National Institute of Standards and Technology
. . .working with industry to develop and apply technology, measurements and standards

Development, Characterization and Performance of New MiniSTR Loci for Typing Degraded Samples

Michael Coble
Becky Hill, Peter Vallone, and John Butler

June 26, 2006
NIJ DNA Grantees meeting (Crystal City, VA)

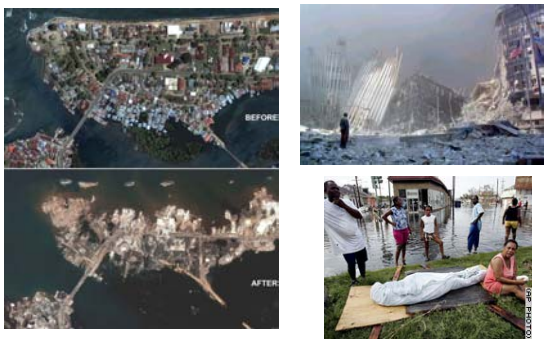
National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Research Effort

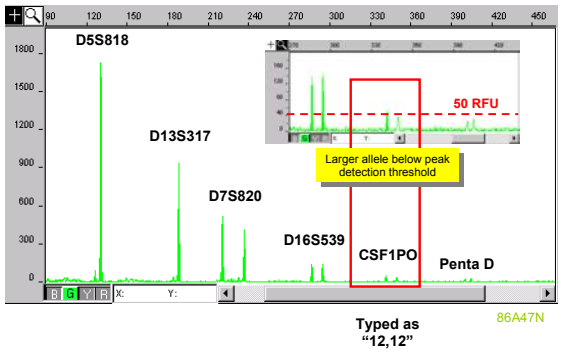
- **Resources for "Challenging Samples"**
- **Standard Reference Materials** (SRM 2391 DNA Profiling Standard)
- **Information on New Loci** (SNPs, Y-Chromosome, new STRs)
- **Standard Information Resources** (STRBase website, training materials/review articles, validation standardization)
- **Allele Sequencing and Interlaboratory Studies** (Real-time qPCR, mixture interpretation)

Visit NIST table during lunchtime on Tuesday to see latest projects and get a copy of the STRBase website content

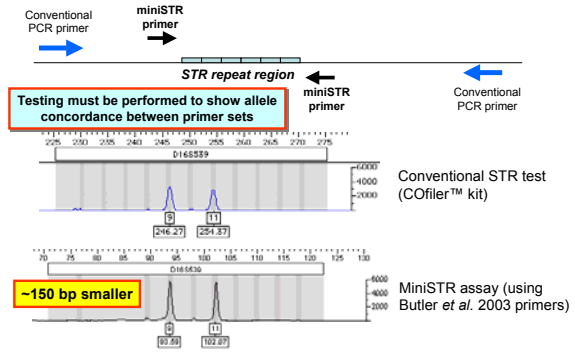
Highly Degraded DNA



PowerPlex 16 Result on Aged Blood Stain
(15 years at room temperature storage)



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

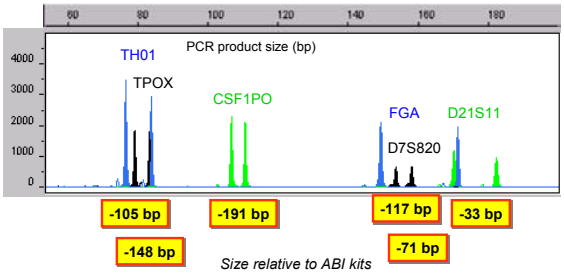
Spanish
Malaysian
Austrian
Japanese
U.S. groups

J. Forensic Sci. Sept 2003 issue

J Forensic Sci, September 2003, Vol. 48, No. 5
Paper ID JFS2003043_485
Available online at: www.astm.org

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*



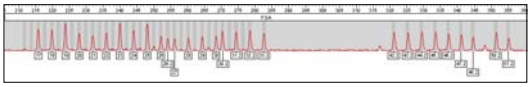
Timeline for miniSTRs

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
- 2004 – Work at NIST with non-CODIS miniSTRs
- 2006 – Applied Biosystems plans to release a 9plex miniSTR kit

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

Why go beyond CODIS loci?

