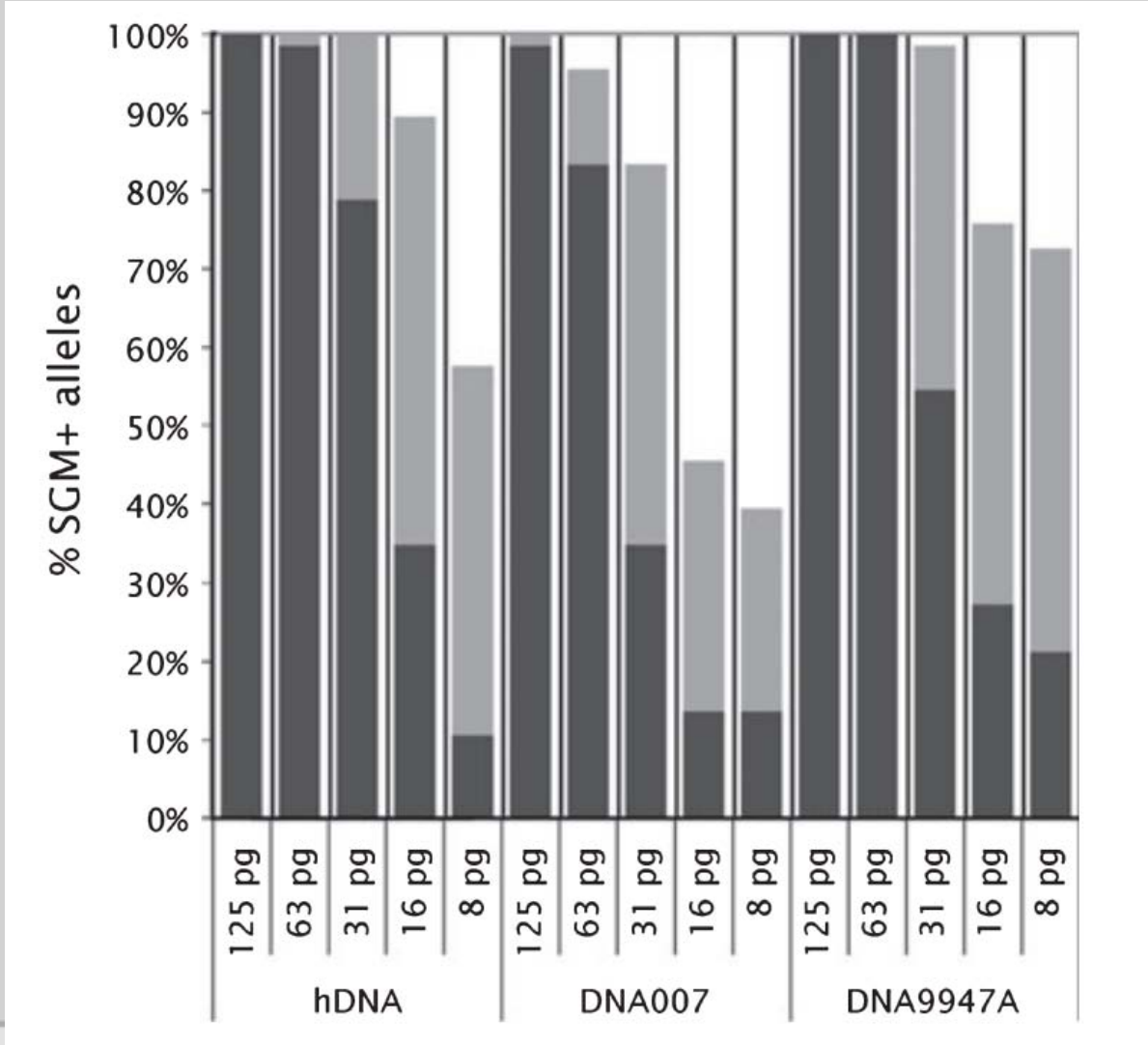
- **Increased CE injection/voltage**
  - **Advantages: More amplicons are electrophoretically injected into the capillary**
  - **Disadvantages: Can increase the analytical and stochastic thresholds**

  - **Westen *et al*. (2009) *J. Forensic Sci*. 54: 591-598**

Higher Capillary Electrophoresis Injection Settings as an Efficient Approach to Increase the Sensitivity of STR Typing

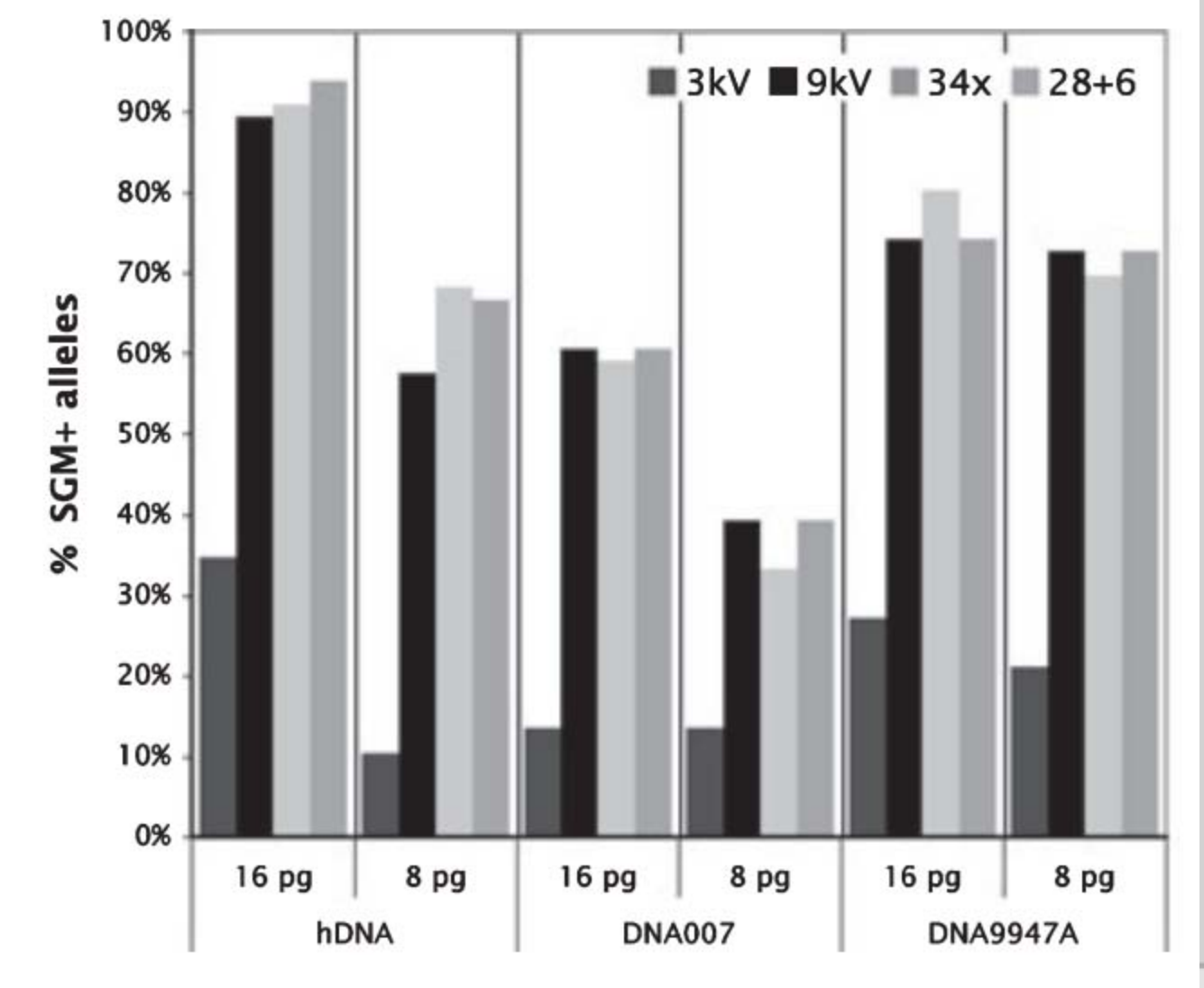
NIJ National Institute of Justice

Westen et al. (2009)

