Topics and Techniques for Forensic DNA Analysis

miniSTRs

Florida Statewide Training Meeting

Indian Rocks Beach, FL
May 12-13, 2008

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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

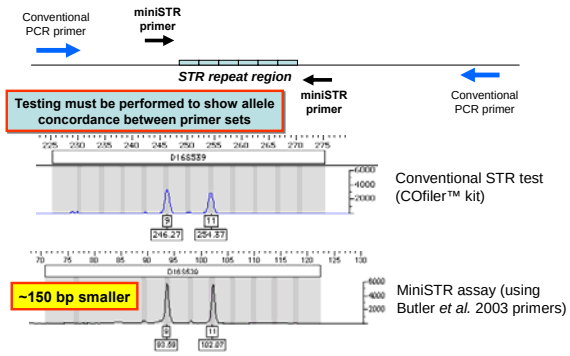
- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development, expert system review
- **Training Materials**
  - Review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

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

Technology: Research Programs

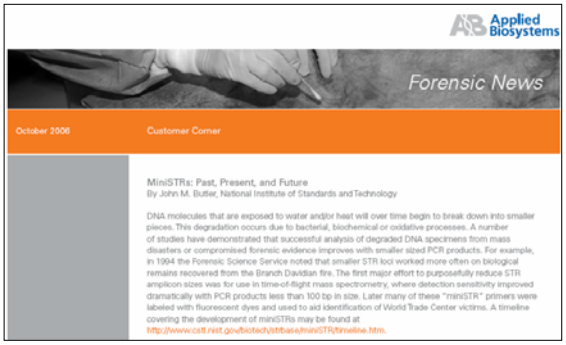
- miniSTRs
- Y-chromosome STRs
- mtDNA
- SNPs
- qPCR for DNA quantitation
- DNA stability studies
- Variant allele characterization and sequencing
- Software tools
- Expert System review
- Assay development with collaborators

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

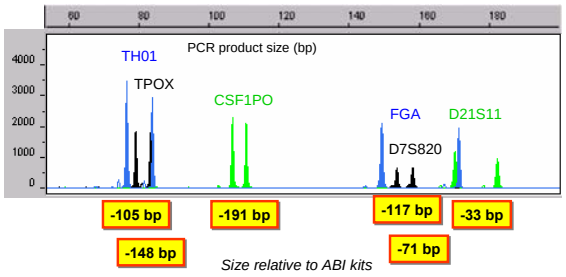
- 1994 – FSS finds that smaller STR loci work best with burned bone and tissue from Branch Davidian fire
- 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- 2004 – Work at NIST with **non-CODIS (NC) miniSTRs**
- 2007 – Applied Biosystems releases 9plex MiniFiler

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

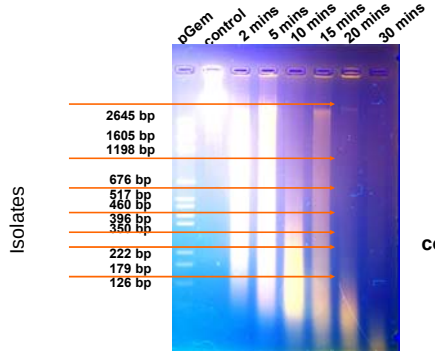
J. Forensic Sci. Sept 2003 issue

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*

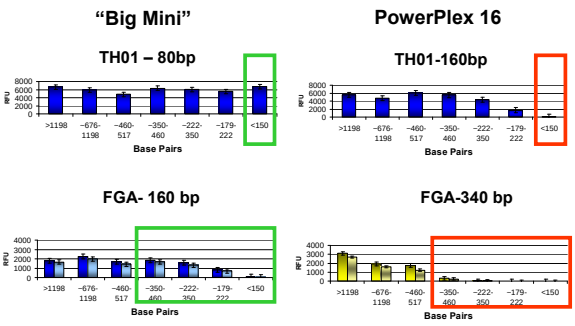


DNA Degraded With DNase I



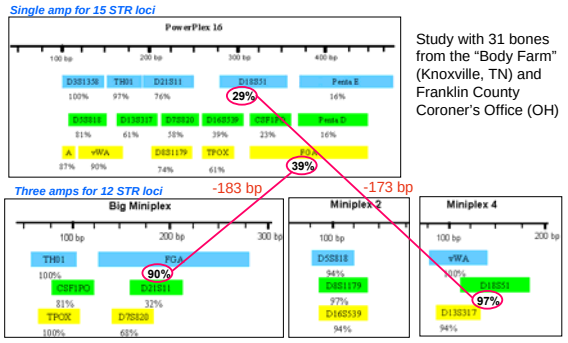
Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.

