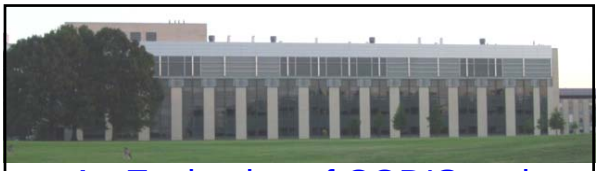


An Evaluation of CODIS and Non-CODIS MiniSTR Loci

Becky Hill

National Institute of Standards and Technology

Mid-Atlantic Association of Forensic Sciences Meeting
Washington D.C.
May 24, 2007

Disclaimers

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice.

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

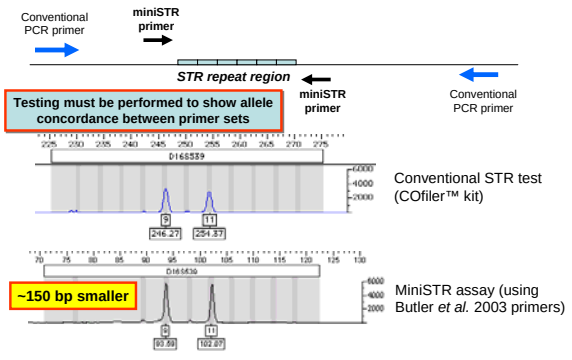
Our publications and presentations are made available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Outline of Topics to Discuss

- Introduction to miniSTR loci
- Concordance study with ABI MiniFiler™ kit (including 7 CODIS loci) using U.S. population samples
- The value of non-CODIS miniSTR loci
- The characterization of additional miniSTR loci
- The "miniMegaplex" 5-dye single amplification multiplex system: *in development*…

Introduction to MiniSTR Loci

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

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

NIJ 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons

NIJ 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002

NIJ 2004 – Work at NIST with **non-CODIS (NC) miniSTRs**

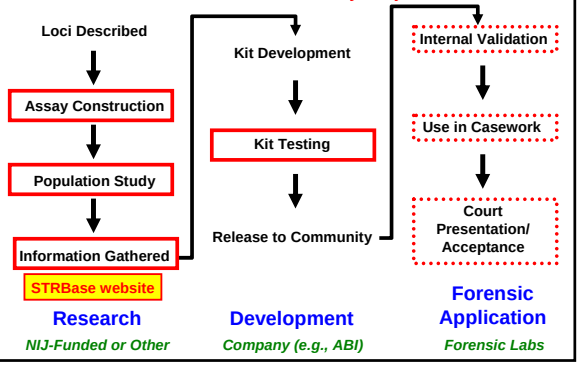
- 2007 – Applied Biosystems releases 9plex MiniFiler

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

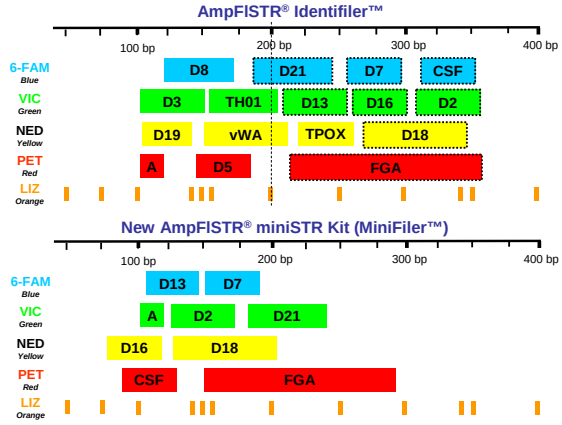
How Do We See Our Role at NIST?

