NIST Research Update

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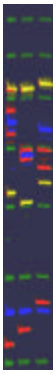
Public SWGDAM Meeting – Phoenix, AZ
September 28, 2003

Outline for Presentation



- STRBase Updates
- Variant Allele Cataloging and Characterization
- SRM 2395 Y-Chromosome Standard
- mtDNA Typing Work
- miniSTR Work
- Future Work
- Another NIST Interlaboratory Study

STRBase

Short Tandem Repeat DNA Internet Database

Recent Additions

- **Forensic SNP Information** (will be official site for ISFG SNP information) …/SNP.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

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

201 total variants reported as of 09/05/03

- CSF1PO (9)
- D2S1338 (2)
- D3S1358 (15)
- D5S818 (5)
- D7S820 (19)
- D8S1179 (4)
- D13S317 (5)
- D16S539 (10)
- D18S51 (27)
- D19S433 (4)
- D21S11 (19)
- FESFPS (1)
- FGA (60)
- HUMTH01 (4)
- Penta E (2)
- TPOX (7)
- VWA (5)

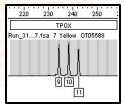
Currently **201** at 13/13 CODIS loci

Tri-Allelic Patterns

49 total patterns reported as of 09/15/03

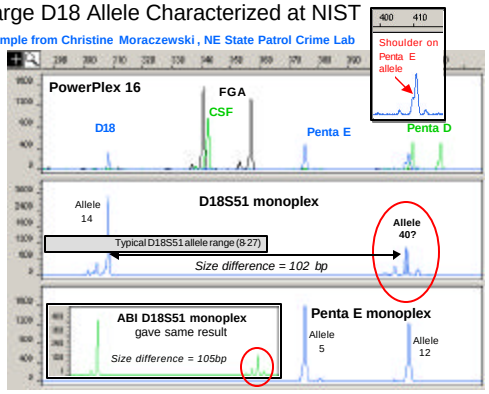
- CSF1PO (3)
- D3S1358 (4)
- D5S818 (1)
- D7S820 (0)
- D8S1179 (3)
- D13S317 (3)
- D16S539 (1)
- D18S51 (4)
- D21S11 (4)
- FGA (7)
- HUMTH01 (1)
- TPOX (12)
- VWA (7)

Currently **49** at 12/13 CODIS loci



Large D18 Allele Characterized at NIST

Sample from Christine Moraczewski, NE State Patrol Crime Lab



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)

New Y-Chromosome NIST SRM

Human Y-Chromosome DNA Profiling Standard

- **5 male samples** + 1 female sample (neg. control)
- **100 ng of each (50 µL at ~2 ng/µL)**
- **22 Y STR markers sequenced**
- **9 additional Y STR markers typed**
- **42 Y SNPs typed** with Marligen kit

$245

Certified for all loci in commercial Y-STR kits:

Y-PLEX 6
Y-PLEX 5
Y-PLEX 12
PowerPlex Y

SWGDAM recommended loci: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

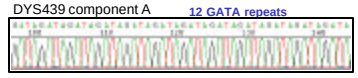
Come see Poster #32 on NIST SRM 2395 and Other Y-Chromosome Work

SRM 2395

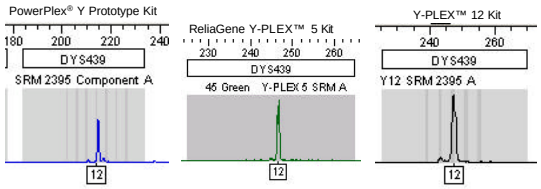
NIST Y-Chromosome DNA Profiling Standard

Helps establish allele nomenclature and confirm/calibrate typing data

DYS439 component A — 12 GATA repeats

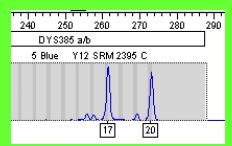


22 Y-STRs sequenced
42 Y-SNPs typed



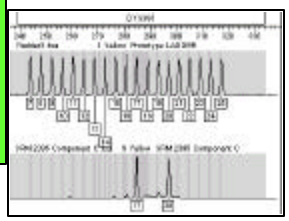
Warning with SRM 2395 Component C and DYS385 Locus Using Original Y-PLEX 6 Macro

DYS385 allele 20 is called correctly with new Y-PLEX 12 kit



DYS385 allele 20 is not called with old Y-PLEX 6 kit macro as it is outside the defined range

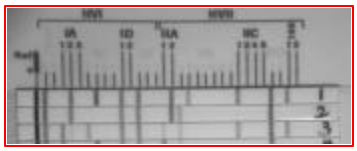
Promega PowerPlex Y Prototype Kit



DYS385 allele 20 is called correctly

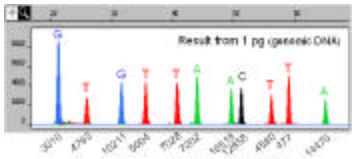
Y6_TYPER310V4.0

NIST mtDNA Work



Roche Linear Arrays
(probes for HVI/HVII)

Beta-test/Population Study



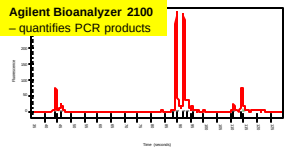
Coding Region
mtSNP 11plex
(minisequencing assay)

Developed with AFDIL to resolve mtDNA most common types

Int. J. Legal Med., submitted

Semi-Automation of mtDNA LINEAR ARRAYS

Agilent Bioanalyzer 2100
– quantifies PCR products



Tecan Profiblot – processes sample through wash steps

Analysis of probe results is still manual!

