


NIST Research Update
Michael Coble
Peter M. Vallone, Margaret C. Kline, Amy E. Decker, Janette W. Redman, Becky Hill, David L. Duewer, John Butler
April 4, 2005
EDNAP Meeting – Glasgow, Scotland

NIST Human Identity Project Team

			
John Butler (Project Leader)	Pete Vallone	Margaret Kline	Jan Redman
			
Amy Decker	Mike Coble	Dave Duewer	Becky Hill (new hire)

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

- **Standard Information Resources** (STRBase information, training materials/review articles, validation standardization, calibration datasets)
- **Interlaboratory Studies** (Real-time PCR, mixture interpretation)
- **Resources for “Challenging Samples”** (miniSTRs for degraded DNA)
- **Information on New Loci** (Y-Chromosome, new STRs)



Standard Information Resources

STRBase, training materials, variant allele sequencing etc.


STRBase Updates
(since July 2004)

- Validation section
- miniSTR section
- Y-chromosome information (multiplexes & databases)
- Population data summary & OmniPop program download (courtesy of Brian Burritt)
- Reference Sequences for Commonly Used STR Markers

More minor additions

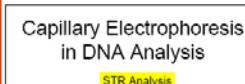
- Additional commercial STR kit schematics (Yfiler, PowerPlex Y)
- Published Promega primers (added PP16)
- Additional NIST publications/presentations (14 new talks, 12 new papers)
- Additional variant alleles & scientist addresses

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


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
	Outline for Workshop
	<ul style="list-style-type: none"> • Introductions • STR Analysis - Introduction to CE and ABI 310 - CE 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

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JOHN M. BUTLER
FORENSIC DNA TYPING
Biology and Technology behind STR Markers
- Second Edition

John M. Butler

Listed on amazon.com

Published February 2005

ACADEMIC PRESS
Forensic Science

Validation Standardization Efforts

Presentation at Promega meeting (October 2004)

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

We have been contacted by NFSTC with the desire to collaborate on validation standardization—they do not plan to produce validation kits now but rather have a workbook to help members of the community with validation...

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

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 validation information from the literature. The purpose of this effort is to facilitate the validation of new products tested, and the number of samples tested, by forensic DNA laboratories. The SWGDAM Revised Validation Guidelines are documented and summarized.

PowerPlex Y Validation

Reference: Krenke et al. (2005) *Forensic Sci. Int.* 148:13-14

Study Completed	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male/female)	6 labs x 2 M/F mixture series x 11 ratios (0.1:1, 1:1, 1:10, 1:100, 0.5:300, 0.25:300, 0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
Mixture Ratio (male:male)	6 labs x 2 M/M mixtures series x 11 ratios (1:1, 1:10, 1:100, 1:1000)	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5, 0.25/0.125, 0.0625/0.03, 0.0125/0.00625, 0.0025/0.00125, 0.0005/0.00025, 0.000125/0.000125)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (29/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (480/240/09600/7000) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 filtration series (0-500 ng female DNA) x 5 amounts each	10
TagGold polymerase titration	5 amounts (1.382/0.625/753.444/13.1U) X 4 quantities (10.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (10.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (10.5/0.25/0.13 ng DNA)	20

TOTAL SAMPLES EXAMINED **1269**

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	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (0.1:1, 1:1, 1:10, 1:100, 0.5:300, 0.25:300, 0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
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Non-Human	24 animals	24
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Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (10.5/0.25/0.13 ng DNA)	20

Krenke et al. (2005) *Forensic Sci. Int.* 148: 1-14

TOTAL SAMPLES EXAMINED **1269**

Laboratory Internal Validation Summaries

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

We invite updates to this table. Please contact John Butler <john.butler@nist.gov> if you would like to add a summary of your laboratory's validation studies with a particular forensic DNA test, instrument, or software program. Please submit information in a standard format summarizing the studies conducted, a description of samples run, and the number of samples examined using the downloadable Excel file ([click here](#)).

