Development of New miniSTR Loci for Improved Analysis of Degraded DNA Samples

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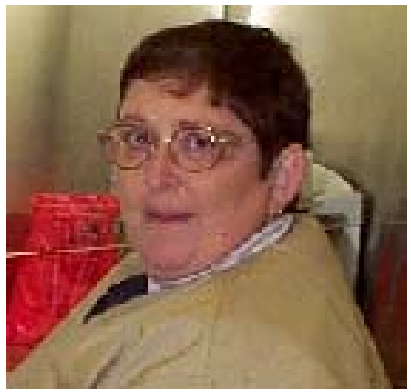
NIST Human Identity Project Team

John Butler



Margaret Kline

Jan Redman

Pete Vallone

Dave Duewer

Amy Decker

Mike Coble

Former (Honorary) Project Team Members

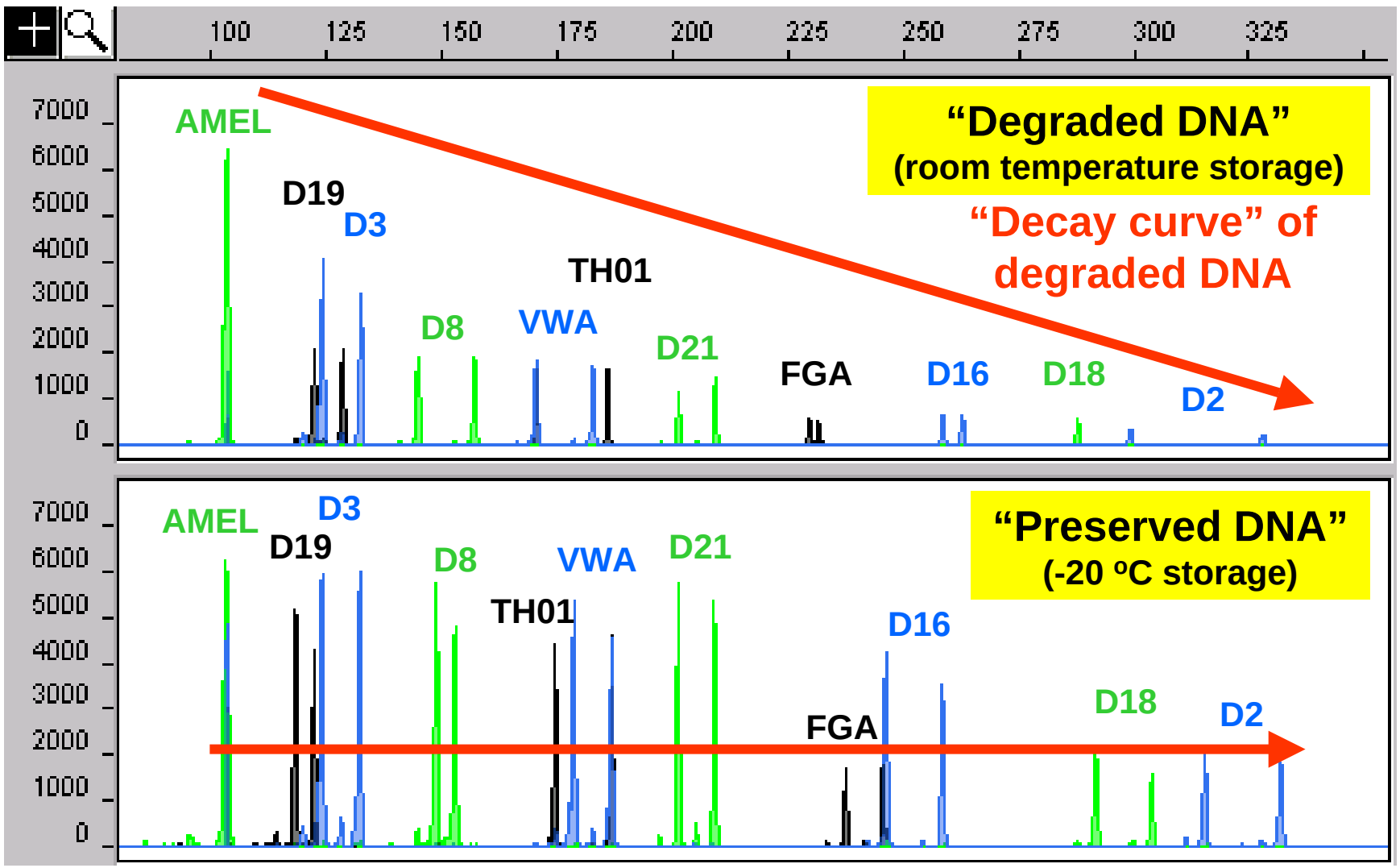
Rich Schoske

Christian Ruitberg

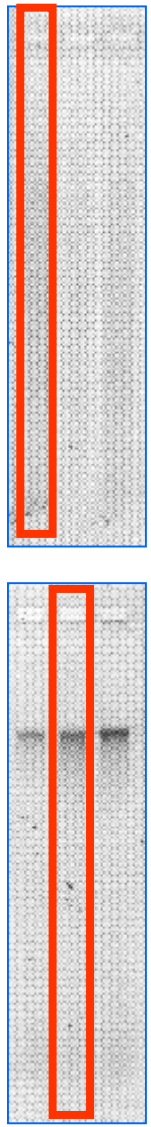
Jill Appleby

Degraded DNA

Loss of Signal for Larger PCR Products



Yield gel results



Results with SGM Plus STR kit (Applied Biosystems)

Data from a study done at NIST in May 2001

STR Size Reduction

Through Moving Primer Positions Closer to Repeat

Primer positions define PCR product size
Repeat information is independent of amplicon size

<u>Advantages of Approach</u>:

Size reduction enhances success rate with degraded DNA

Retains same marker information (database compatibility)

Uses highly polymorphic STR loci (high discriminatory power)

Selected References on STRs with Degraded DNA

- Whitaker, J.P., et al. (1995) Short tandem repeat typing of bodies from a mass disaster: high success rate and characteristic amplification patterns in highly degraded samples. *BioTechniques* 18: 670-677

- Clayton, T.M., et al. (1995) Further validation of a quadruplex STR DNA typing system: a collaborative effort to identify victims of a mass disaster. *Forensic Sci.Int.* 76: 17-25

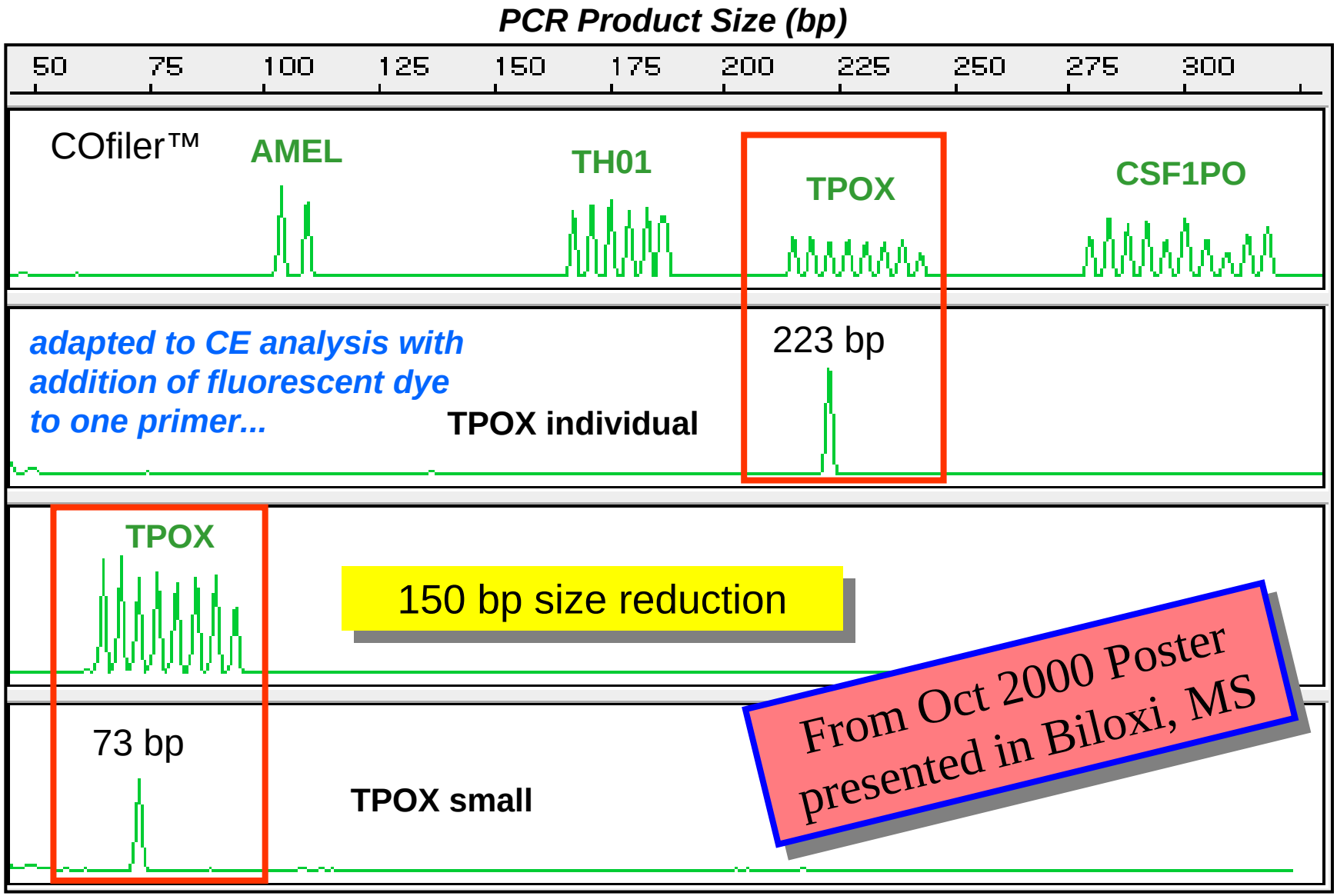
- Yoshida, K., et al. (1997) Evaluation of new primers for CSF1PO. *Int.J.Legal Med.* 110: 36-38

- Schmerer, W.M., et al. (1999) Optimized DNA extraction to improve reproducibility of short tandem repeat genotyping with highly degraded DNA as target. *Electrophoresis* 20: 1712-1716

- Wiegand, P. and Kleiber, M. (2001) Less is more--length reduction of STR amplicons using redesigned primers. *Int.J.Legal Med.* 114: 285-287

Smaller PCR product size improves success rates with degraded DNA

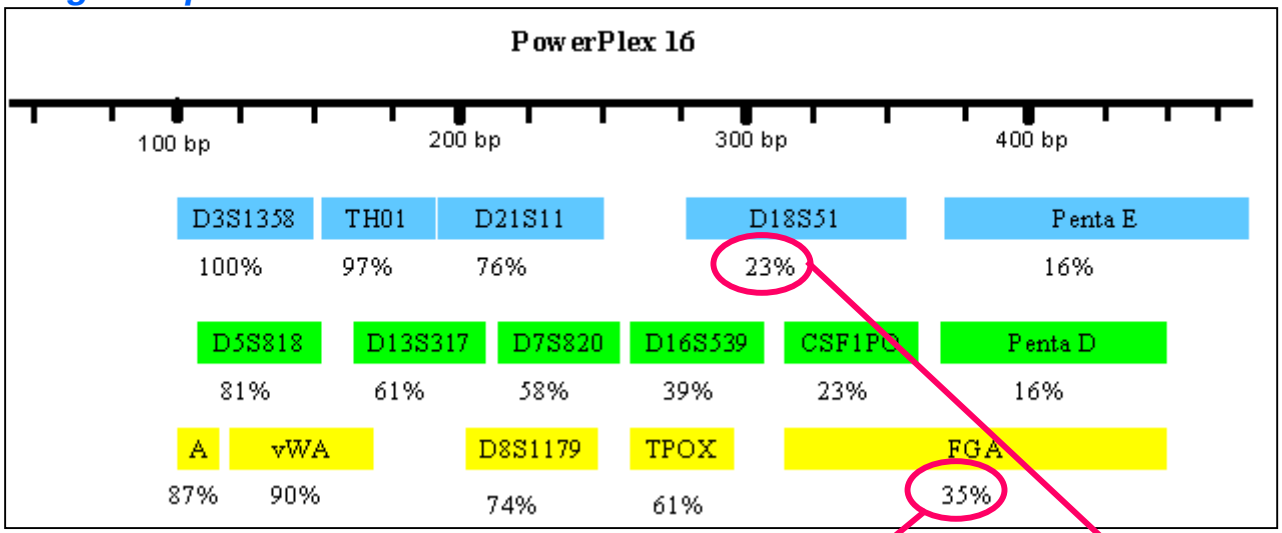
Development of miniSTRs: Past Work



*New primer sets are intended **to aid with typing degraded DNA samples** as well as future microchip CE and mass spectrometry applications…*

Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays

Single amp for 15 STR loci

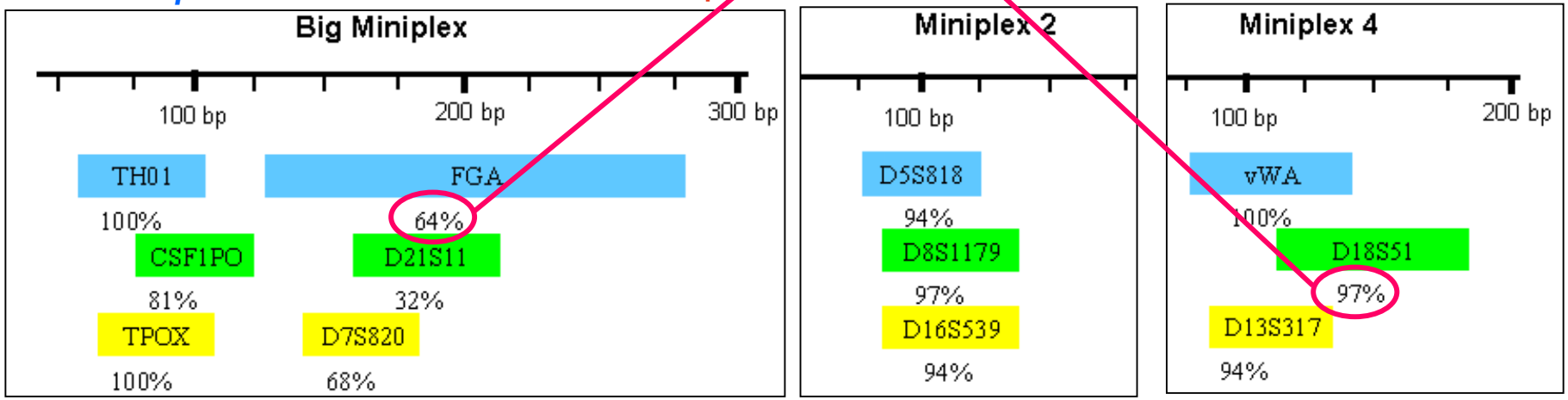


Study with 31 bones from the “Body Farm” (Knoxville, TN) and Franklin County Coroner’s Office (OH)

Three amps for 12 STR loci

-183 bp

-173 bp



Chung, et al., The application of miniplex primer sets in the DNA profiling of human skeletal remains, submitted

