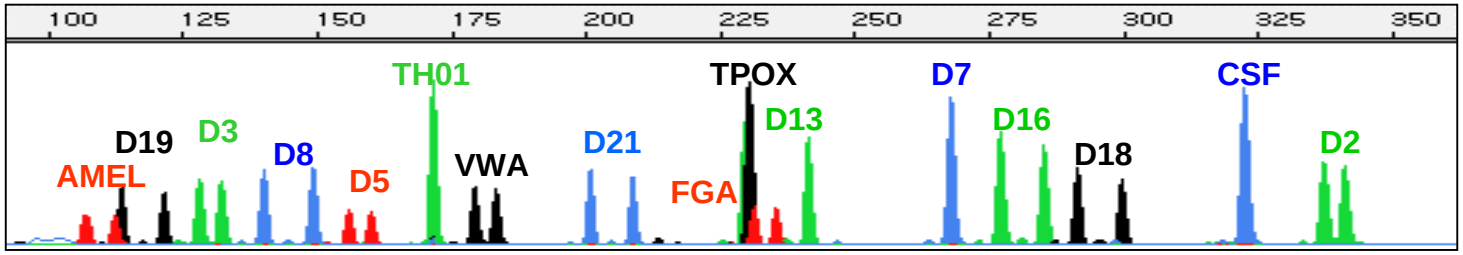


NIST Projects in Human Identity Testing

Peter Vallone

John Butler, Margaret Kline, Mike Coble

Jan Redman, Amy Decker, Becky Hill, Chris DeAngelis

Dave Duewer (NIST Analytical Chemistry Division)

AFDAA Summer Meeting Austin, TX August 4, 2005

NIST Human Identity Project Team

John Butler
(Project Leader)

Margaret Kline

Pete Vallone

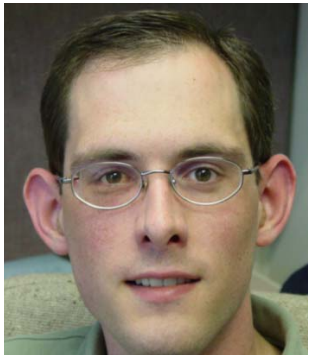
Mike Coble

Dave Duewer
Anal. Chem. Division

Jan Redman

Amy Decker

Becky Hill



Chris DeAngelis

Funding: **Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)**

Current Areas of NIST Research Effort

- **Resources for "Challenging Samples"** (**miniSTRs**)
- **Information on New Loci** (**SNPs**, Y-Chromosome, new STRs)
- **Standard Information Resources** (STRBase website, training materials/review articles, validation standardization)
- **Allele Sequencing and Interlaboratory Studies** (**Real-time qPCR**, mixture interpretation)


Mike Coble


Becky Hill

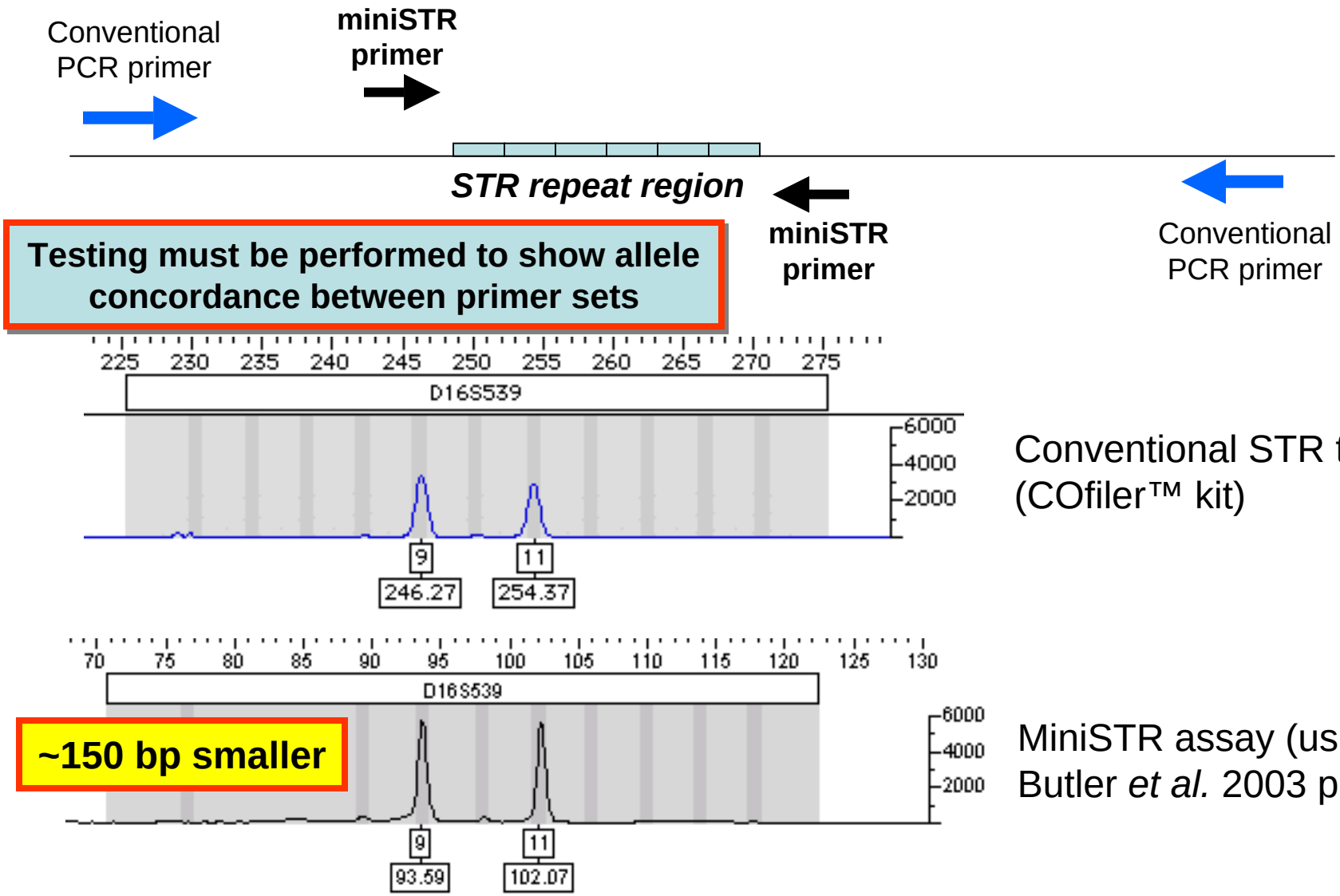

John Butler

miniSTRs for Degraded DNA

- Original miniSTR paper with CODIS loci, D2, D19, Penta D, Penta E
  - Butler *et al.* (2003) *J. Forensic Sci.* 48: 1054-1064
- Many CODIS loci are too big and make poor miniSTRs
- New miniSTRs and assays: **NC01, NC02**
  - Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50:43-53
- New miniSGM miniplex: AMEL, TH01, FGA, D18, D16, D2
- Creation of miniSTR information on STRBase

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

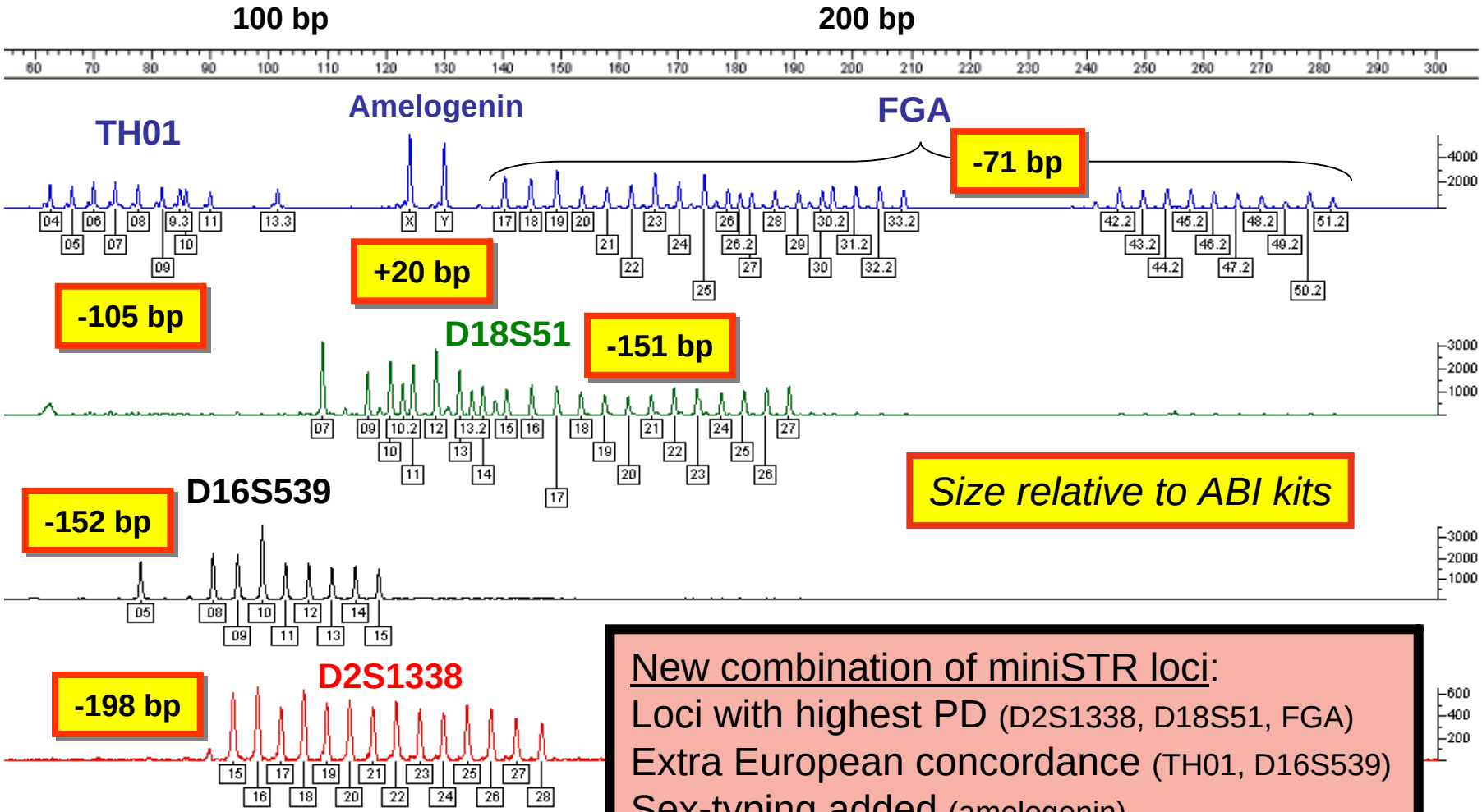
A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition,* Figure 7.2, ©Elsevier Science/Academic Press

New miniSGM miniplex assay

Provided to EDNAP/ENFSI group for degraded DNA study (Fall 2004)



Retains same miniSTR primers from Butler et al. (2003) *J. Forensic Sci* 48(5): 1054-1064

Many CODIS Loci Make Poor miniSTRs

- Large allele range (e.g., FGA)
- Large alleles (e.g., D21S11 and FGA)
- Poor flanking regions prohibiting reliable primer annealing immediately adjacent to the repeat region (e.g., D7S820)

Why go beyond CODIS loci

"STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

Why go beyond CODIS loci

"To achieve this purpose, either **new STRs could be developed**, or alternatively, existing STRs could be supplemented with a SNP panel."

