Topics and Techniques for Forensic DNA Analysis

Continuing Education Seminar

STR Markers & CE Instrumentation

NYC OCME
Dept of Forensic Biology

New York City, NY
April 18, 2012

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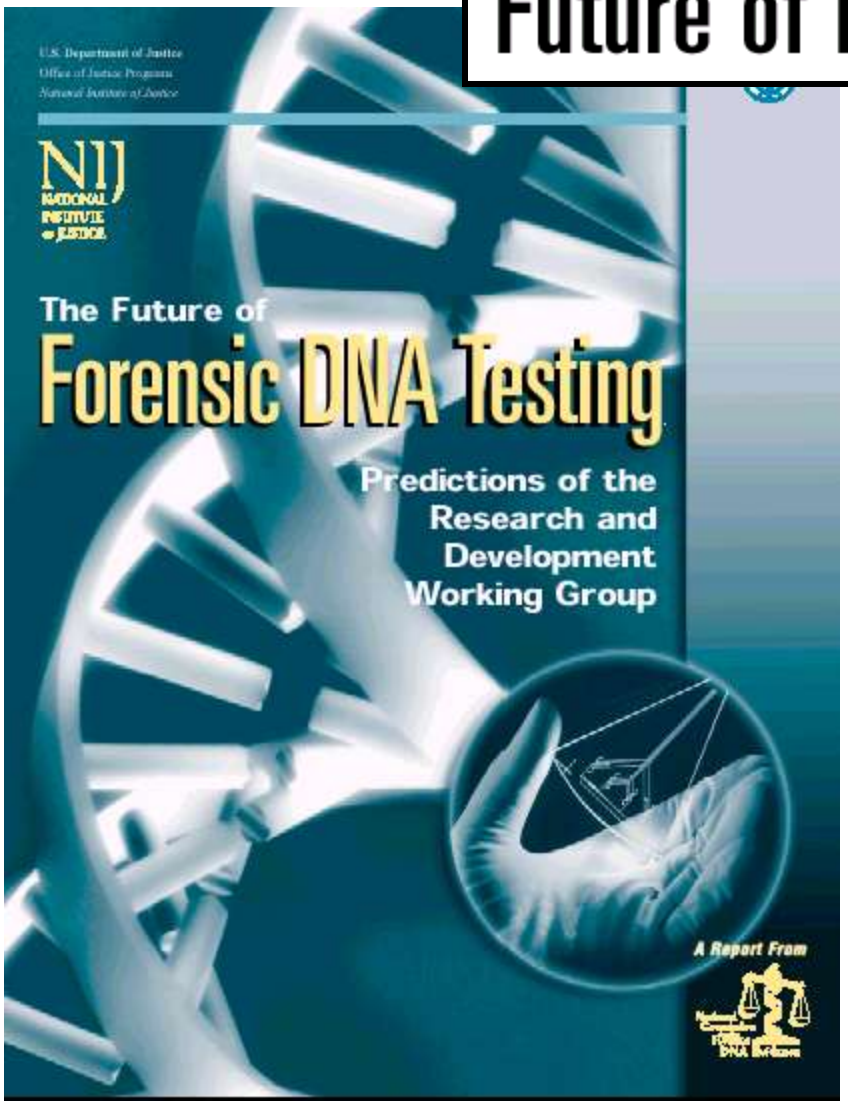
National Commission on the Future of DNA Evidence



- Report published in Nov 2000

- Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles

http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

What topic are you most interested in learning about today? (select only one)

1. Troubleshooting
2. Rapid DNA testing
3. Expanded CODIS core loci
4. ABI 3500
5. Promega-ABI lawsuit over STRs

Planned Presentation Outline

- STR measurement issues
- Expanded CODIS core loci
  - European STR locus expansion in 2009
  - Our recent *Forensic Science Review* article
- New STR kits & recent Promega-ABI lawsuit

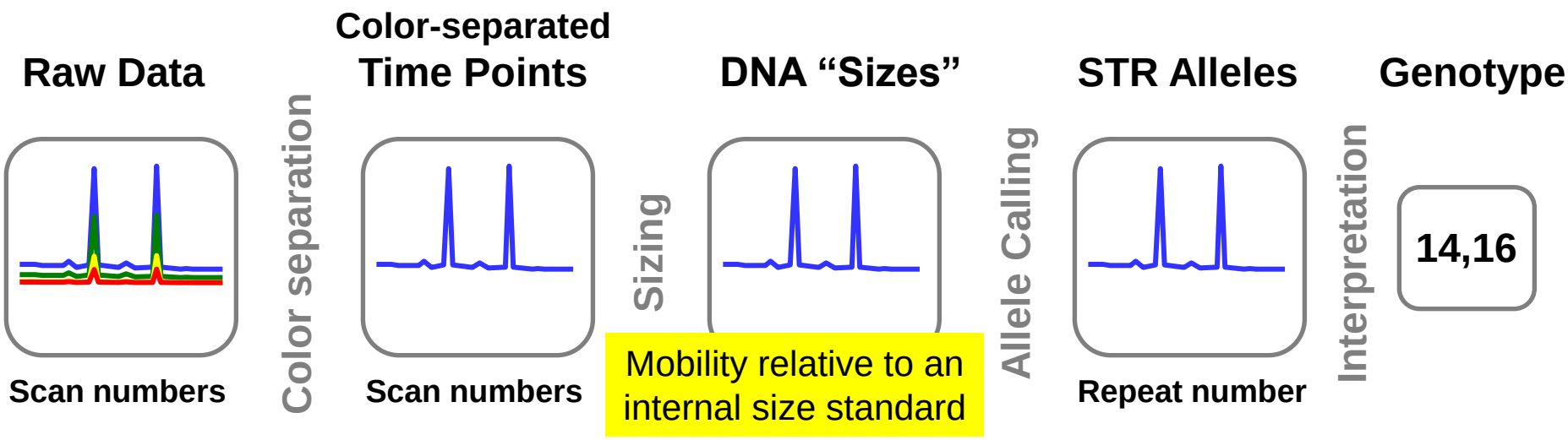
- CE fundamentals & troubleshooting issues
- ABI 3500 differences with ABI 3130
- Efforts towards rapid PCR & DNA testing

CE Peak Position is Primarily Determined by … (select only one)

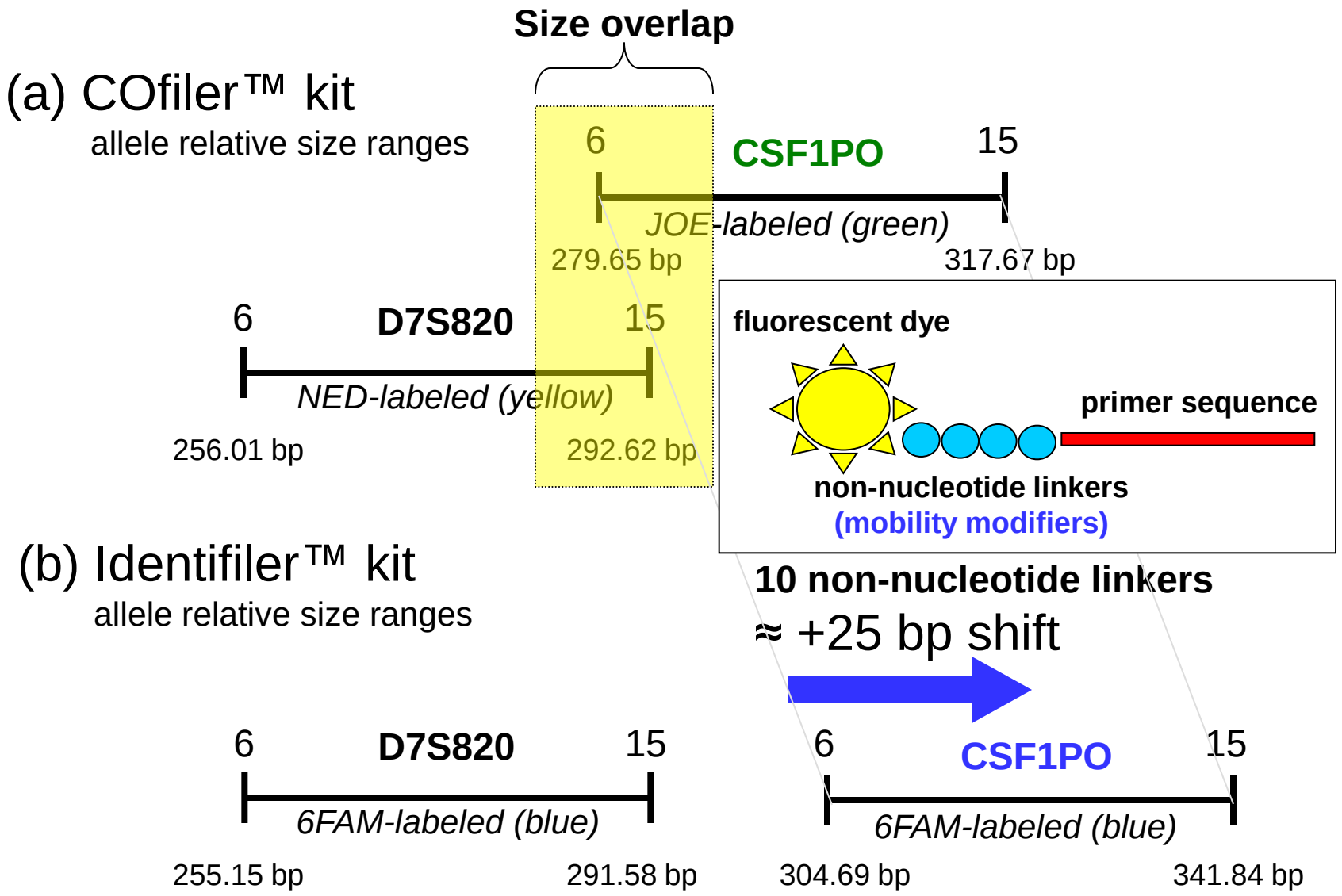
1. DNA length
2. Mobility (time from injection to detection)

What is Being Measured with STR Alleles during CE Separation

- Mobility of a PCR product with a fluorescent tag is being measured
- Mobility is the time it takes for the DNA molecule to move from the injection point to the detection point
- Mobility modifiers are used in some ABI STR kits
  - Identifiler has five loci with mobility modifiers



Mobility Modifiers Permit Shifting Allele Sizes without Changing Primer Binding Sites

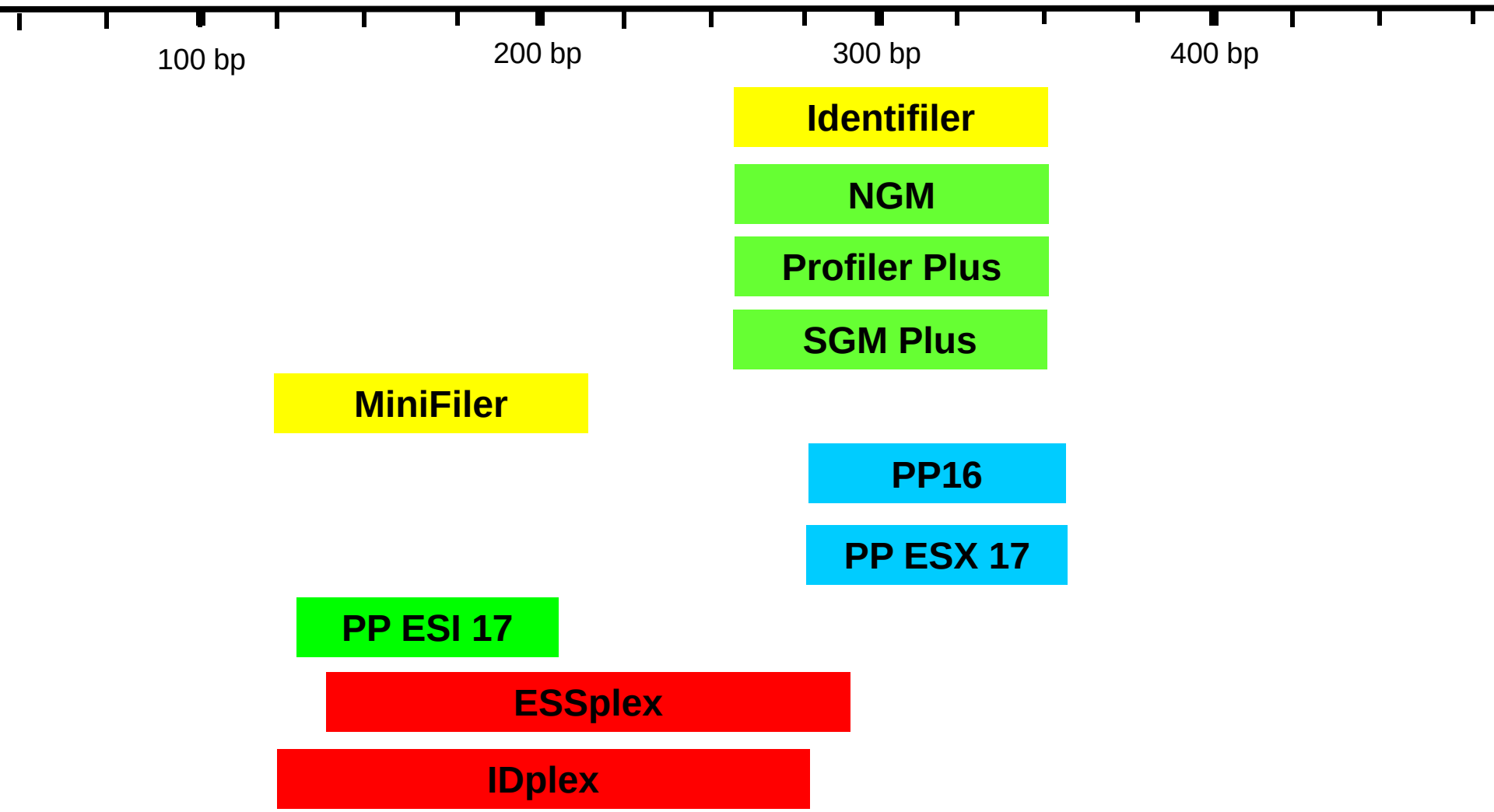


To Avoid Overlapping PCR Product Size Ranges with STR Loci in the Same Dye Channel

- Applied Biosystems Strategy
  - **Maintains primer sequences** (except MiniFiler & NGM kits)
  - Utilizes mobility modifiers or additional dyes, no primer redesign is necessary
  - Enables comparison to legacy data with earlier kits but null alleles may go undetected with the potential for incorrect genotypes within data sets

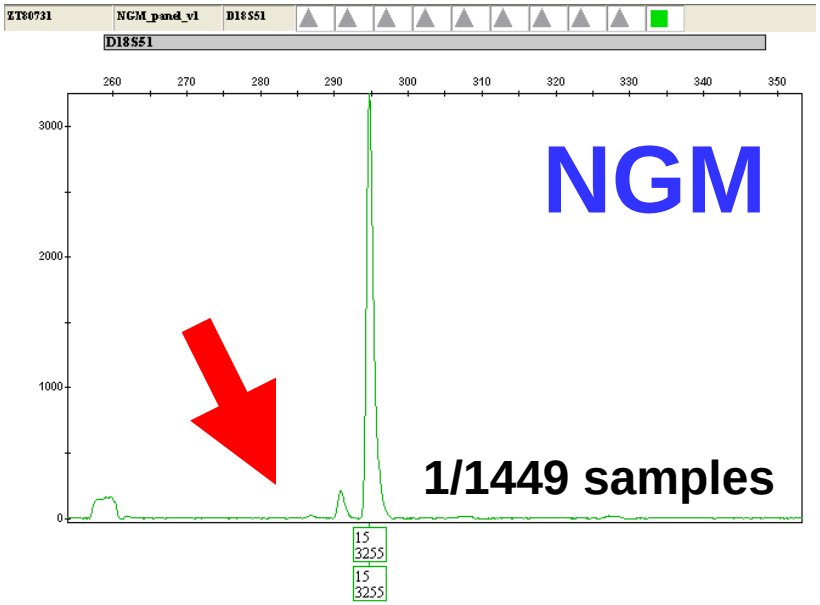
- Promega Corporation Strategy
  - Moves primer sequences to change PCR product size ranges
  - Primer redesign can be difficult, but can be moved from primer-binding-site mutations
  - **Requires concordance studies to check for potential allele dropout**

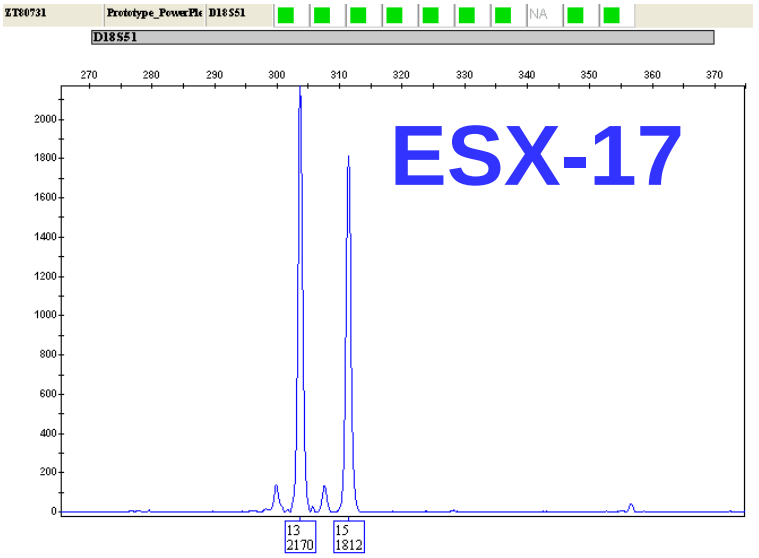
D18S51 Concordance Checking

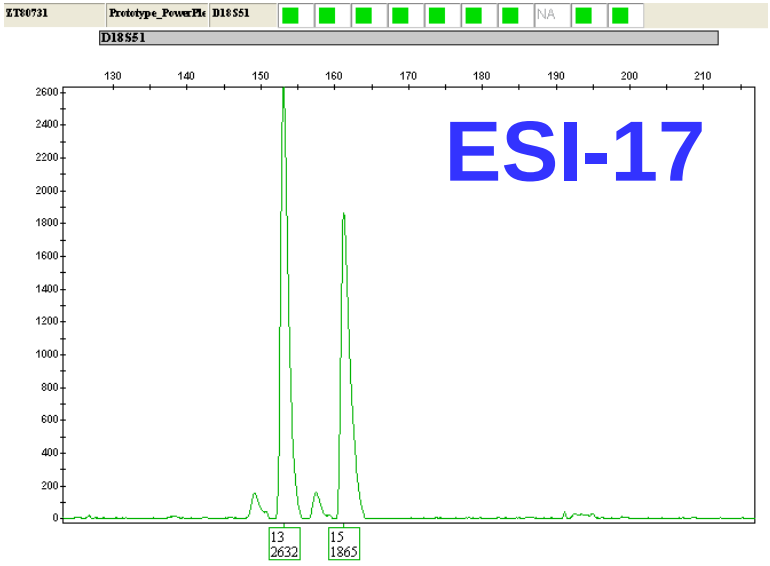


D18S51 marker positions for all kits tested

D18S51 Null Allele







Correct type (13,15)

C→T SNP 172 bp downstream from repeat

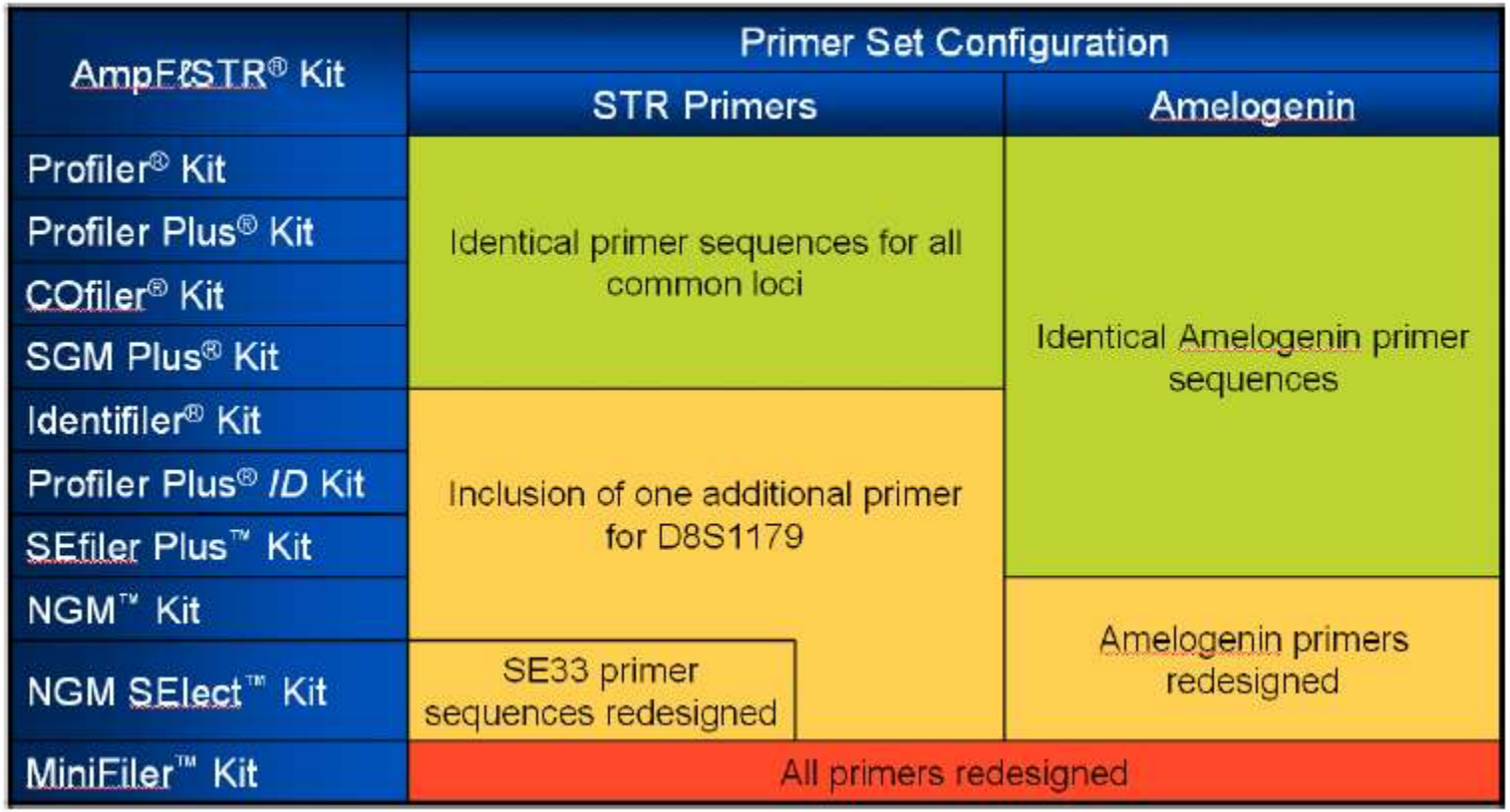
Primer Binding Site Mutations Causing Allele Dropout, Not Corrected by ABI

From >1400 U.S. population samples tested:

- **D18S51** – 1 difference (Hispanic); loss of allele 13 with ID/NGM/ProPlus/SGM+ while ESX/ESI showed full 13,15 type
- **D3S1358** – 1 difference (Caucasian); loss of allele 17 with ID/ProPlus/SGM+/NGM while ESX/ESI showed full 14,17 type
- **D19S433** – 2 differences (Asian); loss of allele 13 with ID/NGM/SGM+ while ESX/ESI showed full 13,14 or 13,14.2 type
- **D8S1179** – 1 difference (Asian); loss of allele 15 with ProPlus/SGM+ while ID/NGM/ESX/ESI showed full 14,15 type

http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm

Primer Changes with ABI Kits

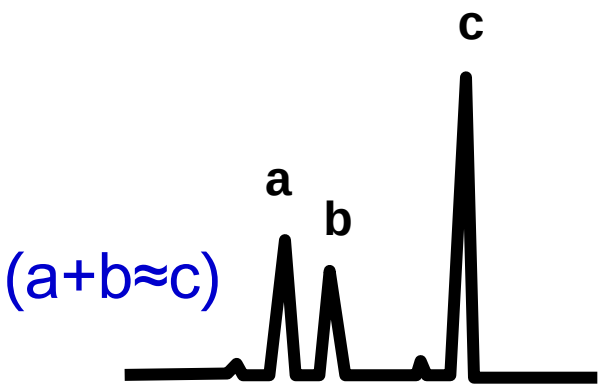


D2S441 and D22S1045 have an additional primer in NGM and NGM SElect

Table 4 from "Development of the AmpFℓSTR NGM SElect Kit: New Sequence Discoveries and Implications for Genotype Concordance", Forensic News (January 2011)

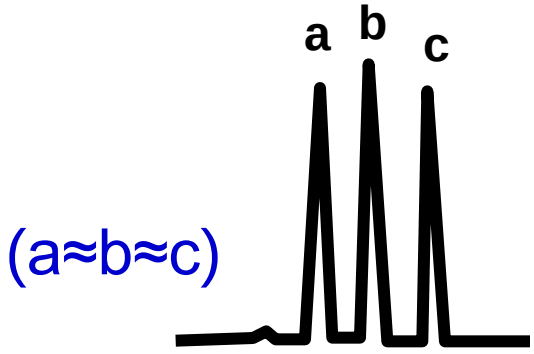
Tri-Allelic Patterns

- Tri-alleles are Copy Number Variants (CNVs) in the human genome detected as three peaks at a single locus rather than the expected single (homozygous) or double (heterozygous) peak



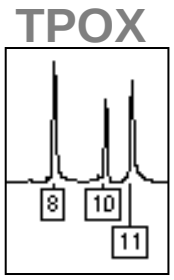
Type 1

Sum of peak heights for two alleles is almost equal to the third allele



Type 2

Fairly balanced peak heights are observed



On average, how many times have you observed a tri-allelic pattern over a year of casework?

