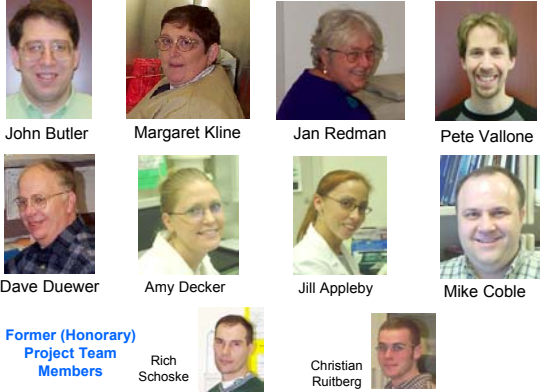




Development of New miniSTR Loci for Improved Analysis of Degraded DNA Samples

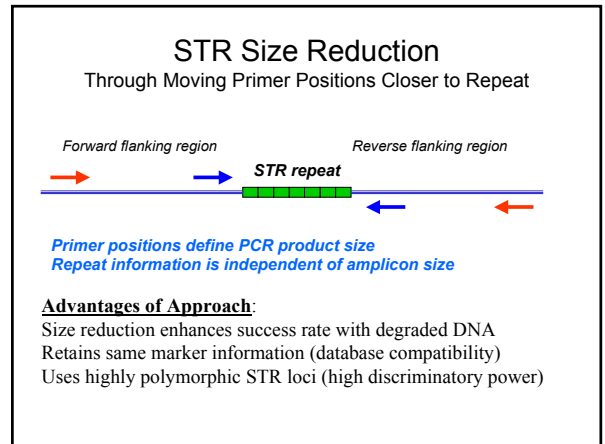
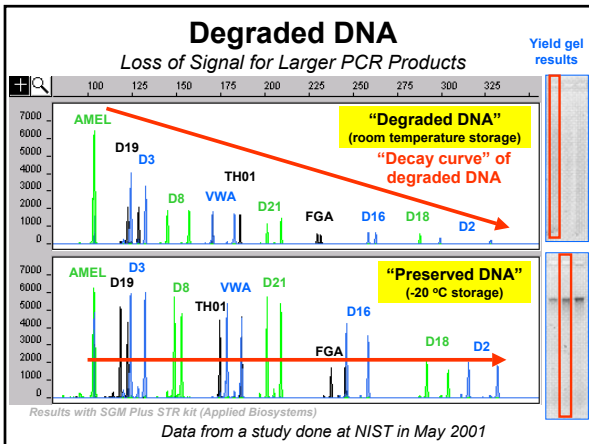
Michael D. Coble,
Peter M. Vallone and John M. Butler
National Institute of Standards and Technology

NIST Human Identity Project Team



John Butler Margaret Kline Jan Redman Pete Vallone
Dave Duewer Amy Decker Jill Appleby Mike Coble

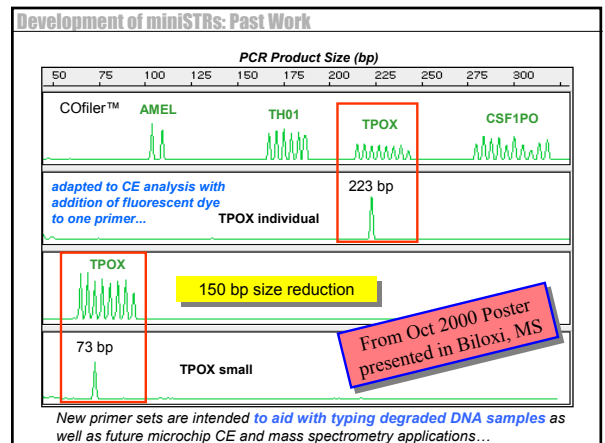
Former (Honorary) Project Team Members
Rich Schoske Christian Ruitberg

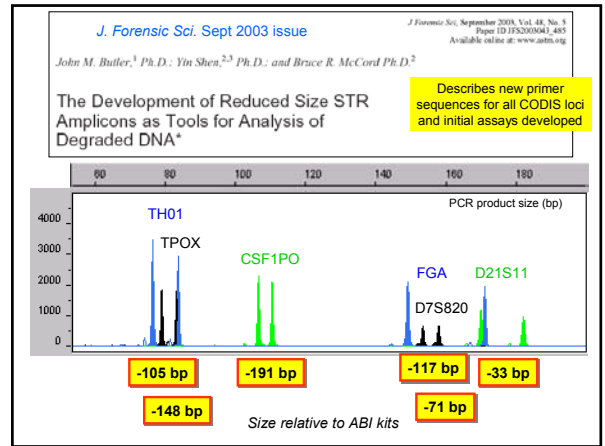
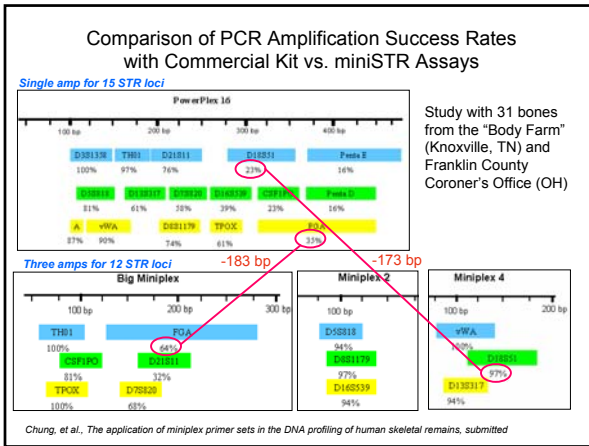