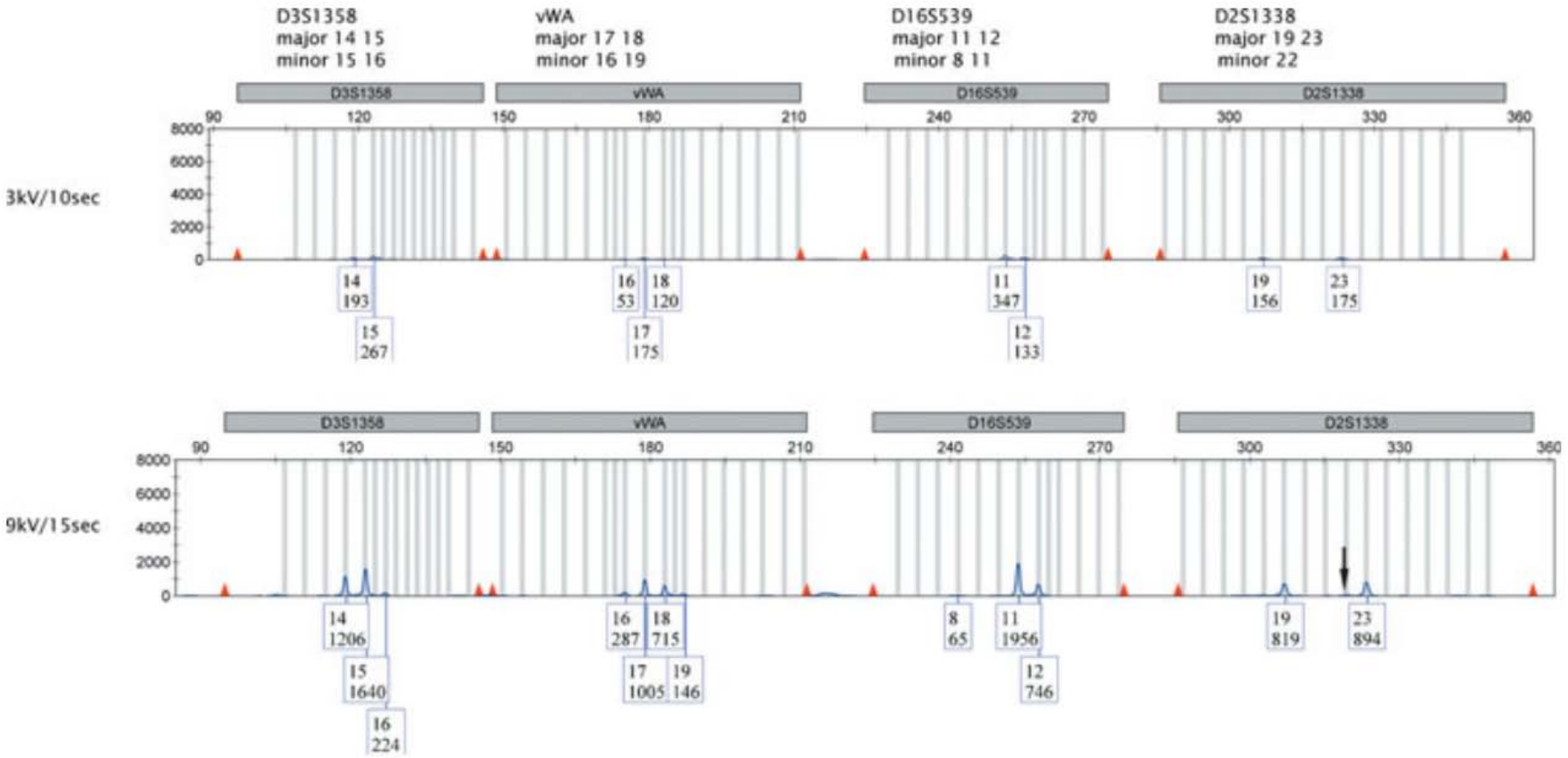


NIJ National Institute of Justice

Westen et al. (2009)



Westen et al. (2009)

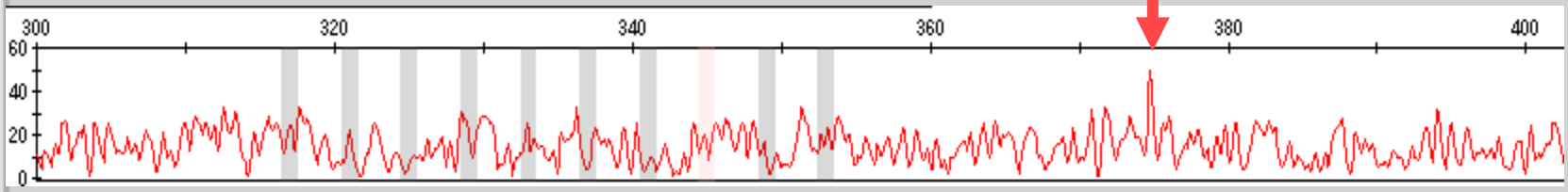


10:1 mixture SGM+ 28 cycles

Changes in Thresholds…

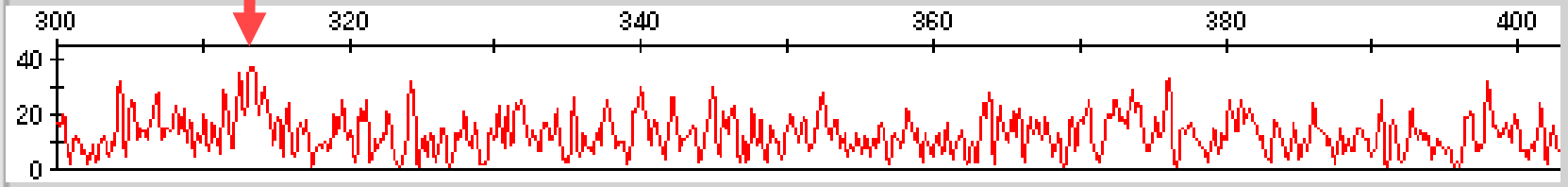
ABI 3500
1.2 kV, 15 sec (Default)

50 RFUs



37 RFUs

ABI 3500
1.2 kV, 10 sec



NIJ National Institute of Justice

Modified Procedures to Increase Sensitivity

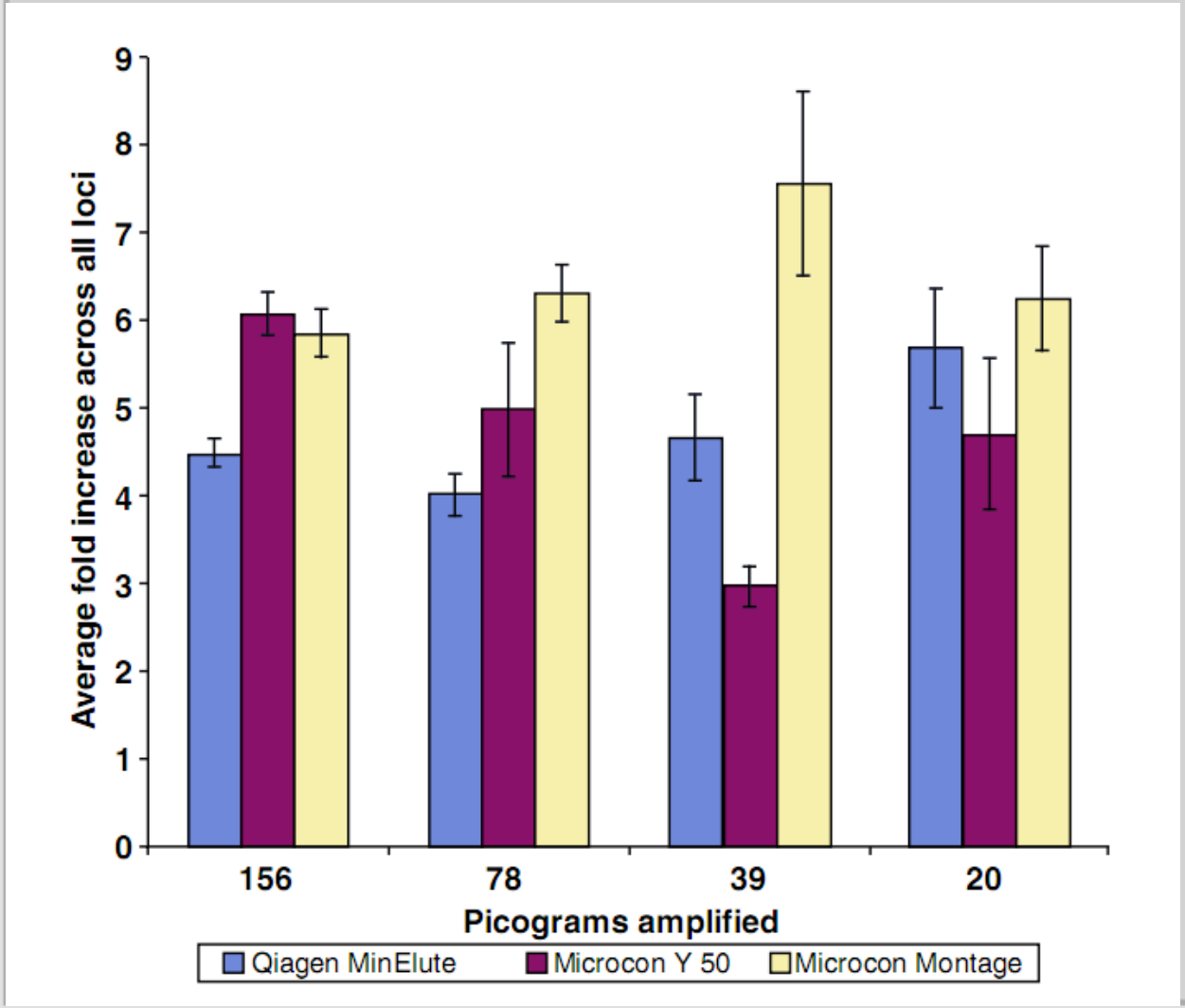
- **Post-PCR Removal of Salts**
  - **Advantages: Less competition of charged ions and amplicons electrophoretically injected into the capillary**
  - **Disadvantages: Can increase the stochastic threshold, added expense and time for processing**

  - **Smith and Ballantyne (2007) *J. Forensic Sci*. 52: 820-829**

Simplified Low-Copy-Number DNA Analysis by Post-PCR Purification

NIJ National Institute of Justice

Smith and Ballantyne (2007)



