Topics in Forensic DNA Analysis & Interpretation

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

Background on the Presenter

John M. Butler has a B.S. in chemistry from Brigham Young University and a Ph.D. in analytical chemistry from the University of Virginia. His dissertation research, which was conducted at the FBI Academy in Quantico, VA, involved pioneering work in applying capillary electrophoresis to STR typing. While a postdoc at NIST, he designed and built STRBase, the widely used Short Tandem Repeat Internet Database (http://www.cstl.nist.gov/biotech/strbase) that contains a wealth of standardized information on STRs used in human identity applications. He worked for several years as a staff scientist and project leader at a California startup company named GeneTrace System developing rapid DNA analysis technologies involving time-of-flight mass spectrometry. In the fall of 1999, he returned to NIST to lead their efforts in human identity testing with funding from the National Institute of Justice.

Dr. Butler is currently a NIST Fellow and Group Leader of Applied Genetics in the Biochemical Science Division at the National Institute of Standards and Technology. He is a regular invited guest of the FBI's Scientific Working Group on DNA Analysis Methods (SWGDAM) and a member of the Department of Defense Quality Assurance Oversight Committee for DNA Analysis. Following the terrorist attacks of 11 September 2001, he aided the DNA identification efforts and served as part of the distinguished World Trade Center Kinship and Data Analysis Panel (WTC KADAP). He is a member of the International Society of Forensic Genetics and serve as an Associate Editor for *Forensic Science International: Genetics*.

Dr. Butler has received numerous awards including the Presidential Early Career Award for Scientists and Engineers (2002), the Department of Commerce Silver Medal (2002) and Gold Medal (2008), the Arthur S. Flemming Award (2007), the Edward Uhler Condon Award (2010), Brigham Young University's College of Physical and Mathematical Sciences Honored Alumnus (2005), and the Scientific Prize of the International Society of Forensic Genetics (2003).

He has more than 100 publications describing aspects of forensic DNA testing and is one of the most prolific active authors in the field with articles appearing regularly in every major forensic science journal. Dr. Butler has been an invited speaker to numerous national and international forensic DNA meetings and in the past few years has spoken in Germany, France, England, Canada, Mexico, Denmark, Belgium, Poland, Portugal, Cyprus, The Netherlands, Argentina, Japan, and Australia. In addition to his busy scientific career, he and his wife serve in their community and church and are the proud parents of six children, all of whom have been proven to be theirs through the power of DNA typing.

For listing of publications, see http://www.cstl.nist.gov/biotech/strbase/butler.htm.

Topics in Forensic DNA Analysis & Interpretation

DNA Workshop for Indiana State Police Laboratory

Instructor: John M. Butler (NIST)

Indianapolis, IN
March 28, 2011

Proposed Agenda (to start at 8:00 a.m.)

Introductions (15 minutes)

CE Fundamentals & Troubleshooting (60 minutes)
ABI 3500 (15 minutes)

- BREAK (15 minutes) -

Low-level DNA Issues (30 minutes)

Validation Discussion (60 minutes) – discuss specific on-going validation studies

Thoughts on the Future Directions of the Field (15 minutes)

- LUNCH (60 minutes) -

Mixtures & SWGDAM Interpretation Guidelines (120 minutes)

- BREAK (15 minutes) -

Y-STRs (30 minutes)

Relationship Testing & Parentage Statistics (30 minutes)

Additional Q & A (15 minutes)

Please ask questions throughout the presentations!

Recommended Literature

Compiled by John Butler (March 2011)

CE Fundamentals and Troubleshooting

Butler, J.M., et al. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.

Lazaruk, K., et al. (1998) Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis* 19: 86-93.

McCord, B.R. (2003) Troubleshooting capillary electrophoresis systems. *Profiles in DNA* 6(2): 10-12. Available at: http://www.promega.com/profiles/.

Moretti, T.R., et al. (2001) Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *J. Forensic Sci.* 46: 647-660.

Low-level DNA Issues (http://www.cstl.nist.gov/biotech/strbase/LTDNA.htm)

Benschop, C.C.G., et al. (2010) Low template STR typing: effect of replicate number and consensus method on genotyping reliability and DNA database search results. *Forensic Sci. Int. Genet. (in press)*. doi:10.1016/j.fsigen.2010.06.006

Butler, J.M., & Hill, C.R. (2010) Scientific issues with analysis of low amounts of DNA. *Profiles in DNA* 13(1). Available at http://www.promega.com/profiles/1301/1301_02.html.

Whitaker, J. P., et al. (2001) A comparison of the characteristics of profiles produced with the AMPFlSTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. *Forensic Sci. Int.* 123: 215-223.

Validation (http://www.cstl.nist.gov/biotech/strbase/validation.htm)

Butler, J.M. (2006) Debunking some urban legends surrounding validation within the forensic DNA community. *Profiles in DNA* 9(2): 3-6. Available at http://www.promega.com/profiles/.

ENFSI DNA Working Group (2010) Recommended minimum criteria for the validation of various aspects of the DNA profiling process. Available at http://www.enfsi.eu.

SWGDAM. (2004) Revised validation guidelines. *Forensic Science Communications, 6(3)*. Available at http://www2.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

Future Directions of the Field

Butler, J.M., et al. (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. *Forensic Sci. Med. Pathol.* 3: 200-205.

Kayser, M., & de Knijff, P. (2011) Improving human forensics through advances in genetics, genomics and molecular biology. *Nature Rev. Genet.* 12: 179-192.

Mixtures and SWGDAM Interpretation Guidelines (http://www.cstl.nist.gov/biotech/strbase/mixture.htm)

Clayton, T.M., et al. (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Sci. Int.* 91: 55-70.

Gill, P., et al. (2006) DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101.

SWGDAM. (2010) SWGDAM interpretation guidelines for autosomal STR typing by forensic DNA testing laboratories. Available at http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines.

Y-STRs (http://www.cstl.nist.gov/biotech/strbase/y_str.htm)

Brenner, C.H. (2010) Fundamental problem of forensic mathematics—the evidential value of a rare haplotype. *Forensic Sci. Int. Genet.* 4: 281-291.

Buckleton, J.S., et al. (2011) The interpretation of lineage markers in forensic DNA testing. *Forensic Sci. Int. Genet.* 5: 78-83.

Gusmão, L. et al. (2006) DNA Commission of the International Society of Forensic Genetics (ISFG): an update of the recommendations on the use of Y-STRs in forensic analysis. *Forensic Sci. Int.* 157: 187-197.

Krenke, B.E., et al. (2005) Validation of male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex. *Forensic Sci. Int.* 151: 111-124.

Relationship Testing (http://www.cstl.nist.gov/biotech/strbase/kinship.htm)

Gjertson, D.W. et al. (2007) ISFG: recommendations on biostatistics in paternity testing. *Forensic Sci. Int. Genet.* 1(3-4): 223-231.

Topics in Forensic DNA Analysis & Interpretation

Introductions

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Dr. John M. Butler

NIST Fellow & Applied Genetics Group Leader

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

Experience

- University of Virginia/FBI Laboratory (1992-1995)
  - Work performed in Bruce McCord's lab
- NIST NRC Postdoc (1995-1997)
- GeneTrace Systems Inc (1997-1999)
- NIST Human Identity Project Leader (1999-present)

Contact Information
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301-975-4049

- Ph.D. dissertation (Aug 1995): "Sizing and quantitation of polymerase chain reaction products by capillary electrophoresis for use in DNA typing"
- *Forensic DNA Typing* textbook (now in its 3rd Edition)
- STRBase website: http://www.cstl.nist.gov/biotech/strbase/
- Family: wife Terilynne and 6 children
- Hobbies: reading, writing, and making PowerPoint slides

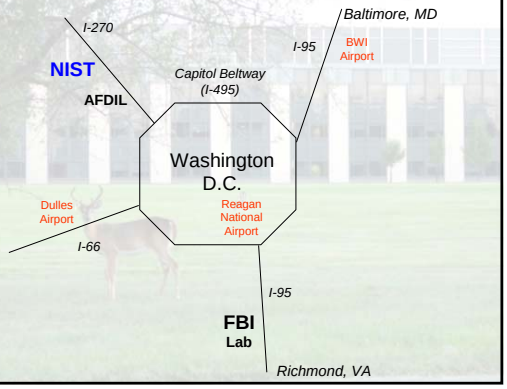
NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government **use in calibration of measurements**.
- **NIST defines time for the U.S.**

$686 for 3 jars

DNA typing standard

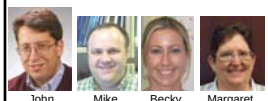
Location of NIST



NIST Human Identity Project Teams

within the Applied Genetics Group

Forensic DNA Team

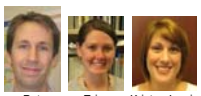


John Butler, Mike Coble, Becky Hill, Margaret Kline

Data Analysis Support

Dave Duewer

DNA Biometrics Team



Pete Vallone, Erica Butts, Kristen Lewis O'Connor

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

Funding from the **FBI S&T Branch** through NIST Information Access Division

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

Contributors to These Workshop Slides


Bruce McCord
Florida International University
CE


Erica Butts
NIST
ABI 3500

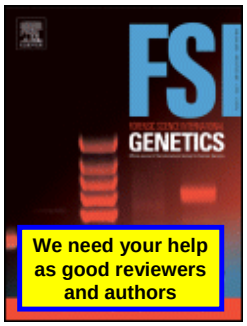

Becky Hill
NIST
low level DNA


Mike Coble
NIST
DNA mixtures


Kristen O'Connor
NIST
kinship analysis

Forensic Science International: Genetics

http://www.fsigenetics.com/



Editor-in-Chief:
Angel Carracedo (Spain)
Associate Editors:
Peter M. Schneider (Germany)
John M. Butler (USA)

FSI: Genetics is a new journal dedicated exclusively to the field of forensic genetics. It has been launched in 2007 by Elsevier Publishers in affiliation with the International Society of Forensic Genetics. **All members of the ISFG receive a free subscription of this journal** (print and online version) as part of their membership benefits.

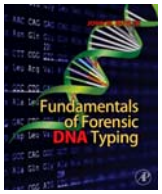
Primary Sources for Material Covered in this Workshop

- See recommended reference list

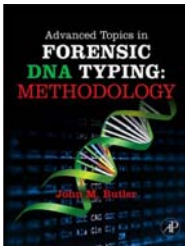
- **Butler, J.M. (2009) *Fundamentals of Forensic DNA Typing.* Elsevier Academic Press**
- **Butler, J.M. (2011) *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press**
- NIST STRBase website: http://www.cstl.nist.gov/biotech/strbase/

These workshop materials are available at http://www.cstl.nist.gov/biotech/strbase/training.htm

3rd Edition is Three Volumes



Sept 2009

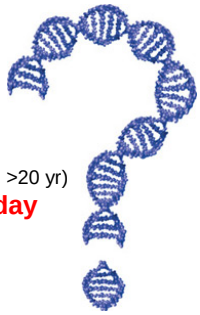


Sept 2011

Fall 2012

Participants' Backgrounds

Your name
Your organization/lab
Experience level (e.g., <1 yr, 5 yr, >20 yr)
What you hope to learn today



NIST and NIJ Disclaimer

Funding: Interagency Agreement 2008-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

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.

Our publications and presentations are made available at: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Topics in Forensic DNA Analysis & Interpretation

Capillary Electrophoresis: Fundamentals & Troubleshooting

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Presentation Outline

- NIST experience with DNA analysis using CE
- CE separation, injection, detection process
- Troubleshooting tips and suggestions

J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, Table 6.1

Genetic Analyzers from Applied Biosystems

ABI Genetic Analyzer	Years Released for Human ID	Number of Capillaries	Laser	Polymer delivery	Other features
373 (gel system)	1992-2003	--	40 mW Ar+ (488/514 nm)	--	PMTs and color filter wheel for detection
377 (gel system)	1995-2006	--	40 mW Ar+ (488/514 nm)	--	CCD camera
310	1995-	1	10 mW Ar+ (488/514 nm)	syringe	Mac operating system & Windows NT (later)
3100	2000-2005	16	25 mW Ar+ (488/514 nm)	syringe	
3100-Avant	2002-2007	4	25 mW Ar+ (488/514 nm)	syringe	
3130	2003-2011	4	25 mW Ar+ (488/514 nm)	pump	
3130xl	2003-2011	16	25 mW Ar+ (488/514 nm)	pump	
3500	2010-	8	10-25 mW diode (505 nm)	new pump	110V power; **RFID-tagged reagents**; **.hid files**; normalization & 6-dye detection possible
3500xl	2010-	24			
3700	2002-2003	96	25 mW Ar+ (488/514 nm)	cuvette-based	Split beam technology
3730	2005-	48	25 mW Ar+ (488/514 nm)	pump	
3730xl	2005-	96	25 mW Ar+ (488/514 nm)	pump	

Information courtesy of Michelle S. Shepherd, Applied Biosystems, LIFE Technologies.

ABI Genetic Analyzer Usage at NIST

(All instruments were purchased using NIJ funds)

ABI 310 Single capillary

- 1st was purchased in 1996 as Mac (A230, now B233)
- 2nd was purchased in June 2002 as NT (B261)

ABI 3100 → 3130xl 16 capillaries

- 1st purchased in April 2001 as ABI 3100
  - upgraded to 3130xl in Sept 2005
  - Located in a different room (A230, now B219)
- 2nd purchased in June 2002 as ABI 3100
  - Original data collection (v1.0.1) software retained
  - updated to 3130xl in Jan 2007 (B219, now B261)

ABI 3500 8 capillaries

- Purchased Nov 2010 (B233)

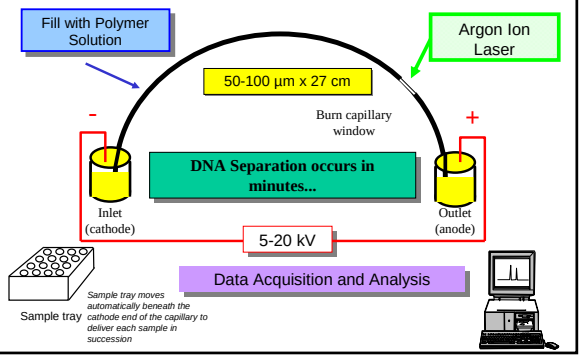
DNA Samples Run at NIST

we have **processed >100,000 samples** (from 1996-present)

- **STR kits**
  - Identifiler, PP16, PP16HS, Identifiler Plus, Identifiler Direct, Profiler Plus, Cofiler, SGM Plus, ESI/ESX 17, SE33 monoplex
- **Research & development on new assays**
  - **STRs**: Y-STR 20plex, MeowPlex, miniSTRs, 26plex
  - **SNPs**: SNaPshot assays: mtDNA (one 10plex), Y-SNPs (four 6plexes), Orchid SNPs (twelve 6plexes), ancestry SNPs (two 12plexes), SNPforID (one 29plex), SNPplex (one 48plex)
- **DNA sequencing**
  - Variant allele sequencing

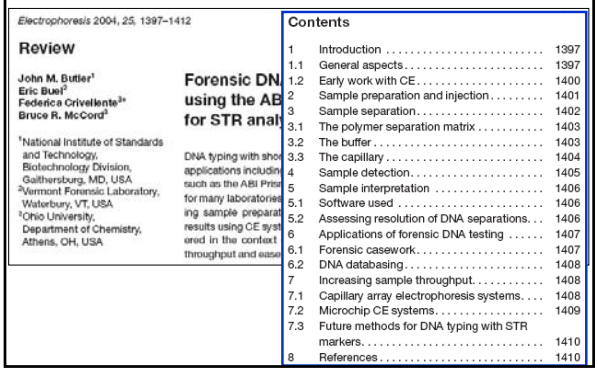
We have a unique breadth and depth of experience with these instruments…

Capillary Electrophoresis (CE)



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[1]
Eric Buel[2]
Federica Crivellente[3]*
Bruce R. McCord[3]

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[2]Vermont Forensic Laboratory, Waterbury, VT, USA
[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN
using the AB
for STR anal

DNA typing with sho
applications includin
such as the ABI Prisr
for many laboratories
ing sample preparat
results using CE syst
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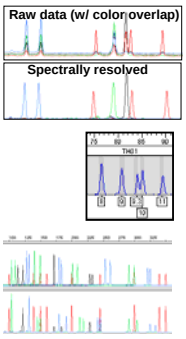
throughput and ease

Contents

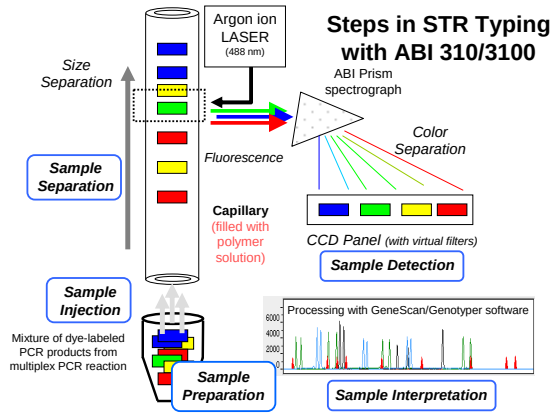
Analytical Requirements for STR Typing

Butler *et al.* (2004) Electrophoresis 25: 1397-1412

- Fluorescent dyes must be **spectrally resolved** in order to distinguish different dye labels on PCR products
- PCR products must be **spatially resolved** – desirable to have single base resolution out to >350 bp in order to distinguish variant alleles
- High **run-to-run precision** – an internal sizing standard is used to calibrate each run in order to compare data over time



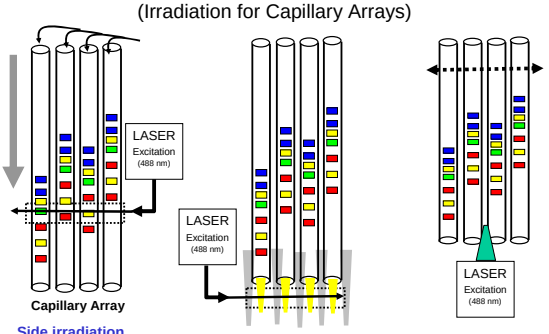
Steps in STR Typing with ABI 310/3100



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 13.8, © Elsevier Science/Academic Press

Detection with Multiple Capillaries

(Irradiation for Capillary Arrays)



Capillary Array

Side irradiation (on-capillary)
ABI 3100, 3130, 3100*Avant*

Sheath flow detection
ABI 3700

Fixed laser, moving capillaries
MegaBACE

Process Involved in 310/3100 Analysis

- Separation
  - Capillary – 50um fused silica, 43 cm length (36 cm to detector)
  - POP-4 polymer – Polydimethyl acrylamide
  - Buffer - TAPS pH 8.0
  - Denaturants – urea, pyrolidinone
- Injection
  - electrokinetic injection process (formamide, water)
  - importance of sample stacking
- Detection
  - fluorescent dyes with excitation and emission traits
  - CCD with defined virtual filters produced by assigning certain pixels

Separation

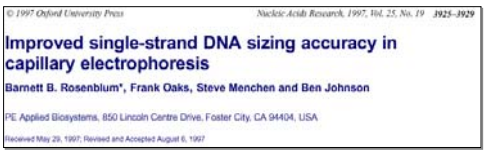
Ohm's Law

- V = IR (where V is voltage, I is current, and R is resistance)
- Current, or the flow of ions, is what matters most in electrophoresis
- CE currents are much lower than gels because of a higher resistance in the narrow capillary
- CE can run a higher voltage because the capillary offers a higher surface area-to-volume ratio and can thus dissipate heat better from the ion flow (current)

Separation Issues

- Electrophoresis buffer –
  - Urea for denaturing and viscosity
  - Buffer for consistent pH
  - Pyrolidinone for denaturing DNA
  - EDTA for stability and chelating metals
- Polymer solution -- POP-4 (but others work also)
- Capillary wall coating -- dynamic coating with polymer
  - Wall charges are masked by methyl acrylamide
- Run temperature -- 60 °C helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

What is in POP-4 and Genetic Analyzer Buffer?