"There are also efforts for modifying existing STR panels by decreasing the size amplicons by designing new primers."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

Why go beyond CODIS loci

- Desirable to have markers unlinked from CODIS loci (different chromosomes) for some applications

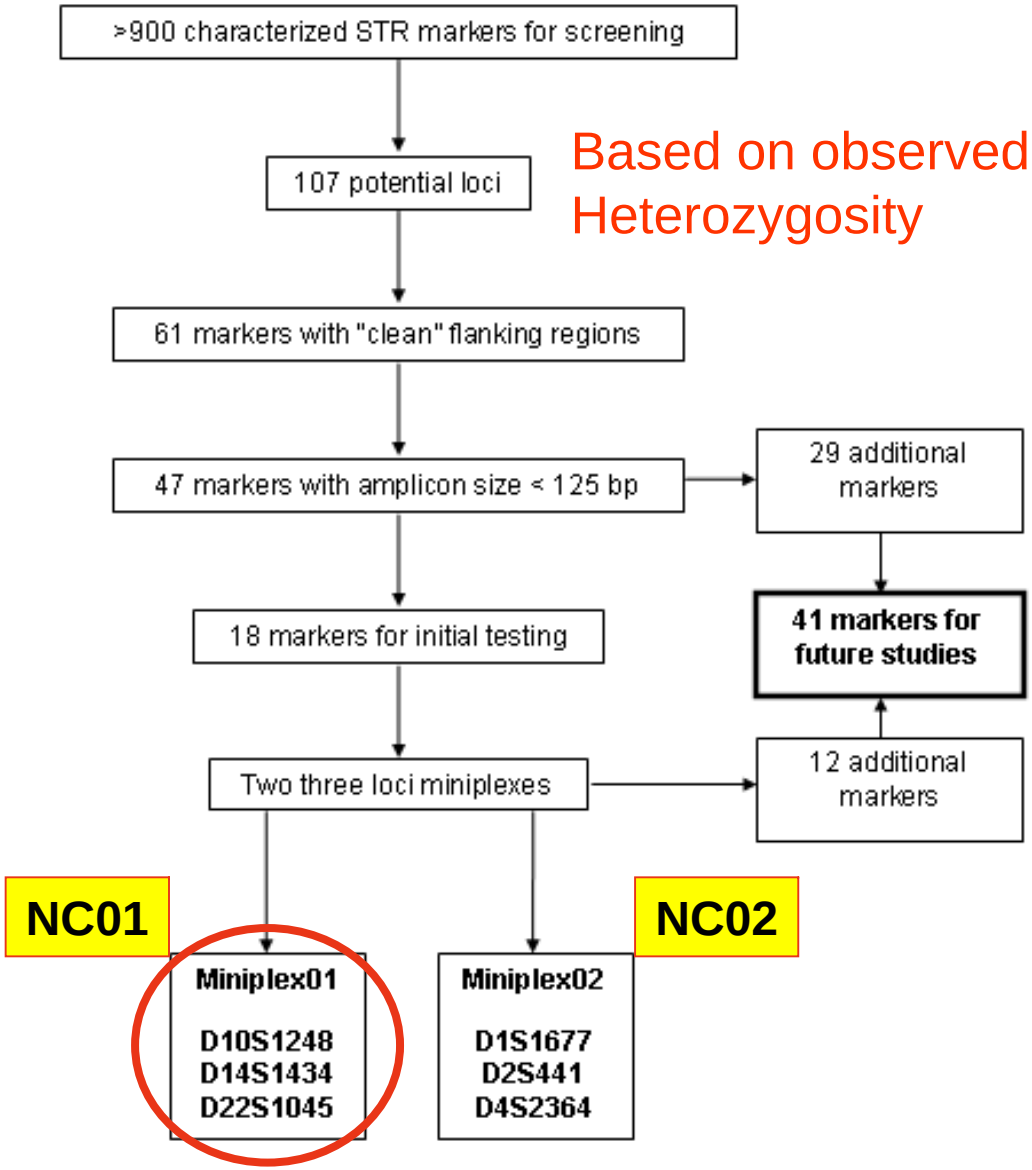
- Small size ranges to aid amplification from degraded DNA samples

- **New miniSTR loci will benefit missing persons investigations and paternity testing (and perhaps national databases in the future)**

Characterization of New miniSTR Loci

- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance

Initial Testing Results with Potential miniSTR Loci

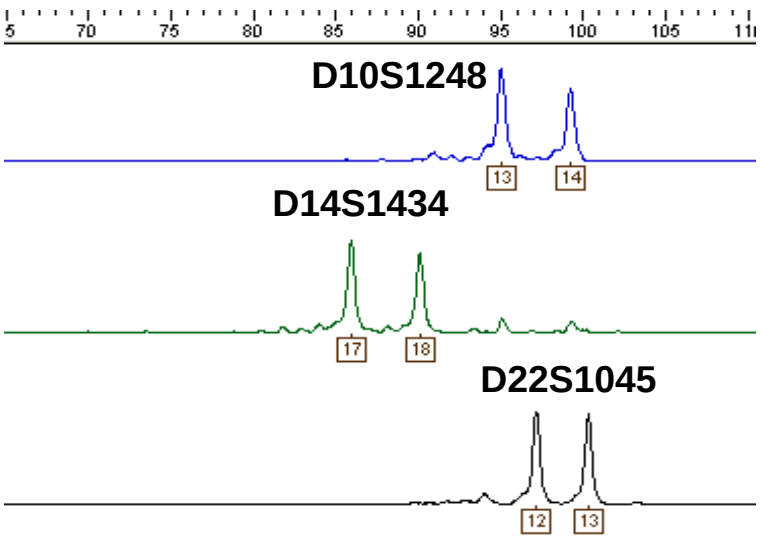


Based on observed allele range
Heterozygosity

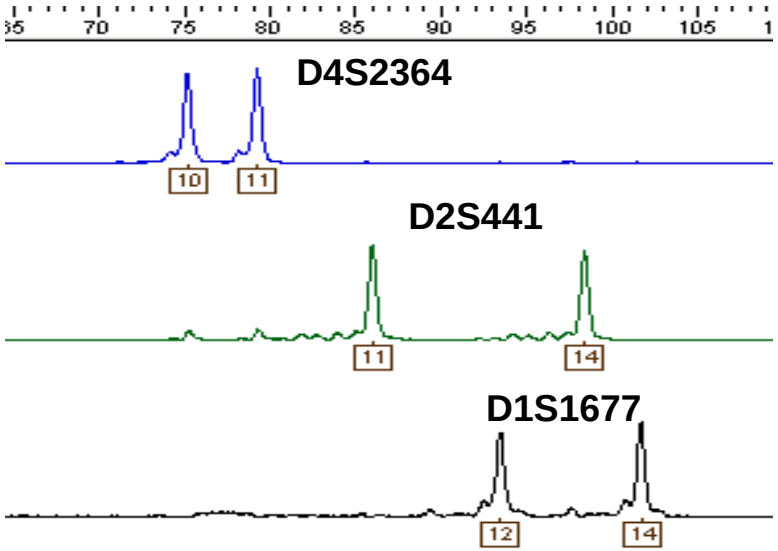
7 miniplexes (21 markers) are currently under investigation

Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Miniplex NC01



Miniplex NC02



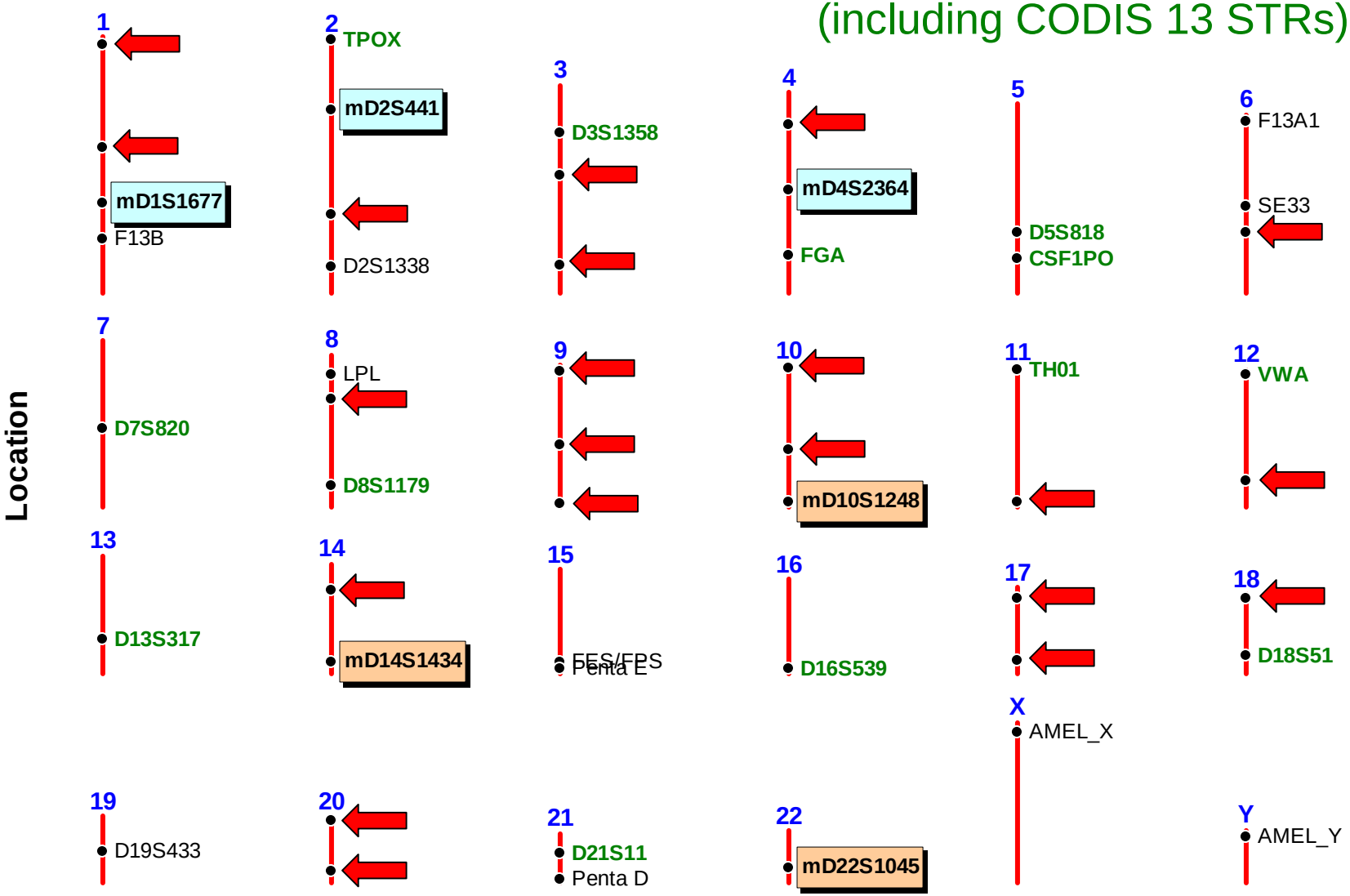
Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Some Marker Characteristics

Chr.	Marker Name	(Motif)	Ref. Repeat	Amplicon Size	Primer distance from repeat
10	**D10S1248**	**TETRA**	13	102	1
	GGAA23C05N	GGAA			0
14	**D14S1434**	**TETRA**	10	88	1
	GATA168F06	GATA			0
22	**D22S1045**	**TRI**	13	105	3
	ATA37D06	ATA			6
1	**D1S1677**	**TETRA**	15	103	0
	GGAA22G10N	GGAA			0
2	**D2S441**	**TETRA**	12	92	0
	GATA8F03	GATA			0
4	**D4S2364**	**TETRA**	7	78	2
	GAAT1F09	GAAT			1

STR Loci Positions

(including CODIS 13 STRs)



Chromosome

Positions determined along May 2004 Human Genome Reference Sequence (NCBI Build 35)

Standard U.S. Population Dataset

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

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

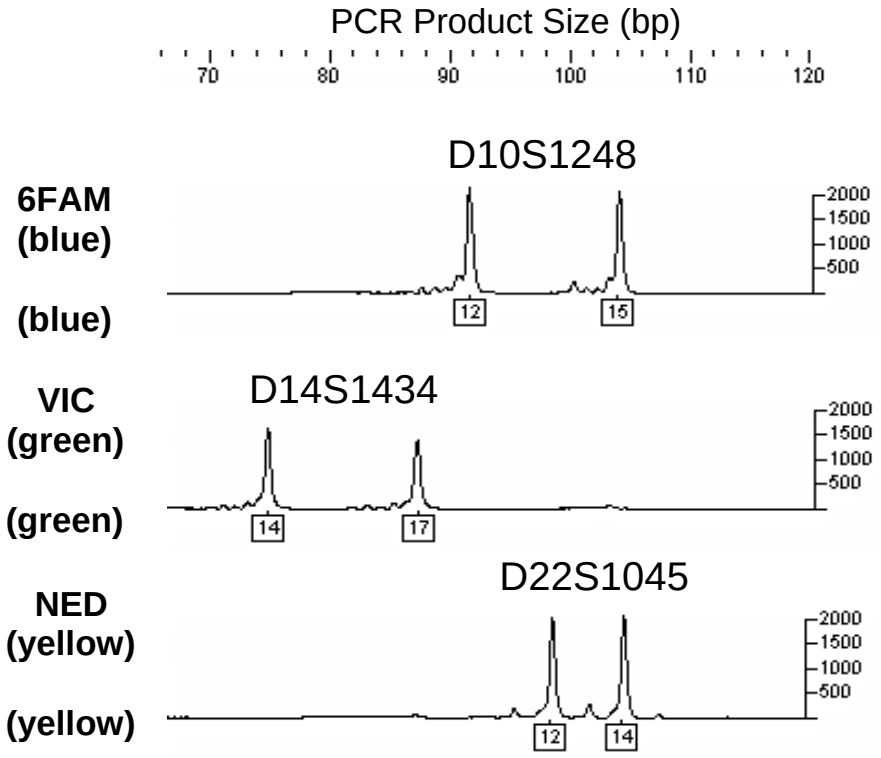
DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)