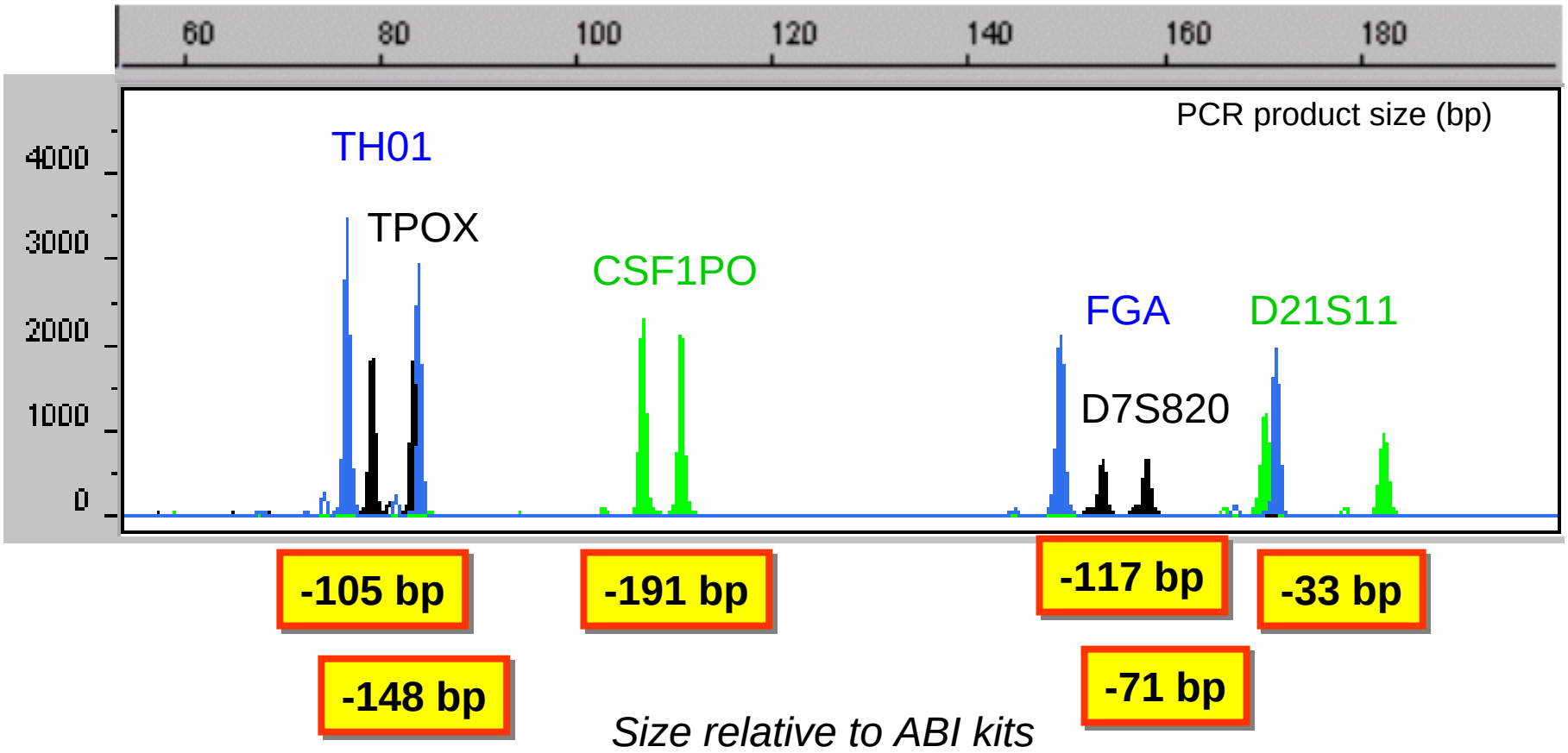
J. Forensic Sci. Sept 2003 issue

J Forensic Sci, September 2003, Vol. 48, No. 5
Paper ID JFS2003043_485
Available online at: www.astm.org

John M. Butler,[1] Ph.D.; Yin Shen,[2,3] Ph.D.; and Bruce R. McCord Ph.D.[2]

The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

Describes new primer sequences for all CODIS loci and initial assays developed



Size relative to ABI kits

Reduction in PCR Product Size

Locus	Size Difference (relative to ABI kits)
TH01	-105 bp
FGA	-71 bp
CSF1PO	-191 bp
D21S11	-33 bp
TPOX	-148 bp
D7S820	-117 bp

Not as much size reduction as other STR loci…

How Close Can a Stable Primer be Designed to the STR Repeat Region?

Locus		Distance 3'end from Repeat	Comment
CSF1PO	F	**14**	*partial repeat just 5' of repeat*
	R	6	
FGA	F	3	
	R	**23**	*partial repeat just 3' of repeat*
TH01	F	0	
	R	1	
TPOX	F	-4	
	R	5	
VWA	F	0	
	R	0	
D3S1358	F	-1	
	R	-1	
D5S818	F	4	
	R	-5	
D7S820	F	4	
	R	**65**	*polyA stretch just 3' of repeat*

Problems with Large Allele Spreads

STR Locus	GenBank Accession	GenBank Allele	Allele Range	Allele Spread
CSF1PO	X14720	12	6–16	40 bp
FGA	M64982	21	12.2–51.2	156 bp
TH01	D00269	9	3–14	44 bp
TPOX	M68651	11	5–14	36 bp
vWA	M25858	18	10–25	60 bp
D3S1358	NT_005997	18	8–20	48 bp
D5S818	AC008512	11	7–16	36 bp
D7S820	AC004848	13	5–15	40 bp
D8S1179	AF216671	13	7–19	48 bp
D13S317	AL353628	11	5–16	44 bp
D16S539	AC024591	11	5–15	40 bp
D18S51	AP001534	18	7–27	80 bp
D21S11	AP000433	29	24–38.2	58 bp
Penta D	AP001752	13	2.2–17	73 bp
Penta E	AC027004	5	5–24	95 bp
D2S1338	AC010136	20	15–28	52 bp

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 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), *in press*.

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

Characterization of New miniSTR Loci

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- Chromosomal locations and marker characteristics
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Candidate STR marker selection

http://research.marshfieldclinic.org/genetics/

http://www.decode.com/

http://www.cidr.jhmi.edu/

Candidate STR marker selection

SCIENCE VOL 298 20 DECEMBER 2002

Genetic Structure of Human Populations

Noah A. Rosenberg,[1]* Jonathan K. Pritchard,[2] James L. Weber,[3] Howard M. Cann,[4] Kenneth K. Kidd,[5] Lev A. Zhivotovsky,[6] Marcus W. Feldman[7]

1: Am J Phys Anthropol. 2003 Nov;122(3):259-68.

Reconstruction of human evolutionary tree using polymorphic autosomal microsatellites.

Ayub Q, Mansoor A, Ismail M, Khaliq S, Mohyuddin A, Hameed A, Mazhar K, Rehman S, Siddiqi S, Papaioannou M, Piazza A, Cavalli-Sforza LL, Mehdi SQ.

Characterization of New miniSTR Loci

Rosenberg *et al.* 2002 – 1062 samples; 377 STRs; diverse populations

Locus name	Alternate name	Heterozygosity	Number of alleles	Chromosome
D6S1017	GGAT3H10	0.748	9	6
D6S1009	GATA32B03	0.748	13	6
D8S261	AFM123XG5	0.747	17	8
D18S1390	18QTEL11	0.747	15	18
D13S1807	GATA11C08	0.747	9	13
D12S1052	GATA26D02	0.747	8	12
D16S2616	ATA41E04	0.746	11	16
D2S1780	GATA72G11	0.746	13	2
NA-D1S-3	GATA133A08	0.745	12	1
NA-D8S-2	GAAT1A4	0.745	9	8
D4S1625	GATA107	0.745	10	4
D18S851	GATA6D09	0.745	12	18
D8S1136	GATA41A01	0.745	11	8
NA-D5S-1	ATA52D02	0.744	27	5
D3S3038	GATA73D01	0.743	14	3
D15S1515	GATA197B10	0.743	9	15
D16S2624	GATA81D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D13S895	GGAA22G01	0.740	11	13
D11S1998	GATA23E06	0.740	9	11

Focus on:

High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

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D18S1897	GATA11C08	0.747	9	18
D12S1052	GATA26D02	0.747	8	12
D16S2616	ATA41E04	0.746	11	16
D2S1780	GATA72G11	0.746	13	2
NA-D1S-3	GATA133A08	0.745	12	1
NA-D8S-2	GAAT1A4	0.745	9	8
D4S1625	GATA107	0.745	10	4
D18S851	GATA6D09	0.745	12	18
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NA-D5S-1	ATA52D02	0.744	27	5
D3S3038	GATA73D01	0.743	14	3
D15S1515	GATA197B10	0.743	9	15
D16S2624	GATA81D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D13S895	GGAA22G01	0.740	11	13
D11S1998	GATA23E06	0.740	9	11
D16S3027	GGAT1A4	0.740	9	16

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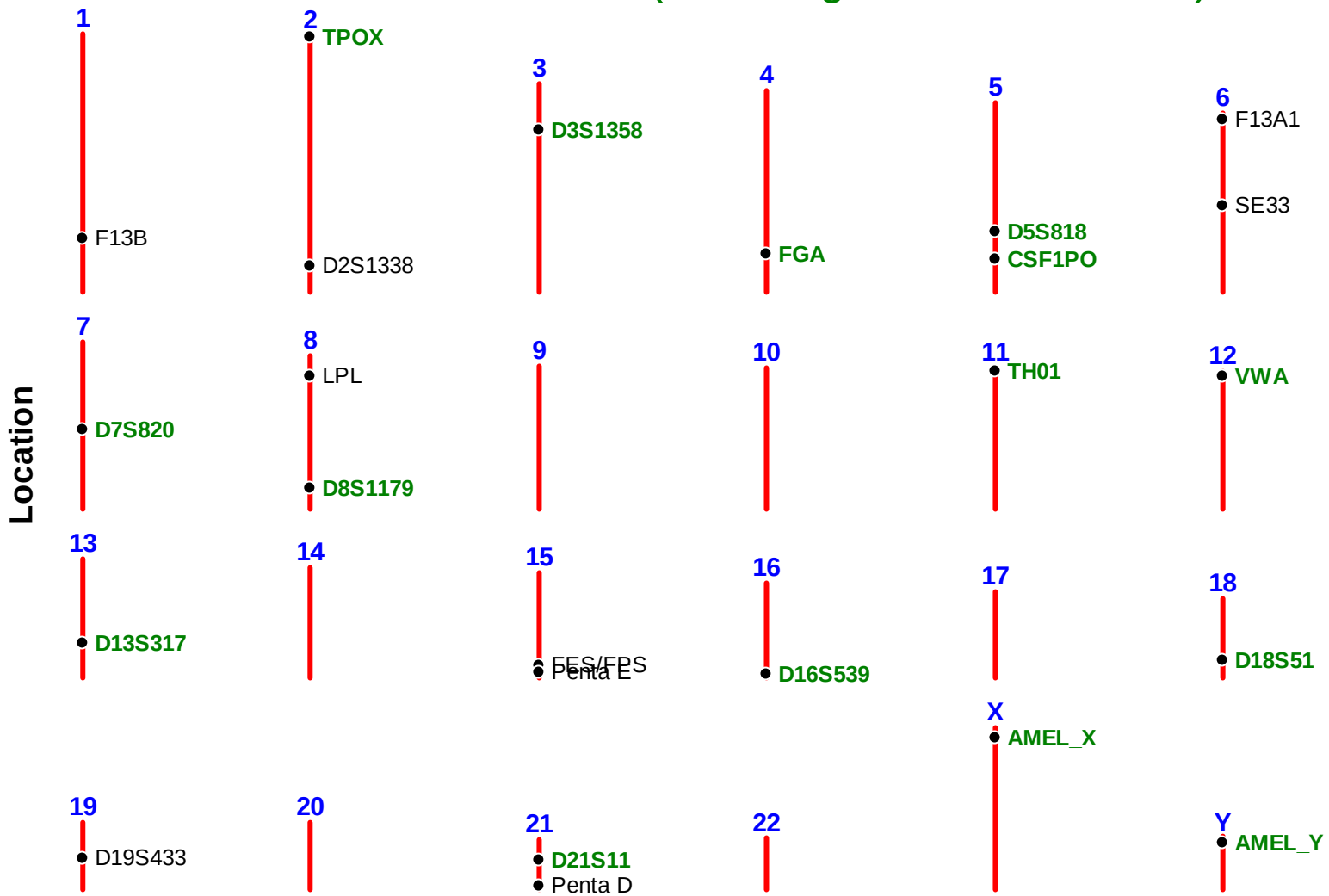
Commercial STR Kit Loci Positions

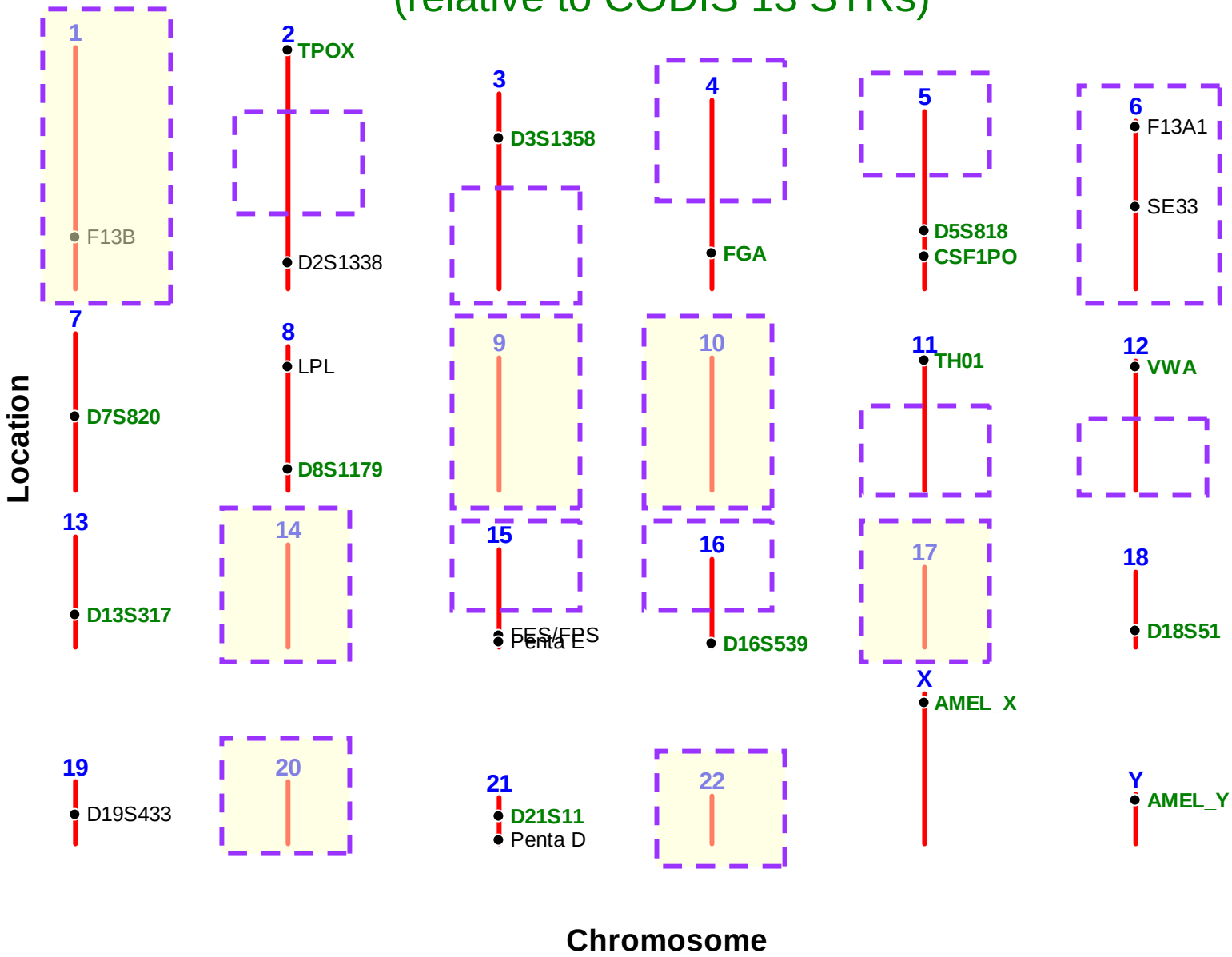
(including CODIS 13 STRs)



Positions determined along July 2003 Human Genome Reference Sequence (NCBI Build 34)

Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



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PCR Primer Design

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/

Primer3

pick primers from a DNA sequence (see NEW)

disclaimer | source code

cautions

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library): NONE

☑ Pick left primer or use left primer below.