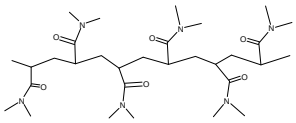


See also Wenz *et al.* (1998) *Genome Research* 8: 69-80

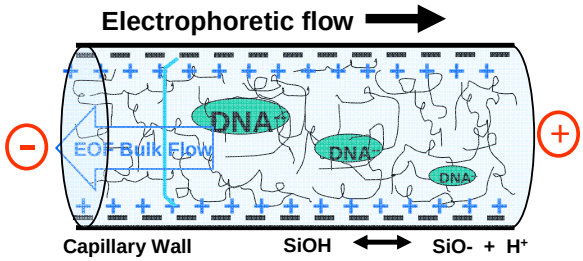
POP-4 (4% poly-dimethylacrylamide, 8 M urea, 5% 2-pyrrolidinone)

US Patent 5,552,028 covers POP-4 synthesis

Running buffer contains 100 mM TAPS and 1 mM EDTA (adjusted to pH 8.0 with NaOH) *TAPS = N-Tris-(hydroxymethyl)methyl-3-aminopropane-sulfonic acid*

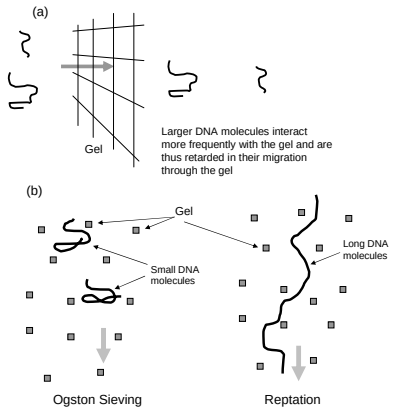


Capillary Wall Coatings Impact DNA Separations



Electroosmotic flow (EOF)

Solvated ions drag solution towards cathode in a flat flow profile

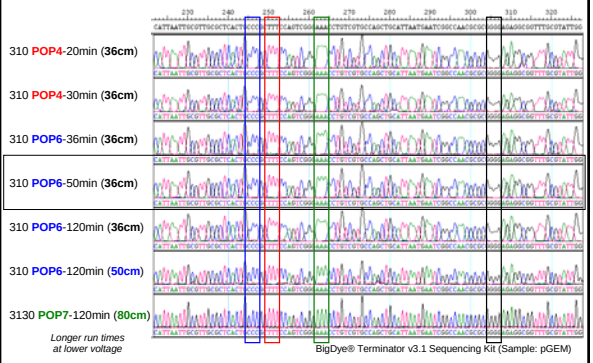


How to Improve Resolution?

1. **Lower Field Strength**
2. **Increase Capillary Length**
3. **Increase Polymer Concentration**
4. **Increase Polymer Length**

All of these come at a cost of longer separation run times

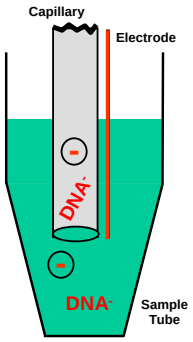
Impact of Capillary Length and Polymer Concentration on DNA Sequencing Resolution



Data collected at NIST by Tomohiro Takamaya (Japanese guest researcher, fall 2007)

Injection

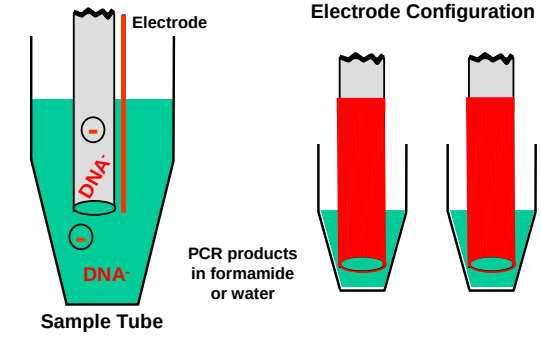
Electrokinetic Injection Process



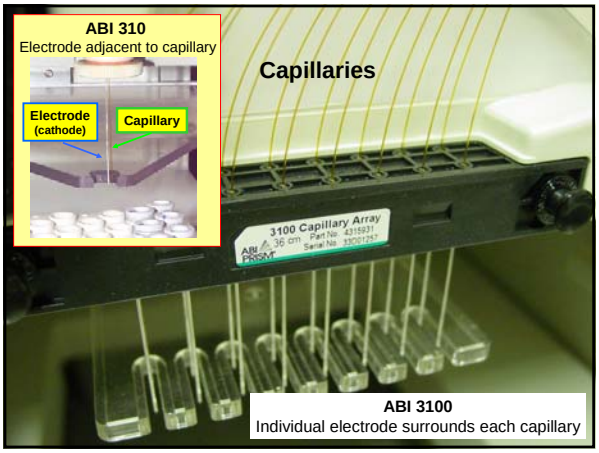
Amount of DNA injected is inversely proportional to the ionic strength of the solution

Salty samples result in poor injections

Capillary and Electrode Configurations



ABI 310 / ABI 3100



Sample Conductivity Impacts Amount Injected

$$[DNA_{inj}] = \frac{Et(\pi r^2)(\mu_{ep} + \mu_{eof})[DNA_{sample}](\lambda_{buffer})}{\lambda_{sample}}$$

$[DNA_{inj}]$ is the amount of sample injected

E is the electric field applied

t is the injection time

r is the radius of the capillary

μ_{ep} is the mobility of the sample molecules

μ_{eof} is the electroosmotic mobility

$[DNA_{sample}]$ is the concentration of DNA in the sample

λ_{buffer} is the buffer conductivity

λ_{sample} is the sample conductivity

Cl- ions and other buffer ions present in PCR reaction contribute to the sample conductivity and thus will compete with DNA for injection onto the capillary

Butler *et al.* (2004) Electrophoresis 25: 1397-1412

Steps Performed in Standard Module

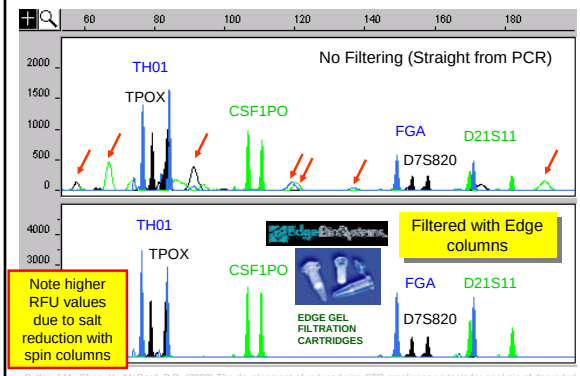
See J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition; Chapter 14

- **Capillary fill** – polymer solution is forced into the capillary by applying a force to the syringe
- **Pre-electrophoresis –** the separation voltage is raised to 10,000 volts and run for 5 minutes;
- **Water wash of capillary – capillary is dipped several times in deionized water to remove buffer salts that would interfere with the injection process**
- **Sample injection** – the autosampler moves to position A1 (or the next sample in the sample set) and is moved up onto the capillary to perform the injection; a voltage is applied to the sample and a few nanoliters of sample are pulled onto the end of the capillary; the default injection is 15 kV (kilovolts) for 5 seconds
- **Water wash of capillary** – capillary is dipped several times in waste water to remove any contaminating solution adhering to the outside of the capillary
- **Water dip** – capillary is dipped in clean water (position 2) several times
- **Electrophoresis** – autosampler moves to inlet buffer vial (position 1) and separation voltage is applied across the capillary; the injected DNA molecules begin separating through the POP-4 polymer solution
- **Detection** – data collection begins; raw data is collected with no spectral deconvolution of the different dye colors; the matrix is applied during Genescan analysis

Comments on Sample Preparation

- Use high quality formamide (<100 μS/cm)
- Denaturation with heating and snap cooling is not needed (although most labs still do it…)
- Post-PCR purification reduces salt levels and leads to more DNA injected onto the capillary

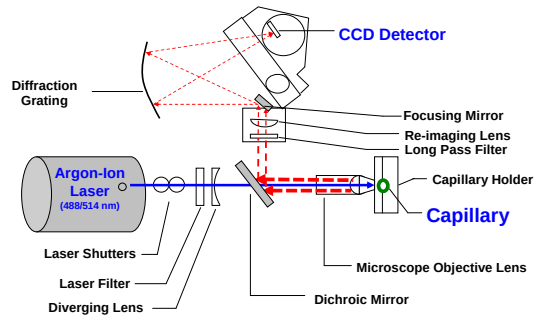
Removal of Dye Artifacts Following PCR Amplification



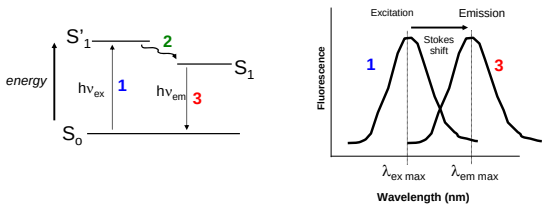
Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5) 1054-1064.

Detection

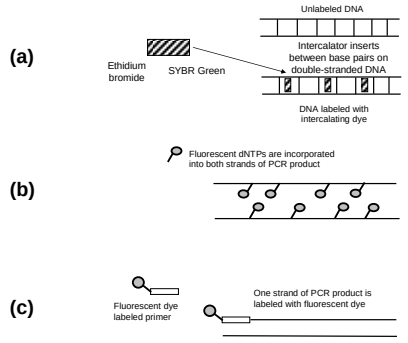
Optics for ABI 310



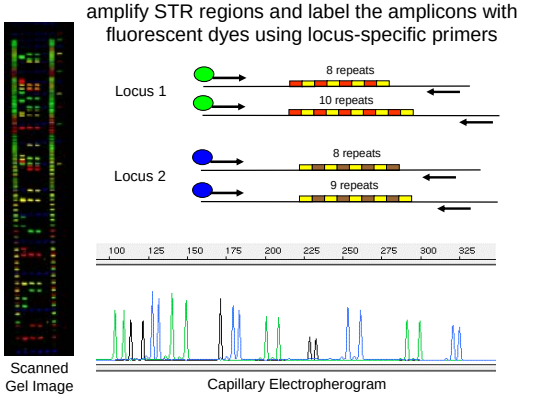
Fluorescence



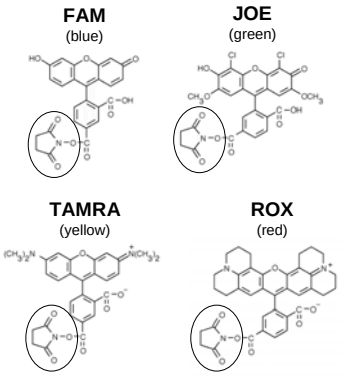
Methods for Fluorescently Labeling DNA



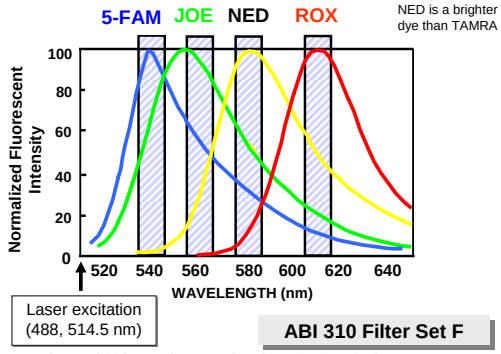
The polymerase chain reaction (PCR) is used to amplify STR regions and label the amplicons with fluorescent dyes using locus-specific primers



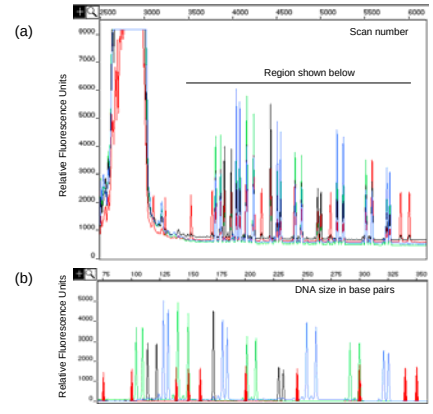
ABI Fluorescent Dyes Used in Four-Color Detection



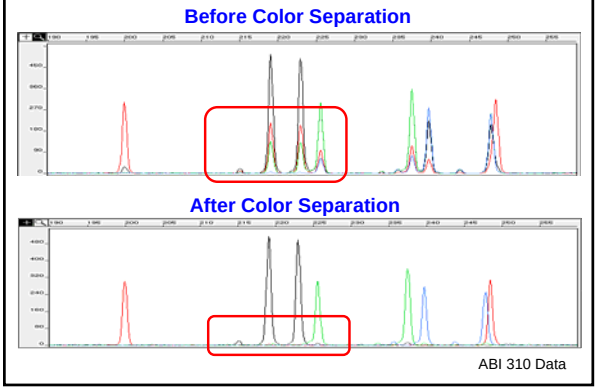
Fluorescent Emission Spectra for ABI Dyes



Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.4, ©Academic Press



Importance of Spectral Calibration



ABI 310 Data

Matrix with 4 Dyes on ABI 310

$I_{540} = bx_b + gy_b + yz_b + rw_b$ intensity of blue
$I_{560} = bx_g + gy_g + yz_g + rw_g$ intensity of green
$I_{580} = bx_y + gy_y + yz_y + rw_y$ intensity of yellow
$I_{610} = bx_r + gy_r + yz_r + yw_r$ intensity of red

Where
b is the %blue labeled DNA
g is the %green labeled DNA, etc.

x,y,z,w are the numbers in the matrix (sensitivity to each color)

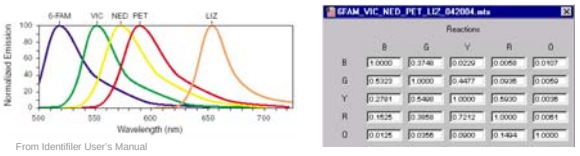
POP4STRMODF

Reactions

	B	G	Y	R
B	1.0000	0.8502	0.1380	0.0009
G	0.8300	1.0000	0.7622	0.0051
Y	0.6416	0.8324	1.0000	0.1102
R	0.4493	0.6484	0.7851	1.0000

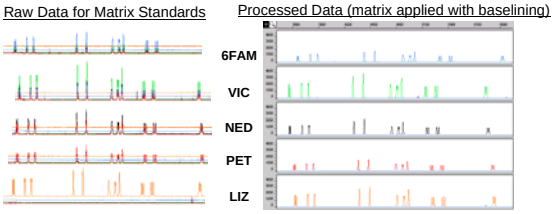
If you solve xyzw for each dye individually
Then you can determine dye contribution for any mixture

5 x 5 matrix for 5-dye analysis on ABI 310

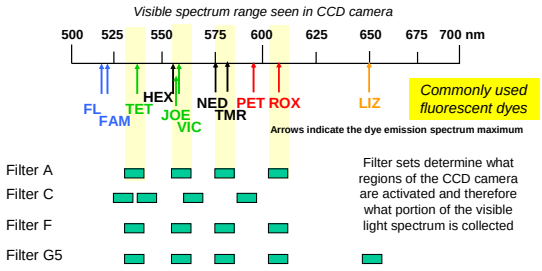


Raw Data for Matrix Standards

Processed Data (matrix applied with baselining)



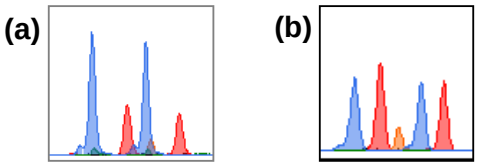
Virtual Filters Used in ABI 310



	Blue	Green	Yellow	Red	Orange	Used with These Kits
Filter A	FL	JOE	TMR	CXR		PowerPlex 16
Filter C	6FAM	TET	HEX	ROX		in-house assays
Filter F	5FAM	JOE	NED	ROX		Profiler Plus
Filter G5	6FAM	VIC	NED	PET	LIZ	Identifiler

Variable Binning Increases Red Peaks

Comparison of Data Collection Versions

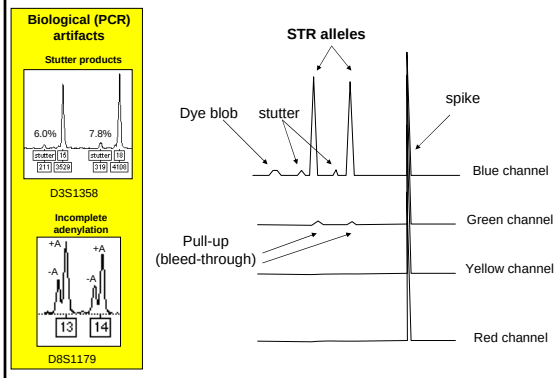


ABI 3100
Data Collection v1.0.1

ABI 3130xl
Data Collection v3.0

The same PCR products examined with different data collection versions. In (a) there is an equal number of pixels of light collected from the CCD camera for the blue-labeled and red-labeled peaks. In (b) the signal increase in the red dye-labeled PCR products is accomplished with 'variable binning' where more pixels of light are collected from the CCD camera in the red-channel to help balance the less sensitive red dye with blue dye-labeled amplicons.

