Advanced Topics in STR DNA Analysis

STR Biology, Markers, and Methods

AAFS 2006 Workshop #6
Seattle, WA
February 20, 2006

john.butler@nist.gov
NIST

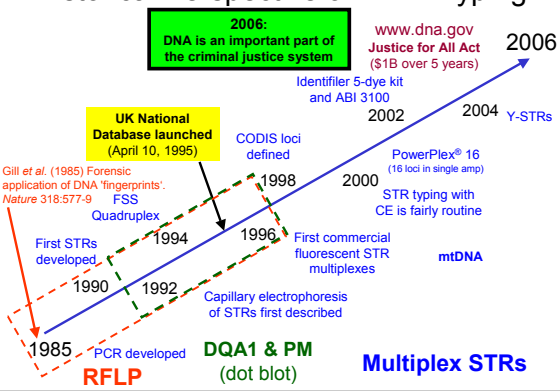
Dr. John M. Butler
Dr. Bruce R. McCord

mccordb@fiu.edu
FLORIDA INTERNATIONAL UNIVERSITY

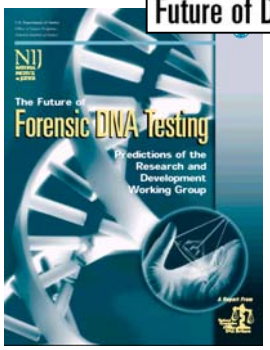
Outline of This Section

- Timeline of field and growth of STR use
- STR characteristics and biology
- STR core loci and commonly used kits
- miniSTRs
- STR protocols
- Reduced volume reactions
- STRBase resources

Historical Perspective on DNA Typing



National Commission on the Future of DNA Evidence



http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

- Report published in Nov 2000
- Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles

National DNA Index System (NDIS)

FBI LABORATORY

CODIS
COMBINED DNA INDEX SYSTEM

http://www.fbi.gov/hq/lab/codis/index1.htm

Combined DNA Index System (CODIS)

Launched in October 1998 and now links all 50 states
Used for linking serial crimes and unsolved cases with repeat offenders
Convicted offender and forensic case samples along with a missing persons index
Requires 13 core STR markers
>27,000 investigations aided nationwide as of Sept 2005
Contains more than 2.8 million DNA profiles

Advantages for STR Markers

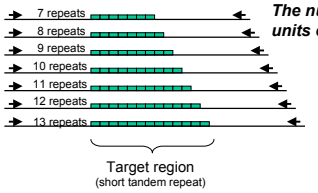
- Small product sizes are generally compatible with degraded DNA and PCR enables recovery of information from small amounts of material
- Multiplex amplification with fluorescence detection enables high power of discrimination in a single test
- Commercially available in an easy to use kit format
- Uniform set of core STR loci provide capability for national and international sharing of criminal DNA profiles

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTG**GATAGATAGATAGATAGATAGATAGATAGATAGATAGA**
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

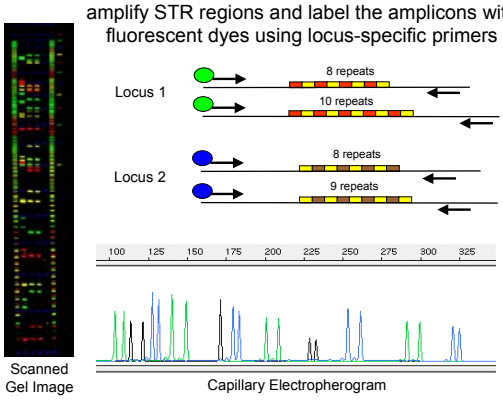
= 12 GATA repeats ("12" is all that is reported)



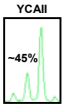
The number of consecutive repeat units can vary between people

The FBI has selected **13 core STR loci** that must be run in all DNA tests in order to provide a common currency with DNA profiles

The polymerase chain reaction (PCR) is used to amplify STR regions and label the amplicons with fluorescent dyes using locus-specific primers



Types of STR Repeat Units

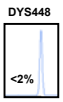


Requires size based DNA separation to resolve different alleles from one another

High stutter

- **Di**nucleotide (CA)(CA)(CA)(CA)
- **Tri**nucleotide (GCC)(GCC)(GCC)
- **Tetra**nucleotide (AATG)(AATG)(AATG)
- **Penta**nucleotide (AGAAA)(AGAAA)
- **Hexa**nucleotide (AGTACA)(AGTACA)

Low stutter



Short tandem repeat (STR) = microsatellite = simple sequence repeat (SSR)

Categories for STR Markers

Category	Example Repeat Structure	13 CODIS Loci
Simple repeats – contain units of identical length and sequence	(GATA)(GATA)(GATA)	TPOX, CSF1PO, D5S818, D13S317, D16S539
Simple repeats with non-consensus alleles (e.g., TH01 9.3)	(GATA)(GAT-)(GATA)	TH01, D18S51, D7S820
Compound repeats – comprise two or more adjacent simple repeats	(GATA)(GATA)(GACA)	VWA, FGA, D3S1358, D8S1179
Complex repeats – contain several repeat blocks of variable unit length	(GATA)(GACA)(CA)(CATA)	D21S11

These categories were first described by **Urquhart *et al.* (1994) *Int. J. Legal Med.* 107:13-20**

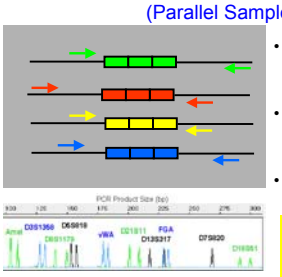
How many STRs in the human genome?