Miniplexes improve detection of degraded DNA



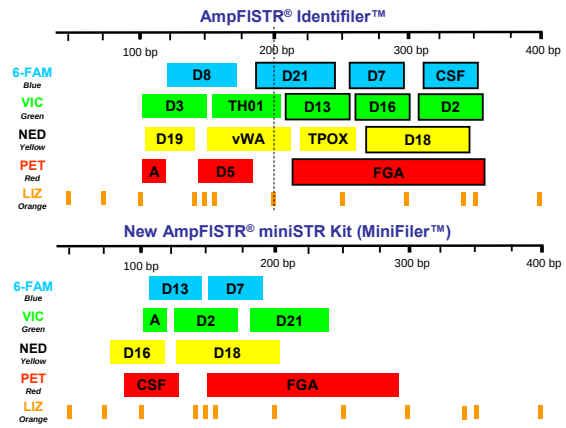
Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.

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



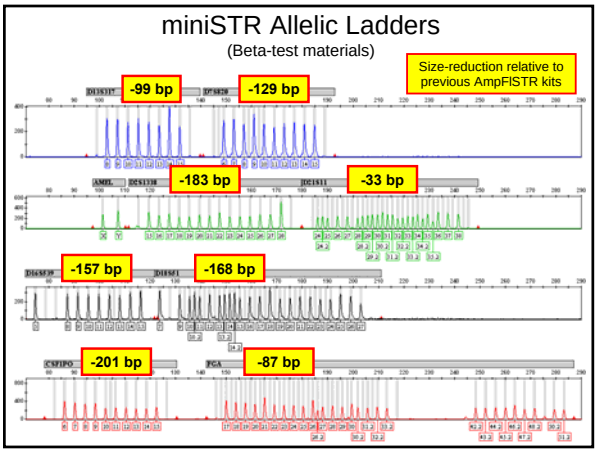
Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

Opel K. L.; Chung, D. T.; Drábek, 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.



miniSTR Allelic Ladders

(Beta-test materials)



Summary of Samples Typed with ABI MiniFiler kit at NIST and ABI

- Primarily only population samples examined – no extensive sensitivity or degraded DNA tests were performed

1,308 samples Allele concordance = 10,437/10,464 = 99.7%

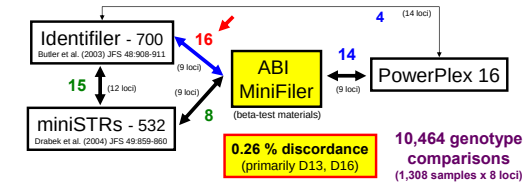
- **656 NIST U.S. population samples**
  - 260 Caucasian, 253 African American, 140 Hispanic, 3 Asian
  - Previously examined with **Identifiler; also with PowerPlex 16**
  - Also tested with Butler *et al.* (2003) **published miniSTR primers**
  - http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm
- **481 father-son pairs**
  - 184 Caucasian, 196 African American, 101 Asian samples (provided by paternity testing company DDC)
  - Previously examined with **Identifiler**
- **171 samples from Applied Biosystems**

Hill *et al.* (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.* 52(4): 870-873.

Concordance Conducted at NIST

27 Discordant Calls

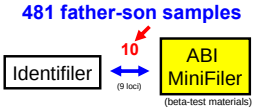
656 NIST U.S. population samples



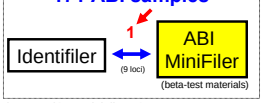
0.26 % discordance (primarily D13, D16)

10,464 genotype comparisons (1,308 samples x 8 loci)

481 father-son samples



171 ABI samples

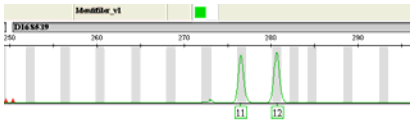


Hill *et al.* (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.* 52(4): 870-873.