Fold-increase in signal strength

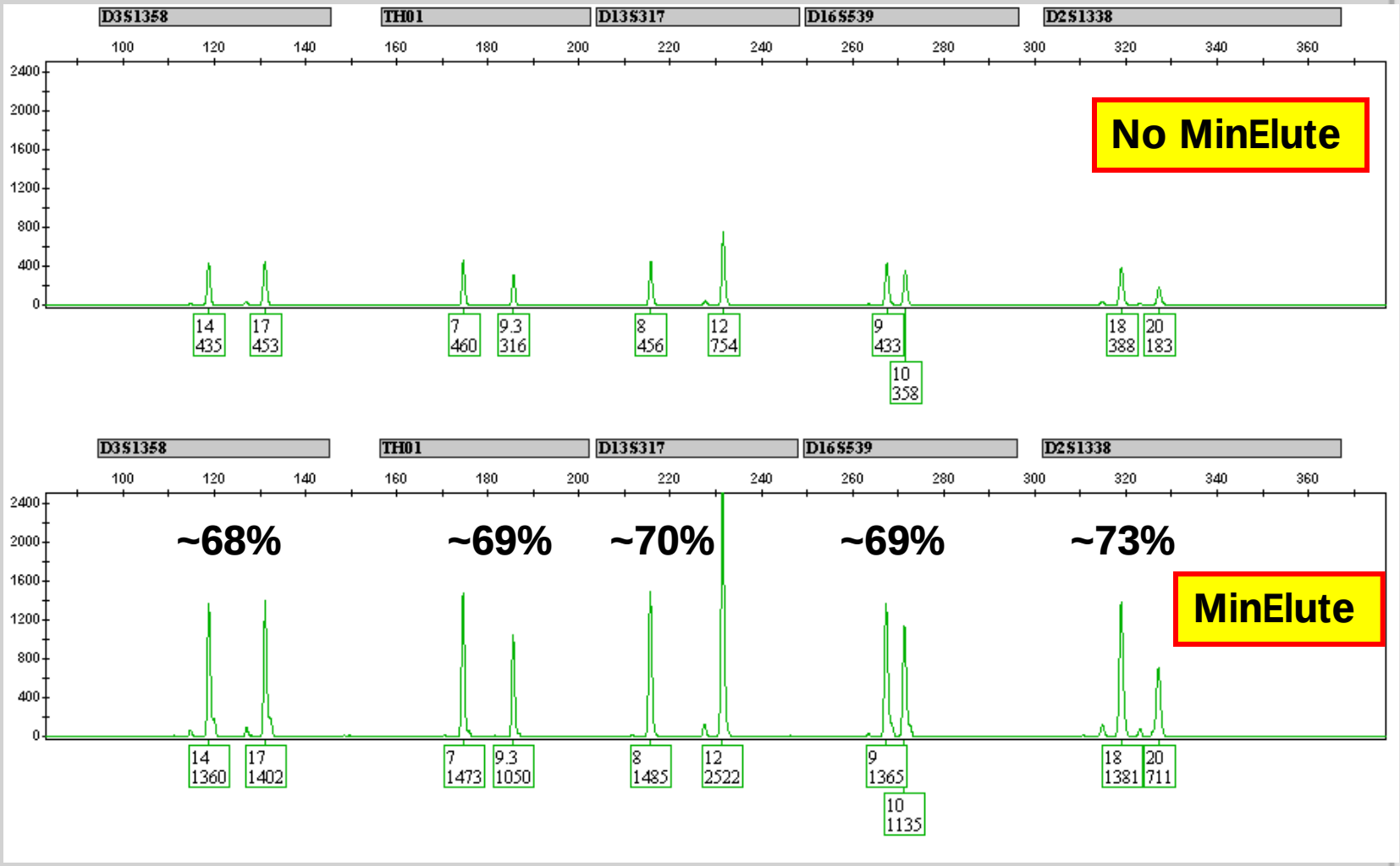
Smith and Ballantyne (2007)

TABLE 1—*Increased sensitivity with post-PCR purification.*

PCR product	156 pg	78 pg	39 pg	20 pg	10 pg	5 pg
1.5 μL unpurified	30	15–25	5–9	0–1	0	0
1.5 μL purified	30	30	27–28	9–19	5–13	0–5
Entire purified product	N/D	30	30	30	22–28	12–27

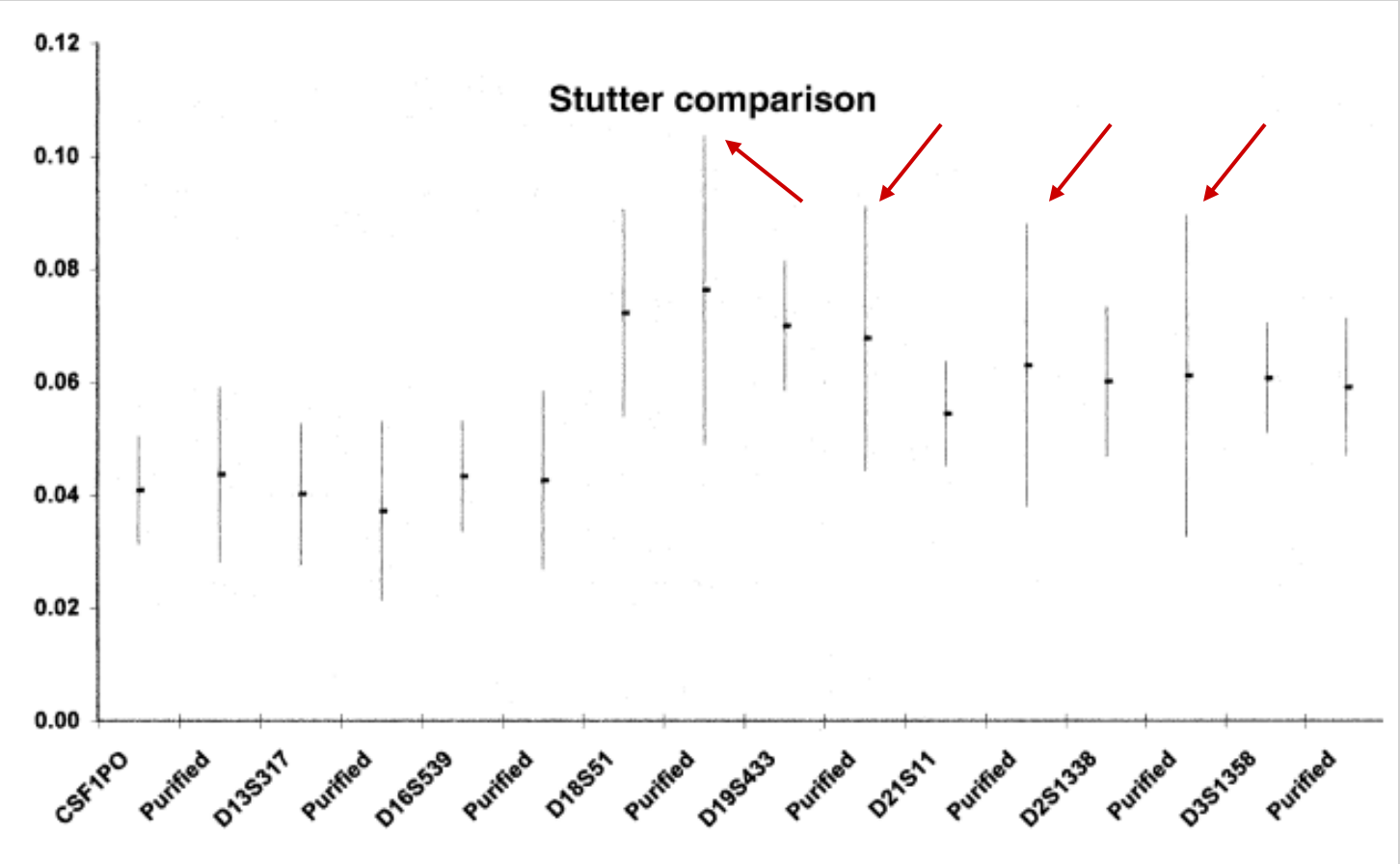
PCR, polymerase chain reaction.