Selected References on STRs with Degraded DNA

- 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





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: 0	
	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	
D5S11	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
D5S11	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 (SWGDM). *Science & Justice*, 44(1), in press.

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 (SWGAM). *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

- 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

- **Candidate STR marker selection**
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Candidate STR marker selection

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



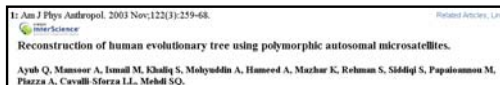
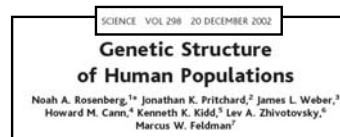
<http://www.decode.com/>



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



Candidate STR marker selection



Characterization of New miniSTR Loci

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

Locus name	Alternate name	Heterozygosity	Number of alleles	Chromosome
D8S1017	GGAT3B10	0.748	9	6
D8S1099	GATA32B01	0.748	13	6
D8S261	AFM123VG5	0.747	17	8
D14S1490	19QTEL11	0.747	15	18
D18S1827	GATA11506	0.747	9	13
D12S1032	GATA26702	0.747	8	12
D16S2616	ATA1104	0.746	11	16
D2S1780	GATA79C11	0.746	13	2
NA-D18S13	GATA133A06	0.745	12	1
NA-D8S12	GAAT1A1	0.745	9	8
D4S1625	GATA107	0.745	10	4
D18S811	GATAG09	0.745	12	18
D8S1136	GATA41A01	0.745	11	8
NA-D3S14	ATA5202	0.744	27	5
D3S308	GATA73E01	0.743	14	3
D15S115	GATA109B10	0.743	9	15
D16S2624	GATA41D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D11S895	GCAAT2201	0.740	11	11
D11S1998	GATA23E06	0.740	9	11

Focus on:
High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

Characterization of New miniSTR Loci

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

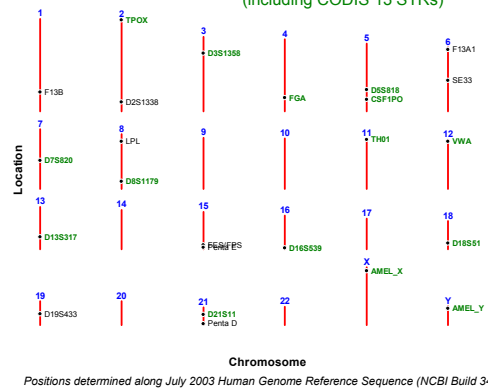
Locus name	Alternate name	Heterozygosity	Number of alleles	Chromosome
D8S1017	GGAT3B10	0.748	9	6
D8S1099	GATA32B01	0.748	13	6
D8S261	AFM123VG5	0.747	17	8
D14S1490	19QTEL11	0.747	15	18
D18S1827	GATA11506	0.747	9	13
D12S1032	GATA26702	0.747	8	12
D16S2616	ATA1104	0.746	11	16
D2S1780	GATA79C11	0.746	13	2
NA-D18S13	GATA133A06	0.745	12	1
NA-D8S12	GAAT1A1	0.745	9	8
D4S1625	GATA107	0.745	10	4
D18S811	GATAG09	0.745	12	18
D8S1136	GATA41A01	0.745	11	8
NA-D3S14	ATA5202	0.744	27	5
D3S308	GATA73E01	0.743	14	3
D15S115	GATA109B10	0.743	9	15
D16S2624	GATA41D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D11S895	GCAAT2201	0.740	11	11
D11S1998	GATA23E06	0.740	9	11

Focus on:
High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

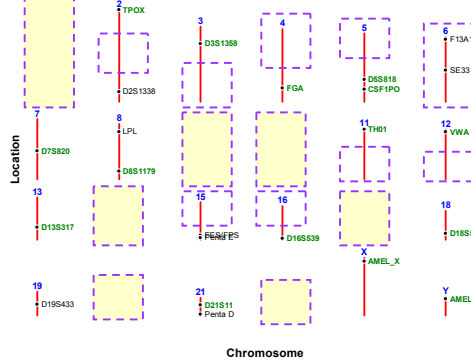
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- Miniplex assay performance

Commercial STR Kit Loci Positions
(including CODIS 13 STRs)



Locations of Focus for New miniSTR Loci
(relative to CODIS 13 STRs)



Characterization of New miniSTR Loci

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PCR Primer Design

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

Primer3 [disclaimer](#) [source code](#)

pick primers from a DNA sequence (see [help](#))

disclaimer contains

Paste source sequence below (5'>3', string of ACGTNaigh -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a [Minipriming 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

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. ...ATCTCCCTCCAT, 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.