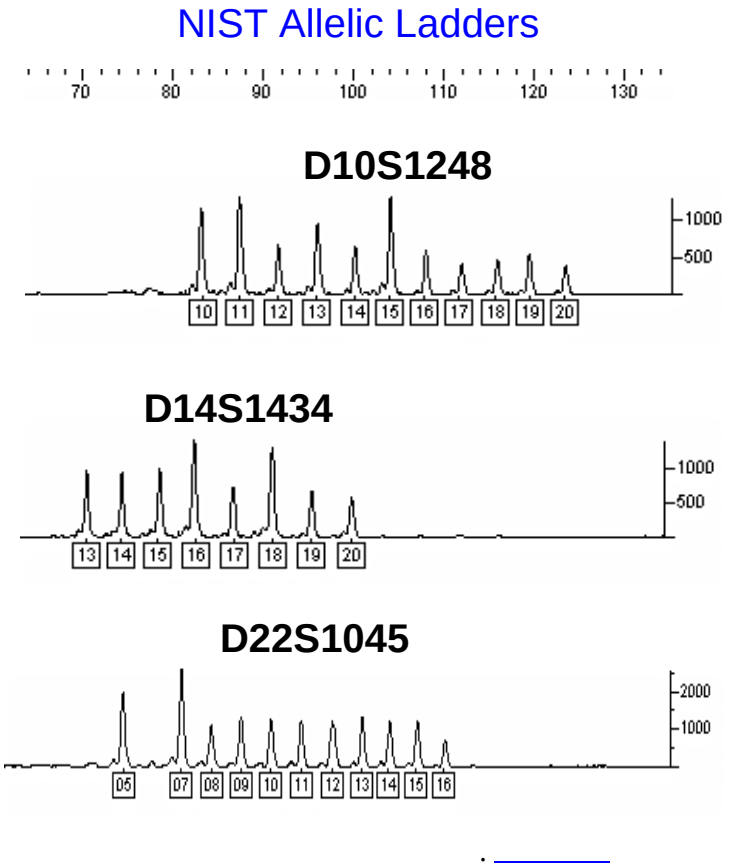
To date: (~95,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y STRs 27 new loci (14,535)
Yfiler kit 17 loci (11,237)
Y SNPs 50 markers on sub-set of samples (11,498)
Orchid 70 autosomal SNPs on sub-set (13,230)
miniSTR testing-new loci and CODIS concordance (9,228)
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers

Allele Ladders for Miniplex "NC01"





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

Coble and Butler (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA *J. Forensic Sci.* 50(1): 43-53

miniSTR Assay Sensitivity (D10S1248)

28 cycles – 1U Taq

32 cycles – 2U Taq

200 pg

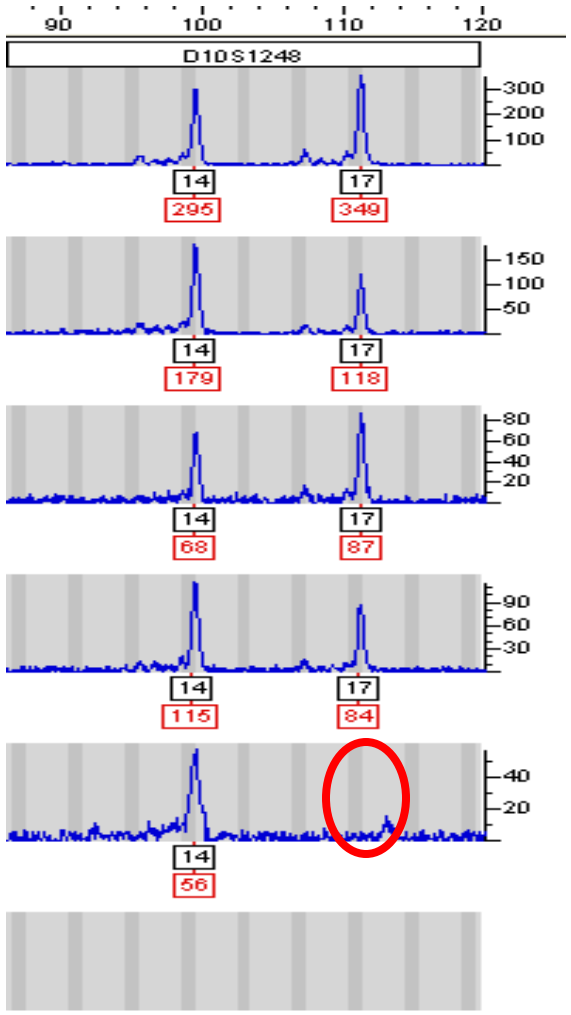
100 pg

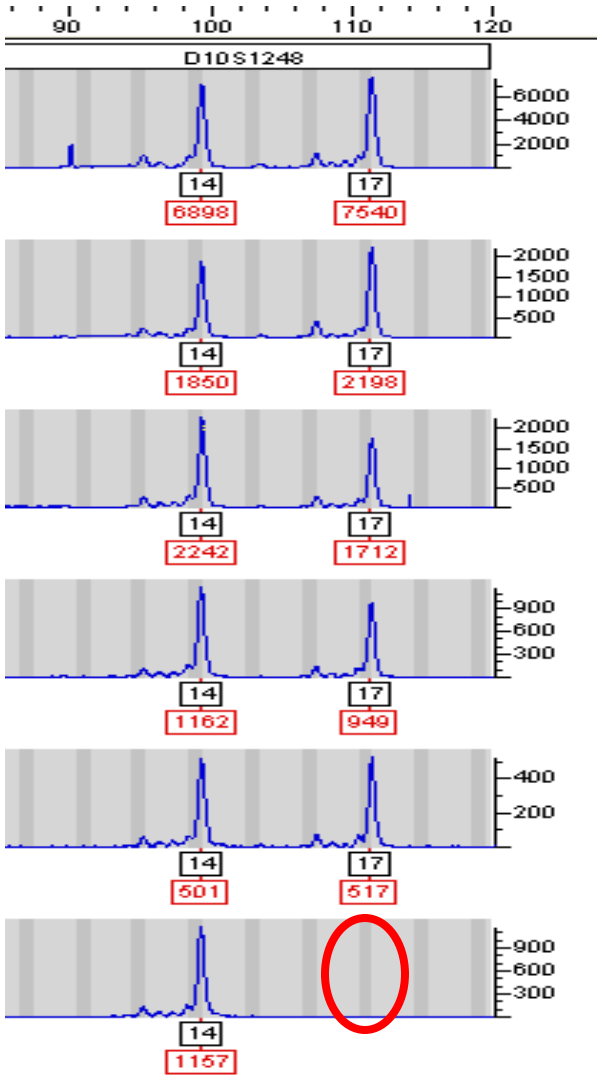
50 pg

20 pg

10 pg

5 pg





Protocol for Using the miniSTR System "miniNC01" on the ABI 3100 Instrument

PCR Conditions

PCR Conditions:

Preparation of Master Mix:

___ (# reactions) x 10.5 µL PCR mix (from an ABI kit) = _____

___ (# reactions) x 0.4 µL Taq Gold = _____

___ (# reactions) x 5.5 µL primer mix (green-topped tube) = _____
16.4 µL – includes overfill for pipetting

Preparation of Individual PCR Reactions:

15 µL master mix *(from above)*
10 µL DNA template (or dI H_2O to bring up the volume)

Thermal Cycling

Thermal cycling was performed with the GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e., ramp speeds of 1 °C/s):

95 °C for 10 minutes
32 cycles: 94 °C for 1 minute
55 °C for 1 minute
72 °C for 1 minute

60 °C for 45 minutes
25 °C forever

Primer Sequences

Primer Sequences (Coble and Butler, JFS, in press)*

Locus		MiniNC01 Primer Sequences (5'-3')	Distance 3'end from STR repeat
D10S1248	F	6FAM-TTAATGAATTGAACAAATGAGTGAG	1
	R	GCAACTCTGGTTGTATTGTCTTCAT	0
D14S1434	F	VIC-TGTAATAACTCTACGACTGTCTGTCTG	-11
	R	GAATAGGAGGTGGATGGATGG	0
D22S1045	F	NED-ATTTTCCCCGATGATAGTAGTCT	3
	R	GCGAATGTATGATTGGCAATATTTTT	6

*A PDF copy of this paper can be downloaded at the STRBase website:
http://www.cstl.nist.gov/biotech/strbase/miniSTR/CobleandButlerJFS .pdf

Pos. Control Results

Expected Control Results

STR Locus	Control DNA 007 Genotype	Control DNA 9947A Genotype
D10S1248	13, 16	14, 16
D14S1434	15, 18	15, 17
D22S1045	8, 13	8, 11

http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniNC01_Protocol.pdf

New Autosomal miniSTR Loci

- NC01 loci: **D10**S1248**, D14**S1434**, D22**S1045
- Peter Gill and the EDNAP/ENFSI group have recommended the NC01 loci as an extension of current European core loci
- Population data, locus characterization, and allelic ladders for **27 new autosomal STRs** under development as new miniSTRs
- All new STR loci are physically unlinked to CODIS core loci

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


Pete Vallone


Amy Decker


John Butler

Work with SNP Loci

- U.S. population frequencies with 70 autosomal SNPs
  - Vallone *et al.* (2005) *Forensic Sci. Int.* 149: 279-286
- U.S. population information with 50 Y-SNPs
  - Vallone *et al.* (2004) *J. Forensic Sci.* 49: 723-732
- Coding Region Mitochondrial SNPs
  - Vallone et al., (2004) Int. J. Legal Med. 118: 147-157
- Construction of 12plex autosomal SNP assay

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

SNPs

Why are we interested in using SNPs?

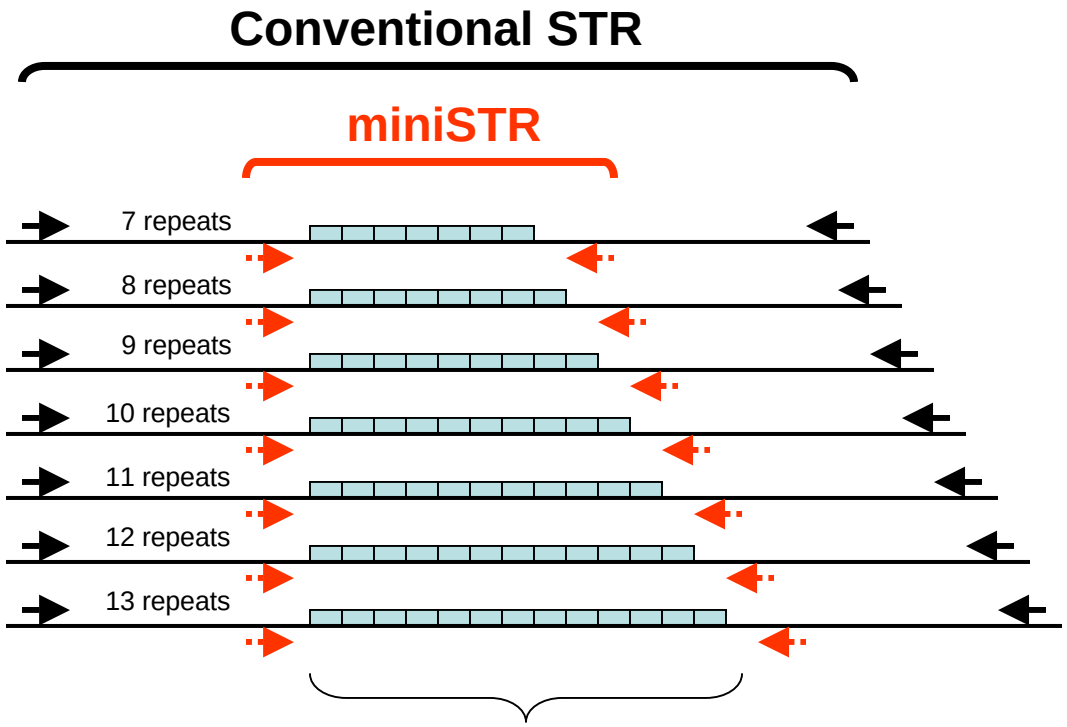
- Use on degraded samples (WTC), low copy number, or telogenic (shed) hairs
- Lower mutation rate (Paternity testing)
- Easier data interpretation (no microvariants or stutter)
- Amenable to high throughput analysis