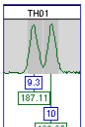
Deciphering Artifacts from the True Alleles



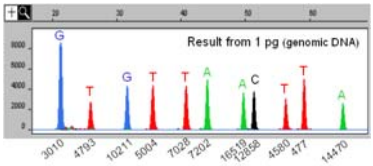
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 15.4, © Elsevier Science/Academic Press

NIST ABI 3100 Analysis Using POP-6 Polymer

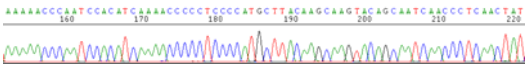
High Resolution STR Typing



SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)



mtDNA Sequencing (HV1)



Maintenance of ABI 310/3100/3130

- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – **it dries, it dies!**
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

YOU MUST BE CLEAN AROUND A CE!

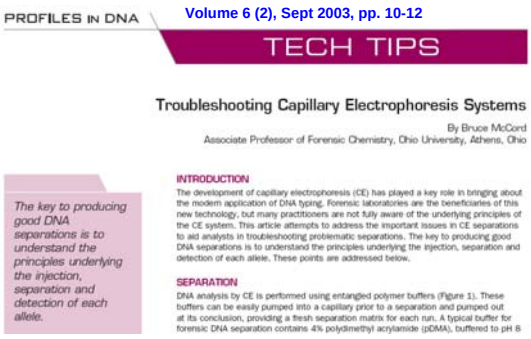
Protocols Used for STR Typing

- 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
  - POP-4 polymer lasts much longer than 5 days on an ABI 310
  - **Validation does not have to be an overwhelming task**

Troubleshooting

Bruce McCord's *Profiles in DNA* Article



PROFILES IN DNA

Volume 6 (2), Sept 2003, pp. 10-12

TECH TIPS

Troubleshooting Capillary Electrophoresis Systems

By Bruce McCord
Associate Professor of Forensic Chemistry, Ohio University, Athens, Ohio

The key to producing good DNA separations is to understand the principles underlying the injection, separation and detection of each allele.

INTRODUCTION

The development of capillary electrophoresis (CE) has played a key role in bringing about the modern application of DNA typing. Forensic laboratories are the beneficiaries of this new technology, but many practitioners are not fully aware of the underlying principles of the CE system. This article attempts to address the important issues in CE separations to aid analysts in troubleshooting problematic separations. The key to producing good DNA separations is to understand the principles underlying the injection, separation and detection of each allele. These points are addressed below.

SEPARATION

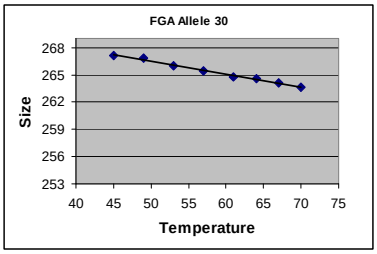
DNA analysis by CE is performed using entangled polymer buffers (Figure 1). These buffers can be easily pumped into a capillary prior to a separation and pumped out at its conclusion, providing a fresh separation matrix for each run. A typical buffer for forensic DNA separation contains 4% polydimethyl acrylamide (pDMA), buffered to pH 8

External Factors

- Room temperature
  - Variations in room temperature can cause mobility shifts with band shifts and loss of calibration
  - Temperature is also important due to effects of high humidity on electrical conductance

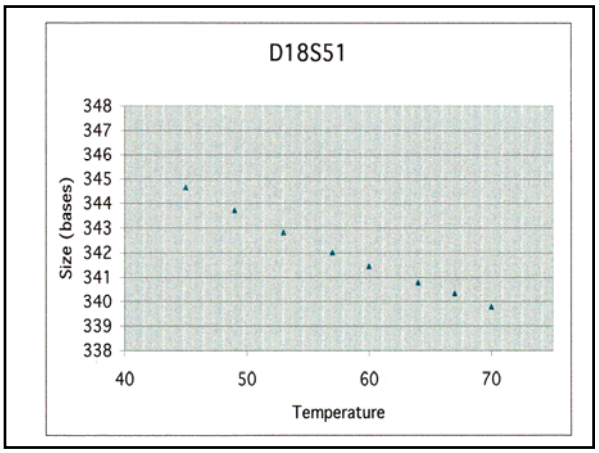
- Cleanliness
  - Urea left in sample block can crystallize and catalyze further crystal formation causing spikes, clogs and other problems.
  - Best bet is to keep polymer in system and not remove or change block until polymer is used up.

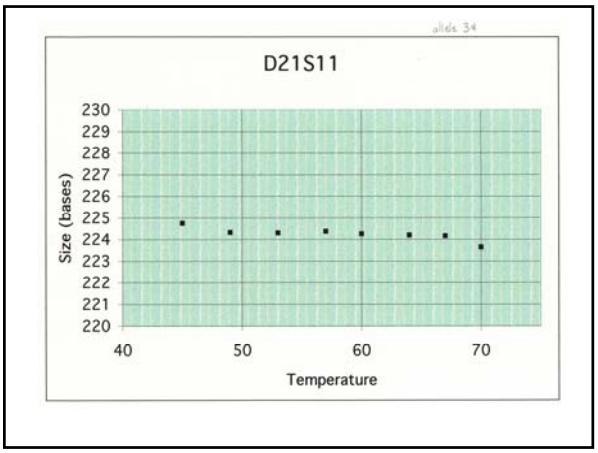
Effect of temperature on allele size



Slope is 0.14 bases/degree centigrade
Therefore a small change in temperature has a big effect
(A 1-2 degree shift in temperature of the heat plate can produce an OL allele)

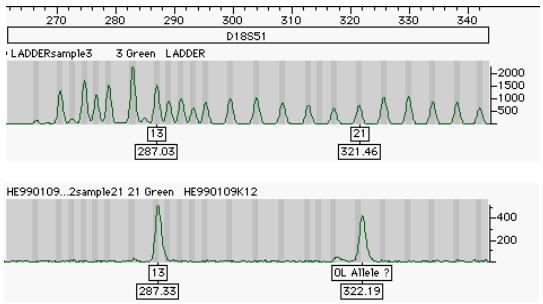
Hartzell, B., et al. (2003). Response of short tandem repeat systems to temperature and sizing methods. *Forensic Science International, 133,* 228-234.



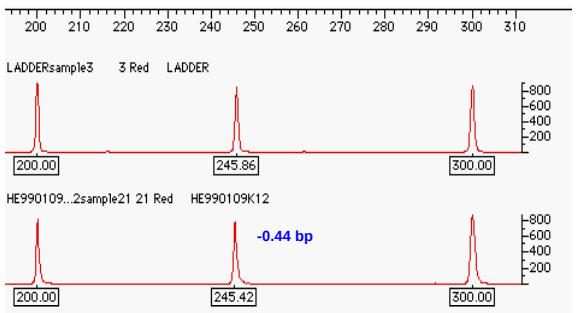


Temperature Effects

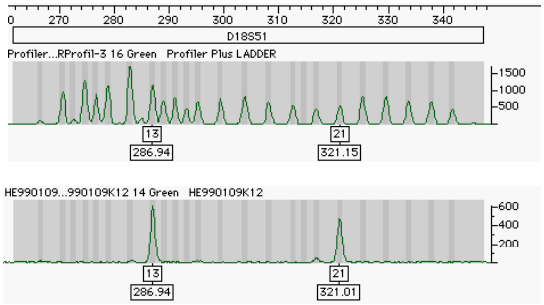
Off-Ladder "OL Alleles"



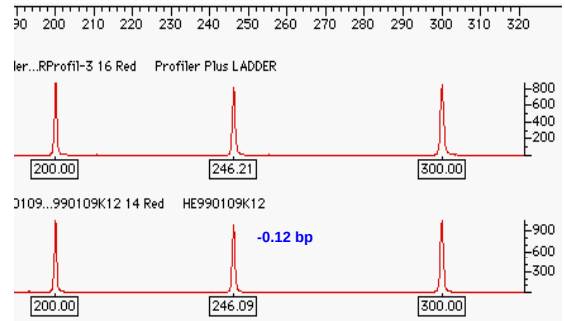
"OL alleles " - look at the 250 peak



"OL allele re-injected"



And the 250 peak...



Monitoring Room Temperature Over Time

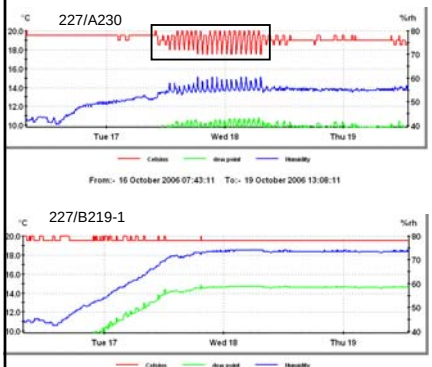
Temperature Probes

Refrigerator and freezer monitoring

Frig/Freeze Monitors $240
#DT-23-33-80 – USB Temperature Datalogger
PLUS Software $79.00 (#DT-23-33-60)
Room Monitors, # DT-23039-52 – USB Temperature-Humidity Datalogger $91.00
(Cole Parmer, Vernon Hills IL)

Room temperature monitoring

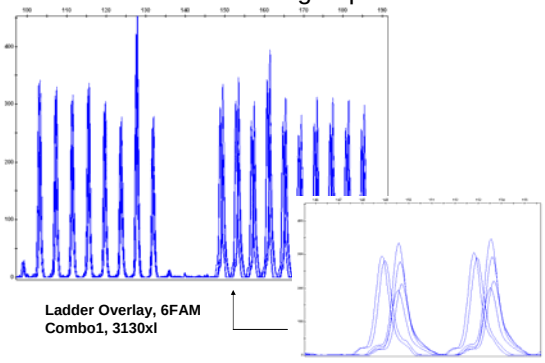
Monitoring Instrument Room Temperature Fluctuations



Temperature Monitoring of two separate instrument rooms.

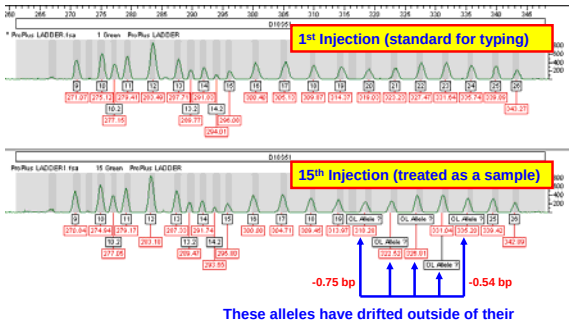
Box area is a 24 hour period where temperature control is not stable.

Poor Temperature Control Causes DNA Sizing Imprecision



Ladder Overlay, 6FAM Combo1, 3130xl

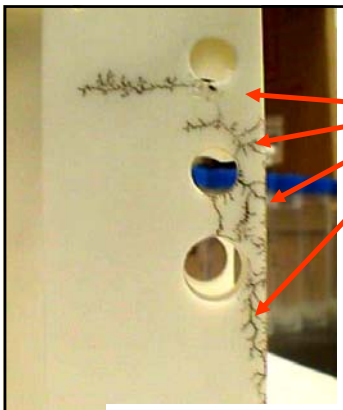
Use of Second Allelic Ladder to Monitor Potential Match Criteria Problems



These alleles have drifted outside of their genotyping bins due to temperature shifting over the course of the sample batch

Cleanliness

- Urea sublimates and breaks down to ionic components - these find a path to ground
- Similarly wet buffer under a vial creates paths to ground
- Capillary windows must be clear or matrix effects will occur
- Laser will often assist in this process
- Vial caps will transfer low levels of DNA to capillary

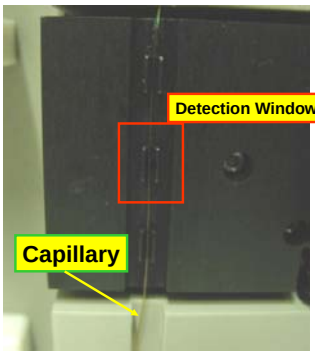


Keep Your System Clean!

Instrumental Factors

- Optical System
  - Sensitivity changes with age, capillary diameter, capillary cleanliness, instrument calibration
- Fluidic System
  - Effects of bubbles, dust, urea crystals, leaks in syringe and capillary ferrule
- Matrix Calculations
  - Changes in buffer, optics, sample dye can alter the software calibrations
- Capillary Problems
  - Chemisorbed materials on capillary surface can produce osmotic flow, DNA band broadening and inconsistent resolution (meltdowns)

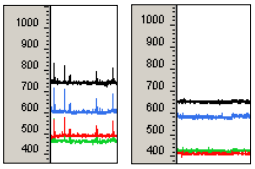
The Detection Window



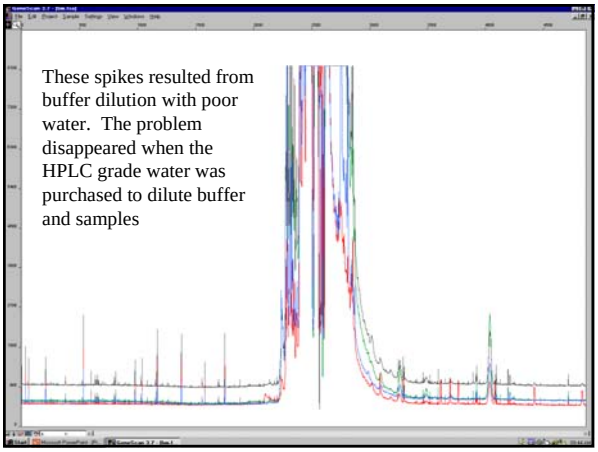
Make sure that the capillary window is lined up (if it is not, then no peaks will be seen)

Window may need to be cleaned with ethanol or methanol

Review Start of Raw Data Collection

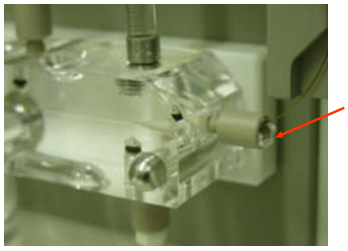


Little spikes indicate need to change buffer… check current



These spikes resulted from buffer dilution with poor water. The problem disappeared when the HPLC grade water was purchased to dilute buffer and samples

Beware of Urea Crystals



Urea crystals have formed due to a small leak where the capillary comes into the pump block

Urea sublimates and can evaporate to appear elsewhere

Use a small balloon to better grip the ferrule and keep it tight

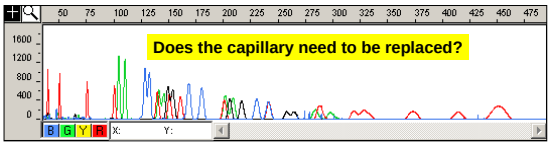
Pump block should be well cleaned to avoid problems with urea crystal formation

Buffer Issues

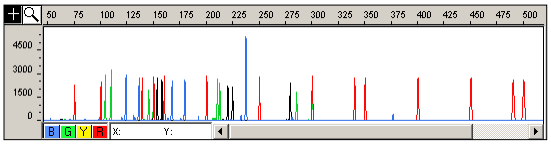
- The buffer and polymer affect the background fluorescence- affecting the matrix
- Urea crystals and dust may produce spikes
- High salt concentrations may produce reannealing of DNA
- High salt concentrations affect current
- Low polymer concentrations affect peak resolution

Meltdowns can be permanent or transitory

as we have seen these may result from sample contamination effects



No! The next injection looks fine…



Meltdowns may be the result of

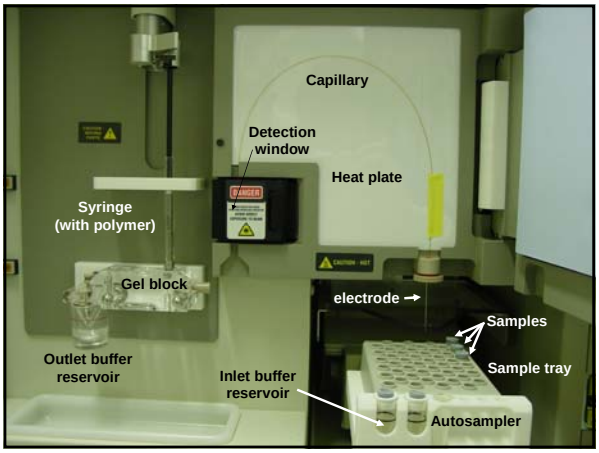
- Bad formamide
- Excess salt in sample/renaturation
- Water in the polymer buffer
- **Syringe leak** or bottom out
- Poisoned capillary
- Conductive polymer buffer due to urea degradation
- Crack/shift in capillary window
- Detergents and metal ions

Troubleshooting benchmarks

- **Monitor run current**
- Observe syringe position and movement during a batch
- Examine ILS (ROX) peak height with no sample
- Observe "250 bp" peak in GS500 size standard
- Monitor resolution of TH01 9.3/10 in allelic ladder and size standard peak shapes
- **Keep an eye on the baseline signal/noise**
- Measure formamide conductivity
- Reagent blank – are any dye blobs present?
- See if positive control DNA is producing typical peak heights (along with the correct genotype)

Measurement of Current

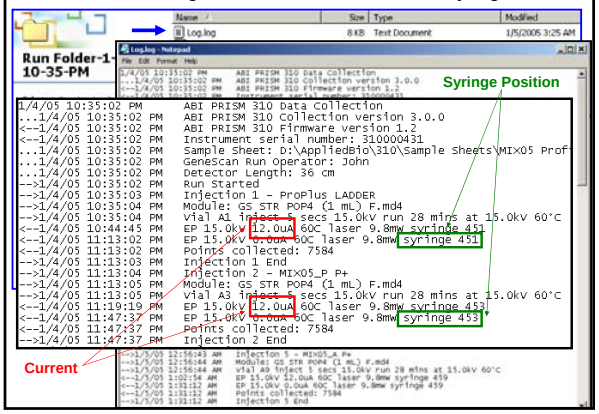
- V/I = R where R is a function of capillary diameter, [buffer], and buffer viscosity
- In a CE system the voltage is fixed, thus changes in resistance in the capillary will be reflected in the current observed
- Air bubbles, syringe leaks, alternate paths to ground, changes in temperature, changes in zeta potential, and contamination, will be reflected in the current
- A typical current for a CE system with POP4 buffer is **8-12 µA** (microamps)



Syringe Travel

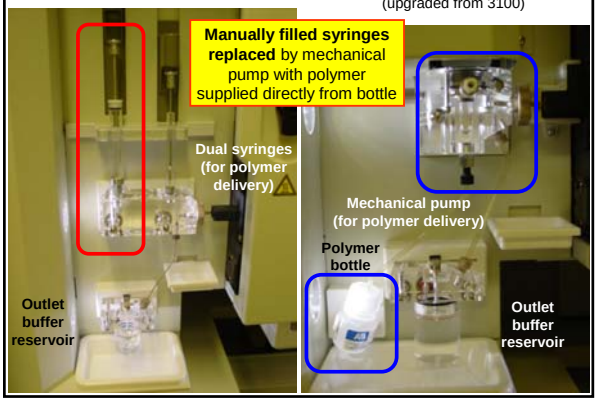
- The ABI 310 instrument also keeps track of the position of the syringe (in the log file)
- Depending on the resistance to flow, the syringe will travel different lengths
- Syringe leaks may be reflected in a longer distance traveled prior to each injection
- These leaks occur around the barrel of the syringe and at the connection to the capillary block

Use of ABI 310 Log File to Monitor Current and Syringe Travel

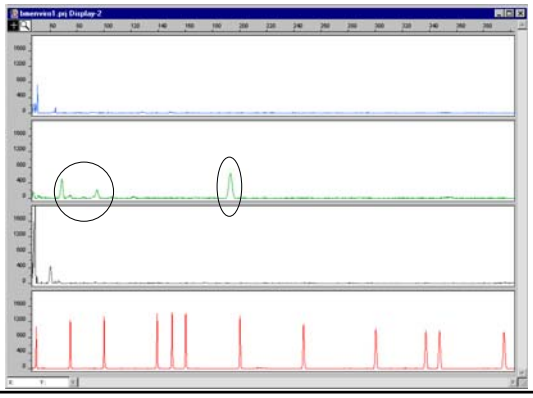


ABI 3100 / ABI 3130xl

ABI 3130xl (upgraded from 3100)



Dye Blobs in the Negative Control Sample



Measuring Formamide Conductivity

(not this way)

The key is to measure the bottle when it comes in or buy the good stuff and immediately pipette it out into small tubes with or without ROX already added. Then freeze the tubes.