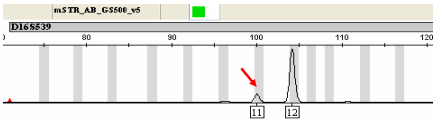
Concordance Studies Reveal Potential Primer Binding Site Mutations with Different Primer Sets

D16S539

Identifiler



miniSTR Kit (beta-test)



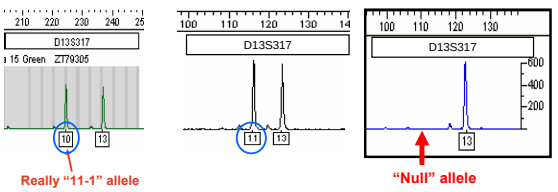
Appears to be an allele 11 dropout/reduction due to primer binding site mutation

Examination of **D13S317** Concordance:

African American sample ZT79305

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.

NIST Identifiler data | Ohio U miniSTR data | **AB miniSTR beta-test**

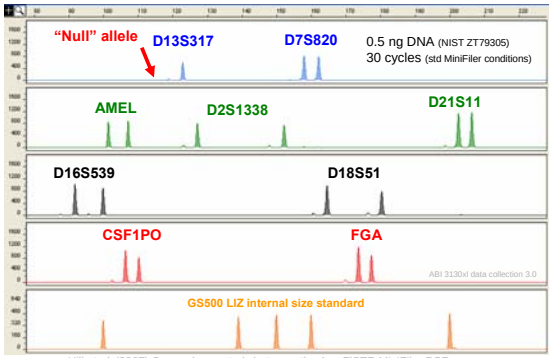


10,13 **11,13** **13,13**

Reverse primer is outside deletion | **Reverse primer is inside deletion** | **Reverse primer is on top of deletion**

Hill *et al.* (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.* 52(4): 870-873.

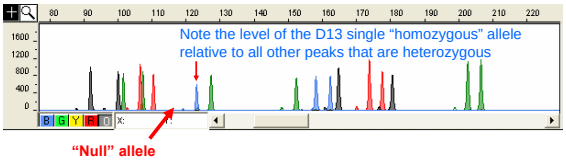
Full MiniFiler Profile for NIST Sample with D13S317 Allele Dropout

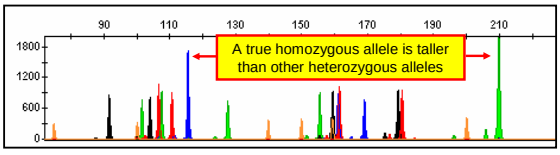


Hill *et al.* (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.* 52(4): 870-873.

Note the Relative D13 Peak Heights

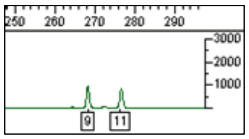
(Suggests Allele Dropout)



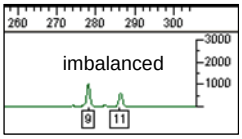


D16S539 SRM 2391b Genomic 8

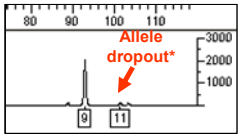
Identifiler



PowerPlex 16



MiniFiler



Due to primer binding site mutation

Example of a SNP in a primer region causing peak imbalances and Allele dropout

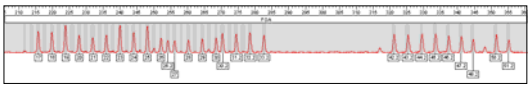
More Loci are Useful in Situations Involving Relatives

- **Missing Persons** and Disaster Victim Identification (kinship analysis)
- Immigration Testing (often limited references)
  - Recommendations for 25 STR loci
- Deficient Parentage Testing
  - often needed if only one parent and child are tested

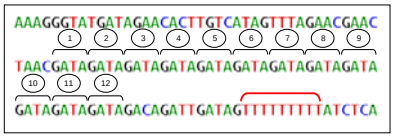
Relationship testing labs are being pushed to answer more difficult genetic questions…and **we want to make sure the right tools are in place**

Why Go Beyond the CODIS Loci?

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



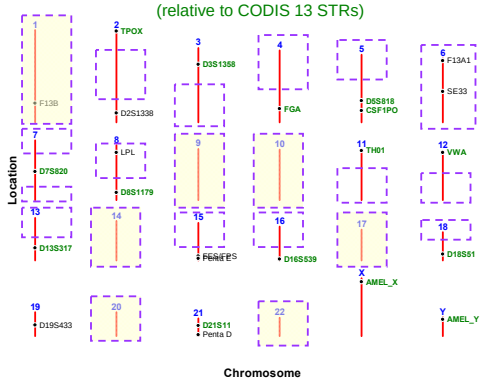
(2) "Unclean" Flanking Sequences (e.g. D7S820)

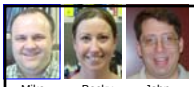


Butler, JM, Shen, Y., McCord, BR (2003) *JFS* 48(5): 1054-1064

Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)