Come see Poster #30 on Semi-Automation of mtDNA Arrays

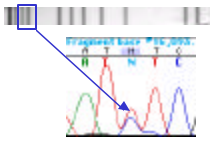
Typing frequencies for 666 NIST population samples

#*	Freq	% Types	% People
1	185	65.6	27.8
2	46	16.3	13.8
3	18	6.4	8.1
4	4	1.4	2.4
5	3	1.1	2.3
6	4	1.4	3.6
7	1	0.4	1.1
8	9	3.2	10.8
9	2	0.7	2.7
10	4	1.4	6.0
11	1	0.4	1.7
12	1	0.4	1.8
18	1	0.4	2.7
23	1	0.4	3.5
28	1	0.4	4.2
51	1	0.4	7.7

Some Results with Roche mtDNA LINEAR ARRAYS

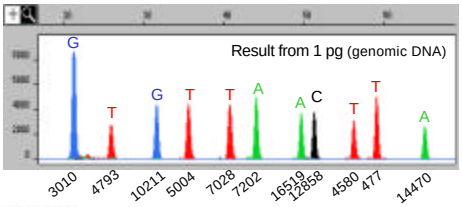
- 282 different types
- 185 were unique (occurred only once)
- 51 samples had "Most Common Type"

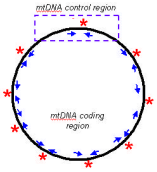
Accurate Detection of Heteroplasmy at 16093



"Most Common Type" evaluated further with mtDNA coding region SNP assay

mtDNA Coding Region 11plex SNaPshot Assay





11plex PCR and 11plex SNP detection

Sites are polymorphic in Caucasians (H1) and useful in resolving most common HV1/HV2 types

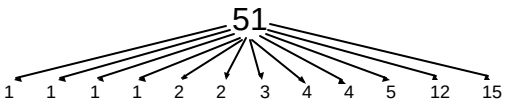
Multiplex PCR used to co-amplify all regions of interest at once

PCR product sizes kept under 200 bp to enable success with degraded DNA samples

"Most Common mtDNA Type"

Examined with mtDNA Coding SNP 11plex

51 samples were identical by Roche mtDNA Linear Arrays
111111AT



Improved resolution of mtDNA samples with coding region SNPs

12 haplogroups were observed
4 haplogroups were unique

miniSTR Work

- miniSTRs (*a.k.a. BodePlexes*) are being used successfully in WTC effort
- Collaboration with Bruce McCord (NIJ-funded) to further develop reduced size STR amplicons
- Starting a new project this fall with Mike Coble (NRC postdoc) to develop new miniSTR loci

J. Forensic Sci. Sept 2003 issue

The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

Describes new primer sequences for all CODIS loci and initial assays developed

Future Work

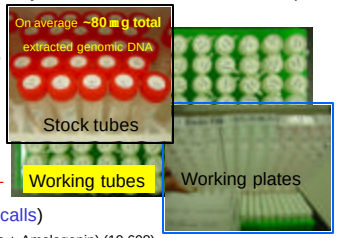
- Quality assurance testing software
  - Dave Duewer in collaboration with NCBI
- Quantitation standard SRM 2372
- Evaluation of real-time PCR methodologies for DNA quantitation
- More miniSTR work for degraded DNA
- Locus-specific brackets for alternative Y-STR typing
- Comparison of new SNP markers to STRs in NIST U.S. population sample set

NIST U.S. Population Samples

As of 06/2003 666 males (anonymous; self-identified ethnicities)

260 Caucasians
260 African Americans
143 Hispanics
3 Asians

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



Working tubes/plates 1 ng/uL

To date: (~50,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y SNPs 50 markers on sub-set of samples (11,498)

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

Another NIST Interlaboratory Test

- Solicitation for 2004 NIST Quantitation Study
  - Plan to ship in Nov 2003
  - Data due by March 1, 2004
- Purpose to examine accuracy of current DNA quantitation methods and measure stability of prototype NIST quantitation SRM
- Open to all human identity testing labs
- Will involve only quantifying 6-8 samples
- Handouts available

Acknowledgments

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NIST Project Team:
John Butler (project leader)
Pete Vallone
Jan Redman
Rich Schoske (American U)
Dave Duewer

Collaborators:
Mike Hammer and Alan Redd (U. AZ) for Y STR sequences
Tom Parsons and Mike Coble (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA linear arrays
Bruce McCord and students (Ohio U) for miniSTR work

This presentation available as pdf file from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Publications from Our Group this Past Year

Available as pdf files from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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