Advanced Topics in Forensic DNA Analysis

miniSTRs and Degraded DNA

New Jersey State Police
Training Workshop

Hamilton, NJ
December 5-6, 2006

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Outline for This Section

- NIST projects funded by NIJ
- Background on miniSTRs
- MiniFiler kit and concordance studies performed
- New non-CODIS (NC) miniSTR loci

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development, expert system review
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

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

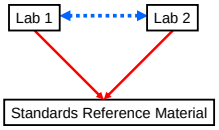
Standard Reference Materials

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

Traceable standards to ensure accurate measurements in our nation's crime laboratories

SRM 2391b – CODIS STRs
SRM 2392-I – mtDNA
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation

Helps meet DAB Std. 9.5 and ISO 17025



Calibration with SRMs enables confidence in comparisons of results between laboratories

Information Resource

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

Includes information on:

- Core STR loci
- Validation
- STR reference list
- NIST publications
- miniSTRs
- Forensic SNPs
- Variant STR alleles
- Population data resources
- Addresses of scientists

Provides up-to-date information and has been used in court cases to support application of DNA technology

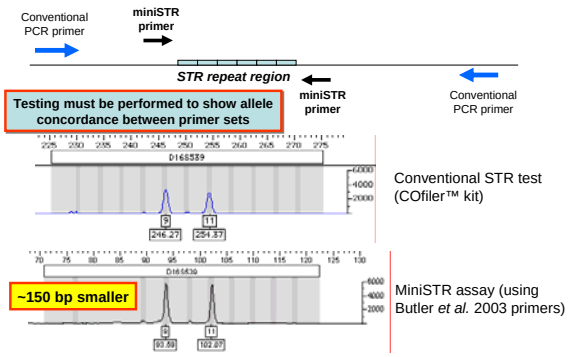
Recent STRBase Updates…

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

Technology: Research Programs

- miniSTRs
- Y-chromosome STRs
- mtDNA
- SNPs
- qPCR for DNA quantitation
- DNA stability studies
- Variant allele characterization and sequencing
- Software tools
- Expert System review
- Assay development with collaborators

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

miniSTR Overview Article

http://marketing.appliedbiosystems.com/images/enews/ForensicNews_Vol7/PDF/02A_CustomerCorner_Butler.pdf

Timeline for miniSTRs

and Demonstrating the Value of Using Reduced Size Amplicons for Degraded DNA

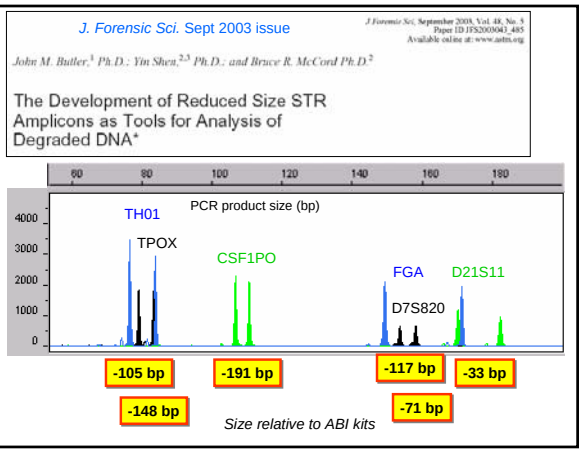
- 1994 – FSS finds that smaller STR loci work best with burned bone and tissue from Branch Davidian fire
- 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- 2004 – Work at NIST with **non-CODIS (NC) miniSTRs**
- 2006/07 – Applied Biosystems to release 9plex MiniFiler

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

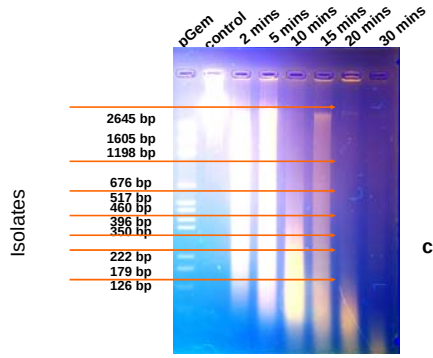
Recent Publications on miniSTRs

- Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5): 1054-1064.
- Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.
- Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.
- 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.

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



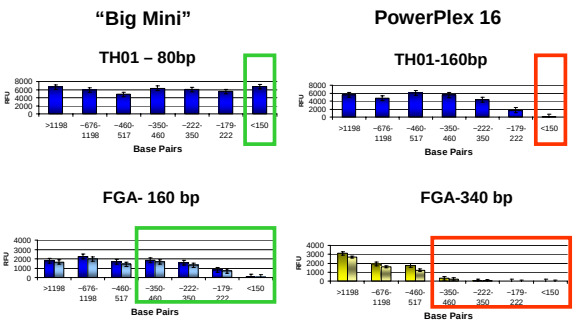
DNA Degraded With DNase I



Dnase concentration: 0.01 U/ µL

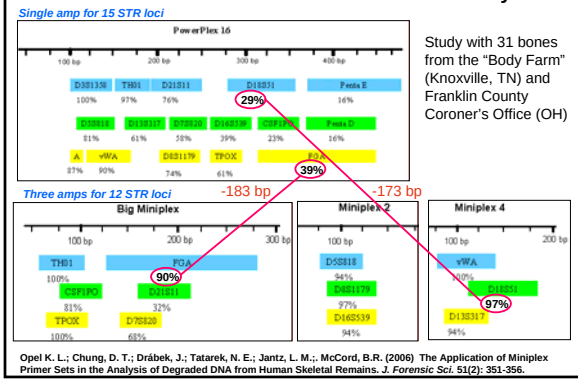
Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.