☐ Pick hybridization probe (internal oligo) or use oligo below.

☑ Pick right primer or use right primer below (5'->3' on opposite strand).

Pick Primers | Reset Form

Sequence Id: A string to identify your output.

Targets: E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.

Excluded Regions: E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.

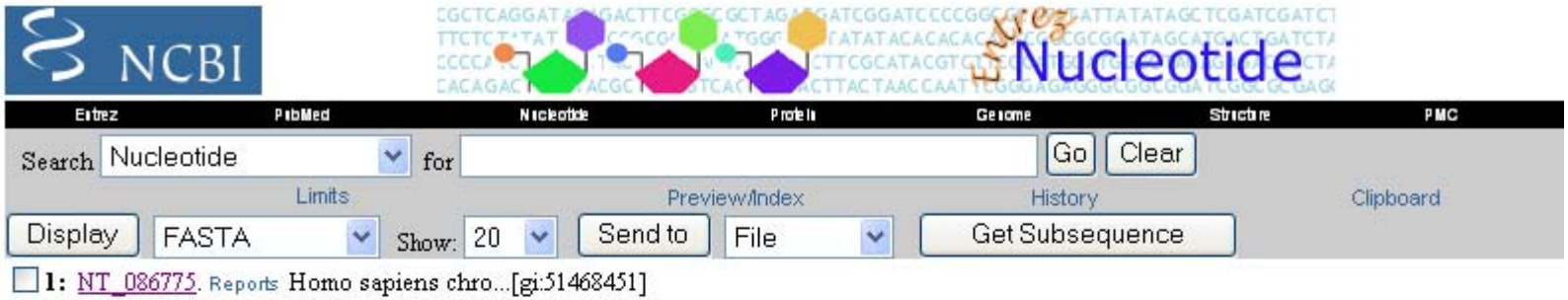
NEW Product Size Ranges 150-250 100-300 301-400 401-500 501-600 601-700 701-850 851-1000

Click here to specify the min, opt, and max product sizes only if you absolutely must. Using them is too slow (and too computationally intensive for our server).

Number To Return: 5 Max 3' Stability: 9.0

Max Mispriming: 12.0 Pair Max Mispriming: 24.0

PCR Primer Design

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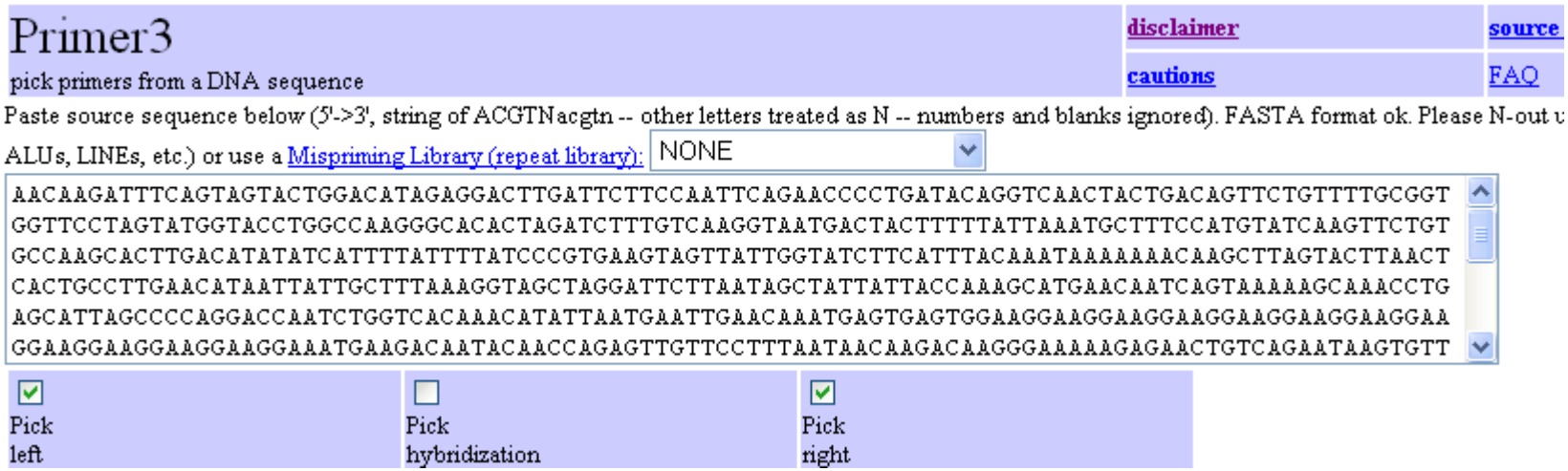
Copy sequence data around the repeat from GenBank

http://www.ncbi.nlm.nih.gov/

PCR Primer Design

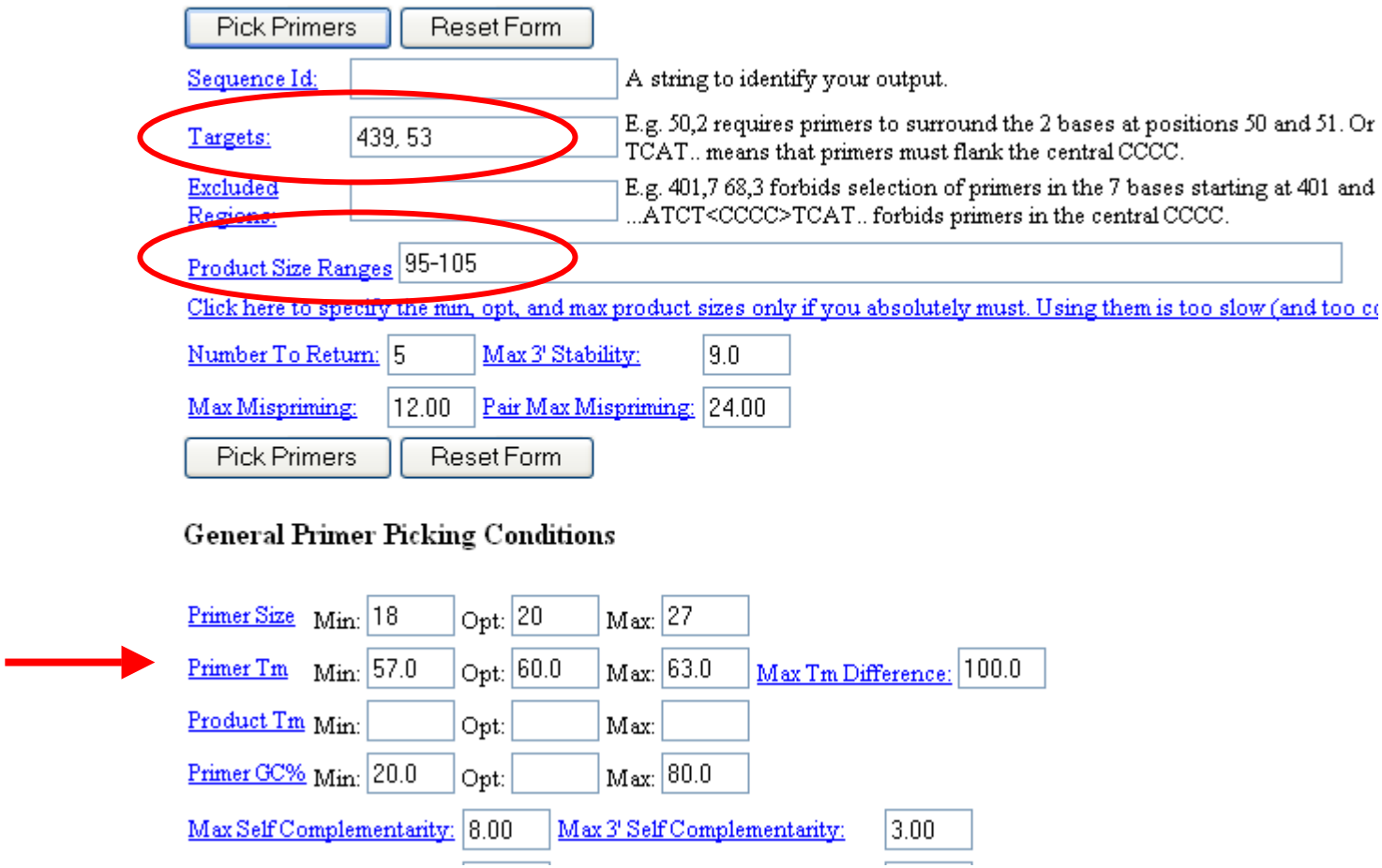
http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/



Paste into Primer3

PCR Primer Design

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/



PCR Primer Design

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/

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No mispriming library specified
Using 1-based sequence positions
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RIGHT PRIMER       516   25   59.16   36.00  7.00  1.00 AACAACTCTGGTTGTATTGTCTTCA
SEQUENCE SIZE: 1050
INCLUDED REGION SIZE: 1050
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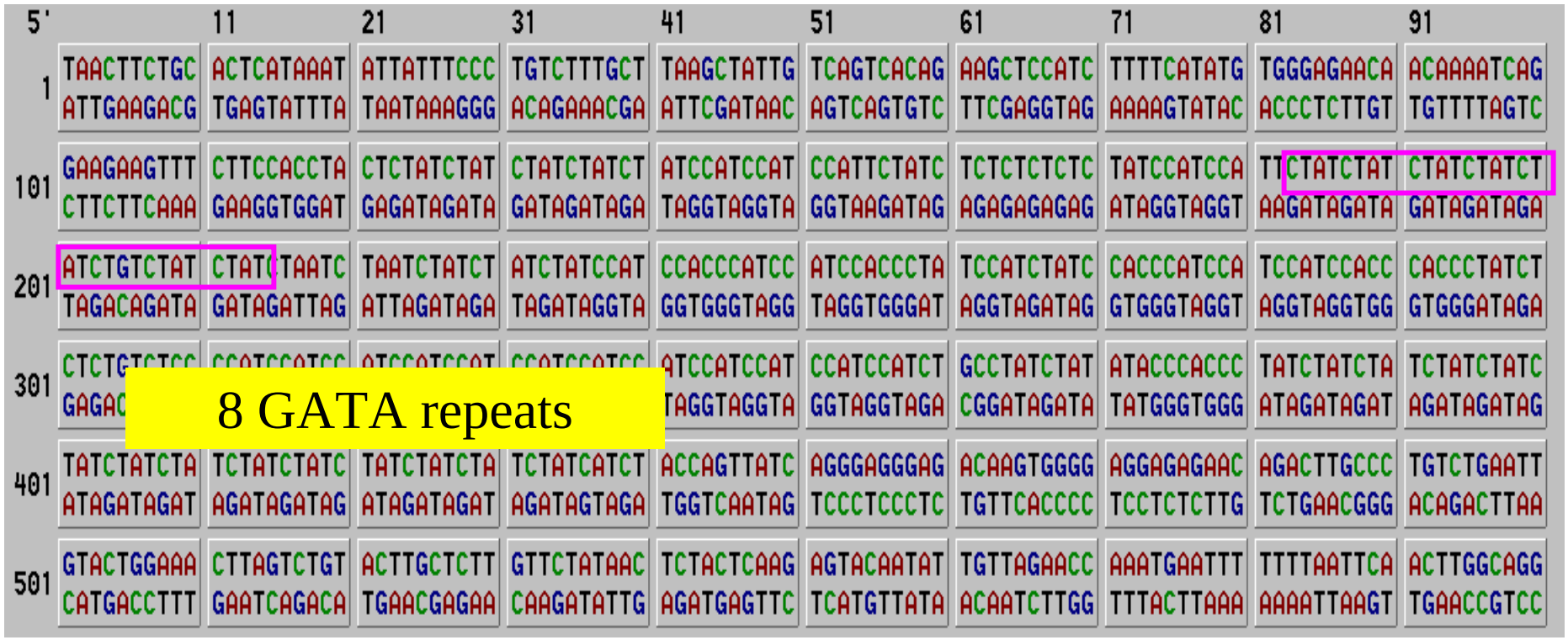
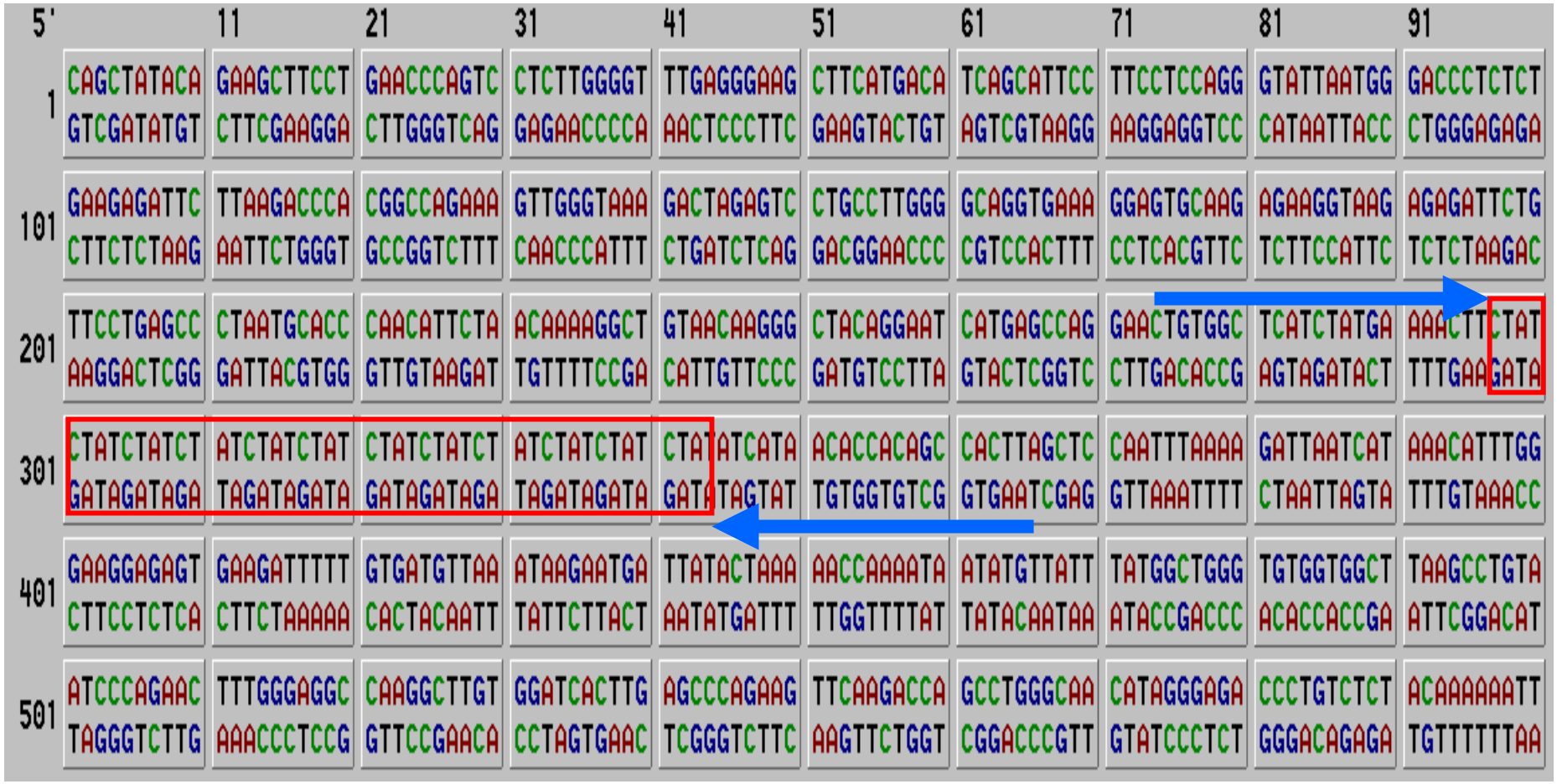
PCR Primer Design