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

Kit/Assay or Instrument	Laboratory	Submitter
PowerPlex 16 Kit with ABI 310	Pennsylvania State Police	Christina Tomasz
Quantifiler with ABI 3100	Alabama Department of Forensic Sciences	Angelo Della Monica

Affiliations

Soliciting Information on Studies Performed by the Community

String Parameters	Description of conditions used with reference to 10 validation	Number of validation	
Sample Source (Concordance)	8 samples (Primate concordance) + 200 samples (part of population concordance study)	100	
Mutates	46	10	
Mixture Ratio	1 sample x 11 ratios (1.0, 1/2, 1/4, 1/8, 1/16, 0.01 x 2 injections (570 seconds))	22	33
Sensitivity	5 samples x 8 seconds (501.60-50.256-125.066-0.03 ng) + (5 samples x 3 points (relative/absolute dropout))	55	33
Non-Template	-	0	0
NIST SRM 2991b	12 injections	12	12
Precision (ABI 310)	(5 samples x 10 injections each) + 10 injections of allelic ladders	60	60
Non-Probative Cases	5 cases x 4 samples each (excluding STRBase/Architect project)	20	20
Strains	200 samples (data used from population samples)	-	-
Peak Height Ratio	-	-	-
Cycling Parameters	14 samples x 2 different cycle numbers (3000s) x 2 injection times (35 seconds)	56	-
Annealing Temperature	3 samples x 4 concentrations (2.01, 0.05, 0.25 ng) x 5 temperatures (55088602054)	60	0
Primer	-	30	12
Substrate	9 common substrates x 1 sample each	9	0
Environment	5 conditions (outside/inside/50/50/Cat/CR) x 3 time points (36/12/24/48/88 days)	30	0
Various tissues	Bone, hair, teeth, semen, perspiration, urine, blood, saliva, vaginal swab (inclusion of one sample each)	0	0
	TOTAL SAMPLES RUN: 693 / 296		

NIST Goals of this Validation Standardization Project

- To help the community gain a better understanding of the validation process and how others have implemented validation in their labs so that validation in one's own lab may be performed more quickly
- To help with establishing uniformity throughout the field to aid auditors in their inspections

NIST QA/QC Software

Tool being developed by Dave Duewer for STR Process Control

Tracks internal size standard in samples

Will be available soon for beta-testing; still working on user's manual (and will need NCBI file conversion program to be more easily accessible)

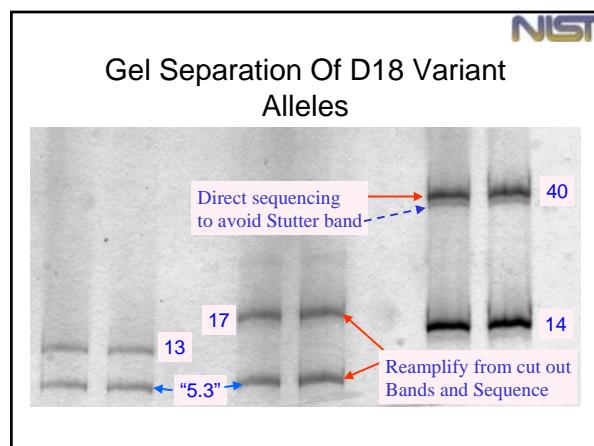
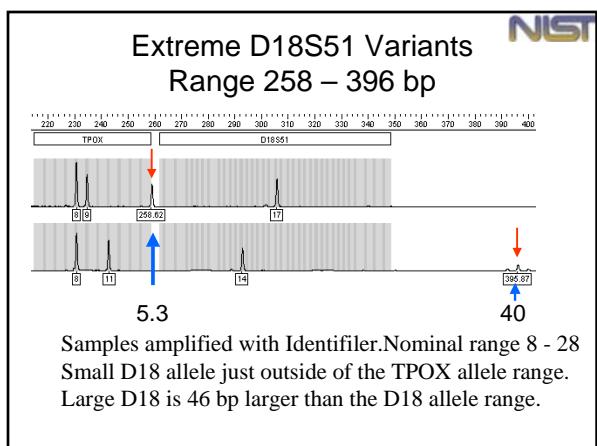
NCBI Program → Peak Height, Area, Size → NIST Multiplex QAC Software → Date vs Resolution (or Signal/Noise)