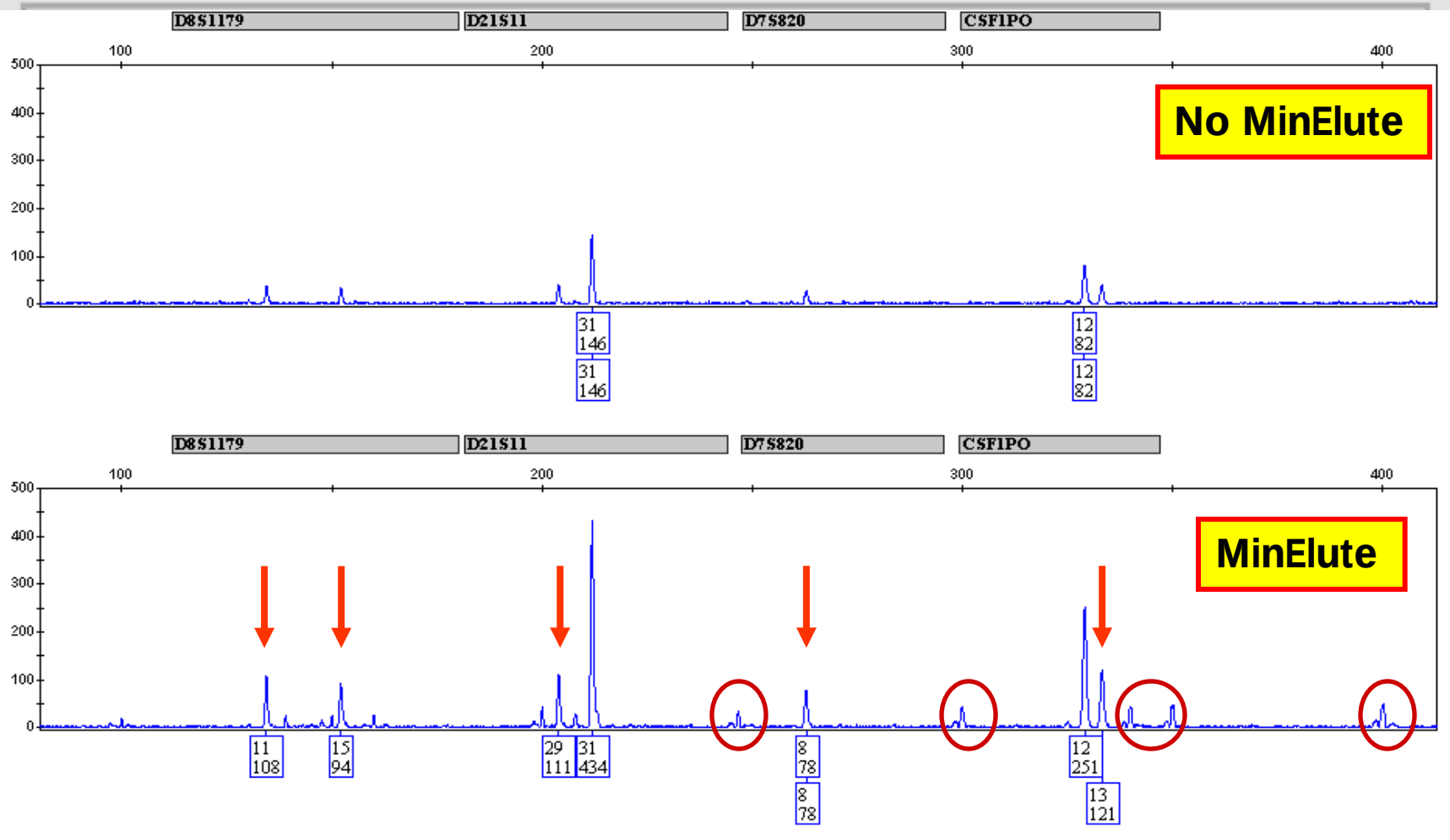
The number of alleles detected out of 30 possible alleles. Data indicate the range of alleles detected from four amplifications (two extractions amplified in duplicate). Complete profiles with or without purification were obtained for all samples amplified with 625–312 pg of DNA.



Identifiler Plus, 29 cycles, 30 pg

Smith and Ballantyne (2007)





Identifiler Plus, 29 cycles, 10 pg

NIJ National Institute of Justice

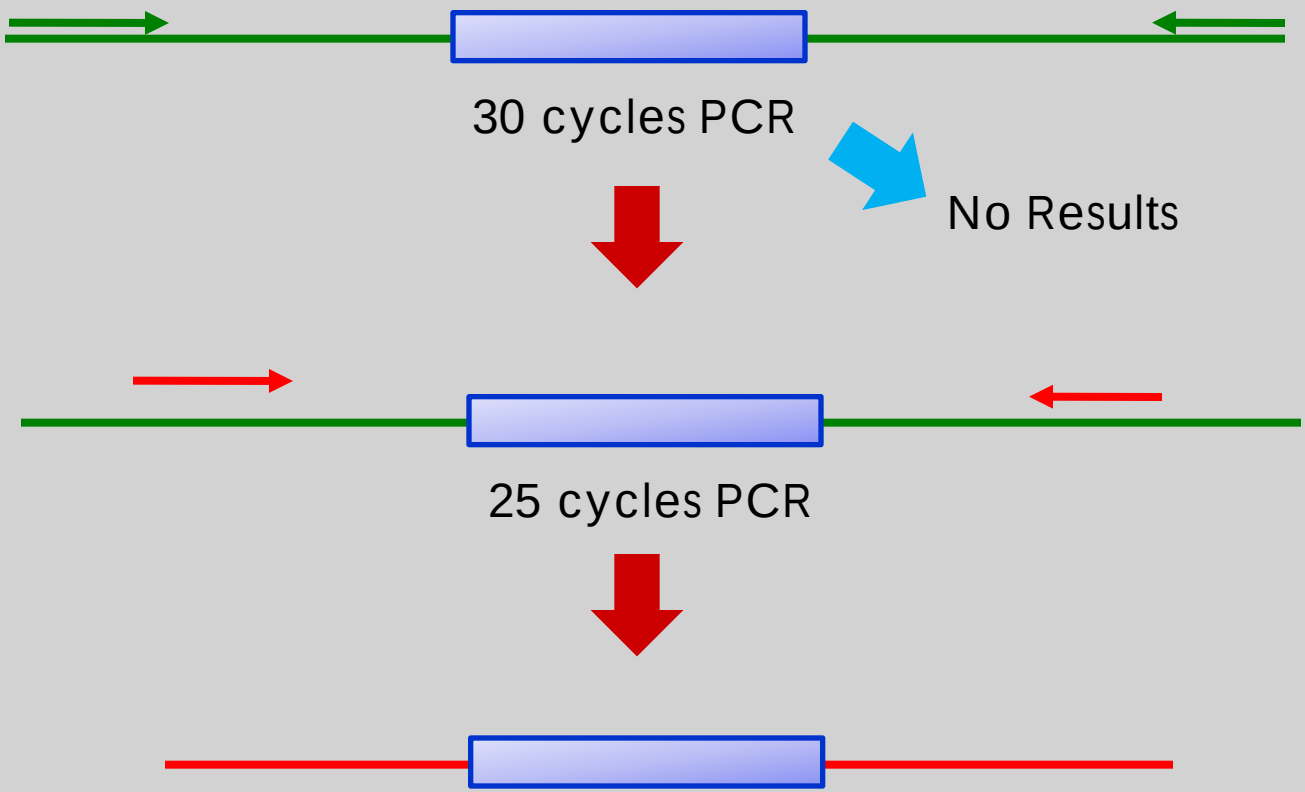
Modified Procedures to Increase Sensitivity

- **Nested PCR**
  - **Advantages: Increases the number of target amplicons**
  - **Disadvantages: Can increase the probability of contamination/allelic drop in**

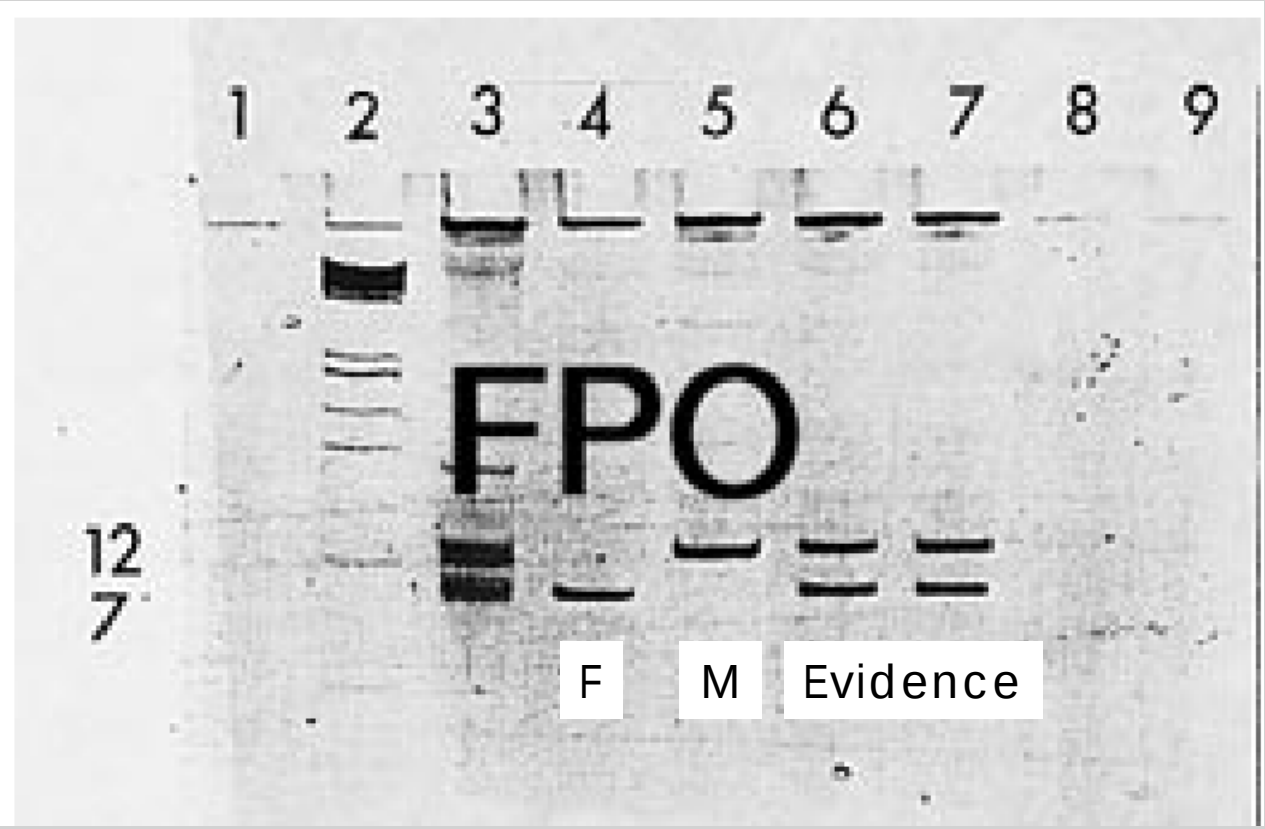
    - **Strom & Rechitsky (1998) *J. Forensic Sci*. 43: 696-700**