Product Size Ranges: [150-250 100-200 201-400 401-500 501-600 601-700 701-800 801-1000]

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 P. Stability: [9.0]

Max Minipriming: [12.0] Pair Max Minipriming: [4.0]

PCR Primer Design

5' 11 21 31 41 51 61 71 81 91

1 TACCTTCCTG ACTCATAAAT ATATTATCCC TGTTTTCCTG TAGCTATTGC TCGACTACAG AACCTCCATC TTTTATATG TGGAGACCA ACAGAAATCG
 ATTGGAGGCG TGAGTATTTA TAAATAGGGG ACAGAAACCA ATCTCATAGC AGTAGTGTGC TTTCAGAGTAG AAGAGATATG ACCCTCTTGT TGTTTATGTC

101 GAGGAGATTT CTTCCACCTA CTTATCTATC ATCATCTATG ATCCATCAT CCATCTATC TCTCTCTCTC TATCATCCCA TTCTATCTAT CTATCTATCT
 CTTCTCTAAG GAGAGTGGAT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT AAGATAGATA GATAGATAGA

201 ATCTGTCTAT CTATCTATC TAGCTATTGC ATCATCTATG CACCCATCC CACCCATCC TCCATCTATC CACCCATCC TCCATCTATC CACCCATCC
 TAGACAGATA GATAGATAGA ATTAGATAGA TAGATAGATA GATGGTAGC TAGGTGGAT AGATAGATAG GTGGATAGCC AGATAGATAG CTTGATCTAT CTATCTATCT

301 CTTCTCTAAG GAGAGTGGAT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT AAGATAGATA GATAGATAGA
 GAGAC 8 GATA repeats

401 TATCTATCTA TCTATCTATC TATCTATCTA TCTATCTATC ACAGATTATC AGGAGAGGAG ACAGATGGGG AGGAGAGAC AGACTGTCCC TGCTGTAATT
 ATAGATAGAT AAGATAGATAG ATAGATAGAT AAGATAGATA TGATCTATAG TCCCTCTCTC TTTTACCCCC TCTCTCTCTC TTTGATAGGG AAGAGATTAA

501 GTACTGGAAA CTTAGTCTGT ACTTGTCTCT GTTCTATAGC TCTACTGAGC AGTAGACAGC AGTAGACAGC TTTTATAGAT TTTTATAGAT ACTTGGACAT
 CATGACCTTT GATTCAGACA TGACAGACAA CAGATATTC AAGATAGTTC TCTATGTATA ACATCTTGG TTTACTTAAA AAGATTAGT TGACCTCTCC

PCR Primer Design

5' 11 21 31 41 51 61 71 81 91

1 TACCTTCCTG ACTCATAAAT ATATTATCCC TGTTTTCCTG TAGCTATTGC TCGACTACAG AACCTCCATC TTTTATATG TGGAGACCA ACAGAAATCG
 ATTGGAGGCG TGAGTATTTA TAAATAGGGG ACAGAAACCA ATCTCATAGC AGTAGTGTGC TTTCAGAGTAG AAGAGATATG ACCCTCTTGT TGTTTATGTC

101 GAGGAGATTT CTTCCACCTA CTTATCTATC ATCATCTATG ATCCATCAT CCATCTATC TCTCTCTCTC TATCATCCCA TTCTATCTAT CTATCTATCT
 CTTCTCTAAG GAGAGTGGAT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT AAGATAGATA GATAGATAGA

201 ATCTGTCTAT CTATCTATC TAGCTATTGC ATCATCTATG CACCCATCC CACCCATCC TCCATCTATC CACCCATCC TCCATCTATC CACCCATCC
 TAGACAGATA GATAGATAGA ATTAGATAGA TAGATAGATA GATGGTAGC TAGGTGGAT AGATAGATAG GTGGATAGCC AGATAGATAG CTTGATCTAT CTATCTATCT

301 CTTCTCTAAG GAGAGTGGAT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT AAGATAGATA GATAGATAGA
 GAGAC 8 GATA repeats