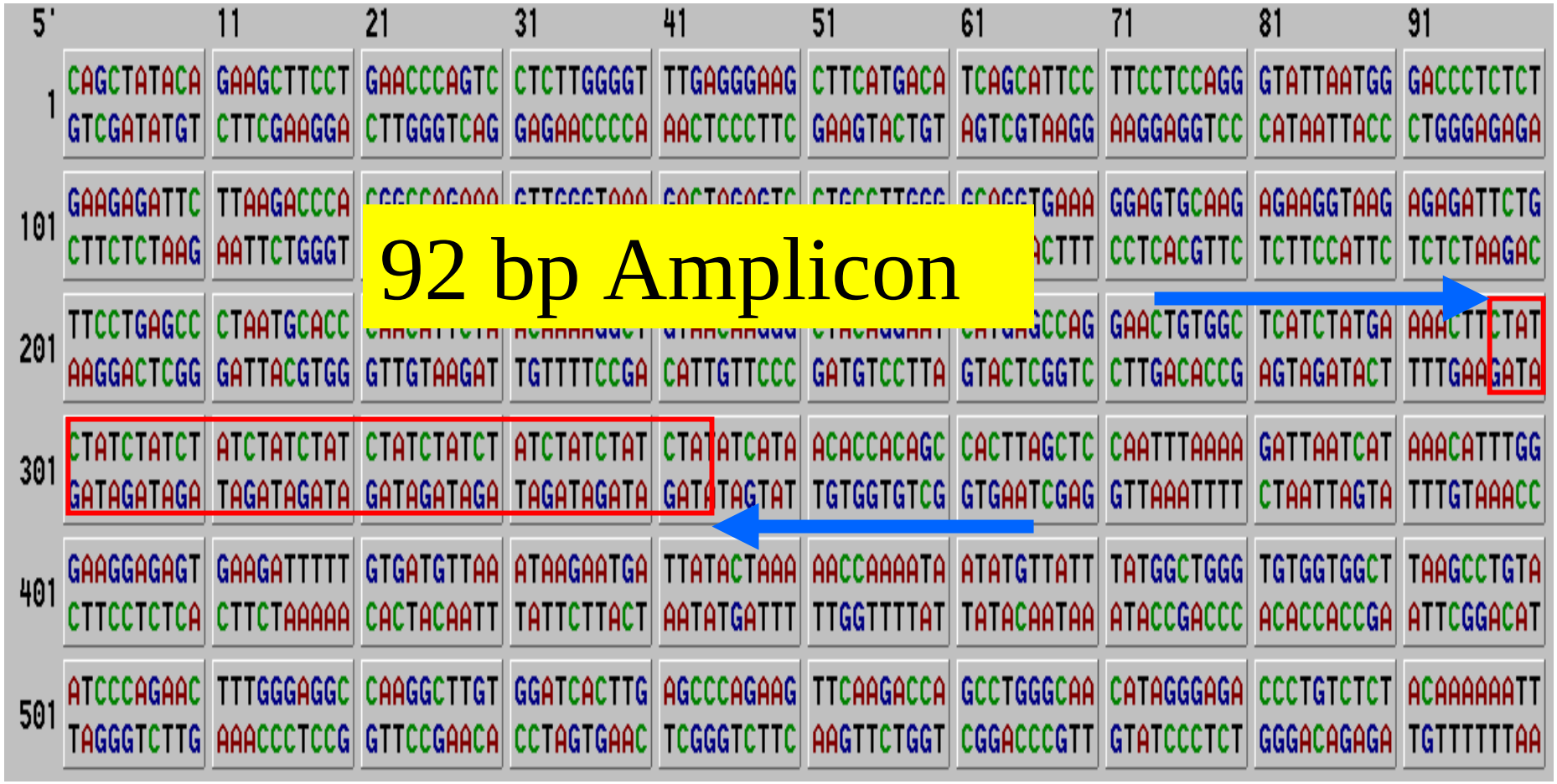
PCR Primer Design

PCR Primer Design



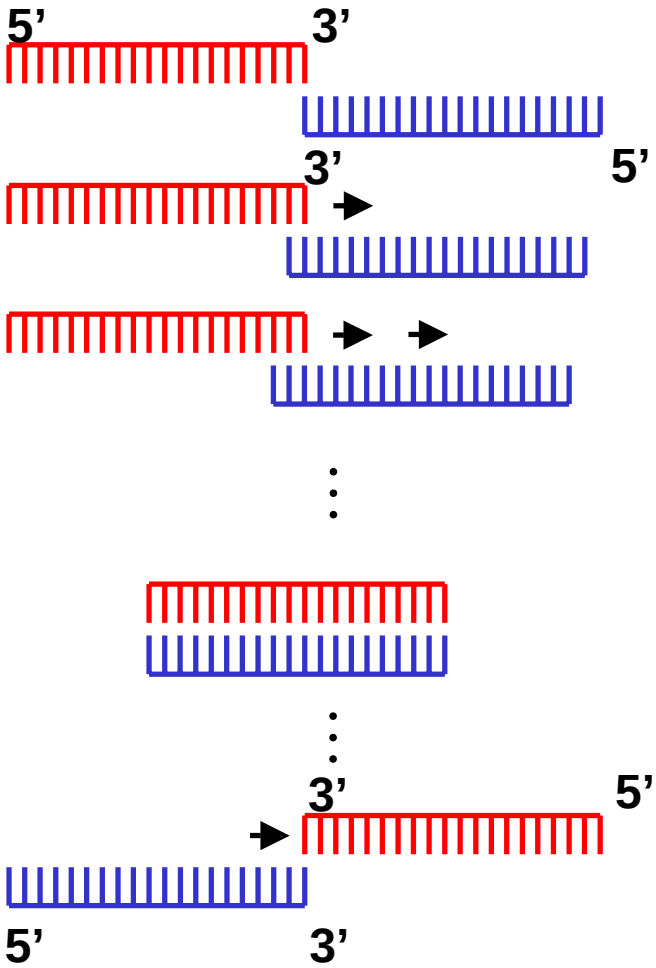
D2S441

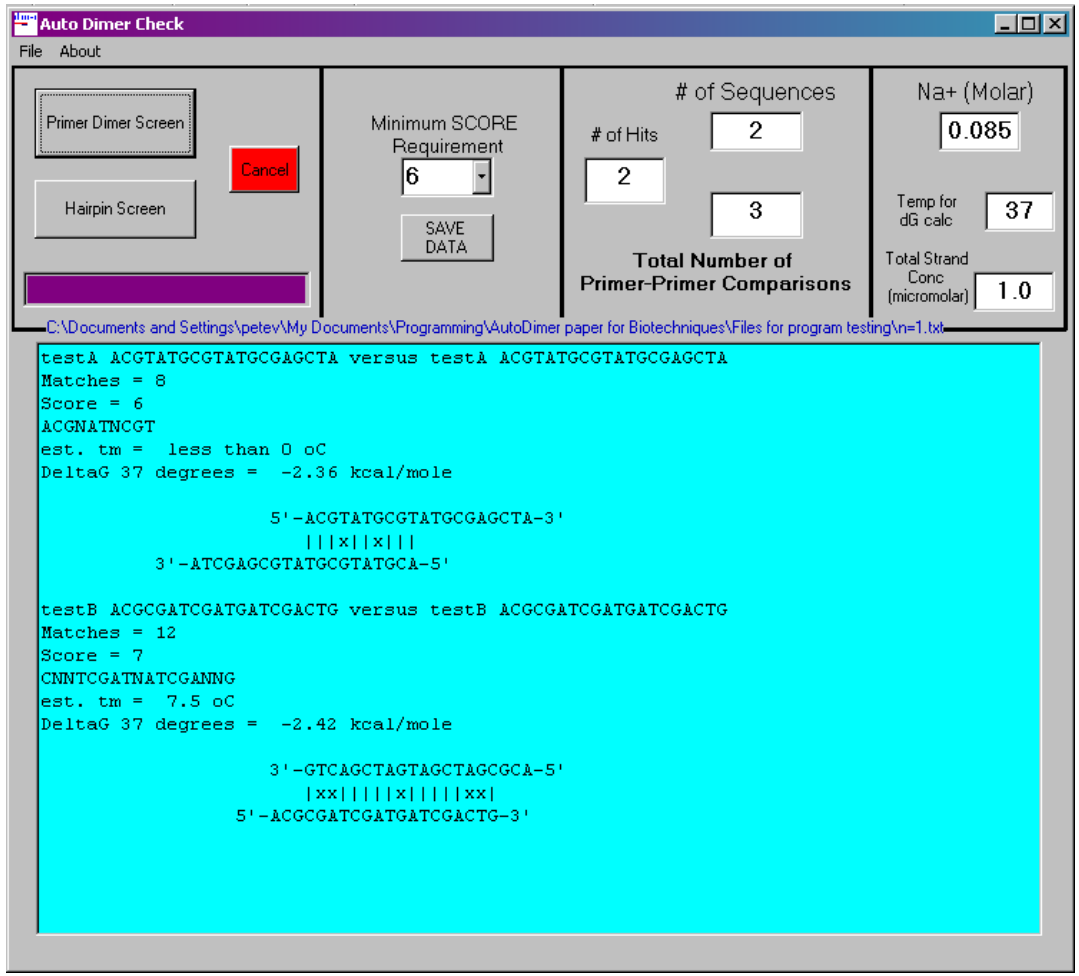
PCR Primer Design



D2S441

AutoDimer – primer screening software is now freely available





http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm BioTechniques (2004) 37: 226-231

Characterization of New miniSTR Loci

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Initial Testing Results

107 potential markers

↓

61 markers with “clean” flanking regions

↓

43 markers with amplicon size < 125bp

↓

18 markers for initial testing

↓

2 three loci miniplexes

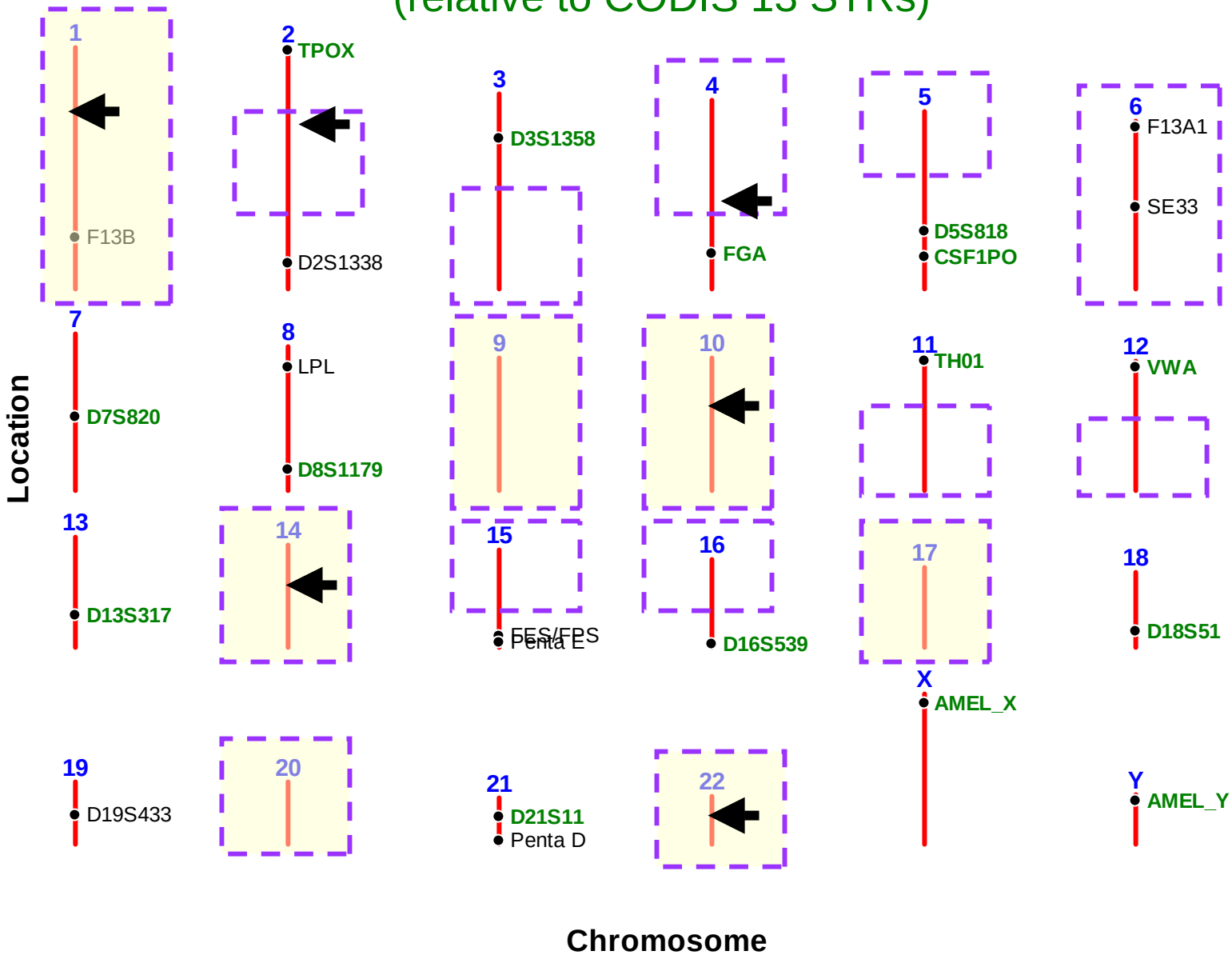
Initial Testing Results

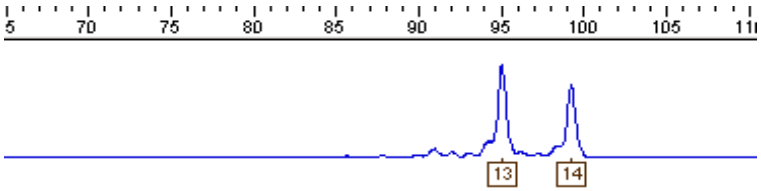
Miniplex01- mD10S1248 - FAM
mD14S1434 - VIC
mD22S1045 - NED

Miniplex02- mD4S2364 - FAM
mD2S441 - VIC
mD1S1677 - NED

Locations of Focus for New miniSTR Loci

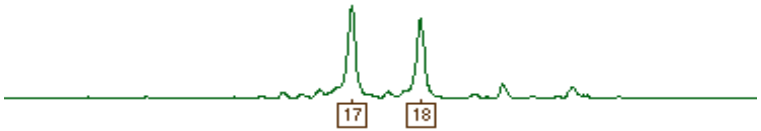
(relative to CODIS 13 STRs)