1. Zero times
2. Maybe once
3. 2-3 times
4. >5 times
5. Only at TPOX

Slide from Steven Myers, CA DOJ Data from Missouri Highway Patrol DNA Lab

Frequency of Tri-Allelic Patterns

- Database Size:

  69,000

- Overall Average Occurrence:

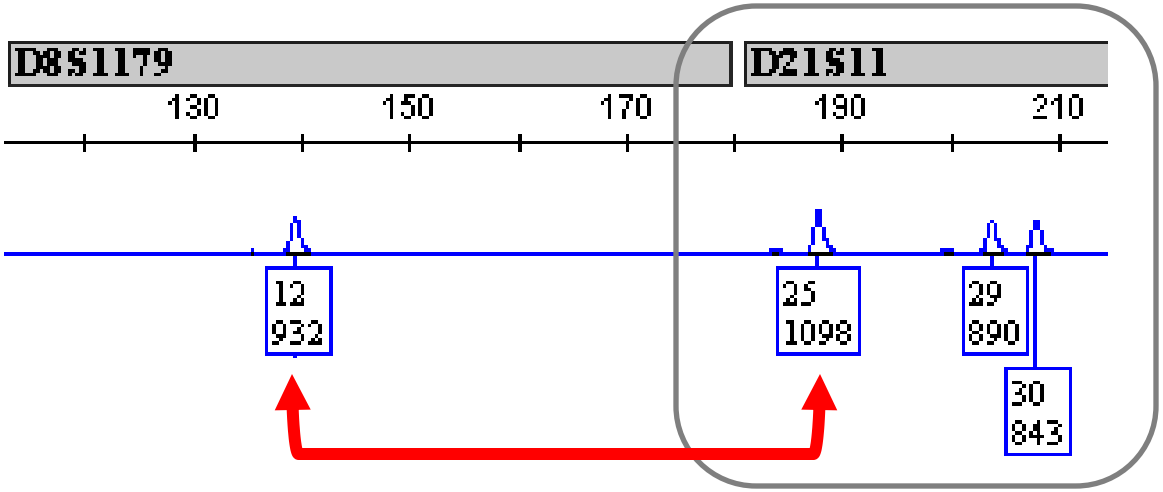
  1 in 1,000

Note:
This is Steven's summary of Missouri's data. You won't find this table on STRBase.

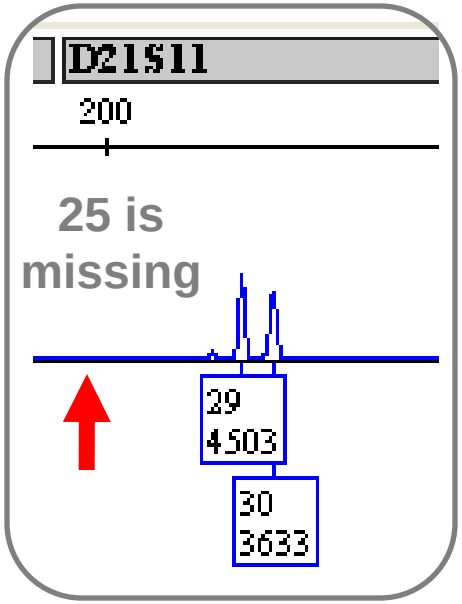
Locus	Observations	1 in...
D3S1358	2	35,000
VWA	10	6,900
FGA	11	6,300
D8S1179	2	35,000
D21S11	9	7,700
D18S51	3	23,000
D5S818	1	69,000
D13S317	4	17,000
D7S820	0	
D16S539	3	23,000
TH01	0	
TPOX	9	7,700
CSF1PO	1	69,000
Penta D	3	23,000
Penta E	10	6,900
Combined	68	1,000

How Do You Characterize Your Tri-Allelic Patterns?

Identifiler



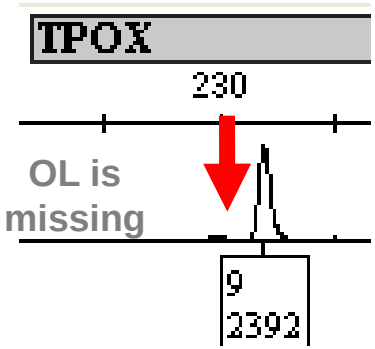
PowerPlex 16 HS

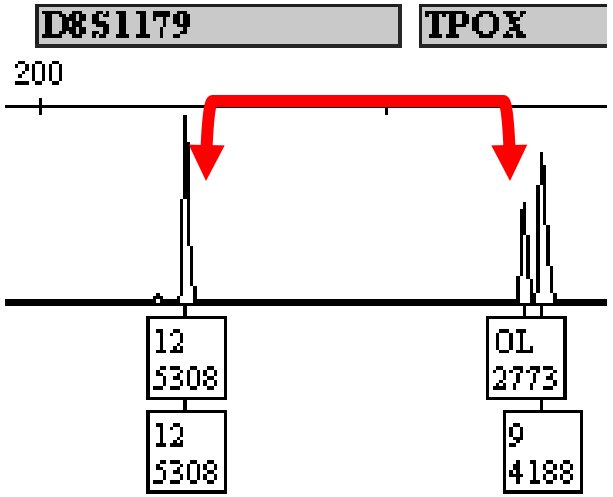


You re-amplify it…
It's Reproducible!

Check STRBase…
It has never been observed before!

A New Large D8S1179 Allele is Discovered – with "24" repeats!
(sequence analysis shows duplication in flanking region)





D8S1179

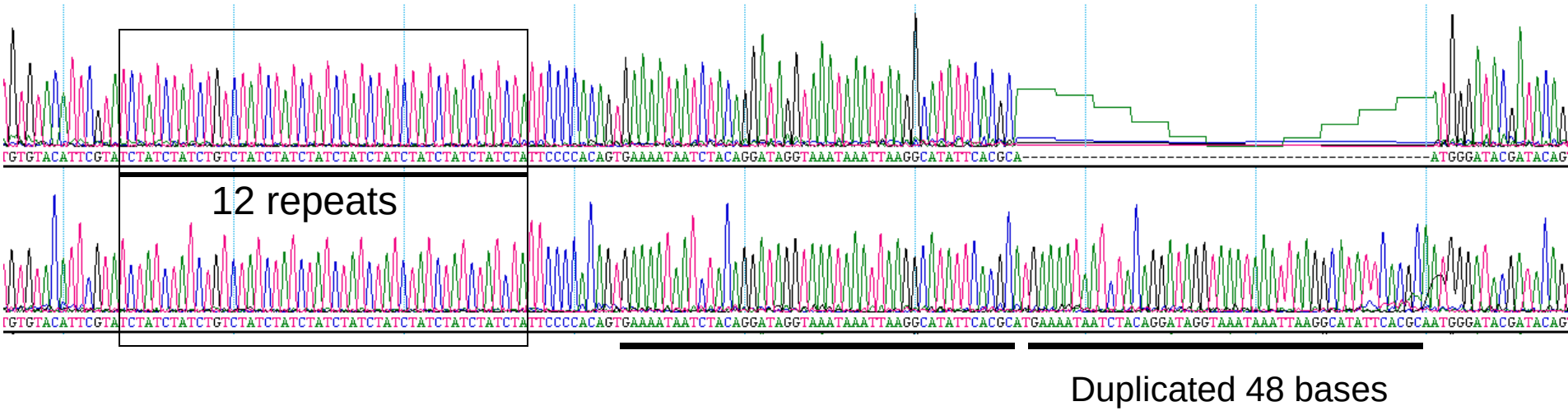
All Previously Known Alleles

Many alleles sequences are not known

We just set the new world record for the largest D8 allele (24)

Allele (Repeat #)	Promega PowerPlex 16	ABI Identifiler	Repeat Structure $[TCTR]_n$	Reference
6	199 bp	119 bp	Not published	STRBase
7	203 bp	123 bp	$[TCTA]_7$	Griffiths *et al.* (1998)
8	207 bp	127 bp	$[TCTA]_8$	Barber and Parkin (1996)
9	211 bp	131 bp	$[TCTA]_9$	Barber and Parkin (1996)
10	215 bp	135 bp	$[TCTA]_{10}$	Barber and Parkin (1996)
10.1	216 bp	136 bp	Not published	STRBase
10.2	217 bp	137 bp	Not published	STRBase
11	219 bp	139 bp	$[TCTA]_{11}$	Barber and Parkin (1996)
12	223 bp	143 bp	$[TCTA]_{12}$	Barber and Parkin (1996)
12.1	224 bp	144 bp	Not published	STRBase
12.2	225 bp	145 bp	Not published	STRBase
12.3	226 bp	146 bp	Not published	STRBase
13 (a)	227 bp	147 bp	$[TCTA]_1[TCTG]_1[TCTA]_{11}$	Barber and Parkin (1996)
13 (b)	227 bp	147 bp	$[TCTA]_2[TCTG]_1[TCTA]_{10}$	**Kline *et al.* (2010)**
13 (c)	227 bp	147 bp	$[TCTA]_1[TCTG]_1TGTA[TCTA]_{10}$	**Kline *et al.* (2010)**
13 (d)	227 bp	147 bp	$[TCTA]_{13}$	**Kline *et al.* (2010)**
13.1	228 bp	148 bp	Not published	STRBase
13.2	229 bp	149 bp	Not published	STRBase
13.3	230 bp	150 bp	Not published	STRBase
14	231 bp	151 bp	$[TCTA]_2[TCTG]_1[TCTA]_{11}$	Barber and Parkin (1996)
14.1	232 bp	152 bp	Not published	STRBase
14.2	233 bp	153 bp	Not published	STRBase
15	235 bp	155 bp	$[TCTA]_2[TCTG]_1[TCTA]_{12}$	Barber and Parkin (1996)
15.1	236 bp	156 bp	Not published	STRBase
15.2	237 bp	157 bp	Not published	STRBase
15.3	238 bp	158 bp	Not published	STRBase
16	239 bp	159 bp	$[TCTA]_2[TCTG]_1[TCTA]_{13}$	Barber and Parkin (1996)
16.1	240 bp	160 bp	Not published	STRBase
17	243 bp	163 bp	$[TCTA]_2[TCTG]_2[TCTA]_{13}$	Barber and Parkin (1996)
17.1	244 bp	164 bp	Not published	STRBase
17.2	245 bp	165 bp	Not published	STRBase
18	247 bp	167 bp	$[TCTA]_2[TCTG]_1[TCTA]_{15}$	Barber and Parkin (1996)
19	251 bp	171 bp	$[TCTA]_2[TCTG]_2[TCTA]_{15}$	Griffiths *et al.* (1998)
20	255 bp	175 bp	Not published	**STRBase**

D8S1179 12,“24”



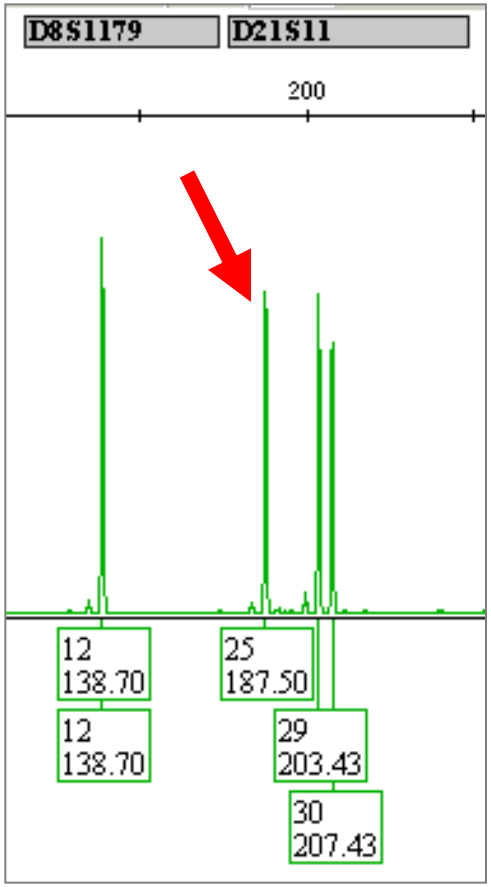
Allele 12 : $[TCTA]_2$ TCTG $[TCTA]_9$

Allele “24” : $[TCTA]_2$ TCTG $[TCTA]_9$ **duplication of the 48 bases 10 bases downstream of the repeat**

Result with This Large D8S1179 Allele

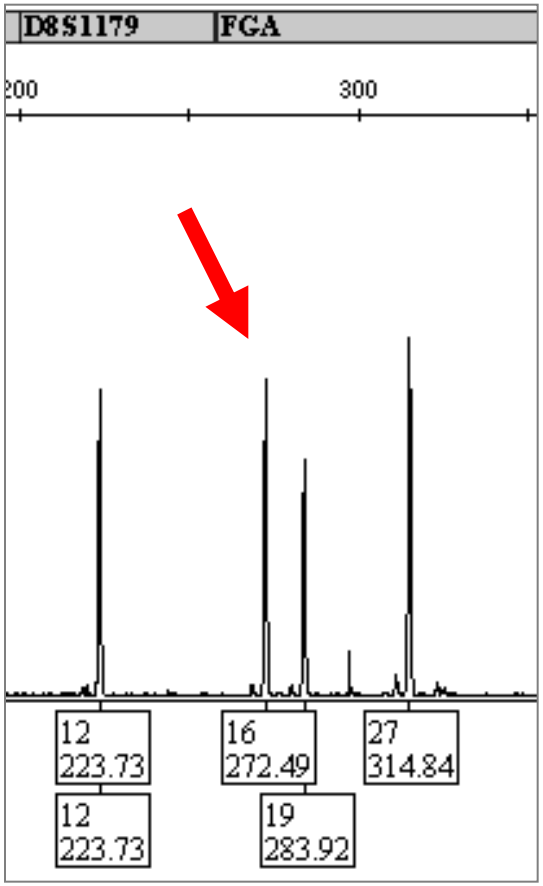
Using European STR Kits

NGM SElect



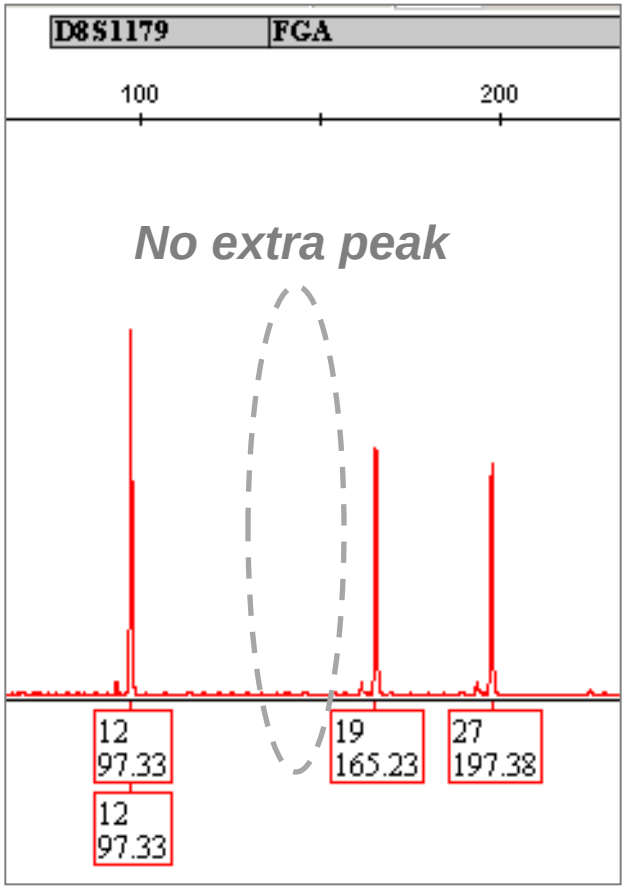
False D21S11 tri-allele

PP ESX 17



False FGA tri-allele

PP ESI 17

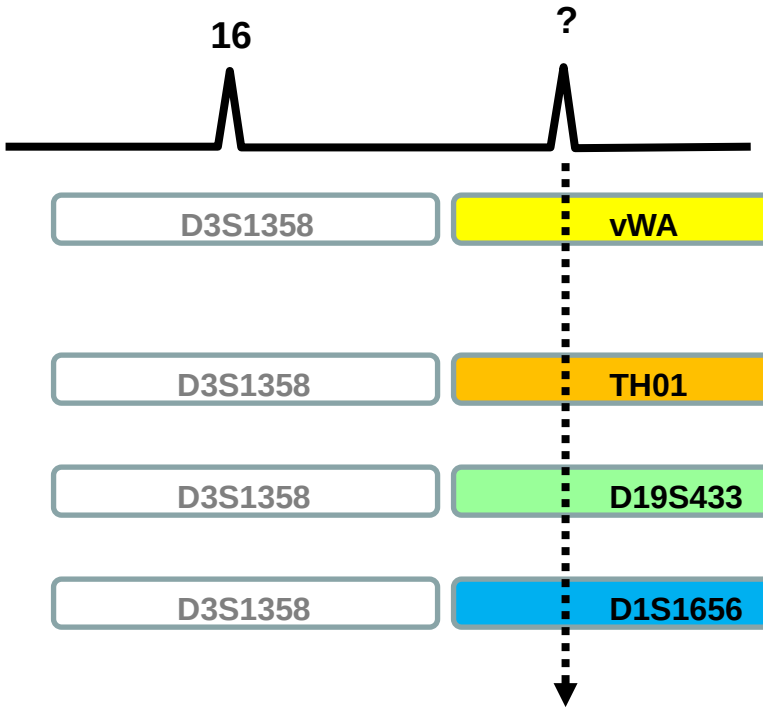


Reverse primer internal to duplicated flanking region

Recommendations for Tri-Allelic Patterns

- **Re-injecting a sample with the same STR kit does not help answer the question**
- **Run a different STR kit with loci in different configurations**
- This duplicate testing will help confirm that you have a true tri-allele rather than an extremely small or large allele that is out of the STR kit defined allele bins for a locus
- **Recording tri-allelic patterns correctly improves database searching comparability when states are using different STR kits**

Tale of a Large D3S1358 Allele



Appearance in each kit

SGM Plus	D3: 16,16	**vWA: OL, 17, 18** *(tri-allele?)*
ESX 17	D3: 16,16	**TH01: OL, 9, 9.3** *(tri-allele?)*
ESI 17	D3: 16,16	**D19: OL, 13, 14** *(tri-allele?)*
NGM	D3: 16,16	**D1: 9, 16, 17** *(tri-allele?)*

22 repeats from sequence analysis

TCTA $(TCTG)_3$ $(TCTA)_4$ TCTG $(TCTA)_{13}$

D3S1358 Allele 22

Initially measured as 22.1 or 22.2

Raziel et al. (2012) *FSI Genetics* 6: 108-112

How do you current handle variant alleles that fall “off-ladder”?

1. Accept first result obtained
2. Re-inject only
3. Re-amplify sample and re-test
4. Send sample to NIST for allele sequencing
5. Something else

Variant STR Allele Sequencing

Margaret Kline

Main Points:

- **STR allele sequencing has been provided free to the community** for the past ten years thanks to NIJ-funding
- Article provides primer sequences (outside of all known kit primers) for 23 autosomal STRs & 17 Y-STRs and full protocol for gel separations and sequencing reactions
  - 111 normal and variant alleles sequenced (at 19 STR & 4 Y-STRs)
  - 17 null alleles sequenced (with impact on various STR kit primers)

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Short communication

STR sequence analysis for characterizing normal, variant, and null alleles

Margaret C. Kline *, Carolyn R. Hill, Amy E. Decker [1], John M. Butler

National Institute of Standards and Technology, 100 Bureau Drive, M/S 8312, Gaithersburg, MD 20899, USA

Presentations/Publications:

- FSI Genetics article (Aug 2011) and numerous talks

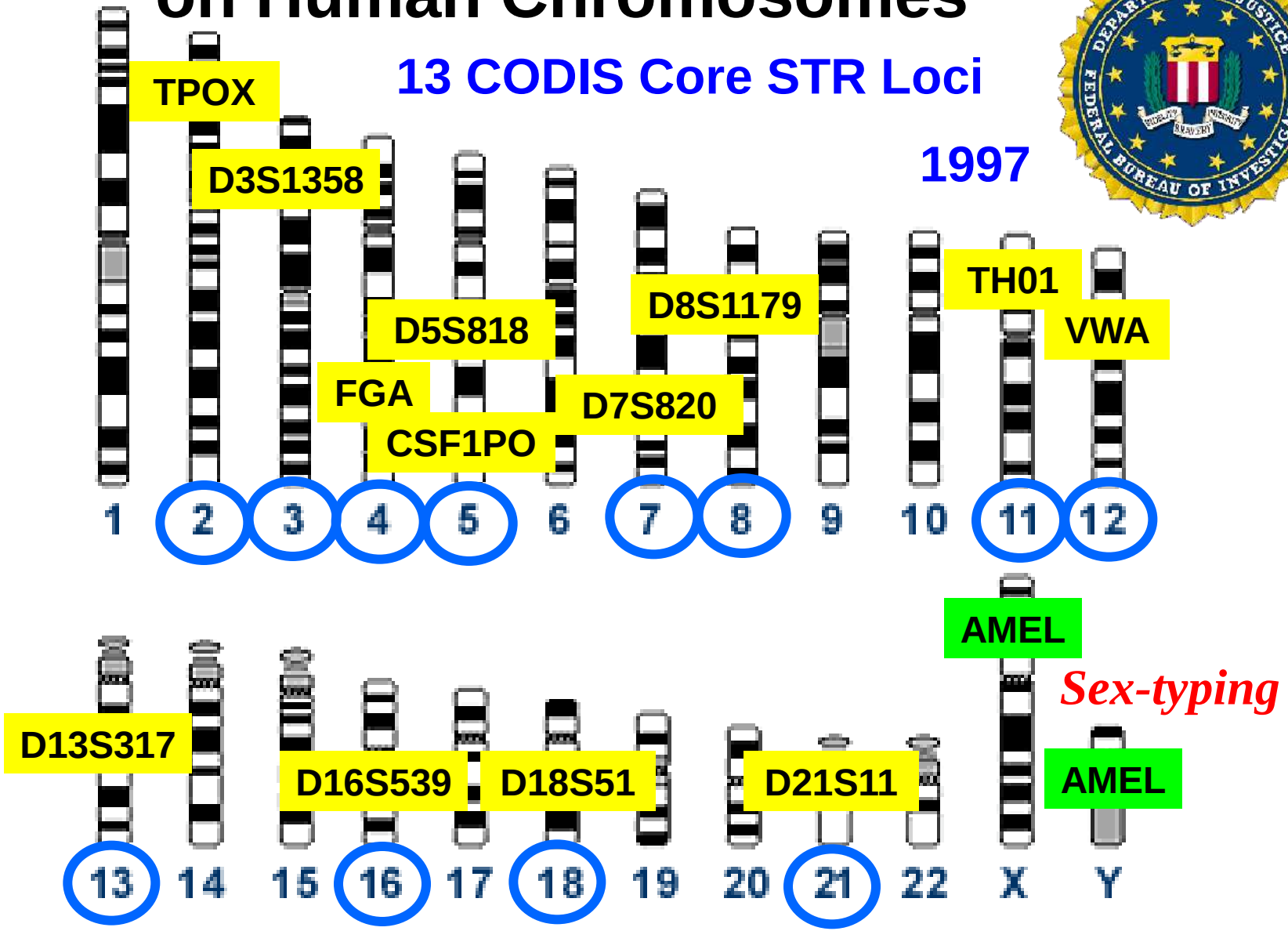
Expanding CODIS Core Loci

Position of Forensic STR Markers on Human Chromosomes

Core STR Loci for the United States

13 CODIS Core STR Loci

1997



Expanding the CODIS Core Loci

D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6: e52-e54
Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) doi:10.1016/j.fsigen.2012.01.003

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Letter to the Editor

Expanding the CODIS core loci in the United States

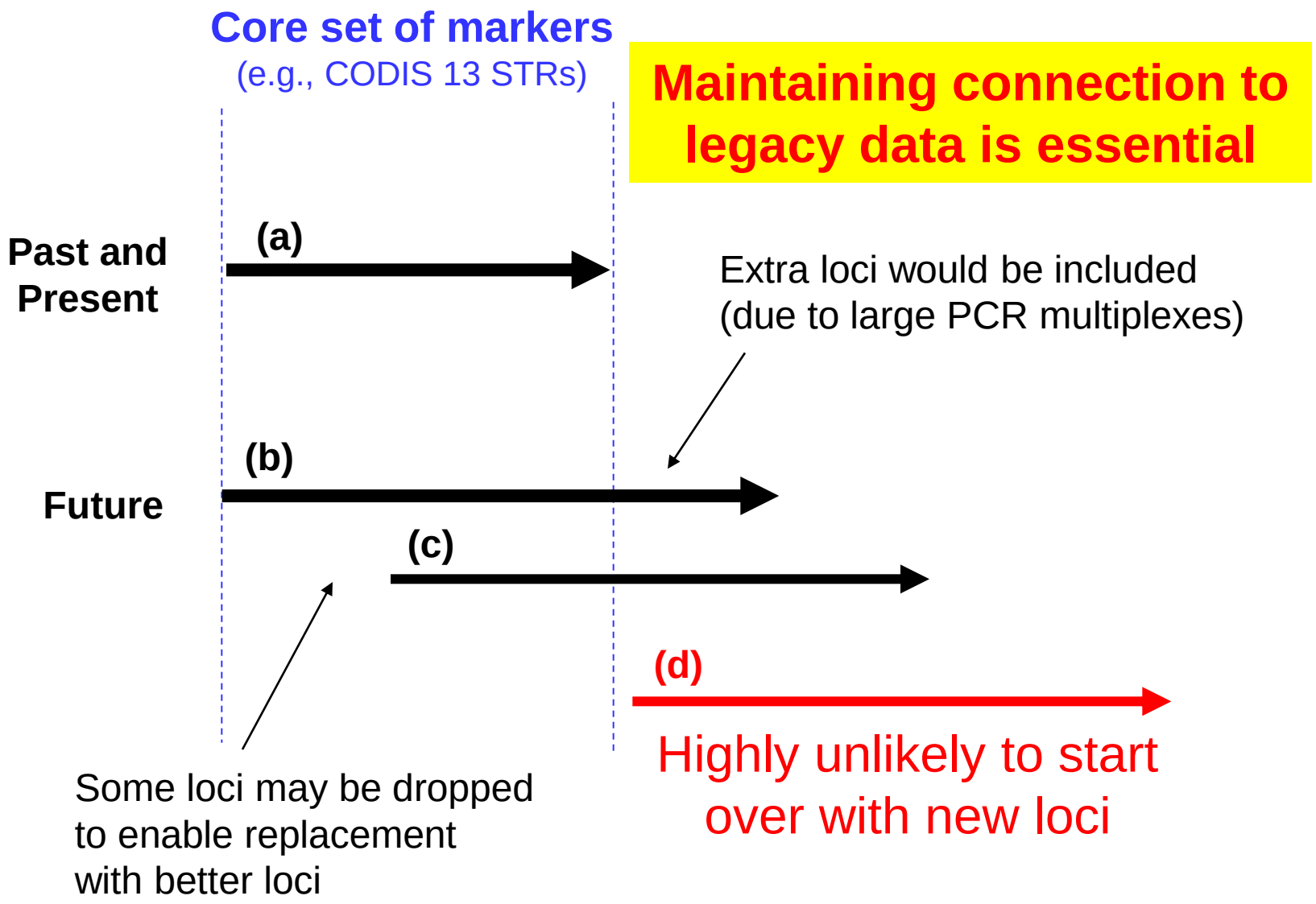
CODIS Core Loci Working Group
Formed in May 2010 to make recommendations to FBI CODIS Unit

Douglas Hares (Chair) – FBI
John Butler – NIST
Cecelia Crouse – FL PBSO
Brad Jenkins – VA DFS
Ken Konzak – CA DOJ
Taylor Scott – IL SP

major reasons for expanding the CODIS core loci in the United States:

(1) To reduce the likelihood of adventitious matches [7] as the number of profiles stored at NDIS continues to increase each year (expected to total over 10 million profiles by the time of this publication). There are no signs that this trend will slow down as States expand the coverage of their DNA database programs and increase laboratory efficiency and capacity.
(2) To increase international compatibility to assist law enforcement data sharing efforts.
(3) To increase discrimination power to aid missing persons cases.