Steps in Applying Forensic DNA Technology

Role of the NIST Human Identity Project Team

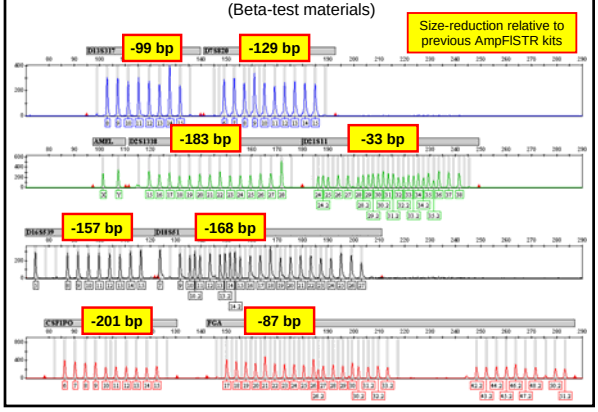


Concordance Study with ABI MiniFiler kit



MiniFiler Allelic Ladders

(Beta-test materials)



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

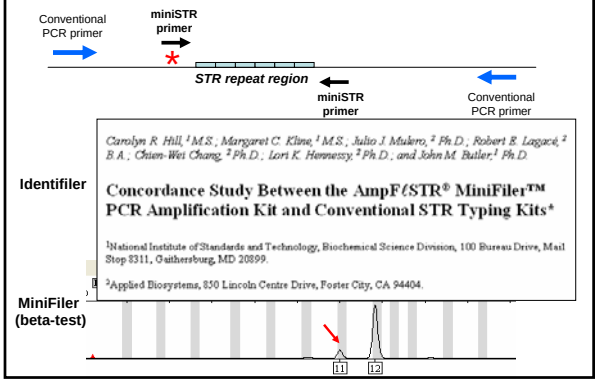
- Primarily only population samples examined – no extensive sensitivity or degraded DNA tests were performed

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, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci., in press.*

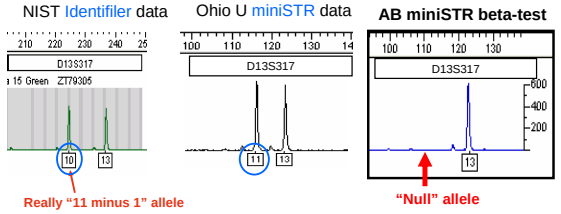
Concordance Studies Reveal Potential Primer Binding Site Mutations with Different Primer Sets



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.



Really "11 minus 1" allele

"Null" allele

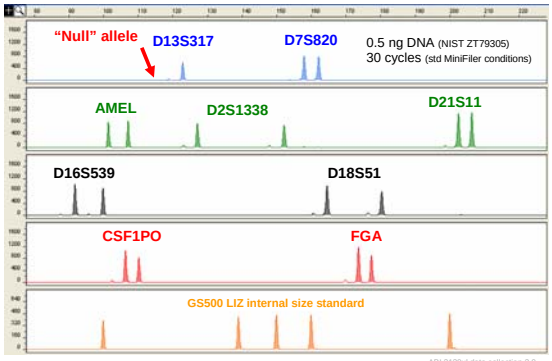
10,13 | **11,13** | **13,13**

Reverse primer is outside 4 bp deletion

Reverse primer is inside 4 bp deletion

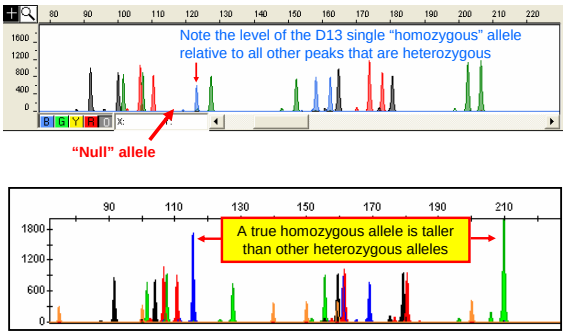
Reverse primer is on top of 4 bp deletion

Full MiniFiler Profile for NIST Sample with D13S317 Allele Dropout



Note the Relative D13 Peak Heights

(Suggests Allele Dropout)



Apparent Null Alleles Observed During Concordance Studies

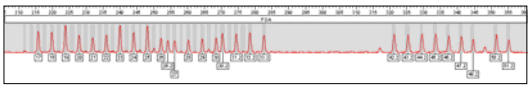
New Section of STRBase (launched to track MiniFiler discordance and allele dropout frequency):
http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm

Locus	STR Kits/Assays Compared	Results	Frequency of Primer Binding Site Mutation	Source
CSF1PO	MiniFiler vs ID vs PP16	MF: 11,11 and ID: 11,11.1 One base insertion in Identifiler amplicon outside of MiniFiler and PP16 primers	1/1308	Hill *et al.* (2007)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with COfiler, fine with PP16	2/1537	Budowle *et al.* (2001)
FGA	SGM vs SGM Plus	Loss of allele 26 with SGM Plus; weak amp of same allele with SGM		Cotton *et al.* (2000)
FGA	PP16 vs ProPlus	Loss of allele 22 with ProPlus; fine with PP16		Budowle and Sprecher (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with COfiler, fine with PP16	1/1537	Budowle *et al.* (2001)
TH01	SGM vs SGM Plus	Loss of allele 6 with SGM Plus; fine with SGM	1/4045	Clayton *et al.* (2004)
VWA	PP1.1 vs ProPlus	Loss of allele 19 with ProPlus; fine with PP1.1	2/1483	Kline *et al.* (1998) and Walsh (1998)
VWA	PP16 vs ProPlus	Loss of alleles 15 and 17 with ProPlus; fine with PP16	2/1537	Budowle *et al.* (2001)
VWA	ID vs miniplexes	Loss of alleles 12, 13, and 14 with miniplex assay; fine with ID	9/532	Drabek *et al.* (2004)

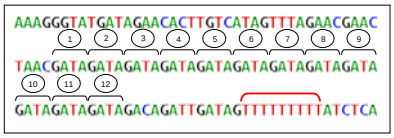
The Value of Non-CODIS MiniSTR Loci

Why Go Beyond the CODIS Loci?

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

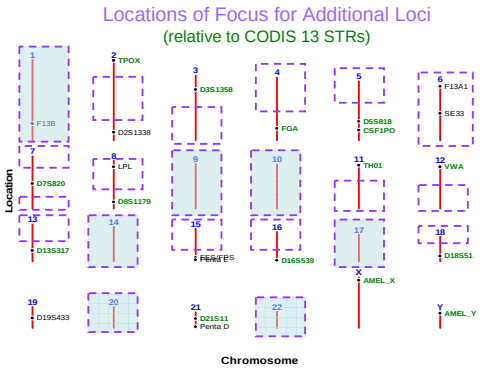


(2) "Unclean" Flanking Sequences (e.g. D7S820)



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

(3) A large amount of unused chromosomal locations

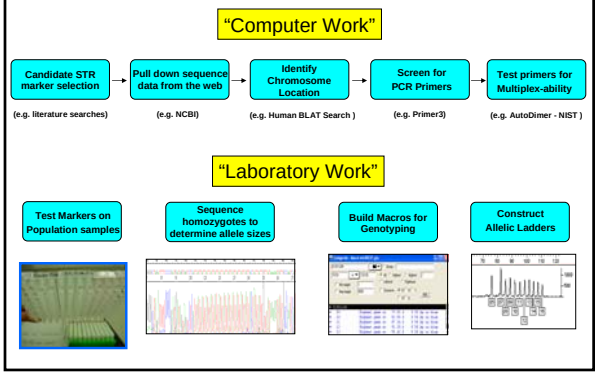


Uses of Additional Non-CODIS miniSTR Loci in the Forensic Community

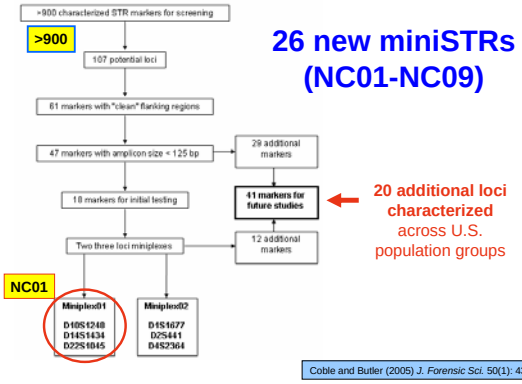
- Obtaining additional information with degraded DNA samples
- Missing persons investigations
- Identification of mass disaster victims
- Paternity testing