Use of Nested PCR to Identify Charred Human Remains and Minute Amounts of Blood

Nested PCR



Strom & Rechitsky (1998)



Modified Procedures to Increase Sensitivity

- **Increased cycles, Additional *Taq*, Improved buffers**
  - **Advantages: Increases the number of target amplicons**
  - **Disadvantages: Increased risk of contamination/allelic drop in, increased thresholds may be necessary, stochastic effects**

    - **Gill *et al.* (2000) *FSI* 112: 17-40.**

Low Template DNA Work

- **Early work on touched objects and single cells:**
  - **van Oorschot, R. A. and Jones, M. K. (1997) DNA fingerprints from fingerprints. *Nature*. 387(6635): 767**
  - **Findlay, I., Taylor, A., Quirke, P., Frazier, R., and Urquhart, A. (1997) DNA fingerprinting from single cells. *Nature*. 389(6651): 555-556**
- **Application to routine forensic casework was pioneered by the Forensic Science Service:**
  - **Gill, P., Whitaker, J., Flaxman, C., Brown, N., and Buckleton, J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 112(1): 17-40**
  - **Whitaker, J. P., Cotton, E. A., and Gill, P. (2001) A comparison of the characteristics of profiles produced with the AMPFlSTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. *Forensic Sci. Int.* 123(2-3): 215-223**
  - **Gill, P. (2001) Application of low copy number DNA profiling. *Croatian Medical Journal* 42(3): 229-32**

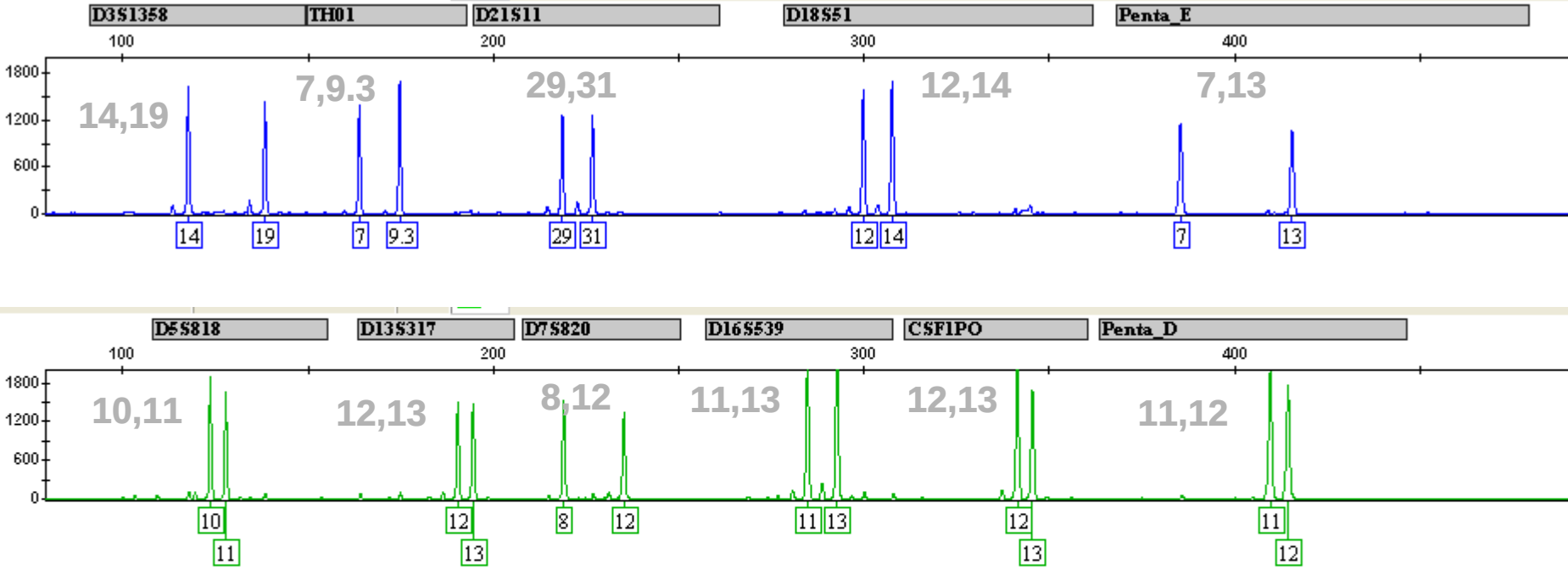
NIJ National Institute of Justice

New STR kits with increased sensitivity

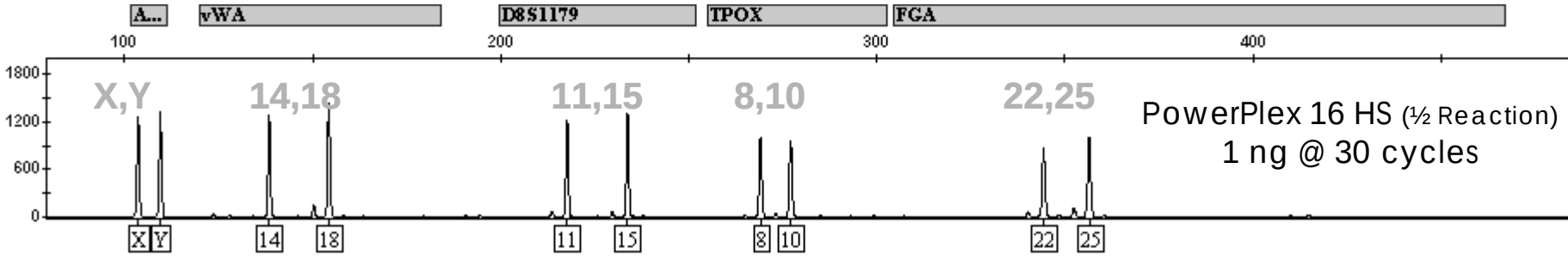
- **New STR kits are now available with new buffer formulations and polymerase already part of the master mix.**

- **These kits tend to be more sensitive compared to those currently used by most forensic labs.**

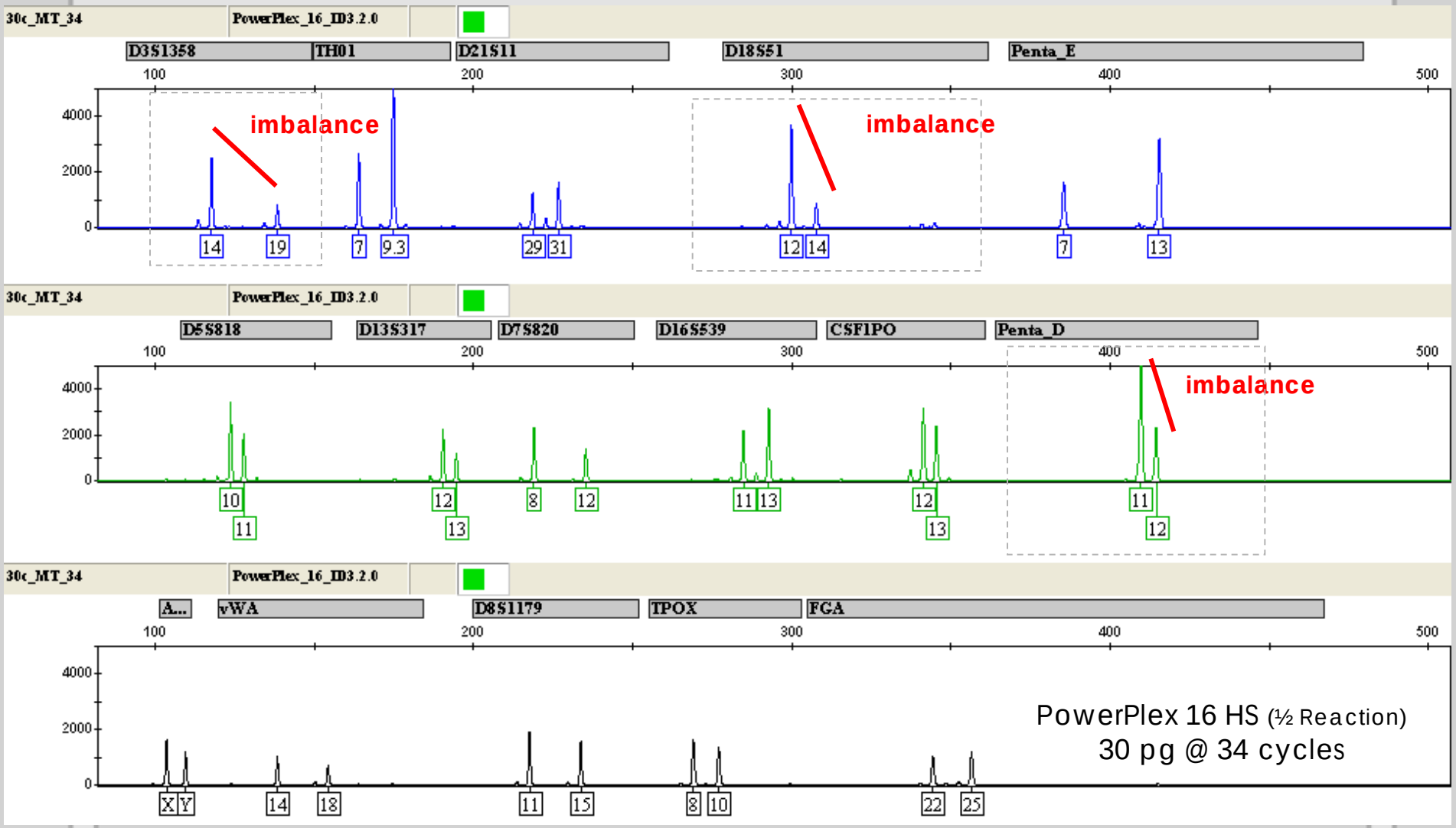
High signal, balanced peak heights (>0.80), no artifacts, low stutter