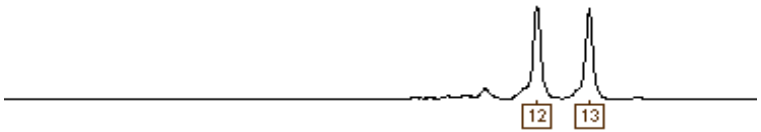
D10S1248

6-FAM



D14S1434

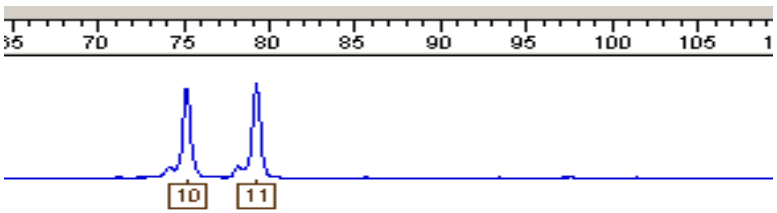
VIC



D22S1045

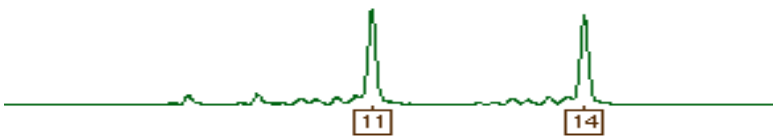
NED

Miniplex01



D4S2364

6-FAM



D2S441

VIC

D1S1677

NED

Miniplex02

miniSTR characteristics

STR Locus	Sequence Motif	Allele Range	Size Range (bp)	Observed Heterozygosity
D1S1677	$(GGAA)_n$	9-18	81-117	0.75
D2S441	$(TCTA)_n$	9-17	78-110	0.76
D4S2364	$(GAAT)(GGAT)(GAAT)_n$	8-12	67-83	0.53
D10S1248	$(GGAA)_n$	10-20	83-123	0.78
D14S1434	$(GATA)_n(GACA)_n$	13-20	70-98	0.68
D22S1045	$(TAA)_n$	5-16	76-109	0.77

Characterization of New miniSTR Loci

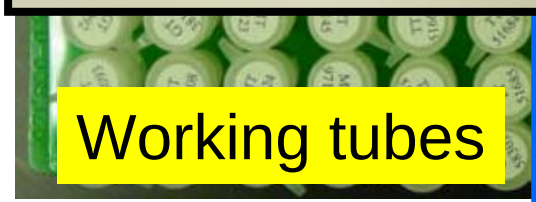
- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance

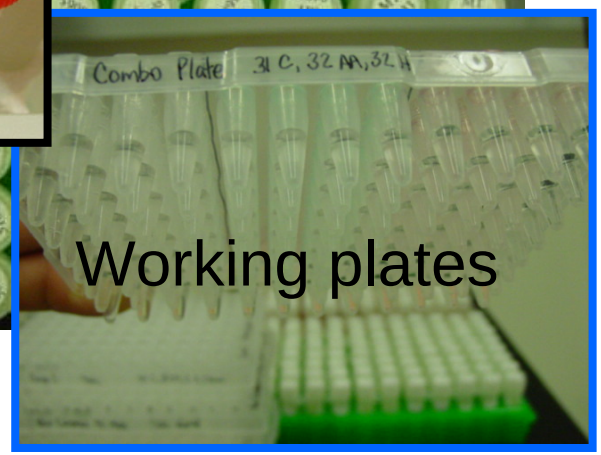
NIST U.S. Population Samples

As of 06/2003 666 males (anonymous; self-identified ethnicities)

286 Caucasians
252 African Americans
128 Hispanics

Whole blood received from
Interstate Blood Bank (Memphis, TN)





Working tubes/plates 1 ng/uL

To date: (~50,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)

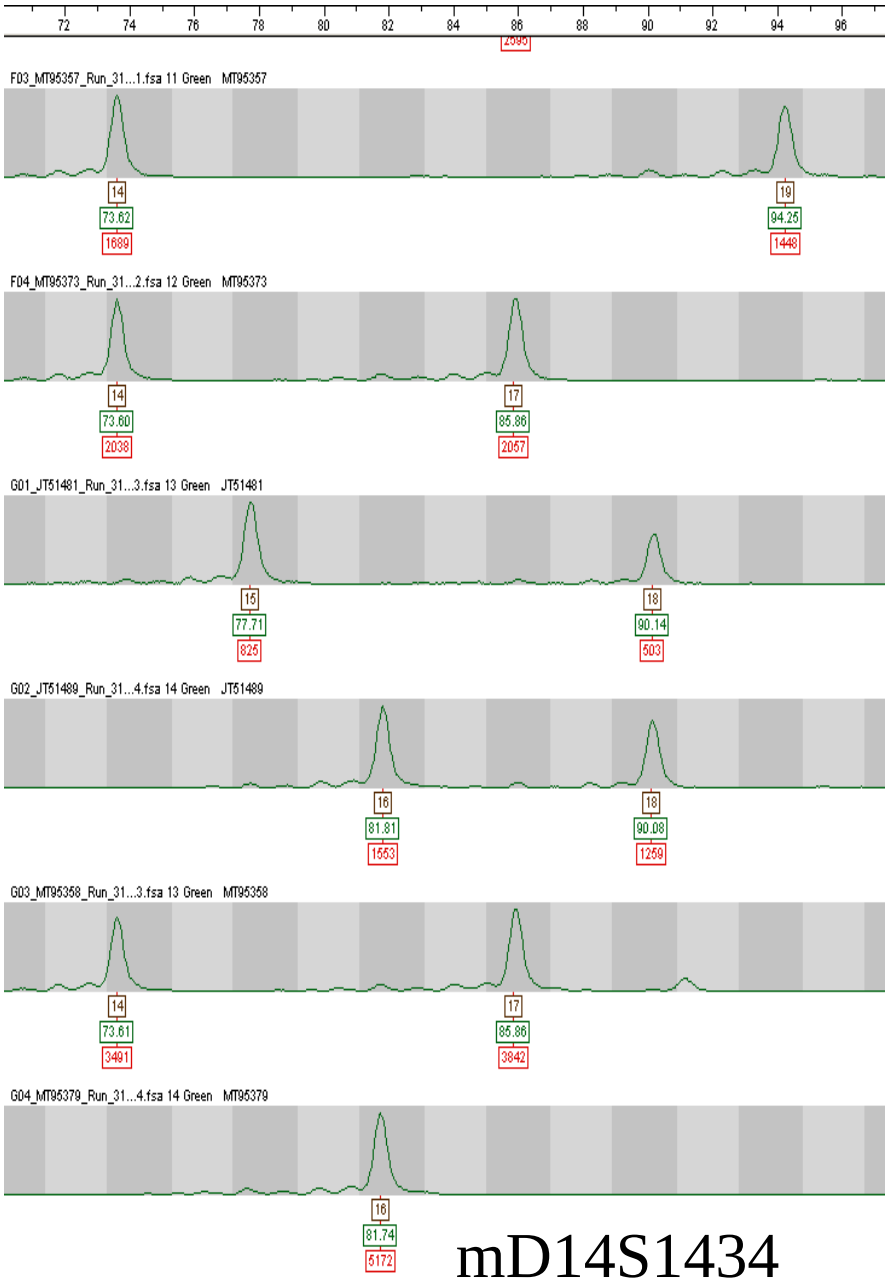
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)

Y STRs 22 loci—27 amplicons (17,388)

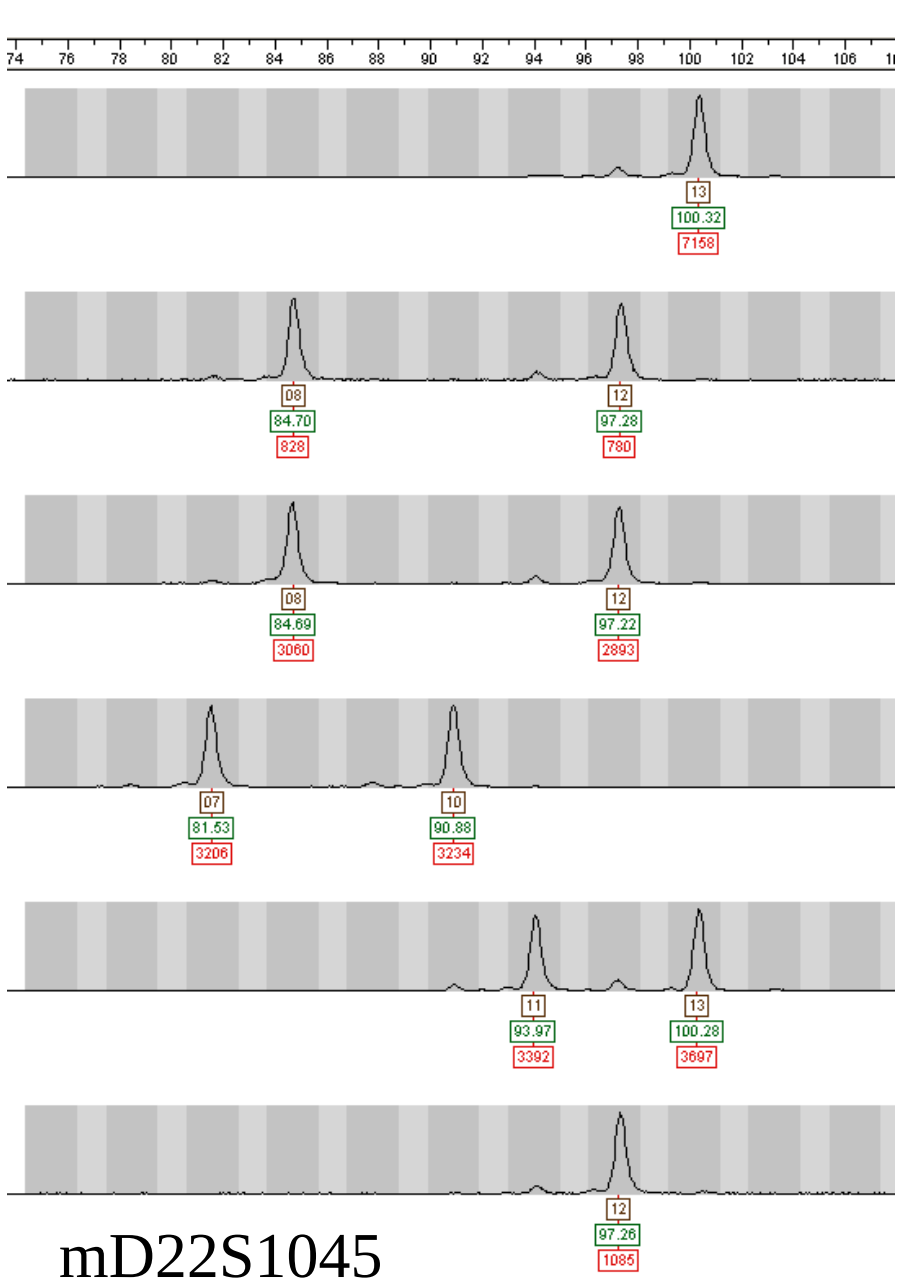
Y SNPs 50 markers on sub-set of samples (11,498)

Samples supplied to **OhioU** for miniSTR typing and **AFDIL** for whole mtGenome sequencing