SNPs

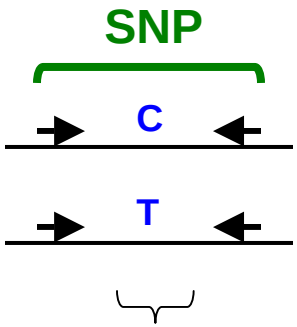
General issues that need to be addressed

- How many SNPs = STR
- Multiplexing (50-plex < 1ng DNA)
- Databases
- Platform for SNP typing?
- Unique interpretation issues – mixtures
- Validation
- Sensitivity
- Cost

Comparison of STRs and SNPs



Target region
(short tandem repeat)



Target region
(single nucleotide polymorphism)

Larger target region (miniSTR targets same region)
More possible variants than SNPs
Only need a moderate number of STR markers
Range of sizes examined (e.g., 28 bp spread if 4 bp/repeat)

Smaller target region
Fewer possible variants
Need more SNP markers
Constant size examined

SNP Typing Instrumentation

PCR & primer extension

Multi-Color Capillary Electrophoresis (ABI 310 or 3100)

Luminex Beads hybridization

Luminex 100 Flow Cytometer

Primer Extension

Time-of-Flight Mass Spectrometer

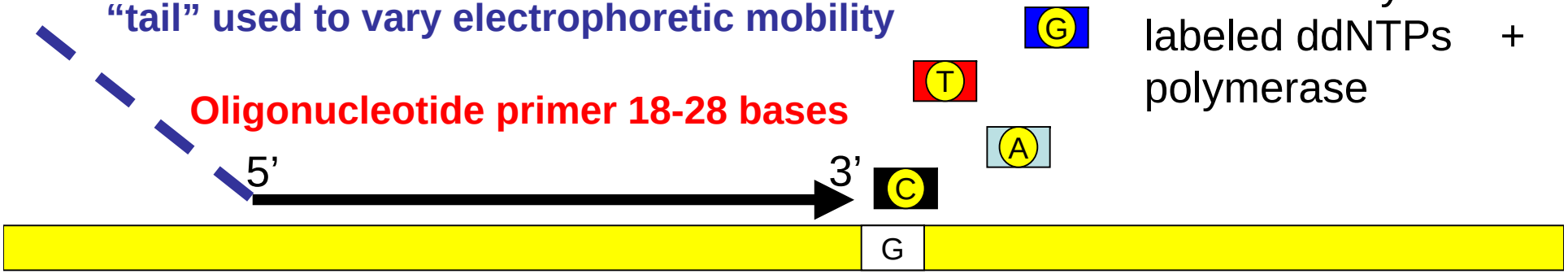
TaqMan

ABI 7000 SDS

Allele-Specific Primer Extension

SNP Primer is extended by one base unit

ABI PRISM® SNaPshot™ Multiplex System



Fluorescently labeled ddNTPs + polymerase

PCR Amplified DNA Template

ddNTP	Dye label	Color
A	dR6G	Green
C	dTAMRA	Black
G	dR110	Blue
T	dROX	Red

25 Cycles
96°C 10s
50°C 5s
60°C 30s

Utility of SNP Markers

Replace Autosomal STRs?

"It is unlikely that SNPs will replace STRs as the preferred method of testing of forensic samples in the near to medium future."

Specialized applications

mtDNA – coding region and linear arrays

Y-SNPs – lineage, population study, sample discrimination

Autosomal SNPs – highly degraded samples, shed hairs, physical characteristics, ethnic/geographical determination

Gill, P., Werret, D.J., Budowle, B., and Guerreri, R. Science and Justice 2004 44: 51-53

SNP Assay Results

70 were typed for 189 U.S. samples (self identified ethnicities)
74 Caucasians + 71 African Americans AA + 44 Hispanics

Total of 13,230 possible genotypes

42 Samples were re-injected to confirm ambiguous results
(99.7 %) success rate on first pass

Allele distribution ranged from (0.25 – 0.74)

P-value was < 5% for 10 loci

Results described in manuscript *(Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Forensic Sci. Int., 2005)*

Results on a 12-plex panel of SNPs to follow…

Allele Frequencies for 70 SNP Loci in U.S. Populations

Hispanic	
N = 44	1
CC	0.455
TT	0.068
CT	0.477
He	0.425
p	0.723
	13
CC	0.068
TT	0.568
CT	0.364
He	0.375
p	1.000
	25
CC	0.318
TT	0.114
CT	0.568
He	0.479
p	0.326
	37
CC	0.523
TT	0.045
CT	0.432
He	0.386
p	0.694
	49
CC	0.068
TT	0.636
CT	0.295
He	0.339
p	0.381
	61
CC	0.068
TT	0.455
CT	0.477
He	0.425
p	0.721

African American	
N = 71	1
CC	0.648
TT	0.070
CT	0.282
He	0.333
p	0.278
	13
CC	0.141
TT	0.451
CT	0.408
He	0.452
p	0.298
	25
CC	0.099
TT	0.394
CT	0.507
He	0.456
p	0.310
	37
CC	0.324
TT	0.183
CT	0.493
He	0.490
p	1.000
	49
CC	0.085
TT	0.549
CT	0.366
He	0.392
p	0.369
	61
CC	0.310
TT	0.225
CT	0.465
He	0.496
p	0.634

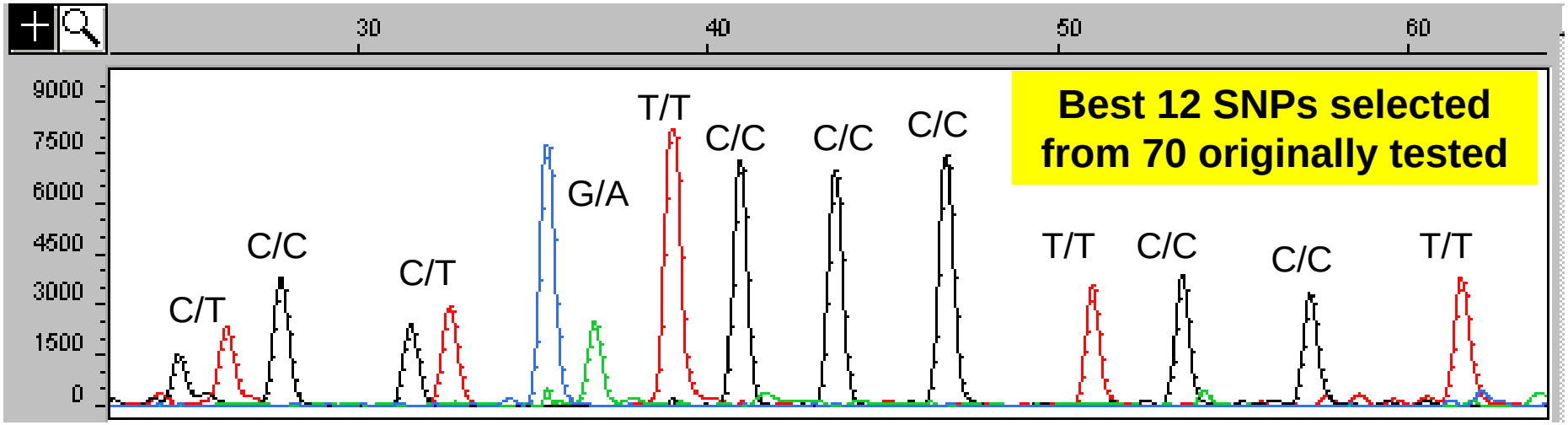
Caucasian												
N = 74	1	2	3	4	5	6	7	8	9	10	11	12
CC	0.243	0.405	0.068	0.581	0.311	0.149	0.486	0.108	0.203	0.068	0.257	0.054
TT	0.243	0.135	0.514	0.135	0.189	0.338	0.122	0.378	0.284	0.459	0.216	0.365
CT	0.514	0.459	0.419	0.284	0.500	0.514	0.392	0.514	0.514	0.473	0.527	0.581
He	0.500	0.463	0.401	0.401	0.493	0.482	0.433	0.463	0.497	0.423	0.499	0.452
p	0.816	1.000	1.000	**0.008**	0.816	0.475	0.413	0.305	1.000	0.269	0.818	**0.021**
	13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
CC	0.068	0.243	0.392	0.446	0.243	0.162	0.473	0.365	0.270	0.108	0.270	0.203
TT	0.514	0.311	0.122	0.149	0.284	0.324	0.054	0.203	0.189	0.432	0.176	0.270
CT	0.419	0.446	0.486	0.405	0.473	0.514	0.473	0.432	0.541	0.459	0.554	0.527
He	0.401	0.498	0.463	0.456	0.499	0.487	0.412	0.487	0.497	0.447	0.496	0.498
p	1.000	0.220	0.805	0.183	0.663	0.818	0.163	0.348	0.485	1.000	0.362	0.650
	25	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
CC	0.243	0.176	0.162	0.068	0.257	0.432	0.419	0.527	0.122	0.581	0.257	0.108
TT	0.203	0.446	0.432	0.689	0.284	0.149	0.122	0.122	0.311	0.068	0.203	0.243
CT	0.554	0.378	0.405	0.243	0.459	0.419	0.459	0.351	0.568	0.351	0.541	0.649
He	0.499	0.463	0.463	0.307	0.500	0.460	0.456	0.418	0.482	0.368	0.499	0.491
p	0.480	0.135	0.327	0.119	0.350	0.302	0.797	0.092	0.088	0.538	0.642	**0.008**
	37	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
CC	0.378	0.095	0.378	0.149	0.297	0.311	0.257	0.473	0.122	0.189	0.162	0.351
TT	0.122	0.473	0.149	0.514	0.216	0.149	0.216	0.095	0.446	0.284	0.351	0.162
CT	0.500	0.432	0.473	0.338	0.486	0.541	0.527	0.432	0.432	0.527	0.486	0.486
He	0.467	0.428	0.474	0.433	0.497	0.487	0.499	0.428	0.447	0.496	0.482	0.482
p	0.444	0.790	0.806	0.060	0.827	0.491	0.822	0.791	0.790	0.635	0.803	0.802
	49	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
CC	0.137	0.205	0.178	0.356	0.096	0.288	0.446	0.419	0.149	0.081	0.081	0.351
TT	0.562	0.370	0.247	0.178	0.534	0.192	0.135	0.108	0.392	0.662	0.527	0.203
CT	0.301	0.425	0.575	0.466	0.370	0.521	0.419	0.473	0.459	0.257	0.392	0.446
He	0.410	0.486	0.498	0.484	0.404	0.495	0.452	0.452	0.470	0.331	0.401	0.489
p	**0.040**	0.230	0.226	0.630	0.401	0.639	0.472	0.612	0.618	0.068	0.785	0.331
	61	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**		
CC	0.068	0.473	0.189	0.162	0.233	0.378	0.486	0.324	0.216	0.284		
TT	0.608	0.027	0.486	0.284	0.219	0.162	0.054	0.108	0.351	0.149		
CT	0.324	0.500	0.324	0.554	0.548	0.459	0.459	0.568	0.432	0.568		
He	0.354	0.401	0.456	0.493	0.500	0.477	0.407	0.477	0.491	0.491		
p	0.326	**0.043**	**0.011**	0.232	0.500	0.805	0.401	0.142	0.346	0.263		

Vallone *et al.* (2005) *Forensic Sci. Int.* 149:279-286

NIST Autosomal 12plex SNP Assay

Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Allele frequencies for 70 autosomal SNP loci with U.S. Caucasian, African American, and Hispanic Samples. *Forensic Sci. Int.* 149:279-286.