- The efforts of the Human Genome Project have increased knowledge regarding the human genome, and hence there are many more STR loci available now than there were 10 years ago when the 13 CODIS core loci were selected.
- **More than 20,000 tetranucleotide STR loci have been characterized in the human genome** (Collins et al. An exhaustive DNA micro-satellite map of the human genome using high performance computing. Genomics 2003;82:10-19)
- There may be more than a million STR loci present depending on how they are counted (Ellegren H. Microsatellites: simple sequences with complex evolution. Nature Rev Genet 2004;5:435-445).
- STR sequences account for approximately 3% of the total human genome (Lander et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921).

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci., in press.*

Multiplex PCR

(Parallel Sample Processing)

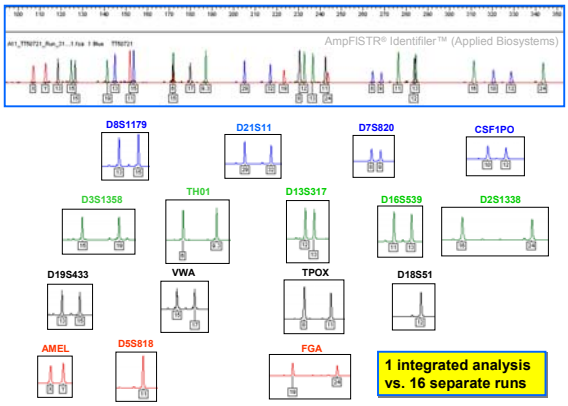


- **Compatible primers are the key to successful multiplex PCR**
- **STR kits are commercially available**
- **15 or more STR loci can be simultaneously amplified**

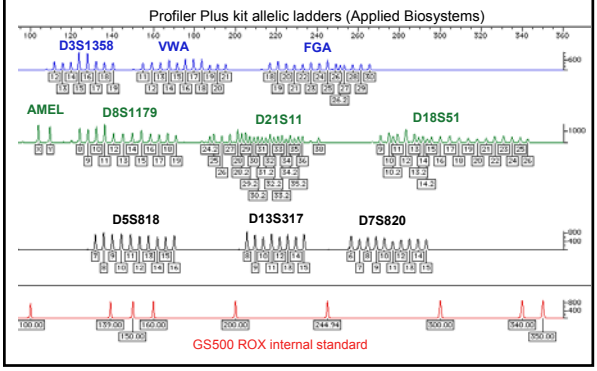
Challenges to Multiplexing
- primer design to find compatible primers (no program exists)
- reaction optimization is highly empirical often taking months

Advantages of Multiplex PCR
- –Increases information obtained per unit time (increases power of discrimination)
- –Reduces labor to obtain results
- –Reduces template required (smaller sample consumed)

Information is tied together with multiplex PCR and data analysis



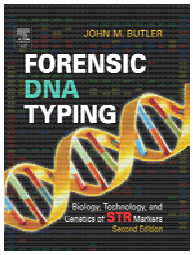
Companies Supply **Allelic Ladders** in STR Kits to **Aid Interlaboratory Consistency**



Biological "Artifacts" of STR Markers

- Stutter Products
- Non-template nucleotide addition
- Microvariants
- Tri-allelic patterns
- Null alleles
- Mutations

Chapter 6 covers these topics in detail



Stutter Products

- Peaks that show up primarily one repeat less than the true allele as a result of strand slippage during DNA synthesis
- Stutter is less pronounced with larger repeat unit sizes (dinucleotides > tri- > tetra- > penta-)
- Longer repeat regions generate more stutter
- Each successive stutter product is less intense (allele > repeat-1 > repeat-2)
- Stutter peaks make mixture analysis more difficult

STR Alleles with Stutter Products

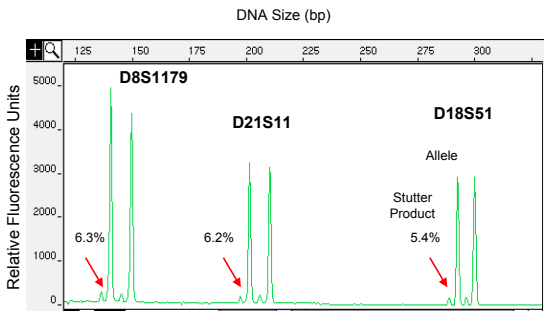
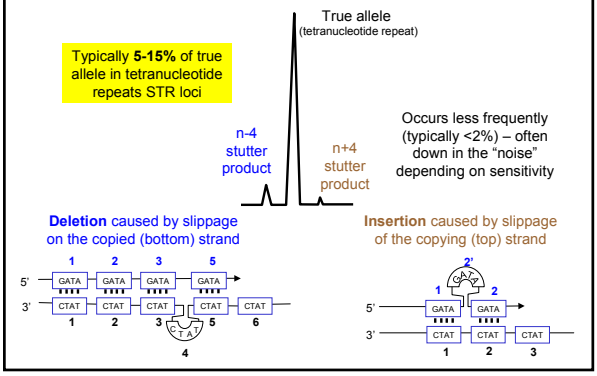


Figure 6.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Stutter Product Formation

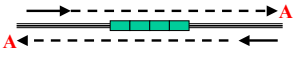
Repeat unit bulges out when strand breathing occurs during replication

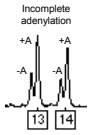


Non-Template Addition

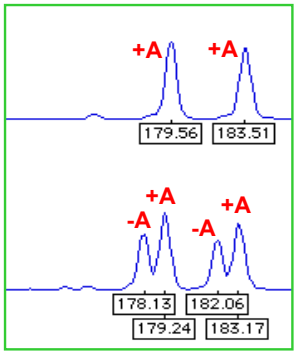
- Taq polymerase will often add an extra nucleotide to the end of a PCR product; most often an "A" (termed "adenylation")
- **Dependent on 5'-end of the reverse primer; a "G" can be put at the end of a primer to promote non-template addition**
- Can be enhanced with extension soak at the end of the PCR cycle (e.g., 15-45 min @ 60 or 72 ºC) – to give polymerase more time
- Excess amounts of DNA template in the PCR reaction can result in incomplete adenylation (not enough polymerase to go around)