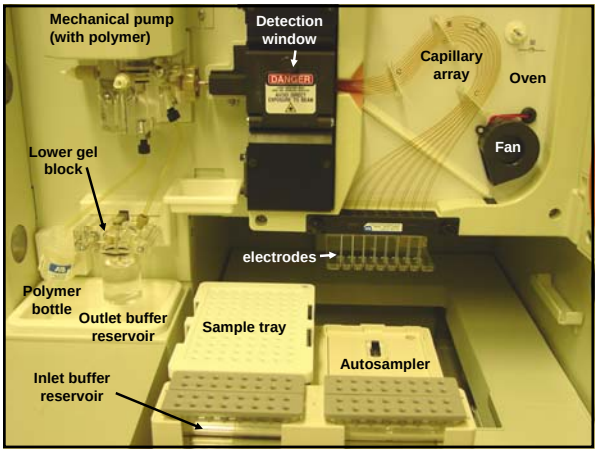
Do not ever open a cold bottle of formamide. Water will condense inside and aid in the formation of conductive formic acid.

Conclusion:

Troubleshooting is more than following the protocols

It means keeping watch on all aspects of the operation

1. Monitoring conductivity of sample and formamide
2. Keeping track of current and syringe position in log.
3. Watching the laser current
4. Watching and listening for voltage spikes
5. Monitoring room temperature and humidity



Topics in Forensic DNA Analysis & Interpretation

ABI 3500
Genetic Analyzer

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

Presentation Outline

- ABI 31xx series retirement
- ABI 3500 features and concerns
- Open letter to Applied Biosystems
- NIST early validation experiments with ABI 3500 instrument

ABI 31xx Retirement

No ABI 3100/3100 Avant instruments after Dec 31, 2011
No more sales of ABI 3130/3130xl after June 30, 2011

Angela Wang
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Angela.Wang@lifetech.com

November 18, 2010

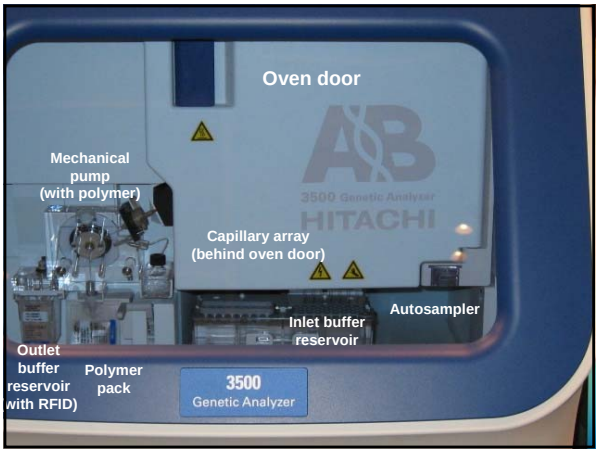
Dear Valued Customer:

We are pleased to be your partner in performing human identification testing with validated, industry-leading Capillary Electrophoresis instrumentation from Applied Biosystems. In order to help plan for your laboratory's future needs, we wish to inform you that Applied Biosystems has planned the retirement of the 3100 Avant and 3100 Capillary Electrophoresis instruments ("3100 System") effective **December 31, 2011**.

Please note that the 3130/3130xl Genetic Analyzers for Human Identification will no longer be available for upgrade or sale by June 30, 2011. However, we intend to use commercially reasonable efforts to support current 3130 Genetic Analyzer HID users with related software, reagents and consumables, as well as maintenance and repair service and technical and applications support for a minimum of five years from the date of discontinuance. Please note that the level of support we are able to provide may be limited by circumstances beyond our reasonable control, including but not limited to our inability to source supply. If any changes to the level of support we can offer should be necessary, you will be notified at that time.

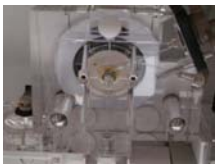
ABI 3500

- With the upcoming retirement of the 31xx series, the entire community will be forced to go to the 3500 series
- How will this impact validation and interpretation guidelines?
- How many labs have experience with the 3500? Are any on-line doing casework?

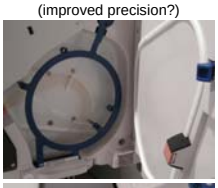


Details of the new ABI 3500

No lower pump block
(less polymer waste)



Improved sealing for better temperature control
(improved precision?)



ABI 3500 Evaluation

- ***NIJ will likely be requested to use federal grants to supply state and local labs with this new instrument***
- ***It needs to be assessed for advantages and costs***

New Features of the ABI 3500 CE

- an improved polymer delivery pump design,
- ready-to-use consumables and containers,
- Radio Frequency Identification (RFID) consumable tracking,
- quality control software features for rapid identification and re-injection of failed samples,
- increased throughput,
- new laser technology,
- reduced power requirements,
- peak height normalization,
- intuitive user software, and integrated primary analysis software,
- improved peak height uniformity across capillaries, runs and instruments
- **6-dye channel capability**

DNA Community Moving to ABI 3500s

Advantages

- Smaller footprint and 110V power requirement
- Better polymer delivery and temperature control
  - Improved success rates?
- New capabilities
  - between instrument normalization
  - 6-dye detection (bigger kits with more loci)
- Simpler software

Disadvantages

- Up-front cost of new instruments
  - Federal government (NIJ) will likely be expected to foot the bill
- Generates .hid files
  - Requires new analysis software
- Validation down-time
  - New RFU thresholds
- Higher per run cost with RFID tags & limited expiration
  - many labs cannot purchase reagents rapidly throughout the year
- Creating technicians not scientists
  - Plug and play approach leading to loss of understanding for process
  - Less flexible *(impacts research with it)*

Cost for the Forensic DNA Community to Switch from ABI 3100s to 3500s

1. **Instrument up-front cost**
   - Will likely be requested from federal grant funds (NIJ)
2. **New software purchase**
   - Will likely be requested from federal grant funds (NIJ)
   - new .hid file format will not work on current software (GMIDv3.2)
   - 3500 will not create .fsa files with 36cm arrays (HID applications)
3. **Validation time & expense**
   - Relative fluorescent scales are completely different…
4. **Operational cost**
   - ABI claims that the running costs are equivalent to 3130s…

Consumable Costs for the ABI 3500

POP polymer pack with RFID tag

$180 (384 samples)
$455 (960 samples)

Buffer pack with RFID tag

$60 ($25+$35)

8-capillary array

$1200 (160 injections)

"Expires" after 1-week on the instrument

Thus, if you run 1 sample or 960 samples (or 384) in that week, the consumable cost will be the same…

Likely Cost Increase… and Backlog Increase?

- ABI 3500 reagents are RFID-tagged and made to work under very limited time windows (e.g., 1 week expiration for the polymer)
- If a lab is not running at full capacity, reagents will expire and add to the true cost of performing forensic DNA testing (i.e., can be a similar total cost whether running a few or a few hundred samples)
- Casework **throughput efficiencies are best when small batches are run frequently** – **to save money, will labs store samples to amass enough for a busy week of running samples through the 3500 instrument?**

Cost Comparison

ABI 3130xl (current)

- **16-capillary array**
  - $898 (for 100+ injections)
- **POP4 polymer**
  - $468 (1760 samples)
  - "Expires" after 3 months
  - **$0.27 per sample**
- **Buffer**
  - $5 (for one week)

ABI 3500 (new/future)

- **8-capillary array**
  - $1200 (for 160 injections)
- **POP4 polymer**
  - $180 (384 samples) or $455 (960 samples)
  - "Expires" after 1 week
  - **$0.47 per sample**
- **Buffer**
  - $60 (25+35) for one week

ABI 3500 Reagent Costs for 90 samples

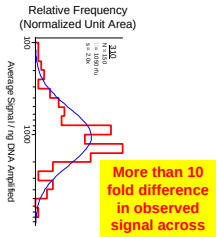
(90 samples +6 controls: allelic ladders, positive, negative**)**

	Quantity Provided	Total Cost	
Capillary array	1 array of 8-capillaries (36 cm)	$90	12 injections on array
3500 POP-4 polymer	384 sample pouch ($180)	$45	~4 plates per pouch
ABC Buffer	4 pack ($100)	$3	assume 2 plates/day
CBC Buffer	4 pack ($140)	$4	assume 2 plates/day
Formamide, Hi-Di	25 mL (for $25)	$1	1 mL used
Pipet tips	960 tips for $124	$14	106 tips used
96well plate	10 plates for $57 (not ABI)	$12	2 plates
Septa	20 septum	$16	1 septum
Identifiler matrix standards DS-33	6FAM, VIC, NED, PET, LIZ	$2	assume recalibration every 50 plates
GS500 LIZ size standard	800 tests/pk	$33	35 µL each plate
	Subtotal	$220	$2.44 per sample
Identifiler STR kit	200 tests/kit	$1,715	25 µL PCR (full reaction)
~90% of cost is the STR kit		$1,935	$21.50 per sample

Reduced Inter-Instrument Variability

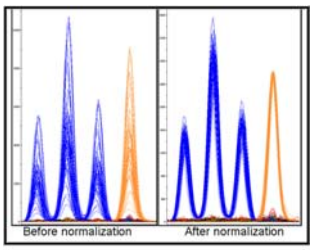
Through Normalization Function

150 different ABI 310 instruments
(2001 NIST interlab study)

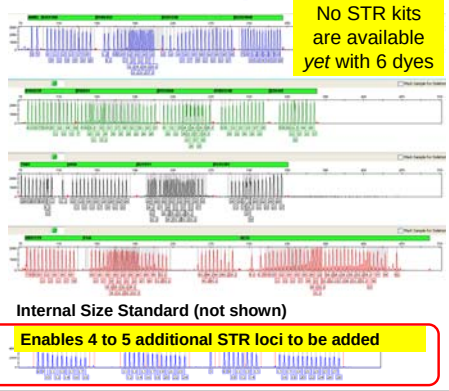


More than 10 fold difference in observed signal across instruments

3 different ABI 3500 instruments



6-dye Detection Capability



No STR kits are available *yet* with 6 dyes

Enables 4 to 5 additional STR loci to be added

Open Letter to Applied Biosystems on Concerns with ABI 3500

- **3/14/11 - emailed ~900 forensic DNA scientists** (SWGDAM, forens-dna, ENFSI, EDNAP) inviting them to sign onto a letter that will be sent to Applied Biosystems expressing concern with ABI 3500
- **Very positive response and over 50 have already agreed to sign the letter**
- Letter to be sent March 31 to president of ABI and scientists involved with ABI 3500
- **Community will be notified of ABI's response**

Concerns Expressed in Open Letter

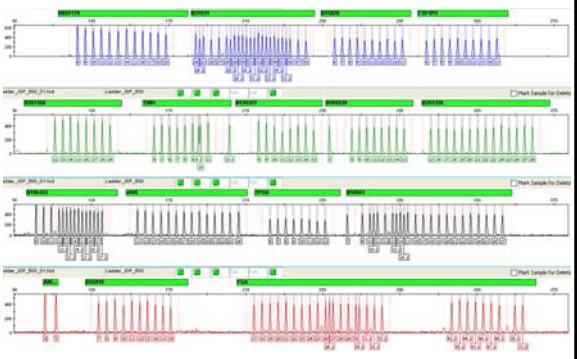
- RFID tags
- New .hid file structure requires new software
- Short shelf life of reagents

Hopefully a change will result…

Work performed by
Erica Butts and Becky Hill (NIST)

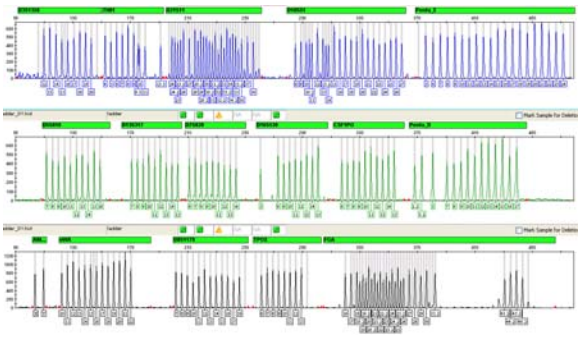
Some Data from ABI 3500 (NIST) and ABI 3500xl (AFDIL) Instruments

Identifiler Plus Ladder on AFDIL 3500*xl*



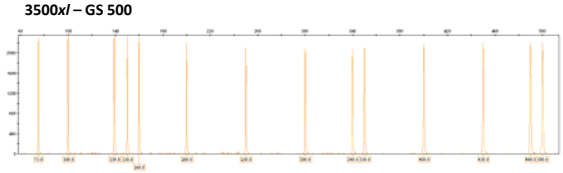
Excellent peak height balance, uniform peak heights between dye channels

PP16HS Ladder on AFDIL 3500*xl*

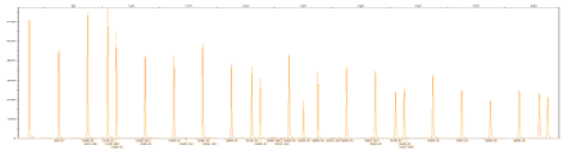


Different Size Standards (3500*xl*)

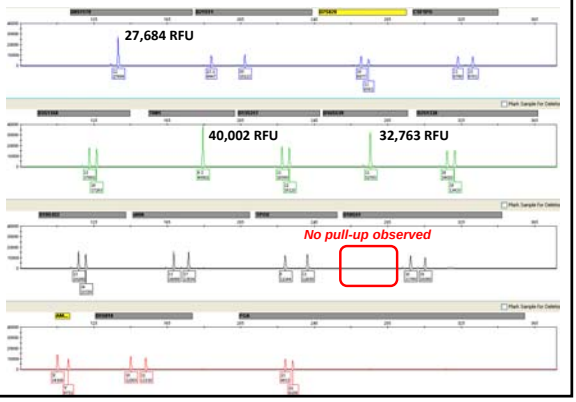
3500*xl* – GS 500



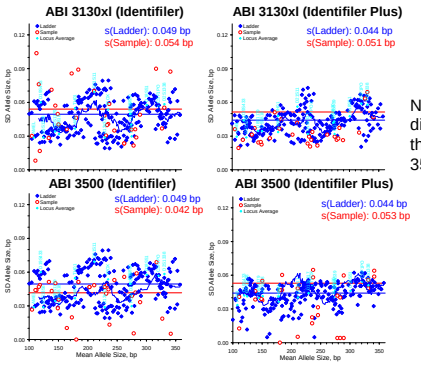
3500*xl* – GS 600 v2



3500xl Low Injection, GS 600 v2



Precision



No significant difference between the 3130*xl* and 3500 for precision

n=16 ladders
n=6 samples

Summary

- The ABI 3500 and 3500xl instruments work fine for Applied Biosystems and Promega STR kits.
- The 3500 series instruments offer some improved capabilities for inter-instrument normalization and 6-dye detection. **Only time will tell how helpful these capabilities are…**
- The cost for the forensic DNA community to switch from ABI 3130xl vs ABI 3500/3500xl instruments will involve more than just the initial purchase price – **reagents are expensive**.

Topics in Forensic DNA Analysis & Interpretation

Low Level DNA Issues

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

Some Definitions of Low Template (LT) DNA

- Working with **<100-200 pg genomic DNA**
- Considered to be data below stochastic threshold level where PCR amplification is not as reliable (determined by each laboratory; typically 150-250 RFUs)
- Enhancing the sensitivity of detection (increasing PCR cycles, PCR product clean-up, increasing CE injection/voltage)
- Having too few copies of DNA template to ensure reliable PCR amplification (allelic or full locus drop-out)
- Can often be the minor component of mixture samples consisting of low level DNA template amounts

Low Template DNA situations exist in many samples

- In a 1:1 mixture, each DNA source is LT when the total amount of DNA in the amplification reaction is ~ 0.125 ng.
- In a 1:9 mixture, the minor component could be LT **even when the total amount of DNA in the amplification is 1 ng**.