New miniSTR Non-CODIS (NC) Loci

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

- 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

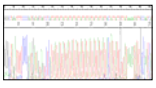
Characterization of New miniSTR Loci

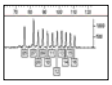
"Computer Work"

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)

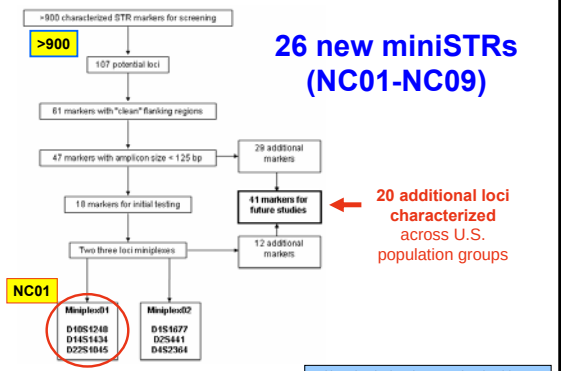
"Laboratory Work"

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



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

New STR Loci Characterized

Hill *et al.* (2008) *J. Forensic Sci.* 53(1):73-80

J Forensic Sci, January 2008, Vol. 53, No. 1
doi: 10.1111/j.1556-4029.2008.00595.x
Available online at: www.blackwell-synergy.com

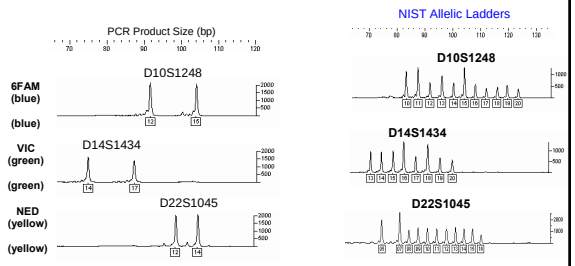
Carolyn R. Hill, M.S.; Margaret C. Kline, M.S.; Michael D. Coble,† Ph.D.; and John M. Butler, Ph.D.

Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

- Primer sequences (for miniplexes), GeneMapper bins and panels, genotypes on common samples, and allele frequency information **available on STRBase**

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_NC_loci_types.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_Panels_Panels.txt
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_Panels_NC_bins_bins.txt

Miniplex "NC01"



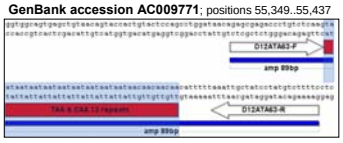
http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

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

Characterization of miniSTR **D12ATA63**

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

[FAM] – GAGCGAGACCCTGTCTCAAG
GAAAAGACATAGGATAGCAATTT



Chr 12 106.825 Mb (12q23.3)

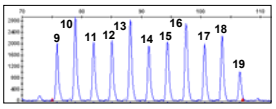
Trinucleotide [TAA][CAA] repeat
76 -106 bp
Alleles 9 -19

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

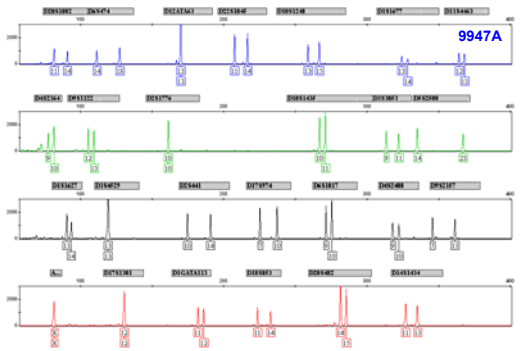
U.S. Caucasian	**0.842**
African American	**0.788**
U.S. Hispanic	**0.879**

D12ATA63 Allelic Ladder



"Autoplex" (26plex)

See Hill et al. AAFS 2008 talk (Washington, DC) and poster PP50 at DNA in Forensics 2008 meeting (Ancona)



Gender identification + 25 autosomal STR loci in a single amplification

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"**
- **MiniFiler concordance** testing performed
- **New miniSTR loci are being characterized** at NIST – 26 loci developed

Thank you for your attention…

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

Becky Hill

miniSTRs and 26plex work

Bruce McCord

Early miniSTR work

Mike Coble

Original NC miniSTR work

Margaret Kline

STR allele sequencing

http://www.cstl.nist.gov/biotech/strbase
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Rob Lagace
Chien-Wei Chang