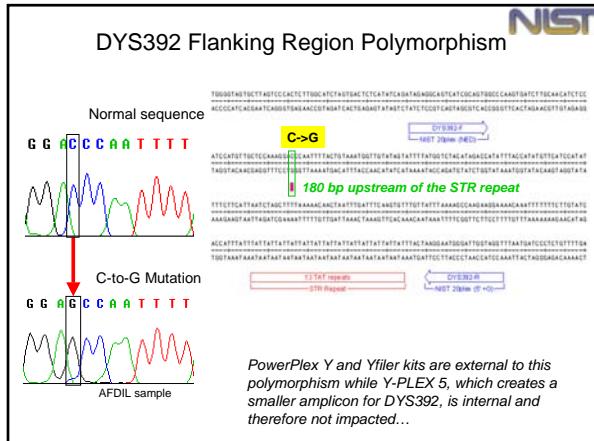
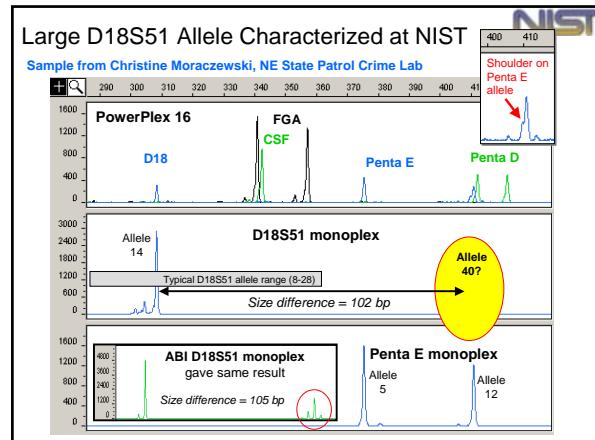
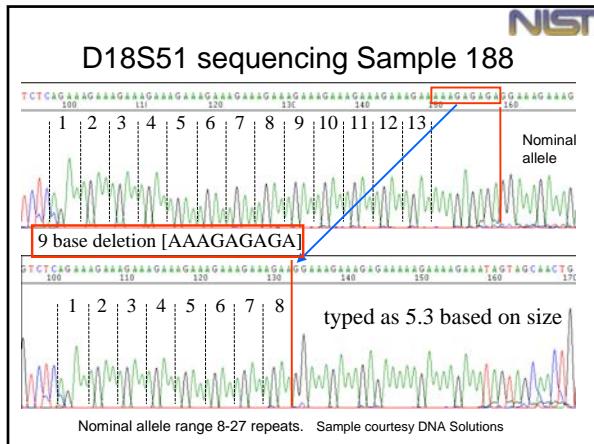
We welcome data sets to help calibrate this software tool

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

NIST Variant Allele Sequencing

- Recent examples:
 - D18 null alleles
 - D18 large allele
 - DYS392 variant
- AAFS talk (Feb 26, 2005) by Margaret Kline on sequencing methods and applications
- We are happy to sequence unusual variant alleles for laboratories

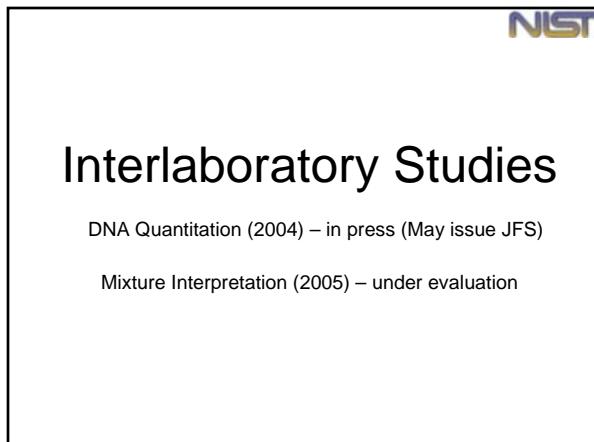
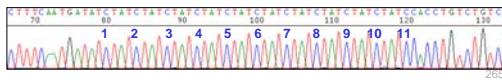