Best if there is NOT a mixture of "+/- A" peaks
(desirable to have full adenylation to avoid split peaks)





Impact of the 5' Nucleotide on Non-Template Addition



5'-ACAAG…

Last Base for Primer Opposite Dye Label

(PCR conditions are the same for these two samples)

5'-CCAAG…

Promega includes an ATT sequence on the 5'-end of many of their unlabeled PP16 primers to promote adenylation
see Krenke *et al.* (2002) *J. Forensic Sci.* 47(4): 773-785
http://www.cstl.nist.gov/biotech/strbase/PP16primers.htm

Higher Levels of DNA Lead to Incomplete Adenylation

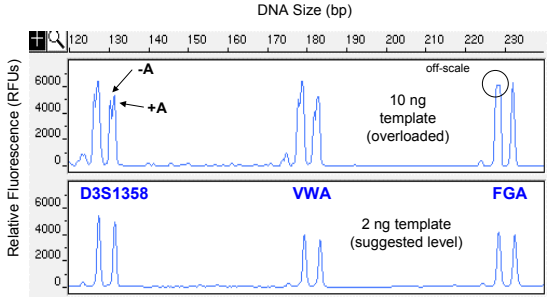


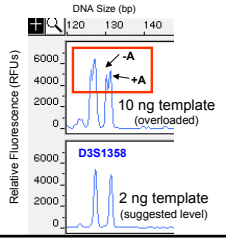
Figure 6.5, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

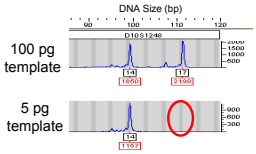
Impact of DNA Amount into PCR

Reason that DNA Quantitation is Important Prior to Multiplex Amplification

Generally 0.5 – 2.0 ng DNA template is best for STR kits

- Too much DNA
  - Off-scale peaks
  - Split peaks (+/-A)
  - Locus-to-locus imbalance
- Too little DNA
  - Heterozygote peak imbalance
  - Allele drop-out
  - Locus-to-locus imbalance

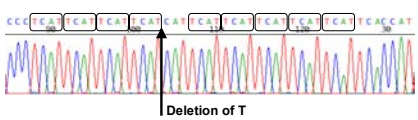




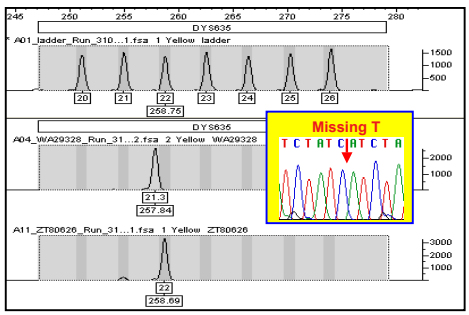
Stochastic effect when amplifying low levels of DNA produces allele dropout

Microvariant "Off-Ladder" Alleles

- Defined as alleles that are not exact multiples of the basic repeat motif or sequence variants of the repeat motif or both
- Alleles with partial repeat units are designated by the number of full repeats and then a decimal point followed by the number of bases in the partial repeat (Bar *et al. Int. J. Legal Med.* 1994, 107:159-160)
- Example: **TH01 9.3 allele**: $[TCAT]_4$ -CAT $[TCAT]_5$



An Example of an "Off-Ladder" Microvariant at the Yfiler Locus DYS635



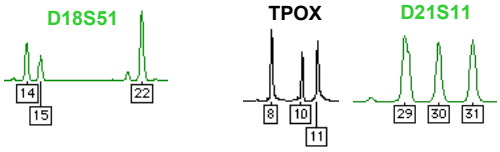
Allele 22 bin
258.75 +/- 0.5
= 258.25 to 259.25

Allele 21.3
257.84
(-0.41 from bin)

$[TCTA]_4(TGTA)_2[TCTA]_2(TGTA)_2[TCTA]_2(TGTA)_2[TCTA]_5$ TC-A $[TCTA]_2$

Three-Peak Patterns

Clayton *et al.* (2004) A genetic basis for anomalous band patterns encountered during DNA STR profiling. *J Forensic Sci.* 49(6):1207-1214



"Type 1"

Sum of heights of two of the peaks is equal to the third

Most common in D18S51 and .....

"Type 2"

Balanced peak heights

Most common in TPOX and D21S11

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

328 total variants reported as of 10/04/05

- CSF1PO (15)
- D2S1338 (5)
- D3S1358 (23)
- D5S818 (7)
- D7S820 (23)
- D8S1179 (6)
- D13S317 (13)
- D16S539 (12)
- D18S51 (32)
- D19S433 (12)
- D21S11 (24)
- FESFPS (1)
- FGA (83)
- F13A01 (1)
- HUMTH01 (11)
- Penta D (23)
- Penta E (16)
- TPOX (14)
- VWA (7)

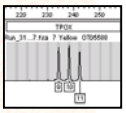
Currently **328** at 13/13 CODIS loci + F13A01, FES/FPS, Penta D, Penta E, D2S1338, D19S433

Tri-Allelic Patterns

80 total patterns reported as of 11/03/05

- CSF1PO (3)
- D3S1358 (4)
- D5S818 (2)
- D7S820 (6)
- D8S1179 (5)
- D13S317 (4)
- D16S539 (4)
- D18S51 (9)
- D21S11 (7)
- FGA (12)
- FES/FEP (1)
- HUMTH01 (1)
- TPOX (13)
- VWA (9)

Currently **80** at 13/13 CODIS loci + FES/FPS

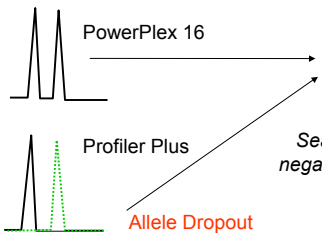


Null Alleles

- Allele is present in the DNA sample but fails to be amplified due to a **nucleotide change in a primer binding site**
- Allele dropout is a problem because a heterozygous sample appears falsely as a homozygote
- Two PCR primer sets can yield different results on samples originating from the same source
- This phenomenon impacts DNA databases
- Large concordance studies are typically performed prior to use of new STR kits

For more information, see J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, pp. 133-138

Concordance between STR primer sets is important for DNA databases



Search results in a false negative (miss samples that should match)

Reduced match stringency is a common solution

vWA Primer Position Comparisons

Promega STR Kit

Polymorphism outside of forward PP16 primer

33 nt | 11 bp | 9 bp | 30 nt | TMR

T→A

155 bp

PowerPlex® 16

Krenke *et al.* (2002) *J. Forensic Sci.* 47:773-785

GenBank = 18 repeats

ABI STR Kit

Polymorphism impacts 2nd base from the 3'end of ProPlus primer

50 bp | 11 bp | A | G | FAM | FAM

T→A

184 bp

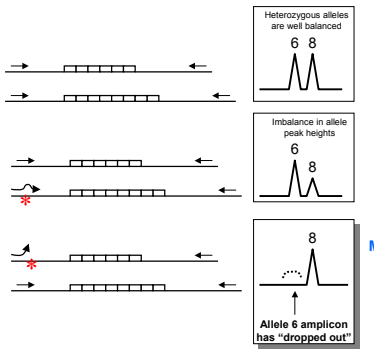
Profiler Plus™

Walsh, P.S. (1998) *J. Forensic Sci.* 43: 1103-1104

In 2 out of 1,483 individuals tested = 0.067%

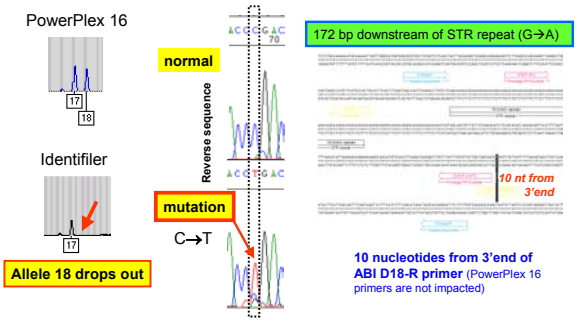
Lazaruk *et al.* (2001) *Forensic Sci Int.* 119:1-10

Impact of DNA Sequence Variation in the PCR Primer Binding Site



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 6.9, ©Elsevier Academic Press

D18S51 Null Allele from Kuwait Samples with ABI Primers



Clayton et al. (2004) **Primer binding site mutations affecting the typing of STR loci contained within the AMPFISTR SGM Plus kit.** *Forensic Sci Int.* 139(2-3): 255-259

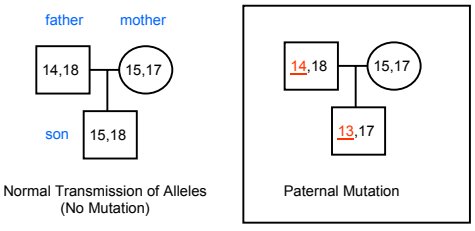
Apparent Null Alleles Observed During Concordance Studies

10/13 CODIS loci affected so far

Locus	STR Kits/Assays Compared	Results	Reference
VWA	PP1.1 vs ProPlus	Loss of allele 19 with **ProPlus**; fine with PP1.1	Kline et al. (1998)
D5S818	PP16 vs ProPlus	Loss of alleles 10 and 11 with **PP16**; fine with ProPlus	Alves et al. (2003)
D13S317	Identifiler vs miniplexes	Shift of alleles 10 and 11 due to deletion outside of **miniplex assay**	Butler et al. (2003), Drabek et al. (2004)
D16S539	PP1.1 vs PP16 vs COfiler	Loss of alleles with **PP1.1**; fine with PP16 and COfiler	Nelson et al. (2002)
D8S1179	PP16 vs ProPlus	Loss of alleles 15, 16, 17, and 18 with **ProPlus**; fine with PP16	Budowle et al. (2001)
FGA	PP16 vs ProPlus	Loss of allele 22 with **ProPlus**; fine with PP16	Budowle and Sprecher (2001)
D18S51	SGM vs SGM Plus	Loss of alleles 17, 18, 19, and 20 with **SGM Plus**; fine with SGM	Clayton et al. (2004)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with **COfiler**; fine with PP16	Budowle et al. (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with **COfiler**; fine with PP16	Budowle et al. (2001)
D21S11	PP16 vs ProPlus	Loss of allele 32.2 with **PP16**; fine with ProPlus	Budowle et al. (2001)

From Table 6.2 in J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 136

Mutation Observed in Family Trio



Butler, J.M. (2001) *Forensic DNA Typing*, Figure 6.9, ©Academic Press

STR Measured Mutation Rates

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

	STR Locus	Maternal Meioses (%)	Paternal Meioses (%)	Either Parent	Total Mutations	Rate
13 CODIS core loci	CSF1PO	70/179,353 (0.04)	727/504,342 (0.14)	303	1,100/683,695	0.16%
	FGA	134/238,378 (0.06)	1,481/473,924 (0.31)	495	2,110/712,302	0.30%
	TH01	23/189,478 (0.01)	29/346,518 (0.008)	23	75/535,996	0.01%
	TPOX	16/299,186 (0.005)	43/328,067 (0.01)	24	83/627,253	0.01%
	VWA	133/400,560 (0.03)	907/646,851 (0.14)	628	1,668/1,047,411	0.16%
	D3S1358	37/244,484 (0.02)	429/336,208 (0.13)	266	732/580,692	0.13%
	D5S818	84/316,102 (0.03)	537/468,366 (0.11)	303	924/784,468	0.12%
	D7S820	43/334,886 (0.01)	550/461,457 (0.12)	218	811/796,343	0.10%
	D8S1179	54/237,235 (0.02)	396/264,350 (0.15)	225	675/501,585	0.13%
	D13S317	142/348,395 (0.04)	608/435,530 (0.14)	402	1,152/783,925	0.15%
	D16S539	77/300,742 (0.03)	350/317,146 (0.11)	256	683/617,888	0.11%
	D18S51	83/130,206 (0.06)	623/278,098 (0.22)	330	1,036/408,304	0.25%
	D21S11	284/258,795 (0.11)	454/306,198 (0.15)	423	1,161/564,993	0.21%
	Penta D	12/18,701 (0.06)	10/15,088 (0.07)	21	43/33,789	0.13%
	Penta E	22/39,121 (0.06)	58/44,152 (0.13)	55	135/83,273	0.16%
	D2S1338	2/25,271 (0.008)	61/81,960 (0.07)	31	94/107,231	0.09%
	D19S433	22/28,027 (0.08)	16/38,983 (0.04)	37	75/67,010	0.11%
	F13A01	1/10,474 (0.01)	37/65,347 (0.06)	3	41/75,821	0.05%
	FES/FPS	3/18,918 (0.02)	79/149,028 (0.05)	None reported	82/167,946	0.05%
	F13B	2/13,157 (0.02)	8/27,183 (0.03)	1	11/40,340	0.03%
	LPL	0/8,821 (<0.01)	9/16,943 (0.05)	4	13/25,764	0.05%
	SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	0.64%

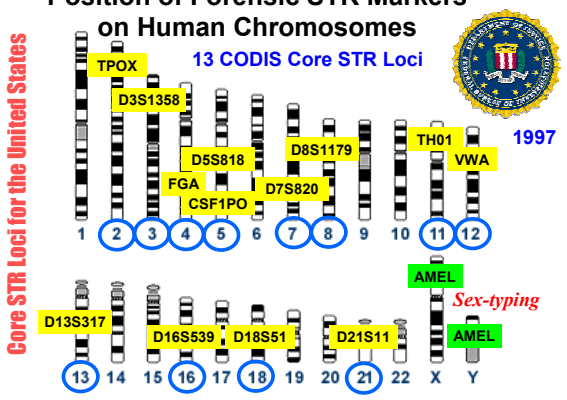
*Data used with permission from American Association of Blood Banks (AABB) 2002 Annual Report.

Summary of STR Mutations

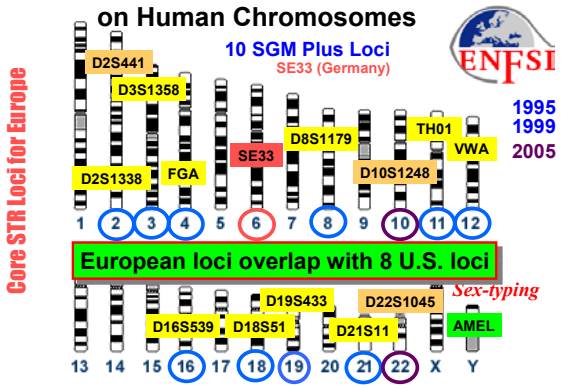
Mutations impact paternity testing and missing persons investigations but not forensic direct evidence-suspect matches…

- Mutations happen and need to be considered
- Usually 1 in ~1000 meioses
- Paternal normally higher than maternal
- VWA, FGA, and D18S51 have highest levels
- TH01, TPOX, and D16S539 have lowest levels

Position of Forensic STR Markers on Human Chromosomes



Position of Forensic STR Markers on Human Chromosomes



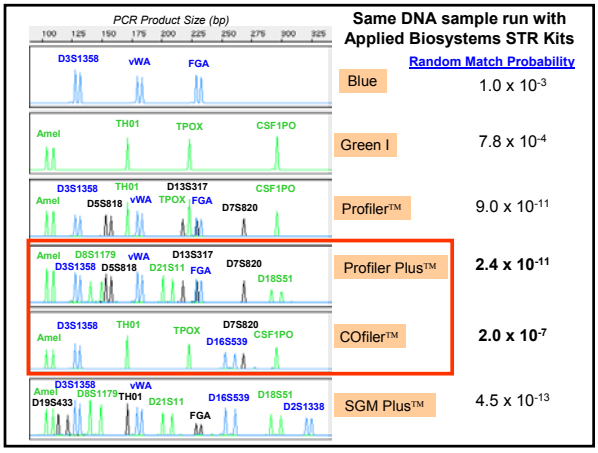
Position of Each CODIS STR Locus in Human Genome

Locus Name	Chromosomal Location	Physical Position[a]
CSF1PO	5q33.1 c-fms proto-oncogene, 6th Intron	Chr 5 149.484 Mb
FGA	4q31.3 alpha fibrinogen, 3rd Intron	Chr 4 156.086 Mb
TH01	11p15.5 tyrosine hydroxylase, 1st Intron	Chr 11 2.156 Mb
TPOX	2p25.3 thyroid peroxidase, 10th Intron	Chr 2 1.436 Mb
VWA	12p13.31 von Willebrand Factor, 40th Intron	Chr 12 19.826 Mb
D3S1358	3p21.31	Chr 3 45.543 Mb
D5S818	5q23.2	Chr 5 123.187 Mb
D7S820	7q21.11	Chr 7 83.401 Mb
D8S1179	8q24.13	Chr 8 125.863 Mb
D13S317	13q31.1	Chr 13 80.52 Mb
D16S539	16q24.1	Chr 16 86.168 Mb
D18S51	18q21.33	Chr 18 59.098 Mb
D21S11	21q21.1	Chr 21 19.476 Mb

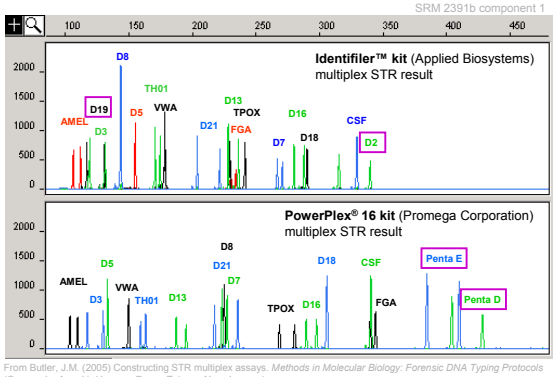
Review article on core STR loci genetics and genomics to be published March 2006

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci., in press.*

From Table 5.2, *Forensic DNA Typing*, 2nd Edition, p. 96 (J.M. Butler, 2005)



Commercial STR 16plex Kits



From Butler, J.M. (2005) Constructing STR multiplex assays. *Methods in Molecular Biology: Forensic DNA Typing Protocols* (Carracedo, A., ed.), Humana Press: Totowa, New Jersey, *in press.*

Value of STR Kits

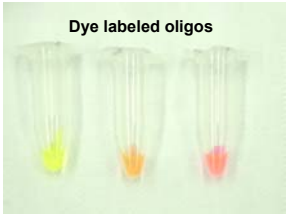
Advantages

- Quality control of materials is in the hands of the manufacturer (saves time for the end-user)
- Improves consistency in results across laboratories – same allelic ladders used
- Common loci and PCR conditions used – aids DNA databasing efforts
- Simpler for the user to obtain results

Disadvantages

- Contents may not be completely known to the user (e.g., primer sequences)
- Higher cost to obtain results

PCR Primer Quality Control



6FAM (yellow), VIC (orange), NED (red)

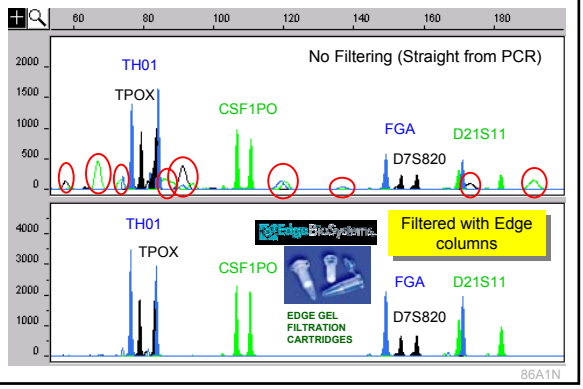
- UV Spec to determine concentration
- HPLC to evaluate purity
- TOF-MS to confirm correct sequence
- CE (ABI 310) to determine presence of residual dye molecules ("dye blobs")

Butler *et al.* (2001) *Forensic Sci. Int.* 119: 87-96

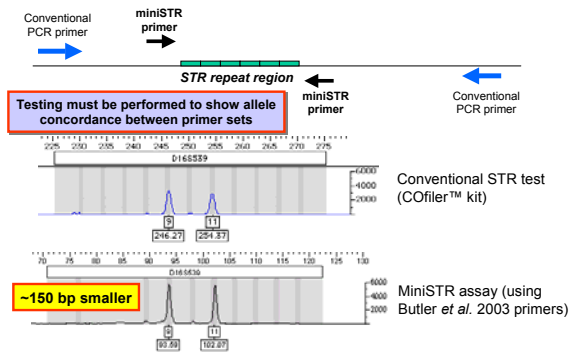
Primer Synthesis and Dye Blobs

- Oligonucleotide primers are synthesized from a 3'-to-5' direction on solid-phase supports using phosphoramidite chemistry
- The fluorescent dye is attached at 5'end of the primer (it is the last component added)
- The coupling reaction at each step of primer synthesis is not 100%, which can lead to some minor level impurities
- Left-over dye molecules that are not removed by post-synthesis purification can be carried through the PCR amplification step and injected onto the capillary to produce "dye blobs" or "dye artifacts" in CE electropherograms (wider than true allele peaks)

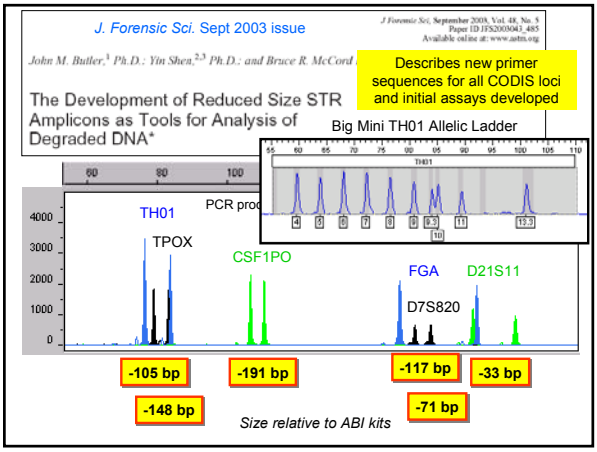
Problems with Dye Artifacts from Fluorescent Primers



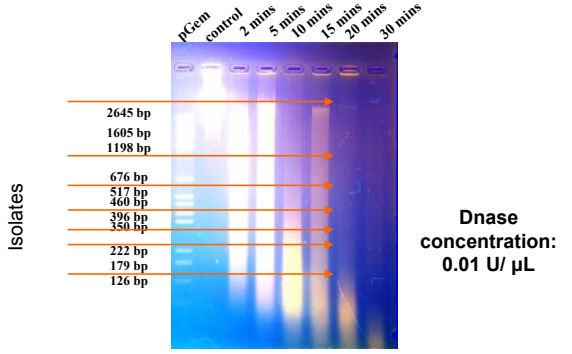
A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 7.2, ©Elsevier Science/Academic Press

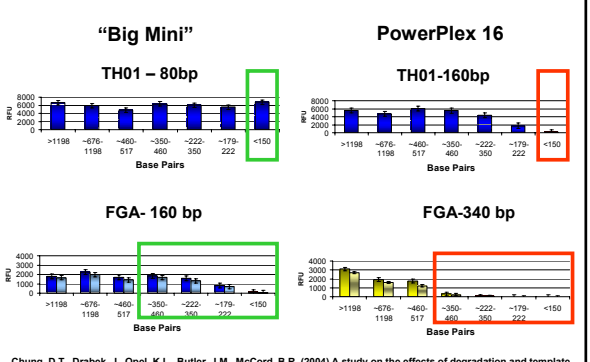


DNA Degraded With DNase I



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.

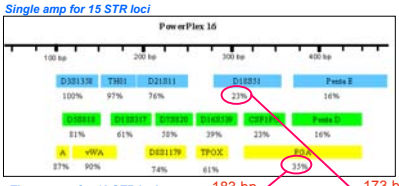
Miniplexes improve detection of degraded DNA



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

Single amp for 15 STR loci

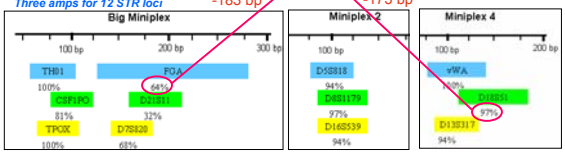


Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

Three amps for 12 STR loci

-183 bp

-173 bp



Opel K. L.; Chung, D. T.; Drábek, J.; Tatarek, N. E.; Jantz, L. M.;. McCord, B.R.; The Application of Miniplex Primer Sets in the Analysis of Degraded DNA from Human Skeletal Remains, *J. Forensic Sciences*, in press.

Bruce McCord's miniSTR work

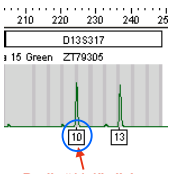
- Collaborated with NIST on original miniSTR paper with CODIS loci, D2, D19, Penta D, Penta E
  - Butler *et al.* (2003) *J. Forensic Sci.* 48: 1054-1064
- Bone work
  - Opel K. L *et al.*, *J. Forensic Sciences*, in press.
- Developmental validation of initial miniSTR assays
  - Drábek *et al.* (2004) *J. Forensic Sci.* 49: 859-860
  - Chung *et al.* (2004) *J. Forensic Sci.* 49: 733-740

Kerry Opel Jiri Drabek Denise Chung Yin Shen Bruce McCord

Examination of Concordance:

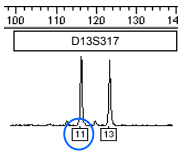
African American sample ZT79305

NIST Identifiler data



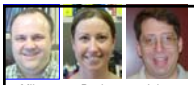
Really "11-1" allele

Ohio U miniSTR data



This problem has been seen multiple times by NYC OCME review of WTC BodePlex data

A deletion *outside* the miniSTR primers causes the kit produced allele to appear one repeat smaller…



Mike Coble Becky Hill John Butler

NIST miniSTR work

- Collaborated with Bruce McCord's group on original miniSTR paper with CODIS loci, D2, D19, Penta D, Penta E
  - Butler *et al.* (2003) *J. Forensic Sci.* 48: 1054-1064
- Many CODIS loci are too big and make poor miniSTRs
- New miniSTRs and assays: NC01, NC02
  - Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50:43-53
- New miniSGM miniplex: AMEL, TH01, FGA, D18, D16, D2
- EDNAP/ENFSI degraded DNA study coordinated by Peter Gill
- Creation of miniSTR information on STRBase

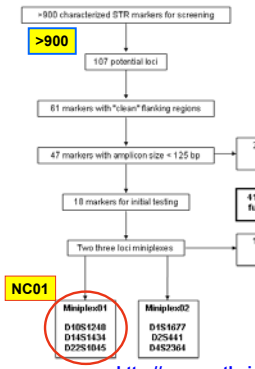
http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

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

Initial Testing Results with Potential miniSTR Loci



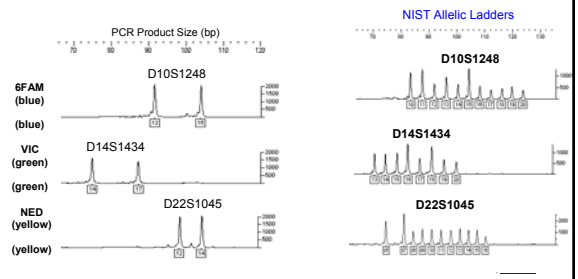
Coble and Butler (2005)
***J. Forensic Sci.* 50(1): 43-53**

Under investigation currently

Plan to characterize ~27 new miniSTRs

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

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

Forthcoming Article Advocating miniSTRs

They recommend that miniSTRs "be adopted as the way forward to increase both the robustness and sensitivity of analysis."

ELSEVIER

Forensic Science International xxx (2005) xxx–xxx

Science International

www.elsevier.com/locate/forsciint

Short communication

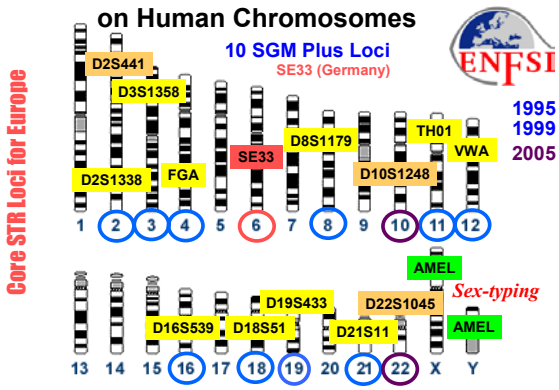
The evolution of DNA databases—Recommendations for new European STR loci

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

They recommend that European laboratories adopt three new mini-STR loci, namely: D10S1248, D14S1434 and D22S1045. (update exchanges D2S441 for D14S1434)

Position of Forensic STR Markers on Human Chromosomes



Protocols Used for STR Typing

- Most forensic DNA laboratories follow PCR amplification and CE instrument protocols provided by the manufacturer
- Comments
  - Lower volume reactions may work fine and reduce costs
  - No heat denaturation/snap cooling is required prior to loading samples into ABI 310 or ABI 3100
  - Capillaries do not have to be thrown away after 100 runs
  - **Validation does not have to be an overwhelming task**

Reduced Volume PCR Amplifications

Advantages

- **Lower cost** since kit contents are stretched
- Improved sensitivity perceived due to use of concentrated PCR products (since 1 uL out of a 5 uL reaction is 20% while 1 uL out of a 50 uL reaction is 2%)

Disadvantages

- Less volume of input DNA
  - **Tighter control (improved precision) required in DNA quantitation**
  - If low amount of DNA, then potential for allelic dropout (LCN conditions)
  - If PCR inhibitor is present, then less opportunity for dilution of inhibitor
- Evaporation impacts PCR amplification performance

Publications:

Gaines et al. *J Forensic Sci* 2002; 47(6):1224-1237. Reduced volume PCR amplification reactions using the AmpFISTR Profiler Plus kit.

Leclair et al. *J Forensic Sci* 2003; 48(5):1001-1013. STR DNA typing: increased sensitivity and efficient sample consumption using reduced PCR reaction volumes.

Fregeau et al. *J Forensic Sci* 2003; 48(5):1014-1034. AmpFISTR profiler Plus short tandem repeat DNA analysis of casework samples, mixture samples, and nonhuman DNA samples amplified under reduced PCR volume conditions (25 microL).

ABI 310 Reagents and Operating Costs

ABI 310 Reagent Costs		for 500 samples		factor for 500	Total Cost
	Part Number	Quantity Provided	Cost	1000 runs with P+C	
Capillaries	402839	5/pk (47cm x 50 um uncoated)	$294	2	$588
POP-4 polymer	402838	5 mL	$196	2	$392
Buffer, Genetic Analyzer 10X	402824	25 mL	$78	1	$78
Sample tubes (0.5 mL)	401957	500/pk	$52	2	$104
Septa for tubes	401956	500/pk	$163	2	$326
Formamide, Hi-Di	4311320	25 mL (for ~1000-1500 samples)	$29	1	$29
GS500-ROX size standard	401734	800 tests/pk	$260	1.25	$325
Matrix standards	4312131	5FAM, JOE, NED, ROX	$70	1	$70
PCR tubes, strips	N801-0580	1000/pk	$76	1	$76
PCR tube caps	N801-0535	1000/pk	$60	1	$60
Pipet tips		~$0.10/tip x 550 tips	$55	2	$110
Profiler Plus STR kit	4303326	100 tests/kit	$2,018.94	5	$10,095
COfiler STR kit	4305246	100 tests/kit	$1,816.54	5	$9,083
Syringe, Kloehn 1.0 mL	4304471	each	$82	1	$82
Genetic Analyzer vials, 4 mL	401955	50/pk	$62	1	$62
48-tube sample tray kit	402867	each	$230	1	$230

Costs may not be fully up-to-date

**following manufacturer's protocols (based on 500 samples total)*

Total per Sample Cost to Obtain Result on 13 CODIS core loci (with Profiler Plus and COfiler STR kits): $43.42

(materials other than STR kits = $5.06)

10 uL PCR (1/5 vol) = $12.73

Identifiler 5 µL PCR Protocol

Identifiler PCR amplification was carried out on a GeneAmp® 9700 using 1 ng of DNA according to kit protocols with the exception of **reduced volume reactions** (5 µL instead of 25 µL) and **reduced cycles** (26 instead of 28).

Amplification products were diluted 1:15 in Hi-Di™ formamide and GS500-LIZ internal size standard (0.3 uL) and analyzed on the 16-capillary ABI Prism® 3100 Genetic Analyzer without prior denaturation of samples.

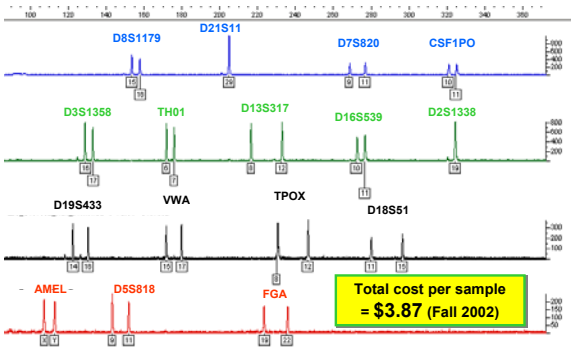
POP™-6 (3700 POP6) rather than POP™-4 was utilized for higher resolution separations.

Allele calls were made in Genotyper® 3.7 by comparison with kit allelic ladders using the Kazaam macro (20% filter).

Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J Forensic Sci* 2003; 48(4):908-911.

Identifiler 5 µL PCR

(lower 3100 injection; 5s@2kV instead of 10s@3kV)



STRBase

Short Tandem Repeat DNA Internet Database

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

General Information	Forensic Interest Data	Supplemental Info
•Intro to STRs (downloadable PowerPoint)	•FBI CODIS Core Loci	•Reference List **>2500**
•STR Fact Sheets	•DAB Standards	•Technology Review
•Sequence Information	•NIST SRMs 2391	•Addresses for Scientists
•Multiplex STR Kits	•Published PCR Primers	•Links to Other Web Sites
•Variant Allele Reports	•Y-Chromosome STRs	•DNA Quantitation
•Training Slides	•Population Data	•mtDNA
	•Validation Studies	•New STRs
	•miniSTRs	

New information is added regularly…

Acknowledgments

NIST Human Identity Project Team

John Butler (Leader) | Margaret Kline | Pete Vallone | Mike Coble | Jan Redman | Amy Decker | Becky Hill | Chris DeAngelis | Dave Duewer

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

Many wonderful collaborators from industry, university, and government laboratories.