401 TATCTATCTA TCTATCTATC TATCTATCTA TCTATCTATC ACAGATTATC AGGAGAGGAG ACAGATGGGG AGGAGAGAC AGACTGTCCC TGCTGTAATT
 ATAGATAGAT AAGATAGATAG ATAGATAGAT AAGATAGATA TGATCTATAG TCCCTCTCTC TTTTACCCCC TCTCTCTCTC TTTGATAGGG AAGAGATTAA

501 GTACTGGAAA CTTAGTCTGT ACTTGTCTCT GTTCTATAGC TCTACTGAGC AGTAGACAGC AGTAGACAGC TTTTATAGAT TTTTATAGAT ACTTGGACAT
 CATGACCTTT GATTCAGACA TGACAGACAA CAGATATTC AAGATAGTTC TCTATGTATA ACATCTTGG TTTACTTAAA AAGATTAGT TGACCTCTCC

PCR Primer Design D2S441

5' 11 21 31 41 51 61 71 81 91

1 CAGCTATACA GAACTCTCCT GAACTCCAGT CTTCTGGGGT TTGAGGGAGG CTTTATGACA TCGATGCCCT TTCTCCAGG GATTATAGG GACCTCTCT
 CTCGATATGT CTTGGAAGCA CTTGGGTCAG GAGACCCCA AACCTCCCTC GATGACTACT AGCTGTAGAG AAGAGATGCC CATATATACC CTGGAGAGA

101 GAGGAGATTT TTAGACCCCA CTTCTCTAAG AATCTGGGT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT
 CTTCTCTAAG AATCTGGGT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT AAGATAGATA GATAGATAGA

201 TCTCTGAGCC CTAATCTACC CAACATTCTA ACARAAGGCT GTACACAGGC CTAAGAGATT CATAGAGCCG AACTGTGCC CTAATCTATG AACTCTTAT
 AAGAGATCCG GATTACCTGG GTTGTAGAT TGTITTCGCA CATTTCTCCC GATCTCCTTA GTACTCGGC CTTGACAGCC AGTAGATACT TTTGATAGC

301 TATCTATCT ATCTATCTAT CTATCTATCT ATCTATCTAT CTAATCTATA ACACACAGC CACTTAGCTC CAATTTAAA GATTAACTG AAGACTTTG
 AAGAGATCCG GATTACCTGG GTTGTAGAT TGTITTCGCA CATTTCTCCC GATCTCCTTA GTACTCGGC CTTGACAGCC AGTAGATACT TTTGATAGC

401 GAGGAGATTT GAAATTTTTT GTAAATTTAA ATAGAAATGA TTAATATAAA AACCAAAATA ATATGTTTAT TATGCTGGG TGTGATGCT TAAAGCTGTA
 CTTCTCTCTA CTTCTAABAA CACTACAAAT TATCTTACT AATATGATTT TTGGTTTTAT TATACATARA ATACAGACC ACACACAGCA ATTCCGACAT

501 ATCCACAGAC TTTGGAGAGC CAGGCTTGT GATCACTTG GATCCAGAGC GATCACTTG GATCCAGAGC CTTGAGGAGA CCTGTCTCT ACARAARAT
 TAGGCTCTG AACCCTCCG GTCCGACAA CCTAGTAGAC TGGGCTCTC AAGTCTGGT CCGACCCGTT GTATCCCTCT GGGACAGAGA TGTITTTTAA

PCR Primer Design D2S441

5' 11 21 31 41 51 61 71 81 91

1 CAGCTATACA GAACTCTCCT GAACTCCAGT CTTCTGGGGT TTGAGGGAGG CTTTATGACA TCGATGCCCT TTCTCCAGG GATTATAGG GACCTCTCT
 CTCGATATGT CTTGGAAGCA CTTGGGTCAG GAGACCCCA AACCTCCCTC GATGACTACT AGCTGTAGAG AAGAGATGCC CATATATACC CTGGAGAGA

101 GAGGAGATTT TTAGACCCCA CTTCTCTAAG AATCTGGGT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT
 CTTCTCTAAG AATCTGGGT GAGATAGATA GATAGATAGA TAGGTAGGTA GGTAGATAGC AGAGAGAGAG ATAGATAGGT AAGATAGATA GATAGATAGA

201 TCTCTGAGCC CTAATCTACC CAACATTCTA ACARAAGGCT GTACACAGGC CTAAGAGATT CATAGAGCCG AACTGTGCC CTAATCTATG AACTCTTAT
 AAGAGATCCG GATTACCTGG GTTGTAGAT TGTITTCGCA CATTTCTCCC GATCTCCTTA GTACTCGGC CTTGACAGCC AGTAGATACT TTTGATAGC

301 TATCTATCT ATCTATCTAT CTATCTATCT ATCTATCTAT CTAATCTATA ACACACAGC CACTTAGCTC CAATTTAAA GATTAACTG AAGACTTTG
 AAGAGATCCG GATTACCTGG GTTGTAGAT TGTITTCGCA CATTTCTCCC GATCTCCTTA GTACTCGGC CTTGACAGCC AGTAGATACT TTTGATAGC