Population Sample Typing with Genotyper Macos

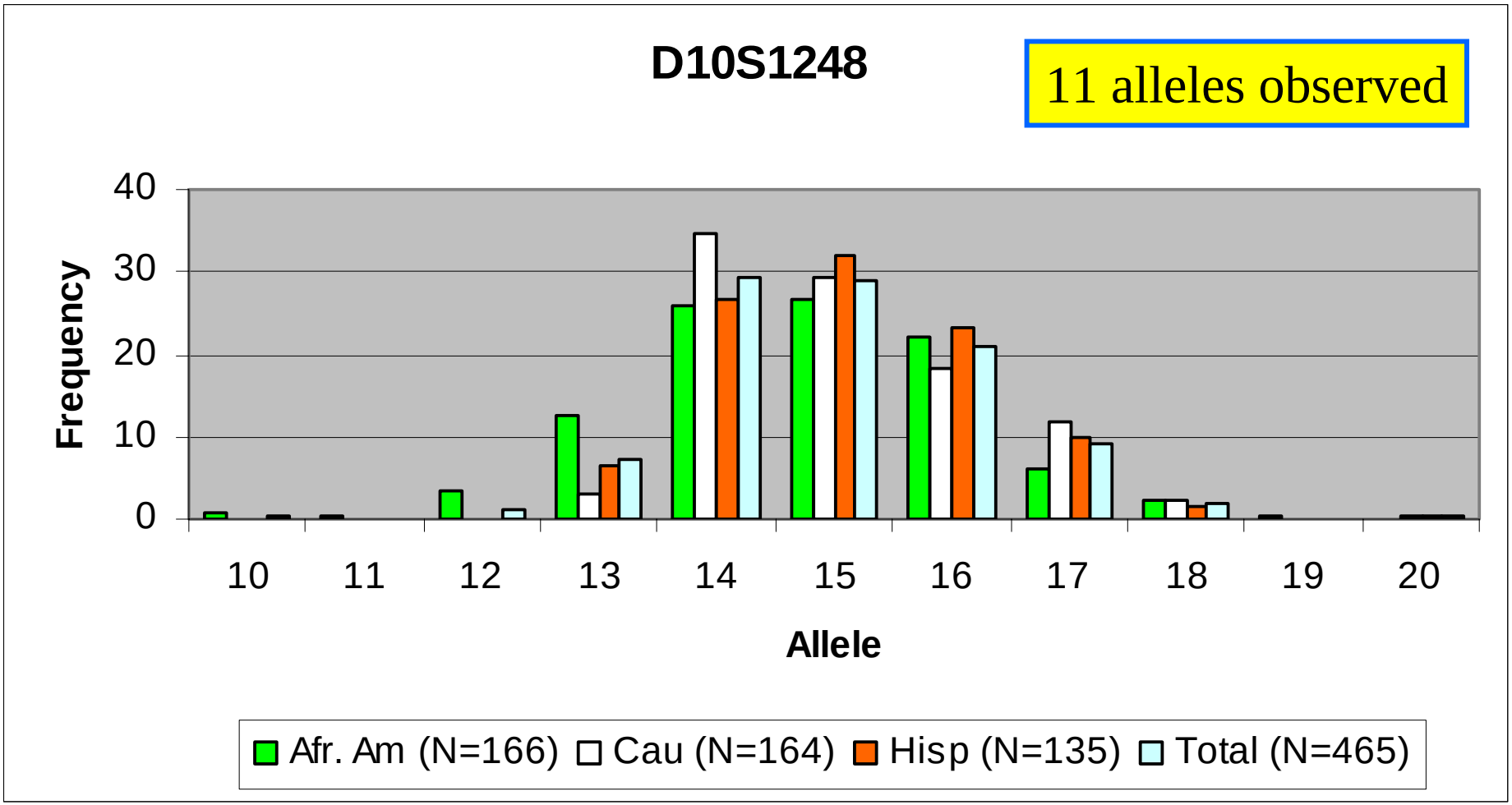


mD14S1434

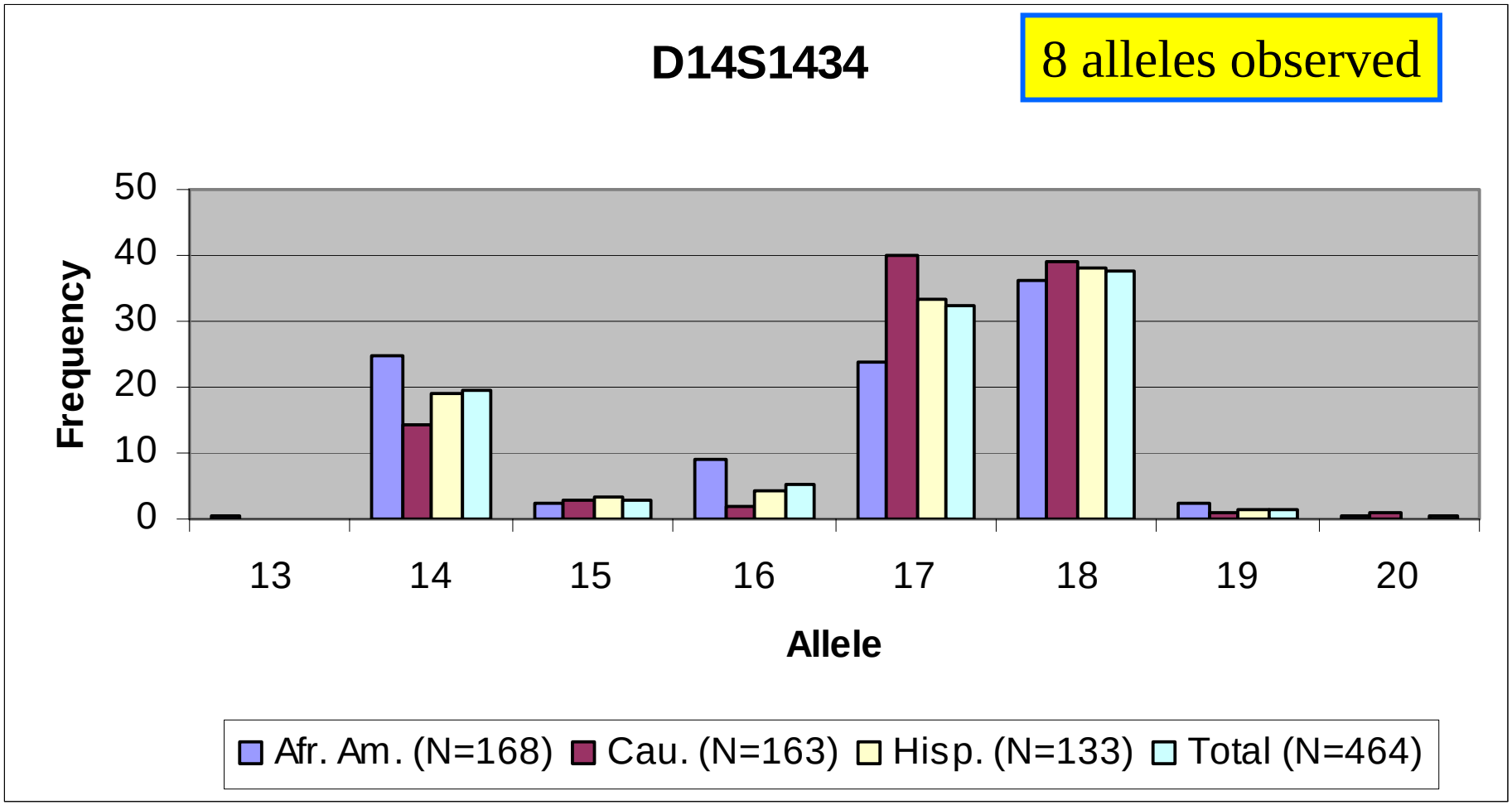


mD22S1045

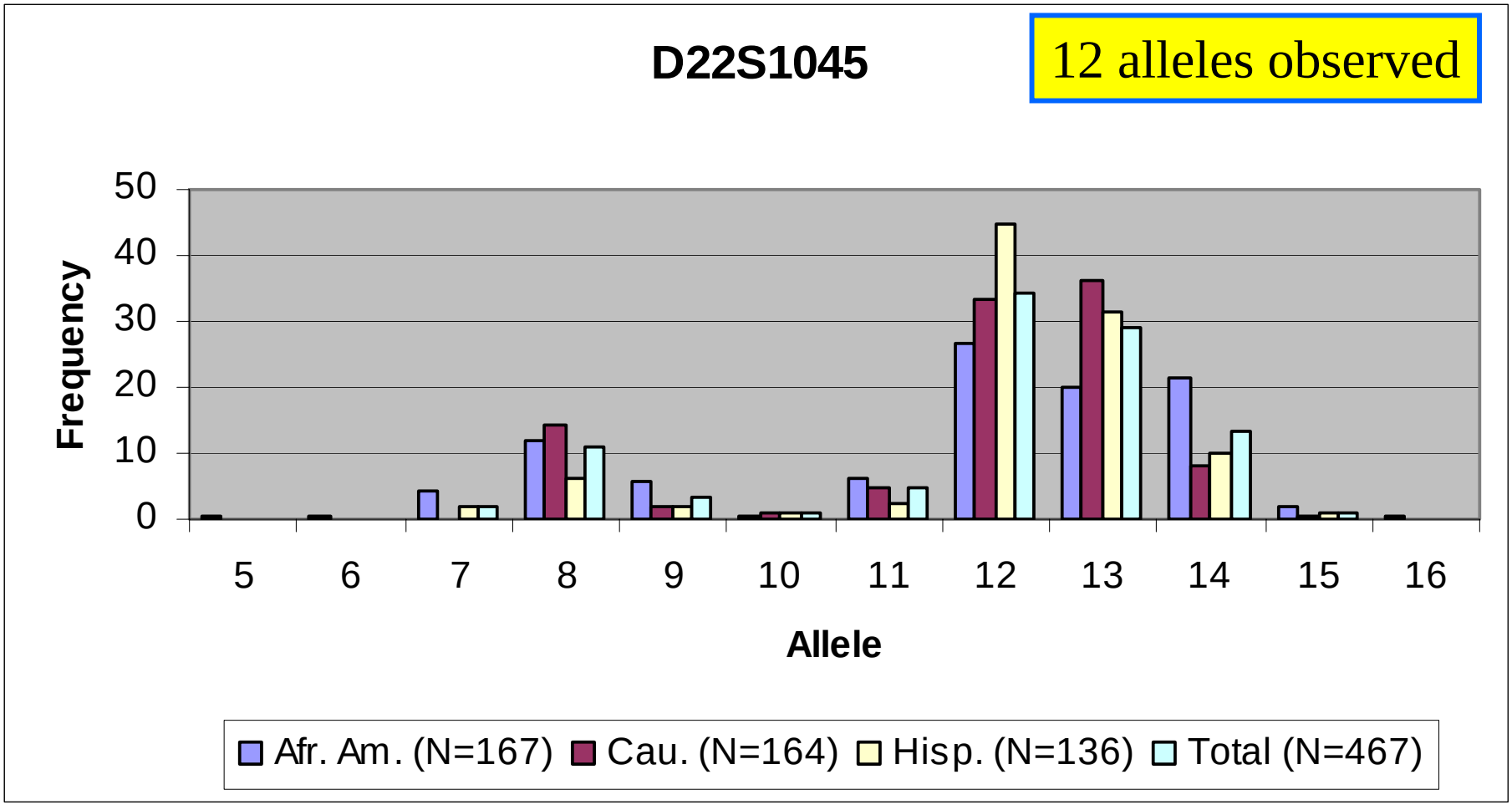
Population Data Analysis



Population Data Analysis



Population Data Analysis



Population Testing –Miniplexes vs. Identifiler

Heterozygosity	Marker
0.8784	D2S1338
0.8753	D18S51
0.8710	FGA
0.8393	D21S11
0.8245	vWA
0.8076	D7S820
0.7970	D19S433
0.7759	mD10S1248 - mini01
0.7759	D16S539
0.7674	mD22S1045 - mini01
0.7674	D8S1179
0.7590	mD2S441 - mini02
0.7548	D3S1358
0.7526	D13S317
0.7463	mD1S1677 - mini02
0.7378	CSF1PO
0.7378	TH01
0.7294	D5S818
0.7146	TPOX
0.6765	mD14S1434 - mini01
0.5307	mD4S2364 - mini02

N = 474 Individuals

164 African Americans

170 Caucasians

140 Hispanics

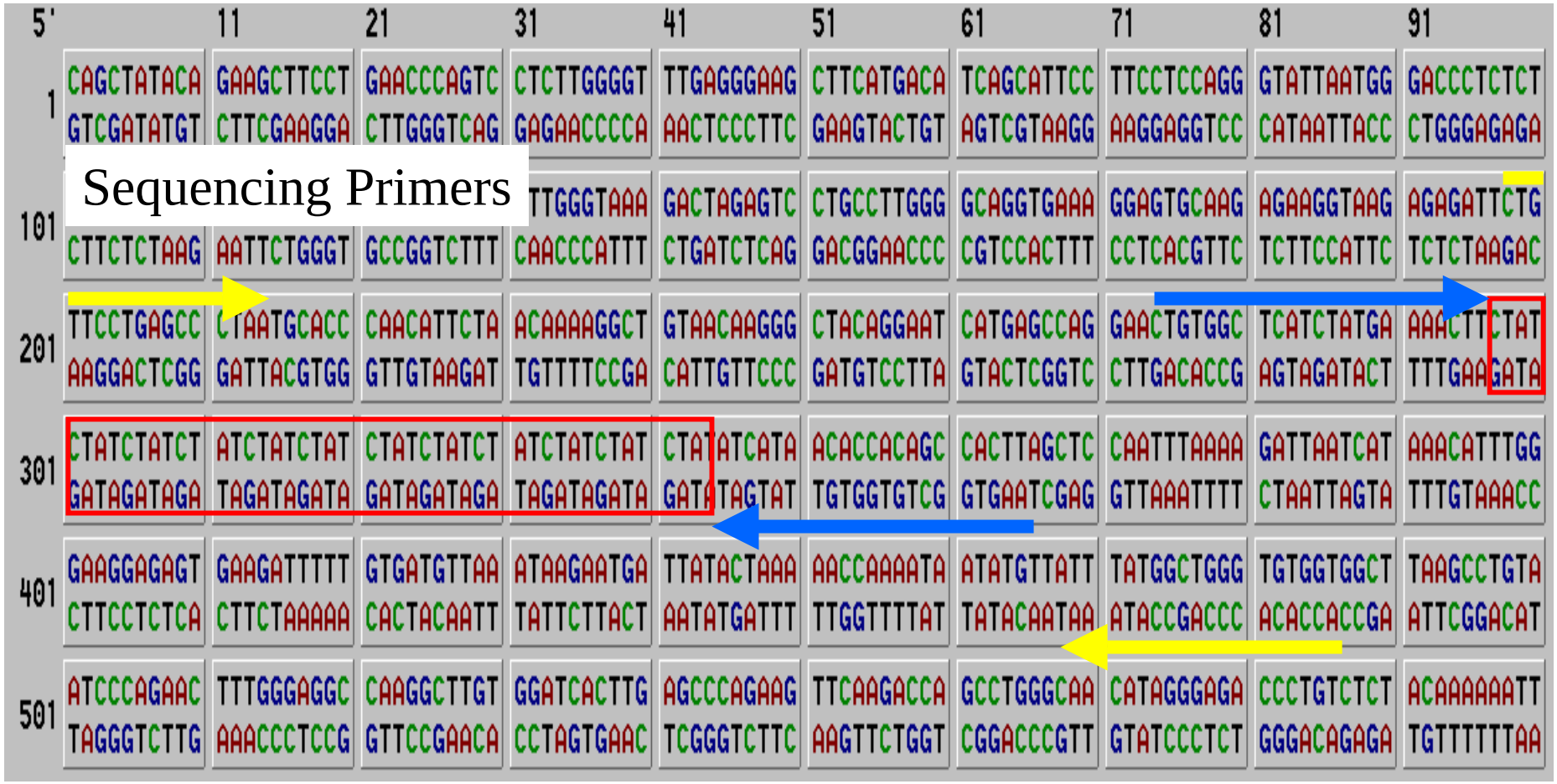
Coble and Butler, *J. Forensic Sci, submitted*