Possible scenarios for extending sets of genetic markers to be used in national DNA databases



Proposed Expanded CODIS Core Loci

D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

20 required loci
Amelogenin (for sex-typing)
18 autosomal STRs
1 Y-STR (DYS391)

	Locus
Section A (required)	**Amelogenin**
	D18S51
	FGA
	D21S11
	D8S1179
	vWA
	D13S317
	D16S539
	D7S820
	TH01
	D3S1358
	D5S818
	CSF1PO
	D2S1338
	D19S433
	D1S1656
	D12S391
	D2S441
	D10S1248
	~~**Penta E**~~
	DYS391

	Locus
Section B (in order of preference)	**TPOX**
	D22S1045
	SE33
	~~**Penta D**~~

Current CODIS 13 loci in red font

Criteria for Acceptance of Additional Loci

D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

Considered only short tandem repeat (STR) loci due to need for compatibility to existing database of >10 million STR profiles

- **STR Loci**
  - **No known association to medical conditions or defects**
  - **Low mutation rate**
  - **High level of independence**
  - **High level of discrimination**
  - **Use by international forensic DNA community**
  - **Number of loci vs. discrimination factor**
  - **Compliance with Quality Assurance Standards (QAS)**

- **Kit performance**
  - **Balance between loci**
  - **Reliable**
  - **Reproducible**
  - **Sensitive**
  - **Quality results**
  - **Adaptable for use by NDIS laboratories (# of amplifications, ability of kit manufacturers to produce)**
  - **QAS compliant (documentation and availability of validation requirements)**

Three major reasons for expanding the CODIS core loci in the United States

D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

- **To reduce the likelihood of adventitious matches** as the number of profiles stored at NDIS continues to increase each year

- **To increase international compatibility** to assist law enforcement data sharing efforts

- **To increase discrimination power to aid missing persons cases**

Adventitious Matches

- The only published account of a false match from a DNA database came in 1999 when the UK database then consisting of 660,000 profiles with only 6 STR loci (SGM assay) lead to a "hit" between two individuals whose 6-locus random match probability was 1 in 37 million (R. Willing, *USA Today*, Feb 8, 2000; "Mismatch calls DNA test into question").

- Further testing with four additional STRs (SGM Plus loci) showed that the samples were from different individuals. **The UK expanded the number of core loci from 6 to 10 with the adoption of the SGM Plus kit to try and prevent another adventitious match**.

- The growth of DNA databases necessitates the inclusion of additional loci to avoid this problem.

For further information, see D.N.A. Box 8.3 in Butler, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*, p. 251

International Comparability

Currently there are 24 autosomal STR markers present in commercial kits

ESS = European Standard Set

13 CODIS loci

U.S.	Europe
TPOX	
CSF1PO	
D5S818	
D7S820	
D13S317	
FGA	FGA
vWA	vWA
D3S1358	D3S1358
D8S1179	D8S1179
D18S51	D18S51
D21S11	D21S11
TH01	TH01
D16S539	D16S539
D2S1338	D2S1338
D19S433	D19S433
Penta D	
Penta E	

7 ESS loci

5 loci adopted in 2009 to expand to 12 ESS loci

D12S391
D1S1656
D2S441
D10S1248
D22S1045
SE33 → Core locus for Germany

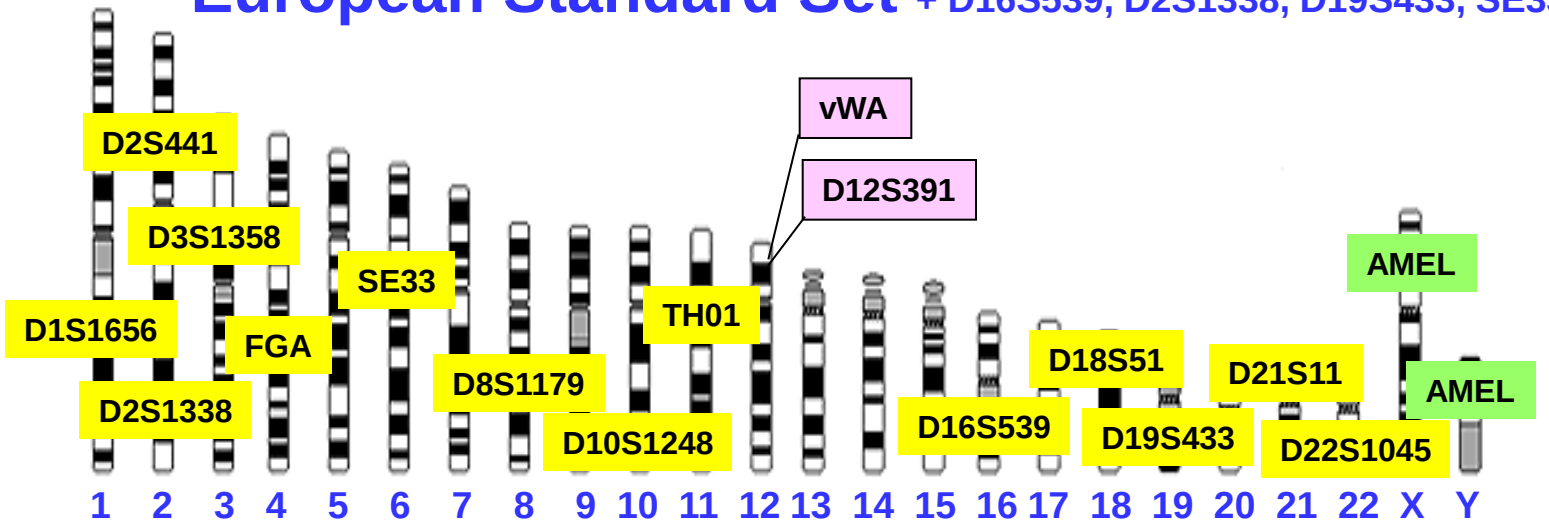
3 miniSTR loci developed at NIST

Locus used in China ← **D6S1043**

Common Forensic STR Loci

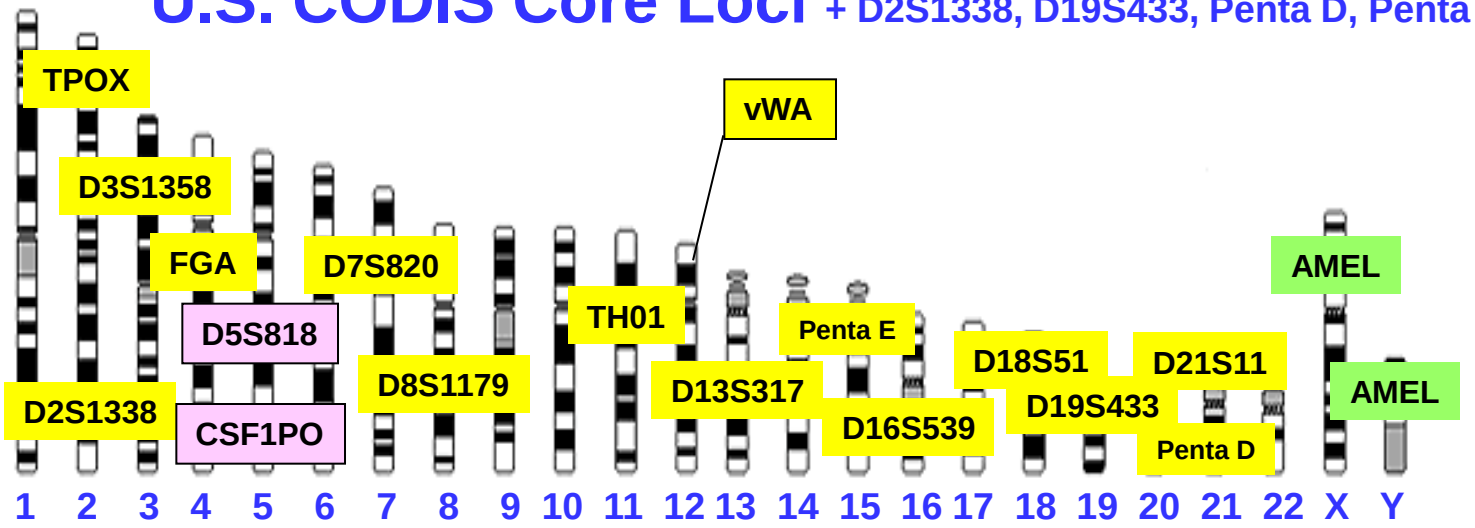
Europe

European Standard Set + D16S539, D2S1338, D19S433, SE33



United States

U.S. CODIS Core Loci + D2S1338, D19S433, Penta D, Penta E



What can we learn from European Standard Set (ESS) expansion experience?

PROFILES IN DNA

March 2009 issue

THE ESS LOCI

Expansion of the European Standard Set of DNA Database Loci—the Current Situation

Peter M. Schneider
Institute of Legal Medicine, University Hospital of Cologne, Germany

http://www.promega.com/resources/articles/profiles-in-dna/2009/expansion-of-the-european-standard-set/

ELSEVIER

Forensic Science International 119 (2001) 225–231

Forensic Science International

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A brief history of the formation of DNA databases in forensic science within Europe

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Interpol European Working Party on DNA Profiling

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Forensic Science International 119 (2001) 232–238

Forensic Science International

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Criminal DNA databases: the European situation

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Received 10 September 2000; accepted 14 November 2000

DNA Databases for Offender Id
The Need for Technical, Legal an

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http://www.promega.com/products/pm/genetic-identity/ishi-conference-proceedings/2nd-eshi-oral-presentations/

European Expansion Efforts

More loci added as databases grew…

European Standard Set (ESS) of Loci
1998: 4 STRs
1999: 7 STRs
2009: 12 STRs
(but 15 with most kits)

1995
UK National Database launched

1998
UK began with 6 STRs (SGM)

1999
ESS increased to 7 STRs; UK went to 10 STRs (SGM Plus)

2001
Leriche et al. (1998)
Initial Interpol ESS selection (4 STRs)

2004
Degraded DNA interlab study (EDNAP)

2005
Martin et al. (2001)
Multiple countries have established national databases

Prüm treaty
(data sharing)

EDNAP/ENFSI recommend new loci

2006
Gill et al. (2006a, 2006b)
Letters to editor (1) & (2) announcing proposed new loci - miniSTRs advocated

Dixon et al. (2006)
EDNAP interlab published

2007
Prototype kits developed & tested

2009
ESS expanded to 12 loci

2010
NGM and NGM SElect; Qiagen kits

PowerPlex ESI/ESX 16/17 kits released

Implementation required

2011

With expanded loci selections, focus is on casework capabilities not familial searching potential

Lessons from European ESS Expansion

- **Data studies should drive decisions**
  - Interlaboratory study with degraded DNA (Dixon et al. 2006 article was key)
- Casework capabilities are a primary goal
  - miniSTRs and desire for kits with ability to overcome inhibitors
- **Initial locus selection announced through Letters to the Editor** of the leading forensic DNA journal (Gill et al. 2006a, 2006b)
- Companies responded with prototype kits for evaluation
- Expanded ESS loci were selected and voted upon after data review by ENFSI labs **(4 years after initial recommendations were made)**
- EU adopted recommendations of ENFSI
- Commercial kits became available to meet expanded ESS requirements
- Population data gathered and software developed
- European labs must be compliant by Nov 30, 2011 (**2 years after adoption**)
- Casework capabilities not familial searching potential were the intent of the core loci selection

EDNAP Study Showed Value of miniSTRs

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Forensic Science International 164 (2006) 33–44

Forensic Science International

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Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise

L.A. Dixon [a,*], A.E. Dobbins [a], H.K. Pulker [a], J.M. Butler [b], P.M. Vallone [b], M.D. Coble [b], W. Parson [c], B. Berger [c], P. Grubwieser [c], H.S. Mogensen [d], N. Morling [d], K. Nielsen [d], J.J. Sanchez [d], E. Petkovski [e], A. Carracedo [f], P. Sanchez-Diz [f], E. Ramos-Luis [f], M. Briõn [f], J.A. Irwin [g], R.S. Just [g], O. Loreille [g], T.J. Parsons [g], D. Syndercombe-Court [h], H. Schmitter [i], B. Stradmann-Bellinghausen [j], K. Bender [j], P. Gill [a]

[a] *The Forensic Science Service, Research and Development, Trident Court, Birmingham, UK*
[b] *National Institute of Standards and Technology, Gaithersburg, MD, USA*
[c] *Institute of Legal Medicine, Innsbruck Medical University, Austria*

“Recently, there has been much debate about what kinds of genetic markers should be implemented as new core loci that constitute national DNA databases. The choices lie between conventional STRs, ranging in size from 100 to 450 bp; mini-STRs, with amplicon sizes less than 200 bp; and single nucleotide polymorphisms (SNPs)…Results were collated and analysed and, in general, **mini-STR systems were shown to be the most effective**…”

Data Driven Decisions

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Forensic Science International 156 (2006) 242–244

Forensic Science International

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Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill [a,*], Lyn Fereday [b], Niels Morling [c], Peter M. Schneider [d]

[a] *Forensic Science Service, Birmingham, UK*
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[c] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*
[d] *Institute of Legal Medicine, University of Cologne, Germany*

Received 25 May 2005; accepted 26 May 2005
Available online 5 July 2005

"Following a recent meeting by the ENFSI and EDNAP groups on the 4–5 April, 2005, in Glasgow, UK, it was unanimously agreed that the process of standardization within Europe **should take account of recent work that unequivocally demonstrated that chance of obtaining a result from a degraded sample was increased when small amplicons (mini-STRs) were analysed**…"

Characterizing New STR Loci


John Butler


Becky Hill

Main Points:

- In April 2011, the FBI announced plans to expand the core loci for the U.S. beyond the current 13 CODIS STRs

- Our group is collecting U.S. population data on new loci and characterizing them to aid understanding of various marker combinations

- We are collecting all available information from the literature on the 24 commonly used autosomal STR loci

Presentations/Publications:

- AAFS 2011 presentation
- Hill et al (2011) *FSI Genetics* 5(4): 269-275
- Hares (2012) Expanding the U.S. core loci… *FSI Genetics* 6(1): e52-e54
- Butler & Hill (2012) *Forensic Sci Rev* 24(1): 15-26

Article in the January 2012 issue of *Forensic Science Review*

Available at **http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm**

Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis

REFERENCE: Butler JM, Hill CR: Biology and genetics of new autosomal STR loci useful for forensic DNA analysis; *Forensic Sci Rev* 24:15; 2012.

ABSTRACT: Short tandem repeats (STRs) are regions of tandemly repeated DNA segments found throughout the human genome that vary in length (through insertion, deletion, or mutation) with a core repeated DNA sequence. Forensic laboratories commonly use tetranucleotide repeats, containing a four base pair (4-bp) repeat structure such as GATA. In 1997, the Federal Bureau of Investigation (FBI) Laboratory selected 13 STR loci that form the backbone of the U.S. national DNA database. Building on the European expansion in 2009, the FBI announced plans in April 2011 to expand the U.S. core loci to as many as 20 STRs to enable more global DNA data sharing. Commercial STR kits enable consistency in marker use and allele nomenclature between laboratories and help improve quality control. The STRBase website, maintained by the U.S. National Institute of Standards and Technology (NIST), contains helpful information on STR markers used in human identity testing.

Key Words: Autosomal genetic markers, CODIS STRs, core loci, DNA typing, European Standard Set, expanded U.S. core loci, short tandem repeat (STR), STR kits.

Discusses the 24 autosomal STR loci available in commercial kits

The 11 STR Loci Beyond the CODIS 13

STR Locus	Location	Repeat Motif	Allele Range*	# Alleles*
D2S1338	2q35	TGCC/TTCC	10 to 31	40
D19S433	19q12	AAGG/TAGG	5.2 to 20	36
Penta D	21q22.3	AAAGA	1.1 to 19	50
Penta E	15q26.2	AAAGA	5 to 32	53
D1S1656	1q42	TAGA	8 to 20.3	25
D12S391	12p13.2	AGAT/AGAC	13 to 27.2	52
D2S441	2p14	TCTA/TCAA	8 to 17	22
D10S1248	10q26.3	GGAA	7 to 19	13
D22S1045	22q12.3	ATT	7 to 20	14
SE33	6q14	AAAG‡	3 to 49	**178**
D6S1043	6q15	AGAT/AGAC	8 to 25	25

5 new European loci

**Allele range and number of observed alleles from Appendix 1, J.M. Butler (2012) Advanced Topics in Forensic DNA Typing: Methodology; ‡SE33 alleles have complex repeat structure*

25 Alleles Reported in the Literature for **D1S1656**

15 NIST observed alleles circled in red

Allele (Repeat #)	Promega ESX 17	Promega ESI 17	ABI NGM	Repeat Structure $[TAGA]_4[TGA]_{0-1}[TAGA]_nTAGG[TG]_5$	Reference
8	133 bp	222 bp	171 bp	$[TAGA]_8[TG]_5$	Phillips *et al.* (2010)
9	137 bp	226 bp	175 bp	$[TAGA]_9[TG]_5$	Phillips *et al.* (2010)
10 (a)	141 bp	230 bp	179 bp	$[TAGA]_{10}[TG]_5$	Lareu *et al.* (1998)
10 (b)	141 bp	230 bp	179 bp	$[TAGA]_{10}TAGG[TG]_5$	Phillips *et al.* (2010)
11	145 bp	234 bp	183 bp	$[TAGA]_{11}[TG]_5$	Lareu *et al.* (1998)
12 (a)	149 bp	238 bp	187 bp	$[TAGA]_{12}[TG]_5$	Lareu *et al.* (1998)
12 (b)	149 bp	238 bp	187 bp	$[TAGA]_{11}TAGG[TG]_5$	Lareu *et al.* (1998)
13 (a)	153 bp	242 bp	191 bp	$[TAGA]_{12}TAGG[TG]_5$	Lareu *et al.* (1998)
13 (b)	153 bp	242 bp	191 bp	$[TAGA]_{13}[TG]_5$	Phillips *et al.* (2010)
13.3	156 bp	245 bp	194 bp	$[TAGA]_1TGA[TAGA]_{11}TAGG[TG]_5$	Phillips *et al.* (2010)
14 (a)	157 bp	246 bp	195 bp	$[TAGA]_{13}TAGG[TG]_5$	Lareu *et al.* (1998)
14 (b)	157 bp	246 bp	195 bp	$[TAGA]_{14}[TG]_5$	Phillips *et al.* (2010)
14.3	160 bp	249 bp	198 bp	$[TAGA]_4TGA[TAGA]_9TAGG[TG]_5$	Phillips *et al.* (2010)
15	161 bp	250 bp	199 bp	$[TAGA]_{14}TAGG[TG]_5$	Lareu *et al.* (1998)
15.3	164 bp	253 bp	202 bp	$[TAGA]_4TGA[TAGA]_{10}TAGG[TG]_5$	Lareu *et al.* (1998)
16	165 bp	254 bp	203 bp	$[TAGA]_{15}TAGG[TG]_5$	Lareu *et al.* (1998)
16.3	168 bp	257 bp	206 bp	$[TAGA]_4TGA[TAGA]_{11}TAGG[TG]_5$	Lareu *et al.* (1998)
17	169 bp	258 bp	207 bp	$[TAGA]_{16}TAGG[TG]_5$	Lareu *et al.* (1998)
17.1	170 bp	259 bp	208 bp	Not published	Schröer *et al.* (2000)
17.3	172 bp	261 bp	210 bp	$[TAGA]_4TGA[TAGA]_{12}TAGG[TG]_5$	Lareu *et al.* (1998)
18	173 bp	262 bp	211 bp	$[TAGA]_{17}TAGG[TG]_5$	Phillips *et al.* (2010)
18.3	176 bp	265 bp	214 bp	$[TAGA]_4TGA[TAGA]_{13}TAGG[TG]_5$	Lareu *et al.* (1998)
19	177 bp	266 bp	215 bp	Not published	Asamura *et al.* (2008)
19.3	180 bp	269 bp	218 bp	$[TAGA]_4TGA[TAGA]_{14}TAGG[TG]_5$	Lareu *et al.* (1998)
20.3	184 bp	273 bp	222 bp	Not published	Gamero *et al.* (2000)

from Appendix 1, J.M. Butler (2011) Advanced Topics in Forensic DNA Typing: Methodology

NIST **U.S. Population Allele Frequencies**

D1S1656 (15 different alleles)

15 different alleles

Allele	African American (N = 341)	Caucasian (N = 361)	Hispanic (N = 236)
10	0.01433	0.00277	0.00630
11	0.04871	0.07756	0.02731
12	0.06304	0.11773	0.08824
13	0.10029	0.06648	0.11555
14	**0.25788**	0.07895	0.11765
14.3	0.00716	0.00277	0.00420
15	0.15616	**0.14820**	0.13866
15.3	0.03009	0.05817	0.05042
16	0.11032	0.13573	**0.17437**
16.3	0.10029	0.06094	0.05462
17	0.02865	0.04709	0.04202
17.3	0.05014	0.13296	0.14496
18	0.00287	0.00554	0.00630
18.3	0.02436	0.05125	0.02521
19.3	0.00573	0.01385	0.00420

N = 938

(only unrelated samples used; fathers removed from this sample set)

< 5/2N

D1S1656 Characteristics

- **15 alleles** observed
- **92 genotypes** observed
- **>89% heterozygotes** (heterozygosity = 0.8934)
- **0.0220 Probability of Identity** (P_I)

$$P_I = \sum (genotype\ frequencies)^2$$

These values have been calculated for all 24 STR loci across the U.S. population samples examined

Loci sorted on Probability of Identity (P_I) values

STR Locus	Alleles Observed	Genotypes Observed	Het. (obs)	P_I value N = 938
SE33	53	292	0.9360	0.0069
Penta E*	20	114	0.8799	0.0177
D2S1338	13	68	0.8785	0.0219
D1S1656	15	92	0.8934	0.0220
D18S51	21	91	0.8689	0.0256
D12S391	23	110	0.8795	0.0257
FGA	26	93	0.8742	0.0299
D6S1043*	25	91	0.8627	0.0343
Penta D*	16	71	0.8754	0.0356
D21S11	25	81	0.8358	0.0410
D19S433	16	76	0.8124	0.0561
D8S1179	11	45	0.7878	0.0582
vWA	11	38	0.8060	0.0622
D7S820	11	32	0.8070	0.0734
TH01	8	24	0.7580	0.0784
D16S539	9	28	0.7825	0.0784
D13S317	8	29	0.7655	0.0812
D10S1248	12	39	0.7825	0.0837
D2S441	14	41	0.7772	0.0855
D3S1358	11	30	0.7569	0.0873
D22S1045	11	42	0.7697	0.0933
CSF1PO	9	30	0.7537	0.1071
D5S818	9	34	0.7164	0.1192
TPOX	9	28	0.6983	0.1283

24 STR Loci in STR kits rank ordered by their variability

Better for mixtures (more alleles seen)

There are several loci more polymorphic than the current CODIS 13 STRs

Better for kinship (low mutation rate)