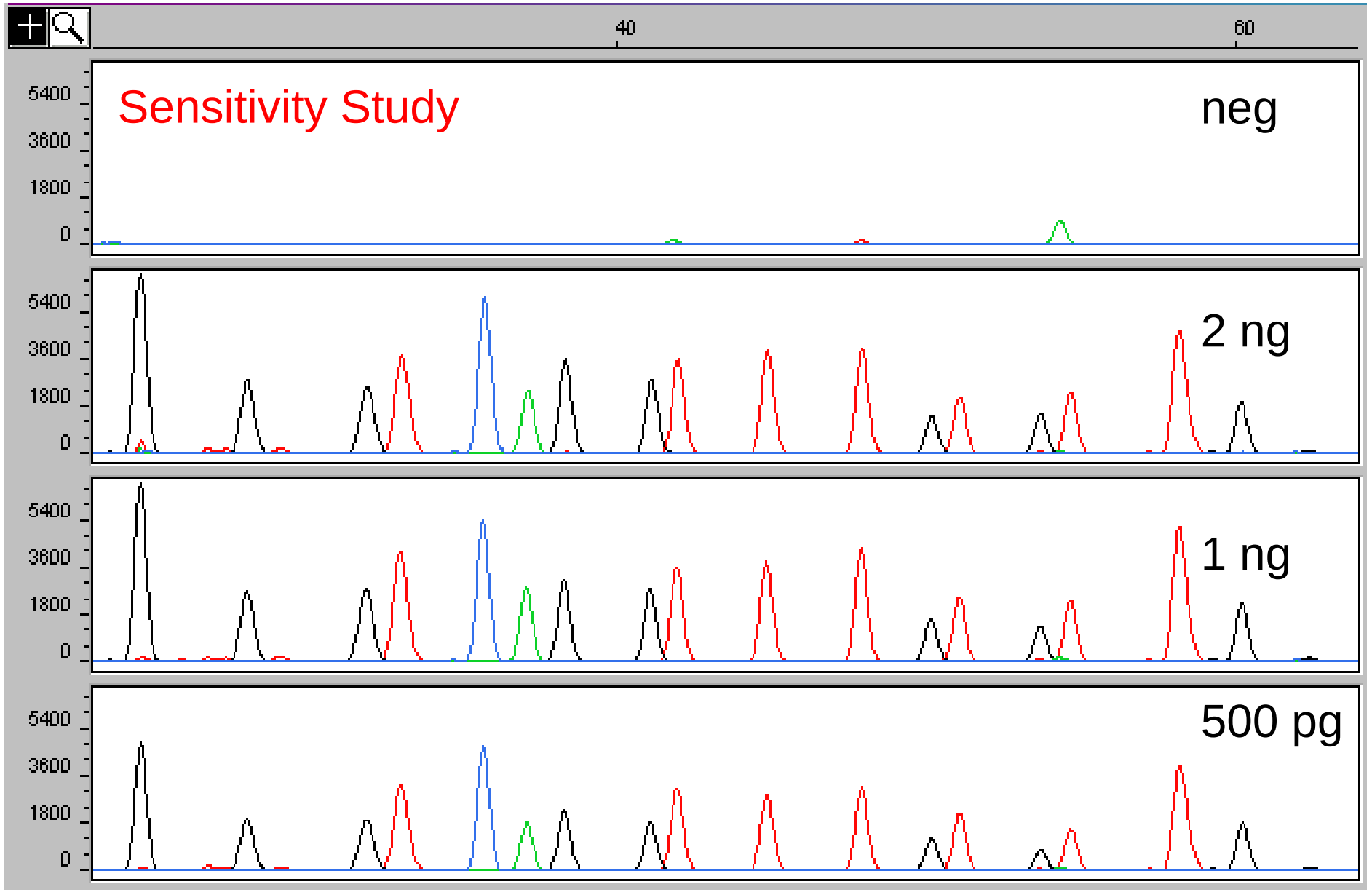
SNaPshot result



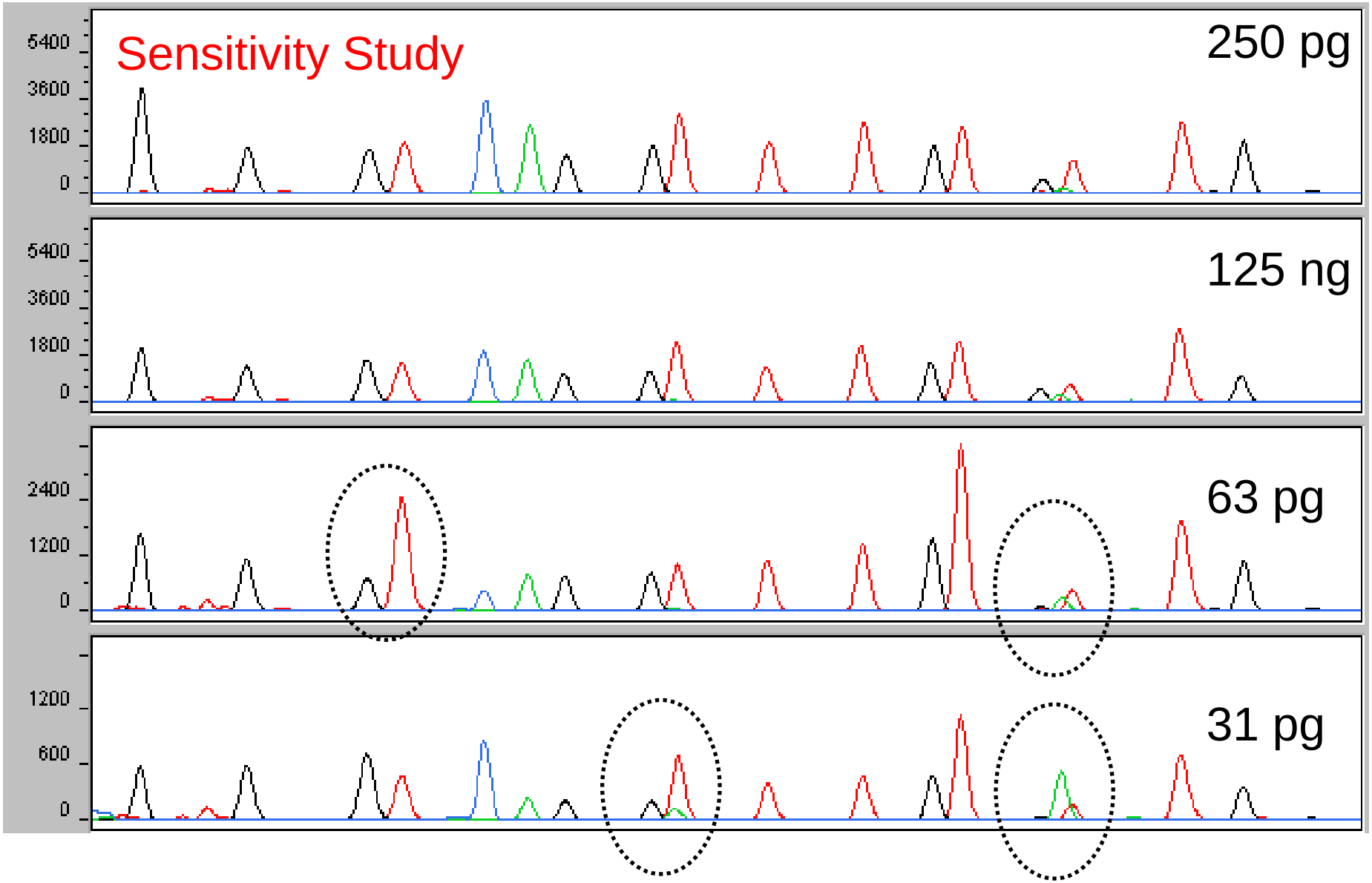
CHR:13 15 10 01 17 13 17 01 06 11 20 15

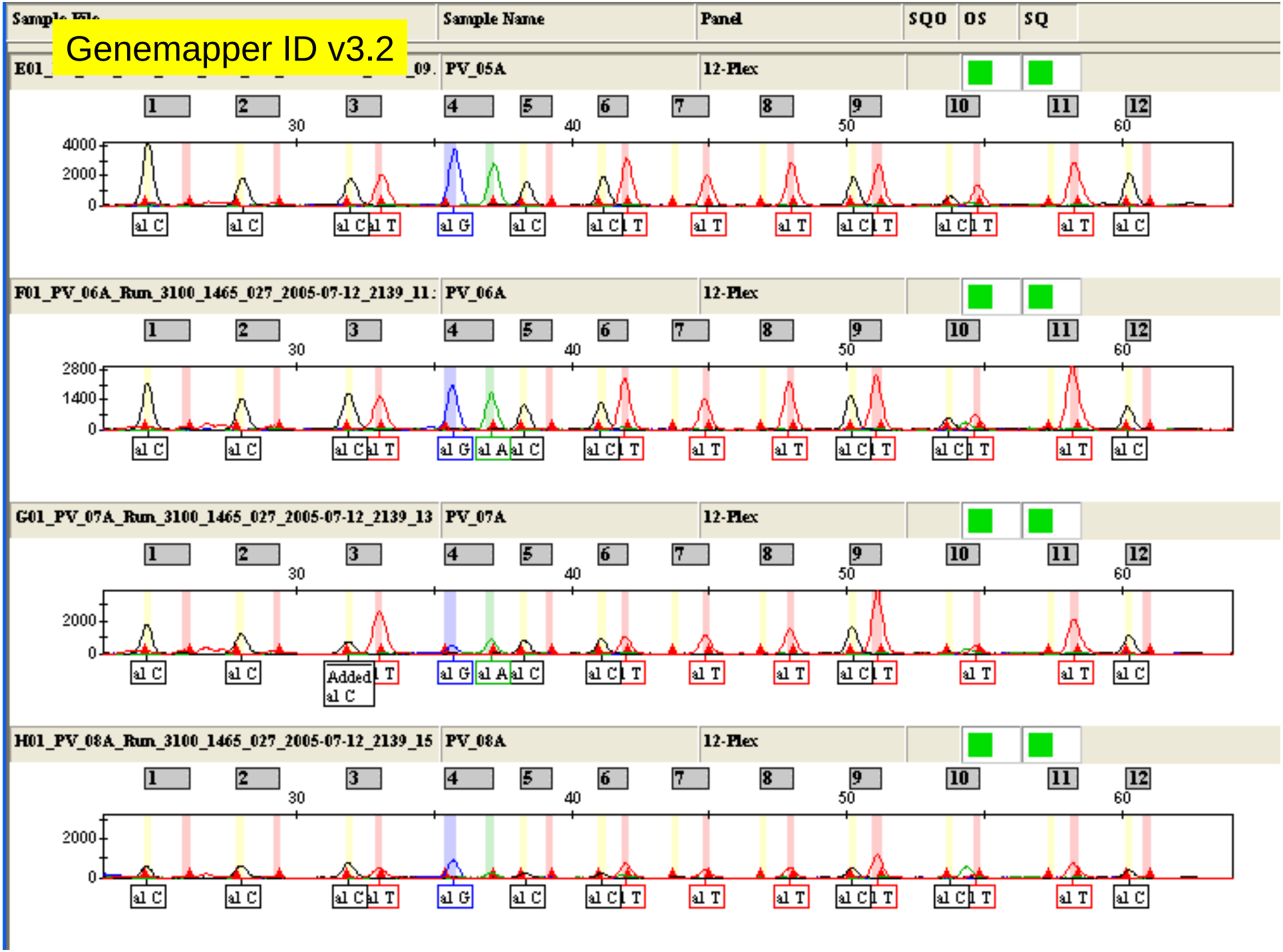
12plex PCR followed by 12-plex ASPE
Fragments separated on a ABI 3100 in 35 minutes
A Genotyper macro has been developed to type data
The 12plex assay has been run on over 600 samples
Works well on 0.5 to 1 ng of template
Sensitivity studies are underway