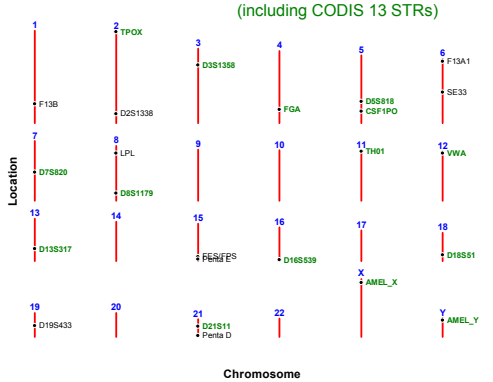
"STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association."

"To achieve this purpose, either ***new STRs could be developed***, or alternatively, existing STRs could be supplemented with a SNP panel."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM). *Science&Justice*, **44(1):** 51-53.

Commercial STR Kit Loci Positions

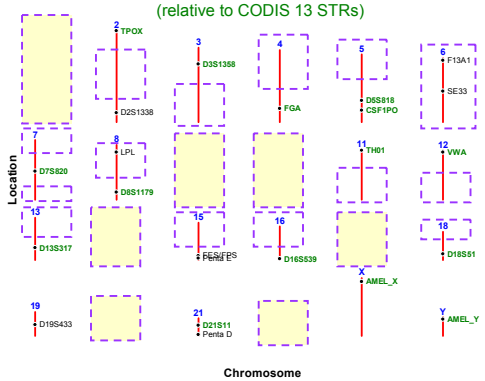
(including CODIS 13 STRs)



Positions determined along May 2004 Human Genome Reference Sequence (NCBI Build 35)

Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



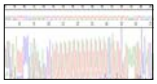
Characterization of New miniSTR Loci

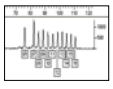
"Computer Work"

- 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



Candidate STR marker selection

Marshfield Clinic Research Foundation GENETICS

Reconstruction of Human Evolutionary Tree Using Polymorphic Autosomal Microsatellites

deCODE genetics

SCIENCE VOL 298 20 DECEMBER 2002
Genetic Structure of Human Populations

CIDR Center for Inherited Disease Research

Characterization of New miniSTR Loci

Rosenberg *et al.* 2002 – 1062 samples; 377 STRs; diverse populations

Locus name	Alternate name	Heterozygosity	Number of alleles	Chromosome
D6S1017	GCATH10	0.748	9	6
D6S1009	GATA32E03	0.748	13	6
D8S261	AFM123XG5	0.747	17	8
D18S1390	18QTEL11	0.747	15	18
D13S1817	GATA11C08	0.747	9	13
D12S1052	GATA26D02	0.747	8	12
D16S2616	ATA41E04	0.746	11	16
D2S1780	GATA72G11	0.746	13	2
NA-D1S-3	GATA133A08	0.745	12	1
NA-D8S-2	GAAT1A4	0.745	9	8
D8S1025	GATA10F	0.745	10	4
D18S851	GATA6D09	0.745	12	18
D8S1136	GATA41A01	0.745	11	8
NA-D5S-1	ATA52D02	0.744	27	5
D3S3038	GATA73D01	0.743	14	3
D15S1515	GATA109H10	0.743	9	15
D16S2624	GATA81D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D13S895	GGAA22C01	0.740	11	13
D11S1998	GATA23E06	0.740	9	11

Focus on:

High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

Characterization of New miniSTR Loci

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D16S2624	GATA81D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D13S895	GGAA22C01	0.740	11	13
D11S1998	GATA23E06	0.740	9	11

Focus on:

High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

Identification of PCR Primers

Drop in sequence from GenBank

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

Identification of PCR Primers

PCR Primer Design

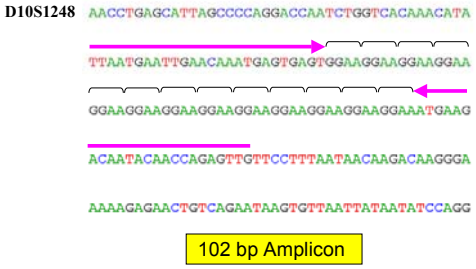
PCR Primer Design

REJECT!