Determination of Additional CODIS Core Loci

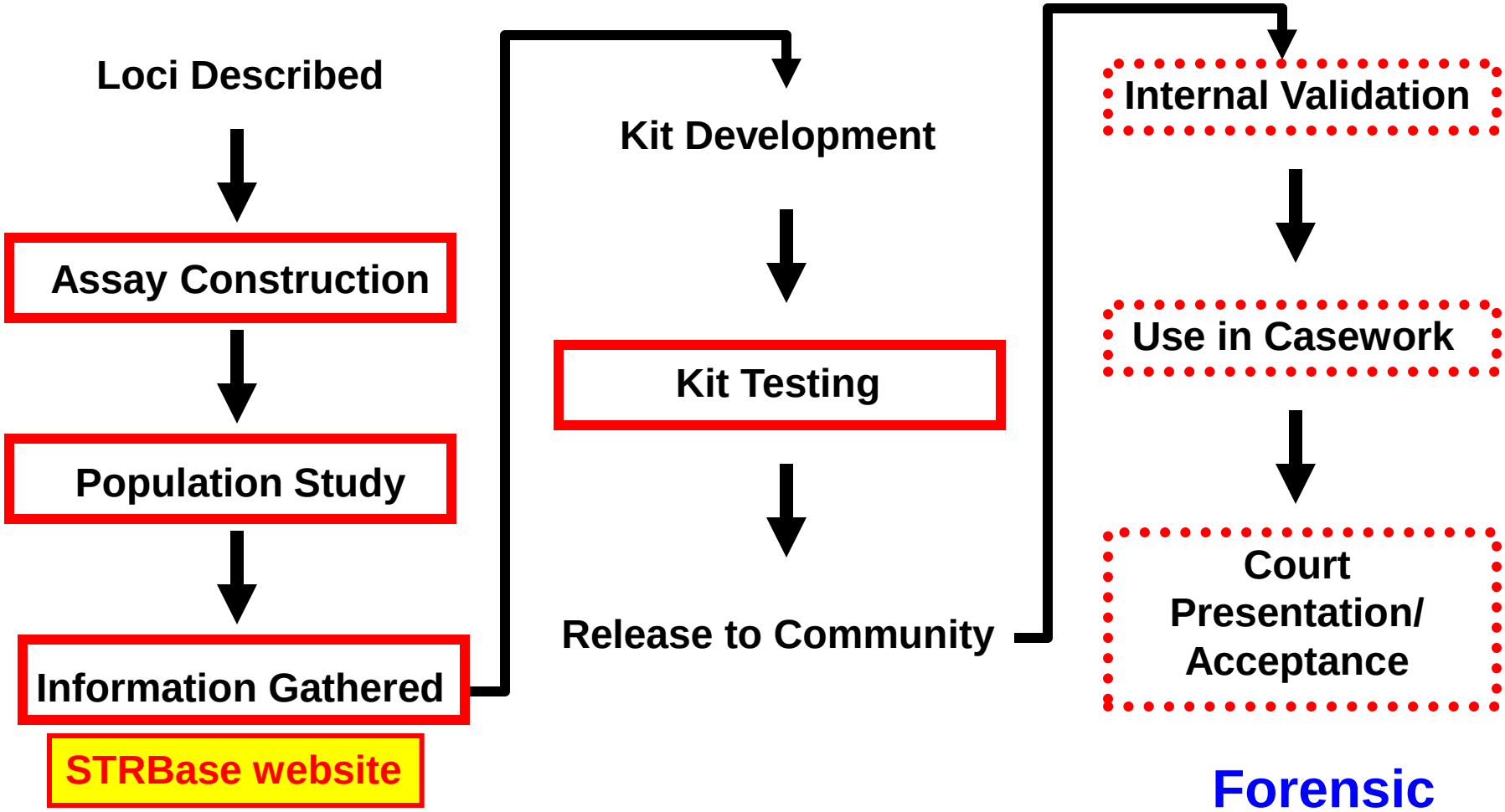
D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6: e52-e54
Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) doi:10.1016/j.fsigen.2012.01.003

What	Why	Who/How	When
Form a Working Group (WG) to discuss initial selection	Establishes target goals	CODIS Core Loci Working Group with FBI Chair and 5 members; Web meetings	May 2010 - present
Announce proposed additional CODIS core loci	Sets desired target goals and informs manufacturers	WG Chair; Publish proposed listing of CODIS core loci	April 2011 online (published Jan 2012)
Ongoing Progress Reports	Provides updates for DNA community	WG Chair; Present updates on status of CODIS Core Loci project at meetings	2010-2012
Implementation Considerations & Strategy	Identify issues for implementation and timeline	WG	June 2011 - present
Manufacturers develop prototype kits	Creates tools to meet target goals	Manufacturers; Provide status reports to WG for timeline	2011-2012
Test and validate prototype kits	Examines if target goals can be met	Validation Laboratories; Follow QAS compliant validation plan	Beginning in 2012
Review and evaluate data from validation	Evaluates if desired performance is obtained	NIST, SWGDAM and FBI; Provide feedback, if any, to Manufacturers	In conjunction with and at the conclusion of validation
Selection of new CODIS core loci	Allows protocols to be established	FBI; seek input from DNA community and stakeholders; Notify Congress	After evaluation of validation data and kit production factors
Implementation of new CODIS core loci at the National DNA Index System	Enables target goals to be met	All NDIS-participating labs	~ 24 months after selection of new CODIS core loci

http://www.fbi.gov/about-us/lab/codis/planned-process-and-timeline-for-implementation-of-additional-codis-core-loci

Steps in Adopting Genetic Markers

Role of the NIST Human Identity Project Team



Which autosomal STR kit do you use?
(select only one)

1. Identifiler
2. MiniFiler
3. Profiler/Cofiler
4. Identifiler & MiniFiler
5. All of the above
6. Promega kits

Recent Court Decision Impacting Sale of STR Typing Kits

Disclaimer: The information contained herein is only as accurate as my understanding of the information available to me at the time this presentation was given. ***Things are still evolving with this case…***

http://www.appliedbiosystems.com

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Human Identification & Forensic DNA

- 3500 Series Genetic Analyzers for

Products > Human Identification & Forensic DNA > Kits > PCR Human DNA Identification Kits

PCR Amplification Kit

AmpFℓSTR® Identifiler® PCR Amplification Kit

Ordering Information | Product Description | Specifications | Literature/Support

The Identifiler kit is the single most discriminating and widely used STR based kit for human identification applications from Applied Biosystems. The Identifiler kit amplifies 15 loci and Amelogenin in a single tube and provides loci consistent with major world-wide STR databasing standards.

Note: For Forensic or Paternity Use Only.

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IMPORTANT NOTICE

The UNITED STATES DISTRICT COURT FOR THE WESTERN DISTRICT OF WISCONSIN ruled that certain products (listed below) sold by Life Technologies Corporation ("Life") can only be used by customers for forensic and paternity uses ("Licensed Use"). Specifically, the Court held that the license Life holds from Promega Corporation ("Promega") does not include the following applications: (1) chimerism (which involves determining the relative amount present of two different types of DNA); (2) classifying molar specimens (which involves determining whether a mole is present and what type it is); (3) cell line authentication (which involves a determination of whether two cell lines are unique); (4) determination of fetal sex;(5) cancer analysis; (6) genetic research; (7) non-casework-related forensic applications such as general research in forensics or teaching and training of persons not employed in a forensic laboratory; (8) maternal cell contamination; and (9) sample tracking. Accordingly, this notice replaces any other label license or use statement for the listed products only as those labels or

Notice on ABI STR Kits

IMPORTANT NOTICE

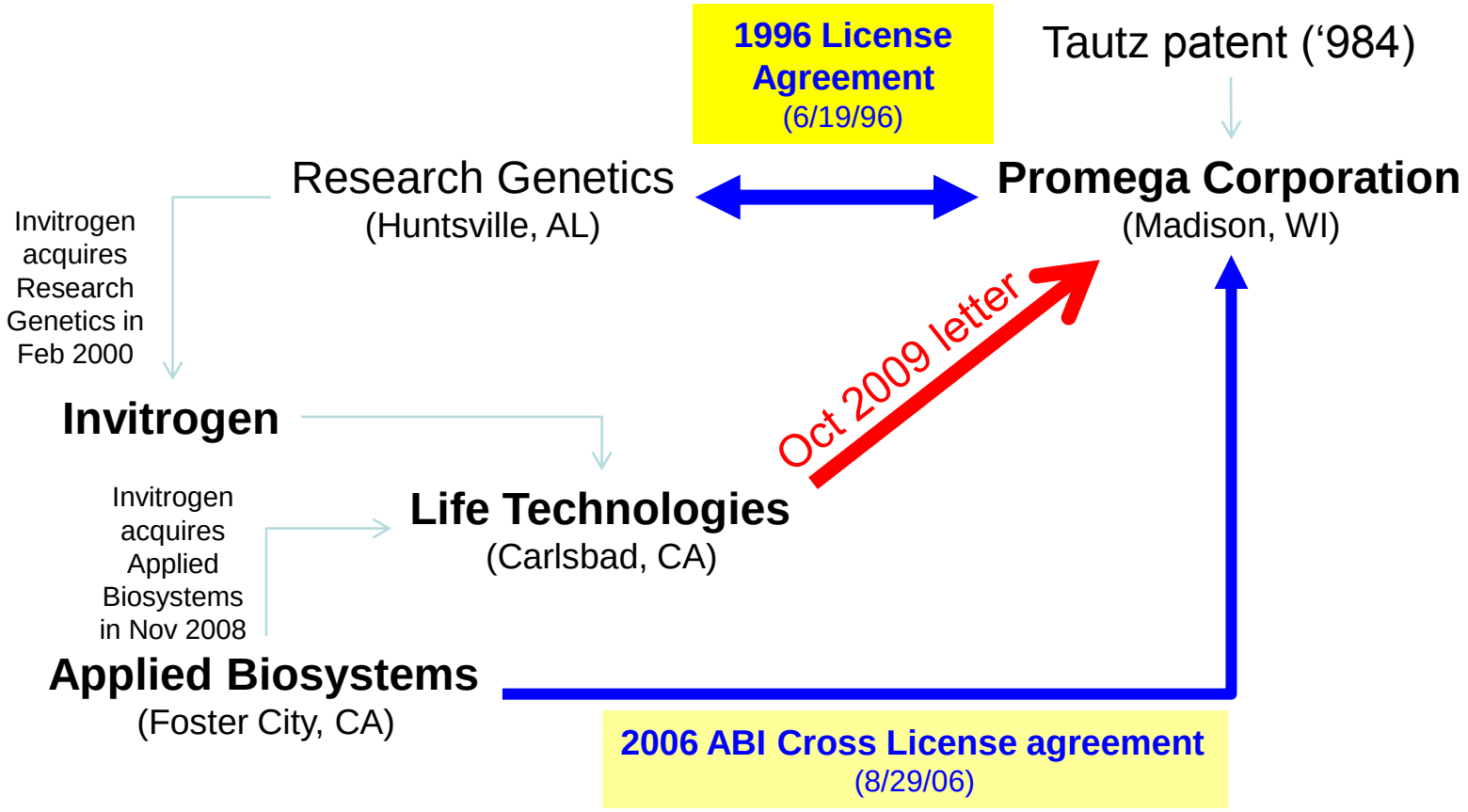
The UNITED STATES DISTRICT COURT FOR THE WESTERN DISTRICT OF WISCONSIN ruled that certain products (listed below) sold by Life Technologies Corporation ("Life") can only be used by customers for forensic and paternity uses ("Licensed Use"). Specifically, the Court held that the license Life holds from Promega Corporation ("Promega") does not include the following applications: (1) chimerism (which involves determining the relative amount present of two different types of DNA); (2) classifying molar specimens (which involves determining whether a mole is present and what type it is); (3) cell line authentication (which involves a determination of whether two cell lines are unique); (4) determination of fetal sex;(5) cancer analysis; (6) genetic research; (7) non-casework-related forensic applications such as general research in forensics or teaching and training of persons not employed in a forensic laboratory; (8) maternal cell contamination; and (9) sample tracking. Accordingly, this notice replaces any other label license or use statement for the listed products only as those labels or statements relate to the use of such products under the Promega license. Any other restrictions, such as regulatory restrictions, related to the use of these products are not affected by this notice. If a customer has any question regarding whether their intended use is within or outside the Licensed Use, please contact LicenseQuery@lifetech.com .

The following products are subject to this notice:
4322288 AmpFℓSTR® Identifiler® PCR Amplification Kit …

The following products are subject to this notice:

4322288 AmpFℓSTR® Identifiler® PCR Amplification Kit
4408580 AmpFℓSTR® Identifiler® Direct PCR Amplification Kit (1000 tests)
4467831 AmpFℓSTR® Identifiler® Direct PCR Amplification Kit (200 tests)
4427368 AmpFℓSTR® Identifiler® Plus PCR Amplification Kit
4373872 AmpFℓSTR® MiniFiler™ PCR Amplification Kit
4415021 AmpFℓSTR® NGM™ PCR Amplification Kit (1000 rxn)
4415020 AmpFℓSTR® NGM™ PCR Amplification Kit (200 rxn)
4457890 AmpFℓSTR® NGM SElect™ PCR Amplification Kit (1000 rxn)
4457889 AmpFℓSTR® NGM SElect™ PCR Amplification Kit (200 rxn)
403038 AmpFℓSTR® Profiler® PCR Amplification Kit
4303326 AmpFℓSTR® Profiler Plus® PCR Amplification Kit
4330284 AmpFℓSTR® Profiler Plus® ID PCR Amplification Kit
4305246 AmpFℓSTR® COfiler® PCR Amplification Kit
4307133 AmpFℓSTR® SGM Plus® PCR Amplification Kit
4382699 AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit
4305979 AmpFℓSTR® Profiler Plus® and AmpFLSTR® Cofiler® Kits
4330621 AmpFℓSTR® Profiler Plus® ID Kit and AmpFLSTR® Cofiler ® Kit
4359513 AmpFℓSTR® Yfiler® PCR Amplification Kit
4382306 AmpFℓSTR® Sinofiler™ PCR Amplification Kit
4382324 AmpFℓSTR® Sinofiler™ PCR Amplification Kit Primer Set

Summary of the Facts from My Understanding of Court Documents



United States Patent [19]
Schumm et al.

[11] **Patent Number: 5,843,660**
[45] **Date of Patent: Dec. 1, 1998**

[54] **MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI**

‘660

(12) **United States Patent**
Schumm et al.

(10) **Patent No.: US 6,221,598 B1**
(45) **Date of Patent: *Apr. 24, 2001**

‘598

(12) **United States Patent**
Schumm et al.

(10) **Patent No.: US 6,479,235 B1**
(45) **Date of Patent: Nov. 12, 2002**

‘235

(12) **United States Patent**
Schumm et al.

(10) **Patent No.: US 7,008,771 B1**
(45) **Date of Patent: Mar. 7, 2006**

‘771

(19) **United States**
(12) **Reissued Patent**
Jäckle et al.

(10) **Patent Number: US RE37,984 E**
(45) **Date of Reissued Patent: Feb. 11, 2003**

‘984

(54) **PROCESS FOR ANALYZING LENGTH POLYMORPHISMS IN DNA REGIONS**

(75) Inventors: **Herbert Jäckle**, Göttingen (DE); **Diethard Tautz**, Köln (DE)

(73) Assignee: **Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V.**, Gottingen (DE)

(21) Appl. No.: **09/591,383**

(22) Filed: **Jun. 9, 2000**

Related U.S. Patent Documents

H. Chen et al., *Human Mutation*, 4:208–211 (1994).
X. Y. Hauge et al., *Human Molecular Genetics*, 2(4):411–415 (1993).
J. M. Hite et al., *Nucleic Acids Research*, 1996, vol. 24, No. 12, pp. 2429–2434.
D. Tautz, *Nucleic Acids Research*, 1989, vol. 17, No. 16, pp. 6463–6471.
A. Edwards et al., *Trans. Assoc. Am. Phys., 102nd Session*, vol. 102:185–194 (1989).
M. Litt et al., *Am. J. Hum. Genetics*, 1989, vol. 44, pp. 397–401.
J. S. Chamberlain, *Nucleic Acids Research*, 1988, vol. 16, No. 23, pp. 11141–11155.
S. S. Chong et al., *Nature Genetics*, 1995, vol. 10. pp.

This Patent was Previously Licensed to Both Promega and Applied Biosystems

United States Patent [19]

Caskey et al.

[11] **Patent Number: 5,364,759**

[45] **Date of Patent: Nov. 15, 1994**

[54] **DNA TYPING WITH SHORT TANDEM REPEAT POLYMORPHISMS AND IDENTIFICATION OF POLYMORPHIC SHORT TANDEM REPEATS**

[75] Inventors: **Charles T. Caskey; Albert O. Edwards,** both of Houston, Tex.

[73] Assignee: **Baylor College of Medicine,** Houston, Tex.

[21] Appl. No.: **647,655**

[22] Filed: **Jan. 31, 1991**

map of the mouse genome using PCR-analysed microsatellites; Nucleic Acids Res., 18:4123–4130 (1990).
Zuliani, et al: A High Frequency of Length Polymorphisms in Repeated Sequences Adjacent to Alu Sequences; Am. J. Hum. Genet. 46:963–969 (1990).
Sinnett, et al; Alumorphs-Human DNA Polymorphisms Detected by Polymerase Chain Reaction Using *Alu*-Specific Primers; Genomics, 7:331–334 (1990).
Turner, et al; Genetic variation in clonal vertebrates

(List continued on next page.)

Patent expired after 17 years
on November 15, 2011

Timeline to Court Case

- On **October 20, 2009**, Life Technologies (LTI = ABI) sent a letter to Promega asserting new interpretation of the 1996 License Agreement which would have required Promega to pay >$50M within 60 days of demand (>20X what has previously been paid)
- During January 2010 meeting, Promega and ABI agreed to conduct audits about royalty payments
- In a February 10, 2010 letter, LTI conceded it had no documentary evidence to support its novel claim
- In a May 4, 2010 letter, LTI demanded arbitration of a supposed royalty BUT ABI had breached the 1996 agreement
- In a July 7, 2010 follow-up letter, Promega sought a declaration that LTI and ABI have **willfully infringed 5 patents by selling outside permitted fields** (in clinical diagnostics, clinical research, & research markets)

Trial Dates and Results

- February 6, 7, 8, 9, 10, 13, 14, 15 (2012)
- Jury verdict on February 15, 2012
- **Judgment on February 23, 2012**
- **Promega received $52,009,941 from Life Technologies (Applied Biosystems)**

Jury Verdict on February 15, 2012

IN THE UNITED STATES DISTRICT COURT

FOR THE WESTERN DISTRICT OF WISCONSIN

- -

PROMEGA CORPORATION,

Plaintiff,

SPECIAL VERDICT

10-cv-281-bbc

v.

LIFE TECHNOLOGIES CORPORATION,
INVITROGEN IP HOLDINGS, INC. and
APPLIED BIOSYSTEMS, LLC,

Defendants.

- -

- Ouestion No.1: What is the **total dollar amount of worldwide STR kit sales made between August 29, 2006 through the end of January 2012** by defendants Life Technologies Corporation, Invitrogen IP Holdings. Inc. and Applied Biosystems, LLC?

- Answer: **$ 707,618,247**

Answer Question No. 5.

Question No. 5: What profits, if any, did plaintiff lose as a result of defendants' sales that you found in Question No.4?

Answer: $ 52,009,941

Answer Question No. 7.

Question No. 7: Was defendants' infringement willful?

Answer: YES

(Yes or No)

Daniel J Lynch
Presiding Juror

Madison, Wisconsin
Dated this 15 day of February, 2012

Forensic DNA Labs

- Forensic & paternity testing DNA laboratories performing casework **should not be directly impacted by this court ruling** because ABI has a license to sell for casework applications

Potential Impact on NIST

- Judge has narrowly defined that ***only forensic labs and paternity labs may be sold ABI kits*** **– NOT universities or other research labs**
- I have spoken with lawyers from both Promega and Life Technologies (Applied Biosystems)
- The initial plan was for Promega to work with LTI/ABI to develop a permitted purchase list institution by institution
  - Promega wants to take over cell line authentication market and other clinical DNA applications
- Purchase of ABI STR kits for forensic research and training may not be permitted in the future
- Both companies would like to keep their customers happy…

New STR Kits

Commercially Available STR Kits

Applied Biosystems (17)

- ~~AmpFlSTR Blue (1996)~~
- ~~AmpFlSTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- Yfiler (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- **Identifiler Direct** (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)

Promega Corporation (15)

- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- PowerPlex Y (2003)
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)
- PowerPlex 18D (2011)
- PowerPlex 21 (2012)
- PowerPlex ESI 17 Pro (2012)

Qiagen (2010)

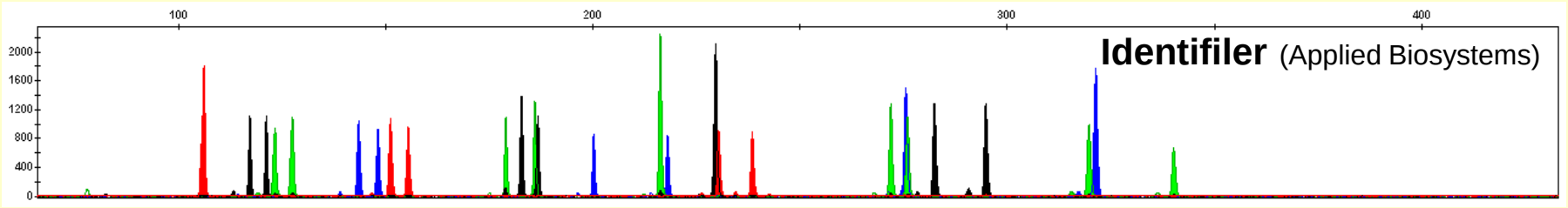
Primarily selling kits in Europe

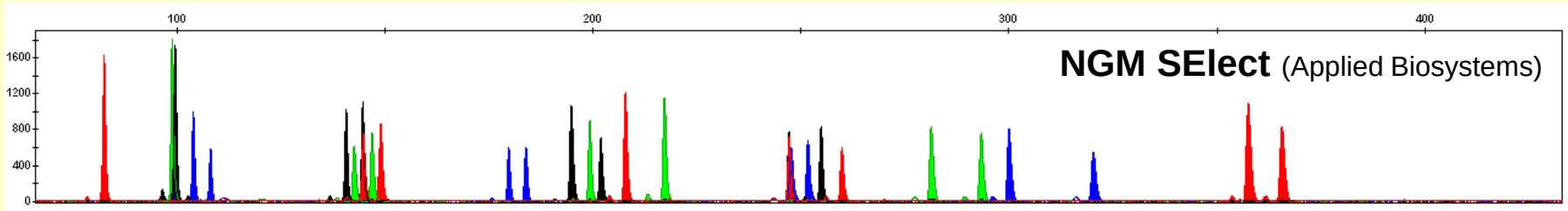
Due to patent restrictions cannot sell in U.S.

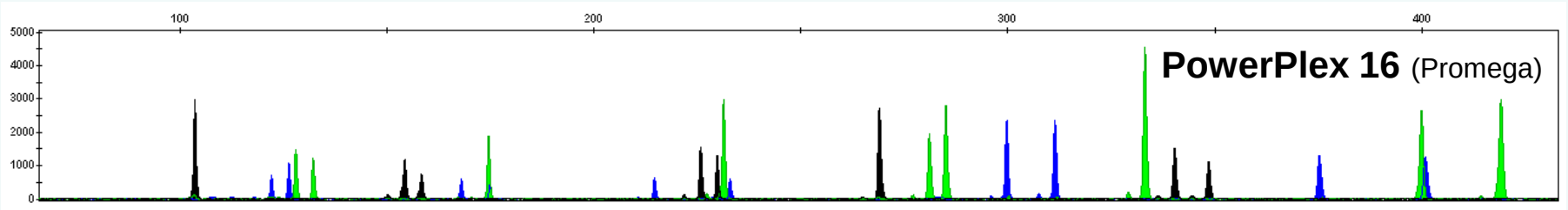
- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HD (Chimera)
- Argus X-12
- Argus Y-12
- DIPlex (30 InDels)

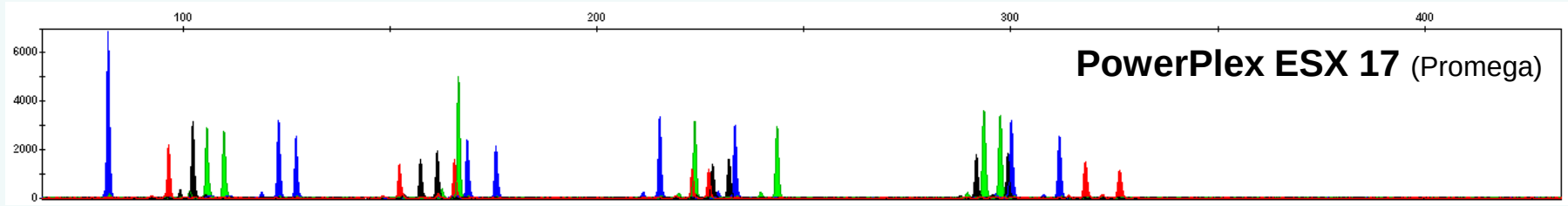
~1/3 of all STR kits were released in the last three years

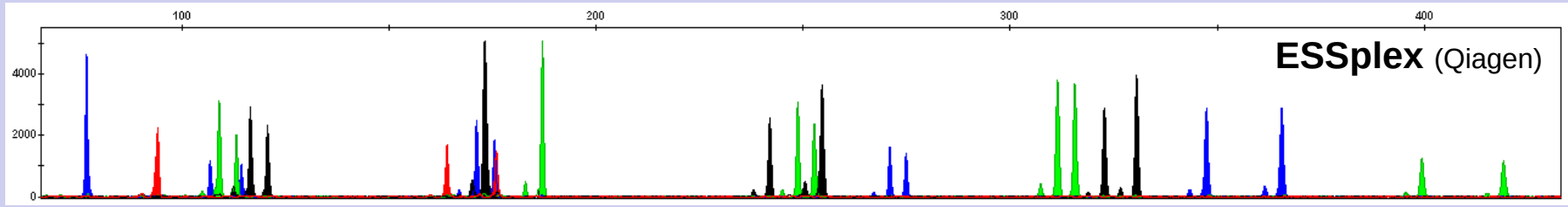
Same DNA Sample Tested with Five STR Kits











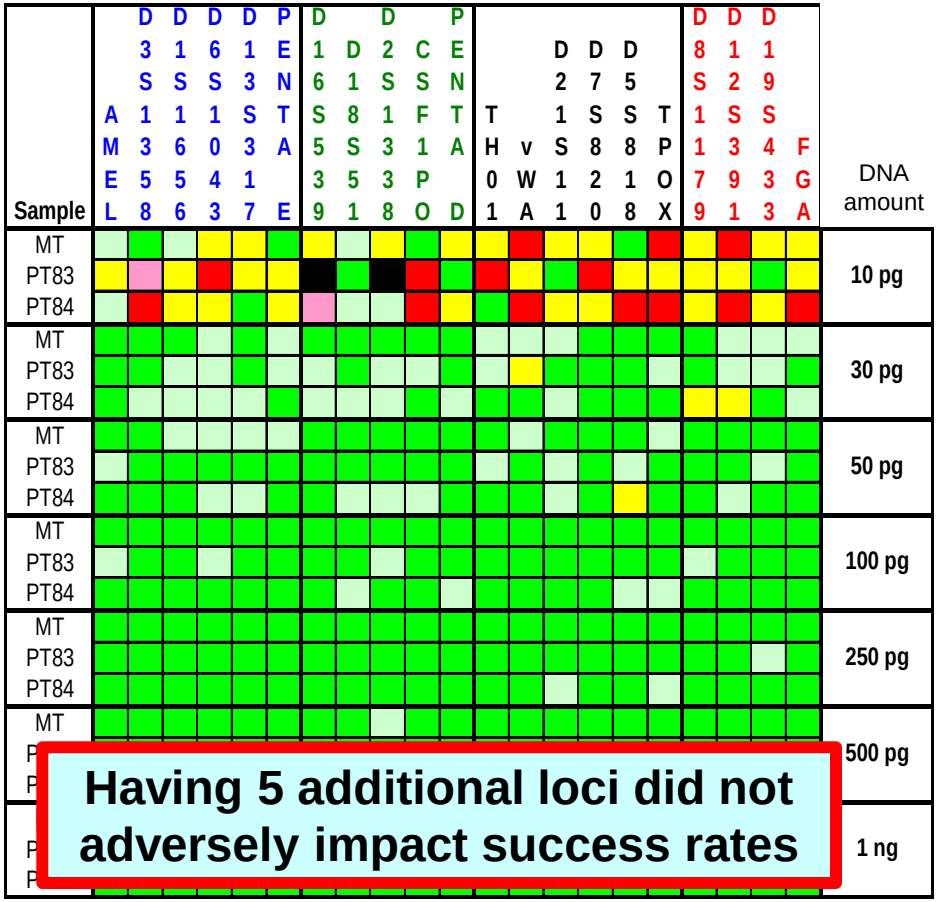
Data courtesy of Becky Hill (NIST)

STR Kit Performance

21plex (PowerPlex 21) vs 16plex (Identifiler Plus)

Three fully heterozygous (except PT83 at Penta D) pristine DNA samples were examined in a dilution series with PowerPlex 21 and Identifiler Plus. Results are ordered by amplicon size and dye color.