Analysis of Common STR Variant Alleles

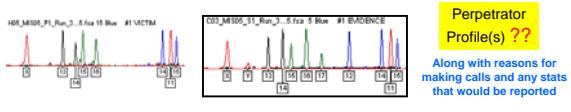
- We have monoplex primers for all common STR loci and kits
- We have sequencing primers that bind outside of STR kit primer sequence positions to enable view of polymorphic nucleotides that cause primer binding site mutations
- NIJ has funded us to characterize STR variants for the forensic DNA community

D16S539 (bottom strand)



Mixture Interpretation Interlab Study (MIX05)

- Only involves interpretation of data
- As of early March, ~97 labs are enrolled for participation (22 from overseas) – Data are currently being evaluated.
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files MAC & NT made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifier, PowerPlex 16 BIO (FMBIO) kits
- Summary of results will involve training materials to illustrate various approaches to solving mixtures



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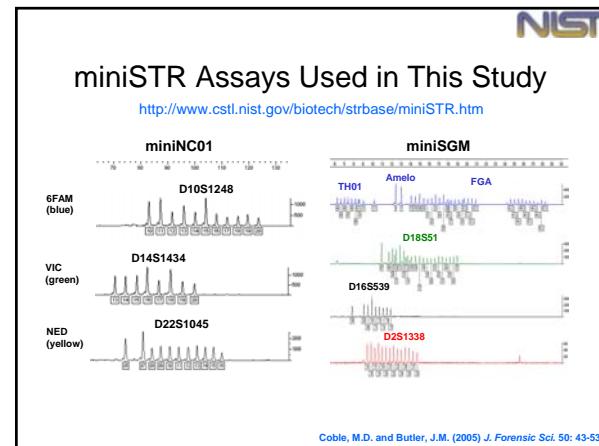
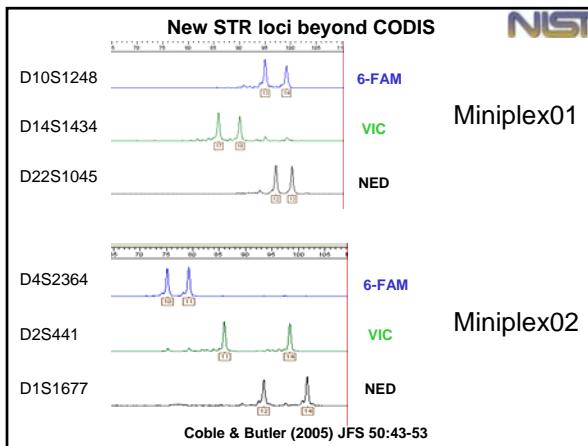
Resources for Challenging Samples

Degraded DNA and Mixtures

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Degraded DNA work

- ENFSI study participation
 - compared STRs, miniSTRs, and autosomal SNPs on same set of degraded DNA samples provided by Peter Gill
- miniSTR website
 - <http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm>
- New miniSTR loci published
 - http://www.cstl.nist.gov/biotech/strbase/pub_pres/Coble2005miniSTR.pdf
- SNP markers and assays
 - <http://www.cstl.nist.gov/biotech/strbase/SNP.htm>
- Performance of miniSTRs on shed hairs
 - Mike Coble spoke at AAFS (Feb 25, 2005)



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Recovered Volumes from DNA Extractions – Sample 02

Sample	Recovered Volume (μL)	Approx. Quantity (ng/μL)	Approx. Final Conc. (ng/μL)
02 blood - day 0	22	3.8	
02 blood - day 0	26	4.36	5.161
02 blood - day 0	22	7.47	
02 blood - week 2	31	0.741	
02 blood - week 2	21	0.884	0.921
02 blood - week 2	16	1.32	
02 blood - week 8	37	0.188	
02 blood - week 8	25	0.207	0.249
02 blood - week 8	21	0.372	
02 blood - week 16	29	1.16	
02 blood - week 16	25	0.224	0.455
02 blood - week 16	38	0.068	
Reagent Blank	18	0	0.000
Reagent Blank	42	0	0.000

