

New Autosomal and Y-Chromosome STR Loci

John M. Butler,
Carolyn R. "Becky" Hill, Amy E. Decker,
Margaret C. Kline, Peter M. Vallone

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Presentation Outline

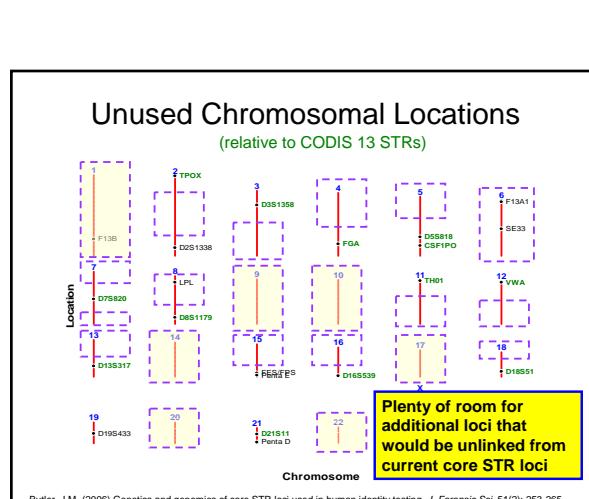
- Why Consider New Loci?
- Selection and Characterization of New STR Loci
- Work with 26 Autosomal STR Loci
- Megaplex vs Miniplex Concordance Studies
- Nomenclature and Standard Sample Typing
- On-going and Future Studies / Publications

Commercial Kits and Current Core Loci

Most forensic DNA laboratories (certainly in the U.S.) will **only** use commercially available kits due to quality control issues

<http://www.cstl.nist.gov/biotech/strbase/coreSTRs.htm>

U.S. Core Loci: CSF1PO, FGA, TH01, TPOX, VWA, D2S1338, D5S818, D13S317, D16S539, D18S51, D21S11, D3S1358, D7S820, D8S1179, D18S433, D20S1200, D22S1043, D23S1230, D24S407, D25S1243, D26S1230, D27S1230, D28S1230, D29S1230, D30S1230, D31S1230, D32S1230, D33S1230, D34S1230, D35S1230, D36S1230, D37S1230, D38S1230, D39S1230, D40S1230, D41S1230, D42S1230, D43S1230, D44S1230, D45S1230, D46S1230, D47S1230, D48S1230, D49S1230, D50S1230, D51S1230, D52S1230, D53S1230, D54S1230, D55S1230, D56S1230, D57S1230, D58S1230, D59S1230, D60S1230, D61S1230, D62S1230, D63S1230, D64S1230, D65S1230, D66S1230, D67S1230, D68S1230, D69S1230, D70S1230, D71S1230, D72S1230, D73S1230, D74S1230, D75S1230, D76S1230, D77S1230, D78S1230, D79S1230, D80S1230, D81S1230, D82S1230, D83S1230, D84S1230, D85S1230, D86S1230, D87S1230, D88S1230, D89S1230, D90S1230, D91S1230, D92S1230, D93S1230, D94S1230, D95S1230, D96S1230, D97S1230, D98S1230, D99S1230, D100S1230, D101S1230, D102S1230, D103S1230, D104S1230, D105S1230, D106S1230, 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Selection of New Autosomal Loci

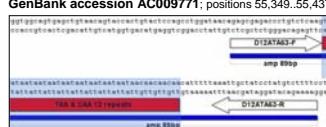
- Aim to have candidate sets for optimal miniSTRs
- Using ~900 STR loci with some literature data as a starting point...
 - Loci with high heterozygosities (>0.7)
 - Loci with small allele ranges (<24 bp) – **low mutation?**
 - Tetra (some tri-)nucleotide repeats without variants
 - Clean flanking regions (PCR products <140 bp)
- 26 loci met criteria and fully characterized...