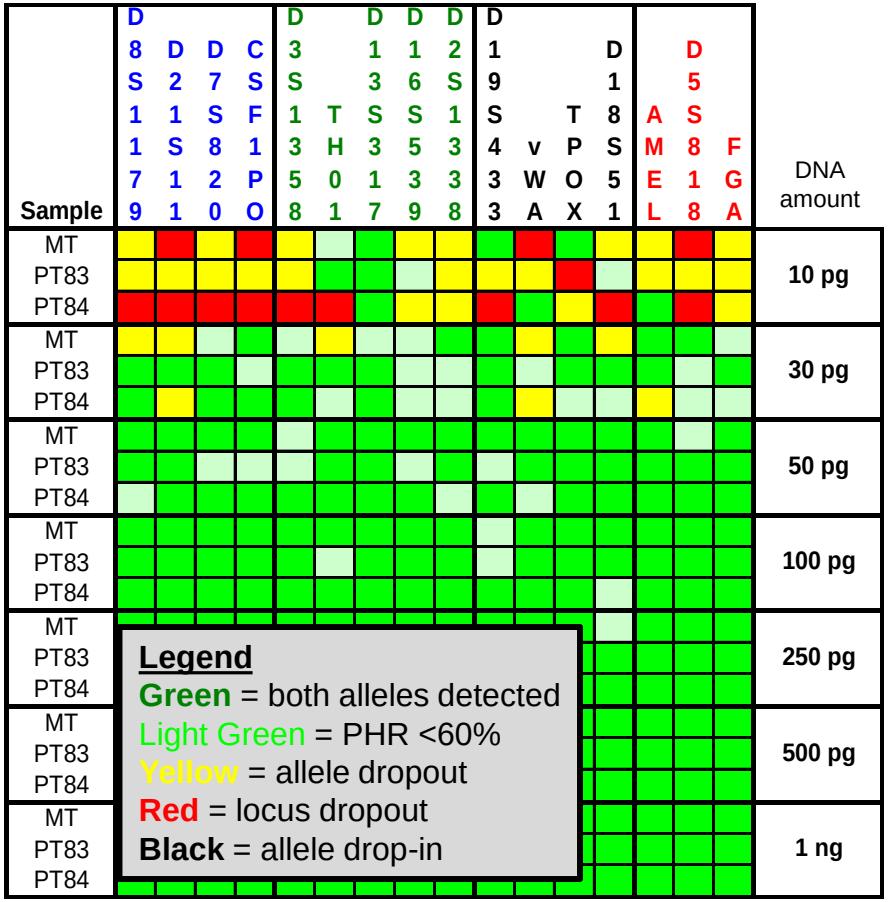
PowerPlex 21 - 30 cycles (5s@2kV)



Total alleles possible = 875
Total alleles present = 805

92% detected

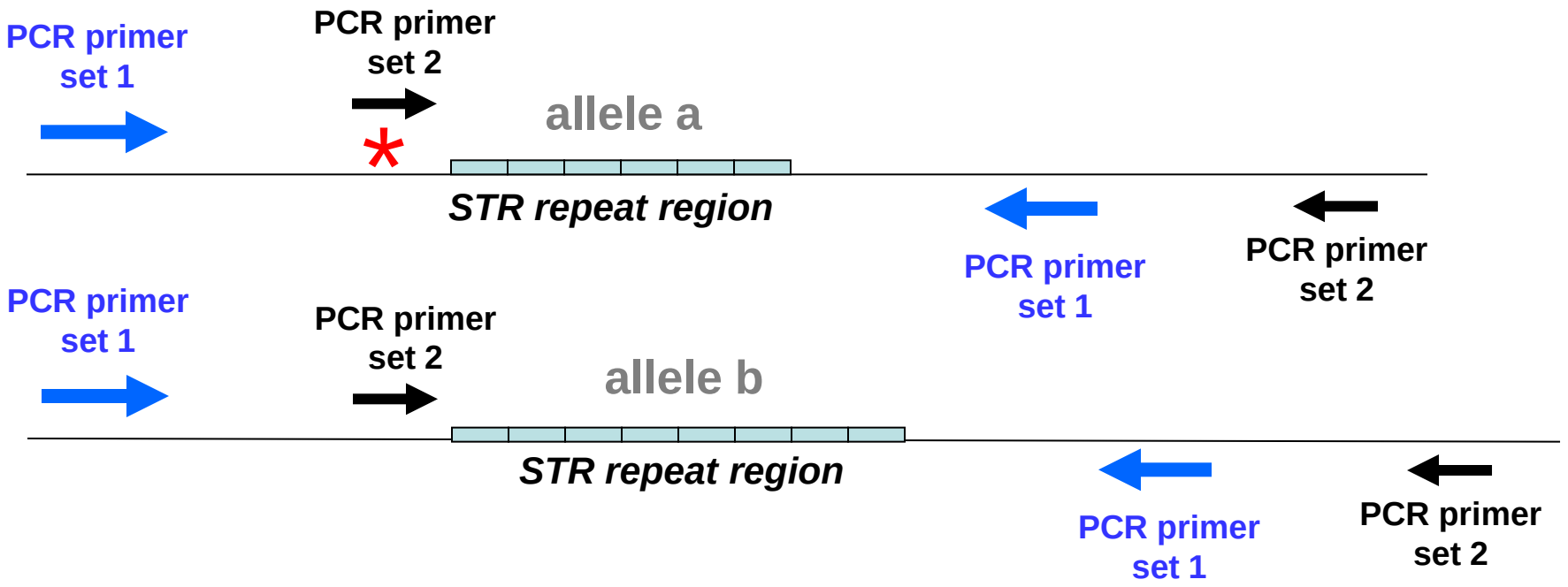
Identifiler Plus - 28 cycles (10s@3kV)



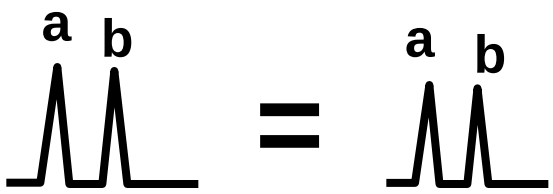
Total alleles possible = 672
Total alleles present = 619

92% detected

STR Kit Concordance Testing

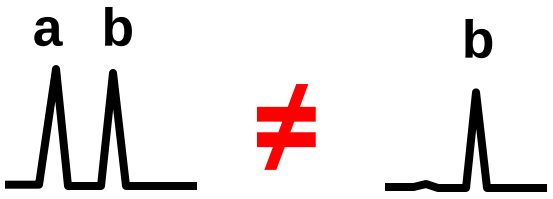


If no primer binding site mutations



Set 1 Amplicons Set 2 Amplicons

If a primer binding site mutation (*)exists



Set 1 Amplicons Set 2 Amplicons

STR Kit Comparisons Searching for Primer Binding Site Mutations

Becky Hill

Kits compared	Samples	Loci compared	Comparisons	# Differences	Concordance (%)
SGM-ID	1436	11	15,796	1	99.994
ID-ProPlus	1427	10	14,270	1	99.993
ID-IDplex	669	16	10,704	19	99.822
ID-PP16	662	14	9,268	4	99.957
ID-MiniFiler	1308	9	11,772	27	99.771
SGM-NGM	1436	11	15,796	4	99.975
ID-NGM	1449				
ProPlus-NGM	1427				
SGM-ESI	1436				
ProPlus-ESX	1427				
ESI-ESX	1455				
ESI-ESSplex	1445				
ESX-ESSplex	1445				
ESI-NGMSElect	715				

128 kit-to-kit comparisons

1,104,031 allele comparisons

1224 differences observed

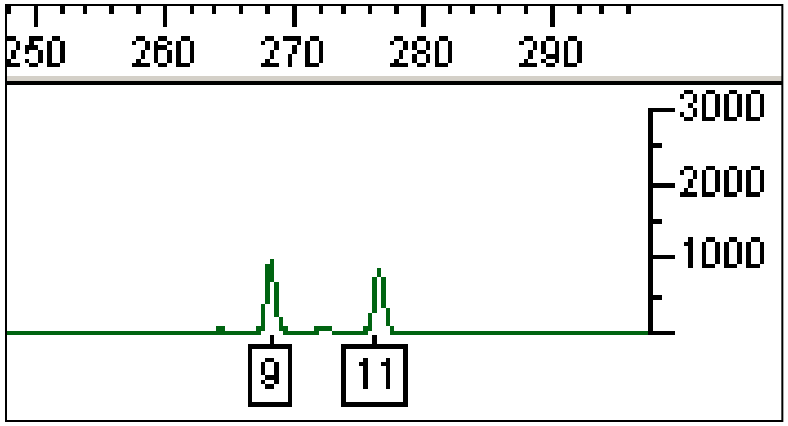
~99.9% concordance

(many corrected now)

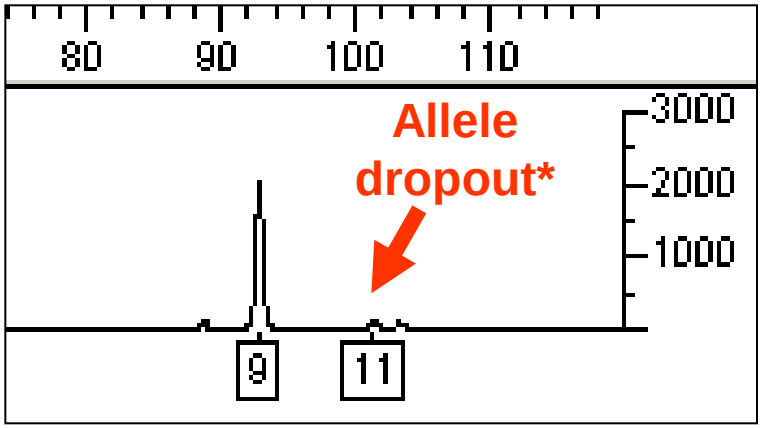
*Kits (except Identifiler) were kindly provided by **Applied Biosystems, Promega, and Qiagen** for concordance testing performed at NIST*

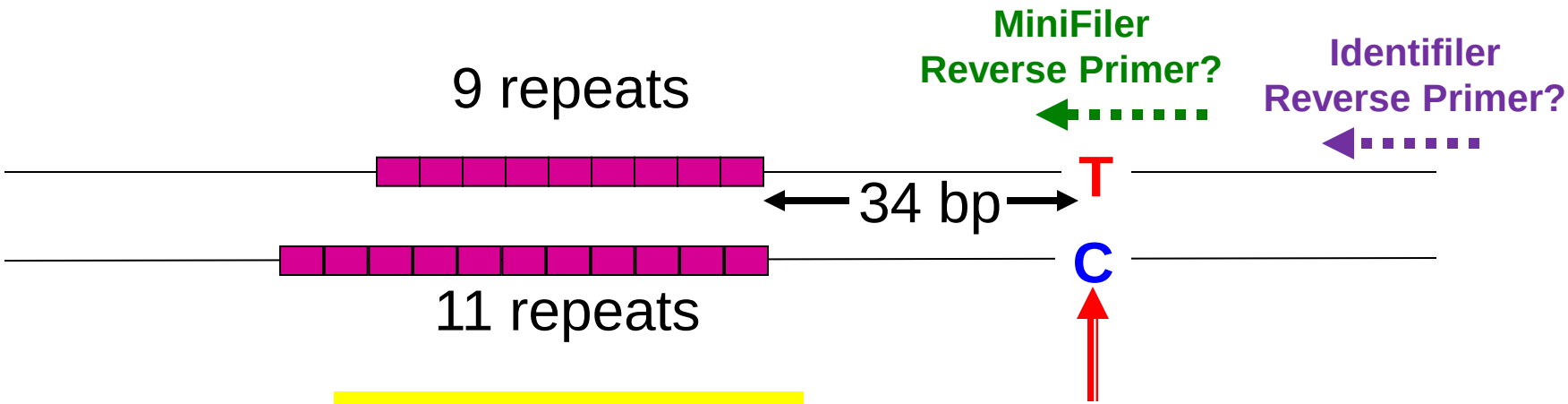
SRM 2391b Genomic 8 with D16S539

Identifiler



MiniFiler





Type 9T,11C

NIST Standard Reference Material (SRM) for Forensic DNA Testing

SRM 2391b (2003-2011)

- **48 autosomal STR loci** with certified values
- **10 liquid genomic DNA** components + **2 punches** (cells on 903 paper)
- All single source samples
- 4 males + 6 females
- 9947A & 9948 included

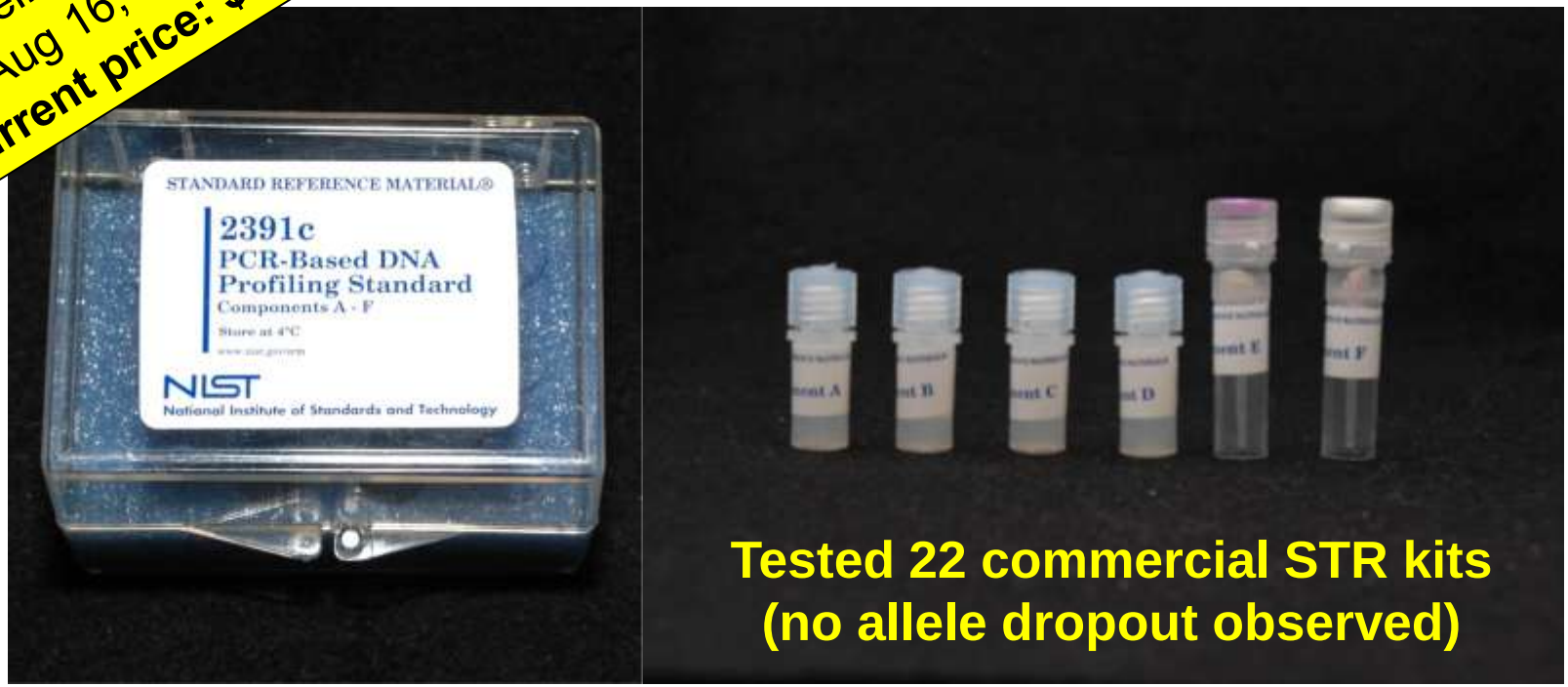
SRM 2391c (2011-future)

- **23 autosomal STR loci and 17 Y-STRs** certified
- **4 liquid genomic DNA** components + **2 punches** (cells on **FTA** & 903 paper)
- 5 single source + 1 mixture
- 3 males + 2 females (unique)
- All new samples
  - no 9947A or 9948

SRM 2391c to replace SRM 2391b and SRM 2395 (for Y-STRs)

NIST SRM 2391c

Selling since
Aug 16, 2011
Current price: $626



Produced with an entirely new set of genomic DNA samples.

9947A & 9948 are NOT included.

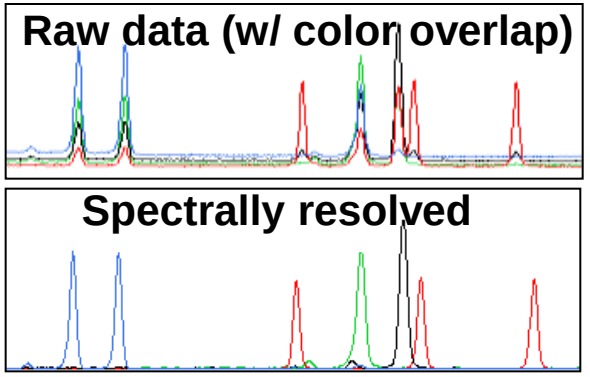
https://www-s.nist.gov/srmors/view_detail.cfm?srm=2391C

CE Fundamentals & Troubleshooting

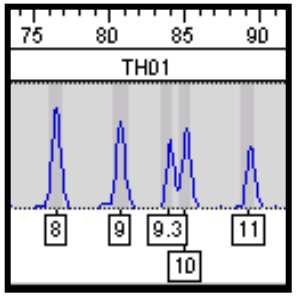
Analytical Requirements for STR Typing

Butler *et al.* (2004) Electrophoresis 25: 1397-1412

- Fluorescent dyes must be **spectrally resolved** in order to distinguish different dye labels on PCR products



- PCR products must be **spatially resolved** – desirable to have single base resolution out to >350 bp in order to distinguish variant alleles



- High **run-to-run precision** – an internal sizing standard is used to calibrate each run in order to compare data over time

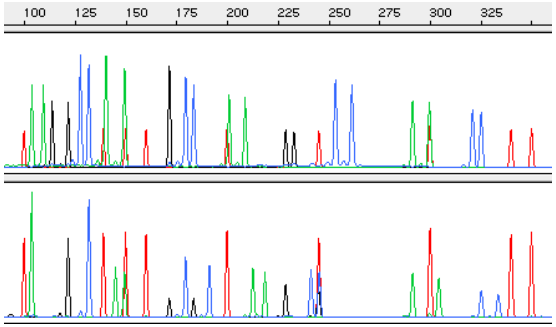
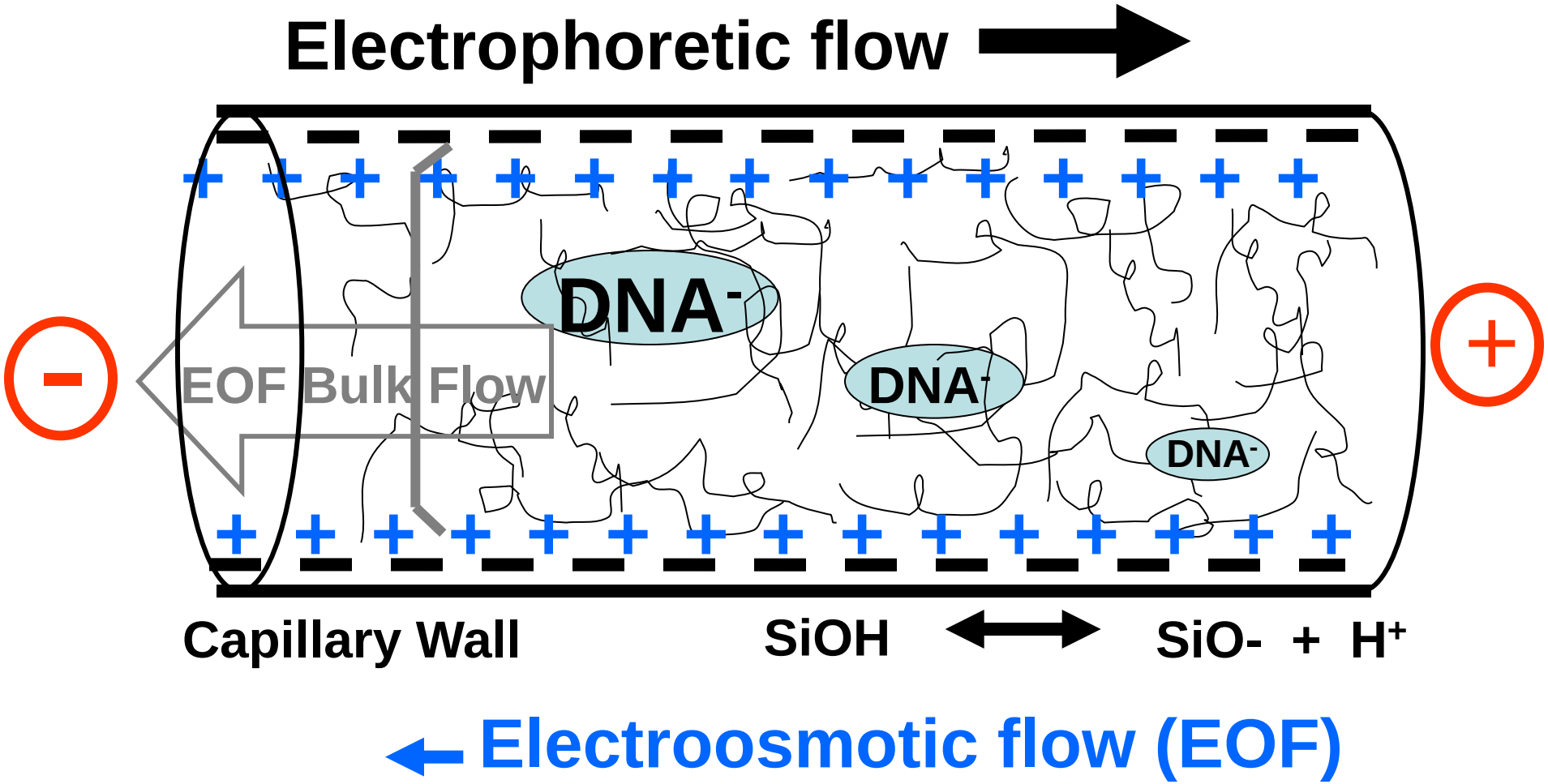
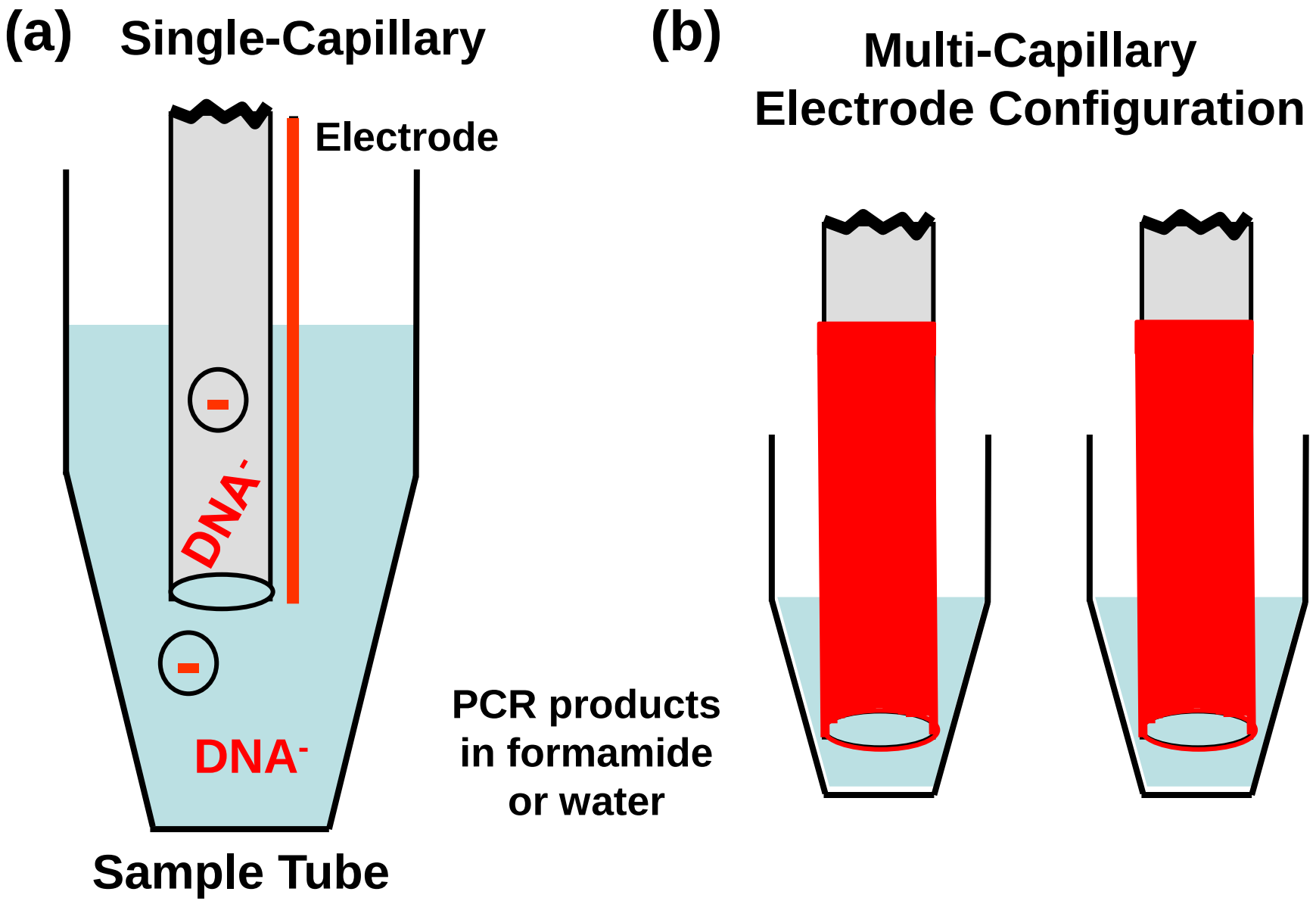