PCR Primer Design

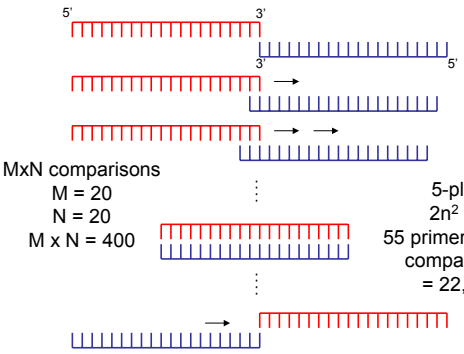
13 GGAA Repeats

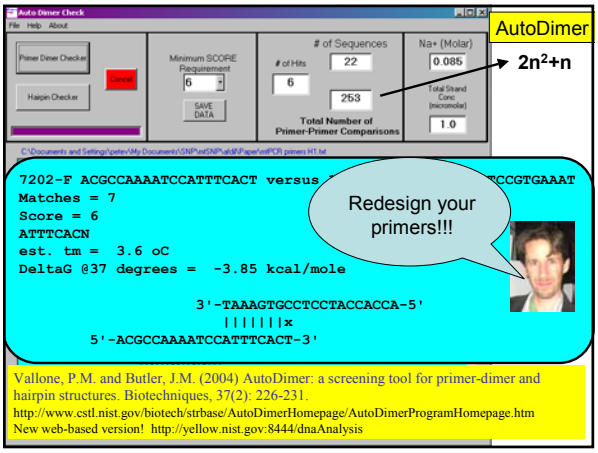
PCR Primer Design



102 bp Amplicon

Basic Sliding Algorithm for Complementarity Check



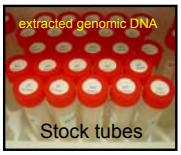


Standard U.S. Population Dataset

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)

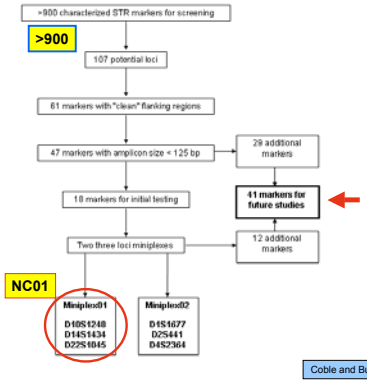


Genotypes with various human identity testing markers

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 11 loci, 7,293 genotypes
mtDNA full control region sequences by AFDIL

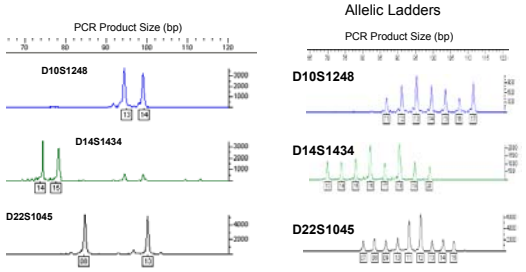
Initial Testing Results with Potential miniSTR Loci



We have just completed our final pass.

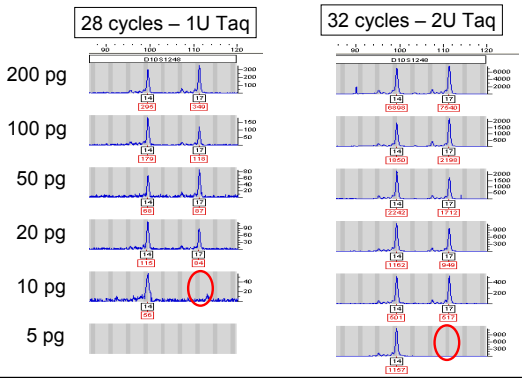
Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Miniplex "NC01"