Two different amplifications would be useful with a 1:9 mixture situation:
Normal level of total DNA (e.g., 1 ng) so that major component is on-scale
High level of total DNA (e.g., 5 ng) so that minor (e.g., ~500 pg) is out of LT realm – yes, the major component will be off-scale…

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

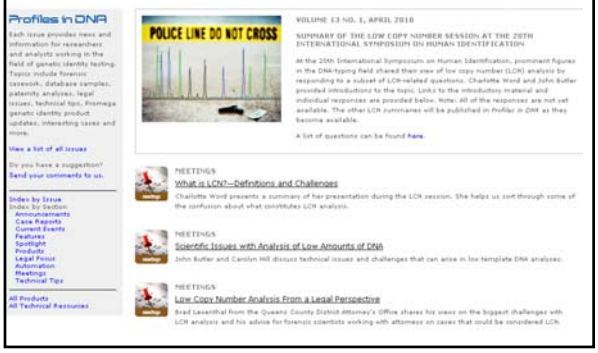
Recent LT-DNA Court Rulings

- "…a challenge to the validity of the method of analysing Low Template DNA by the LCN process should no longer be permitted at trials where the quantity of DNA analysed is above the stochastic threshold of 100-200 picograms…"
  - United Kingdom: Crown vs. Reed & Reed, Dec. 21, 2009
- LT-DNA testing is "…generally accepted as reliable in the forensic scientific community under the standard enunciated in Frye…"
  - NYC OCME: People vs. Megnath, Feb. 8, 2010
- "LCN DNA evidence is not inherently unreliable."
  - New Zealand: Crown vs. Wallace, Mar. 3, 2010

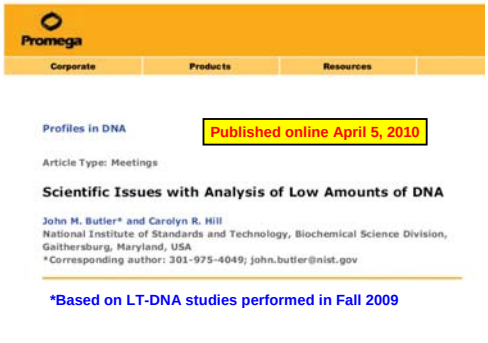
The judge in the Wallace case quotes from John Butler's *Fundamentals of Forensic DNA Typing* in drawing the court's conclusion

Profiles in DNA (April 2010)

http://www.promega.com/profiles/



Publication on Scientific Issues of LT-DNA



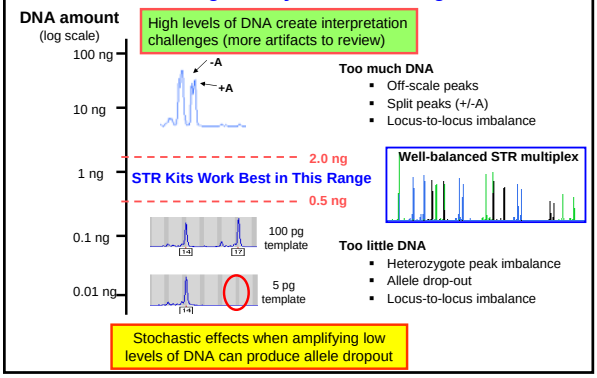
***Based on LT-DNA studies performed in Fall 2009**

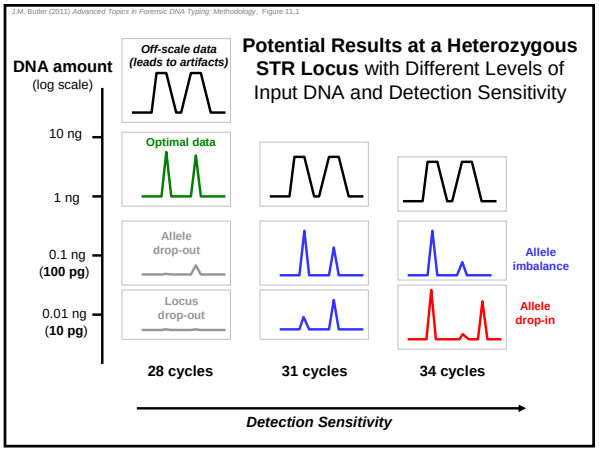
Summary and Overview of Issues

- **Faced with limited evidence that yield low amounts of DNA, forensic analysts will continually have to confront the question of how far to push DNA testing techniques.**
- Low level DNA testing, also known as low copy number (LCN) analysis or low template DNA (LT-DNA) testing, involves enhancing detection sensitivity usually through increasing the number of PCR cycles.
- Stochastic effects inherent with analysis of low amounts of DNA yield allele or locus drop-out.
- Additionally, increasing detection sensitivity can result in a greater potential for contamination or allele drop-in.

Impact of DNA Amount into Multiplex PCR Reaction

We generally aim for 0.5-2 ng





Framing the Issues

- Forensic science methods often **must work close to the edge** of a technique due to the limited nature of the evidence
  - perpetrators are usually not willing to go back and add more biological material to a crime scene…
- **Validation studies** are performed in order to **define the limits of a technique**
  - sensitivity studies to determine at what point a lab cannot obtain reliable results anymore

We would always like improved sensitivity to enable results where ever possible

"Enhanced Interrogation" Techniques to Improve Sensitivity

Are you "waterboarding" your DNA trying to get more information from the sample?

- **Increased PCR cycle number**
  With 100% efficiency:
  - 28 cycles = 67 million copies
  - 31 cycles = 1 billion copies (x16)
  - 34 cycles = 4 billion copies (x64)
- Reduced volume PCR
- Sample desalting (e.g., MinElute) prior to CE
- Extended CE injections

Requires validation to determine appropriate thresholds for reliability

Concern with How Methods Are Used…

"Enhanced Interrogation Techniques" Should Not Be Used for This Purpose

LCN testing should not be used for exculpatory purposes such as post-conviction testing due to potential of the LCN profile not being relevant to the case due to contamination

See J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 154

Low Template DNA Testing

- **Every lab faces samples with low template DNA**
  - Do you choose to attempt an "enhanced interrogation technique" such as increasing the cycle number, desalting samples, etc.?
  - **Next generation kits coming from manufacturers are capable of greater sensitivity – will they be misused without appropriate caution and validation?**
- **At what point do you draw a line and not attempt to analyze data below this line?**
  - A certain amount of input DNA (based on what data?)
  - A pre-determined stochastic threshold (based on what data?)

Comments on DNA Quantitation

- qPCR has enabled lower amounts of DNA to be quantified in recent years – providing in some cases a false sense of confidence in accuracy at these low levels
- Remember that **qPCR is also subject to stochastic effects** and thus DNA quantitation will be less accurate and exhibit more variation at the low end…
- **Next generation STR kits** with their greater sensitivity and ability to overcome inhibition **have the potential to make the current qPCR DNA quantitation kits obsolete as an appropriate gatekeeper** to whether or not to continue with a low level, compromised DNA sample

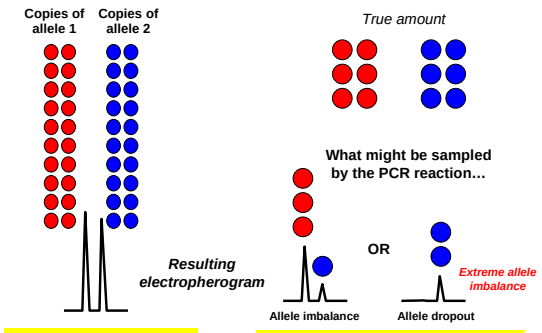
Stochastic = random selection

Stochastic Fluctuation Effects

- Unequal sampling of the two alleles present in a heterozygous individual can occur when low levels of input DNA are used (**results in allele drop-out**)
- Walsh *et al.* (1992) – proposed avoiding stochastic effect by adjusting the number of PCR cycles in an assay so that the sensitivity limit is around 20 or more copies of target DNA (i.e., a full profile is obtained with ~125 pg)

Walsh PS, Erlich HA, Higuchi R. Preferential PCR amplification of alleles: Mechanisms and solutions. *PCR Meth Appl* 1992; 1:241-250.

Stochastic Statistical Sampling

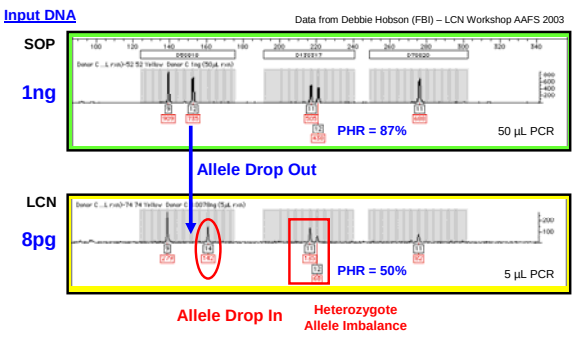


>20 copies per allele

6 copies per allele **(LT-DNA)**

Comparison of STR Kit Amplification SOP with LT-DNA

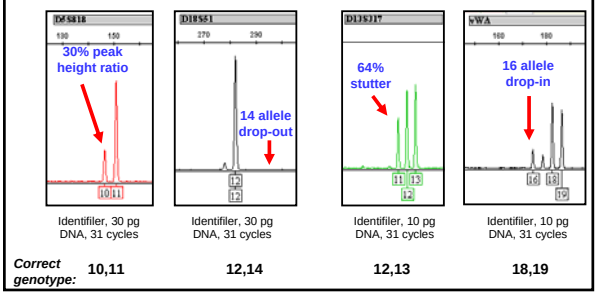
Using the Same DNA Donor



Stochastic (Random) Effects with LT-DNA

When Combined with Higher Sensitivity Techniques

*Loss of True Signal (**False Negative**)* | *Gain of False Signal (**False Positive**)*



Correct genotype: **10,11** **12,14** **12,13** **18,19**

Comparison of Approaches

Replicate Amplification with Consensus Profile

Low amount of DNA examined

Stochastic effects

What "LCN Labs" Are Doing

Amplification #1
Amplification #2
Amplification #3

Consensus Profile Developed
(from repeated alleles observed)

Interpretation Rules Applied
(based on validation experience)
e.g., specific loci may dropout more

Result can be and usually is Reliable & Reproducible

Single Amplification

Low amount of DNA examined

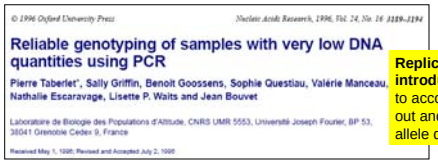
Stochastic effects

Amplification #1 (only a single test)

Result can be Unreliable

Individual results may vary but a consensus profile is reproducible (based on our experience with sensitivity studies and replicate amplifications)

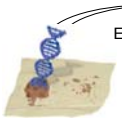
Early Work on Replicate Testing with Low Levels of DNA



Replicate testing introduced (up to 7 times) to account for allele drop-out and avoid miscalling allele drop-in

In conjunction with interpretation rules, duplication of observed alleles in replicates was shown to correctly define the original sample

Replicate Testing and Consensus Profiles



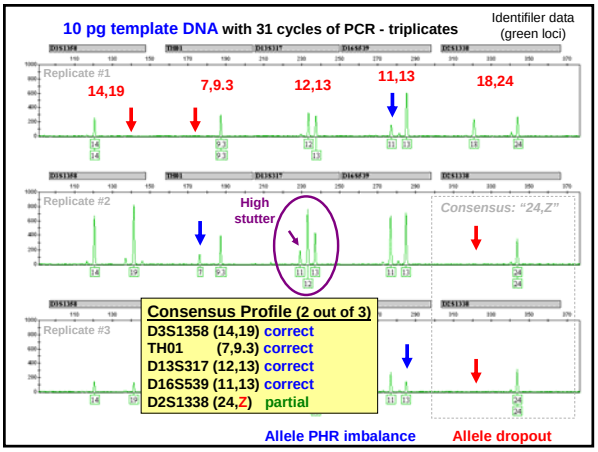
Extract DNA from stain

Quantify Amount of DNA Present

Perform 2 or 3 Separate PCR Amplifications

Interpret Alleles Present

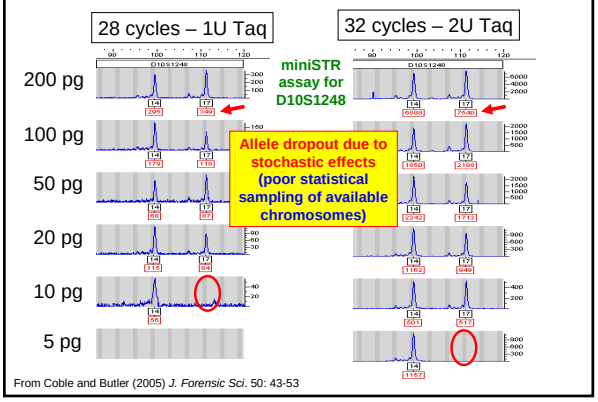
Develop a Consensus Profile
(based on replicate consistent results)



Impact of "Unreliable" Results

- Allele drop-out can be dealt with using moderate stringency searches in CODIS algorithms
  - a homozygote "14" would hit to a heterozygote "11,14"
- **Allele drop-in is most problematic for DNA database searches**
  - **this can be corrected for with replicate testing and consensus profiles to eliminate incorrect alleles**

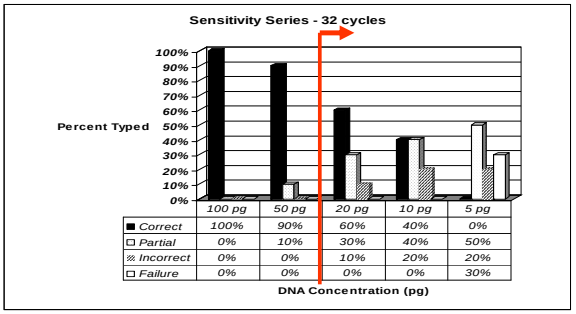
Higher Sensitivity with More Polymerase and Cycle Numbers



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

From John Butler May 3, 2006 MAAFS LCN Workshop presentation (Richmond, VA)
Available at http://www.cstl.nist.gov/biotech/strbase/pub_pres/LCNintro_MAAFSworkshop_May2006.pdf

Problems with Obtaining Correct Allele Calls at Low DNA Levels



	100 pg	50 pg	20 pg	10 pg	5 pg
Correct	100%	90%	60%	40%	0%
Partial	0%	10%	30%	40%	50%
Incorrect	0%	0%	10%	20%	20%
Failure	0%	0%	0%	0%	30%

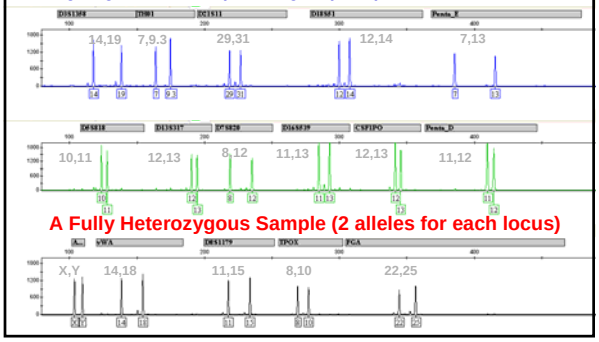
Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50: 43-53

Experimental Design to Study LT-DNA Issues

- Pristine DNA Samples
  - 2 single-source samples (and mixtures created from these)
  - **heterozygous for all loci tested** (permits peak height ratio studies)
- **Low DNA Temple Amounts**
  - Dilutions made after DNA quantitation against NIST SRM 2372
  - **100 pg, 30 pg, and 10 pg** (1 ng tested for comparison purposes)
- Replicates
  - **10 separate PCR reactions** for each sample
- STR Kits
  - **Identifiler and PowerPlex 16 HS** (half-reactions)
- **Increased Cycle Number**
  - Identifiler (**31 cycles**; 28 for 1 ng)
  - PowerPlex 16 HS (**31 cycles and 34 cycles**; 30 for 1 ng)

PowerPlex 16 HS (½ Reaction) 1 ng @ 30 cycles

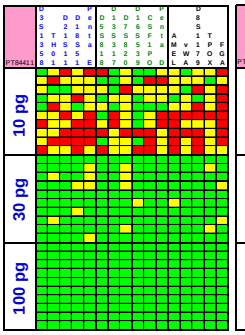
High signal, balanced peak heights (>0.80), no artifacts, low stutter



A Fully Heterozygous Sample (2 alleles for each locus)

Sensitivity & Performance PowerPlex 16 HS

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in



Results broken down by locus

10 replicates of each [DNA]

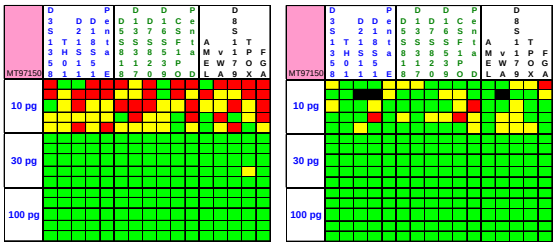
10 pg (~2 cells)
30 pg (~6 cells)
100 pg (~18 cells)

Full (correct) profiles observed in all replicates at 100 pg

31 Cycles **34 Cycles**

Sensitivity & Performance PowerPlex 16 HS

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in



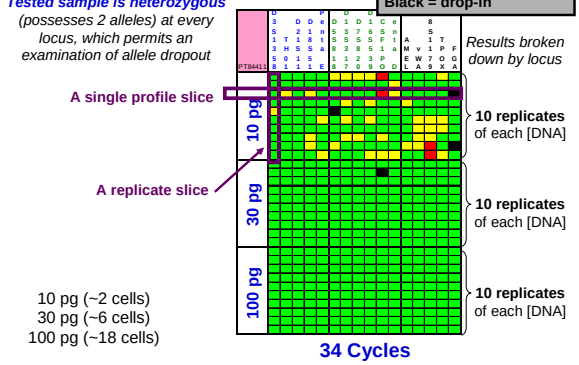
31 Cycles **34 Cycles**

At 10 pg level: 16% vs. 60% full profiles
73% improvement with 3 extra cycles

Sensitivity Comparison

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in

Tested sample is heterozygous *(possesses 2 alleles) at every locus, which permits an examination of allele dropout*



Results broken down by locus

10 replicates of each [DNA]