Illustration of internal capillary environment and forces at play with moving DNA molecules through a sieving polymer buffer



Capillary and Electrode Configurations



Sample Conductivity Impacts Amount Injected

$$[DNA_{inj}] = \frac{Et(\pi r^2)\,(\mu_{ep} + \mu_{eof})[DNA_{sample}]\,(\lambda_{buffer})}{\lambda_{sample}}$$

$[DNA_{inj}]$ is the amount of sample injected

E is the electric field applied

t is the injection time

r is the radius of the capillary

μ_{ep} is the mobility of the sample molecules

μ_{eof} is the electroosmotic mobility

$[DNA_{sample}]$ is the concentration of DNA in the sample

λ_{buffer} is the buffer conductivity

λ_{sample} is the sample conductivity

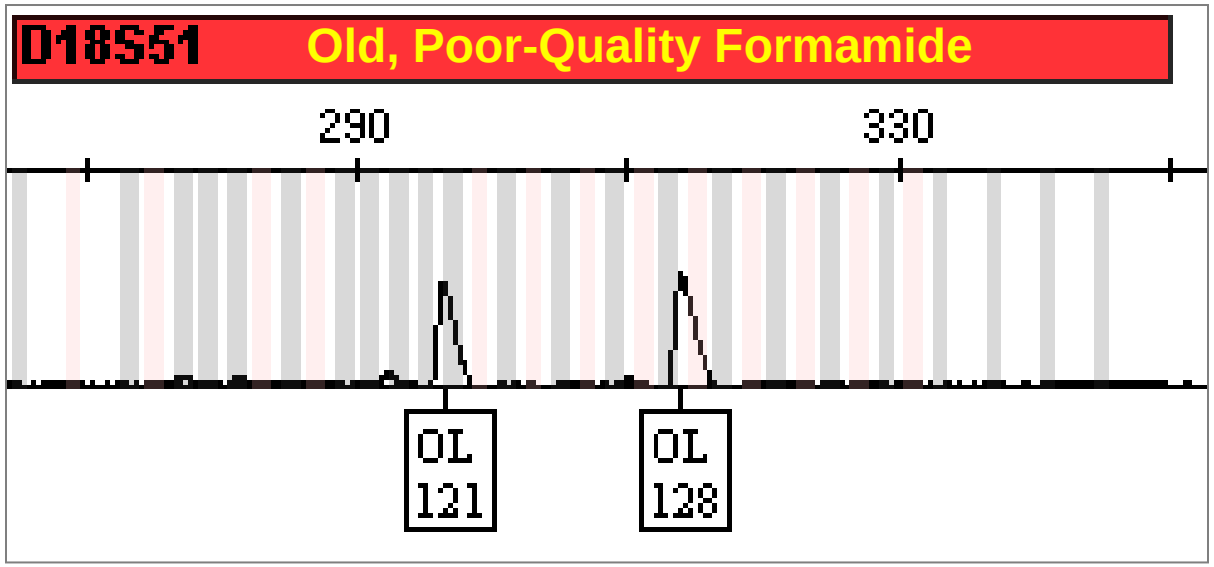
Cl- ions and other buffer ions present in PCR reaction contribute to the sample conductivity and thus will compete with DNA for injection onto the capillary

Butler *et al.* (2004) Electrophoresis 25: 1397-1412

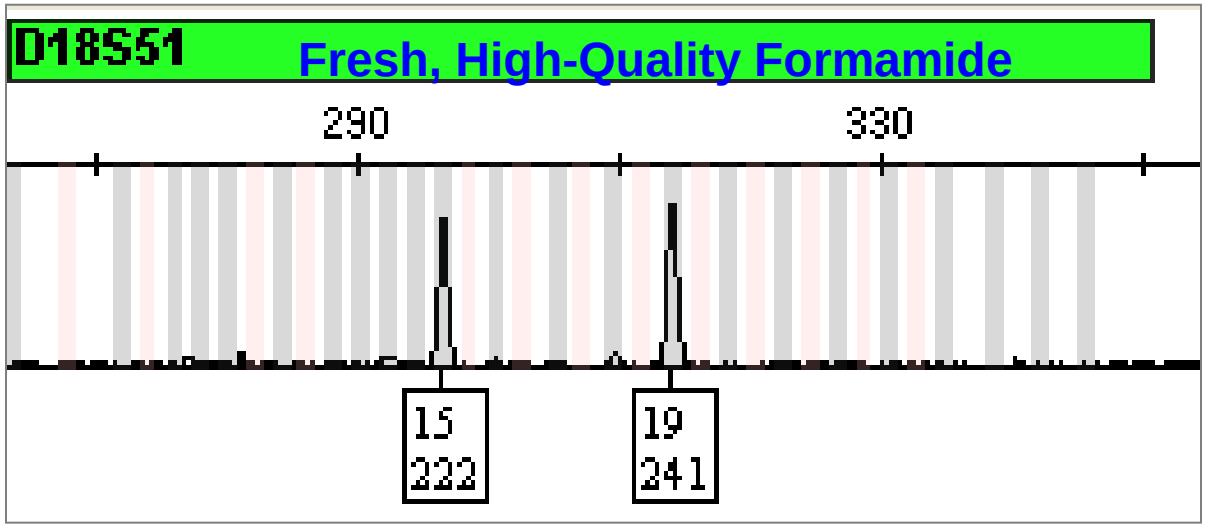
Comments on Sample Preparation

- Use high quality formamide (<100 μS/cm)
- Denaturation with heating and snap cooling is not needed (although most labs still do it…)
- **Post-PCR purification reduces salt levels** and leads to more DNA injected onto the capillary

Importance of Sample Preparation



Peak tailing and wider peaks indicative of an injection with a salty sample



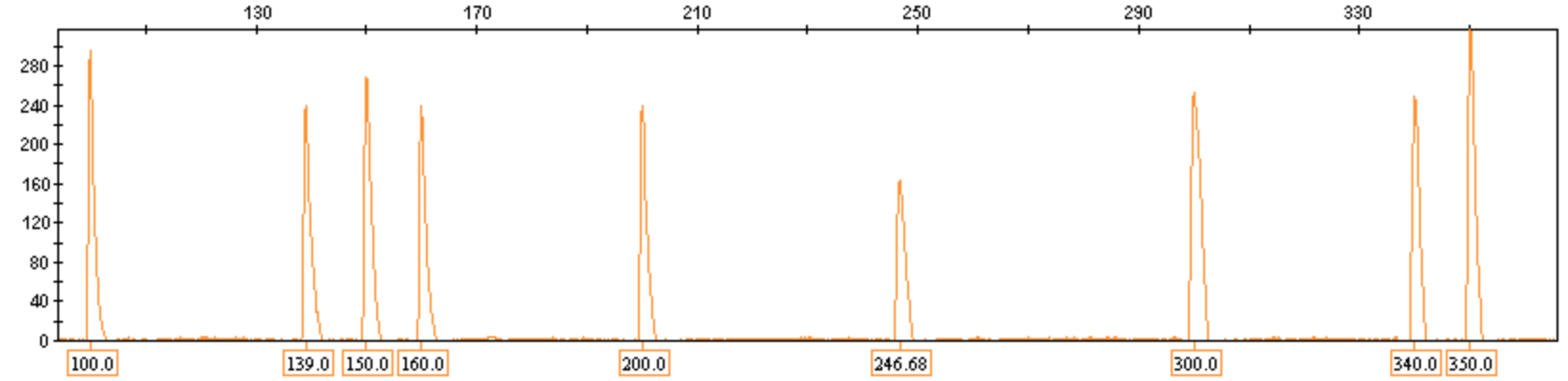
Same DNA sample prepared in fresh, high-quality formamide

Data from Amy Decker (NIST, now AFDIL)

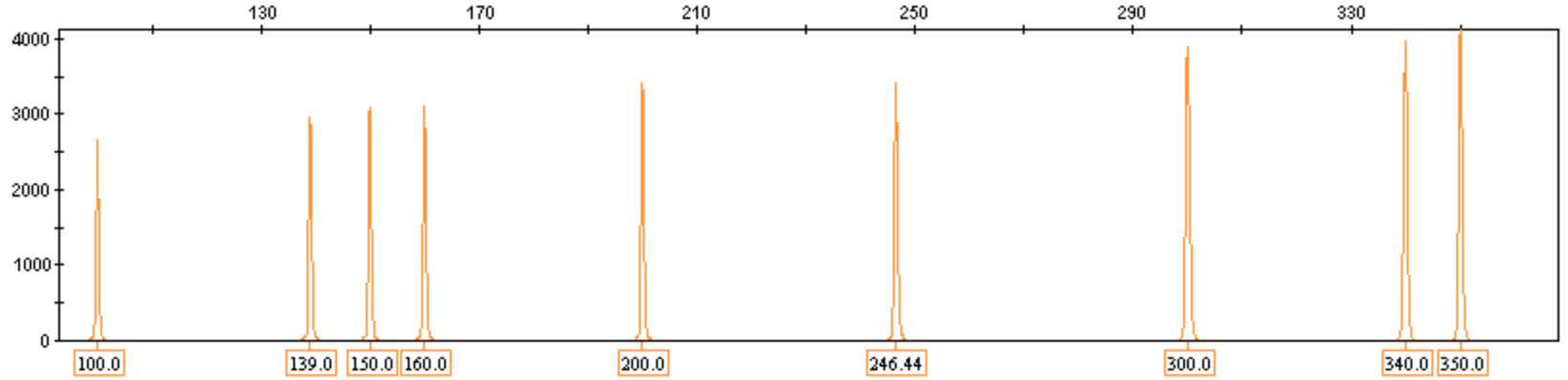
Troubleshooting Hint: **Examine Internal Size Standard**

Comparison of GS500 LIZ Size Standard

Sample diluted in **Old Hi-Di Formamide**



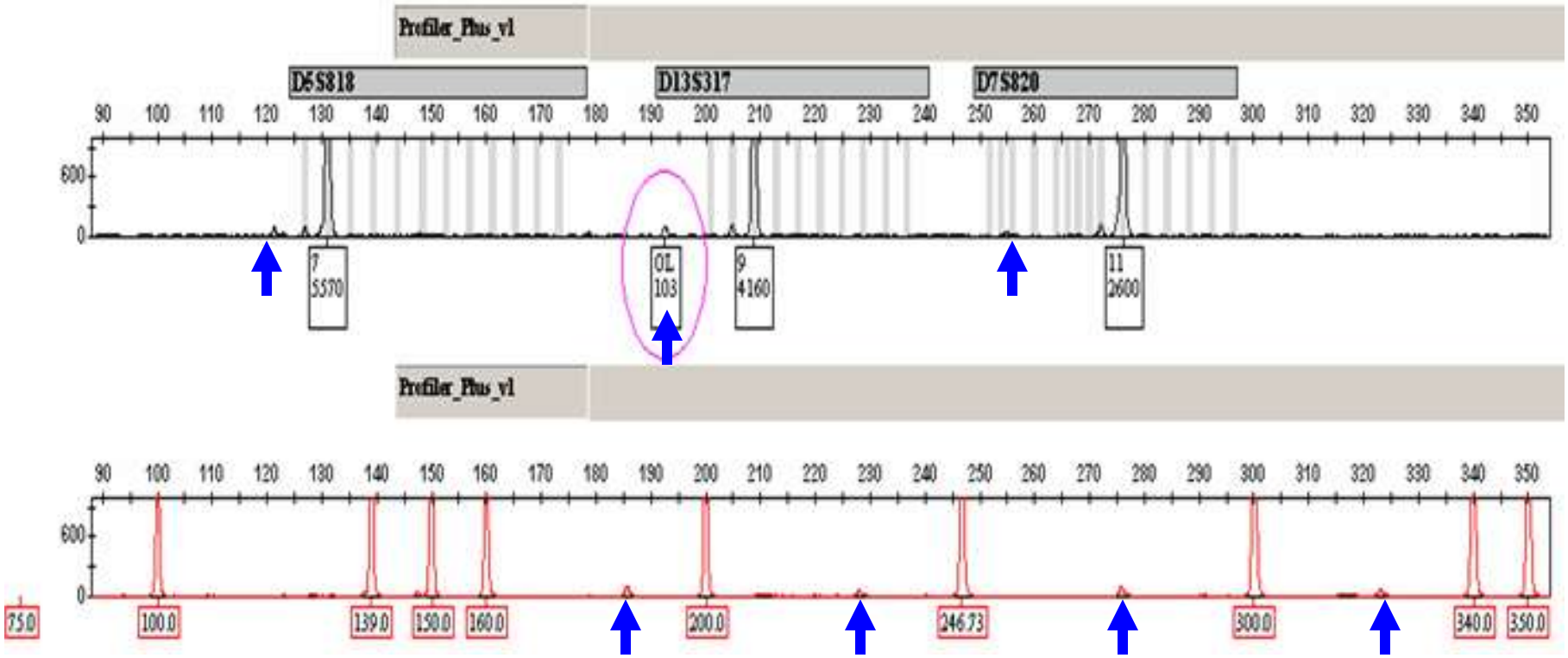
Sample diluted in **Fresh Hi-Di Formamide**



Data from Amy Decker (NIST, now AFDIL)

Sample Renaturation (minor dsDNA peaks running in front of primary ssDNA STR alleles)

ROX Artifacts
Comparison Casework Blood Sample



Data from Peggy Philion (RCMP)

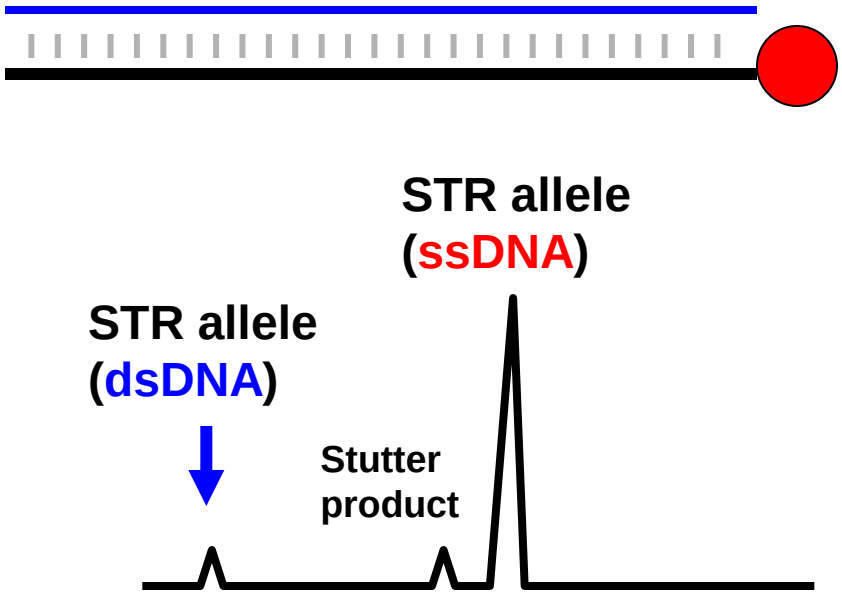
Why dsDNA migrates through CE capillary faster than ssDNA…

- DNA molecule separation depends on interactions with the polymer
  - Higher polymer concentration (or longer polymer molecules) permits more polymer interactions and provides better resolution (i.e., POP-6 vs POP-4)

- **Single-stranded DNA (ssDNA) is more flexible than double-stranded DNA (dsDNA)** and therefore moves more slowly through the capillary because it is interacting with polymer strands more

dsDNA vs ssDNA CE Migration

- If a small amount of the complementary strand re-hybridizes to the labeled STR allele strand, then a little peak will be seen in-front of each internal lane standard peak and



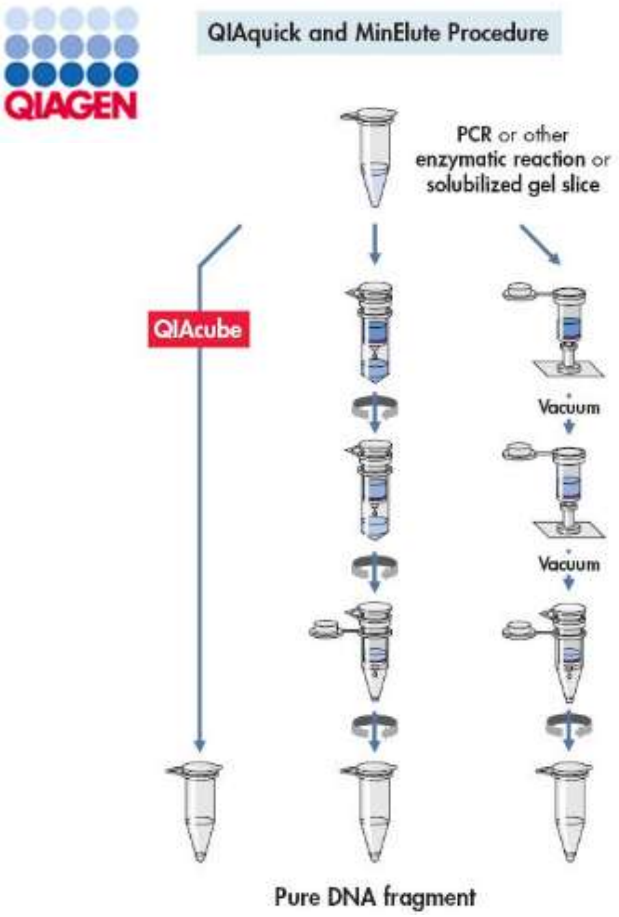
•Height of dsDNA peak will depend on amount of re-hybridization between the two strands (**some loci will re-hybridize more readily giving rise to larger dsDNA peaks**)

•Local temperature environment of capillary impacts amount of re-hybridization (may change over time)

Why MiniElute increases peak heights

- QIAGEN MiniElute **reduces salt levels in samples causing more DNA to be injected**

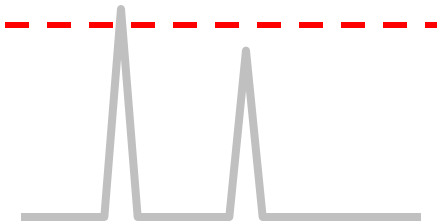
- **Requires setting a higher stochastic threshold** to account for the increased sensitivity



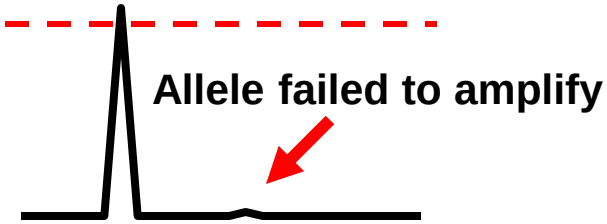
Smith, P.J. and Ballantyne, J. (2007) Simplified low-copy-number DNA analysis by post-PCR purification. *J. Forensic Sci.* 52: 820-829