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

Characterization of New Autosomal Loci

(miniSTR D12ATA63)

GenBank accession AC009771: positions 55,349..55,437
[FAM]-CAGCGAGACCCCTGCTCAAG
GAAAAACATAGGATAGCAATT



Chr 12 106.825 Mb (12q23.3)
 Trinucleotide [TAA][CAA] repeat 76 -106 bp
 Alleles 9 -19

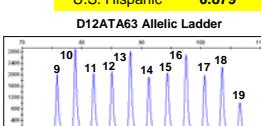
Allele	Caucasian (N = 260)	African Am (N = 259)	Hispanic (N = 140)
9	--	--	0.0036
10	0.0019	0.0154	0.0036
11	0.1385	0.1525	
12	0.2154	0.1000	
13	0.0173		0.0286
14	0.0040		0.2214
15			0.0772
16	0.2981	0.1004	0.2643
17	0.0981	0.0521	0.0679
18	0.0096	0.0058	0.0071
19	0.0019	0.0058	0.0036

~60 U.S. population samples

Heterozygosity Values

U.S. Caucasian	0.842
African American	0.788
U.S. Hispanic	0.879

D12ATA63 Allelic Ladder



Comparison of heterozygosity values on 26 non-CODIS loci across the U.S. samples examined in this study.					
Locus	N	Heterozygosity (Overall)	Rank	African American	Caucasian Hispanic
D952157	661	0.844	1	0.884	0.840 0.779
ATA63 (D12)	659	0.829	2	0.788	0.842 0.879
D10S1248 (NC01)	663	0.792	3	0.825	0.785 0.743 European recommended loci
D22S1045 (NC01)	663	0.784	4	0.817	0.785 0.721
D25441 (NC02)	660	0.774	5	0.798	0.780 0.721
D10S1435	663	0.766	6	0.798	0.770 0.700
D251776	654	0.763	7	0.740	0.801 0.734
D354529	660	0.761	8	0.752	0.723 0.829
D6S474	648	0.761	9	0.765	0.802 0.679
D5S2500	664	0.747	10	0.757	0.747 0.729
D1S1627	660	0.746	11	0.783	0.737 0.693
D1S1677 (NC02)	660	0.746	12	0.743	0.749 0.743
D6S1017	664	0.740	13	0.807	0.698 0.693
D3S3053	648	0.739	14	0.713	0.724 0.814
D9S1122	659	0.734	15	0.753	0.742 0.686
D17S974	664	0.732	16	0.727	0.702 0.672
D11S463	664	0.730			
D4S2408	654	0.722			
D18S853	664	0.711			
D20S1082	664	0.696			
D14S1434 (NC01)	663	0.696			
D20S482	648	0.691			
GATA113 (D1)	654	0.668	23	0.673	0.632 0.727
D8S1115	664	0.663	24	0.629	0.660 0.729
D17S1301	664	0.649	25	0.626	0.717 0.564
D4S2364 (NC02)	660	0.511	26	0.385	0.551 0.664

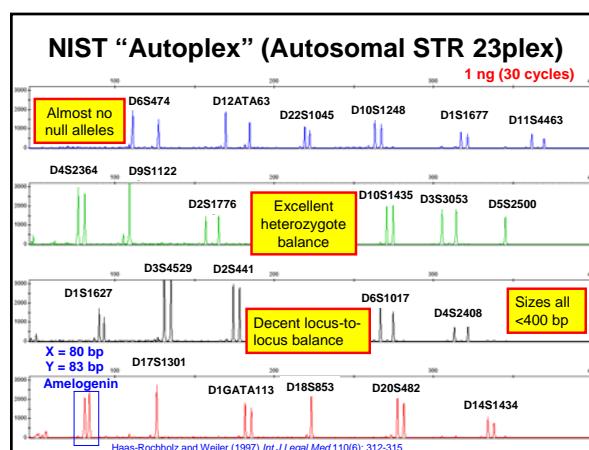
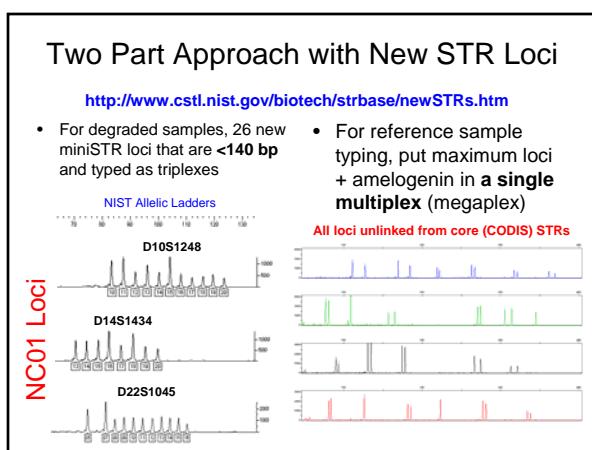
>17,000 genotypes collected to measure these relative heterozygosities