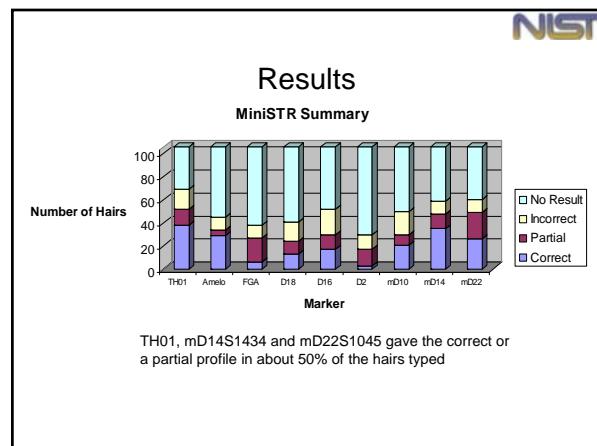
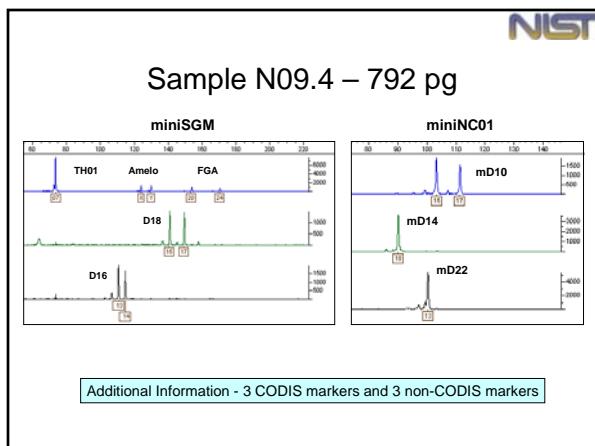
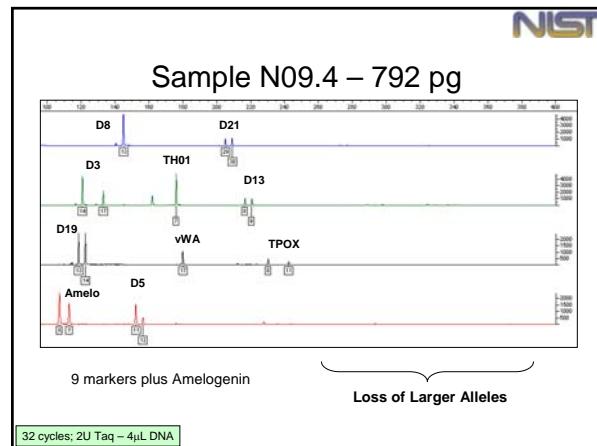
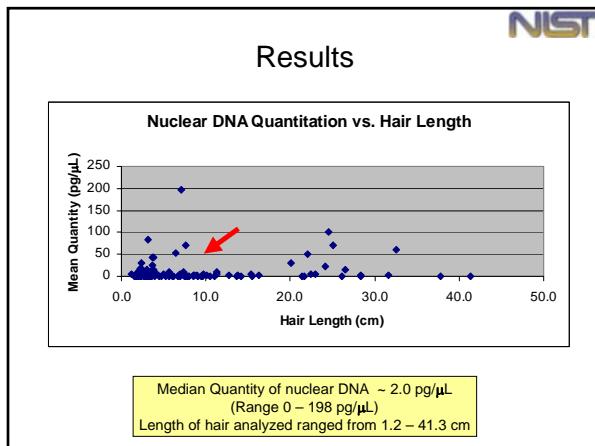
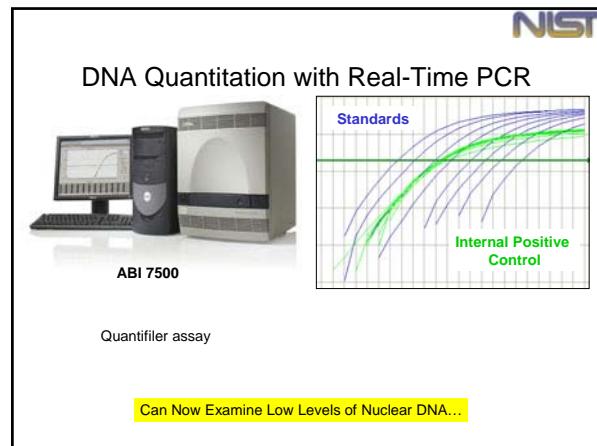
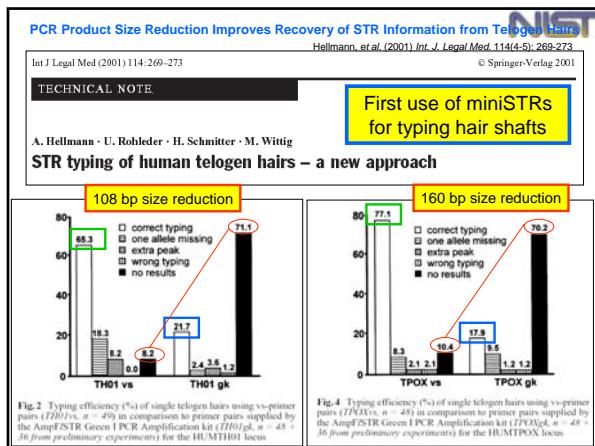
ENSFI Degraded DNA Collaborative Study