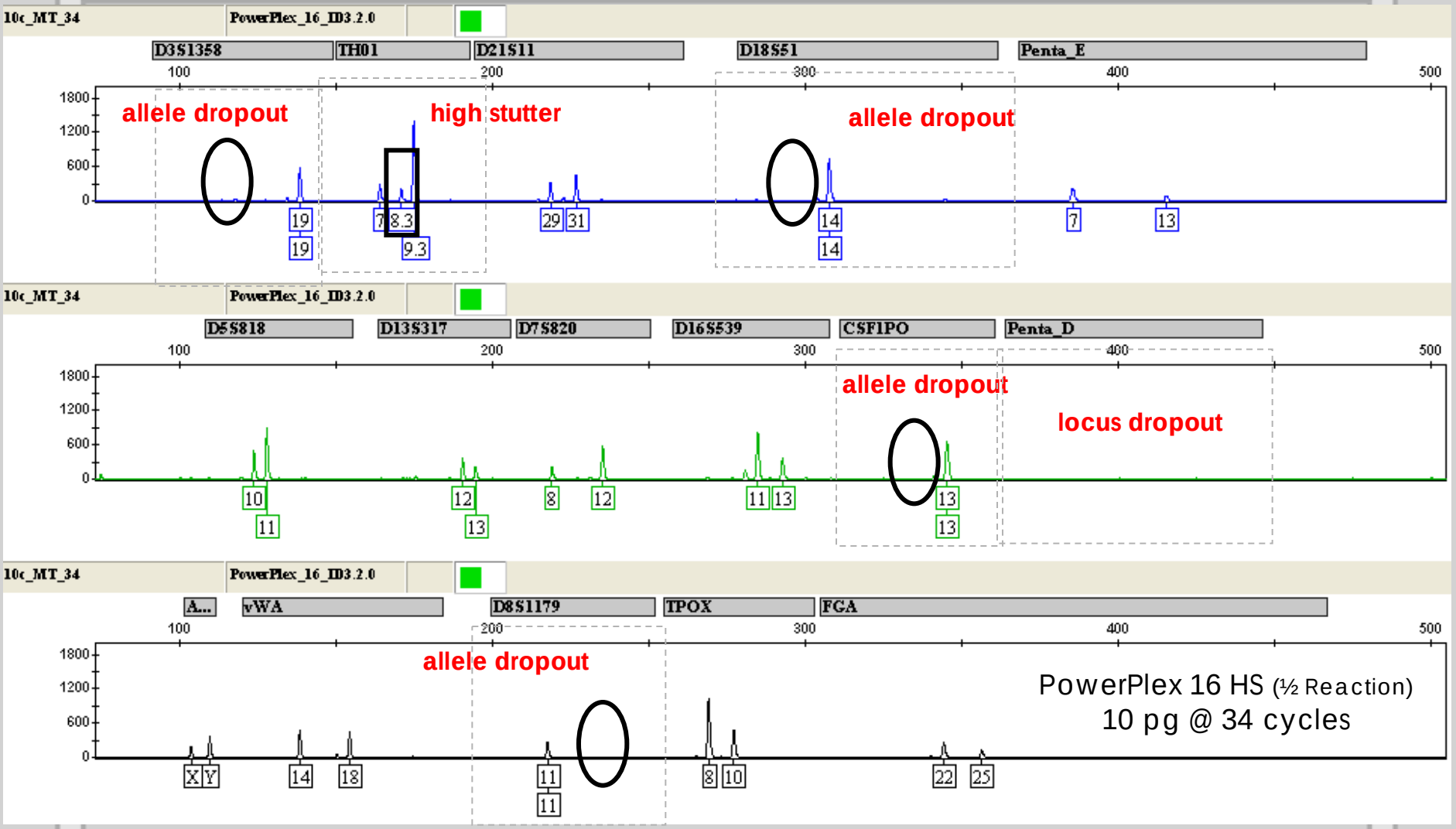


A Fully Heterozygous Sample (2 alleles for each locus)



PowerPlex 16 HS (½ Reaction)
1 ng @ 30 cycles


30c_MT_34
PowerPlex_16_ID3.2.0
D3S1358
TH01
D21S11
D18S51
Penta_E
imbalance
imbalance
14 19 7 9.3 29 31 12 14 7 13
30c_MT_34
PowerPlex_16_ID3.2.0
D5S818
D13S317
D7S820
D16S539
CSF1PO
Penta_D
imbalance
10 11 12 13 8 12 11 13 12 13 11 12
30c_MT_34
PowerPlex_16_ID3.2.0
A...
vWA
D8S1179
TPOX
FGA
X Y 14 18 11 15 8 10 22 25
100
200
300
400
500
4000
2000
0
PowerPlex 16 HS (½ Reaction)
30 pg @ 34 cycles


10c_MT_34
PowerPlex_16_ID3.2.0
D3S1358
TH01
D21S11
D18S51
Penta_E
allele dropout
high stutter
allele dropout
10c_MT_34
PowerPlex_16_ID3.2.0
D5S818
D13S317
D7S820
D16S539
CSF1PO
Penta_D
allele dropout
locus dropout
10c_MT_34
PowerPlex_16_ID3.2.0
A...
vWA
D8S1179
TPOX
FGA
allele dropout
PowerPlex 16 HS (½ Reaction)
10 pg @ 34 cycles

Early Work on Replicate Testing with Low Levels of DNA

Nucleic Acids Research, 1996, Vol. 24, No. 16 3189–3194

Reliable genotyping of samples with very low DNA quantities using PCR

Pierre Taberlet*, Sally Griffin, Benoît Goossens, Sophie Questiau, Valérie Manceau, Nathalie Escaravage, Lisette P. Waits and Jean Bouvet

Laboratoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France

Received May 1, 1996; Revised and Accepted July 2, 1996

Replicate testing introduced (up to 7 times) to account for allele drop-out and avoid miscalling allele drop-in

In conjunction with interpretation rules, duplication of observed alleles in replicates was shown to correctly define the original sample

ELSEVIER

Forensic Science International
112 (2000) 17–40

Forensic Science International

www.elsevier.com/locate/forsciint

An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA

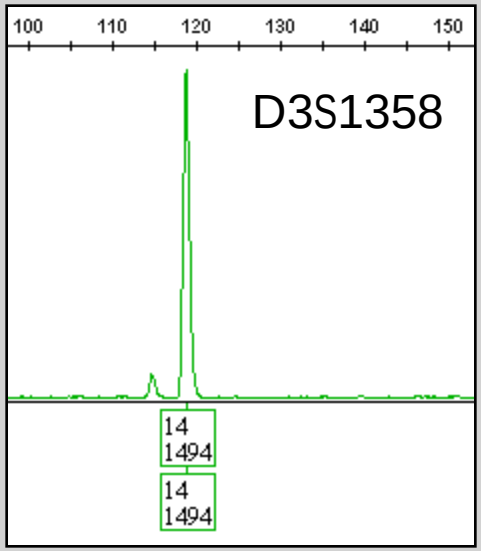
Peter Gill[a,*], Jonathan Whitaker[a], Christine Flaxman[a], Nick Brown[a], John Buckleton[b]

[a]*Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK*
[b]*ESR, Private Bag 92021, Auckland, New Zealand*

Received 9 December 1999; received in revised form 12 February 2000; accepted 13 February 2000

NIJ National Institute of Justice

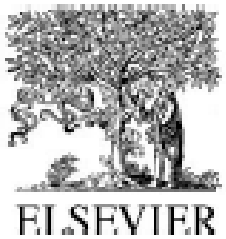
A cautionary note…



Identifiler Plus
32 cycles (3 extra cycles)
10 pg DNA

1494 RFUs

New Interpretation Rules Required for LT-DNA



ELSEVIER

Forensic Science International
112 (2000) 17–40

Forensic Science International

www.elsevier.com/locate/forsciint

An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA

Peter Gill[a,*], Jonathan Whitaker[a], Christine Flaxman[a], Nick Brown[a], John Buckleton[b]

[a]*Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK*
[b]*ESR, Private Bag 92021, Auckland, New Zealand*

Received 9 December 1999; received in revised form 12 February 2000; accepted 13 February 2000

NIJ National Institute of Justice

Replicate LT-DNA Test Results from FSS

Gill, P. (2002) Role of short tandem repeat DNA in forensic casework in the UK--past, present, and future perspectives. *BioTechniques* 32(2): 366-385.

Table 2. Results of Six Replicate PCR Tests of a Sample Under Low Copy Number Analysis Conditions Compared to the Control Sample

	Amelo	D19	D3	D8	THO	VWA	D21	FGA	D16	D18	D2
CONTROL	X X	14,14	18,18	15,15	7 9.3	19,19	28 32.2	20,23	9,12	12,16	17,23
Sample											
1	--	14 F′	--	15 F′	--	--	28 32.2	20 F′	--	16 F′	--
2	X F′	--	18 F′	15 F′	--	19 F′	--	–	12 F′	--	--
3	X F′	--	--	15 F′	--	--	--	–	--	--	17 F′
4	X F′	14 F′	18 F′	--	--	--	--	–	9 12	--	--
5	X F′	--	18 F′	--	--	18 F′	--	–	--	--	--
6	X F′	14 F′	--	--	--	19 F′	28 32.2	20 F′	--	12 F′	--
Consensus	X F′	14 F′	18 F′	15 F′	--	19 F′	28 32.2	20 F′	12 F′	--	--

The consensus result is reported, provided that an allele is observed at least twice. If only one allele is observed, then an F′ designation is given to denote the possibility of allele drop-out.