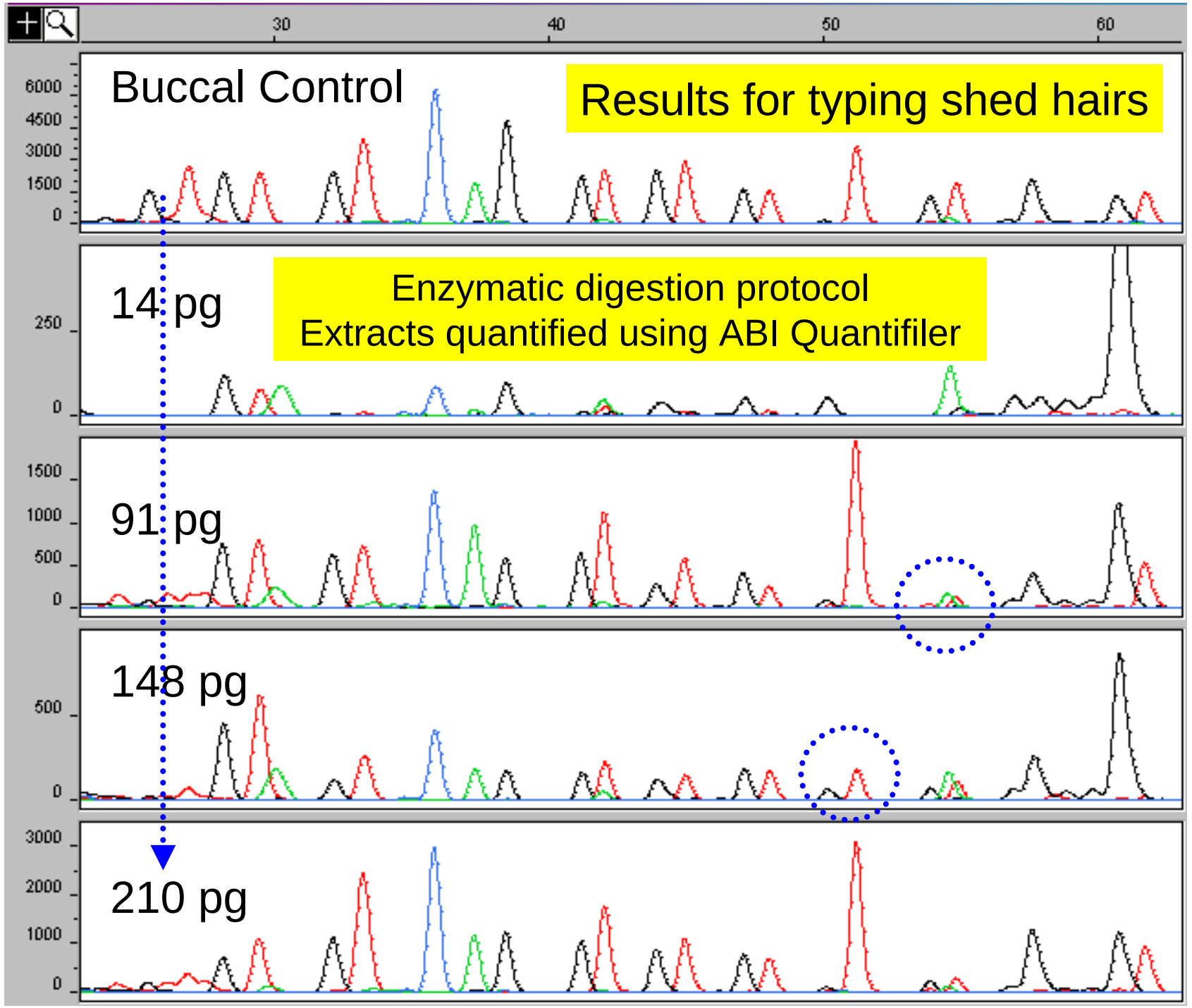
# of SNPs	# of Genotypes
1	3
2	9
3	27
4	64
5	107
6	145
7	160
8	175
9	182
10	186
11	188
12	189



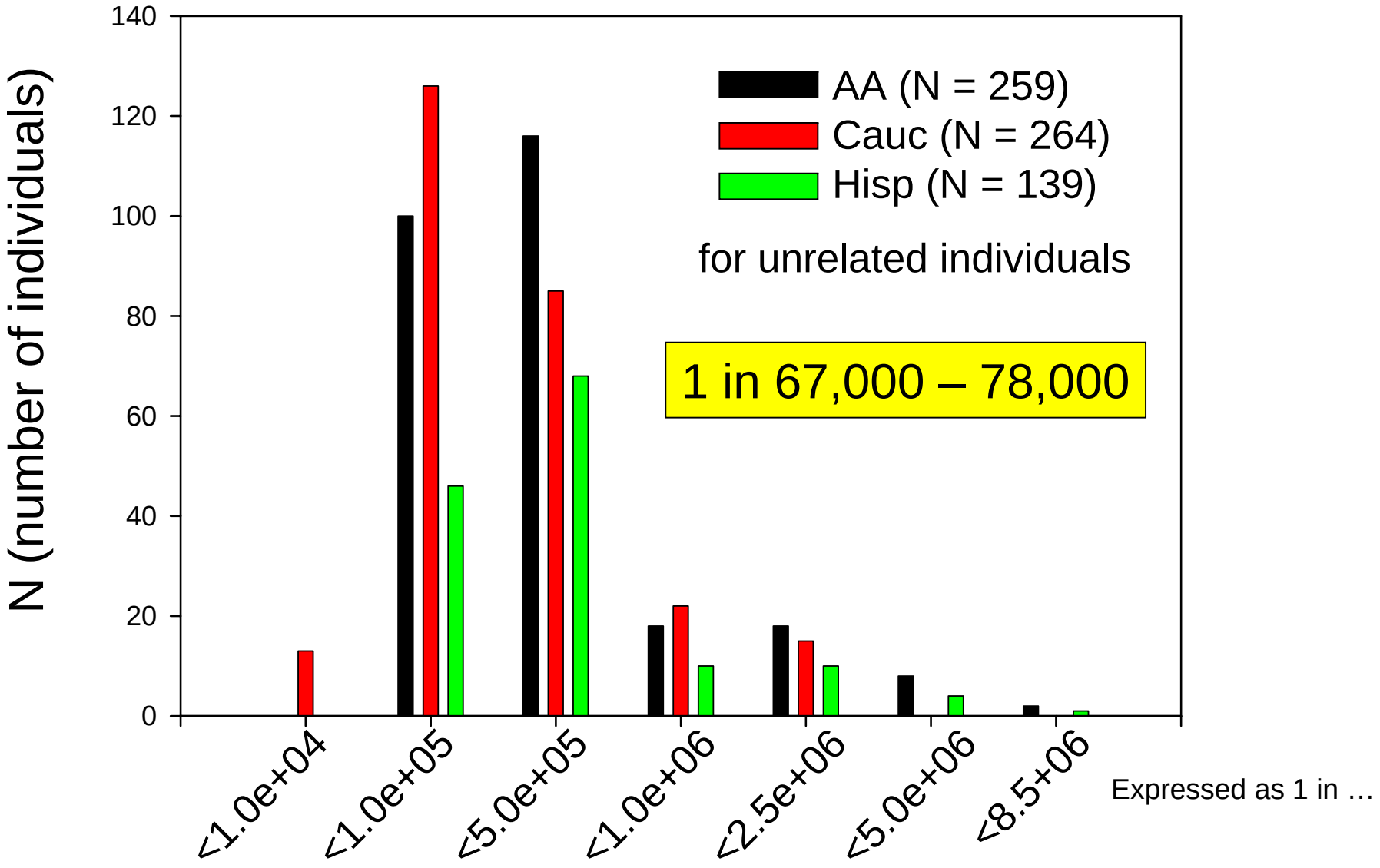
32 cycles PCR; 1.5 U Taq Gold


Sensitivity Study
250 pg
125 ng
63 pg
31 pg
5400
3600
1800
0
2400
1200
600

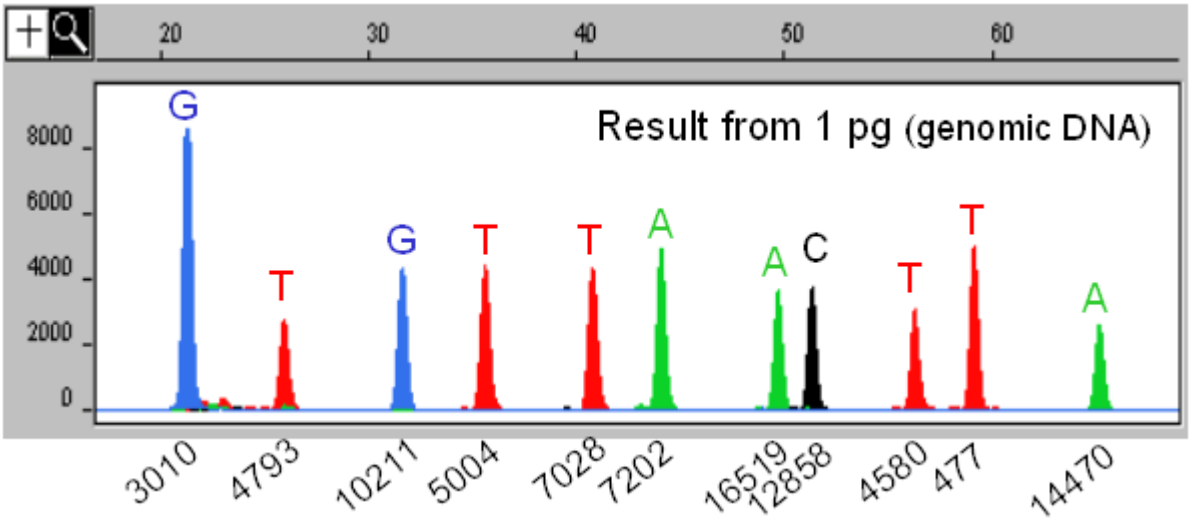

Genemapper ID v3.2
Sample Name
Panel
SQO
OS
SQ
PV_05A
12-Plex
PV_06A
12-Plex
PV_07A
12-Plex
PV_08A
12-Plex
Added
al C


30
40
50
60
Buccal Control
Results for typing shed hairs
6000
4500
3000
1500
0
14 pg
Enzymatic digestion protocol
Extracts quantified using ABI Quantifiler
250
0
91 pg
1500
1000
500
0
148 pg
500
0
210 pg
3000
2000
1000
0

Probability of a Random Match using 12-plex



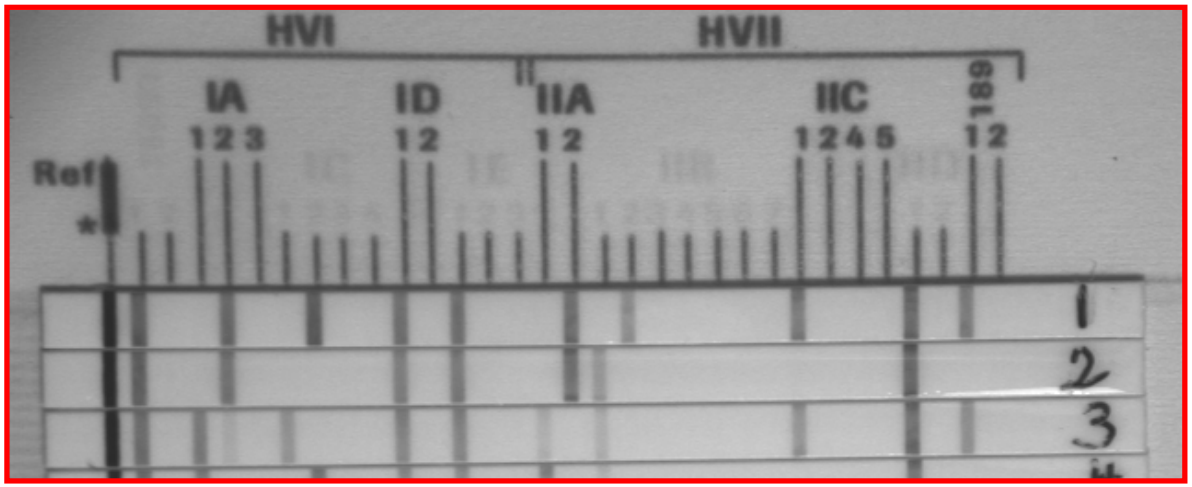
NIST mtDNA Work



Coding Region
mtSNP 11plex
(minisequencing assay)

Developed with AFDIL to resolve mtDNA most common types

Int. J. Legal Med., 2004; 118: 147-157



Roche Linear Arrays
(probes for HVI/HVII)

J. Forensic Sci. 2005, 50(2): 377-385

Automated washing/
Population Study

Typing frequencies for 666 NIST population samples

#*	Freq	% Types	% People
1	185	65.6	27.8
2	46	16.3	13.8
3	18	6.4	8.1
4	4	1.4	2.4
5	3	1.1	2.3
6	4	1.4	3.6
7	1	0.4	1.1
8	9	3.2	10.8
9	2	0.7	2.7
10	4	1.4	6.0
11	1	0.4	1.7
12	1	0.4	1.8
18	1	0.4	2.7
23	1	0.4	3.5
28	1	0.4	4.2
51	1	0.4	7.7

