New Autosomal and Y-Chromosome STR Loci

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Presentation Outline

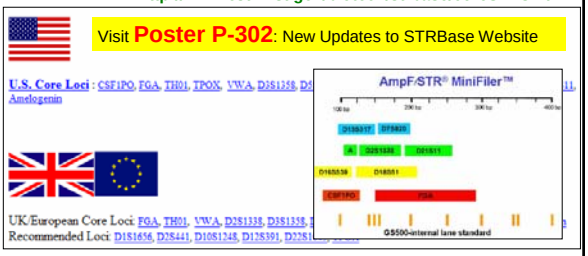
- Why Consider New Loci?
- Selection and Characterization of New STR Loci
- Work with 26 Autosomal STR Loci
- Megaplex vs Miniplex Concordance Studies
- Nomenclature and Standard Sample Typing
- On-going and Future Studies / Publications

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

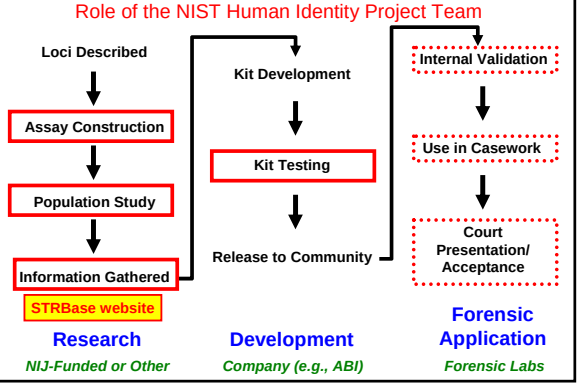
Visit **Poster P-302**: New Updates to STRBase Website



Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.* 51(2): 253-265.

Steps in Applying Forensic DNA Technology

Role of the NIST Human Identity Project Team

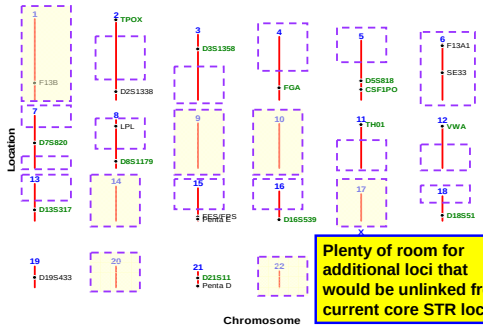


How Would Additional STR Loci Be Useful?

- **Casework**: Obtaining additional information with degraded DNA samples (miniSTRs); rapid screening of multiple crime scene samples
- **Identity Testing**: Kinship analysis, parentage testing, complex criminal paternity, missing persons/mass disasters
- **Databases**: More loci to help resolve relatives in growing national DNA databases (UK went from 6 to 10 STRs in 1999; future Pan-European database will include >10 loci)

Unused Chromosomal Locations

(relative to CODIS 13 STRs)



Plenty of room for additional loci that would be unlinked from current core STR loci

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.* 51(2): 253-265.

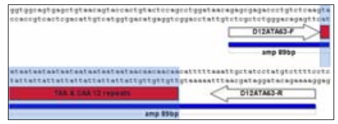
Selection of New Autosomal Loci

- 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] – GAGCGAGACCCTGTCTCAAG
GGAAAAGACATAGGATAGCAATTT

Chr 12 106.825 Mb
(12q23.3)

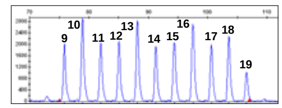
Trinucleotide
[TAA][CAA] repeat
76 -106 bp
Alleles 9 -19

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	[illegible]
12	0.2154	[illegible]	[illegible]
13	0.0173	[illegible]	0.0286
14	[illegible]	**[illegible]**	0.2214
[illegible]	[illegible]	0.0772	0.0714
[illegible]	**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

~660 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
D9S2157	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
D22S1045 (NC01)	663	0.784	4	0.817	0.785	0.721
D2S441 (NC02)	660	0.774	5	0.798	0.780	0.721
D10S1435	663	0.766	6	0.798	0.770	0.700
D2S1776	654	0.763	7	0.740	0.801	0.734
D3S4529	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	0.757	0.702	0.743
D11S4463	664	0.730	[illegible]	[illegible]	[illegible]	[illegible]
D4S2408	654	0.722	[illegible]	[illegible]	[illegible]	[illegible]
D18S853	664	0.711	[illegible]	[illegible]	[illegible]	[illegible]
D20S1082	664	0.696	[illegible]	[illegible]	[illegible]	[illegible]
D14S1434 (NC01)	663	0.696	[illegible]	[illegible]	[illegible]	[illegible]
D20S482	648	0.691	[illegible]	[illegible]	[illegible]	[illegible]
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

European recommended loci

>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

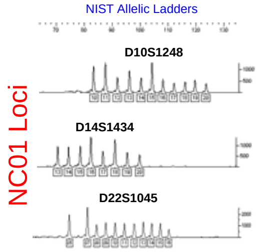
http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm

Two Part Approach with New STR Loci

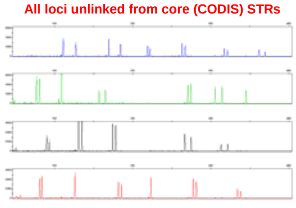
http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm

- For degraded samples, 26 new miniSTR loci that are **<140 bp** and typed as triplexes
- For reference sample typing, put maximum loci + amelogenin in **a single multiplex** (megaplex)

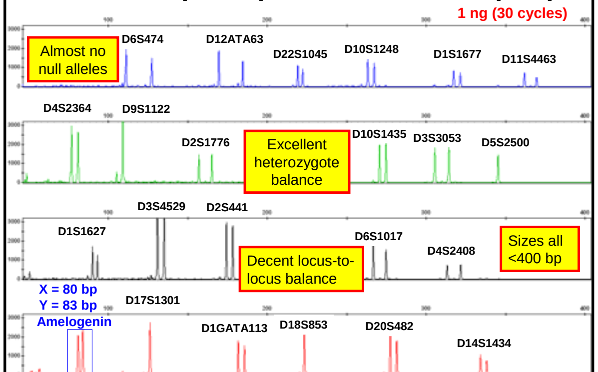
NIST Allelic Ladders



All loci unlinked from core (CODIS) STRs



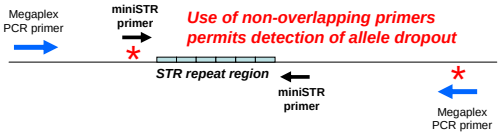
NIST "Autoplex" (Autosomal STR 23plex)



Haas-Rochholz and Weiler (1997) *Int J Legal Med* 110(6): 312-315

Concordance Study to Check for Null Alleles

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



"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

Identifiler 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 9947A	9948	ABI 007	K562	SRM 2391b Components Genomic 1	Genomic 2	Genomic 3	Genomic 4	Genomic 5	Genomic 6	Genomic 7	Genomic 8
D1GATA113	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	13,14	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	11,12	14,15	10,14	11,14	11,14	10,14	12,14	11,14	10,11	11,14	11,11.3
D2S1776	10,10	10,12	8,10	11,11	11,12	11,11	8,10	11,12	12,13	11,12	11,12	11,12
D3S3053	9,11	9,12	9,9	12,12	9,12	10,11	9,11	11,11	11,11	9,9	11,11	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	9,9	9,9	9,10	9,10	9,9	9,10	8,9	9,9	9,9
D4S2408	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	14,16	14,18	14,20	14,18
D6S474	14,18	17,17	14,14	15,18	15,17	14,17	14,15	14,16	15,18	14,17	15,17	17,17
D6S1017	9,10	8,8	10,10	8,11	10,10	10,12	10,12	7,10	8,9	10,10	7,12	10,12
D8S1115	9,18	15,17	15,17	16,16	16,16	16,16	16,17	9,17	9,15	9,16	9,18	15,16
D9S1122	12,13	12,15	12,12	10,14,15	11,12	12,13	12,12	12,12	11,13	11,12	11,12	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	12,15	12,15	12,12	14,16	13,15	13,16	12,12	14,15	14,15	13,14	11,15
D10S1435	10,11	12,13	11,13	10,12	13,13	11,14	13,14	12,12	11,12	12,12	12,12	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
D12ATA63	13,13	13,18	13,17	17,17	14,17	13,17	12,15	16,18	13,15	14,18	16,17	14,15
D14S1434 (NC01)	11,13	13,14	11,14	10,10	13,14	11,13	14,15	10,11	13,14	13,14	10,14	13,13
D17S974	7,10	10,11	9,10	8,8	9,11	9,10	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	11,11	11,12	12,12
D18S853	11,14	11,11	11,11	12,15	11,14	11,11	11,11	11,13	10,15	11,14	14,14	12,13
D20S482	14,15	13,14	14,15	15,15	14,14	14,16	15,15	14,15	14,15	14,14	14,14	15,16
D20S1082	11,14	11,15	12,14	11,11	11,15	14,15	11,11	14,15	11,14	11,15	14,15	11,15
D22S1045 (NC01)	11,14	16,18	11,16	16,16	14,15	11,16	15,16	17,18	11,14	11,15	11,15	16,17

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

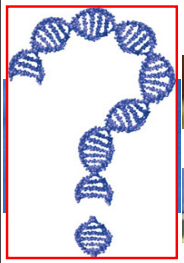
Publications on New Autosomal and Y-STR Loci

- 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* 1(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
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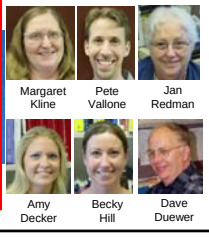
001-301-975-4049



Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

Collaborators

Mike Coble (now AFDIL) – early miniSTR work

Tom Reid (DDC) - father/son samples