Stochastic Effects and Thresholds

Regular Injection

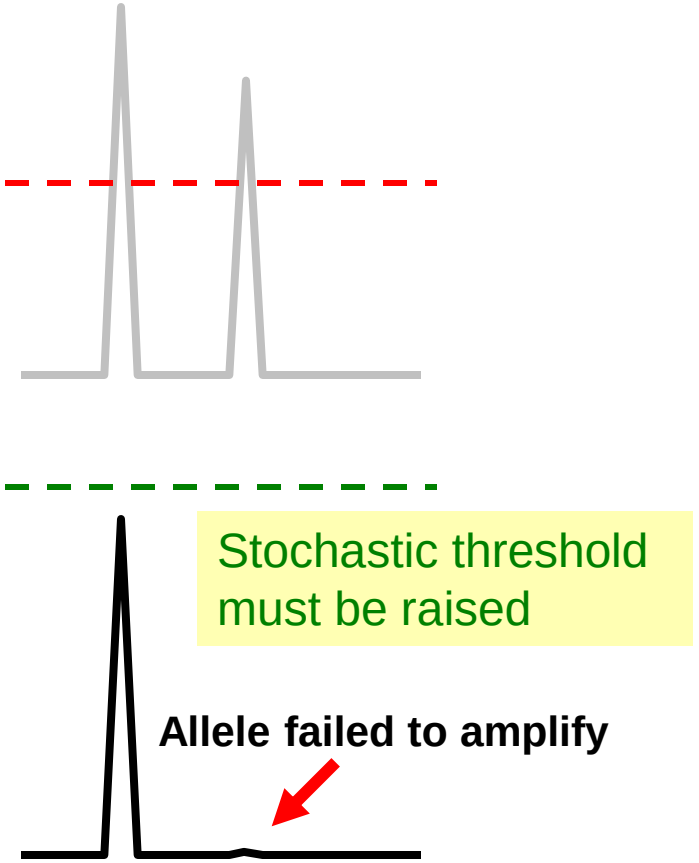


When PCR amplifying low levels of DNA, allele dropout may occur

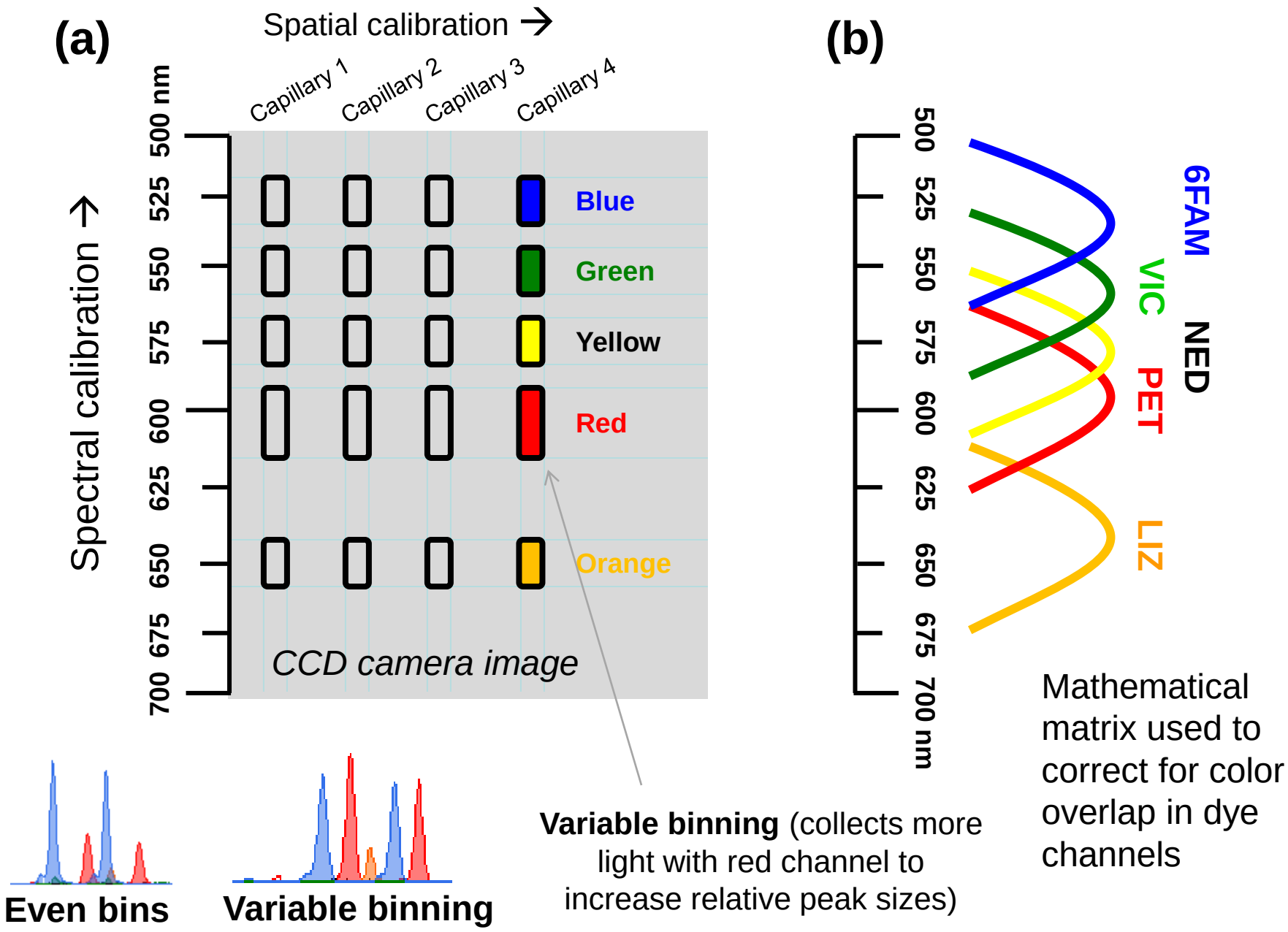


False homozygote

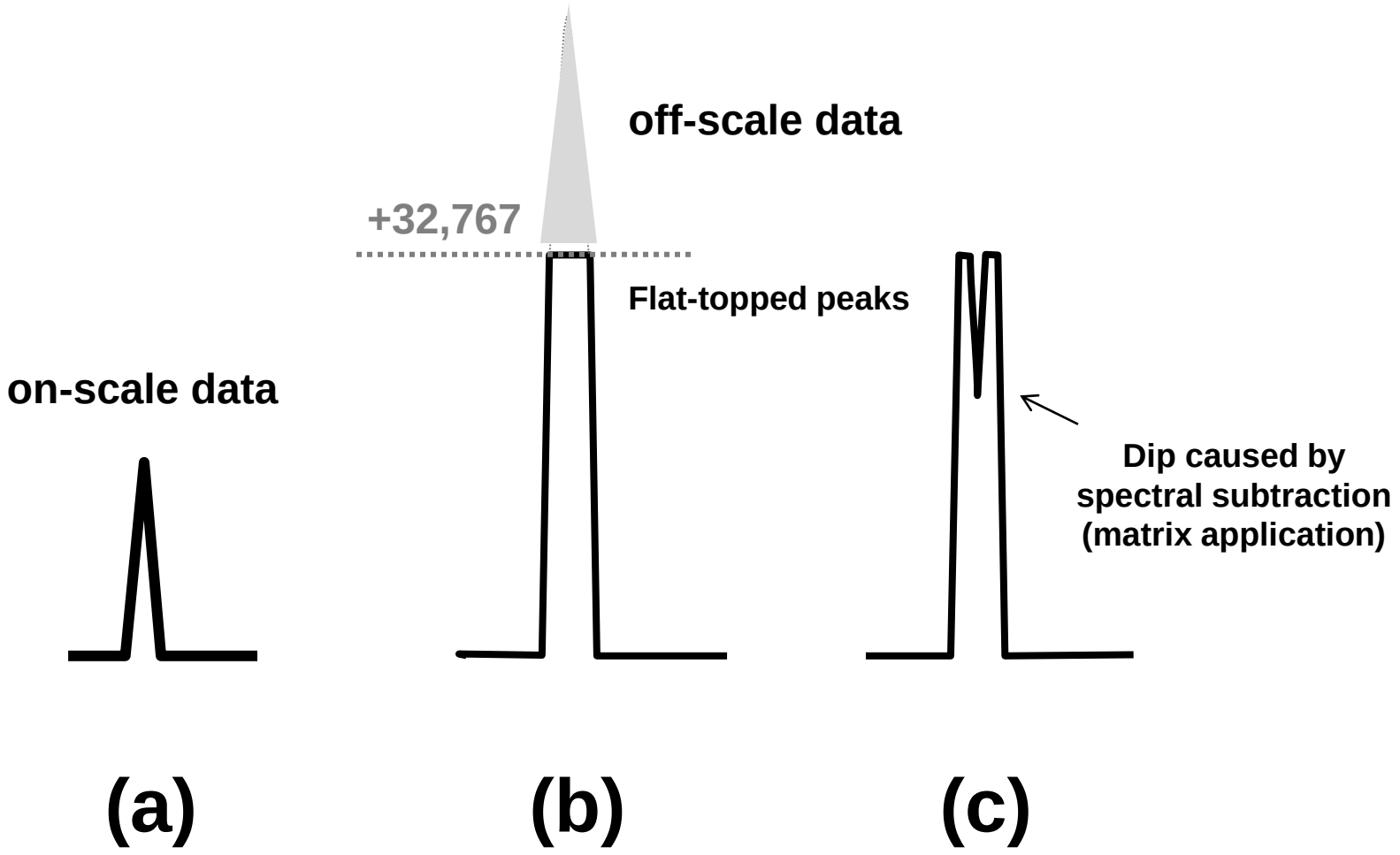
Injection Following Desalting (MiniElute)



Depiction of CCD Camera Image from Multi-Capillary Genetic Analyzer



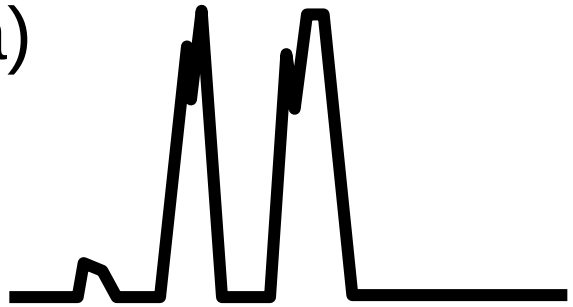
Upper Limit for Data Storage



STR Typing Works Best in a Narrow Window of DNA Template Amounts

Off-scale data with flat-topped peaks

(a)

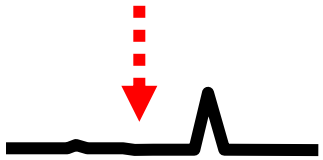


Too much DNA amplified

Or injected onto CE

Allele dropout due to stochastic effects

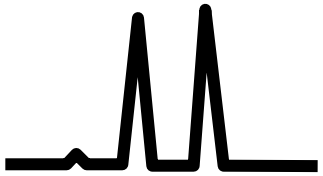
(b)



Too little DNA amplified

"Just right"

(c)



Within optimal range

ABI 3500
Genetic Analyzer

Genetic Analyzers from Applied Biosystems

ABI Genetic Analyzer	Years Released for Human ID	Number of Capillaries	Laser	Polymer delivery	Other features
373 (gel system)	1992-2003	--	40 mW Ar+ (488/514 nm)	--	PMTs and color filter wheel for detection
377 (gel system)	1995-2006	--	40 mW Ar+ (488/514 nm)	--	CCD camera
310	1995-	1	10 mW Ar+ (488/514 nm)	syringe	Mac operating system & Windows NT (later)
3100	2000-2005	16	25 mW Ar+ (488/514 nm)	syringe	
3100-Avant	2002-2007	4	25 mW Ar+ (488/514 nm)	syringe	
3130	2003-2011	4	25 mW Ar+ (488/514 nm)	pump	
3130xl	2003-2011	16	25 mW Ar+ (488/514 nm)	pump	
3500	2010-	8	10-25 mW diode (505 nm)	new pump	110V power; **RFID-tagged reagents**; **.hid files**; normalization & 6-dye detection possible
3500xl	2010-	24			
3700	2002-2003	96	25 mW Ar+ (488/514 nm)	cuvette-based	Split beam technology
3730	2005-	48	25 mW Ar+ (488/514 nm)	pump	
3730xl	2005-	96	25 mW Ar+ (488/514 nm)	pump	

Information courtesy of Michelle S. Shepherd, Applied Biosystems, LIFE Technologies.

Do you currently use the ABI 3500?

1. Yes
2. No

ABI 3500 Genetic Analyzer

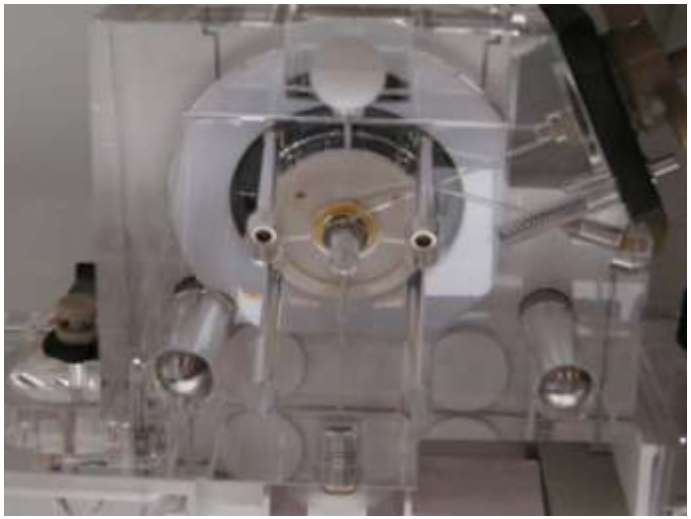
- **3500 (8 capillary)**
- **3500xl (24 capillary)**

New Features of the ABI 3500 CE

- an improved polymer delivery pump design,
- ready-to-use consumables and containers,
- Radio Frequency Identification (RFID) consumable tracking,
- quality control software features for rapid identification and re-injection of failed samples,
- increased throughput,
- new laser technology,
- reduced power requirements,
- peak height normalization,
- intuitive user software, and integrated primary analysis software,
- improved peak height uniformity across capillaries, runs and instruments
- **6-dye channel capability**

Details of the ABI 3500

No lower pump block
(Fewer air bubbles)

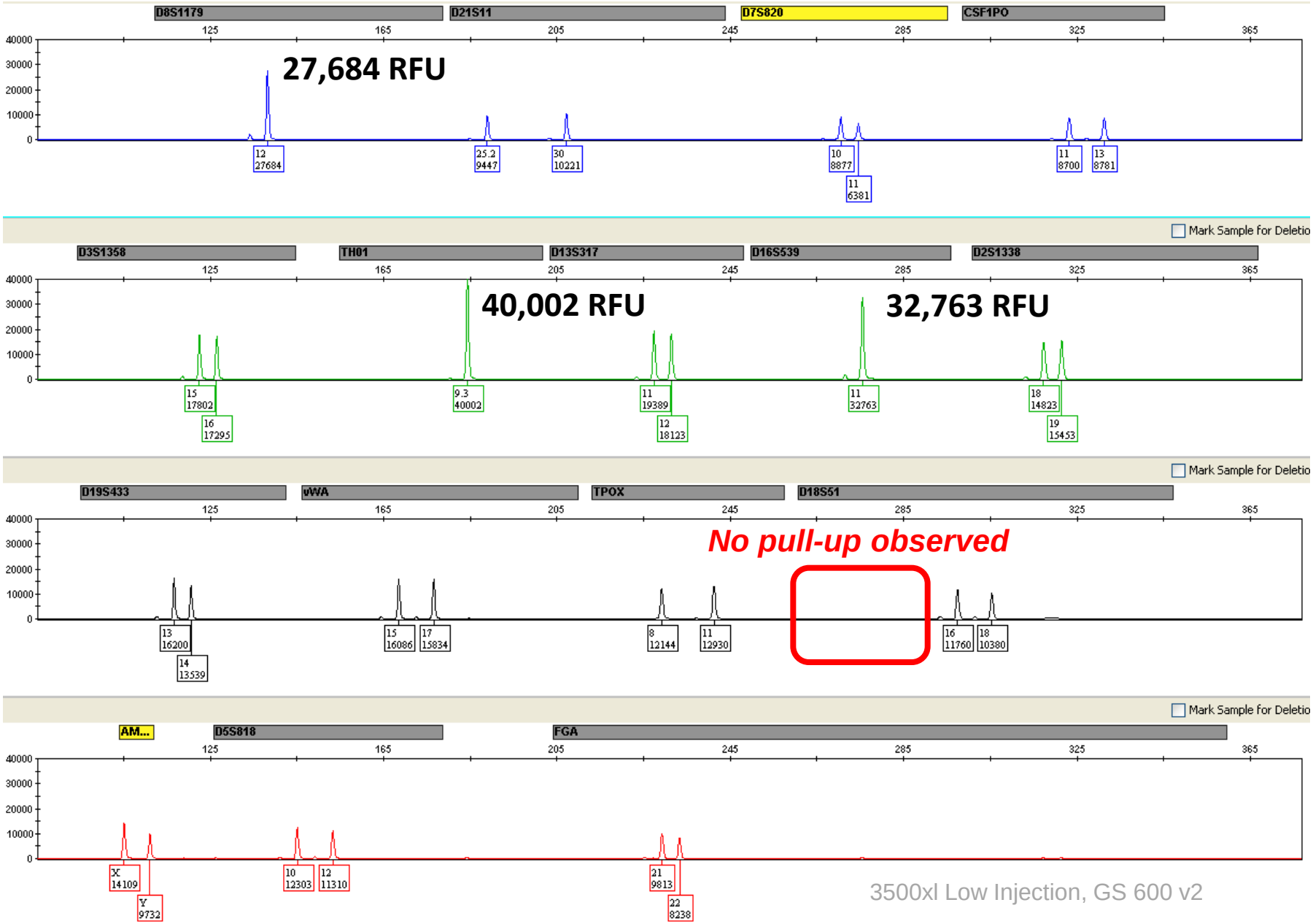


Improved sealing for better temperature control

Primary Differences

	31xx Platforms	**3500 Platforms**
Laser	Argon ion (AR+) with 488/514 nm wavelength	Single-line 505 nm, solid-state, long-life laser
Power Requirement	220V	110V
File Generated	.fsa files	.hid files
Normalization	None	Instrument-to-instrument; only with AB kits
Optimal Signal Intensity	1500-3000 RFU	4x greater than 31xx platforms

Identifiler Result on ABI 3500xl



3500xl Low Injection, GS 600 v2

DNA Community Moving to ABI 3500s

Advantages

- Smaller footprint and 110V power requirement
- Better polymer delivery and temperature control
  - Improved success rates?
- New capabilities
  - between instrument normalization
  - 6-dye detection (bigger kits with more loci)
- Simpler software

Disadvantages

- Up-front cost of new instruments
  - Federal government (NIJ) will likely be expected to foot the bill
- Generates .hid files
  - Requires new analysis software
- Validation down-time
  - New RFU thresholds
- Higher per run cost with RFID tags & limited expiration
  - many labs cannot purchase reagents rapidly throughout the year
- Creating technicians not scientists
  - Plug and play approach leading to loss of understanding for process
  - Less flexible *(impacts research with it)*

Cost for the Forensic DNA Community to Switch from ABI 3100s to 3500s

1. **Instrument up-front cost**
   - Will likely be requested from federal grant funds (NIJ)

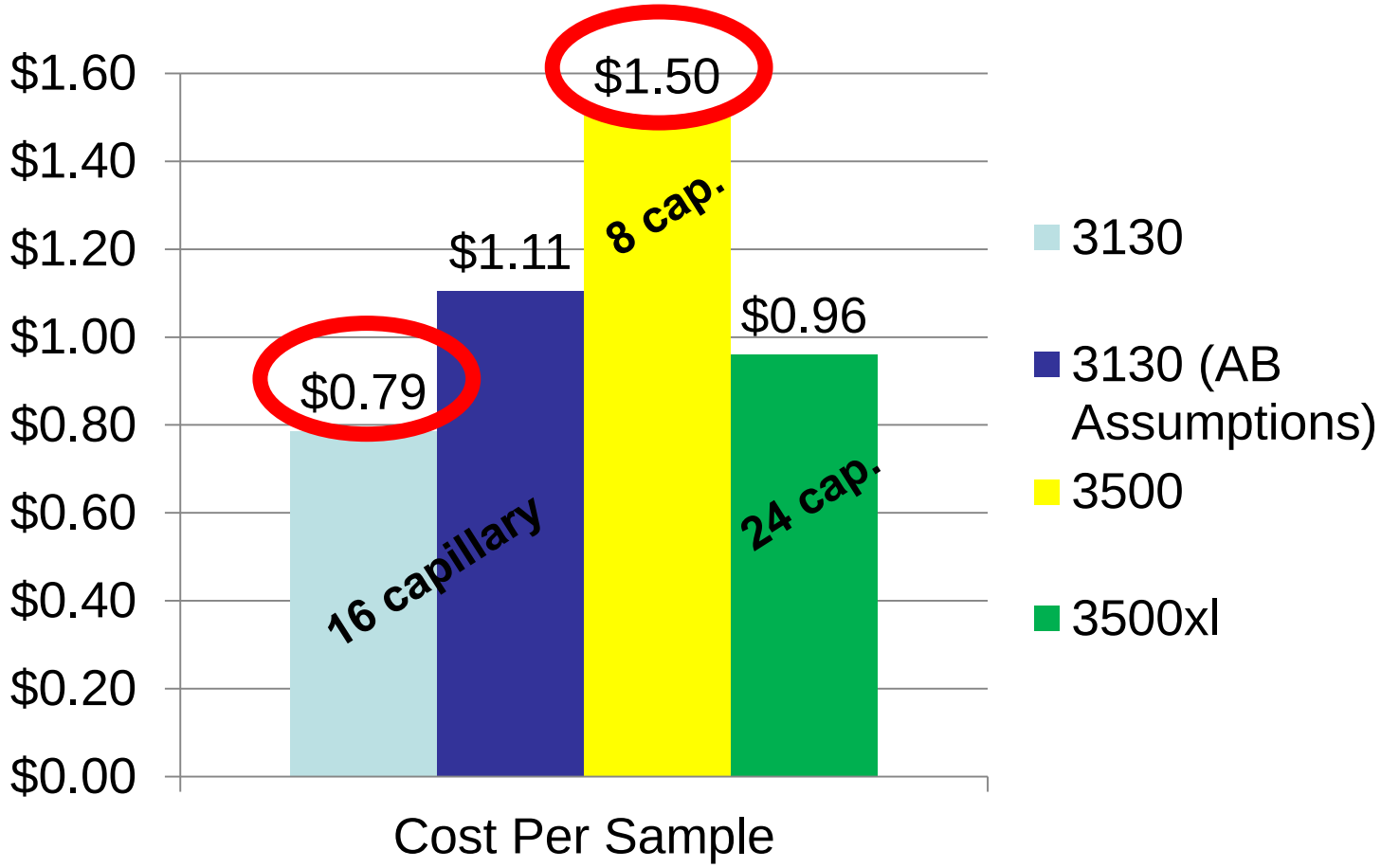
2. **New software purchase**
   - Will likely be requested from federal grant funds (NIJ)
   - new .hid file format will not work on current software (GMIDv3.2)
   - 3500 will not create .fsa files with 36cm arrays (HID applications)

3. **Validation time & expense**
   - Relative fluorescent scales are completely different…

4. **Operational cost**
   - ABI claims that the running costs are equivalent to 3130s…

NIST Calculated Cost per Sample for ABI 3130xl vs. 3500 and 3500xl Reagents

Running two plates per day (10 plates per week)



Consumable Costs for the ABI 3500

POP polymer pack with RFID tag

$180 (384 samples)

$455 (960 samples)

Buffer pack with RFID tag

$60 ($25+$35)

8-capillary array

$1200 (160 injections)

“Expires” after 1-week on the instrument

Thus, if you run 1 sample or 960 samples (or 384) in that week, the consumable cost will be the same…

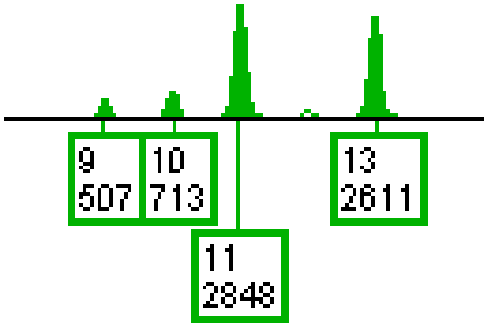
Comparison to a New Car…

- **Instrument cost** – like buying a new car with the latest features
- **New software** – like installing a new navigational system and getting a new drivers license
- **Validation time & expense** – like learning how to drive the new car which will handle differently
- **Operational cost** – like paying for a more expensive high-grade gasoline

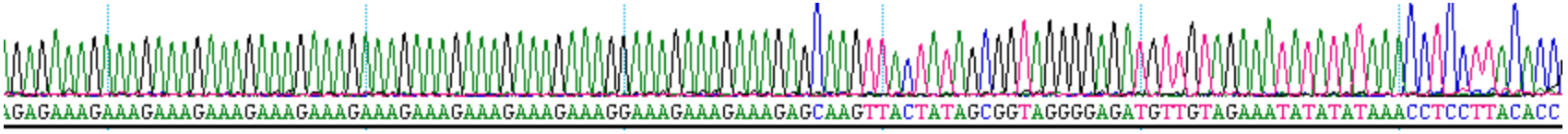
If the current car is already paid for and works fine (e.g., ABI 3130xl), then why are we going to purchase a new car (e.g., ABI 3500)?

ABI 3500 Generates Excellent Data

STR typing with a 1:7 mixture using **36 cm array and POP4**



DNA sequencing of an SE33 allele using **50 cm array and POP7**



ABI 3500 Validation Studies at NIST

Erica Butts

Main Points:

- The 3500 has proven to be reliable, reproducible and robust in our hands – we have provided feedback to ABI to improve use
- Produces excellent DNA sequencing results
- Signal strength is different compared to ABI 3130xl and requires studies to set analytical and stochastic thresholds
- **Dye-specific analytical thresholds** resulted in less allelic and full locus dropout than applying one analytical threshold to all dyes
- RFID tracking decreases flexibility in our research experience

Presentations/Publications:

- MAAFS talk (May 2011)
- ABI road show talks (July & Aug 2011)
- ISFG presentation (Sept 2011)
- *Forensic News* (Spring 2012)

http://marketing.appliedbiosystems.com/mk/get/FORENSICNEWS_HIDINACTION#article5

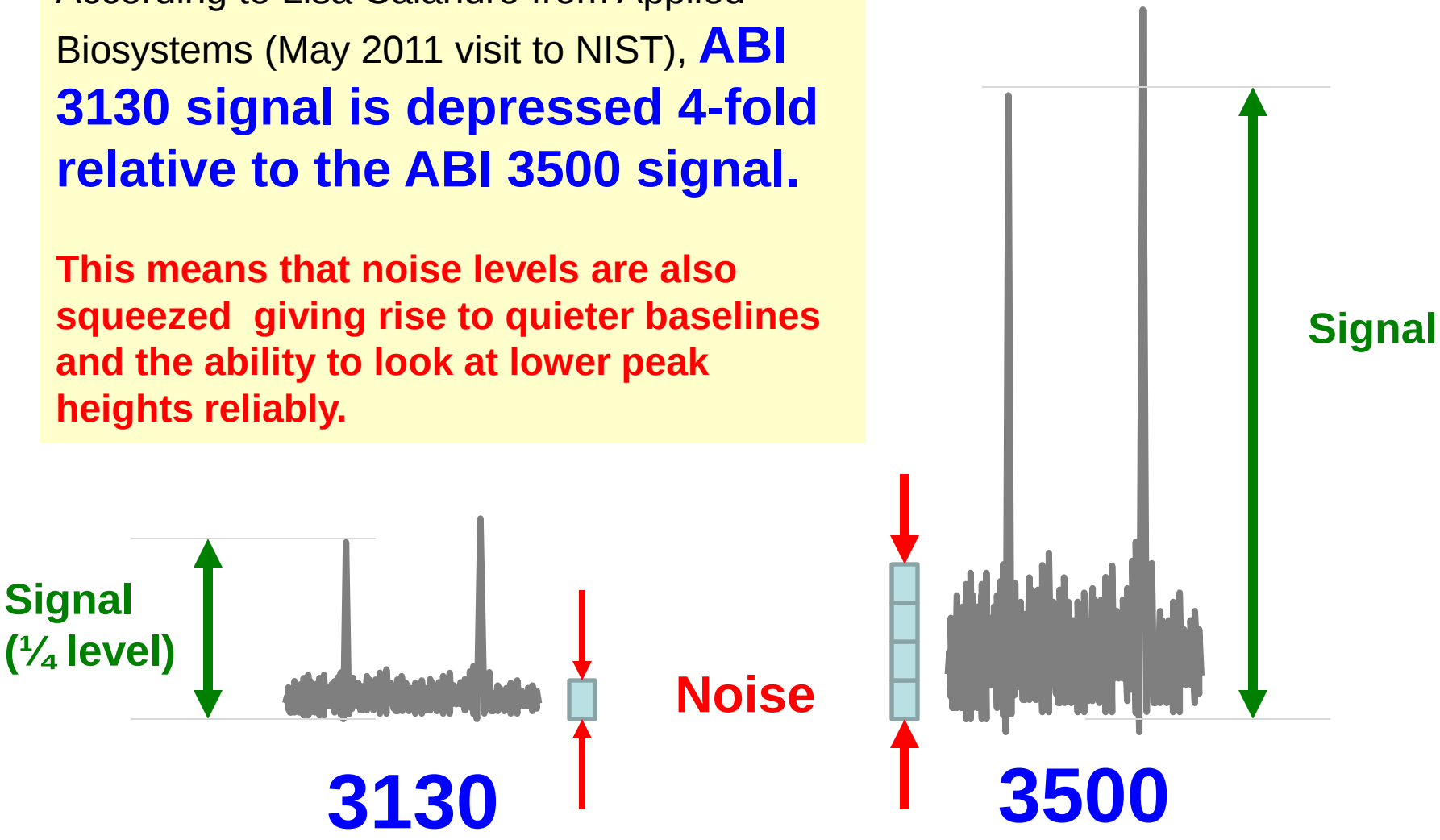
Questions about the ABI 3500

- **Is the 3500 more sensitive** because it shows peaks with higher RFU levels than 3130?
  - Not necessarily → what matters is the signal-to-noise
- **Can we normalize signal across instruments** to generate “equivalent” data between our instruments?
  - I am not aware of anyone using normalization successfully (including Applied Biosystems)
- **Will 6-dye detection be necessary** with the CODIS core loci expansion?