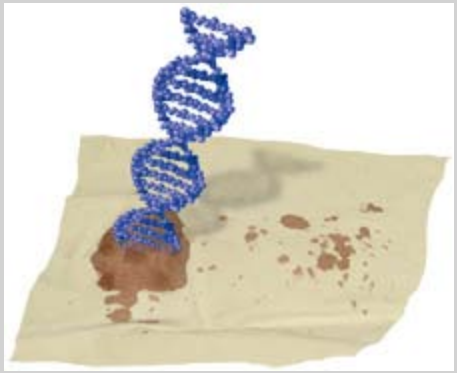
F' used to designate that allele drop-out of a second allele cannot be discounted when only a single allele is observed (OCME uses "Z")

NIJ National Institute of Justice

Suggestions for Optimal Results with LT-DNA

- **Typically at least 2 – 3 PCR amplifications from the same DNA extract are performed to obtain consensus profiles**
- **An allele cannot be scored (considered real) unless it is present at least twice in replicate samples**
- **Extremely sterile environment is required for PCR setup to avoid contamination from laboratory personnel or other sources**

Typical LT-DNA Analysis Procedure



Extract DNA from stain

Quantify Amount of DNA Present

Perform 3 Separate PCR Amplifications

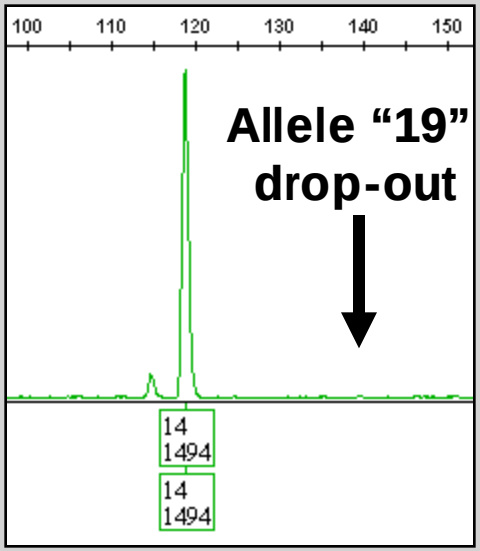
Interpret Alleles Present

Develop a Consensus Profile

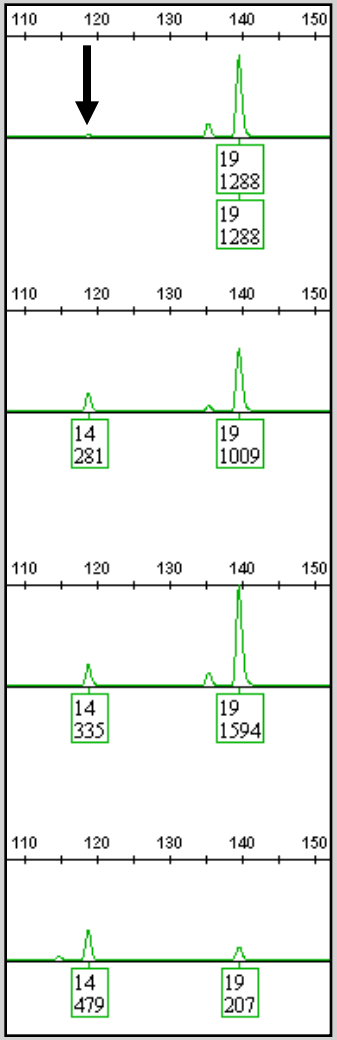
(based on replicate consistent results)

NIJ National Institute of Justice

D3S1358 replicates with 3 extra cycles



Identifiler Plus
32 cycles
10 pg DNA



*Any combination of 3/5 replicates gives the correct genotype (14,19)

Comparison of Approaches

Replicate Amplification with Consensus Profile

Low amount of DNA examined

Stochastic effects

Amplification #1
Amplification #2
Amplification #3

Consensus Profile Developed
(from repeated alleles observed)

Interpretation Rules Applied
(based on validation experience)
e.g., specific loci may dropout more

Result can be and usually is Reliable & Reproducible

What "LCN Labs" Are Doing

Single Amplification

Low amount of DNA examined

Stochastic effects

Amplification #1
(only a single test)

Result can be Unreliable

Individual results may vary but a consensus profile is reproducible
(based on our experience with sensitivity studies and replicate amplifications)

NIJ National Institute of Justice

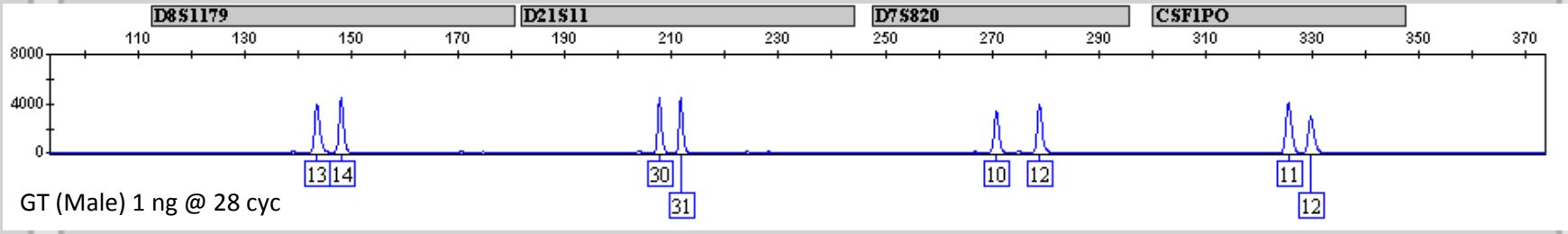
Examination of LT-DNA Mixtures

LT-DNA Mixture Samples

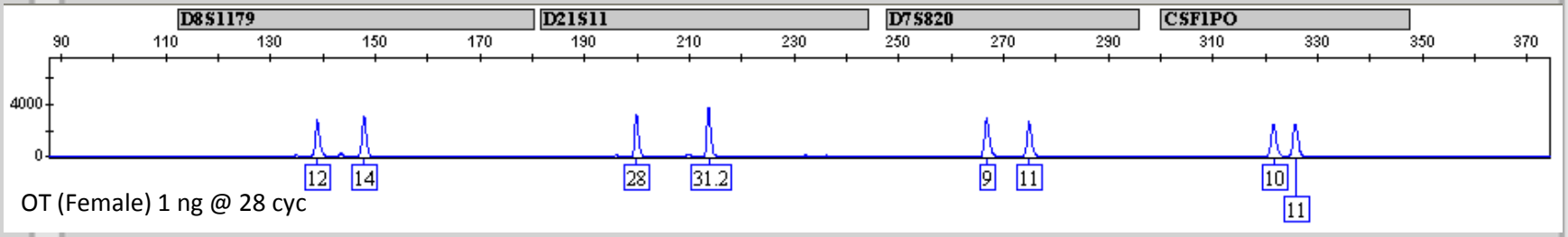
- **2 samples (male and female) were mixed together at 1:3 and 1:5 – 1 ng (1:3 and 1:5) or 100 pg (1:5) or 50 pg (1:3) total DNA**
- **3 person mixture (2 males and female) were mixed together at 1:2:3 – 1 ng or 100 pg total DNA**
- **Identifiler Plus (28 and 31 cycles) was tested (half reactions)**
- **5 replicates with 3 extra cycles**
- **Variability of peak heights in replicates was observed**
- **More minor contributor peaks were called with 3 extra cycles**