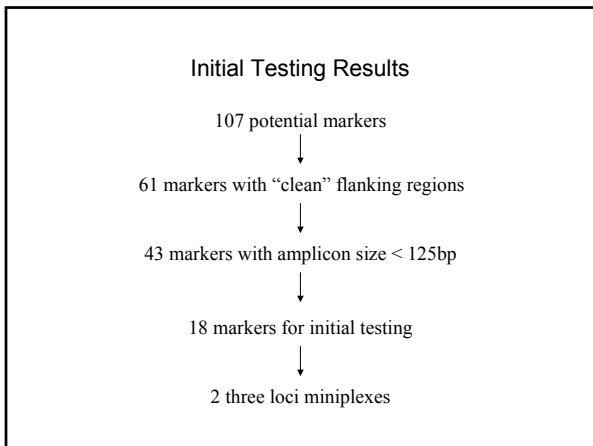
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501 ATCCACAGAC TTTGGAGAGC CAGGCTTGT GATCACTTG GATCCAGAGC GATCACTTG GATCCAGAGC CTTGAGGAGA CCTGTCTCT ACARAARAT
 TAGGCTCTG AACCCTCCG GTCCGACAA CCTAGTAGAC TGGGCTCTC AAGTCTGGT CCGACCCGTT GTATCCCTCT GGGACAGAGA TGTITTTTAA

92 bp Amplicon

Characterization of New miniSTR Loci

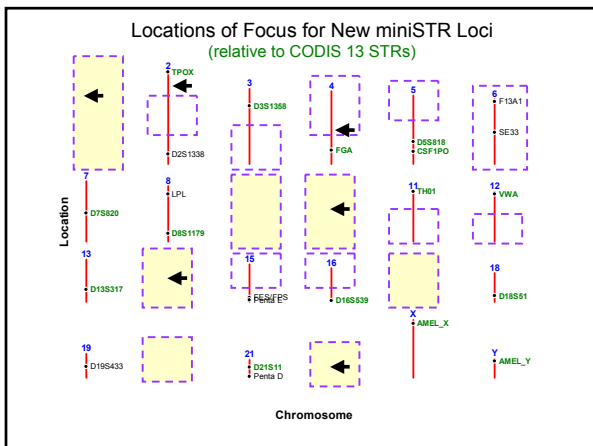
- Candidate STR marker selection
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- Miniplex assay performance



Initial Testing Results

Miniplex01- mD10S1248 - FAM
mD14S1434 - VIC
mD22S1045 - NED

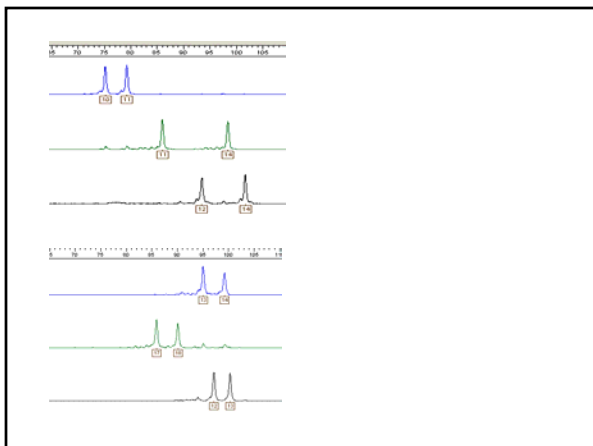
Miniplex02- mD4S2364 - FAM
mD2S441 - VIC
mD1S1677 - NED



Characterization of New miniSTR Loci

Chr.	Marker Name	Het	Allele Size Range	(Motif)	Repeat	Ref. Primer distance from repeat	Amplicon Size
10	D10S1248	0.77	20 bp	TETRA	13	1	102
	GGAA23C05N			GGAA		0	
14	D14S1434	0.72	20 bp	TETRA	10	1	88
	GATA168F06			GATA		0	
22	D22S1045	0.77	18 bp	TRI	13	3	105
	ATA37D06			ATA		6	
1	D1S1677	0.74	35 bp	TETRA	15	0	103
	GGAA22G10N			GGAA		0	
2	D2S441	0.74	18 bp	TETRA	12	0	92
	GATABF03			GATA		0	
4	D4S2364	0.64	18 bp	TETRA	7	2	78
	GAAT1F09			GAAT		1	