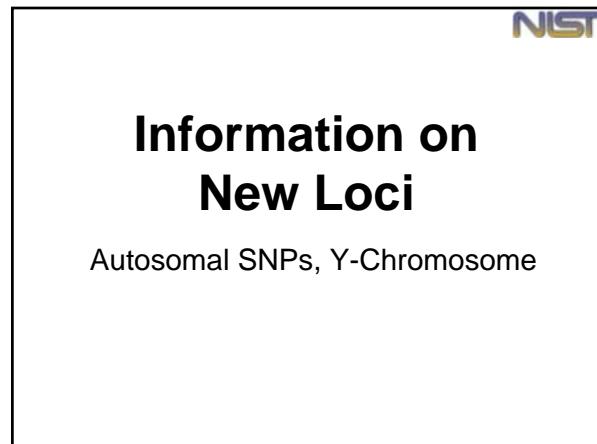
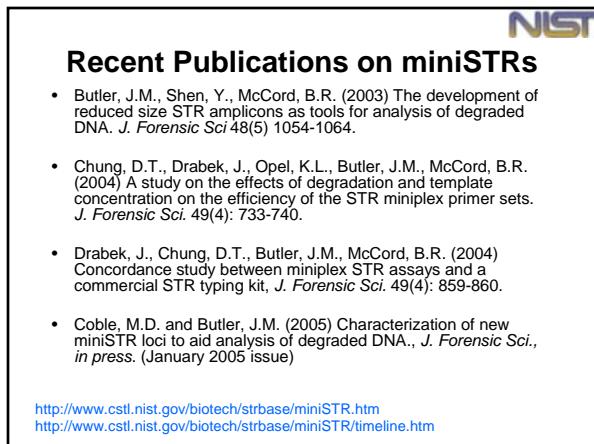
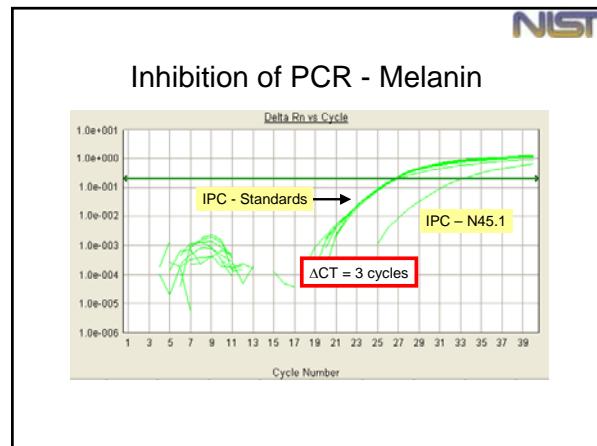
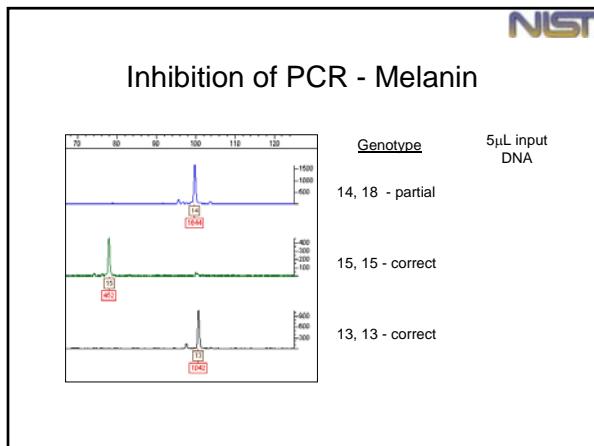
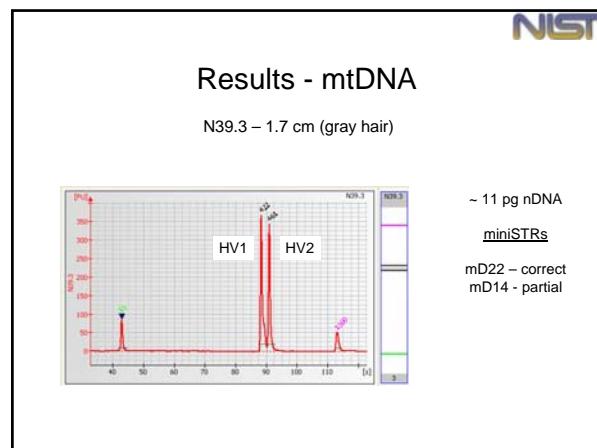
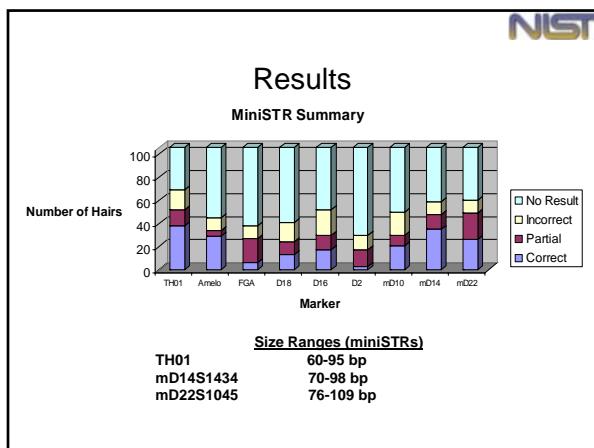
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Summary of Results for Sample 02 Blood (Weeks 2, 8, 16)