Summary of Our Population Typing with Roche mtDNA LINEAR ARRAYS

- 282 different types
- 185 were unique (occurred only once)
- 51 samples had “Most Common Type”

“Most Common Type” evaluated further with mtDNA coding region SNP assay

Affymetrix Genechip Mitochondrial Resequencing Array (2nd gen)

Interrogates >12,000 bases (coding region)
Less than 48h
3 long PCR amplicons
Detection of heteroplasmy

We will be testing 3 - 4 NIST population samples that have been sequenced by AFDIL

Forensic SNP Site
now a part of STRBase


John Butler


Margaret Kline


Pete Vallone


Amy Decker

Work with Y-STRs

- Beta-testing of all commercial Y-STR kits
- Population data supplied to Yfiler haplotype database
- **49 Y-STR loci evaluated with ~650 U.S. samples**
- New Y-chromosome information on STRBase linking to all available haplotype databases
- Nomenclature defined for new loci
- Human Y-Chromosome DNA Profiling Standard Reference Material (SRM 2395) – updates with DYS635 for Yfiler
- **Separation of two brothers with 47 Y-STRs**

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

Y-Chromosome Standard NIST SRM 2395

Human Y-Chromosome DNA Profiling Standard

- **5 male samples** + 1 female sample (neg. control)
- **100 ng of each (50 µL at ~2 ng/µL)**
- **22 Y STR markers sequenced**
- **9 additional Y STR markers typed**
- **42 Y SNPs typed** with Marligen kit

Certified for all loci in commercial Y-STR kits:

Y-PLEX 6
Y-PLEX 5
Y-PLEX 12
PowerPlex Y

SWGDAM recommended loci:
DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

Y-filer - adds DYS635 (C4); now sequenced

Helps meet FBI Standard 9.5 (and ISO 17025)…traceability to a national standard

Margaret Kline

Pete Vallone

Amy Decker

Evaluation of qPCR Assays

- Evaluation of published assays on same samples
- Characterization of DNA Standard lot-to-lot performance
- Additional studies under way utilizing qPCR:
  - Examining the challenge of multiplexing qPCR assays
  - Studies to track DNA recovery from various types of tubes
  - Characterizing potential SRM 2372 components (Human DNA Quantitation Standard)

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

Importance of DNA Quantitation

(prior to multiplex PCR)

DNA amount
(log scale)

100 ng

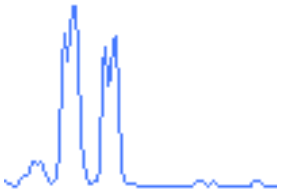
10 ng

1 ng

0.1 ng

0.01 ng

High levels of DNA create interpretation challenges (more artifacts to review)



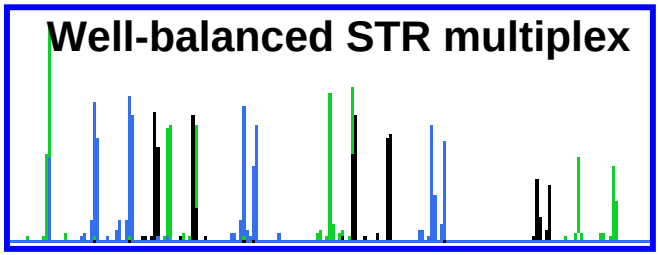
Too much DNA

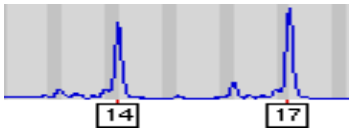
- Off-scale peaks
- Split peaks (+/-A)
- Locus-to-locus imbalance

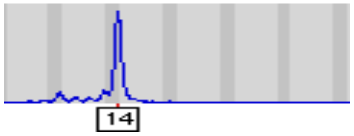
2.0 ng

STR Kits Work Best in This Range

0.5 ng







Too little DNA

- Heterozygote peak imbalance
- Allele drop-out
- Locus-to-locus imbalance

Stochastic effect when amplifying low levels of DNA produces allele dropout

ABI 7500 Real-Time PCR System

We also have access to ABI 7000 and 7900 instruments

- 96-well format thermal cycler
- five-color detection system with CCD camera
- Real-time monitoring of amplification growth curves enabling viewing of runs in progress

Studies Performed

Human ID methods SYBR Green-based

- Alu (high copy #)
  - Nicklas & Buel (2003) J Forensic Sci 48 (5):936-944

Human ID methods Probe based

- CFS-HumRT
  - Richard et al. (2003) J Forensic Sci 48(5):1041-1046
- Quantifiler™ Human DNA Quantification Kit
- Quantifiler™ Y Human Male Quantification Kit
  - ABI Quantifiler Kits User's Manual PN4344790
- CA DOJ Duplex
  - Timken et al., *in press*

Assays Examined

Assay	amplicon	GeneTarget	probe	#Cycles
Alu	124 bp	Alu , Ya5 Subfamilygene	NA	28-35
CFS-HUMRT 11p15.5	62 bp	Human tyrosine hydroxylase	TH01	40
Qfiler Human	62 bp	Human telomerase reverse transcriptase gene (hTERT), 5p15.33	TaqMan MGB	40
Qfiler Y Male	64 bp	Sex determining region Y gene (SRY)	TaqMan MGB	40
CA DOJ nuclear	170-190 bp	TH01	TaqManMGB	45
CA DOJ mito	69 bp	ND1 gene	TaqManMGB	45

Experimental Design

Assay	Std1	Std2	Std3	Std4	Std5	Std6
Quantifiler	1.5	2.3	1.0	1.2	1.0	0.9
Quantifiler Y	1.7	2.0	1.3	f	1.3	1.2
CFS	1.6	1.8	1.3	1.5	1.2	1.1
CA DOJ	1.5	2.0	1.6	2.0	1.6	1.5
ALU	1.7	3.1	1.9	2.0	2.0	1.8

Target concentration 1.6 ng/uL

Experimental Design

Assay	Std1	Std2	Std3	Std4	Std5	Std6
Quantifiler						
Quantifiler Y						
CFS						
CA DOJ						
ALU						

Do the different methods agree for a single genomic DNA standard? (Assay bias)

How do different genomic DNA standards compare? (Standard bias)

Do observed concentration differences translate into significant signal variation in a human ID test? (RFUs)

Target concentration 1.6 ng/uL

SRM 2372

Human DNA Quantitation Standard
(Tentative Information)

3 Samples Male, Female, Mixture

50 ng/μL

50 μL total volume

Available in 2006

Margaret Kline

John Butler

STR Allele Sequencing and Characterization

- Variant characterization
  - TPOX 10.3 (Maryland State Police)
  - D18S51 null alleles (FSS and Kuwait govt)
  - D18S51 allele 40 (Nebraska State Crime Lab)
  - D18S51 allele 5.3 (DNA Solutions)
  - FGA allele 46.2 (Denver Crime Lab)
  - DYS392 allele “10.3” (AFDIL)
- Locus duplication or deletion
  - DYS390 (CFS Toronto)
  - DYS392 (MN BCA)
- **Forensic labs are sending us unusual STR alleles for sequence characterization**

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

Steps in STR Allele Sequencing

DNA Extraction

Amplification with primers external to kit primers

Gel Cutouts with Heterozygotes

Re-Amplification

Amplicon Quantitation

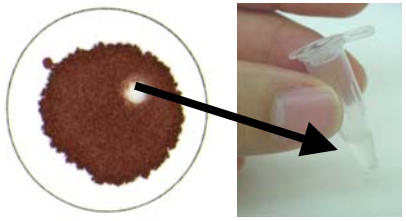
ExoSAP

Cycle Sequencing

Dye Terminator Removal

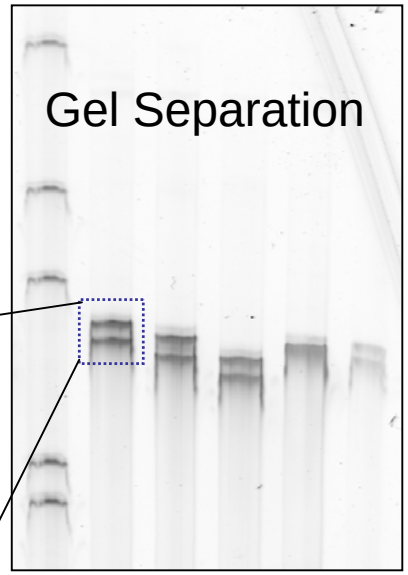
F/R Sequence Alignment to Reference Sequence

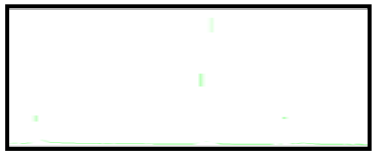
Samples provided by collaborators or forensic practioners