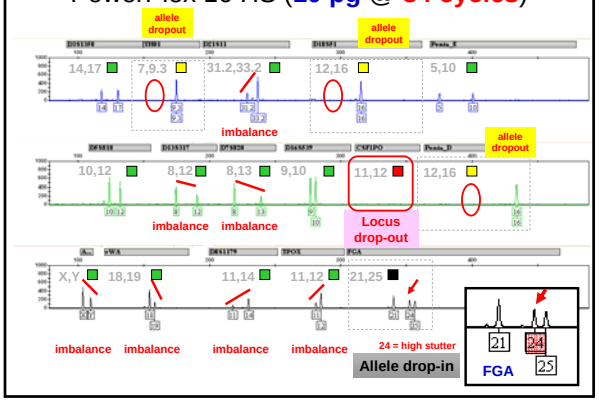
10 replicates of each [DNA]

10 replicates of each [DNA]

10 pg (~2 cells)
30 pg (~6 cells)
100 pg (~18 cells)

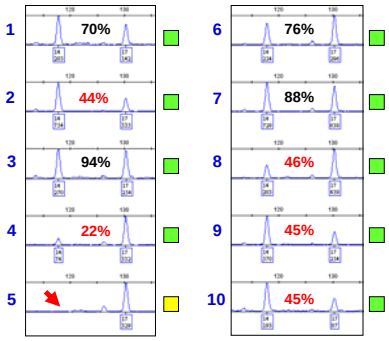
34 Cycles

PowerPlex 16 HS (**10 pg** @ **34 cycles**)



D3S1358 Replicates

PowerPlex 16 HS (**10 pg** @ 34 cycles)



A Few Observations:
Locus-dependent performance

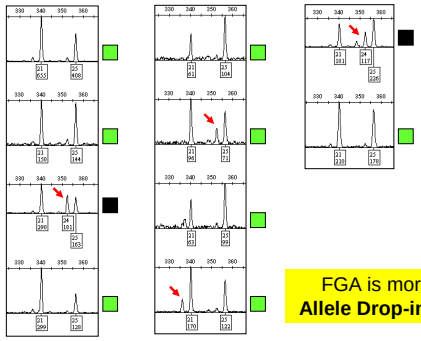
Calling alleles as only those above detection threshold of 50 RFU

Heterozygote balance is many times <60%

From any grouping of three, the correct consensus profile of 14,17 would be made

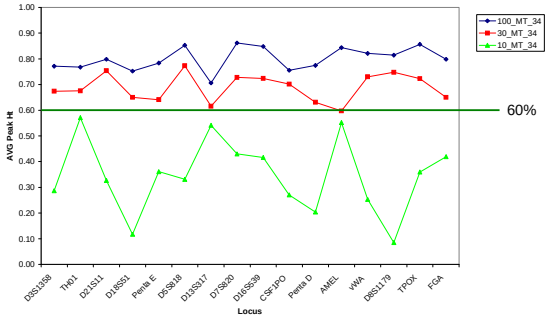
FGA Replicates

PowerPlex 16 HS (**10 pg** @ 34 cycles)

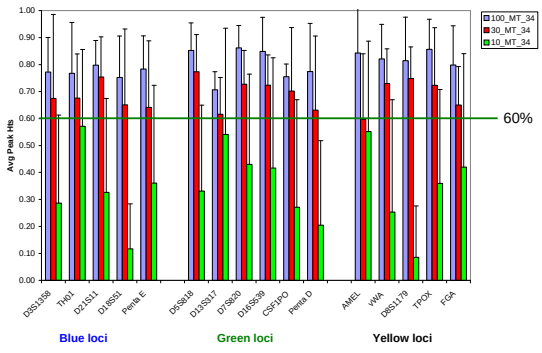


FGA is more prone to **Allele Drop-in** from stutter

Peak Height Ratio: PP16HS, 34 cyc



Peak Height Ratio: PP16HS, 34 cyc

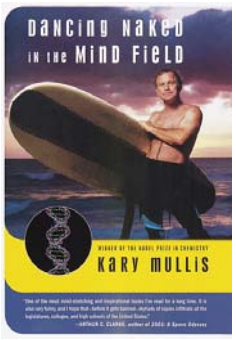


Summary of Data Observed at NIST

- **Increasing the cycle number creates a higher number of full profiles** (note: at both 31 and 34 cycles, 100 pg results were all correct with PowerPlex 16 HS)
- **Across any grouping of 3 replicates, there was never an instance of an incorrect allele being called when two of three replicates matched**
- Certain loci are more prone to allele and locus drop-out (depends on kit and PCR product sizes)

KNOW YOUR SYSTEM THROUGH VALIDATION STUDIES!

Kary Mullis – Inventor of PCR



"If it works, fine; if it works again, even better!"

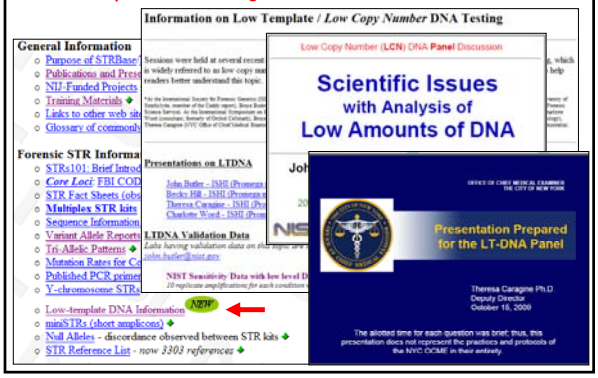
-DTRA Talk 9/30/09

Section of STRBase on LT-DNA

- Recently launched webpage
  - **http://www.cstl.nist.gov/biotech/strbase/LTDNA.htm**
  - Low-template DNA = LT-DNA
- The LT-DNA section includes:
  - **Presentations from past LT-DNA talks and workshops**
  - **Validation data from our sensitivity studies** to illustrate problems and consensus profile solution to low levels of DNA testing
  - **Literature listing of pertinent articles** to help explain the issues involved in this topic

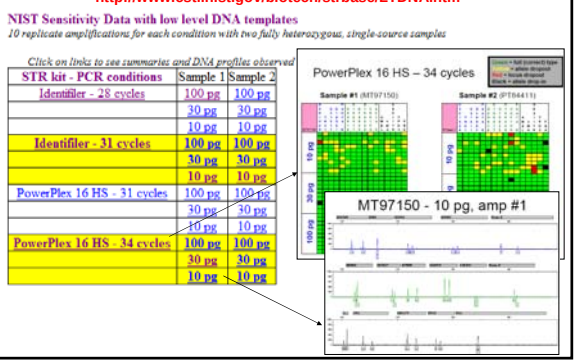
STRBase Website on LT-DNA (LCN)

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



Complete Set of NIST Sensitivity Data Available on New LT-DNA Website

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

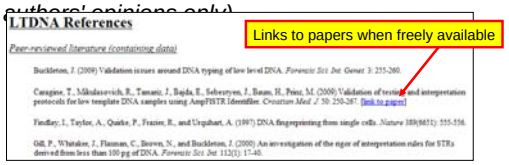


Literature Listing on LT-DNA (LCN)

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

Subdivided into categories

- Peer-reviewed literature *(containing data)*
- Reports *(evaluating the methodology)*
- Review articles *(commenting on other's data)*
- Non-peer reviewed literature *(representing the authors' opinions only)*



Discussion of Submitted Questions

- **Submitted Question #5**
  How would you set a cut-off for qPCR kits?
- **Submitted Question #6**
  What would you consider as LCN or low template (LT-DNA) analysis?

Topics in Forensic DNA Analysis & Interpretation

Validation Discussion

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

NIST

Presentation Outline

- Validation principles
- SWGDAM Revised Validation Guidelines
- Setting thresholds (some ideas)
  - analytical threshold
  - stochastic threshold

Examples from recent ABI 3500 studies at NIST

- **Topics of interest to your labs**

There are no cook books for validation!

Topics in Forensic DNA Analysis & Interpretation

Future Directions of the Field

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

Presentation Overview

1. Additional core STR loci
2. Familial searching
3. Rapid DNA testing & portable devices
4. Phenotyping & biogeographical ancestry
5. Other technologies for DNA analysis
   - Mass spectrometry
   - Next-generation DNA sequencing
   - Expert systems for data interpretation

Additional STR Loci

- Will be needed for more complex kinship analyses and extended applications
  - Example: Y-STRs needed for familial searching
- Immigration testing needs more than 13 STRs (a Sept 2007 article called for 25 loci)
- **Larger DNA databases will require more loci to avoid adventitious matches**

Growth in Numbers of DNA Profiles Present in Various NDIS Indices

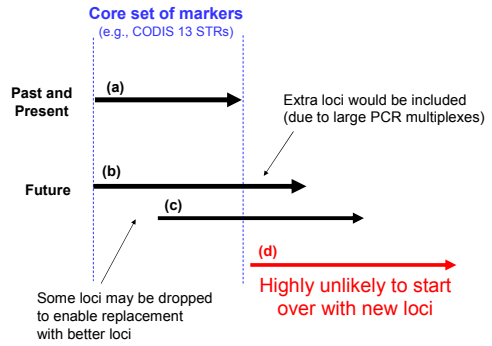
(cumulative totals by year)

Year ending Dec 31	Forensic	Convicted Offender	Arrestee	Total Offender*
2000	21,625	441,181	--	441,181
2001	27,897	750,929	--	750,929
2002	46,177	1,247,163	--	1,247,163
2003				
2004				
2005				
2006				
2007				
2008	248,943	6,398,874	140,719	6,539,919
2009	298,369	7,389,917	351,926	7,743,329
2010	**351,951**	8,559,841	668,849	**9,233,554**

In the last two years (2009, 2010):
103,008 forensic samples added
2,693,635 offender samples added

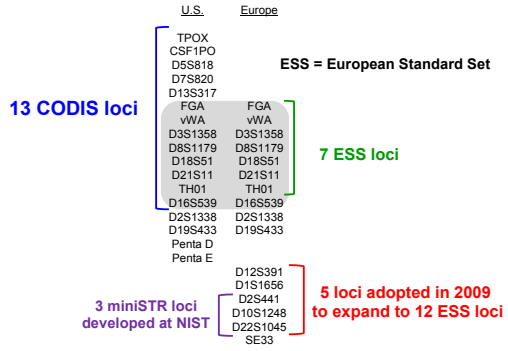
Source: FBI Laboratory's CODIS Unit

Possible scenarios for extending sets of genetic markers to be used in national DNA databases



23 Autosomal STR Markers

Present in Commercial STR Multiplex Kits



Commercially Available STR Kits

Applied Biosystems (17)
- ~~AmpFISTR Blue (1996)~~
- ~~AmpFISTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- Yfiler (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- **Identifiler Direct** (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)

Promega Corporation (13)
- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- PowerPlex Y (2003)
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)
- PowerPlex 18D (2011)

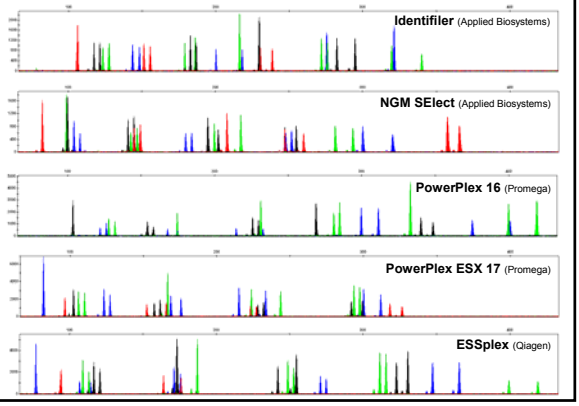
Qiagen (10) kits in 2010

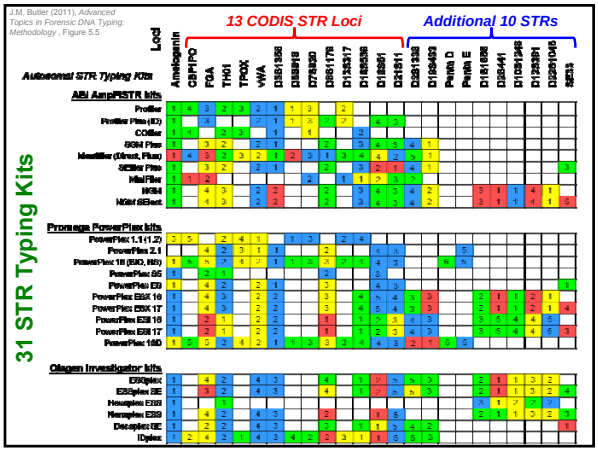
Primarily selling kits in Europe
Due to patent restrictions cannot sell in U.S.

Investigator kits
- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HDplex
- Triplex AFS QS
- Triplex DSF
- Argus X-12

~1/3 of all STR kits were released in the last two years

Same DNA Sample Tested with Five STR Kits





The 10 STR Loci Beyond the CODIS 13

STR Locus	Location	Repeat Motif	Allele Range*	# Alleles*
D2S1338	2q35	TGCC/TTCC	10 to 31	40
D19S433	19q12	AAGG/TAGG	5.2 to 20	36
Penta D	21q22.3	AAAGA	1.1 to 19	50
Penta E	15q26.2	AAAGA	5 to 32	53
D1S1656	1q42	TAGA	8 to 20.3	25
D12S391	12p13.2	AGAT/AGAC	13 to 27.2	52
D2S441	2p14	TCTA/TCAA	8 to 17	22
D10S1248	10q26.3	GGAA	7 to 19	13
D22S1045	22q12.3	ATT	7 to 20	14
SE33	6q14	AAAG‡	3 to 49	**178**

5 new European loci

**Allele range and number of observed alleles from Appendix 1, J.M. Butler (2011) Advanced Topics in Forensic DNA Typing: Methodology; ‡SE33 alleles have complex repeat structure*

Loci sorted on Probability of Identity (P_I) values

STR Locus	Alleles Observed	Genotypes Observed	Het. (obs)	P_I value N = 938
SE33	53	292	0.9360	0.0069
Penta E*	20	114	0.8799	0.0177
D2S1338	13	68	0.8785	0.0219
D1S1656	15	92	0.8934	0.0220
D18S51	21	91	0.8689	0.0256
D12S391	23	110	0.8795	0.0257
FGA	26	93	0.8742	0.0299
Penta D*	16	71	0.8754	0.0356
D21S11	25	81	0.8358	0.0410
D19S433	16	76	0.8124	0.0561
D8S1179	11	45	0.7878	0.0582
vWA	11	38	0.8060	0.0622
D7S820	11	32	0.8070	0.0734
TH01	8	24	0.7580	0.0784
D16S539	9	28	0.7825	0.0784
D13S317	8	29	0.7655	0.0812
D10S1248	12	39	0.7825	0.0837
D2S441	14	41	0.7772	0.0855
D3S1358	11	30	0.7569	0.0873
D22S1045	11	42	0.7697	0.0933
CSF1PO	9	30	0.7537	0.1071
D5S818	9	34	0.7164	0.1192
TPOX	9	28	0.6983	0.1283

23 STR Loci present in STR kits rank ordered by their variability

Better for mixtures (more alleles seen)

There are several loci more polymorphic than the **current CODIS 13 STRs**

Better for kinship (low mutation rate)

Random Match Probability for Various Combinations (assuming unrelated individuals)

STR Marker Combinations	RMP*	1 in …
13 CODIS STRs	**6.0E-16**	**1.7E+15**
15 STRs (+D2S1338, D19S433)	7.3E-19	1.4E+18
18 STRs (+D2S441, D10S1248, D22S1045)	4.9E-22	2.0E+21
20 STRs (+D1S1656, D12S391)	2.8E-25	3.6E+24
23 STRs (+SE33, Penta D, Penta E)	**1.2E-30**	**8.4E+29**

14 orders of magnitude improvement

**RMP values calculated by combining Probability of Identity values for each locus*

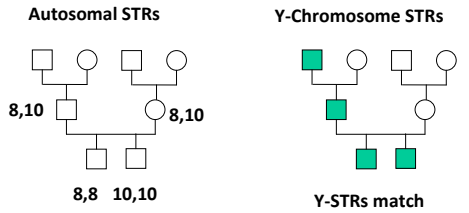
Summary: Additional STR Loci

- Additional autosomal STR loci exist in new STR kits and are being studied at NIST in U.S. population sample sets
- To avoid potential adventitious matches with large DNA databases, enable greater international data sharing, and aid missing persons applications, it is highly likely that **additional loci will be added to the U.S. core in the future**

Familial Searching

- Search unknown evidence profile against offender database to identify a close relative
- No suspect cases, cold cases, violent crimes
- Success in the United Kingdom
  - 2004-2010: 176 submitted, 131 searches, 35 successes
- Recent familial searching programs in the U.S.
  - Colorado (all forensic unknowns, 19 leads, 1 conviction)
  - **California** (**14 searches, 2 arrests**)
- Combining autosomal STR results with Y-STR information helps

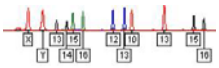
Why Y-STRs Are Needed for Familial Searching



For brothers, autosomal STRs may not match at a locus (or even share a single allele)

December 14, 2003

"We got him!"



DNA Profile

Saddam was known to have many "stunt doubles" that acted as decoys for his own safety

Saddam Hussein's capture was verified with DNA testing conducted in Rockville, MD at the **Armed Forces DNA Identification Laboratory**

Source: www.cnn.com; *The Scientist* Dec 19, 2003

Biological Relatives Served as References

Captured December 13, 2003

Matching Y-STR Haplotype Used to Confirm Identity

(along with allele sharing from autosomal STRs)

Uday and Qusay Hussein

Is this man really Sadaam Hussein?

Killed July 22, 2003

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Box 23.1, p. 534

CRIME & COURTS July 7, 2010

Arrest Made in L.A. 'Grim Sleeper' Killings

Published July 07, 2010 | Associated Press

Print Email Share Comments (0) Recommend 42 Text Size

LOS ANGELES -- A one-time police mechanic was arrested and charged Wednesday in the serial killing of 10 people over 25 years after a DNA sample from his son was found to bear a close resemblance to DNA found on the victims.

Lonnie Franklin Jr., 57, was charged with 10 counts of murder, one count of attempted murder and special circumstance allegations of multiple murders that could make him eligible for the death penalty if convicted, District Attorney Steve Cooley said.

He is charged with 10 counts of murder and one count of attempted murder for a series of killings that date back to 1985.

Lonnie David Franklin Jr.