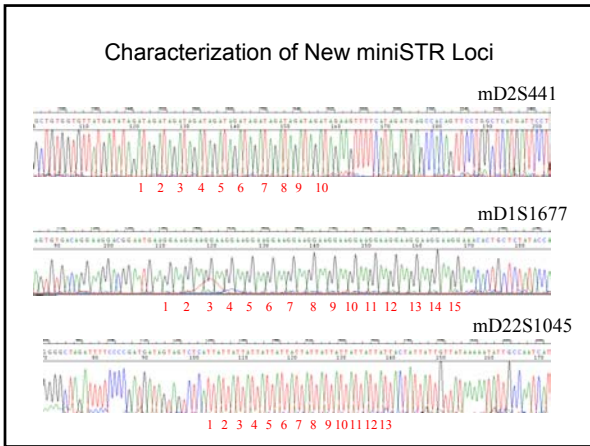
Miniplex01
Miniplex02



Characterization of New miniSTR Loci

D2S441

5'	11	21	31	41	51	61	71	81	91	
1	CAGCTATACG	GAGCCTTCC	GACCCCGATC	CTCTGGGGT	TTGAGGGAG	CTTCATGAC	TCAGCATCC	TTCCCTCAGG	GTATTATGG	GACCCCTCT
101	CTCCATATGT	CTTCGAGGGA	CTTGGGTGAG	GGAGACCCCA	AACCTCCCTC	GAGTACTGT	AGGAGATCG	AGGAGATCC	CATATATACC	CTGGGAGAGA
Sequencing Primers										
	TTGGGTAAA	GACTAGAGTC	CTCCCTGGG	GCGAGTAAA	GGAGTCCAG	AGAGGTAA	AGAGGTAA	AGAGGTAA	AGAGGTAA	AGAGGTAA
261	TTCTCTAAG	AATCTGGGT	GCCGGTCTT	CAACCATTT	CTGATCTGAG	GACGGARCC	CGTCCACTT	CCTCCGTTT	TTCTCCATC	TTCTTAGAC
361	TTCTCTAAG	AATCTGGGT	GCCGGTCTT	CAACCATTT	CTGATCTGAG	GACGGARCC	CGTCCACTT	CCTCCGTTT	TTCTCCATC	TTCTTAGAC
461	GAGGAGAGC	GAGATTTTT	GTGATGTTA	ATAGAGTGA	TTATACTAA	AGCCAAATA	ATATGTTATT	TATGGCTGG	TGTGGTGG	TAGGCTGTA
561	ATCCAGAC	TTGGGAGG	CAGGCTTGT	GGATCTTGT	AGCCAGAG	TTGACACCA	GCCTGGGCA	CATAGGAGA	CCCTCTCT	ACAAAATTT
661	TAGGCTCTG	AAACCCCTCG	GTCCGACCA	CCTAGTAA	TGGGCTCT	AGTCTGGT	GAGACCGTT	GTATCCCTT	GGACAGAGA	TGTTTTTAA



- ### Characterization of New miniSTR Loci
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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)

On average ~80 µg total extracted genomic DNA

Stock tubes

Working tubes/plates 1 ng/µL

Working tubes

Working plates

To date: (~50,000 allele calls)

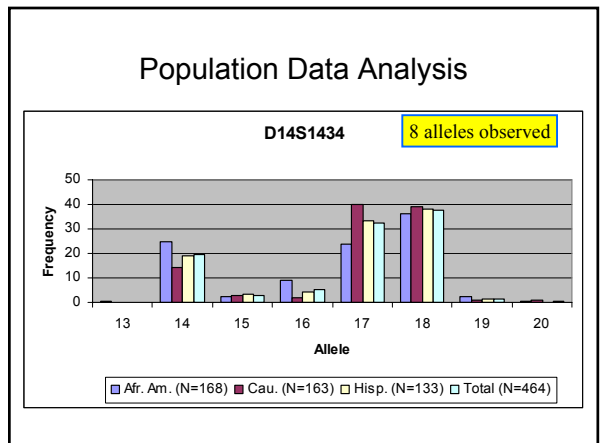
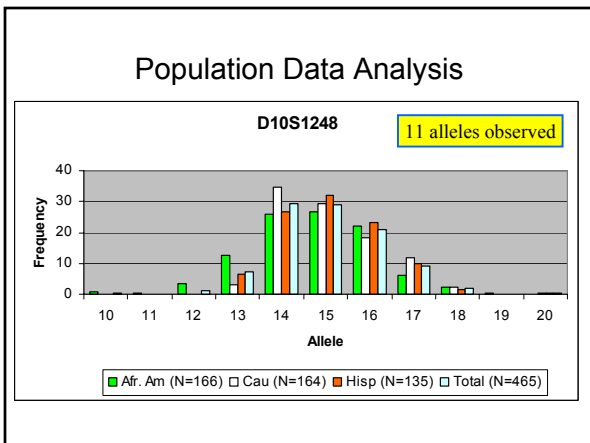
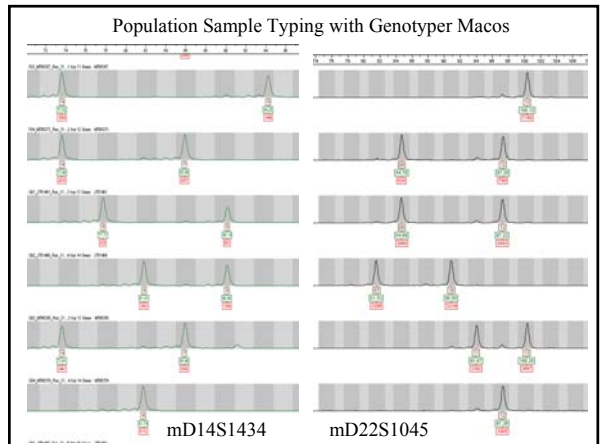
Identifier (15 autosomal markers + Amelogenin) (10,608)

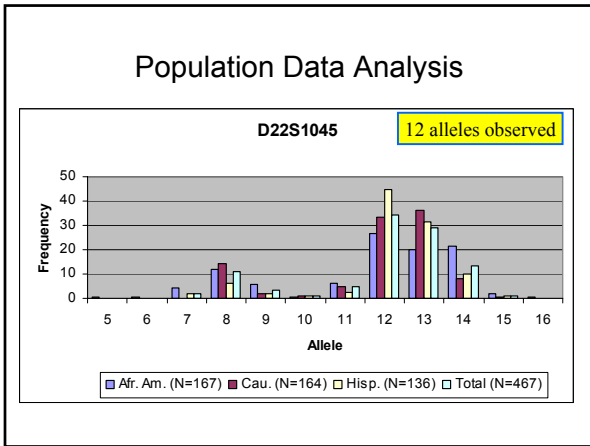
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)

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





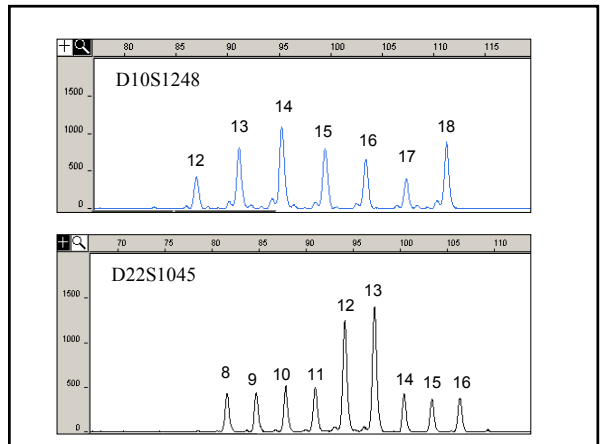
Population Testing –Miniplex01 vs. Identifier

Loci	Heterozygosity
D18S51	0.914
FGA	0.886
D21S1338	0.871
D7S820	0.864
VWA	0.850
D2S1338	0.843
D13S317	0.843
D16S539	0.793
D8S1179	0.786
D19S433	0.764
TH01	0.764
D3S1358	0.757
CSF1PO	0.743
D10S1248	0.733
D5S818	0.729
D22S1045	0.721
TPOX	0.679
D14S1434	0.662

N = 140 Hispanics (Identifier)

N = 135 Hispanics (Mini01)

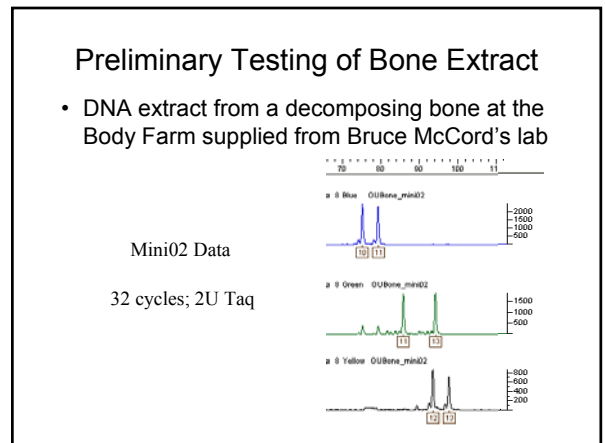
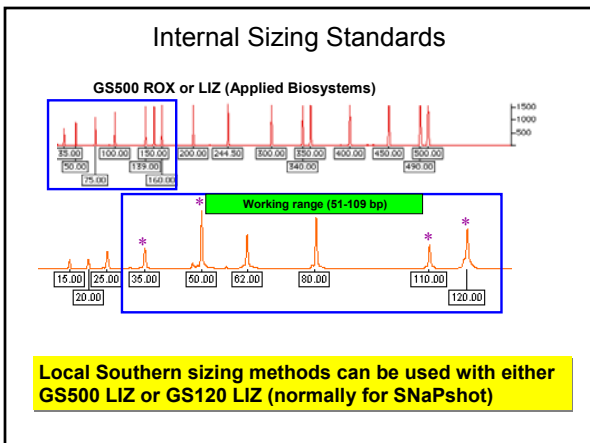
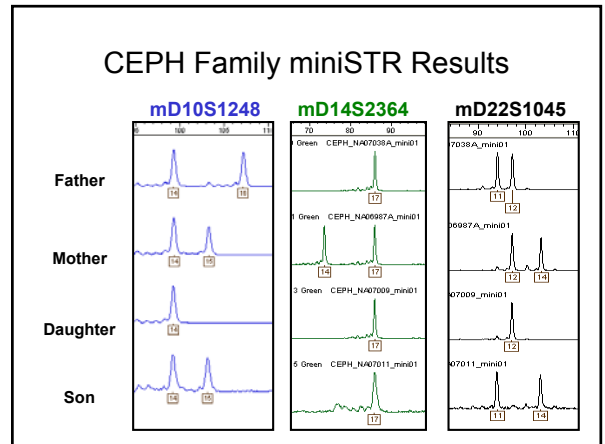
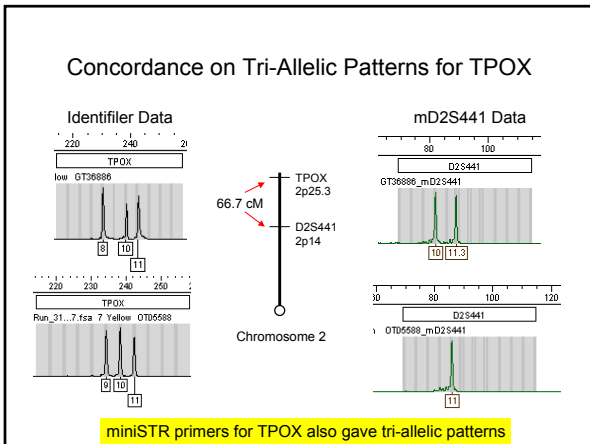
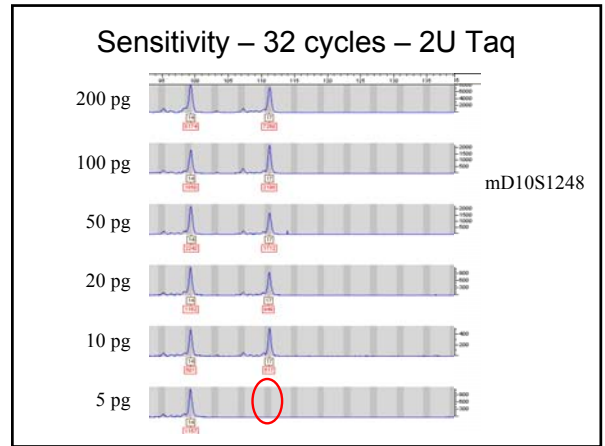
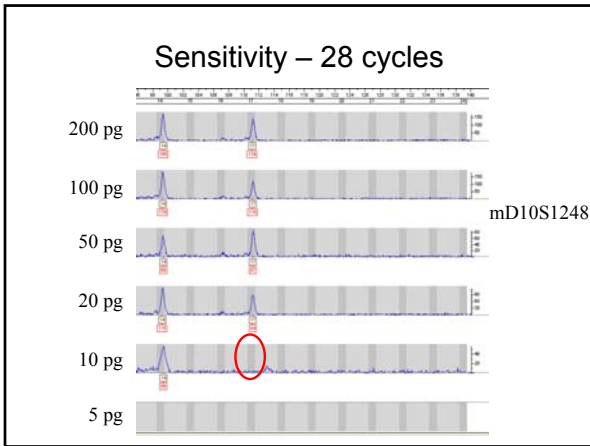
- ### Characterization of New miniSTR Loci
- Candidate STR marker selection
 - Chromosomal locations and marker characteristics
 - PCR primer design
 - Initial testing results
 - Population testing
 - Allelic ladder construction
 - Miniplex assay performance



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



“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.
- **Shed telogen hairs could be typed!**

Future Plans

Testing and characterization of more markers.

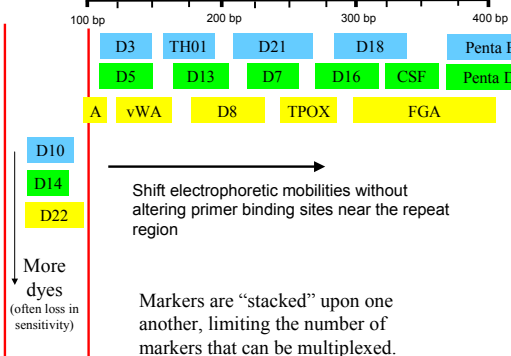
Population Databasing.

Testing on degraded materials.

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

We would welcome collaborations with those wishing to test some of these new miniSTR systems

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>

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 Mike Coble

Collaborators (also funded by NIJ):

[Bruce McCord](#) and students (Ohio U) for miniSTR work