

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

NIST Human Identity Team Projects

John M. Butler, Ph.D.
National Institute of Standards and Technology

EDNAP and 26th ENFSI DNA Working Group Meeting

April 17-20, 2007 Krakow, Poland

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>

Some Workshops Conducted This Past Year

- **qPCR workshop by Vallone and Orrego** (July 2006)
slides available on STRBase
 - <http://www.cstl.nist.gov/biotech/strbase/qPCRworkshop.htm>
- **LCN workshop by Butler, Caragine, and Gill** (May 2006)
Butler slides available on STRBase
 - <http://www.cstl.nist.gov/biotech/strbase/training.htm>Peter Gill's talk covered LoComatioN software (see *Forensic Sci. Int.* 2007, 116: 128-138)
- **Y-STR and mtDNA workshop by Butler and Coble** (Nov 2006)
>600 slides available on STRBase
 - <http://www.cstl.nist.gov/biotech/strbase/YmtDNAworkshop.htm>

Training Workshops Planned

- ISFG Meeting (August 2007, Copenhagen, Denmark)
 - **CE Fundamentals and Troubleshooting**
 - **Validation**
- SAFS Meeting (September 2007, Atlanta, GA)
 - **Mixture Interpretation**

Profiles in DNA (Promega Corporation), vol. 9(2), pp. 3-6

VALIDATION

http://www.promega.com/profiles/902/ProfilesInDNA_902_03.pdf

Debunking Some Urban Legends Surrounding Validation Within the Forensic DNA Community
By John Butler
National Institute of Standards and Technology, Gaithersburg, Maryland, USA

Training Resources on STRBase...

<http://www.cstl.nist.gov/biotech/strbase/training.htm>

STR Training Materials

PowerPoint slides for figures from *Forensic DNA Typing* (2nd Edition) [181 slides, 8.72 Mb file]

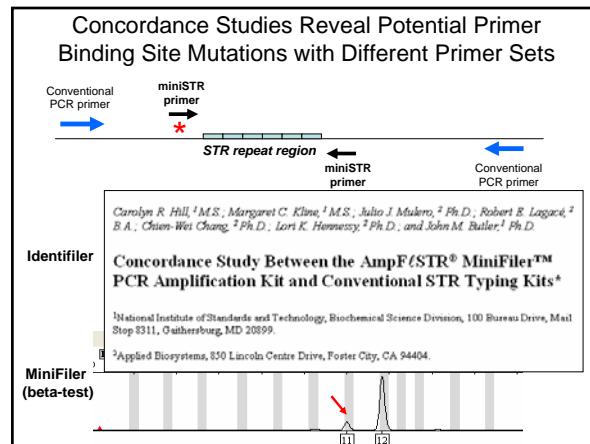
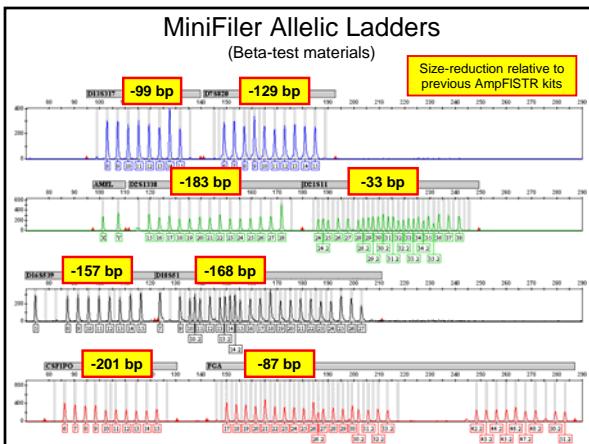
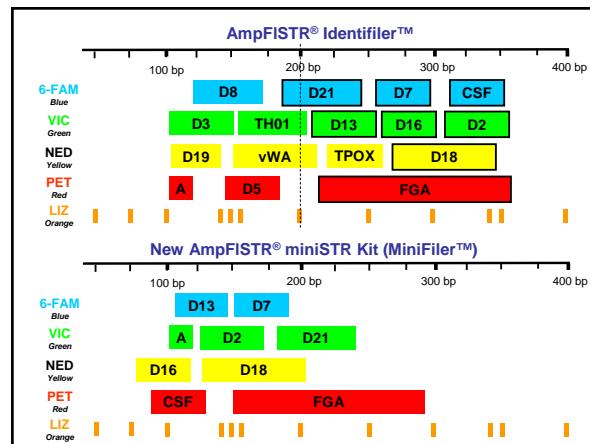
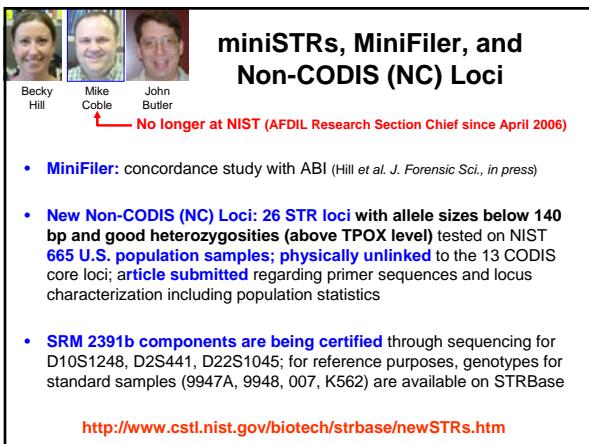
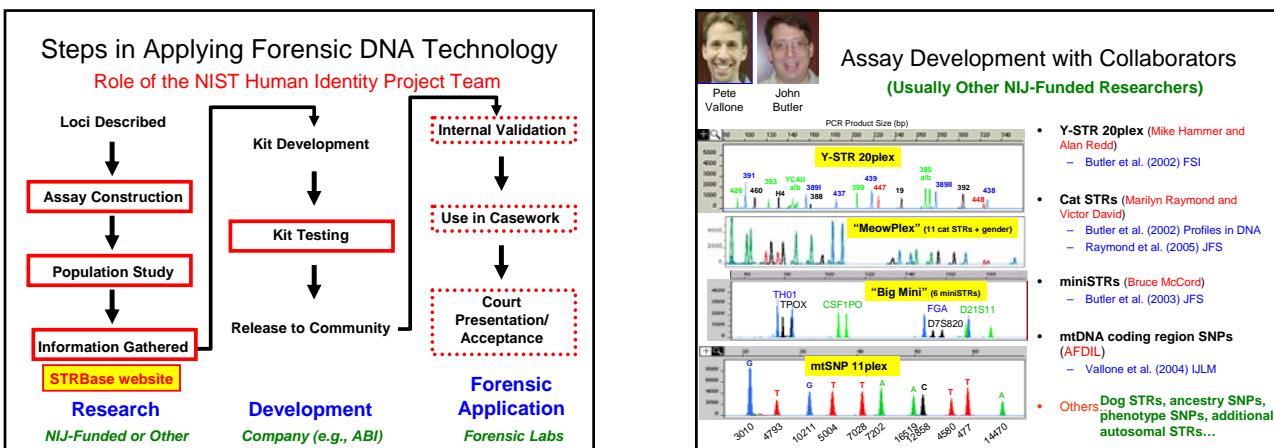
NEW! GMA Section Training Manual (2.1 Mb pdf file) for the Missouri State Highway Patrol Forensic Laboratory - an example of information required reading, expectations for DNA analysts and technicians in training - provided by Ruth Montgomery of the Missouri State Highway Patrol Case Laboratory

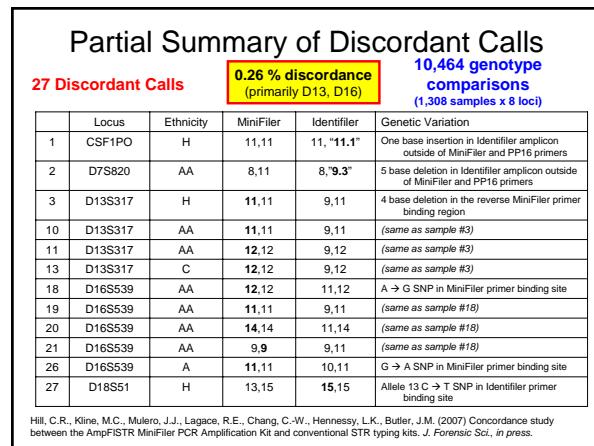
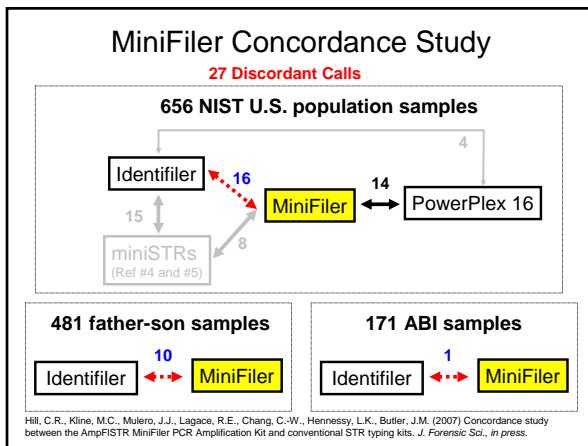
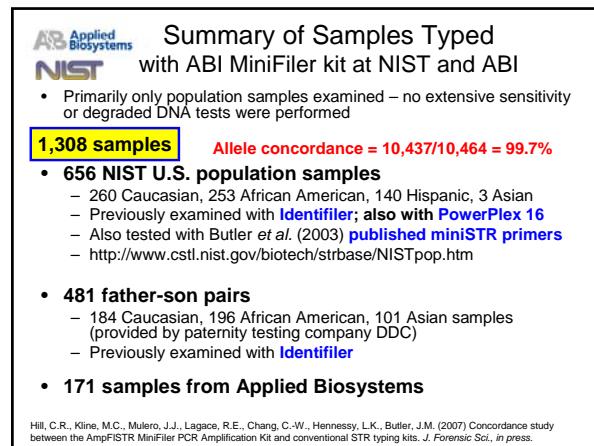
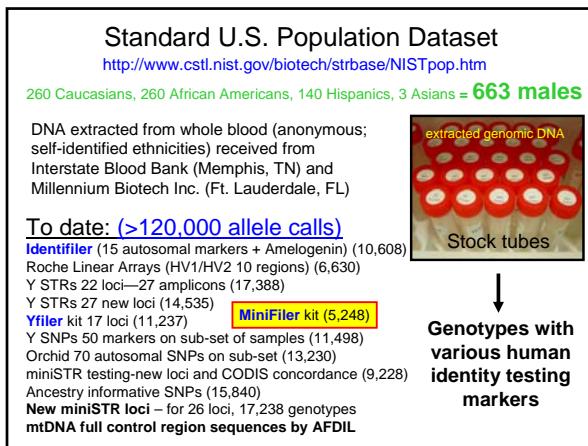
PowerPoint Presentations and Slide Shows

- **Background Information** (20 slides)
- **STR Techniques** (15 slides)
- **Y-STRs and STRs** (45 slides)
- **Amplification and Analysis** (Cambridge HealthTech Institute's Fourth Annual DNA Forensics Meeting June 1, 2000)
- **STRs 2001** (70 slides)
- **STRs 2002** (60 slides)
- **STRs 2003** (60 slides)
- **Using STRs for Identifying Commercial Kits and AB3000/3100** [Eml1 \(44 slides\)](#) [Eml2 \(44 slides\)](#)
- **John Butler Bruce McCord workshop at the American Academy of Forensic Sciences (Seattle, WA), February 20, 2006**
 - o **STR Biology, Methods, and Methods** (69 slides, 5.4 Mb file)
 - o **Capillary Electrophoresis Instrumentation, Theory and Application** (77 slides, 5.4 Mb)
 - o **Amplification and Analysis in the New STRs 2002 Kit "On-Line"** (91 slides, 4.1 Mb)
 - o **CE Troubleshooting** (72 slides, 8.5 Mb)
 - o **STR Mixture Interpretation** (46 slides, 11.1 Mb)
 - o **STRs and STRs with STRs: PCR and Lanes/Copy Number Issues** (63 slides, 13 Mb)
 - o **Y-STRs and mtDNA** (27 slides, 2.8 Mb)

Technology: NIST 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**

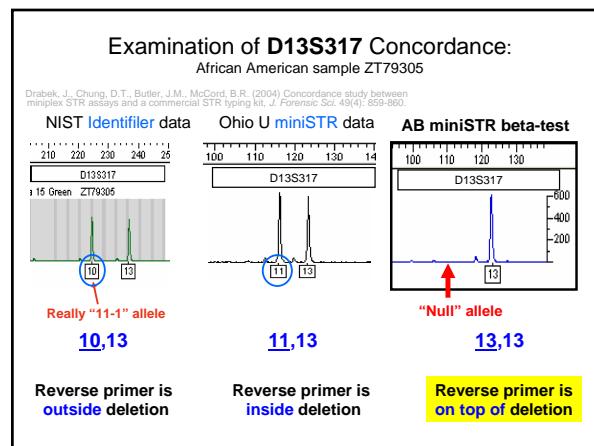


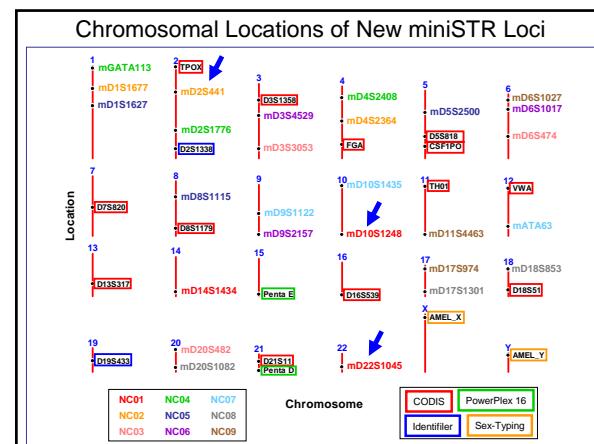
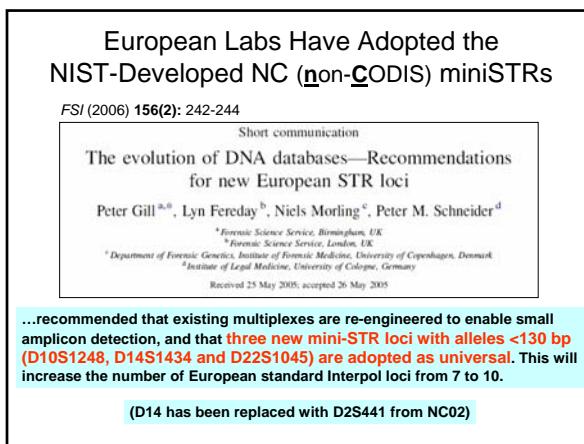


Apparent Null Alleles Observed During Concordance Studies

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

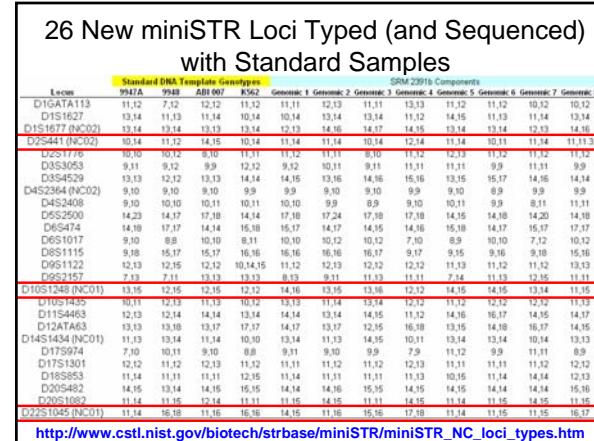
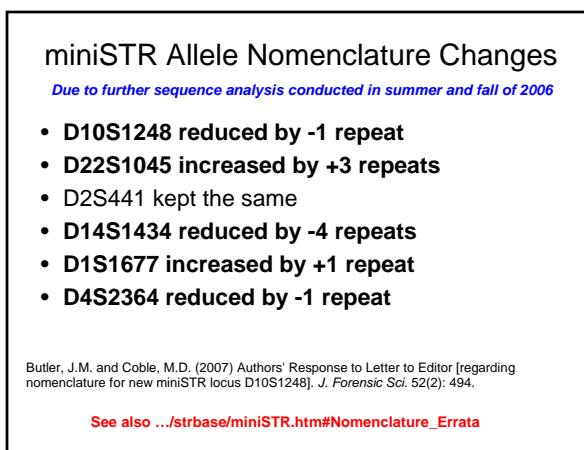
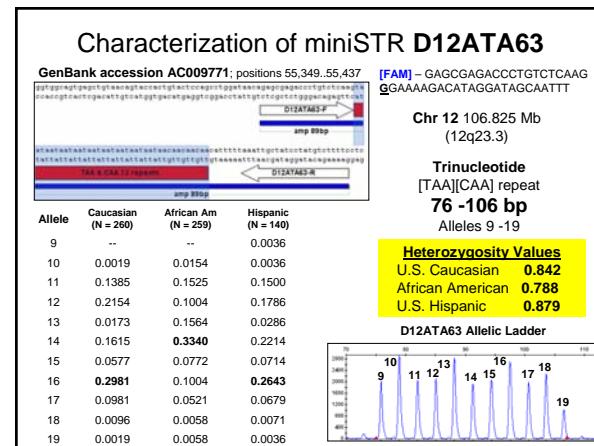
Locus	STR Kit/Assays Compared	Results	Frequency of Primer Binding Site Mutation	Source
CSF1PO	Minifiler vs PP16	PP16: 11,11 and ID: 11,11.1 One base insertion in Identifier amplicon outside of MiniFiler and PP16 primers	1/1308	Hill et al. (2007)
CSF1PO	PP16 vs OCFiler	Loss of allele 14 with OCFiler, fine with PP16	2/1537	Bradwelle et al. (2001)
F0A	SOM vs SOM Plus	Loss of allele 26 with SOM Plus, same as allelic size with SOM	2/1537	Cottrell et al. (2000)
F0A	PP16 vs ProPP16	Loss of allele 22 with ProPP16, fine with PP16	2/1537	Bradwelle and Spencer (2001)
TH01	PP16 vs OCFiler	Loss of allele 9 with OCFiler, fine with PP16	1/1537	Bradwelle et al. (2001)
TH01	SOM vs SOM Plus	Loss of allele 6 with SOM Plus, fine with SOM	1/4245	Clayton et al. (2004)
VWA	PP1.1 vs ProPP1.1	Loss of allele 19 with ProPP1.1, fine with PP1.1	2/1483	Kline et al. (1998) and Walsh (1999)
VWA	PP16 vs ProPP16	Loss of alleles 15 and 17 with ProPP16, fine with PP16	2/1537	Bradwelle et al. (2001)
VWA	ID vs minisamples	Loss of alleles 12, 13, and 14 with minisample assay, fine with ID	9/532	Drabek et al. (2004)





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
AT6A3 (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
D2S176	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
D1T54463	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
D20S1082	648	0.691	22	0.673	0.689	0.729
DAT11A10 (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



Standard Reference Materials

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

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

Helps meet DAB Std. 9.5 and ISO 17025

Working to update 2391b with new miniSTRs and 2395 with new Y-STRs

Calibration with SRMs enables confidence in comparisons of results between laboratories

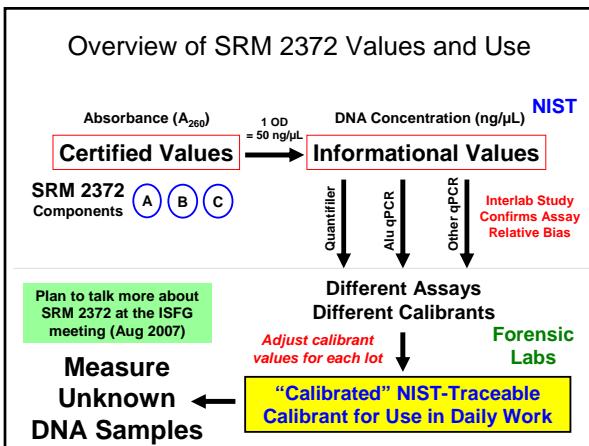
```

    graph TD
      Lab1[Lab 1] --> SRM[Standards Reference Material]
      Lab2[Lab 2] --> SRM
  
```

DNA Quantitation (qPCR)

- Production of SRM 2372 (Human DNA Quant Std)
 - Interlab study completed; final characterization underway
- PDI Training: qPCR Course (July 25-26, 2006)
<http://www.cstl.nist.gov/biotech/strbase/qPCRworkshop.htm>
- Evaluation of published assays on same samples
 - Promega 2005 and AAFS 2006 presentations
 - Trying to educate ABI on quality control of their materials (Quantifier lot-to-lot variation)

<http://www.cstl.nist.gov/biotech/strbase/DNAquant.htm>



Variant Allele Sequencing Service (Free)

Send us any unusual variant or null alleles and we will sequence them...

Address: <http://www.cstl.nist.gov/biotech/strbase/STRseq.htm>

Variant allele characterization			
Locus	Variant Allele	Sample Source	Comments
TPOX	10.3	Maryland State Police	Deletion of a 1 bp that is 157 bp from the repeat region under PowerPlex 1.1 and Identifiler primers does not affect primer binding or allele sizing. However, PowerPlex 2.1 and PowerPlex 16 products are 1 bp smaller because they are further away from the repeat and encompass the deletion.
FDA	46.2	Denver Crime Laboratory	Checked with Identifiler allele ladder.
D18S51	null allele 18	FBI and Kuwait government lab	Base change from C to T transition 172 bp downstream of the repeat region was made with the ABI D18S51 reverse primer but not the PowerPlex 16 D18S51 reverse primer that is internal to this mutation.
D18S51	40	Nebraska State Crime Lab	DNA sequence analysis showed 40 GAAA repeats
D18S51	*5.3*	DNA Solutions	DNA sequence analysis revealed a 5 bp deletion beyond the end of the 5th repeat unit to produce a *5.3* allele

Send 10-20 ng of DNA (or 2-3 FTA bloodstain punches)
Contact margaret.kline@nist.gov or john.butler@nist.gov
Information will be posted on [STRBase .../STRseq.htm](http://www.cstl.nist.gov/biotech/strbase/STRseq.htm)
Sequence details provided back to sender

From:	To:	University:	Repeat:
Peter D	6	Peter de Knijff's lab at Leiden University	[REDACTED]

Software Tools from NIST

Pete Vallone Dave Duewer

- AutoDimer – multiplex PCR primer screening tool
<http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm>
- mixSTR – mixture component resolution tool
- Multiplex_QA** – quality assessment tool for monitoring instrument performance over time
- Tools to aid Expert System data review
 - STR_ConvertFormats.xls** (converts data format)
 - STR_MatchSamples.xls** (compares samples)

<http://www.cstl.nist.gov/biotech/strbase/software.htm>

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards
NIST Human Identity Project Team – Leading the Way in Forensic DNA...

Past and Present Collaborators (also funded by NIJ):

- Mike Hammer and Alan Reid (U. AZ) for Y-chromosome studies
- Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
- Sandy Calloway (Roche) for mtDNA LINEAR ARRAYS
- Bruce McCord and students (FL Inst U) for miniSTR work
- Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
- Artie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones
- Murray Brilliant (U. AZ) for phenotype markers
- Ken Kidd (Yale U.) for SNP typing population samples
- Sree Kanthaswamy (UC Davis) for dog STR multiplex assay
- Tom Reid (DNA Diagnostics Center) for father-son samples

Disclaimer: Points of view expressed are those of the author(s) 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.