Victims of the Grim Sleeper

http://www.laweekly.com/2008-08-28/news/eleven-lives-stolen-and-one-lucky-survivor/

The Grim Sleeper's Victims

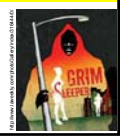
1)Debra Jackson (age 29) – August 10, 1985
2)Henrietta Wright (age 35) – August 12, 1986
3)Thomas Steele (age 36) – August 14, 1986
4)Barbara Ware (age 23) – January 10, 1987
5)Bernita Sparks (age 25) – April 15, 1987
6)Mary Lowe (age 26) – October 31, 1987
7)Lachrica Jefferson (age 22) - January 30, 1988
8)Monique Alexander (age 18) – September 11, 1988
9)Enietra Washington (raped but survived) – November 1988

10) Princess Berthomieux (age 14) – March 19, 2002
11) Valerie McCorvey (age 35) – July 11, 2003
12) Janecia Peters (age 25) – January 1, 2007

Ballistics on bullets recovered from the victim's bodies matched

DNA evidence recovered

Over a 13 year gap in detected crimes, hence the "Sleeper" nickname



http://blogs.laweekly.com/informer/crime/grim-sleeper-son-dna-trail-led/

Putative Relative Is Found

- June 30, 2010: Second familial search of the California database yielded one likely relative
- Database profile belonged to Christopher Franklin (31 years old)
  - Profile added to the database in 2009 after a felony weapons possession charge
- Grim Sleeper profile matched C. Franklin's profile with one allele at all 15 loci
- Both individuals shared the same Y-STR profile, indicating a possible paternal relationship

Identifying the Grim Sleeper

- Given that the murders spanned at least 25 years, the paternal relationship was likely father-son
- Undercover police shadowed C. Franklin's father, Lonnie David Franklin, Jr., who lived in the vicinity of the murders
- Police collected a DNA sample from Lonnie Franklin
  - **Direct match between L. Franklin and the Grim Sleeper**

Familial Searching in the U.S.

High-profile success in the Grim Sleeper case has led other states to consider familial searching

Experts say Texas might solve Twilight Serial Rapist cases with family DNA

http://www.examiner.com/law-enforcement-in-wichita-falls/experts-say-texas-might-solve-twilight-serial-rapist-cases-with-family-dna

DNA DATABASE

Milwaukee police on hunt for serial killer linked to 7 deaths

May 19, 2009 http://articles.cnn.com/2009-05-19/justice/wisconsin.serial.killer_1_dna-technology-dna-database-prostitutes?_s=PM:CRIME

Familial DNA hunt sought in East Coast rape case

http://www2.insidenova.com/news/2010/aug/04/familial_dna_hunt_sought_in_east_coast_rape_case-ar-428231/

March 21, 2011
Virginia announced familial searching capability

Wednesday, December 1, 2010

Virginia could become 3rd state to use familial DNA searches

Some concerned practice could stigmatize those related to criminals

http://www.fairfaxtimes.com/cms/story.php?id=2600

Research Underpins Familial Searching

Forensic Sci Int Genet. 2010 Nov 3. [Epub ahead of print]

Searching for first-degree familial relationships in California's offender DNA database: Validation of a likelihood ratio-based approach.

Myers SP, Timken MD, Piucci ML, Sims GA, Greenwald MA, Weigand JJ, Konzak KC, Buonaiuto MR.

California Department of Justice Jan Bashinski DNA Laboratory, 1001 West Cutting Blvd., Suite 110, Richmond, CA 94804, United States.

Abstract

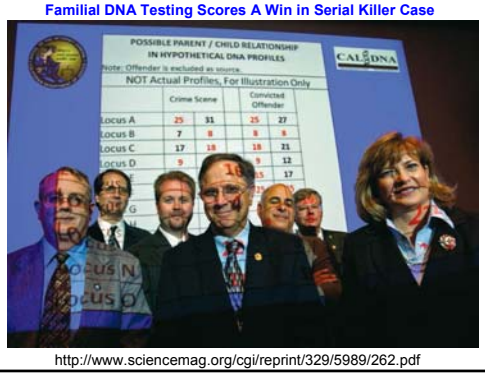
A validation study was performed to measure the effectiveness of using a likelihood ratio-based approach to search for possible first-degree familial relationships (full-sibling and parent-child) by comparing an evidence autosomal short tandem repeat (STR) profile to California's ~1,000,000-profile State DNA Index System (SDIS) database. Test searches used autosomal STR and Y-STR profiles generated for 100 artificial test families. When the test sample and the first-degree relative in the database were characterized at the 15 Identifiler(®) (Applied Biosystems(®), Foster City, CA) STR loci, the search procedure included 96% of the fathers and 72% of the full-siblings. When the relative profile was limited to the 13 Combined DNA Index System (CODIS) core loci, the search procedure included 93% of the fathers and 61% of the full-siblings. These results, combined with those of functional tests using three real families, support the effectiveness of this tool. Based upon these results, the validated approach was implemented as a key, pragmatic and demonstrably practical component of the California Department of Justice's Familial Search Program. An investigative lead created through this process recently led to an arrest in the Los Angeles Grim Sleeper serial murders.

Research is necessary to evaluate the performance of searching algorithms in different state databases

- Size of database
- Number of loci typed
- Types of relationships
- Autosomal vs. lineage markers
- False positives vs. false negatives

California Familial DNA Search Team

Familial DNA Testing Scores A Win in Serial Killer Case



http://www.sciencemag.org/cgi/reprint/329/5989/262.pdf

Rapid DNA Testing

- PCR (~3 hours) is longest step in process
  - **NIST studies have shown that equivalent results can be obtained with 20 minute PCR**
- U.S. government is putting millions of dollars into efforts to reduce time for **DNA typing process to less than one hour** with full automation (buccal swab in, answer out)

How Fast Can We Go?

Steps Involved

Better chemistry has potential to lead to ability to routinely obtain results in < 1 hour with commercially available instruments

- Collection
- Extraction → **Direct PCR (new enzymes & master mix to overcome PCR inhibitors from blood)**
- Quantitation
- Amplification → **Rapid PCR (new enzymes & thermal cyclers)**
- Separation/ Detection → **Improved CE systems (ABI 3500?)**
- Data Interpretation → **Expert system software**

Rapid PCR work published in *FSI Genetics* (Dec 2008)

Full STR profiles in 36 minutes (instead of 3 hour PCR)


Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

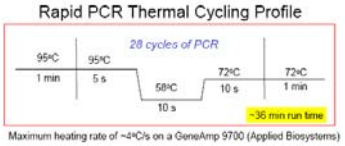
Short communication

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci☆

Peter M. Vallone *, Carolyn R. Hill, John M. Butler

Complete concordance of STR allele calls (for 60 samples) between the rapid and standard thermal cycling protocols were observed although there was incomplete adenylation at several of the loci examined and some PCR artifacts were detected. Using less than **750 pg of template DNA and 28 cycles, STR peaks for all loci were above a 150 relative fluorescent unit (RFU) detection threshold** with fully adequate inter-locus balance and heterozygote peak height ratios of greater than 0.84.

Rapid PCR Work



Maximum heating rate of ~4ºC/s on a GeneAmp 9700 (Applied Biosystems)

- **Much shorter hold times at each temperature**
- **Faster ramp rates between temperatures**

- **Examination of different enzyme mixes**
  - 0.5 x master mix PyroStart (Fermentas) ($0.14/rxn)
  - 0.5 x master mix Premix Ex Taq (Takara) ($0.22/rxn)
  - 0.25 µL = 1.25 units of SpeedStar (Takara) ($1.09/rxn)
- **Evaluation of additional kits**
  - Identifiler, PP16, Yfiler, MiniFiler and Promega S5
- **Testing thermal cyclers with faster ramp rates**

Four Thermal Cyclers Being Evaluated

How fast can we run 28 cycles?

GeneAmp 9700 (Applied Biosystems)

- Heating rate: 4ºC/s
- Heating mechanism: Peltier block (Al)
- Tube format: 0.2 mL - 96 well plate
- 28 cycles = 36 min

36 minutes

SmartCycler (Cepheid)

- Heating rate: 10ºC/s
- Heating mechanism: heating plates and air circulating fan
- Tube format: proprietary 25 µL tubes
- 16 reactions per instrument
- Can also be used for real time PCR
- 28 cycles = 20 min

25 µL PCR rxn volume

20 minutes

Mastercycler pro (Eppendorf)

- Heating rate: 6ºC/s
- Heating mechanism: Peltier block (Ag)
- Tube format: 0.2 mL - 96 well plate
- 28 cycles = 19 min

19 minutes

Rotor-Gene Q (Qiagen)

- Heating rate: 15ºC/s
- Heating mechanism: Air chamber (spinning rotor)
- Tube format: 0.1 mL – 72 tube/rotor
- 28 cycles = 36 min

36 minutes

http://www.cstl.nist.gov/biotech/strbase/pub_pres/ValloneePromega2009poster.pdf

Phenotyping & Biogeographical Ancestry Estimation with SNP Markers

- Hair color prediction
- Eye color prediction


Phenotype prediction

- Efforts by Manfred Kayser's group (Holland)

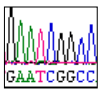

Biogeographical ancestry

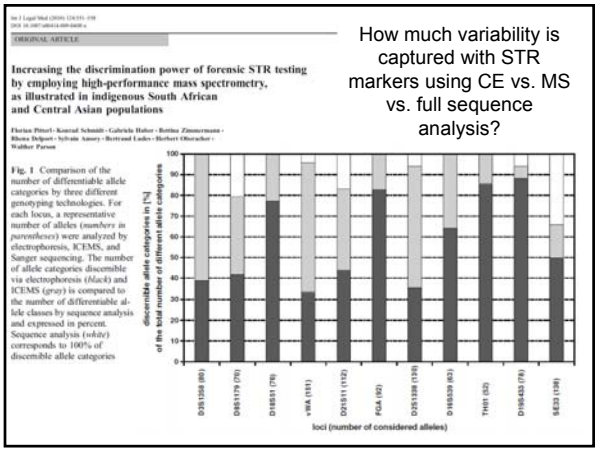
Kayser, M., & de Knijff, P. *Nature Rev. Genet.* 12: 179-192 (March 2011)

Improving human forensics through advances in genetics, genomics and molecular biology

Other Technologies

- Mass spectrometry
- Next-generation DNA sequencing
  - eventually to gain whole genome information with a reasonable cost and effort
- Expert systems for data interpretation
  - To help solve mixtures

	CGCTTTCCA	GAATCGGCC
(a) Electrophoresis (fragment migration)	≈9 nucleotides (compared to size standard)	≈9 nucleotides (compared to size standard)
(b) Mass spectrometry (base composition)	2566 Da $A_1G_1C_4T_3$	2640 Da $A_2G_3C_3T_1$
(c) DNA sequencing (base position)		



Increasing the discrimination power of forensic STR testing by employing high-performance mass spectrometry, as illustrated in indigenous South African and Central Asian populations

Fig. 1 Comparison of the number of differentiable allele categories by three different genotyping technologies. For each locus, a representative number of alleles (*numbers in parentheses*) were analyzed by electrophoresis, ICEMS, and Sanger sequencing. The number of allele categories discernible via electrophoresis (*black*) and ICEMS (*gray*) is compared to the number of differentiable allele classes by sequence analysis and expressed in percent. Sequence analysis (*white*) corresponds to 100% of discernible allele categories

How much variability is captured with STR markers using CE vs. MS vs. full sequence analysis?

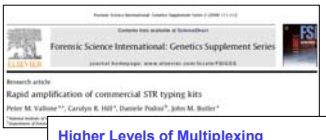
The Future of Forensic DNA

is Similar to the Olympic Motto of
"Swifter, Higher, Stronger"

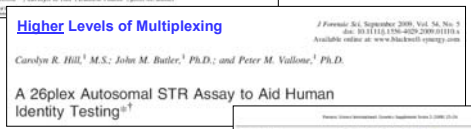
Resources **Training** **Action**

Recent NIST Publications Demonstrating "Swifter, Higher, Stronger" DNA Analysis

Swifter PCR Amplification



Higher Levels of Multiplexing



Stronger Powers of Discrimination

Thank you for your attention

Contact Information

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Group Leader of Applied Genetics
john.butler@nist.gov
301-975-4049

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

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Topics in Forensic DNA Analysis & Interpretation

Mixtures & SWGDAM Interpretation Guidelines

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

To Be Completed…

Topics in Forensic DNA Analysis & Interpretation

Y-STRs

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

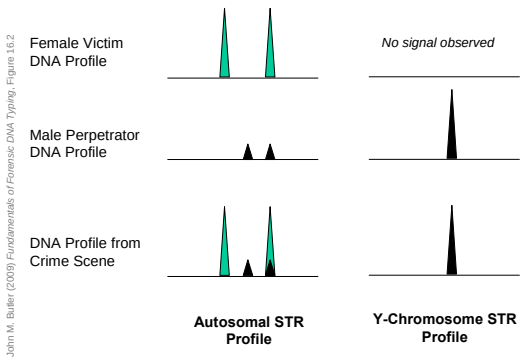
Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

Presentation Outline

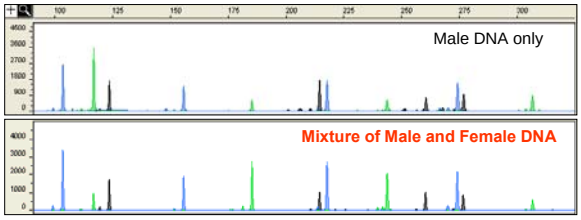
- Why the Y?
- Y-STR Loci & Kits
- Y-STR Databases
- Y-STR Stats

Female-Male Mixture Performance with Autosomal vs. Y-Chromosome DNA Markers



John M. Butler (2009) *Fundamentals of Forensic DNA Typing*, Figure 16.2

Y-STRs Identify the Male Component even with Excess Female DNA



800X female DNA

Scenarios Where Y-STRs Can Aid Forensic Casework

- Sexual assaults by vasectomized or azoospermic males (no sperm left behind for differential extraction)
- Extending length of time after assault for recovery of perpetrator's DNA profile (greater than 48 hours)
- Fingernail scrapings from sexual assault victims
- Male-male mixtures
- Other bodily fluid mixtures (blood-blood, skin-saliva)
- Gang rape situation to include or exclude potential contributors
- Confirmation of amelogenin Y negative males

Confirmation of Amelogenin Negative Males

Normal AMEL X,Y male

AMEL X only

- **Often due to deletion of that entire region of the Y-chromosome rather than a primer binding site mutation**
- Most commonly seen in males of Indian subcontinent origin
- **Y-STRs help demonstrate that the AMEL X sample is really male**
- Chang *et al.* (2007) *Forensic Sci. Int.* 166: 115-120
  - 12/649 Malaysian males showed no AMEL Y
- Cadenas *et al.* (2007) *Forensic Sci. Int.* 166: 155-163
  - 5/77 Nepal males showed no AMEL Y

Covered on the NIST STRBase website
http://www.cstl.nist.gov/biotech/strbase/Amelogenin.htm

NIST Activities with Y-STRs

- SRM 2395 (Human Y Chromosome Standard)
  - http://www.cstl.nist.gov/biotech/strbase/SRM2395.htm
- Characterized duplications and deletions
  - Butler et al. (2005) *J. Forensic Sci.* 50(4): 853-859
- Sequenced variant alleles
  - http://www.cstl.nist.gov/biotech/strbase/STRseq.htm
- Supplied ~20% of Yfiler 3561 database
  - http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm
- Measured mutation rates with Yfiler loci
  - Decker et al. (2008) *FSI Genetics* 2(3): e31-e35

26 publications since 2001 on NIST Y-chromosome work

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

Genetic Genealogy Companies

FamilyTreeDNA
http://www.familytreedna.com
http://www.dna-fingerprint.com

Sorenson Genomics
http://www.sorensongenomics.com

Relative Genetics / GeneTree
http://www.relativegenetics.com

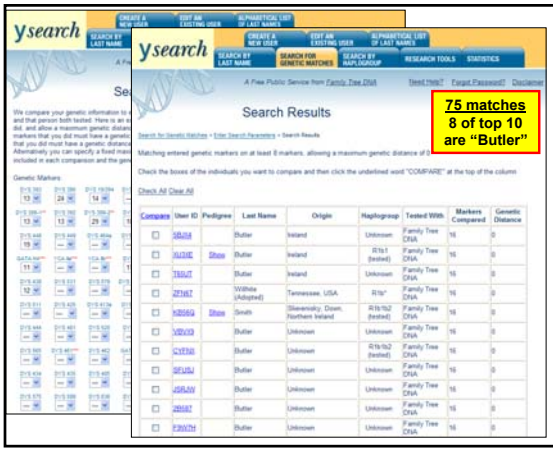
oxford ancestors
http://www.oxfordancestors.com

DNA Heritage
http://www.dnaheritage.com

ETHNO ANCESTRY
http://www.ethnoancestry.com

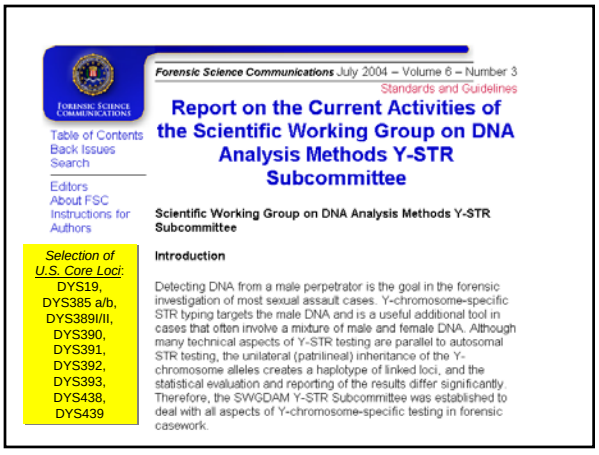
GeoGene
http://www.geogene.com

The rapidly growing field of genetic genealogy is expanding the use of mtDNA and Y-STRs.