Information on 26 Autosomal STRs

Available on STRBase website (NIST Publications and Presentations)

- Hill et al. (2006) Promega poster
 - Describes initial work and miniSTR primer sequences
- Kline et al. (2006) Promega poster
 - Sequence and typing results for SRM 2391b and common samples K562, 007, 9947A, 9948 across all 26 loci
- Butler and Coble (2007) *J. Forensic Sci.* 52(2):494
 - Nomenclature corrections and changes
- Hill et al. (2007) *J. Forensic Sci., in press*
 - Population data, heterozygosities, genomic locations, miniSTR primer sequences
- This meeting (ISFG 2007)
 - Megaplex results and primer concordance study

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



Concordance Study to Check for Null Alleles

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

Use of non-overlapping primers permits detection of allele dropout

"Autoplex" vs miniSTRs
639 samples compared
Total types (639 x 22 loci): 14,058
28 types discordant (0.20%)*
99.80% concordance
*discordance not confirmed yet with sequencing

Identifier vs MiniFiler
1308 samples compared
Total types (1308 x 8 loci): 10,464
27 types discordant (0.26%)*
99.74% concordance
Hill et al. (2007) JFS 52(4): 870-873

Conclusions: (1) Our PCR primers have been well-designed and have almost no primer binding site mutations. (2) Roughly half of dropout is from megaplex primers – flanking regions near STR repeat do not appear to have a higher level of mutation

Nomenclature Changes and Corrections

Due to further sequence analysis conducted in summer and fall of 2006

- D10S1248 reduced by -1 repeat
- D22S1045 increased by +3 repeats
- D2S441 kept the same
- D14S1434 reduced by -4 repeats
- D1S1677 increased by +1 repeat
- D4S2364 reduced by -1 repeat

Butler, J.M. and Coble, M.D. (2007) Authors' Response to Letter to Editor [regarding nomenclature for new miniSTR locus D10S1248]. *J. Forensic Sci.* 52(2): 494.