The Characterization of Additional MiniSTR Loci

Characterization of New Loci

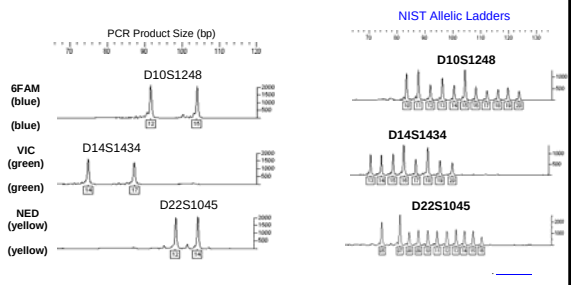


Initial Testing Results with Potential miniSTR Loci



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

Miniplex "NC01"

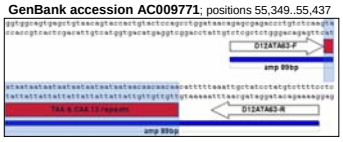


http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

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

Characterization of miniSTR **D12ATA63**

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



[FAM] – GAGCGAGACCCTGTCTCAAG
GAAAAGACATAGGATAGCAATTT

Chr 12 106.825 Mb
(12q23.3)

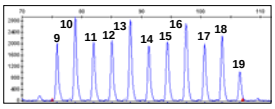
Trinucleotide
[TAA][CAA] repeat
76 -106 bp
Alleles 9 -19

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



European Labs Have Adopted the NIST-Developed NC (**n**on-**C**ODIS) 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)

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

26 New miniSTR Loci Typed (and Sequenced) with Standard Samples

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	14,15	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	12,16	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	12,16	12,16	12,12	14,16	13,15	13,16	12,12	14,16	14,16	13,14	11,16
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

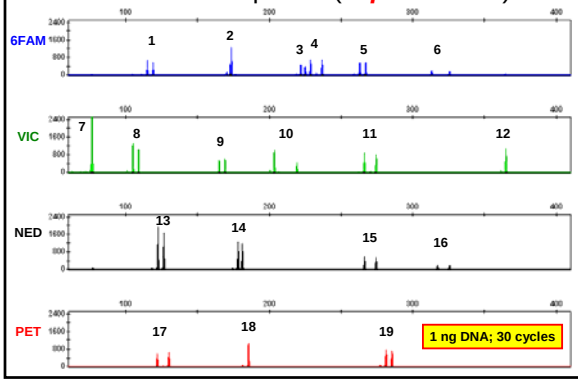
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_NC_loci_types.htm

"miniMegaplex" 5-dye Single Amplification Multiplex System

In Development…

Reference Sample MegaPlex

Under Development (***19plex so far***)



In Summary

Conclusions

- **MiniFiler:** concordance study with ABI (Hill *et al. J. Forensic Sci., in press*)
- **New Non-CODIS (NC) Loci: 26 miniSTR loci** tested on NIST **665 U.S. population samples**
- **European forensic community** has recommended three miniSTRs (D2S441, D10S1248, D22S1045) to be adopted as standard core loci
- **SRM 2391b components are being certified** through sequencing for D10S1248, D2S441, D22S1045; for reference purposes, genotypes for standard samples (9947A, 9948, 007, K562) are available on STRBase
- A **"miniMegaplex"** is in development to type 26 miniSTR loci in a 5-dye single reaction
- **New miniSTR loci information on STRBase**:

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

NIST Publications on miniSTR Loci

- 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.
- 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.
- 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.
- Butler, J.M. (2006) MiniSTRs: past, present, and future. *Forensic News* (Applied Biosystems) October 2006.
- 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.
- Hill, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci., in press.*
- Hill, C.R., Coble, M.D., Butler, J.M. (2007) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *submitted.*

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between the National Institute of Justice and the NIST Office of Law Enforcement Standards

NIST Human Identity Project Team – Leading the Way in Forensic DNA…

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

Tom Reid (DNA Diagnostics Center) – supplying the father-son samples for use in MiniFiler concordance study

Mike Coble (AFDIL Research Section Chief, former member of NIST Human Identity Team)

Applied Biosystems - Lori Hennessy, Julio Mulero, Robert Legacé, and Chien-Wei Chang