Signal-to-Noise Ratio

According to Lisa Calandro from Applied Biosystems (May 2011 visit to NIST), **ABI 3130 signal is depressed 4-fold relative to the ABI 3500 signal.**

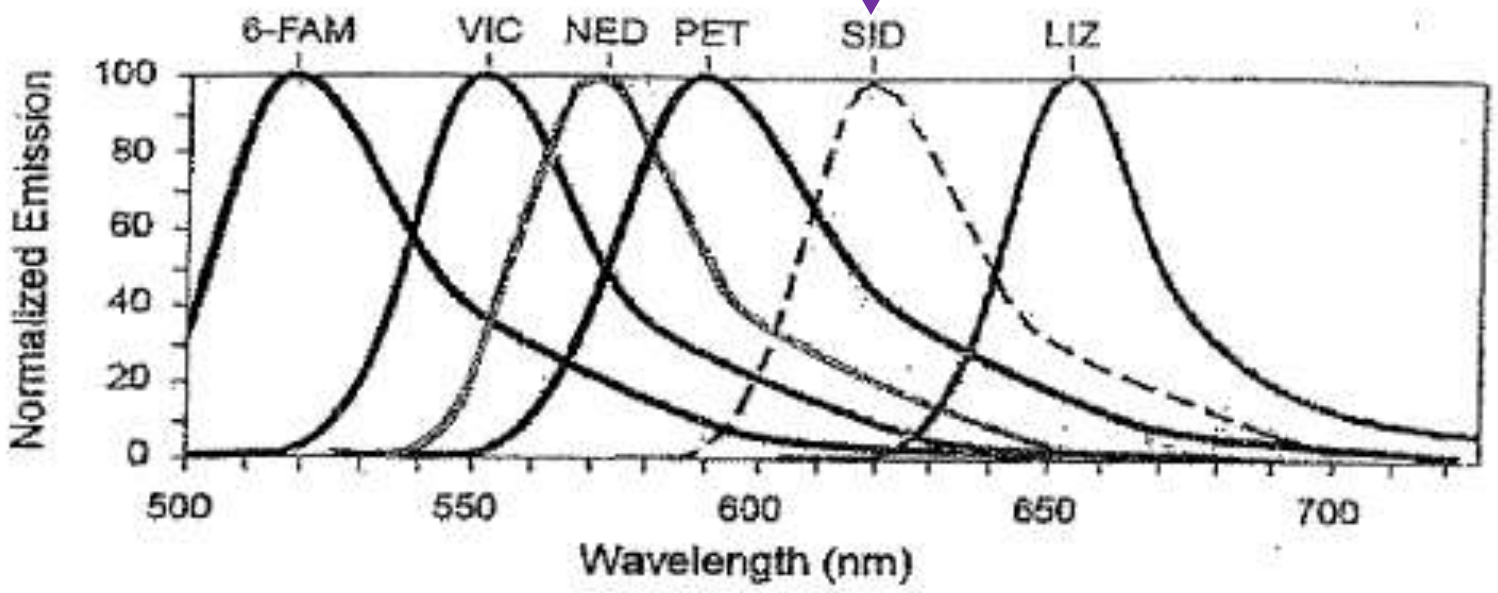
This means that noise levels are also squeezed giving rise to quieter baselines and the ability to look at lower peak heights reliably.



On a relative basis in terms of the signal-to-noise ratio, these data are equivalent

Applied Biosystems' Sixth Dye?

6th Dye named SID
(emission spectrum falls between PET and LIZ)



US Patent Application (filed October 30, 2008 by Lori Hennessy and Robert Green)
"Methods and Kits for Multiplex Amplification of Short Tandem Repeat Loci"

Potential Issues with 6-dye STR kits

- ABI announced in their Spring 2012 issue of *Forensic News* that a 6-dye STR kit was in development
  - Which would enable another 4-6 loci to be added to a multiplex
- **Most labs now have 3130 or 3130xl instruments**
  - Will all labs have to purchase 3500 instruments?
  - Or will the 3130 or 3730 series instruments be made compatible for 6-dyes?
- Spectral calibration issues and potential bleed through across color channels are untested
- FYI: it appears that information from up to 99 different dyes can be stored in .fsa or .hid files (based on current data file structure schema)

Open Letter to Applied Biosystems on Concerns with ABI 3500

- **3/14/11 - emailed ~900 forensic DNA scientists** (SWGDAM, forens-dna, ENFSI, EDNAP) inviting them to sign onto a letter that will be sent to Applied Biosystems expressing concern with ABI 3500
- **Very positive response with 101 who agreed to sign the letter**
- Letter was sent March 31 to the president of ABI and scientists involved with the ABI 3500
- **Community will be notified of ABI's response**

Concerns Expressed in Open Letter

- RFID tags
- New .hid file structure requires new software
- Short shelf life of reagents – would like to see data for expiration times

Hopefully a change will result…

A desire for greater communication with the community – the 3500 FAQ sheet is a good start but does not directly address all of the concerns raised

Brief Timeline of Events

- NIJ requested NIST to explore capabilities, limitations, and cost of ABI 3500 instrument and reagents (May 2010)
- NIST presentations to NIJ (Dec 2010) and SWGDAM (Jan 2011)
- **Open letter support solicited and sent to ABI** (Mar 2011)
- Further discussions between NIST and ABI (Apr-Sept 2011)
- At the Promega ISHI meeting (Oct 2011), ABI announced through a poster at their booth that **polymer and buffer expiration dates will no longer be a hard stop** but only a warning with the future Windows 7 software upgrade

Since May 2011, Erica Butts has presented several validation presentations on our ABI 3500 work – these are available on STRBase

What was learned from the May 11 visit of ABI scientists to NIST…

- RFID over-ride is possible (their R&D lab has instrument that can use “expired” reagents) – they are “considering” making this option available
- New software is required for 3500 .hid or .fsa files due to new file structure
- They do not have ANY data to support short shelf life of 3500 reagents
  - A business decision to set hard stops to keep labs from having failures that lead to ABI having to replace arrays
- ABI 31xx instruments have DEPRESSED signal (i.e., should have a lower analytical threshold)
- **Normalization is not well worked out by ABI or really understood** (although this has been a major selling point for the 3500)
- ABI was shocked that there were concerns with some of the feedback

A Sampling of Feedback I Received…

- **People did not just sign the letter but many have an opinion about the issues or concern about ABI customer support (I have received >100 emails – often with some very strong thoughts)**

- "I think that the AB3500 related issues most likely represent the beginning of a sea of problems, against which every independent lab must take arms. **It is not up to the manufacturer of a machine to decide the basic procedures of a lab - it is up to the lab**" (4/29/11)

- "I greatly appreciate your advocacy on behalf of our community. **Hopefully we will be heard**." (4/1/11)

Response from Dr. Robin Cotton
(shared with her permission)

Sent: Saturday, April 30, 2011 10:39 AM

Dear John,

Thank you for the information and the inclusion of the letter from Dr. Klevan. It is clear that **Dr. Klevan does not consider the substantial time and expense which will be required for each forensic laboratory for instrument and software validation.**

The other point which I feel is significant is the need for the additional software purchase. Since he states that the new software is compatible with .fsa files, I think **the company should make a software exchange available at low cost for any lab purchasing the 3500 instrument**. Many commercially available software companies make new versions available at reduced costs to individuals or groups already running an earlier versions. **Because of the increased number of technical changes the 3500 presents, the validation data may be more extensive than was required for previous instrument change-over and thus the validation time and cost to each laboratory will also be increased.**

Response from Dr. Robin Cotton
(shared with her permission)

It would also be relevant to ask Dr. Klevan to provide figures for the number of current 3500 users without the inclusion of paternity testing laboratories which are all commercial operations. While I am an advocate for private laboratories (both forensic and paternity), these facilities have the option to raise prices and accommodate the need for increased validation time and expense in other ways that do not require federal or other government support.

Additionally, in the Biomedical Forensic Science Masters program here at BU, we feel it is important to teach our students using current instrumentation and techniques. **Introduction of this new instrument will affect many forensic science teaching institutions, both undergraduate and graduate, as well as all current forensic DNA testing laboratories.** These institutions have significantly less access to NIJ funding for large equipment and software than the operating forensic DNA laboratories. Thus **the effect of changes reach into the educational institutions as well.**

Regards,
Robin W. Cotton, Ph.D.
Boston University

ABI 3500 Open Letter Update

Concerns Expressed in 3/31/11 Open Letter

1. RFID tags
2. New .hid file structure requires new software
3. Short shelf life of reagents – would like to see data for expiration times

At the Promega ISHI meeting (Oct 2011), ABI described data for studies around reagent expiration through a poster at their booth. Sailus, Wheaton, Fisher, Calandro. "Understanding the Consumables on the 3500 Genetic Analyzers in the context of a Human Identification (HID) Laboratory"

They have promised that **polymer and buffer expiration dates will no longer be a hard stop** but only a warning with the future Windows 7 software upgrade (3500 Data Collection v1.3).

Should the community try to pursue further action on the ABI 3500 open letter concerns?

1. Yes
2. No

A “Crystal Ball” to the Future?

http://medicalcenter.osu.edu/images/healthconnections/winter2003/dnaCrystalBallIllustration.jpg

Some Thoughts on the Future…

Problems with pushing the envelope (without proper validation)

- **PCR amplification**
  - Faster enzymes to enable rapid PCR
  - More robust enzymes and master mixes to overcome inhibition
- **Instrumentation**
  - More dye colors to aid higher levels of multiplexing
  - Rapid, integrated devices
  - Alternatives to capillary electrophoresis: PLEX-ID and NGS
- **Quantitative information**
  - qPCR and digital PCR
- **Marker systems**
  - Expanding sets of STR loci for growing DNA databases
  - Other marker systems: SNPs, InDels, X-STRs, RM Y-STRs
  - Body fluid identification with mRNA, miRNA, and DNA methylation
  - Phenotyping for external visible characteristics
  - **Challenges with potential whole genome information**
- **Data interpretation**
  - Probabilistic genotyping for low-level DNA and mixture interpretation
  - Probability of dropout

Recent Next-Generation Sequencing (NGS) Meeting Held at NIST

- **Interagency Workshop on the use of Next-Generation DNA Sequencing for Human Identification and Characterization**
- Held January 31, 2012 at NIST
- Presentations by MIT/Lincoln Labs and NIST scientists to government agency representatives
- Minutes of meeting and presentations available at http://www.nist.gov/mml/biochemical/genetics/ngs_hid_workshop.cfm

Concern regarding NGS accuracy:
At this meeting, a NIST researcher (Justin Zook) showed that **across full human genome SNP data** collected on two different NGS platforms and processed with two different alignment algorithms **only about 80% of base calls were the same**

The Future of Forensic DNA

is Similar to the Olympic Motto of

"Swifter, Higher, Stronger"

Resources **Training** **Action**

Recent NIST Publications Demonstrating "Swifter, Higher, Stronger" DNA Analysis

Swifter PCR Amplification

Forensic Science International: Genetics Supplement Series 2 (2009) 111–112

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

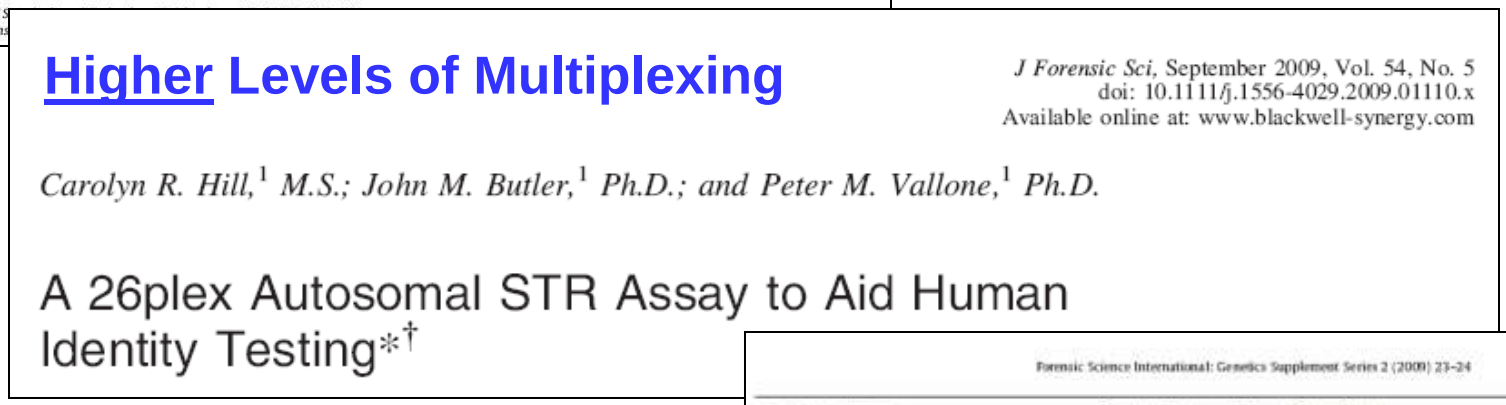
Research article

Rapid amplification of commercial STR typing kits

Peter M. Vallone[a,*], Carolyn R. Hill[a], Daniele Podini[b], John M. Butler[a]

[a] National Institute of S
[b] Department of Forens

Higher Levels of Multiplexing



J Forensic Sci, September 2009, Vol. 54, No. 5
doi: 10.1111/j.1556-4029.2009.01110.x
Available online at: www.blackwell-synergy.com

Carolyn R. Hill,[1] M.S.; John M. Butler,[1] Ph.D.; and Peter M. Vallone,[1] Ph.D.

A 26plex Autosomal STR Assay to Aid Human Identity Testing*†

Stronger Powers of Discrimination

Forensic Science International: Genetics Supplement Series 2 (2009) 23–24

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

Research article

The single most polymorphic STR Locus: SE33 performance in U.S. populations

John M. Butler[a,*], Carolyn R. Hill[a], Margaret C. Kline[a], David L. Duewer[a], Cynthia J. Sprecher[b], Robert S. McLaren[b], Dawn R. Rabbach[b], Benjamin E. Krenke[b], Douglas R. Storts[b]

[a] National Institute of Standards and Technology, Gaithersburg, MD 20899-8312, USA
[b] Promega Corporation, Madison, WI 53711, USA

Rapid PCR and Rapid DNA Testing


Pete Vallone

Main Points:

- **Performing research on reducing the total time required** for STR typing
  - Focusing on the multiplex amplification of commercial STR kits with faster polymerases and thermal cyclers
  - Single-source reference samples (sensitivity > 200 pg)
- **Designing testing plans for rapid DNA typing devices**
  - NIST will be examining rapid DNA instruments with FBI collaboration
- **Exploring direct PCR protocols** with FTA and 903 papers

Presentations/Publications:

- Vallone et al. (2008) FSI Genetics - on rapid PCR
- ISFG 2011 and ISHI 2011 presentations by Tom Callaghan (FBI)
- ISFG 2011 presentation and poster on direct PCR

Common Thermal Cycling Times

Can we reduce PCR cycling times? What are the effects or limitations?

Thermal Cycling Times for Current STR Typing Kits

Year	Run on a 9700 thermal cycler	Hot start	Time per cycle	Cycles	Post soak	Total time
1997/98	Profiler Plus/Cofiler	11 min	3 min	28	60 min	2:52
1999	SGM Plus	11 min	3 min	28	45 min	2:53
2000	PowerPlex 16	12 min	1 min 45 s	32	30 min	3:00
2001	Identifiler	11 min	3 min	28	60 min	2:58
2003	PowerPlex Y	12 min	1 min 45 s	32	30 min	3:18
2004	Yfiler	11 min	3 min	30	80 min	2:45
2007	PowerPlex S5	2 min	4 min	30	45 min	3:21
2007	minifiler	11 min	3 min 20 s	30	45 min	3:16
2009	ESI 16, 17 ESX 16,17	2 min	4 min	30	45 min	3:22
2009	PowerPlex 16 HS	2 min	1 min 45 s	32	30 min	2:42
2009	NGM	11 min	3 min 20 s	29	10 min	2:33
2009	Identifler Direct	11 min	3 min	26	25 min	2:34
2010	Idenfiler Plus	11 min	3 min 20 s	28	10 min	2:18
2011	PowerPlex 18D	2 min	1 min 10s	27	20 min	1:25

Thermal Cyclers

1. GeneAmp 9700 (Applied Biosystems)
2. Mastercycler Pro S (Eppendorf)
   - Peltier based
3. Rotor-Gene Q (Qiagen)
   - Air heated and cooled
4. SmartCycler (Cepheid)
   - Hot plates for heating, fans for cooling

Intended for real-time PCR

- Cycling for most STR kits is run in the
- '9600 emulation mode' (1°C/s)

1

2

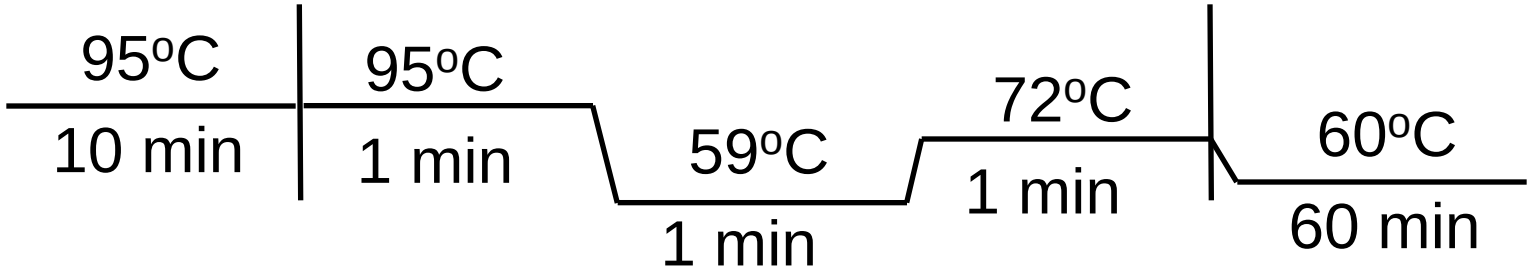
3

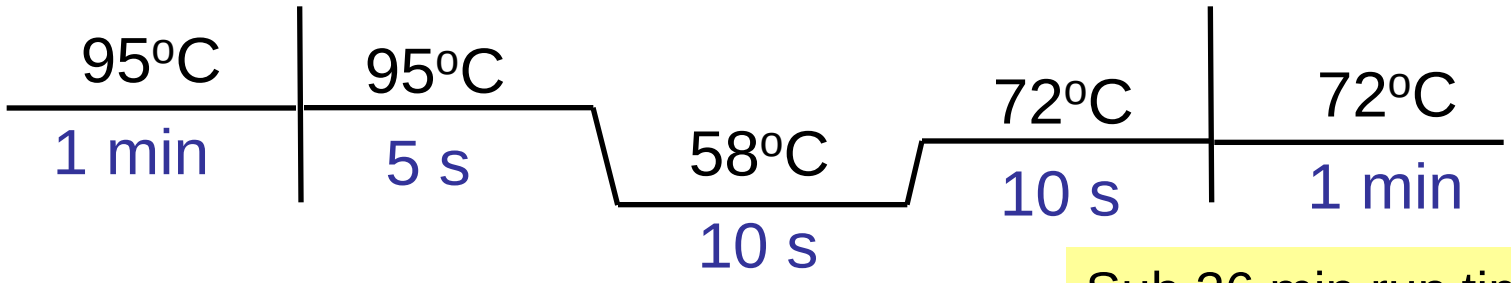
4

PCR Thermal Cycling Profile

Identifiler STR kit

28 cycles of PCR





Sub 36 min run time

Maximum heating/cooling rate of ~2 to 6°C/s (cycler dependent)

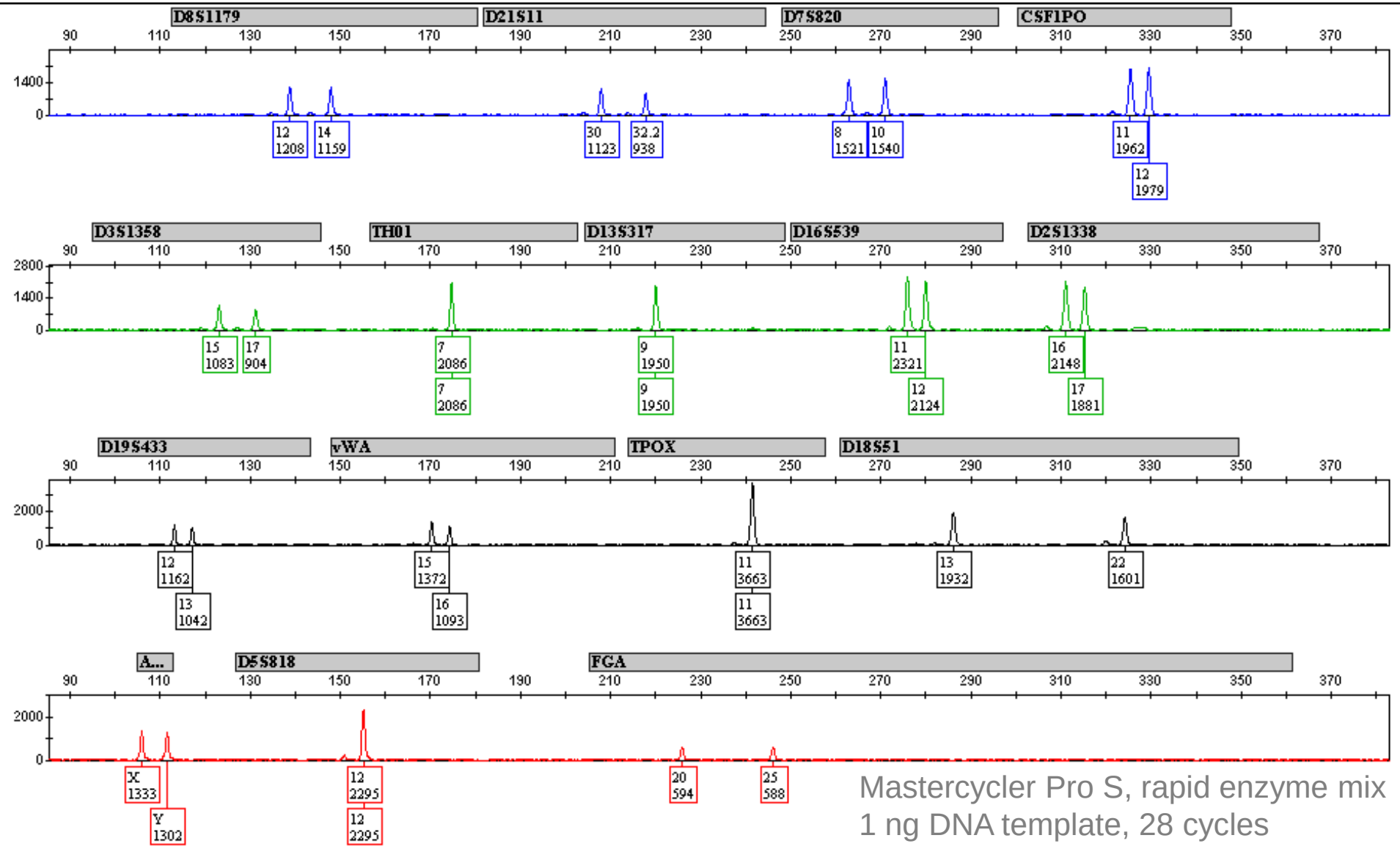
Rapid PCR Conditions

- 1 X Takara PCR mastermix, 1 U SpeedStar polymerase
  - *Premix Ex Taq*™ (Perfect Real Time)
- 10 μL total reaction in a thin walled tube (8-strip)
- 2 μL of Identifiler PCR primer mix
- ~1 ng of template DNA

- Utilize maximum ramp rate on thermal cyclers
  - GeneAmp 9700 = 1.6ºC/s (36 min)
  - Rotor-Gene Q = 1.6ºC/s (36 min)
  - SmartCycler = 5.8ºC/s (20 min)
  - Mastercycler Pro S = 6.8ºC/s (19 min)

Effective heating/cooling rates

Full Identifiler STR Profile with **19 min PCR**



Mastercycler Pro S, rapid enzyme mix
1 ng DNA template, 28 cycles

How Fast Can We Go?

Better chemistry has potential to lead to ability to routinely obtain results in < 1 hour with commercially available instruments

Steps Involved

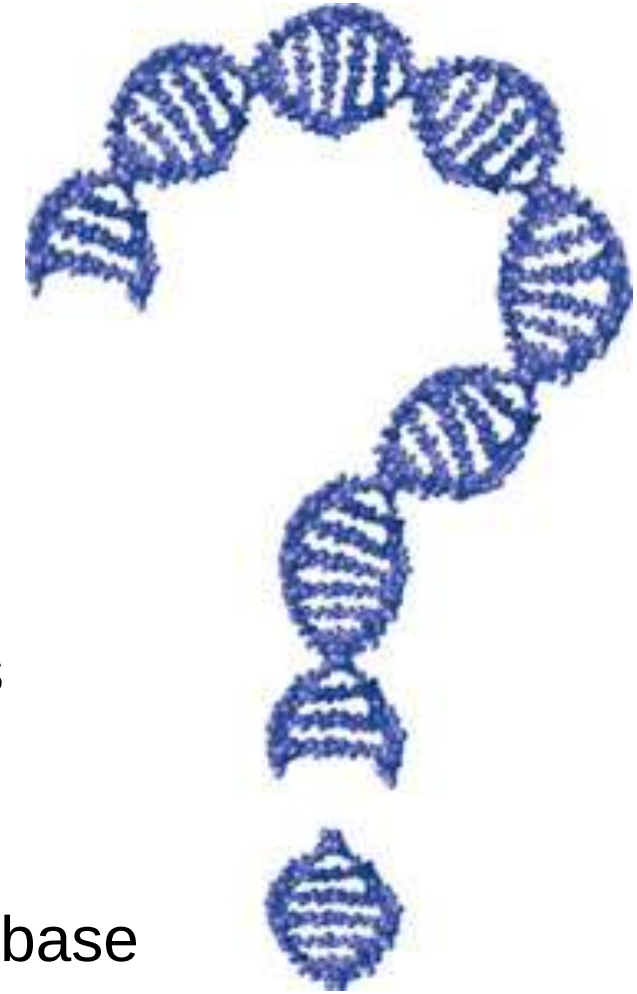
- Collection
- Extraction → **Direct PCR (new enzymes & master mix to overcome PCR inhibitors from blood)**
- Quantitation
- Amplification → **Rapid PCR (new enzymes & thermal cyclers)**
- Separation/ Detection → **Improved CE speed (ABI 3500 has faster runs than 3130)**
- Data Interpretation → **Expert system software**

Take Home Messages

- STR measurements involve assessment of PCR product mobility (not DNA size)
- ABI 3500 works well but will require careful validation for threshold determination because signal and noise levels are different
- Rapid PCR & integrated DNA devices may become a game-changer in the future

Thank you for your attention

Acknowledgments: Applied Biosystems, Promega, and Qiagen for STR kits used in concordance studies



Contact Information

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http://www.cstl.nist.gov/biotech/strbase

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm