


... working with industry to develop and apply technology, measurements and standards

# NIST Update


John M. Butler

CODIS Meeting  
Crystal City, VA  
November 15, 2004




### NIST Human Identity Project Team




John Butler (Project Leader)  
Former Team Member






Rich Schoske  
Amy Decker  
Mike Coble  
Dave Duewer


**Funding:**  
Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards



**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”** (degraded DNA and mixtures)
- Information on New Loci** (Y-Chromosome, new STRs)
- DNA Quantitation** (Interlab studies, Real-time PCR assay comparisons, SRM 2372)
- Tools to Aid State and Local Laboratories** (STRBase information, training materials/review articles, validation standardization, QAC tool, calibration datasets)



### Tools to Aid State and Local Laboratories

- STRBase** – standard information source on STR loci
  - **Training Materials** – review articles, workshop slides
  - **Variant allele** cataloging and characterization
- NIST U.S. Population Samples (typed at many loci)**
- Validation Standardization** Information
- Calibration Data Sets for Expert Systems
- Quality Assurance Tool** – to track analytical performance over time
- Interlaboratory Studies**



# STRBase

## Short Tandem Repeat DNA Internet Database

... working with industry to develop and apply technology, measurements and standards


**Recent Additions**

- Validation** (summary sheets) [.../validation.htm](#)
- NIST publications** and presentations as pdf files [.../NISTpub.htm](#)


**We Regularly Update**

- Reference List
- Variant Alleles
- Addresses for Scientists
- Links to Other Web Sites
- Y-STR Information

We will continue to add downloadable PowerPoint files that can be used for training purposes



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




### Training Materials on STRBase

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

**STR Training Materials** <http://www.cstl.nist.gov/biotech/strbase>

**PowerPoint Presentations**

- **Background Information** (20 slides)
- **STR Technology** (15 slides)
- **Y-Chromosome STRs** (40 slides) from talk given by John Butler at Cambridge Healthtech Institute's Fourth Annual DNA Forensic Meeting June 1, 2000
- **ISEQ 2001 Talk** (33 slides) given by John Butler at 19th Congress of the International Society of Forensic Genetics (Munster, Germany) August 26, 2001
- **Training on STR Typing Using Commercial Kits and ABI 310/3100** Margaret C. Kline, Janette W. Redman, John M. Butler October 22-26, 2001 [Part 1](#) (44 slides) [Part 2](#) (44 slides)

**PDF Versions of Presentations** 

(recommended for lower bandwidths; requires Adobe Acrobat; Click Adobe icon to download)

### NEAFS Workshop Slide Handouts

Handouts available as downloadable pdf files from <http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#NEAFSworkshop>

2 day workshop with **>500 slides** describing STRs and CE (ABI 310 and ABI 3100)

NEAFS CE-DNA Workshop (Butler and McCord) Sept 29-30, 2004

#### Capillary Electrophoresis in DNA Analysis

**STR Analysis**

NEAFS Workshop  
Mystic, CT  
September 29-30, 2004  
Dr. John M. Butler  
Dr. Bruce R. McCord

#### Outline for Workshop

- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Data Interpretation
- Additional Topics - Real-time PCR and miniSTRs
- Higher Throughput Approaches
- Troubleshooting the ABI 310 (Participant Roundtable)
- Additional Topics - Y-STRs, validation, accuracy
- Review and Test

### Review Article on STRs and CE

pdf available from <http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>

*Electrophoresis* 2004, 25, 1397-1412

#### Review

John M. Butler<sup>1</sup>  
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#### Forensic DNA using the ABI for STR anal

DNA typing with short applications including such as the ABI 310 for many laboratories ing sample preparat results using CE system in the context throughput and ease

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Locus Name	Chromosomal Location	Physical Position <sup>a</sup>
CSF1PO	5q33.1 c-fms proto-oncogene, 6 <sup>th</sup> intron	Chr 5 149,484 Mb
FGA	4q31.3 alpha fibrinogen, 3 <sup>rd</sup> intron	Chr 4 156,086 Mb
TH01	11p15.5 tyrosine hydroxylase, 1 <sup>st</sup> intron	Chr 11 2,156 Mb
TPOX	2p25.3 thyroid peroxidase, 10 <sup>th</sup> intron	Chr 2 1,436 Mb
VWA	12p13.31 von Willebrand Factor, 40 <sup>th</sup> 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 89,52 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

Position of Each CODIS STR Locus in Human Genome

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

### 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	Identifier 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)

### STR Measured Mutation Rates

.../strbase/mutation.htm

STR Locus	Maternal Meioses (%)	Paternal Meioses (%)	Either Parent	Total Mutations	Rate
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/PPS	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)	0/30 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	0.64%

<sup>a</sup>Data used with permission from American Association of Blood Banks (AABB) 2002 Annual Report.

### Variant Alleles Cataloged in STRBase

[http://www.cstl.nist.gov/biotech/strbase/var\\_tab.htm](http://www.cstl.nist.gov/biotech/strbase/var_tab.htm)

#### Off-Ladder Alleles

224 total variants reported as of 04/21/04

**Currently 255**  
at 13/13 CODIS loci

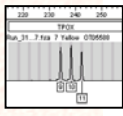
- CSE1PO (10)
- D2S1338 (3)
- D3S1338 (16)
- D5S818 (5)
- D7S820 (20)
- D8S1179 (4)
- D13S317 (3)
- D16S539 (10)
- D18S51 (28)
- D19S433 (3)
- D21S11 (21)
- FESFES (1)
- FGA (62)
- HUMTH01 (4)
- Penta D (3)
- Penta E (6)
- TPOX (7)
- VWA (5)

#### Tri-Allelic Patterns

56 total patterns reported as of 04/09/04

**Currently 58**  
at 13/13 CODIS loci

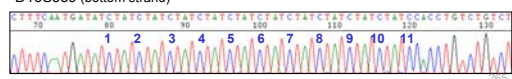
- CSE1PO (2)
- D3S1338 (4)
- D5S818 (1)
- D7S820 (3)
- D8S1179 (3)
- D13S317 (3)
- D16S539 (1)
- D18S51 (4)
- D21S11 (4)
- FGA (9)
- HUMTH01 (1)
- TPOX (12)
- VWA (7)



### Analysis of Common STR Variant Alleles

- We have monoplex primers for all common STR loci and kits
- We have sequencing primers that bind outside of STR kit primer sequence positions to enable view of polymorphic nucleotides that cause primer binding site mutations
- **NIJ has funded us to characterize STR variants for the forensic DNA community**

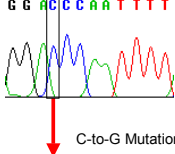
D16S539 (bottom strand)



### DYS392 Flanking Region Polymorphism

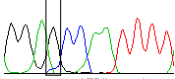
**Normal sequence**

GGG **AGCCAA** TTTT



**C-to-G Mutation**

GGG **AGCCAA** TTTT



AFDIL sample

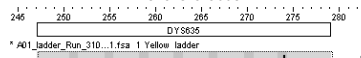
**C->G**

180 bp upstream of the STR repeat

PowerPlex Y and Yfiler kits are external to this polymorphism while Y-PLEX 5, which creates a smaller amplicon for DYS392, is internal and therefore not impacted...

### DYS635 Variant Allele 21.3 (Y-GATA-C4)

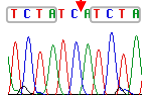
**Yfiler allelic ladder**



\* A01\_ladder\_Run\_310...1.fsa 1 Yellow ladder

**Missing T**

TCTATCATCTA



[TCTA]<sub>4</sub>[TGTA]<sub>2</sub>[TCTA]<sub>2</sub>[TGTA]<sub>2</sub>[TCTA]<sub>2</sub>[TGTA]<sub>2</sub>[TCTA]<sub>5</sub>TC-A [TCTA]<sub>2</sub>

### NIST U.S. Population Samples


As of 06/2003 **663 males** (anonymous; self-identified ethnicities)

- 260 Caucasians
- 260 African Americans
- 140 Hispanics
- 3 Asians

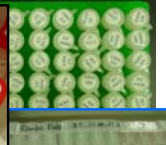
Whole blood received from Interstate Blood Bank (Memphis, TN)

**Working tubes/plates 1 ng/uL**  
To date: (~85,000 allele calls)


Stock tubes



Working tubes



Working plates



Samples supplied to OhioU for miniSTR typing and AFDIL for whole mtGenome sequencing

### 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**

Genetic Markers	Loci Examined	Publications
Common STRs	<b>D2S1338 and D19S433</b> information has been provided to the FBI for inclusion in PopStats to aid statistical calculations	Butler et al. (2003) JFS
miniSTRs		Drabek et al. (2004) JFS
New autosomal STRs		Coble et al. (2005) JFS
Autosomal SNPs	70 C/T SNPs (Orchid panel)	Vallone et al. (2004) FSI
Common Y-STRs	22 loci (27 regions) Yfiler concordance study	Schoske et al. (2004) FSI <i>Data in ABI Yfiler database</i>
<b>New Y-STRs</b>	<b>27 additional loci</b>	<b>Butler et al., submitted</b>
Y-SNPs	50 loci spanning haplogroups A-R	Vallone et al. (2004) JFS
mtDNA	LINEAR ARRAY and coding mtSNPs Full control regions by AFDIL	Kline et al. (2005) JFS <i>inclusion in EMPOP</i>

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## Validation Standardization Efforts

Presentation at Promega meeting  
(October 2004)

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

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Validation Standardization Questionnaire (conducted June-August 2004)

### Can Validation be Standardized?

Statements from survey responders...

**Over 86% (45/52) said yes**

Those who responded "no" said

- "to some degree it can be, however, validation is specific to the platform, kits, ...",
- "a start-up lab should do much more than an experienced lab...",
- "validation builds on previous work by lab or published data",
- "parts of it can be standardized; I don't think the non-probative cases could be", and
- "only in a general way, as with the SWGDAM guidelines. The uniqueness of each new procedure would make standardization difficult."

**Our Conclusion...**

**to a certain extent it can...but everyone will always have a different comfort level...and inflexible, absolute numbers for defined studies will not likely be widely accepted**

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### Revised SWGDAM Validation Guidelines (July 2004)

[http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004\\_03\\_standards02.htm](http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm)

Forensic Science Communications July 2004 – Volume 6 – Number 3  
Standards and Guidelines

**Revised Validation Guidelines**

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Scientific Working Group on DNA Analysis Methods (SWGAM)

**3. Internal Validation**  
...a total of at least 50 samples  
(some studies may not be necessary...)

Program for DNA Analysis by the Technical Working Group on DNA Analysis Methods (Crime Laboratory Digest 1995:22(2):21-43) has been revised due to increased laboratory experience, the advent of new technologies, and the issuance of the Quality Assurance Standards for Forensic DNA Testing Laboratories by the Director of the FBI (Forensic Science Communications available: [www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm](http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm))

The document provides validation guidelines and definitions approved by SWGDAM July 10, 2003.

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### Common Perceptions of Validation

Effort ↑

↓

Time →

Lots of experiments are required

Significant time is required to perform studies

*The goal is not to experience every possible scenario during validation...*

"You cannot mimic casework because every case is different."

Many labs are examining far too many samples in validation and thus delaying application of casework and contributing to backlogs...

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### A Thoughtful Comment from One Interviewee

Before a set of validation experiments is performed...

- The question should be asked "Do we already know the answer to this question from the literature or a previous study performed in-house?"
- If the answer is "yes" and we document how we know this answer, then there is no need to perform that set of validation experiments.

A good example of this scenario is non-human DNA studies.

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### Resources to Aid Future Validation Studies

- STRBase Validation Website
  - <http://www.cstl.nist.gov/biotech/strbase/validation.htm>
  - Validation summary sheets
  - Helpful information on aspects of validation studies
- NIST Calibration Data Set
  - set of ~200 sample data files that can be used to evaluate common STR typing "artifacts" such as stutter, non-template addition, spikes, peak imbalance, tri-allelic patterns, variant alleles, single base resolution
  - will help meet NDIS Appendix B requirements for Expert Systems evaluation
- Quality Control Program (Dave Duewer, NIST)
  - Software to monitor STR electropherogram performance (resolution, sensitivity) over time – can aid performance checks

### New Validation Homepage on STRBase

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

#### Validation Information to Aid Forensic DNA Laboratories

Validation Summary Sheets

We are initiating an effort to catalog the purpose of this effort is to provide a number of samples run as part of the laboratories. These validation summary guidelines 1.2.2.1 that "internal validity"

Below is listed a compilation of reference assays, instrumentation, and a laboratory is listed at the bottom of **Summary Sheet** (note that not all valid)

Kit, Assay, or Instrument	Reference
PowerPlex Y	<a href="#">Krenke et al. (2004) Forensic Sci. Int., in press</a>
Profiler Plus	Tracy et al. (2001)
PowerPlex 16	Tracy et al. (2001)
PowerPlex 16 ES	Tracy et al. (2001)
PowerPlex 16 ES Plus	Tracy et al. (2001)
PowerPlex 16 ES Plus II	Tracy et al. (2001)
PowerPlex 16 ES Plus III	Tracy et al. (2001)
PowerPlex 16 ES Plus IV	Tracy et al. (2001)
PowerPlex 16 ES Plus V	Tracy et al. (2001)
PowerPlex 16 ES Plus VI	Tracy et al. (2001)
PowerPlex 16 ES Plus VII	Tracy et al. (2001)
PowerPlex 16 ES Plus VIII	Tracy et al. (2001)
PowerPlex 16 ES Plus IX	Tracy et al. (2001)
PowerPlex 16 ES Plus X	Tracy et al. (2001)
PowerPlex 16 ES Plus XI	Tracy et al. (2001)
PowerPlex 16 ES Plus XII	Tracy et al. (2001)
PowerPlex 16 ES Plus XIII	Tracy et al. (2001)
PowerPlex 16 ES Plus XIV	Tracy et al. (2001)
PowerPlex 16 ES Plus XV	Tracy et al. (2001)
PowerPlex 16 ES Plus XVI	Tracy et al. (2001)
PowerPlex 16 ES Plus XVII	Tracy et al. (2001)
PowerPlex 16 ES Plus XVIII	Tracy et al. (2001)
PowerPlex 16 ES Plus XIX	Tracy et al. (2001)
PowerPlex 16 ES Plus XX	Tracy et al. (2001)
PowerPlex 16 ES Plus XXI	Tracy et al. (2001)
PowerPlex 16 ES Plus XXII	Tracy et al. (2001)
PowerPlex 16 ES Plus XXIII	Tracy et al. (2001)
PowerPlex 16 ES Plus XXIV	Tracy et al. (2001)
PowerPlex 16 ES Plus XXV	Tracy et al. (2001)
PowerPlex 16 ES Plus XXVI	Tracy et al. (2001)
PowerPlex 16 ES Plus XXVII	Tracy et al. (2001)
PowerPlex 16 ES Plus XXVIII	Tracy et al. (2001)
PowerPlex 16 ES Plus XXIX	Tracy et al. (2001)
PowerPlex 16 ES Plus XXX	Tracy et al. (2001)

Comments: [Other information and conclusions](#)

### Validation Summary Sheet for PowerPlex Y

Study Completed ([17 studies done](#)) Description of Samples Tested ([performed in 7 labs and Promega](#)) # Run

Single Source (Concordance)	5 samples x 8 labs 6 labs x 2 MF mixture series x 11 ratios (1:0.1:1:1:0.1:10:1:100:1:300:1:500:0.5:300, 0.025:300, 0.03:300 ng M.F.)	40
Mixture Ratio (male:female)	6 labs x 2 MM mixtures series x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1.1, 1.2, 1.5, 1.9, 1.19, 0.1)	132
Mixture Ratio (male:male)	6 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Sensitivity	24 amounts	24
Non-Human	6 components of SRM 2395	6
NIST SRM	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Precision (ABI 3100 and ABI 377)	65 cases with 102 samples	102
Non-Probative Cases	412 males used	412
Stutter	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Peak Height Ratio	4 models (480/240/960/60/700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Cycling Parameters	4 models (50/25/15/12.5/6.25) x 5 amounts + 5 concentrations]	50
Annealing Temperature	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
Reaction volume	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts	20
Thermal cycle test	5 amounts (1.382/0.62/753.444/13 U) x 4 quantities (10/50/250/13 ng DNA)	20
Male-specificity	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (10/50/250/13 ng DNA)	20
TaqGold polymerase titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (10/50/250/13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (10/50/250/13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (10/50/250/13 ng DNA)	20
TOTAL SAMPLES EXAMINED		1269

Krenke et al. (2004) Forensic Sci. Int., in press

### Laboratory Internal Validation Summaries

Summaries of Validation Studies Conducted in Individual Laboratories (not published in the literature)

Kit, Assay, or Instrument	Laboratory	Submitter
PowerPlex 16 ES with ABI 310	Pennsylvania State Police	Christine Tomporek

#### Soliciting Information on Studies Performed by the Community

Single Source (Concordance)	8 samples (Promega concordance) + 200 samples (part of population concordance study)	208	100
Mixtures	46	46	10
Mixture Ratio	1 sample x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1.1, 1.2, 1.4, 1.9, 1.19, 0.1) x 2 injections (SFD records)	22	33
Sensitivity	5 samples x 6 amounts (SDF 0.5/0.25/0.125/0.06/0.03 ng) + [5 samples x 3 points (male:female:drop size)]	55	39
Non-Human	61 samples	11	0
NIST SRM 2395	12 components	12	12
Precision (ABI 310)	(5 samples x 10 injections each) + 10 injections of allele ladders	60	60
Non-Probative Cases	5 cases x 8 samples each (infinite BEP/Andromed)	20	20
Stutter	200 samples (80 used from population samples)	-	-
Peak Height Ratio	18 samples x 2 different cycle numbers (50/25) x 2 reaction times (30 seconds)	56	-
Cycling Parameters	3 samples x 4 concentrations (2.0/1.5/0.5/0.25 ng) x 5 temperatures (56/58/60/62/64)	60	0
Annealing Temperature	8 sets x 4 samples per set	36	12
Proficiency	9 common substrates x 1 sample each	9	0
Substrate	5 conditions (outside/inside/curtains) x 6 line pairs (MFL205/48MS allele)	30	0
Environment	0	0	0
Various tissues	(bone, hair, teeth, semen, perspiration, urine, blood, semen, vaginal swab (because of one sample each))	0	0
TOTAL SAMPLES EXAMINED		693	208

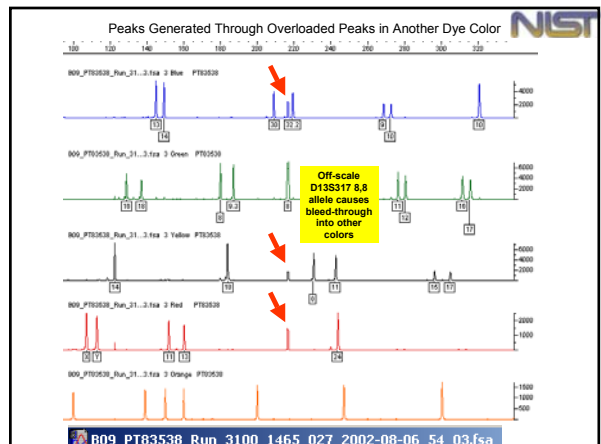
### Expert System Calibration Data Set

"Electronic SRM" to help meet NDIS Appendix B requirements

These data will be able to be used to check software upgrades to ensure reliable performance of the Expert System software

### To Help Meet NDIS Appendix B Requirements for Evaluating Expert Systems...

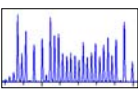
- **200 calibration samples needed**
- Types of challenges (at least 5 of each type)
  - Off-ladder alleles
  - Tri-allelic patterns
  - Non-template addition
  - Spikes and signal overload (bleed-through into another dye channel)
  - Mixtures
  - Degraded DNA
- We welcome suggestions as to other types of challenges to include in the data set
- **Samples are currently being gathered** with plans to generate data using Profiler Plus/Cofiler, Identifier, PowerPlex 16, and SGM Plus (kits have already been purchased)



### NIST QA/QC Software

Tool being developed by Dave Duetter for STR Process Control

**Tracks allelic ladders and positive controls and internal size standards**



NCBI Program

Peak Height, Area, Size

X | Y

103 | 436

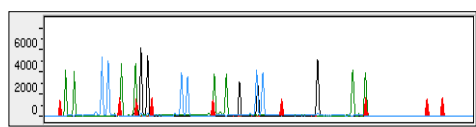
104 | 569

**This software does not perform genotyping. It merely permits a view of analytical parameters over time.**

Date vs Sensitivity, Resolution, Precision

Date vs Signal/Noise

### GeneScan Data Converted with NCBI OSIRIS Program



File Edit View Favorites Tools Help

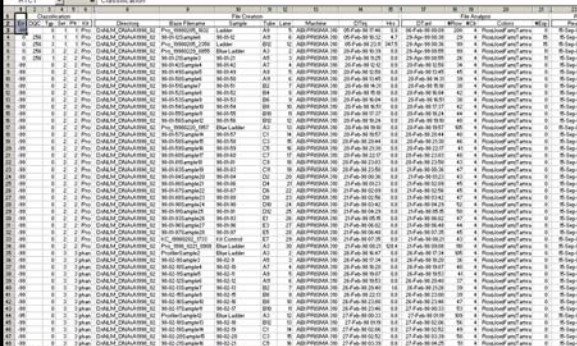
Address: D:\NLM\_DNA\A2000\_05

Name	Size	Type	Modified
00-05-001BG_5_5_00_DyeData.dat	1 KB	DAT File	8/20/2004 11:21 AM
00-05-001BG_5_5_00_PicoData.dat	2 KB	DAT File	8/20/2004 11:21 AM
00-05-001BG_5_5_00_ScanData.dat	3 KB	DAT File	8/20/2004 11:21 AM
00-05-001BG_5_5_00_ScanData.dat	61 KB	DAT File	8/20/2004 11:21 AM
00-05-001BG_5_5_00_StdData.dat	2 KB	DAT File	8/20/2004 11:21 AM

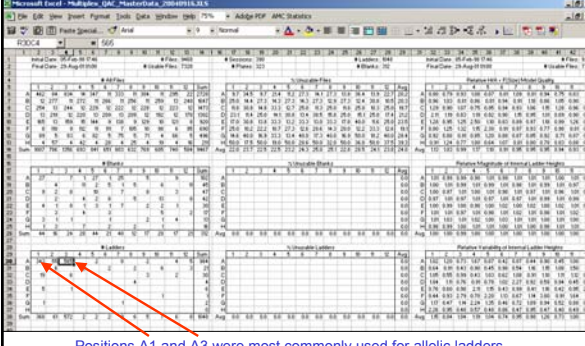
00-05-001BG\_5\_5\_00\_ScanData.dat

File	Edit	Format	Help
1	2	0	0
2	3	4	0
3	5	6	1
4	9	4	2
5	14	0	2
6	16	4	2
7	10	11	1
8	7	17	0

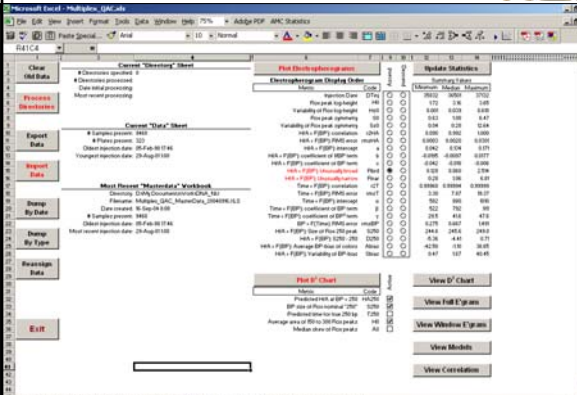
### All files are stored in Excel after conversion process



### View "plate" statistics for ABI 310 runs across 7328 samples spanning 2.5 years (Feb 1998 to July 2001)

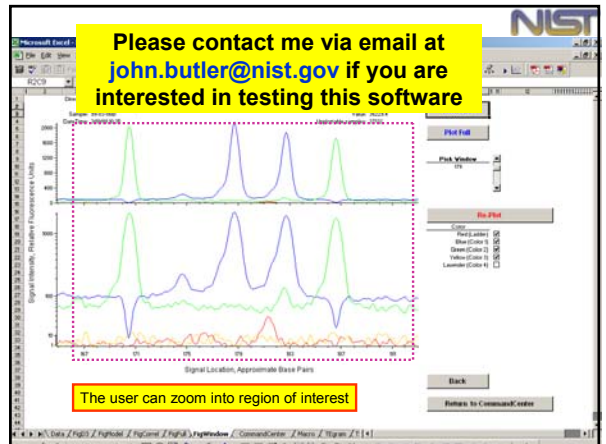
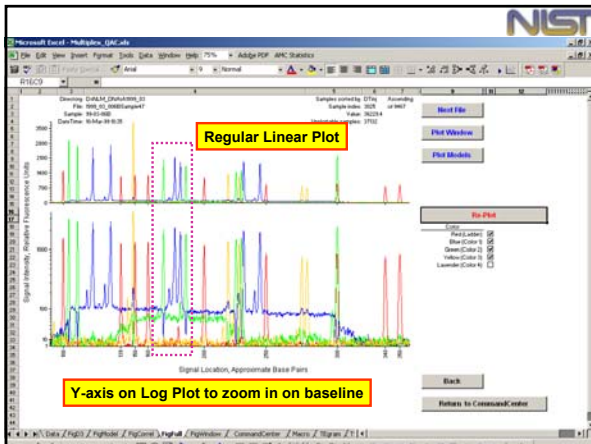
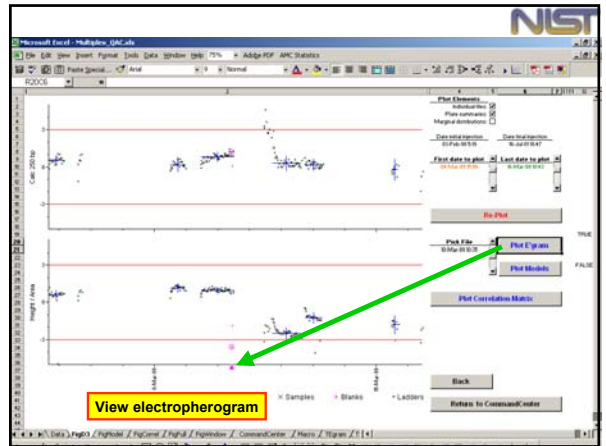
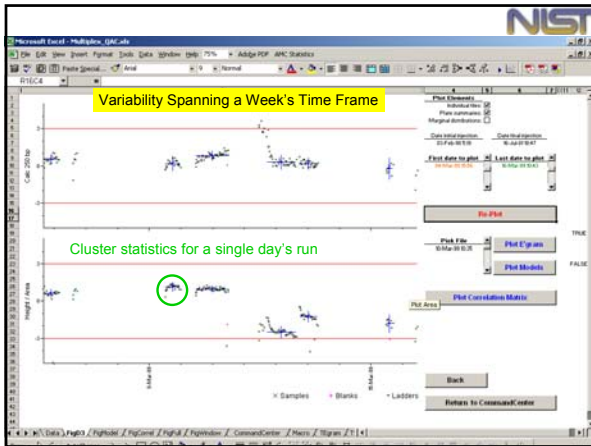


Positions A1 and A3 were most commonly used for allelic ladders



### Variability Spanning a 2.5 Year Time Frame with 7,328 Samples





NIST

## Interlaboratory Studies Conducted by NIST

Goal to help individual labs within forensic community understand their performance relative to other labs

NIST The National Institute of Standards and Technology  
Gratefully Acknowledges the Participation of the

This feedback can be helpful to a laboratory to know where they stand relative to other labs to illustrate opportunities for improvement.

Sample Quantitation: R S T V X U W  
DNA Concentration, ng /  $\mu$ L  
Your Values Interlaboratory Summary  
Quantiles: 97.5%, 75%, Median, 25%, 2.5%

Sample Typing  
Non-Donor Alleles Reported, %  
Donor Alleles (Major + Minor) Identified, %  
You Others

Margaret C. Kline, Study Coordinator

**NIST Interlaboratory Studies Involving Forensic DNA Typing**

Kline MC, et al. (2005) NIST DNA Quantification Study, in press

Duewer et al. (2004) MSS#3 Anal Chem, in press

Kline MC, Duewer DL, Redman JW, Butler JM. NIST Mixed Stain Study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. Anal. Chem. 2003;75:2453-2469.

Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. NIST Mixed Stain Studies #1 and #2: Interlaboratory comparison of DNA Quantification Practice and Short Tandem Repeat Multiplex Performance with Multiple-Source Samples. J. Forensic Sci. 2001;46(5):1199-1210.

Duewer DL, Gary KT, Reeder DJ. RFLP band size standards: Cell line K562 values from 1980 to 1990. J. Forensic Sci. 2000;45(6):1106-1118.

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Duewer DL, Liu H-K, Reeder DJ. Graphical tools for RFLP DNA analysis. J. Forensic Sci. 1999;44(5):969-977.

Gary KT, Duewer DL, Reeder DJ. Graphical tools for RFLP DNA analysis: Performance Charts. J. Forensic Sci. 1999;44(5):978-82.

Duewer DL, Lalonde SA, Aubin RA. Interlaboratory comparison of autoradiographic DNA profiling measurements: precision and accuracy. J. Forensic Sci. 1997;42(3):465-71.

Kline MC, Duewer DL, Redman JW, Butler JM, Richard M. Interlaboratory evaluation of STR triplex CTT. J. Forensic Sci. 1997;42(3):465-71.

Duewer DL, Redman JW, Liu H-K, Mudd JL. Interlaboratory comparison of autoradiographic DNA profiling measurements. Anal Chem 1997;69(10):1882-92.

Sbordone J, Duewer DL, Buel E, Herrin G Jr. Interlaboratory comparison of autoradiographic DNA profiling measurements: 2. Measurement uncertainty and its propagation. Anal Chem 1995;67(7):1220-31.

Kline MC, Redman JW, Reeder DJ, Duewer DL. Intercomparison of DNA sizing ladders in electrophoretic separation matrices and their potential for accurate typing of the D1S80 locus. Applied and Theoretical Electrophoresis 1996;6(1):33-41.

Duewer DL, Currie LA, Reeder DJ, Leigh SD, Liu H-K, Mudd JL. Interlaboratory comparison of autoradiographic DNA profiling measurements. 1. Data and summary statistics. Anal Chem 1994;66(20):3303-17.

**15 papers in the last 10 years**

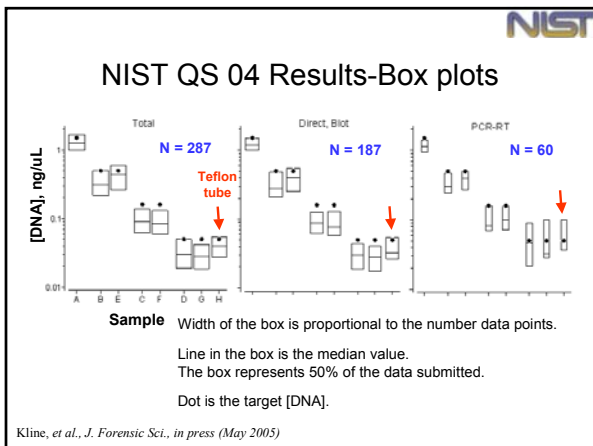
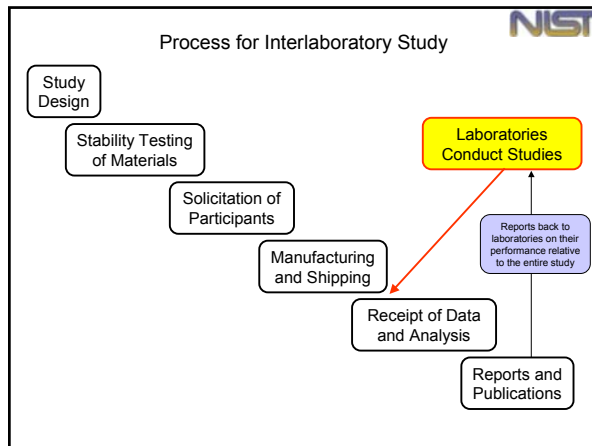
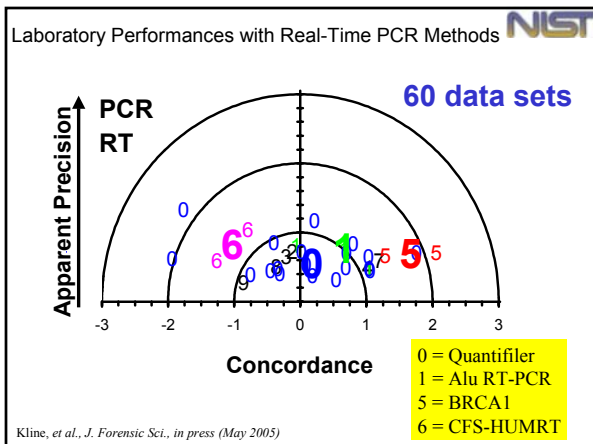


Table 2. The percent success rate reported for a sample.

Target [DNA] ng/μL	Method	N <sub>total</sub>	% Quantitative Results <sup>a</sup>							
			1.5	0.5	0.5	0.16	0.16	0.05	0.05	0.05
	Quantifiler	37	100	100	100	100	100	100	100	100
	Other RT-PCR	23	100	100	100	100	100	100	100	100
	"ACES"	14	100	100	100	100	100	100	100	100
	AluQuant	13	100	100	100	100	100	100	100	100
	PicoGreen	12	100	100	92	100	100	92	83	83
	ECL	75	100	99	99	93	95	84	77	87
	TMB	98	100	100	99	93	94	59	62	63
	Yield gel	14	57	0	0	0	0	0	0	0
		286								

<sup>a</sup> Quantitative results are those that were reported as values, values reported as the range between contiguous calibration standards, values reported as less than the lowest calibration standard if smaller than the target [DNA], or values reported as greater than the highest calibration standard if larger than the target [DNA].

Kline, et al., J. Forensic Sci., in press (May 2005)



- New Interlaboratory Study**
- Mixture Interpretation Study 2005 (MIX05)
  - "Paper challenge" (no lab work required)
  - Purpose to determine "lay of the land" for current practices in solving STR profile mixtures and reporting the results
  - Please pick up a handout pertaining to our SOLICITATION FOR PARTICIPATION...



## MIX05 Study



- Mixture results will be supplied to participants
- 3 or 4 case-like mixture scenarios
- [Data format options](#): Mac, NT, GeneMapper
- [Kit format options](#): Profiler Plus/COfiler, PowerPlex 16, Identifiler, SGM Plus
- Data will be shipped early January 2005
- Responses will be due February 28, 2005

## Plans for Dissemination of MIX05 Results



- Goal is to understand the "lay of the land" regarding mixture analysis across the DNA typing community
- Results will be discussed at NIJ DNA Grantees Meeting (June 2005), SWGDAM (July 2005), and ISFG (Sept 2005)
- We plan to develop training materials to aid in mixture interpretation with available software tools and to help in standardizing reports involving mixture analysis

## Acknowledgements



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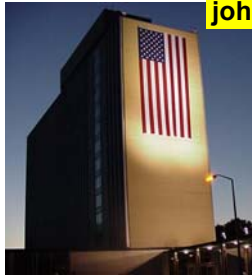


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Standards

### NIST Project Team:

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Margaret Kline     Jan Redman  
Amy Decker     Mike Coble  
                           Dave Duewer



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