Miniplexes improve detection of degraded DNA



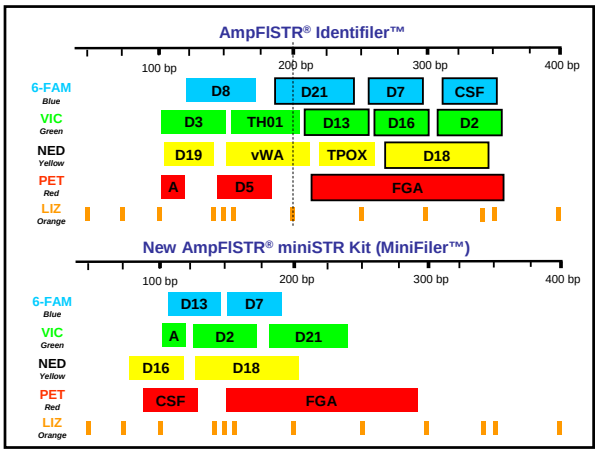
Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.

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



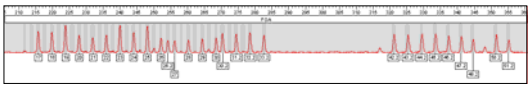
Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

Opel K. L.; Chung, D. T.; Dråbek, J.; Tatarek, N. E.; Jantz, L. M.;. McCord, B.R. (2006) The Application of Miniplex Primer Sets in the Analysis of Degraded DNA from Human Skeletal Remains. *J. Forensic Sci.* 51(2): 351-356.

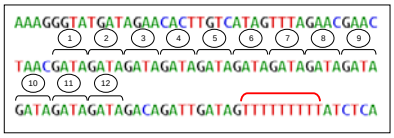


Why Go Beyond the CODIS Loci?

(1) Large Allele Ranges (e.g. FGA)



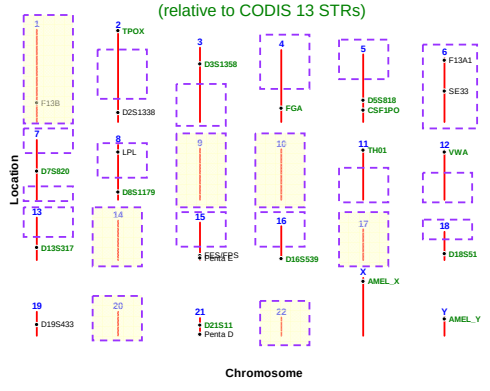
(2) "Unclean" Flanking Sequences (e.g. D7S820)



Butler, JM, Shen, Y., McCord, BR (2003) *JFS* 48(5): 1054-1064

Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



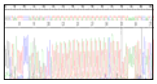
Characterization of New miniSTR Loci

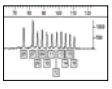
"Computer Work"

Candidate STR marker selection (e.g. Marshfield Clinic Center of Medical Genetics) → Pull down sequence data from the web (e.g. NCBI) → Identify Chromosome Location (e.g. Human BLAT Search) → Screen for PCR Primers (e.g. Primer3) → Test primers for Multiplex-ability (e.g. AutoDimer - NIST)

"Laboratory Work"

Test Markers on Population samples | Sequence homozygotes to determine allele sizes | Build Macros for Genotyping | Construct Allelic Ladders

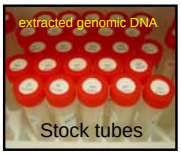


Standard U.S. Population Dataset

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)

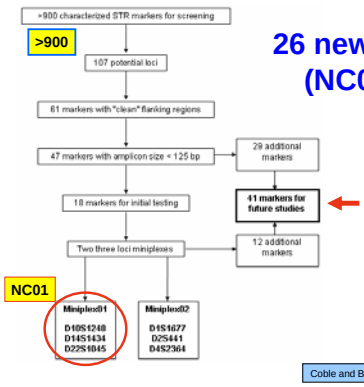


To date: (>100,000 allele calls)

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)
New miniSTR loci – for 26 loci, 17,238 genotypes
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers

Initial Testing Results with Potential miniSTR Loci

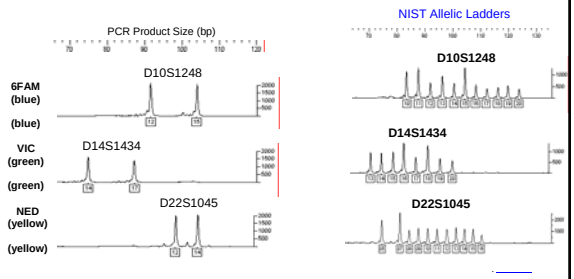


26 new miniSTRs (NC01-NC09)

20 additional loci characterized across U.S. population groups

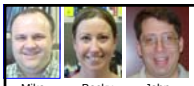
Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

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



Mike Coble | Becky Hill | John Butler

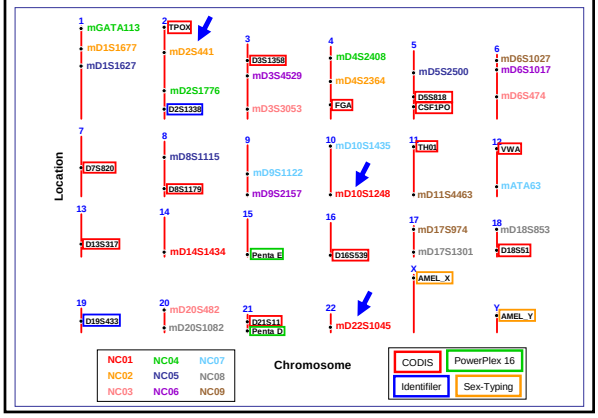
No longer at NIST (AFDIL Research Section Chief since April 2006)

New miniSTR Non-CODIS (NC) Loci

- 32 STR loci tested on NIST **665 U.S. population samples**
- **26 STR loci with allele sizes below 140 bp and good heterozygosities (above TPOX level)**
- All new STR loci are **physically unlinked** to the 13 CODIS core loci
- **Submitted articles** regarding primer sequences and locus characterization including population statistics
- **SRM 2391b components are being certified** through sequencing for D10S1248, D2S441, D22S1045; for reference purposes, genotypes for standard samples (9947A, 9948, 007, K562) will be made available on STRBase

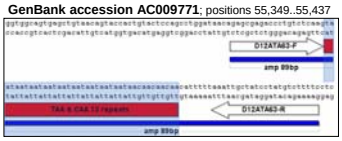
http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm

Chromosomal Locations of New miniSTR Loci



Characterization of miniSTR **D12ATA63**

GenBank accession AC009771; positions 55,349..55,437



[FAM] – GAGCGAGACCCTGTCTCAAG
GGAAAAGACATAGGATAGCAATTT

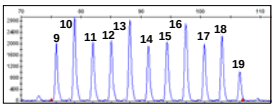
Chr 12 106.825 Mb
(12q23.3)

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	0.1500
12	0.2154	0.1004	0.1786
13	0.0173	0.1564	0.0286
14	0.1615	**0.3340**	0.2214
15	0.0577	0.0772	0.0714
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

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	17	0.780	0.676	0.743
D4S2408	654	0.722	18	0.752	0.709	0.691
D18S853	664	0.711	19	0.772	0.645	0.721
D20S1082	664	0.696	20	0.792	0.653	0.600
D14S1434 (NC01)	663	0.696	21	0.685	0.721	0.650
D20S482	648	0.691	22	0.673	0.689	0.729
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 Labs Have Adopted the NIST-Developed NC miniSTRs

FSI (2006) **156(2):** 242-244

Short communication

The evolution of DNA databases—Recommendations for new European STR loci

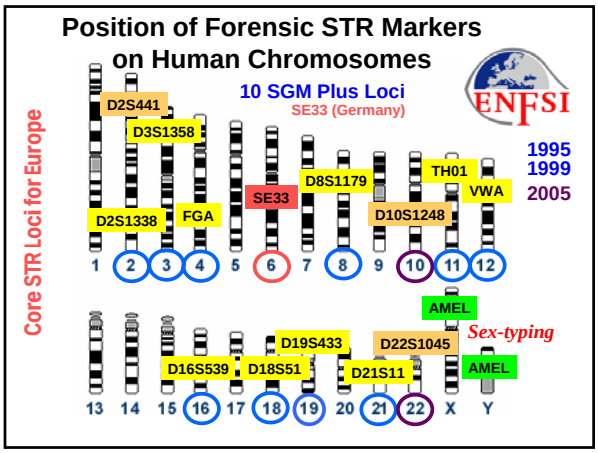
Peter Gill [a,*], Lyn Fereday [b], Niels Morling [c], Peter M. Schneider [d]

[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
[d] Institute of Legal Medicine, University of Cologne, Germany

Received 25 May 2005; accepted 26 May 2005

…recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.

(D14 has been replaced with D2S441 from NC02)



Summary of miniSTRs

- Reduced size amplicons improve success rates with degraded DNA or samples possessing PCR-inhibitors – European leaders view miniSTRs as “the way forward”
- A new kit will be available soon from ABI called MiniFiler – concordance testing done at NIST
- New miniSTR loci are being characterized at NIST – 26 loci in development