Week 2 SGM+ miniSGM		Week 8 SGM+ miniSGM		Week 16 SGM+ miniSGM	
Amel	++	++	Amel	++	++
TH01	++	++	TH01	++	++
FGA	failed	++	FGA	failed	failed
D18	drop out	drop out	D18	failed	failed
D16	++	++	D16	failed	drop out
D2	++	++	D2	failed	drop out
<hr/>					
miniNC01		miniNC01		miniNC01	
D10	++	D10	++	D10	++
D14	++	D14	++	D14	++
D22	++	D22	++	D22	++

ENSFI Degraded DNA Collaborative Study



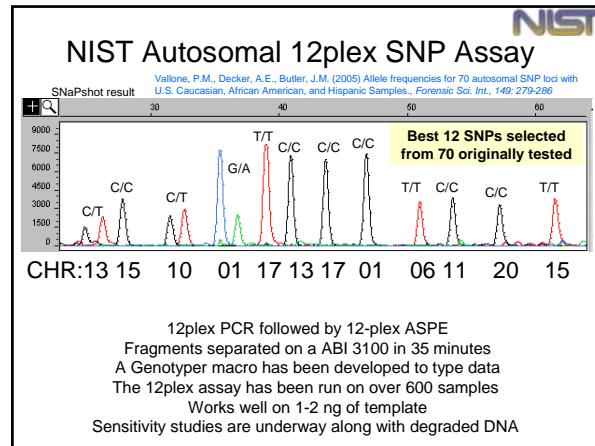


Standard U.S. Population Dataset 

<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	Identifier kit 15 STRs (CODIS + D2S1338 & D19S433)	Butler et al. (2003) JFS
miniSTRs	All CODIS loci except D3S1358	Drabek et al. (2004) JFS
New autosomal STRs	New 6 loci for miniSTRs	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)	Schoske et al. (2004) FSI
	Yfiler concordance study	<i>Data in ABI Yfiler database</i>
New Y-STRs	27 additional loci	<i>Butler et al., in press FSI</i>
Y-SNPs	50 loci spanning haplogroups A-R	Vallone et al. (2004) JFS
mtDNA	LINEAR ARRAY and coding mtSNPs	Kline et al. (2005) JFS
	Full control regions by AFDIL	<i>inclusion in EMPOP</i>

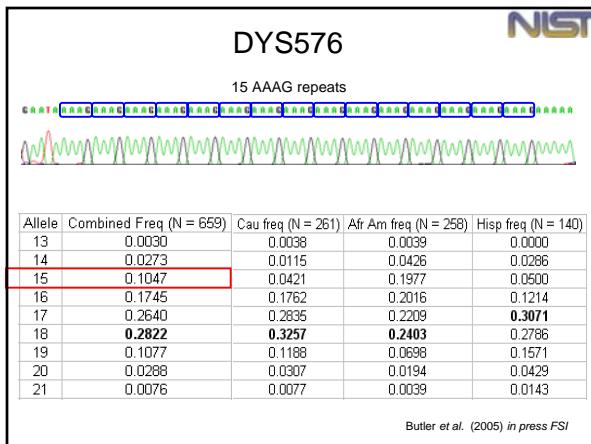


New Y-STR Loci, Issues, and Assays 

- Updates on Y-chromosome information
 - http://www.cstl.nist.gov/biotech/strbase/y_strs.htm
- Testing on 27 new Y-STR loci
 - Butler, J.M., Decker, A.E., Vallone, P.M., Kline, M.C. (2005) Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples, *in press FSI*
- Chromosomal duplication issues
 - Butler, J.M., Decker, A.E., Kline, M.C., Vallone, P.M. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation, *in press JFS*

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- Our Recent Y-Chromosome Work** 
- pdf files available at <http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>
- Schoske, R., Vallone, P.M., Kline, M.C., Redman, J.W., Butler, J.M. (2004) High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays, *Forensic Sci. Int.* 139: 107-121.