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

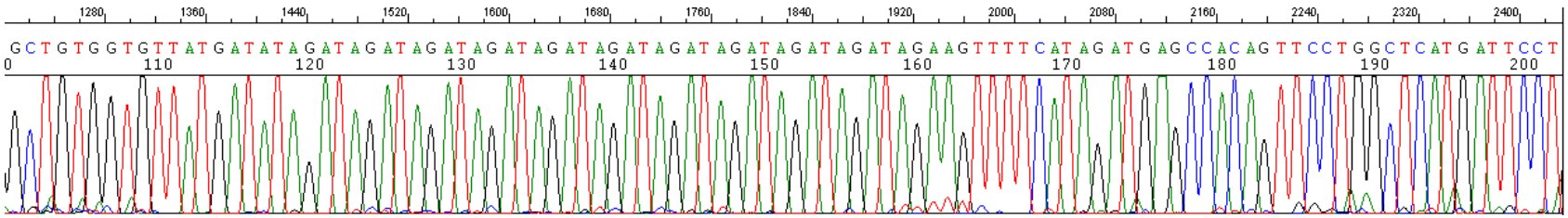
Characterization of New miniSTR Loci

D2S441

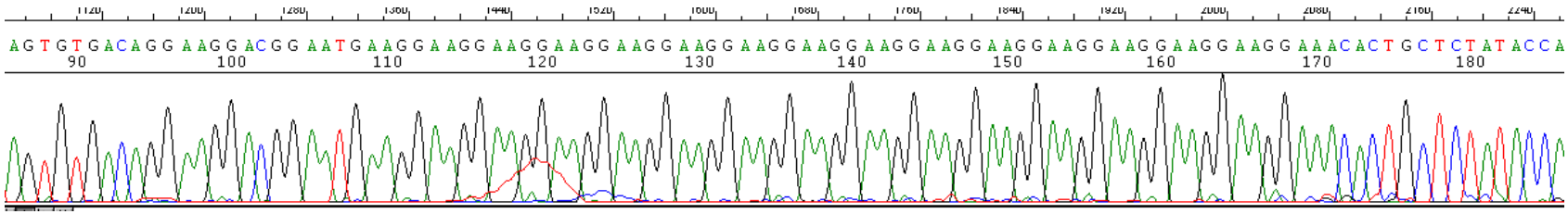


Characterization of New miniSTR Loci

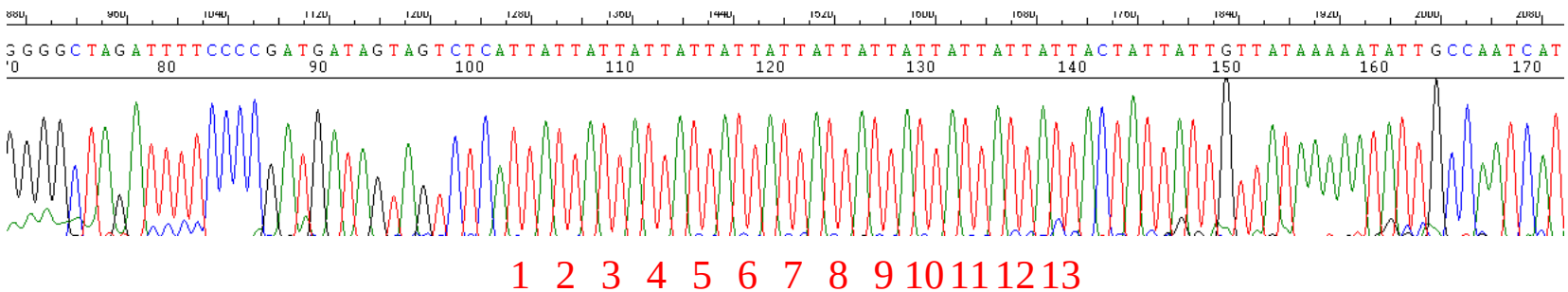
mD2S441



mD1S1677



mD22S1045

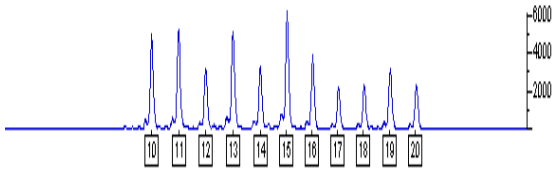


miniSTR Assay

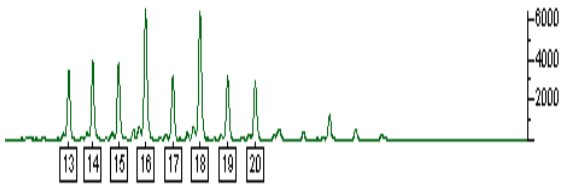
Allelic Ladders

Miniplex01

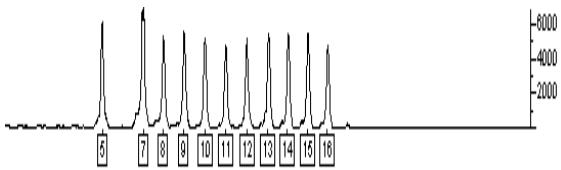
D10S1248



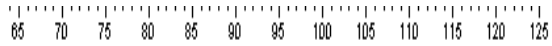
D14S1434



D22S1045

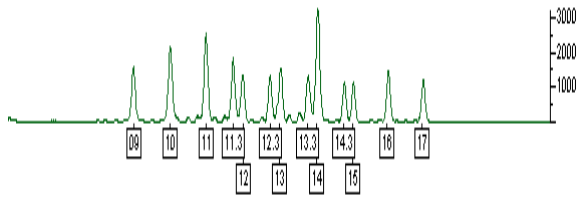


Miniplex02

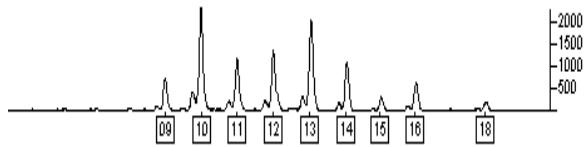


D4S2364

D2S441



D1S1677



```
D10S1248
  09            Highest peak at    79.00 ±    0.50 bp in blue
  10            Highest peak at    83.00 ±    0.50 bp in blue
  11            Highest peak at    87.00 ±    0.50 bp in blue
  12            Highest peak at    91.00 ±    0.50 bp in blue
  13            Highest peak at    95.00 ±    0.50 bp in blue
  14            Highest peak at    99.00 ±    0.50 bp in blue
  15            Highest peak at   103.00 ±    0.50 bp in blue
  16            Highest peak at   107.00 ±    0.50 bp in blue
  17            Highest peak at   111.00 ±    0.50 bp in blue
  18            Highest peak at   115.00 ±    0.50 bp in blue
  19            Highest peak at   119.00 ±    0.50 bp in blue
  20            Highest peak at   123.00 ±    0.50 bp in blue
D14S1434
  13            Highest peak at    70.40 ±    1.00 bp in green
  14            Highest peak at    74.30 ±    1.00 bp in green
  15            Highest peak at    78.20 ±    1.00 bp in green
  16            Highest peak at    82.10 ±    1.00 bp in green
  17            Highest peak at    86.00 ±    1.00 bp in green
  18            Highest peak at    89.90 ±    1.00 bp in green
  19            Highest peak at    93.80 ±    1.00 bp in green
  20            Highest peak at    97.70 ±    1.00 bp in green
D22S1045
  05            Highest peak at    75.40 ±    1.00 bp in yellow
  06            Highest peak at    78.50 ±    1.00 bp in yellow
  07            Highest peak at    81.60 ±    1.00 bp in yellow
  08            Highest peak at    84.70 ±    1.00 bp in yellow
  09            Highest peak at    87.80 ±    1.00 bp in yellow
  10            Highest peak at    90.90 ±    1.00 bp in yellow
  11            Highest peak at    94.00 ±    1.00 bp in yellow
  12            Highest peak at    97.10 ±    1.00 bp in yellow
  13            Highest peak at   100.20 ±    1.00 bp in yellow
  14            Highest peak at   103.30 ±    1.00 bp in yellow
  15            Highest peak at   106.40 ±    1.00 bp in yellow
  16            Highest peak at   109.50 ±    1.00 bp in yellow
```

Macros for analysis have been developed

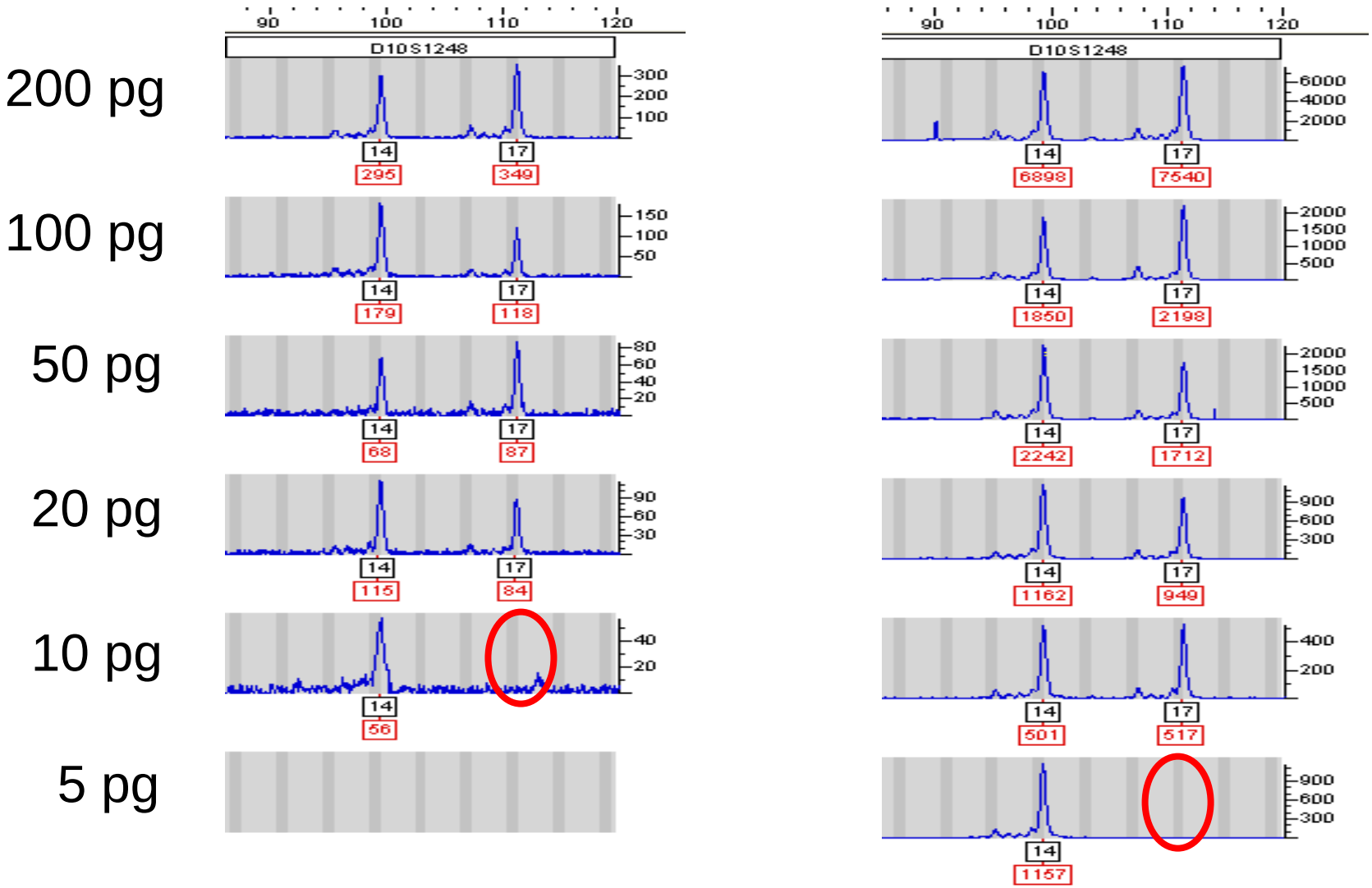
Characterization of New miniSTR Loci

- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance
  - Sensitivity
  - Inheritance with family samples
  - Allele sizing precision
  - Locus stutter percentage characterization
  - Analysis on real-world samples

miniSTR Assay Sensitivity (D10S1248)