See also .../strbase/minISTR.htm#Nomenclature_Errata

26 New miniSTR Loci Typed (not all are sequenced yet) with SRM 2391b Components

Locus	Standard DNA Template Genotypes	SRM 2391b Components
D10ATA113	11,12 7,12 12,12 11,12 11,11 12,13 11,11 13,13 11,12 11,12 10,12 10,12	
D1S1627	11,13 11,14 10,14 10,14 13,14 13,14 11,12 14,15 11,13 11,14 13,14	
D1S1677 (NC02)	13,14 13,14 13,13 13,14 12,13 14,16 14,17 14,15 13,14 13,14 12,13 14,16	
D2S441 (NC02)	10,14 11,15 14,15 10,14 11,14 10,14 12,14 11,14 10,11 11,15 11,13	
D2S500	9,10 9,11 8,9 9,12 9,12 9,12 10,11 9,11 11,11 11,11 9,9 9,9	
D3S053	9,11 9,12 8,9 9,12 9,12 9,12 10,11 9,11 11,11 11,11 9,9 9,9	
D3S4529	13,13 12,12 13,13 14,14 14,15 13,16 14,16 15,16 13,15 15,17 14,16 14,14	
D4S2364 (NC02)	9,10 9,10 9,10 9,10 9,9 9,10 9,10 9,10 9,10 9,10 9,9 9,9	
D4S408	9,10 10,10 10,11 10,11 10,10 9,9 8,9 9,10 10,11 9,9 8,11 11,11	
D5S2500	14,23 14,17 17,18 14,14 17,18 17,24 17,18 17,18 14,15 14,18 14,20 14,18	
D6S474	14,18 17,17 14,14 16,18 16,17 14,17 14,18 14,18 15,18 14,17 15,17 17,17	
D6S517	9,10 9,11 9,11 9,11 9,11 10,10 10,12 10,12 9,10 9,10 9,10 9,10	
D6S115	9,18 16,17 16,17 16,18 16,18 16,18 16,17 9,17 9,16 9,16 9,18 9,18	
D6S1122	12,13 12,15 12,13 12,13 12,13 12,13 12,12 12,12 11,13 11,12 13,13 13,13	
D9S2157	7,13 7,11 13,13 13,13 8,13 9,11 11,13 11,11 7,14 11,13 12,15 11,11	
D10S1248 (NC01)	13,15 9,15 12,15 12,12 14,16 13,16 13,16 12,12 14,15 14,15 13,14 11,15	
D10S1435	10,11 12,13 15,13 15,13 10,12 13,13 11,14 13,14 12,12 11,13 12,13 11,13	
D11S4463	12,13 12,14 14,14 13,14 14,14 13,14 14,15 11,12 14,16 16,17 14,15 14,17	
D12ATAG3	13,13 13,19 13,17 13,17 14,17 13,17 12,18 16,18 13,15 14,18 16,17 14,15	
D14S1610 (NC01)	11,12 11,13 11,14 11,14 10,13 10,14 11,13 14,15 10,14 13,15 13,14 10,13	
D17S274	7,10 10,11 8,10 8,9 9,9 9,9 9,9 7,9 11,12 9,9 11,11 8,9	
D17S1301	12,12 11,12 12,13 11,12 11,11 11,12 11,12 12,13 11,11 11,13 11,12 12,12	
D18S853	11,14 11,11 11,11 11,11 12,15 11,14 11,11 11,11 10,15 10,15 11,14 14,14 12,13	
D20S482	14,13 13,14 14,15 15,15 14,14 14,16 16,16 14,15 14,15 14,15 14,14 14,14 15,16	
D20S1062	11,14 11,15 12,14 11,11 11,15 14,16 11,11 14,15 11,14 11,15 14,15 11,15 11,15	
D22S1045 (NC01)	11,14 16,18 11,16 16,16 14,16 11,16 15,16 17,18 11,14 11,15 11,15 16,17	

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

On-Going and Future Studies

- Rapid PCR with new loci for screening applications
- Mutation rates** on all new autosomal and Y-STR loci using megaplexes
- Publication of all primers for megaplex
- Making allelic ladders and primer information available to interested companies** following publication (do not plan to ship any more allelic ladders to individual researchers—we are not a production lab)

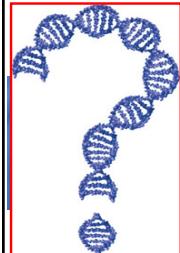
Publications on New Autosomal and Y-STR Loci

- Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* 50: 43-53.
- Butler, J.M., Decker, A.E., Vallone, P.M., Kline, M.C. (2006) Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples. *Forensic Sci. Int.* 156:250-260.
- Coble, M.D., Hill, C.R., Vallone, P.M., Butler, J.M. (2006) Characterization and performance of new miniSTR loci for typing degraded samples. *Progress in Forensic Genetics 11*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1288, 504-506.
- Decker, A.E., Kline, M.C., Vallone, P.M., Butler, J.M. (2007) The impact of additional Y-STR loci on resolving common haplotypes and closely related individuals. *FSI Genetics* 2(2):215-217.
- Hill, C.R., Coble, M.D., Butler, J.M. (2007) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *J. Forensic Sci.* In press.

Thank you for your attention...

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

Questions? <http://www.cstl.nist.gov/biotech/strbase>
john.butler@nist.gov
001-301-975-4049



Margaret Kline	Pete Vallone	Jan Redman
Amy Decker	Becky Hill	Dave Duewer

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Collaborators

Mike Coble (now AFDIL)
– early miniSTR work

Tom Reid (DDC)
- father/son samples