 - Vallone, P.M. and Butler, J.M. (2004) Multiplexed assays for evaluation of Y-SNP markers in U.S. populations. *Progress in Forensic Genetics 10*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1261, 85-87.
 - Butler, J.M. and Schoske, R. (2004) Forensic value of the multi-copy Y-STR marker DYS464. *Progress in Forensic Genetics 10*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1261, 278-280.
 - Butler, J.M. and Schoske, R. (2004) Duplication of DYS19 flanking regions in other parts of the Y chromosome. *Int. J. Legal Med.*, 118: 178-183.
 - Vallone, P.M. and Butler, J.M. (2004) Y-SNP typing of U.S. African American and Caucasian samples using allele-specific hybridization and primer extension. *J. Forensic Sci.* 49(4): 723-732.
 - Butler, J.M., Decker, A.E., Kline, M.C., Vallone, P.M. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation, *J. Forensic Sci.*, *in press*.
 - Butler, J.M., Decker, A.E., Vallone, P.M., Kline, M.C. (2005) Allele Frequencies for 27 Y-STR Loci with U.S. Caucasian, African American, and Hispanic Samples, *Forensic Sci. Int.*, *in press*.
 - Butler, J.M. and Schoske, R. (2005) U.S. population data for the multi-copy Y-STR locus DYS464, *J. Forensic Sci.*, *in press*.

Mitochondrial DNA Work

- Evaluation of Roche LINEAR ARRAY screening assay. Kline et al. (2005) JFS 50: 377-385
- Comparison of LINEAR ARRAY resolution to control region sequencing performed by AFDIL
- Collaboration with AFDIL (Tom Parsons) for developing coding SNP assays using SNaPshot

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AcknowledgementsNIST Project Team:

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

Funding:

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

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