NIJ National Institute of Justice

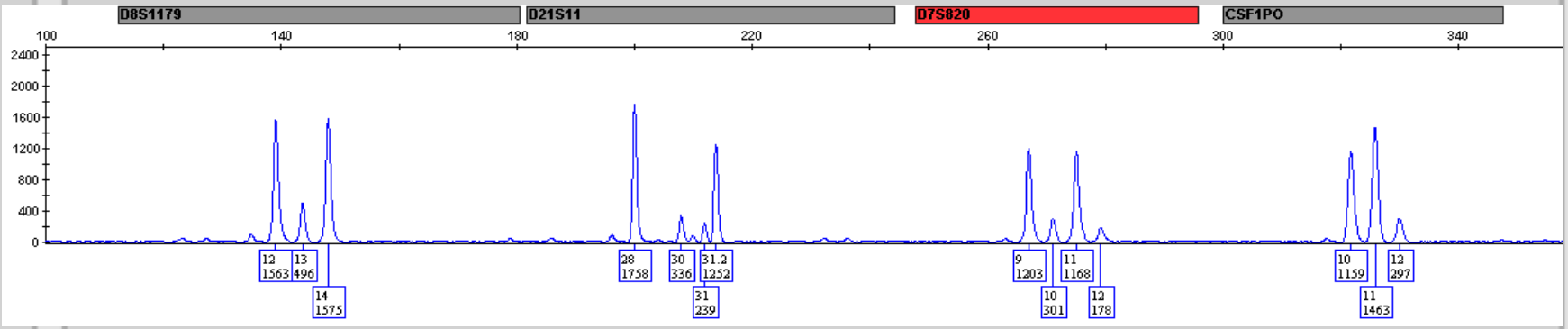
Individual Mixture Components



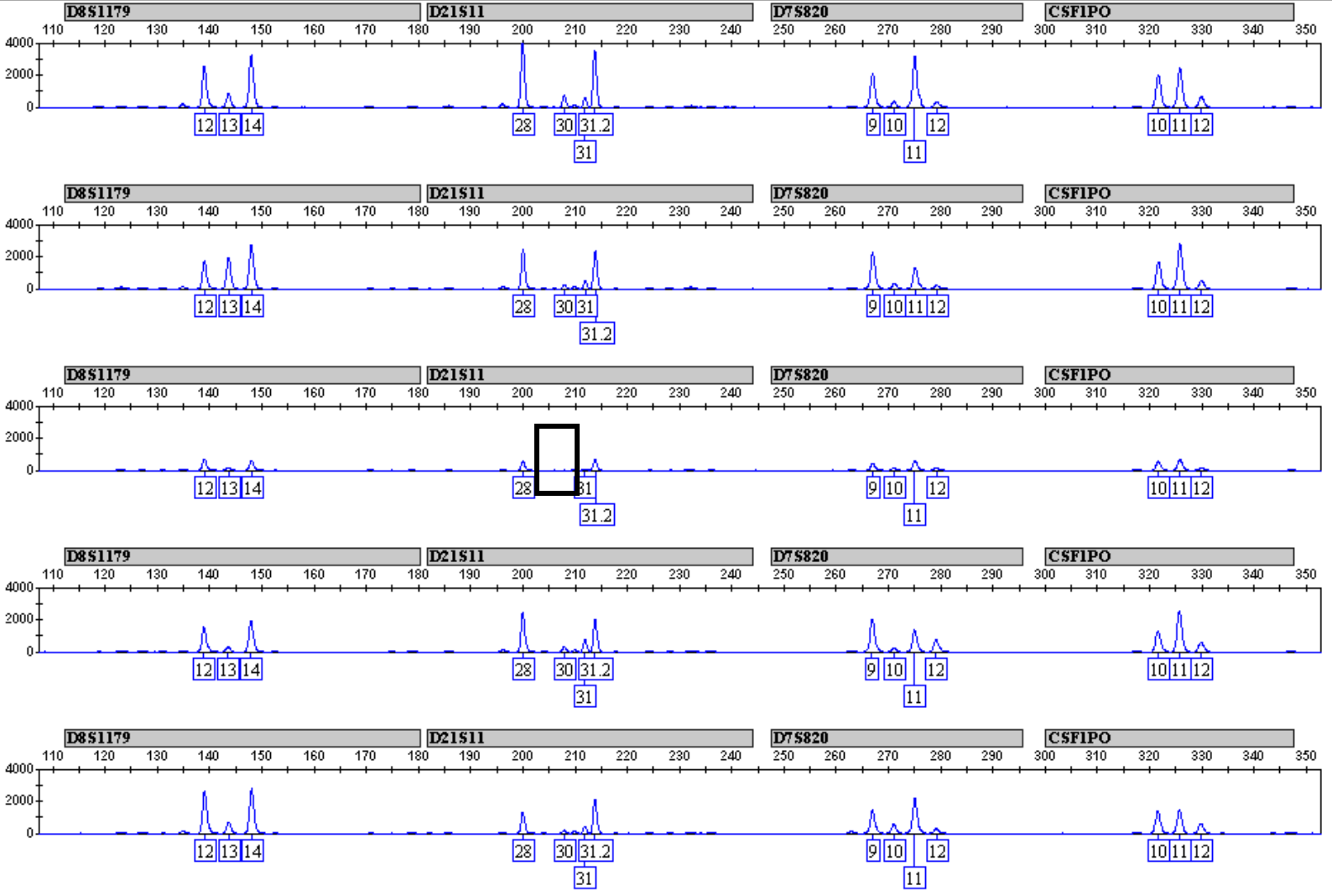
GT (Male) 1 ng @ 28 cyc



OT (Female) 1 ng @ 28 cyc



2-Person Mixture (1GT:5OT) - 1 ng @ 28 cyc

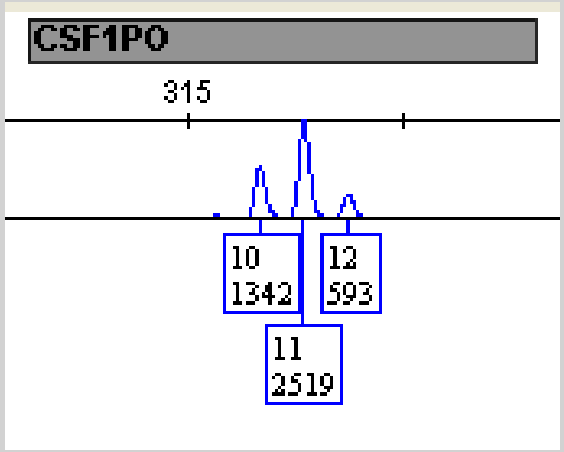


GT:OT, 1:5, 100 pg @ 31 cyc

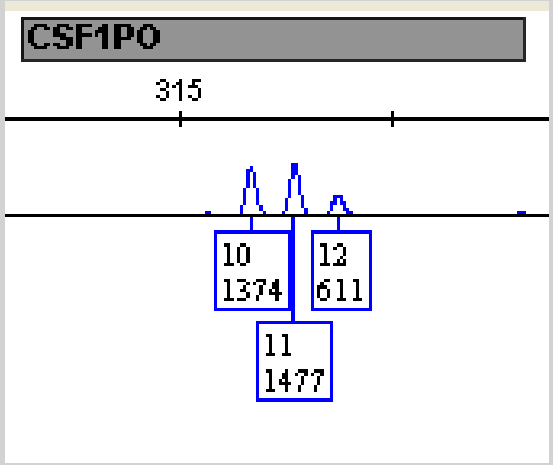
NIJ National Institute of Justice

GT – 11, 12

OT – 10, 11



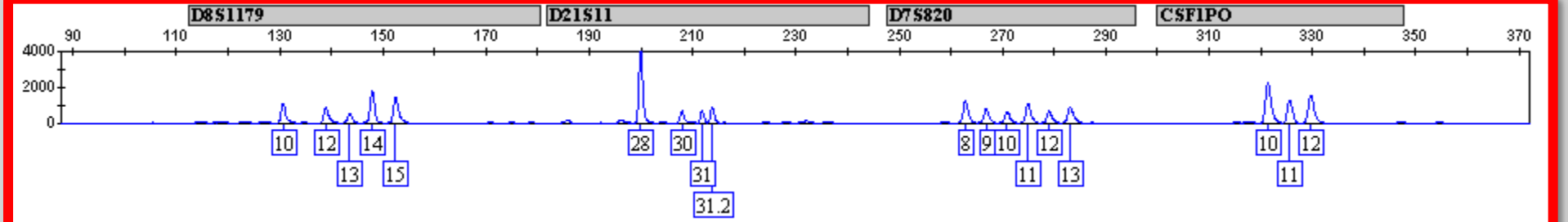
PHR ~~2:1~~



GT:OT, 1:5, 100 pg @ 31 cyc

Individual Mixture Components

3-Person Mixture (1GT:2OT:3UT) - 1 ng @ 28 cyc



Replicate Results from 3-Person Mixture (1GT:2OT:3UT)

100 pg @ 31 cycles

FSI-Genetics, in press

Analysis and interpretation of mixed profiles generated by 34 cycle SGM Plus® amplification

Jon H. Wetton *, John Lee-Edghill, Emily Archer, Valerie C. Tucker, Andrew J. Hopwood, Jonathan Whitaker, Gillian Tully

Forensic Science Service, 2960 Trident Court, Birmingham Business Park, Solihull B37 7YN, UK

NIJ National Institute of Justice

Wetton et al. (in press)

Table 2
The final distribution of major and consensus profiles across the template input and ratio range after completion of the RO requested rework.

Ratio	Total input	Major/minor	Major/ consensus	Consensus only	Unduplicated
5:1	1 ng	12		3	1
	500 pg	10[a]		6	
	250 pg	11[a]	1	4	
	100 pg	3[a]	4	9	
	50 pg	2		14	
	Total	38	5	36	1
2:1	1 ng	1		14	1
	500 pg	1[a]		14	1
	250 pg	1[a]		15	
	100 pg		1	14	1
	50 pg		1[a]	15	
	Total	3	2	72	3
1:1	1 ng			8	
	500 pg			8	
	250 pg			8	
	100 pg			8	
	50 pg		1[a]	7	
	Total	0	1	39	0

[a] One observation in each of these major profile categories was due to a single individual with a possible mutation affecting the accuracy of the Quantifiler® concentration estimate which may have caused the input of this individual to be underestimated by half. This would tend to double its relative representation at each mixture ratio as well as the true amount of template available in the PCR. In the other rows the affected mixture was scored as a consensus.

NIJ National Institute of Justice

Summary

- There are multiple methods to increase sensitivity of low-level mixtures.
- These “enhanced interrogation” techniques should be fully validated to consider changes in thresholds AND interpretational guidelines.
- Improved collection, extraction and re-amplification with more template are potential non-enhancement solutions to bringing peaks above the ST.

Acknowledgements

- **Becky Hill, Erica Butts, Peter Vallone, Dave Duewer and John Butler (NIST)**
- **Robin Cotton (Boston Univ)**
- **Peter Gill (University of Strathclyde)**

Questions?

Contact Information

Michael D. Coble, PhD

NIST - Applied Genetics Group

100 Bureau Drive MS 8314

Gaithersburg, MD 20899-8314

301-975-4330

michael.coble@nist.gov