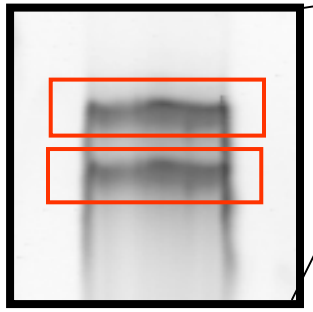
DNA Extraction

PCR Amplification



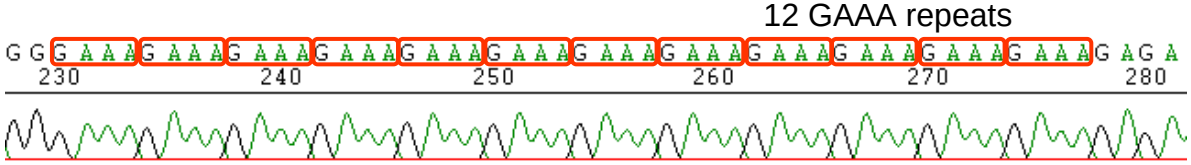


Amplicon Quantitation



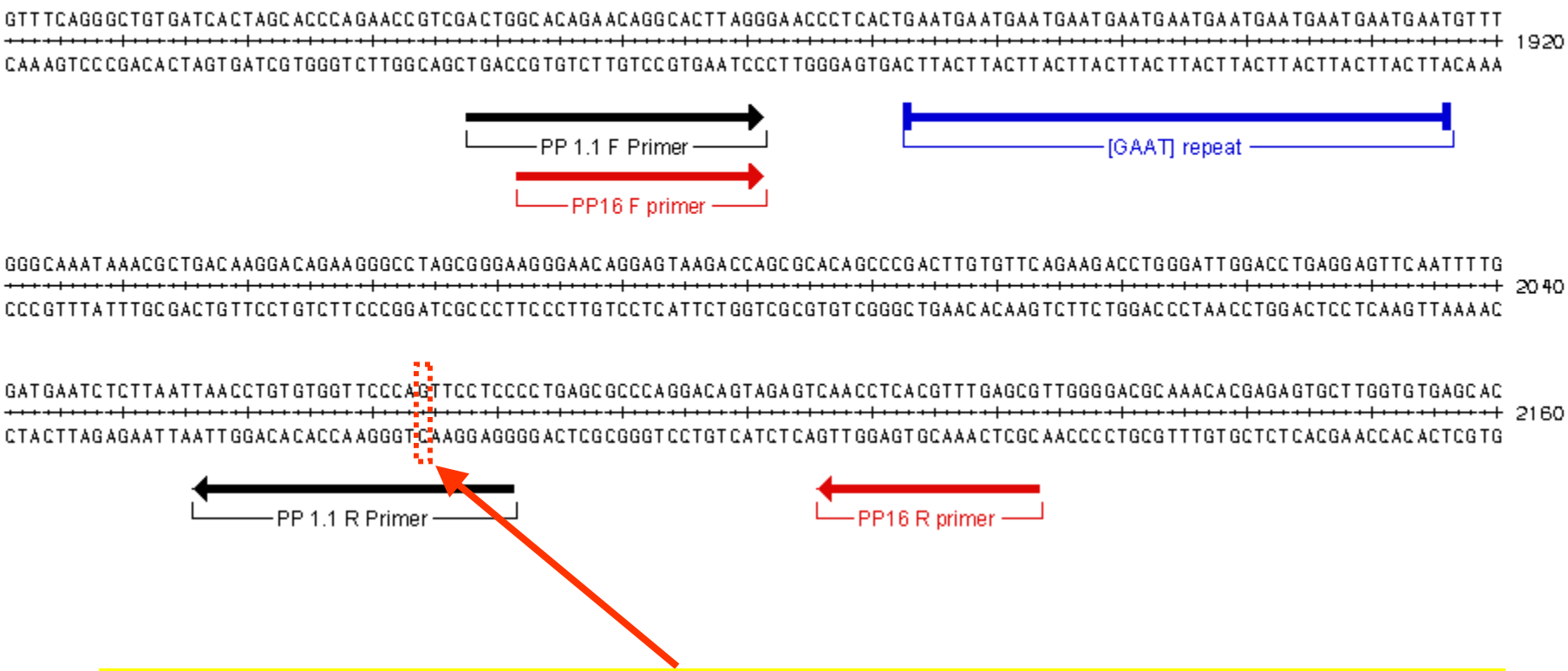
Allele Isolation with gel cutouts

Re-Amplification



DNA sequence analysis

TPOX Flanking Region Deletion Impacting Calls with Different Kits



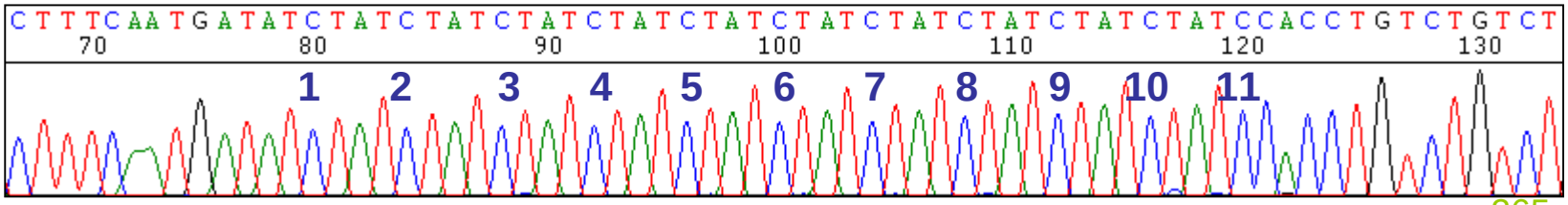
Deletion results in a 10.3 allele call with PP 16 but an allele 11 call with COfiler/Identifiler/PP1.1.

MD state police

Analysis of Common STR Variant Alleles

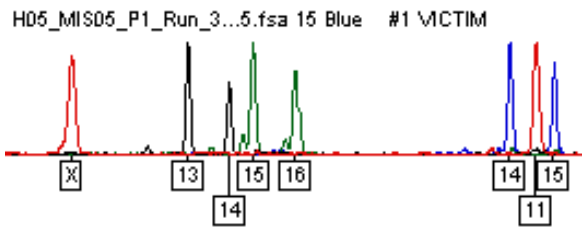
- We have monoplex primers for all common STR loci and kits
- We have sequencing primers that bind outside of STR kit primer sequence positions to enable view of polymorphic nucleotides that cause primer binding site mutations
- NIJ has funded us to characterize STR variants for the forensic DNA community

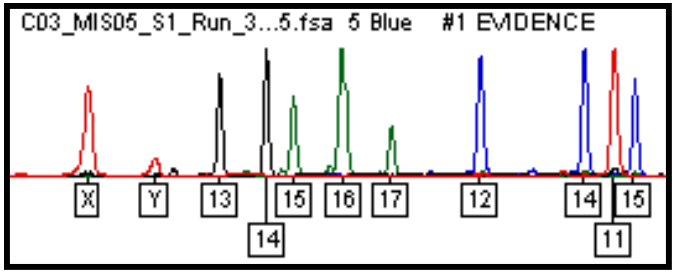
D16S539 (bottom strand)



Mixture Interpretation Interlab Study (MIX05)

- **Only involves interpretation of data**
- **91 labs enrolled** for participation (20 from overseas)
- **64 labs have returned results**
- Four mock cases supplied with “victim” and “evidence” electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits
- Summary of results with involve training materials to illustrate various approaches to solving mixtures





Perpetrator Profile(s) ??

Along with reasons for making calls and any stats that would be reported

Plans for Dissemination of MIX05 Results

- Data shipped in mid-January 2005
- Responses due before March 15, 2005 (but still open)

- **Goal is to understand the "lay of the land" regarding mixture analysis across the DNA typing community**

- Results to be discussed at NIJ DNA Grantees Meeting (June 2005), SWGDAM (June 2005), and ISFG (Sept 2005)

- We plan to develop training materials to aid in mixture interpretation with available software tools and to help in standardizing reports involving mixture analysis


Pete Vallone


Dave Duewer


Chris DeAngelis

Software Tools

- AutoDimer – multiplex PCR primer screening tool

http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm

- mixSTR – mixture component resolution tool

- Multiplex_QA – quality assessment tool for monitoring instrument performance over time

- NIST U.S. population database (internal Access database)

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

AutoDimer Primer Screening Program

SHORT TECHNICAL REPORTS

Vallone, P.M. and Butler, J.M. (2004) *BioTechniques* 37:226-231

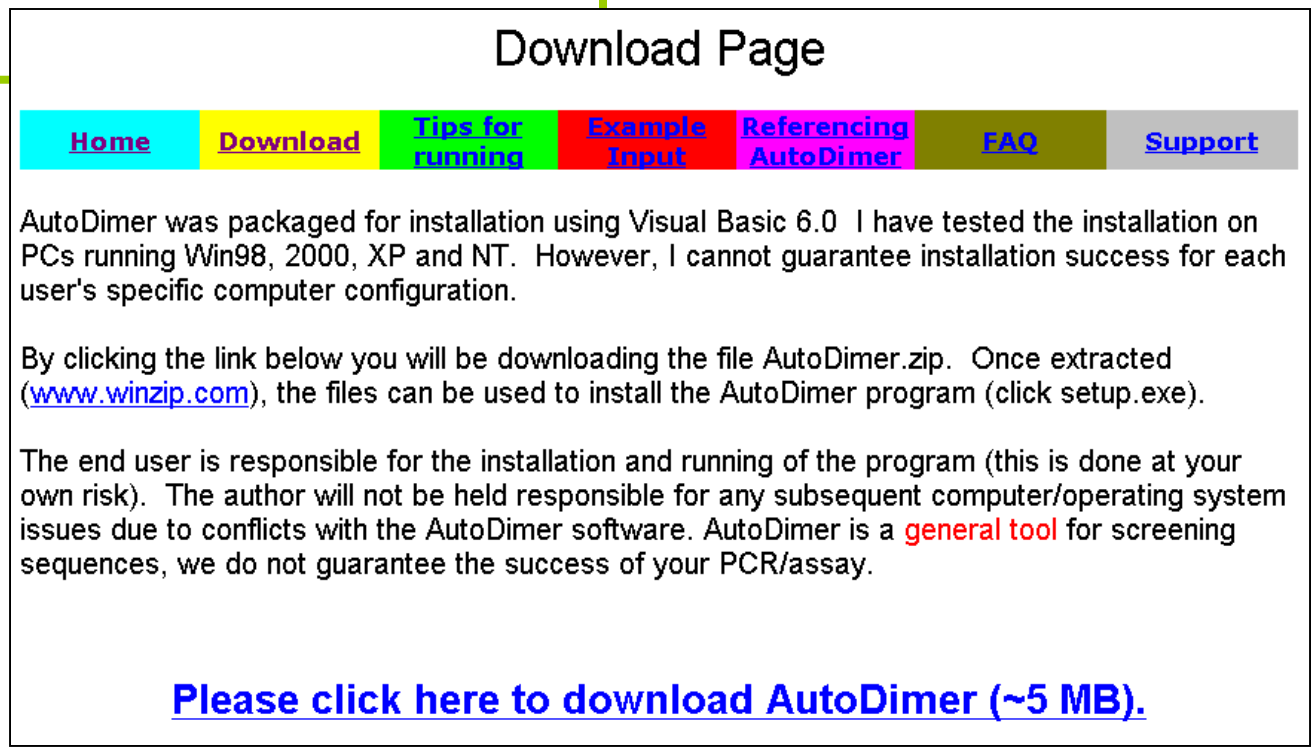
AutoDimer: a screening tool for primer-dimer and hairpin structures

Peter M. Vallone and John M. Butler
National Institute of Standards and Technology, Gaithersburg, MD, USA

BioTechniques 37:226-231 (August 2004)

Available for download from STRBase:
http://www.cstl.nist.gov/biotech/strbase

A web-based interface is in development
(similar to Primer3)



Download Page

Home | Download | Tips for running | Example Input | Referencing AutoDimer | FAQ | Support

AutoDimer was packaged for installation using Visual Basic 6.0 I have tested the installation on PCs running Win98, 2000, XP and NT. However, I cannot guarantee installation success for each user's specific computer configuration.

By clicking the link below you will be downloading the file AutoDimer.zip. Once extracted (www.winzip.com), the files can be used to install the AutoDimer program (click setup.exe).

The end user is responsible for the installation and running of the program (this is done at your own risk). The author will not be held responsible for any subsequent computer/operating system issues due to conflicts with the AutoDimer software. AutoDimer is a general tool for screening sequences, we do not guarantee the success of your PCR/assay.

Please click here to download AutoDimer (~5 MB).

John Butler

Jan Redman

STRBase Updates

<u>Primary updates performed monthly</u>

- Summary of variant alleles and tri-allelic patterns
- List of STR references (Reference Manager database)
- NIST publications and presentations

- New content is being added regularly to aid training and to support forensic DNA laboratories

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

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

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

Content of STRBase Website

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

- …/str_fact.htm STR Fact Sheets on Core Loci
- …/multiplx.htm Multiplex STR Kit Information
- …/y_strs.htm Y-Chromosome Information
- …/var_tab.htm Variant Alleles Reported
- …/mutation.htm Mutation Rates for Common STRs
- …/str_ref.htm Reference List with ~2,300 Papers
- …/training.htm Downloadable PowerPoints for Training
- …/validation.htm Validation Information
- …/miniSTR.htm miniSTR Information
- …/address.htm Addresses for Scientists
- …/NISTpub.htm Publications & Presentations from NIST

John Butler

Training Materials and Review Articles

- Workshops on STRs and CE (ABI 310/3100)
  - Taught with Bruce McCord (Florida Int. Univ.)
  - NEAFS (Sept 29-30, 2004)
  - U. Albany DNA Academy (June 13-14, 2005)

- PowerPoint slides from *Forensic DNA Typing*, 2nd Edition

- Review articles
  - ABI 310 and 3100 chemistry – Electrophoresis 2004, 25, 1397-1412
  - Forensic DNA analysis – Anal. Chem. 2005, 77, 3839-3860
  - STR core loci – J. Forensic Sci., *in press* (Nov 2005)

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

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


Margaret Kline


Dave Duewer


Jan Redman


John Butler

Interlaboratory Studies

- DNA Quantitation Study (QS04)
  - 8 DNA samples supplied
  - 84 laboratories signed up (80 labs returned results)
  - 287 data sets using 19 different methods
  - 60 data sets with real-time qPCR (37 Quantifiler data sets)
  - Publication in May 2005: *J. Forensic Sci.* 50(3): 571-578

- Mixture Interpretation Study (MIX05)
  - 91 labs signed up (64 labs returned data)
  - Interpretation requested of provided e-grams for 4 mock sexual assault cases
  - Data analysis is still on-going…

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

Team Impact on Forensic Community

- **27 publications** since June 2004 (61 since 2000)
- **31 presentations** to the community since June 2004
- All NIST publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
- Training materials: 2 workshops conducted with Bruce McCord
  - NEAFS (Sept 29-30, 2004)
  - Albany DNA Academy (June 13-14, 2005)
  - **AAFS Workshop Seattle 2006 (Advanced Topics in STR DNA Analysis)**
- *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*, 2nd Edition (John Butler)

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards


John Butler


Margaret Kline

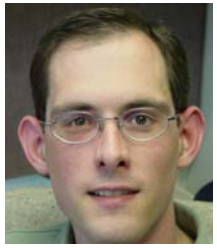

Pete Vallone


Mike Coble


Jan Redman


Amy Decker


Becky Hill


Chris DeAngelis


Dave Duewer

Past and Present Collaborators (also funded by NIJ):

Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies

Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work

Sandy Calloway (Roche) for mtDNA LINEAR ARRAYs

Bruce McCord and students (FL Int. U.) for miniSTR work

Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work

Artie Eisenberg and John Planz (U. North Texas)

Disclaimers and Collaborations

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