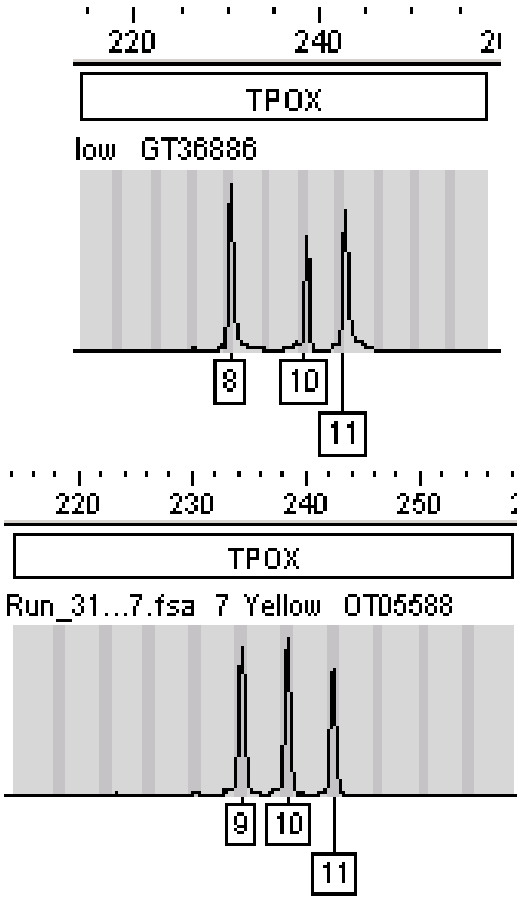
28 cycles – 1U Taq

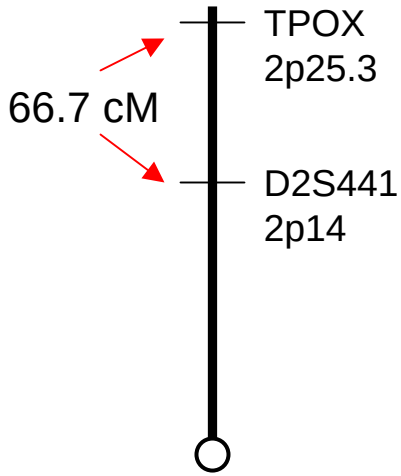
32 cycles – 2U Taq



Concordance on Tri-Allelic Patterns for TPOX

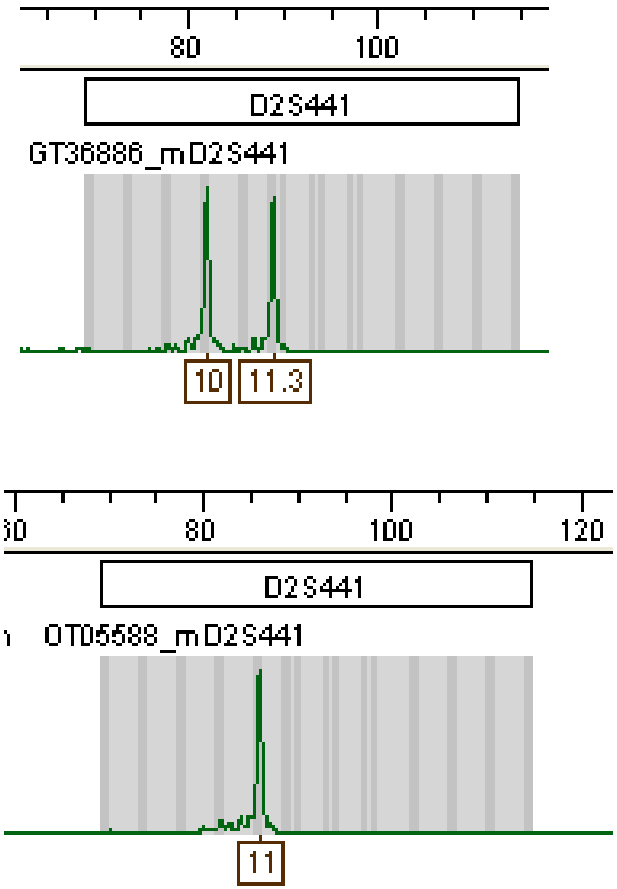
Identifiler Data





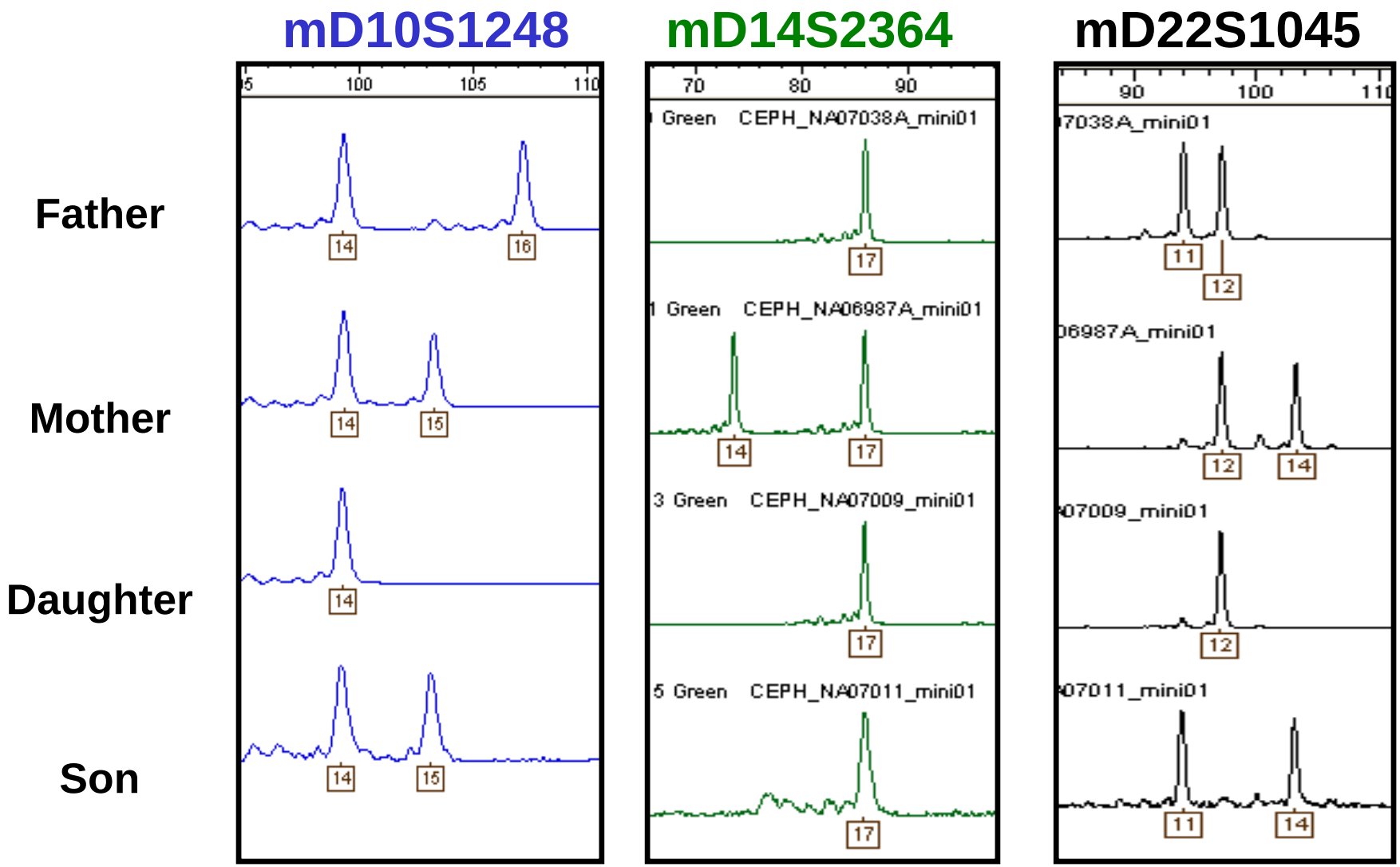
Chromosome 2

mD2S441 Data

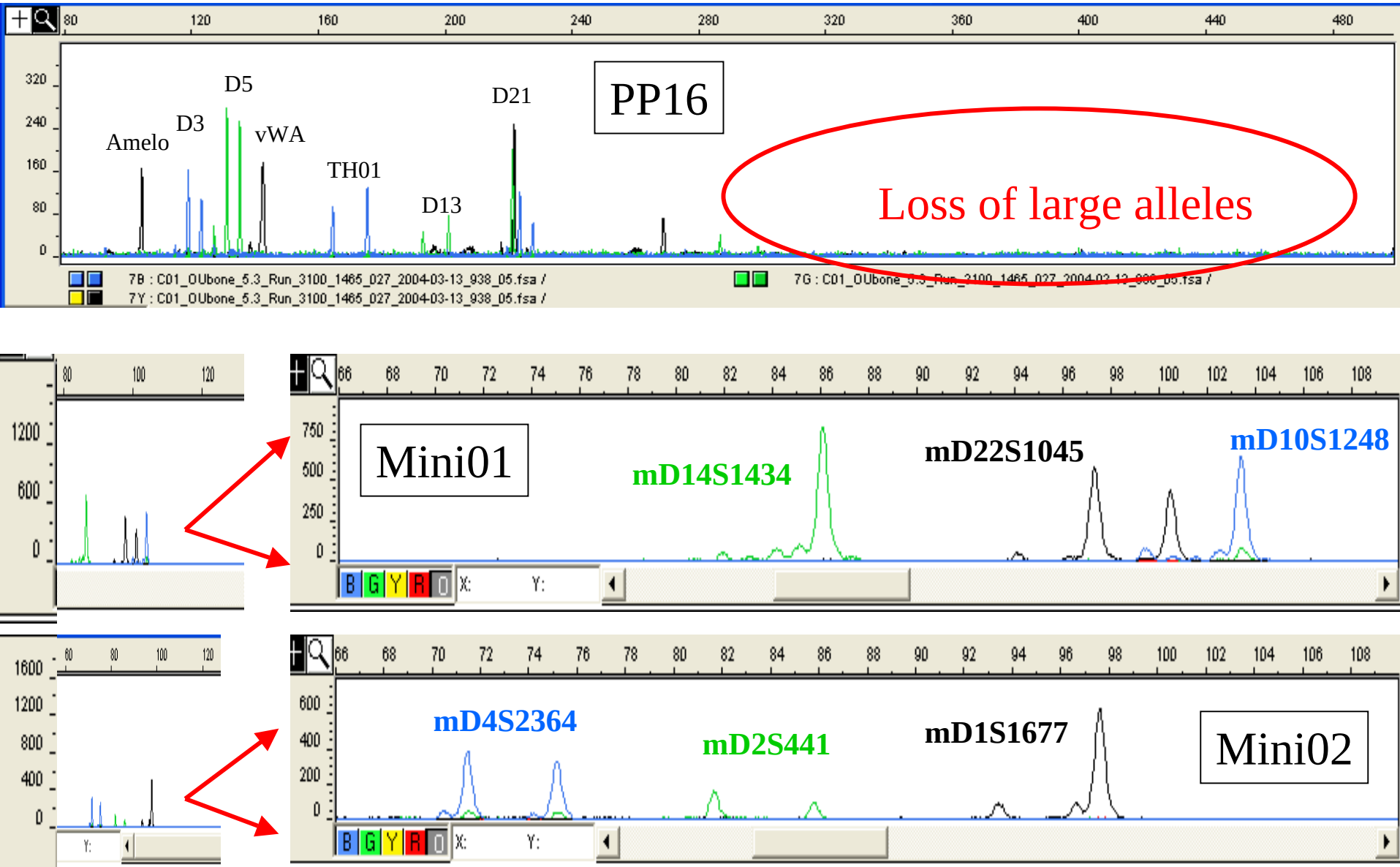


miniSTR primers for TPOX also gave tri-allelic patterns

CEPH Family miniSTR Results



Sensitivity - Degraded DNA from an OU Bone Sample



J Forensic Sci, Jan. 2005, Vol. 50, No. 1
Paper ID JFS2004216
Available online at: www.astm.org

Michael D. Coble,[1] *Ph.D. and John M. Butler,*[1] *Ph.D.*

Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA*

For more information… January issue of JFS

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Coble2005miniSTR.pdf

“STR typing of human telogen hairs -- a new approach”

- Hellmann, *et al.* (2001) *Int. J. Legal Med.* 114(4-5): 269-273
- Primer pairs with annealing positions close to the repeat units of the STR loci FES/FPS, TPOX, and TH01 were used for amplification.
- Complete digestion of hair with increased $CaCl_2$ buffer
- **Shed telogen hairs could be typed!**

		Complete Digestion		
		D10S	D14S	D22S
PV 2.5cm	10ul	-	-	08, 13
	5ul	-	-	08, 08
	2.5ul	-	-	-
PV 2.5cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
PV 2.7cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
PV 2.7cm	10ul	-	-	08, 13 (<50)
	5ul	-	-	13, 13
	2.5ul	-	-	-
PV 5 hairs	10ul	16, 16	17, 18	08, 13
	5ul	16, 16	17, 18	08, 08
	2.5ul	15, 15	-	08, 13
PV 5 hairs	10ul	-	-	08, 13
	5ul	-	-	08, 13
	2.5ul	-	-	13, 13
KEC 20cm	10ul	16, 17	17	13
	5ul	16, 17	17	13
	2.5ul	16, 17	17	13
MK 18cm	10ul	14, 15	17, 18	08, 12
	5ul	14, 15	17, 18	08, 12
	2.5ul	14, 15	17, 18	08, 12

Genotypes	D10S	D14S	D22S
PV	15, 16	17, 18	08, 13
KEC	16, 17	17, 17	13, 13
MK	14, 15	17, 18	08, 12

miniSTR Typing of Hairs

Complete Digestion Protocol

32 cycles; 2U Taq

“Longer” Hairs – greater success

		MicroTissue Grinding		
		D10S	D14S	D22S
PV 2.5cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
PV 2.5cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
PV 2.7cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
PV 2.7cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
PV 5 hairs	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	08, 08
PV 5 hairs	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	-
KEC 20cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	-	-	13, 13
MK 18cm	10ul	-	-	-
	5ul	-	-	-
	2.5ul	14, 15 (<50)	17, 18	08, 12

miniSTR Typing of Hairs

MicroTissue Grinding Protocol

32 cycles; 2U Taq

miniSTR Typing of Hairs

26 hairs (0.8 cm – 3.3 cm)

		32 cycles 5 ul	32 cycles 2.5 ul	36 cycles 2.5 ul (5U Taq)
Dark Hair (Phenol)	JB01 (1.5cm)	18, 18	18, 18	-
	JB02 (1.3cm)	-	-	13, 14
	JB03 (1.5cm)	-	-	08, 08
	JB04 (1.5cm)	17, 17	-	-
	JB07 (1.3cm)	-	-	-
	JB09 (1.0cm)	-	16, 16	-
	JB10 (3.3cm)	-	16, 16; 17, 17; 13, 13	22, 22; 13, 13
Dark Hair (Qiagen)	JB01 (1.1cm)	14,14	-	-
	JB02 (1.2cm)	14, 16	-	13,13
	JB03 (1.8cm)	-	13,13	21,21
	JB04 (1.4cm)	-	-	-
	JB05 (1.2cm)	-	-	16, 16; 13,13
	JB06 (1.5cm)	-	-	-
Gray Hair (Phenol)	JB01 (1.8cm)	14, 16	14, 15; 17,17	14, 16; 18,18; 13,13
	JB02 (2.0cm)	16, 16	16,16; 18,18, 13,13	16, 16; 13,13
	JB03 (1.1cm)	16, 16	17,17	13,13
	JB04 (1.4cm)	-	-	16, 16; 18,18
	JB05 (1.7cm)	15, 15	16, 16	18,18; 13,13
	JB06 (1.0cm)	-	18,18	-
Gray Hair (Qiagen)	JB11 (1.0cm)	13, 13	-	-
	JB14 (1.5cm)	18, 18 13, 13	16, 16	16, 16; 18, 18
	JB15 (1.7cm)	-	-	13, 13
	JB16 (1.1cm)	-	13, 13	13, 13
	JB17 (0.8cm)	18, 18	-	13, 13
	JB19 (1.4cm)	16, 16	-	16, 16
	JB20 (2.0cm)	-	-	14, 14; 18, 18

← 1.8 cm hair

miniSTR Typing of Hairs

	"Correct"	"Partial"	"Incorrect"	"Did Not Type"
Dark Phenol (7)	4/63	2/63	5/63	52/63
%	0.06	0.03	0.08	0.83
Dark Qiagen (6)	4/54	1/54	2/54	47/54
%	0.07	0.02	0.04	0.87
Gray Phenol (6)	12/54	6/54	4/54	32/54
%	0.22	0.11	0.07	0.59
Gray Qiagen (7)	9/63	4/63	2/63	48/63
%	0.14	0.06	0.03	0.76

Greater success with gray hairs

Future Plans

Testing and characterization of more markers.

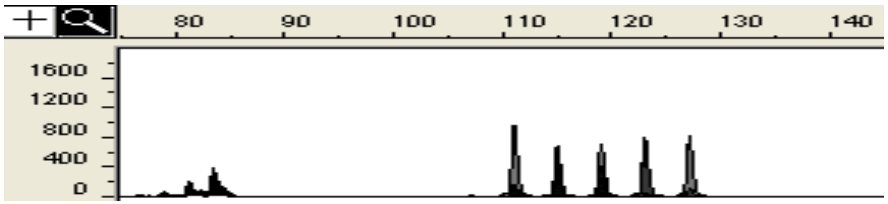
Population Databasing.

Testing on degraded materials.

Information will be posted on STRBase website and published as these loci are characterized

Potential New Miniplex

mD6S474

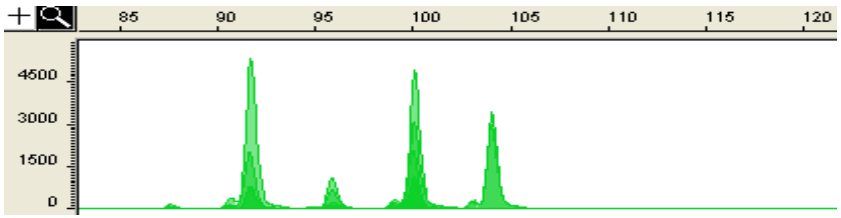


Tetranucleotide repeat – GATA

Exp. Het. – 0.77

Distance to Repeat
F – 0
R – 7

mD3S3053

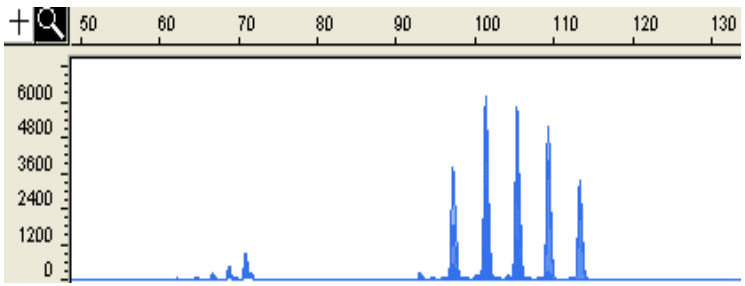


Tetranucleotide repeat – GATA

Exp. Het. – 0.74

Distance to Repeat
F – 14
R – 0

mD20S482

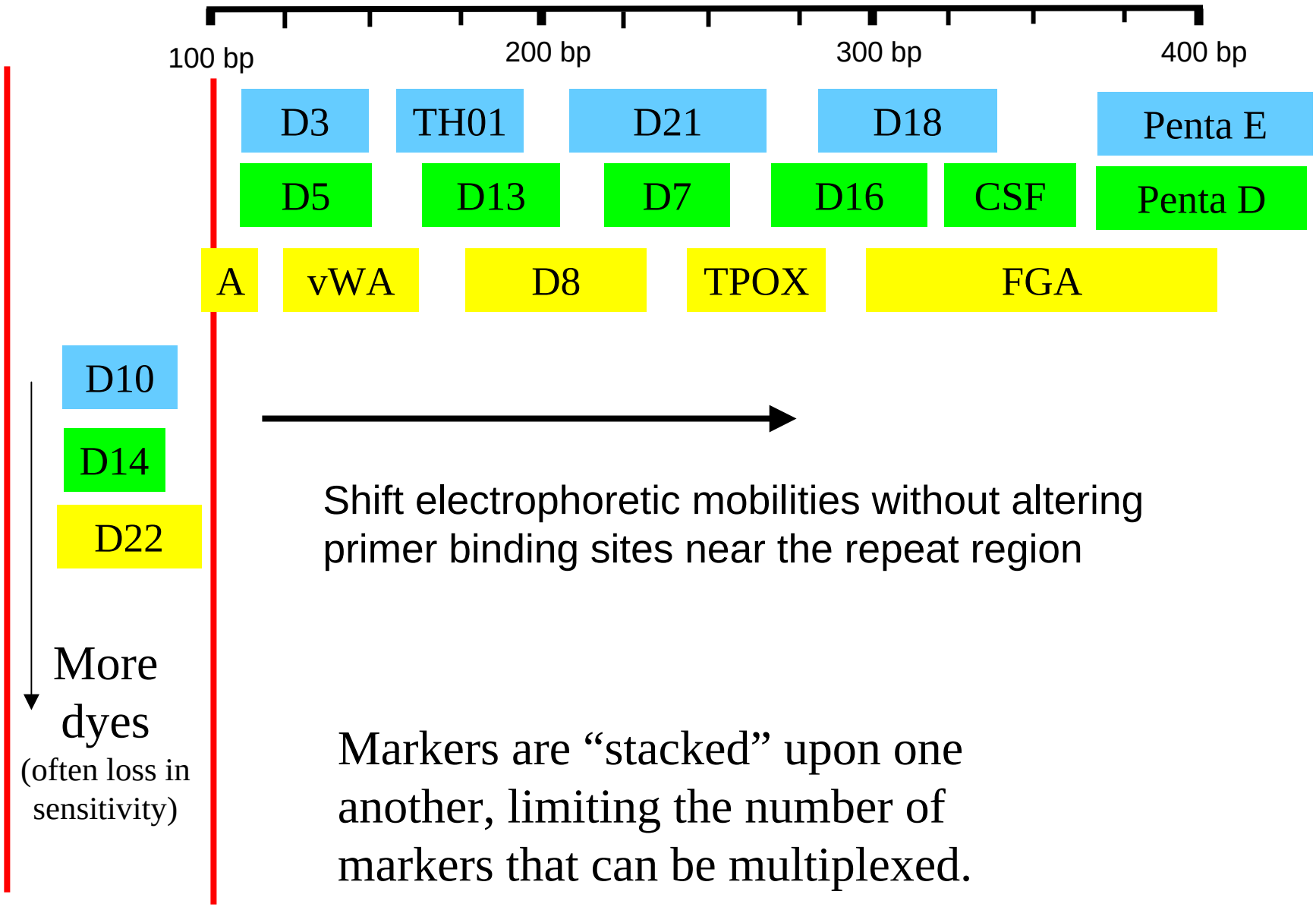


Tetranucleotide repeat – GATA

Exp. Het. – 0.85

Distance to Repeat
F – 3
R – 6

Expanding Multiplex-ability



Funding and Collaborations

We are funded by an Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards

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

Human Identity Project Team
John Butler (Project Leader)
Peter Vallone
Michael Coble
Margaret Kline
Jan Redman
Amy Decker
David Duewer

Collaborators (also funded by NIJ):

Bruce McCord and students (Ohio U) for miniSTR work

http://www.cstl.nist.gov/biotech/strbase/Coble.htm
michael.coble@nist.gov