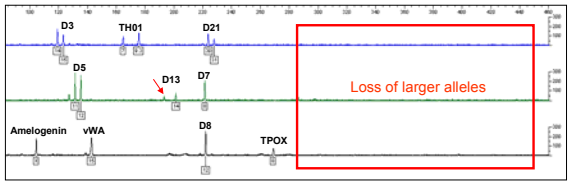
Coble and Butler (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA *J. Forensic Sci.* 50(1): 43-53

miniSTR Assay Sensitivity (D10S1248)

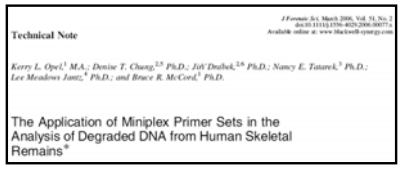


Sensitivity - Degraded DNA from an OU Bone Sample

PP16



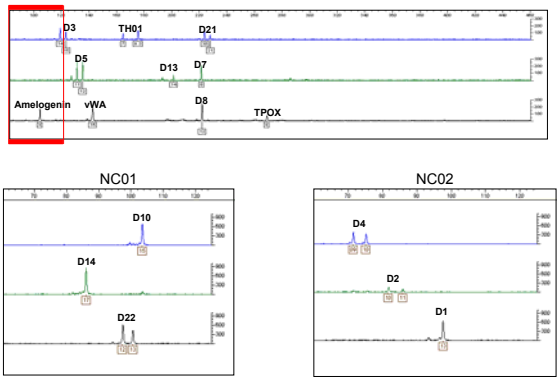
10 pg/µL (30pg input DNA), 32 cycles, 2U Taq



Technical Note

The Application of Miniplex Primer Sets in the Analysis of Degraded DNA from Human Skeletal Remains*

Sensitivity - Degraded DNA from an OU Bone Sample



EDNAP Exercise on Degraded DNA


ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT

Forensic Science International

ELSEVIER

Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise

L.A. Dixon, A.E. Dobbins, H.K. Pulker, J.M. Butler, P.M. Vallone, M.D. Coble, W. Parson, B. Berger, P. Grubwieser, H.S. Mogensen, N. Morling, K. Nielsen, J.J. Sanchez, E. Petkovski, A. Carracedo, P. Sanchez-Diz, E. Ramos-Luis, M. Brión, J.A. Irwin, R.S. Just, O. Loreille, T.J. Parsons, D. Syndercombe-Court, H. Schmitter, B. Stradmann-Bellinghausen, K. Bender, P. Gill

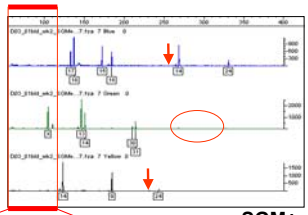
Conducted in the Fall of 2004

MiniSTR primer mixes and allelic ladders were provided by NIST

MiniSTR performance on degraded DNA samples

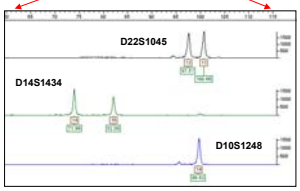
Individual 2
Blood Stain – 2 Weeks

Allelic drop out at D16 and FGA
Failure at D18



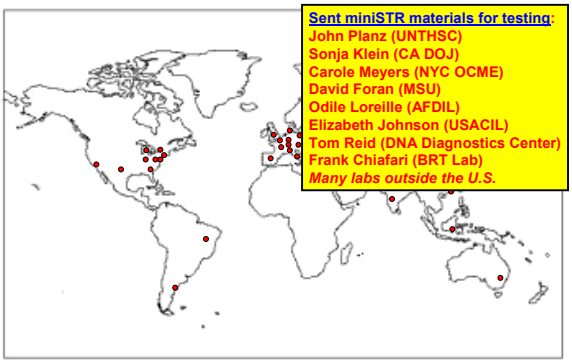
SGM+
32 cycles

NC01
32 cycles



Dixon *et al.*, *FSI, in press*

Global Impact of NC miniSTRs



Sent miniSTR materials for testing:
John Planz (UNTHSC)
Sonja Klein (CA DOJ)
Carole Meyers (NYC OCME)
David Foran (MSU)
Odile Loreille (AFDIL)
Elizabeth Johnson (USACIL)
Tom Reid (DNA Diagnostics Center)
Frank Chiafari (BRT Lab)
Many labs outside the U.S.

The International Commission on Missing Persons (ICMP) is Now Using miniSTRs

100s of bones are tested each week with miniSTRs to help in the re-association of remains

Add details on loci used

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

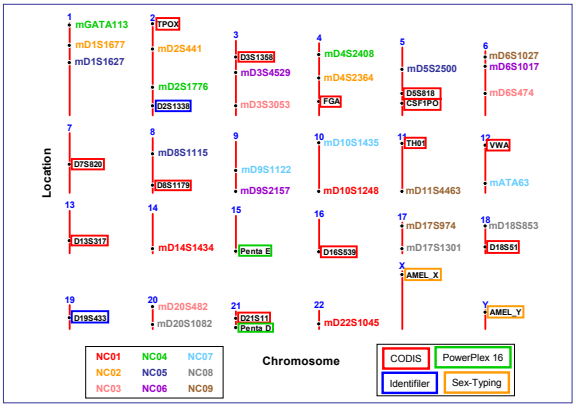
Peter Gill [a,*], Lyn Fereday [b], Niels Morling [c], Peter M. Schneider [d]

[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)



Comparison of heterozygosity values for 28 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

Rank by Heterozygosity (Variability)

	Locus	N	Heterozygosity (Overall)	Rank	Size Range (bp)
	FGA	659	0.886	1	196 - 352 (ProPlus)
	D2S1338	659	0.882	2	288 - 340 (SGM+)
	D18S51	659	0.876	3	264 - 344 (ProPlus)
	D9S2157	*661*	*0.844*	*4*	*71 - 101*
	D21S11	659	0.844	5	186 - 244 (ProPlus)
	ATA63 (D12)	*659*	*0.829*	*6*	*76 - 106*
	vWA	659	0.826	7	152 - 212 (ProPlus)
	D7S820	659	0.806	8	253 - 293 (ProPlus)
	D19S433	659	0.803	9	106 - 140 (SGM+)
EDNAP/ ENFSI suggested markers	**D10S1248 (NC01)**	**663**	**0.792**	**10**	**83 - 123**
	D22S1045 (NC01)	***663***	***0.784***	***11***	***76 - 109***
	D2S441 (NC02)	**660**	**0.774**	**12**	**78 - 110**
	D8S1179	659	0.774	13	123 - 171 (ProPlus)
	D16S539	659	0.766	14	233 - 273 (CoFiler)
	D10S1435	663	0.766	15	82 - 139
	D3S1358	659	0.763	16	97 - 145 (ProPlus)
	D2S1776	654	0.763	17	127 - 161
	D3S4529	660	0.761	18	111 - 139
	D6S474	648	0.761	19	107 - 135
	D5S2500	664	0.747	20	85 - 125
	▪	▪	▪	▪	▪
	▪	▪	▪	▪	▪
	▪	▪	▪	▪	▪
	TPOX	659	0.707	34	213 - 249 (CoFiler)
	D20S1082	*664*	*0.696*	*35*	*73 - 100*
	D14S1434 (NC01)	663	0.696	36	70 - 98

<150 bp

Past and Future Publications

- **Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* 50(1):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.
- Dixon, L.A., Dobbins, A.E., Pulker, H., Butler, J.M., Vallone, P.M., Coble, M.D., et al. (2006) Analysis of artificially degraded DNA using STRs and SNPs--results of a collaborative European (EDNAP) exercise. *Forensic Sci. Int., in press*.
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Conclusions

- MiniSTRs will have a critical role in future forensic DNA investigations (archived samples – post-conviction testing, skeletal remains in missing persons cases, mass disasters)
- Additional markers not linked to the CODIS loci will be helpful for cases involving paternity disputes, or complex criminal investigations (incest)

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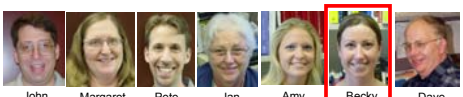

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