Disadvantages of the Y-Chromosome

- Loci are not independent of one another and therefore rare random match probabilities cannot be generated with the product rule; must use haplotypes (combination of alleles observed at all tested loci)
- **Paternal lineages possess the same Y-STR haplotype** (barring mutation) and thus fathers, sons, brothers, uncles, and paternal cousins cannot be distinguished from one another
- **Not as informative as autosomal STR results**
  - **More like addition (10 + 10 + 10 = 30) than multiplication (10 x 10 x 10 = 1,000)**



Core Y-STR Characteristics

11 PCR products
9 primer sets

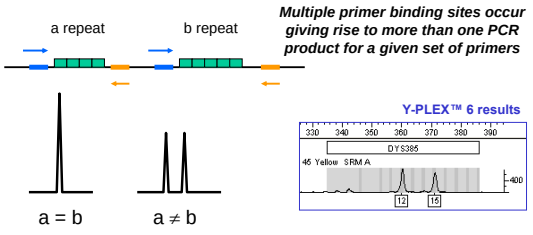
STR Marker	Position (Mb)	Repeat Motif	Allele Range	Mutation Rate
DYS393	3.17	AGAT	8-17	0.05%
DYS19	10.12	TAGA	10-19	0.20%
DYS391	12.54	TCTA	6-14	0.40%
DYS439	12.95	AGAT	8-15	0.38%
DYS389 I/II	13.05	[TCTG] [TCTA]	9-17 / 24-34	0.20%, 0.31%
DYS438	13.38	TTTTC	6-14	0.09%
DYS390	15.71	[TCTA] [TCTG]	17-28	0.32%
DYS385 a/b	19.19, 19.23	GAAA	7-28	0.23%
DYS392	20.97	TAT	6-20	0.05%

Positions in megabases (Mb) along the Y-chromosome were determined with NCBI build 35 (May 2004) using BLAT. Allele ranges represent the full range of alleles reported in the literature. Mutation rates summarized from YHRD (http://www.yhrd.org; accessed 6 Apr 2005).

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.*, 51(2): 253-265

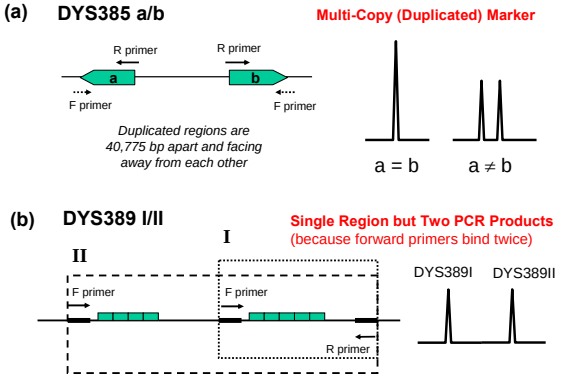
Y-STR Typing of Duplicated Regions

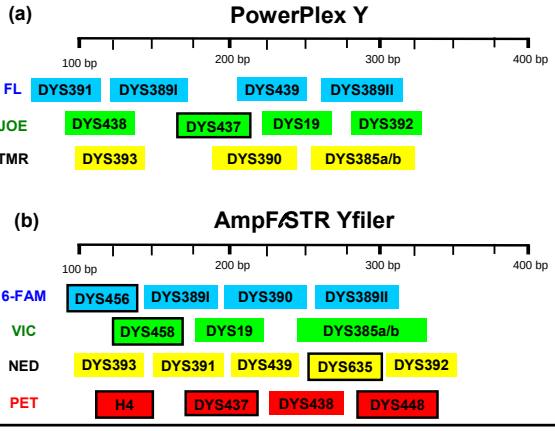
"multi-copy loci"

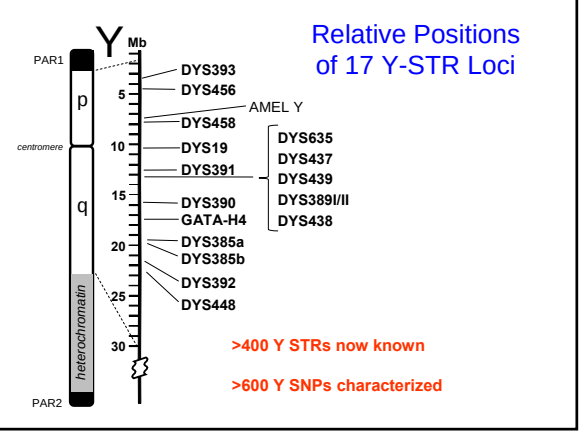


DYS385 a/b and YCAII a/b

Y-STR loci are often counted by the number of amplicons rather than the number of PCR primer pairs





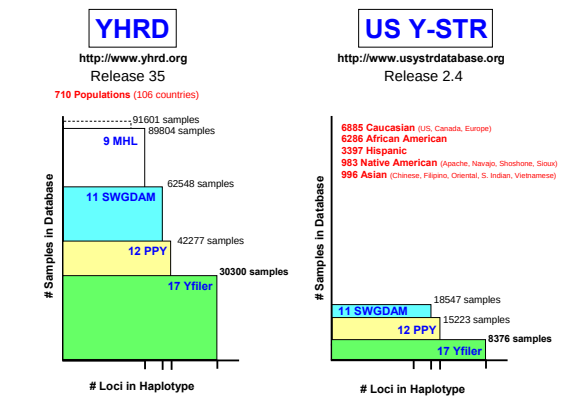


Available Y-STR Loci, Kits and Databases

Loci	Grouping (# Loci)	Available Data
DYS19 DYS389I DYS389II DYS390 DYS391 DYS392 DYS393 DYS385 a/b	Minimal Haplotype (9)	http://www.YHRD.org 89,804 **haplotypes** (>700 populations around the world)
DYS438 DYS439	SWGDAM Core (11)	NIJ-funded **US Database** at UCF: **18,547 haplotypes** **http://www.usystrdatabase.org/**
DYS437	**PowerPlex Y (12)**	http://www.YHRD.org 62,548 haplotypes
DYS448 DYS456 DYS458 DYS635 GATA-H4	Yfiler (17)	**42,277 haplotypes** (YHRD) **15,223 haplotypes** (US YSTR)
		30,300 haplotypes (YHRD) **8,376 haplotypes (US YSTR)**

~400 additional Y-STRs currently known

Hanson & Ballantyne, *Legal Med* 2006;8(2):110-20



Most current information on Y-STR interpretation


Forensic Science International: Genetics 5 (2011) 79–83

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

The interpretation of lineage markers in forensic DNA testing

J.S. Buckleton[a], M. Krawczak[b], B.S. Weir[c,*]

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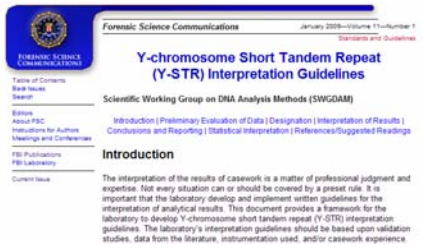
[c] Department of Biostatistics, University of Washington, Box 357232, Seattle, WA 98195-7232, USA

This article reviews and discusses a number of highly relevant topics:

- **Normal vs. binomial (Clopper-Pearson) sampling distributions**
- **Theta corrections**
- **Handling rare haplotypes (Charles Brenner approach)**
- **Combination of lineage and autosomal markers**

SWGDAM Y-STR Interpretation Guidelines

- **Approved July 15, 2008 by SWGDAM**
- Published in *Forensic Sci. Comm.* Jan 2009 issue


Forensic Science Communications

Y-chromosome Short Tandem Repeat (Y-STR) Interpretation Guidelines

Scientific Working Group on DNA Analysis Methods (SWGDAM)

Introduction | Preliminary Evaluation of Data | Designation | Interpretation of Results | Conclusions and Reporting | Statistical Interpretation | References/Suggested Readings

Introduction

The interpretation of the results of casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a preset rule. It is important that the laboratory develop and implement written guidelines for the interpretation of analytical results. This document provides a framework for the laboratory to develop Y-chromosome short tandem repeat (Y-STR) interpretation guidelines. The laboratory's interpretation guidelines should be based upon validation studies, data from the literature, instrumentation used, and/or casework experience.

http://www.fbi.gov/hq/lab/fsc/backissu/jan2009/standards/2009_01_standards01.htm

SWGDAM Y-STR Interpretation Guidelines

Sections 1 – 5

1. Preliminary evaluation of data
2. Allele designation
3. Interpretation of results
4. Conclusions and reporting
5. Statistical interpretation

SWGDAM Y-STR Interpretation Guidelines

Section 5. Statistical Interpretation

(5.1) Population Databases

- Loci on NRY should be considered linked as a single locus
- **Source of population database should be documented**
- Relevant population(s) for which the frequency will be estimated should be identified
- **Consolidated US Y-STR database should be used for population frequency estimation**

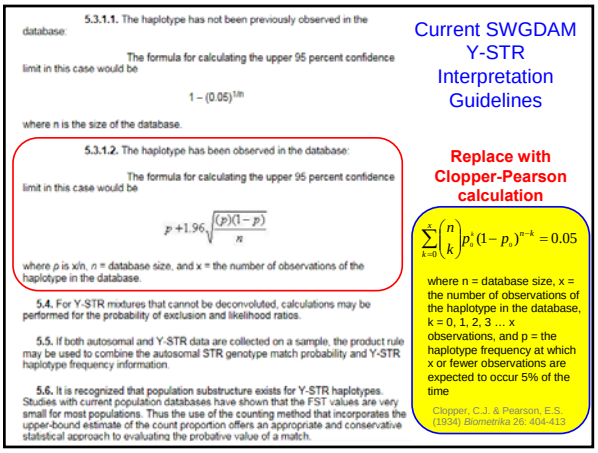
http://www.usystrdatabase.org

SWGDAM Y-STR Interpretation Guidelines

Section 5. Statistical Interpretation

(5.2) Haplotype Searches

- **Should be conducted using all loci for which results were obtained from the evidentiary sample**
- In cases where less information is obtained from the known sample, only those loci for which results were obtained from both the known and evidentiary sample should be used in the population database search


5.3.1.1. The haplotype has not been previously observed in the database:

The formula for calculating the upper 95 percent confidence limit in this case would be

$$1-(0.05)^{1/n}$$

where n is the size of the database.

5.3.1.2. The haplotype has been observed in the database:

The formula for calculating the upper 95 percent confidence limit in this case would be

$$p+1.96\sqrt{\frac{(p)(1-p)}{n}}$$

where p is x/n, n = database size, and x = the number of observations of the haplotype in the database.

5.4. For Y-STR mixtures that cannot be deconvoluted, calculations may be performed for the probability of exclusion and likelihood ratios.

5.5. If both autosomal and Y-STR data are collected on a sample, the product rule may be used to combine the autosomal STR genotype match probability and Y-STR haplotype frequency information.

5.6. It is recognized that population substructure exists for Y-STR haplotypes. Studies with current population databases have shown that the FST values are very small for most populations. Thus the use of the counting method that incorporates the upper-bound estimate of the count proportion offers an appropriate and conservative statistical approach to evaluating the probative value of a match.

Current SWGDAM Y-STR Interpretation Guidelines

Replace with Clopper-Pearson calculation

$$\sum_{k=0}^{x}\binom{n}{k}p_o^k(1-p_o)^{n-k}=0.05$$

where n = database size, x = the number of observations of the haplotype in the database, k = 0, 1, 2, 3 ... x observations, and p = the haplotype frequency at which x or fewer observations are expected to occur 5% of the time

Clopper, C.J. & Pearson, E.S. (1934) *Biometrika* 26: 404-413.

SWGDAM Y-STR Interpretation Guidelines

Section 5. Statistical Interpretation

(5.5) Joint Match Probability

- **The product rule may be utilized** to combine the autosomal STR genotype match probability and Y-STR haplotype frequency information
- Citation to Walsh et al. (2008) Joint match probabilities for Y chromosomal and autosomal markers. *Forensic Sci. Int.* 174: 234-238

SWGDAM Y-STR Interpretation Guidelines

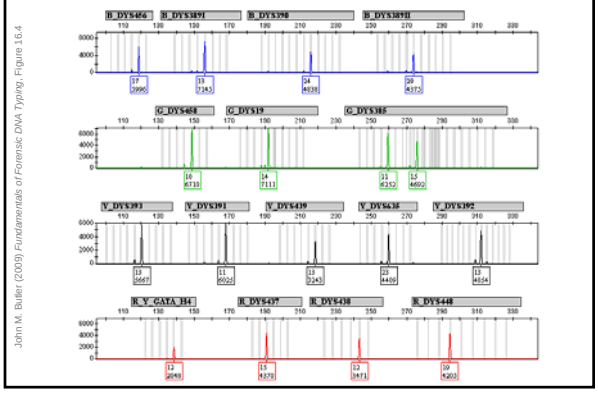
Section 5. Statistical Interpretation

(5.6) Population Substructure

- Studies have shown that F_{st} values are very small for most populations
- **Use of the counting method that incorporates the upper bound estimate of the count proportion offers an appropriate and conservative statistical approach to evaluating the probative value of a match**

No need to use theta correction, but no discussion of partial profiles

Y-STR Result Using Yfiler Kit



J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 13.1

Results of Y-STR Profile Search

The following profile was searched on 15 January 2011 against several databases: DYS19 (14), DYS389I (13), DYS389II (29), DYS390 (24), DYS391 (11), DYS392 (13), DYS393 (13), DYS385 a/b (11,15), DYS438 (12), DYS439 (13), DYS437 (15), DYS448 (19), DYS456 (17), DYS458 (18), DYS635 (23), and GATA-H4 (12).

Database	Minimal haplotype (9 loci)	SWGDAM (11 loci)	PowerPlex Y (12 loci)	Yfiler (17 loci)	3/N for zero observations
YHRD	403/89804 = 0.45 %	29/62548 = 0.046 %	14/42277 = 0.033 %	0/30300 = <0.0033 %	3/30300 = 0.0099 %
US Y-STR	6/18547 = 0.032 %	1/18547 = 0.0054 %	1/15223 = 0.0066 %	0/8376 = <0.012 %	3/8376 = 0.036 %
Yfiler database	64/11393 = 0.56 %	4/11393 = 0.035 %	4/11393 =0.035 %	0/11393 = <0.0088 %	3/11393 = 0.026 %

J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 13.2

Normal vs. Clopper-Pearson

In March 2010 the US Y-STR database changed its 95 % confidence interval calculations to the Clopper-Pearson method.

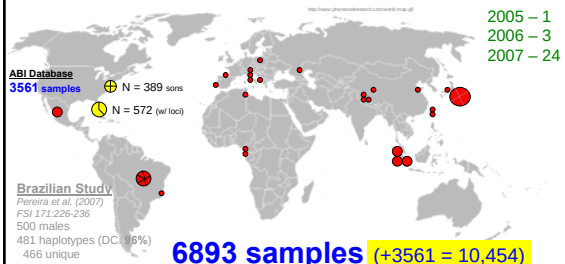
Count values	Frequency p = x/N	Normal 95 % confidence interval	Clopper-Pearson 95 % confidence interval*
YHRD 9 loci: **403**/89804	0.449 %	0.485 %	**0.487 %**
YHRD 12 loci: **14**/42277	0.0331 %	0.0477 %	**0.0518 %**
US Y-STR 12 loci: **1**/15223	0.0657 %	0.0174 %	**0.0317 %**

* Calculation performed with HaploCALc_1.0 Excel spreadsheet kindly provided by Steven P. Myers, CA DOJ

Note that with a large number of observations, such as 403 out of a database of 89804, there is almost no difference between the normal and Clopper-Pearson approaches. However, the normal method is less conservative (i.e., provides a more rare frequency) when the haplotype frequency is low, such as 1 out of 15223 or even 14 out of 42277. Although there are differences in these calculations, re-evaluation by the Clopper-Pearson method will not suddenly change a reported result by orders of magnitude or likely change the outcome of a report significantly.

Sources of Yfiler Worldwide Population Data

28 published population studies with Yfiler data



6893 samples (+3561 = 10,454)

6514 haplotypes (discrimination capacity 94.5%)

6257 single haplotypes **(96.0% singletons)**

The Meaning of a Y-Chromosome Match

Conservative statement for a match report:

The Y-STR profile of the crime sample matches the Y-STR profile of the suspect (at *xxx* number of loci examined). Therefore, ***we cannot exclude the suspect*** as being the donor of the crime sample. In addition, we cannot exclude all patrilineal related male relatives and an unknown number of unrelated males as being the donor of the crime sample.

Y-STR Report Conclusions with Statistics

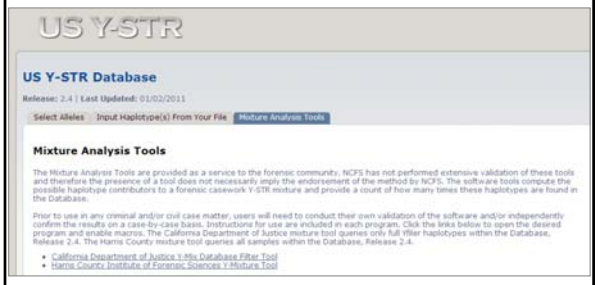
The PowerPlex-Y DNA results are consistent with J. Smith (item #) (or another member of the same paternal lineage) being the source of the Y-DNA profile from item # xx.

The PowerPlex-Y DNA profile detected from item xx has not previously been observed in the National Y-STR population database of 3271 African Americans, 3912 Caucasians, and 1905 Hispanics*. Therefore, the observed PowerPlex-Y DNA profile is not expected to occur more frequently than approximately 1 in 1090 in the African American male population, 1 in 1300 in the Caucasian male population, and 1 in 635 in the Hispanic male population.

*National Y-STR database: See www.usystrdatabase.org

Slide from
Michael Adamowicz, Ph.D.
CT Dept. of Public Safety
Forensic Science Laboratory
DNA Unit

US Y-STR Mixture Analysis Tools



http://www.usystrdatabase.org/ymix.aspx

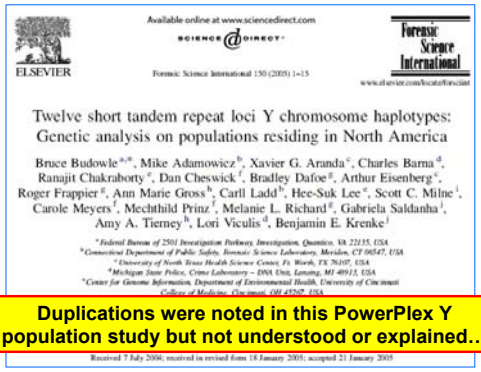
YHRD Mixture Analysis

http://www.yhrd.org/Mixture

Locus Duplication and Deletion

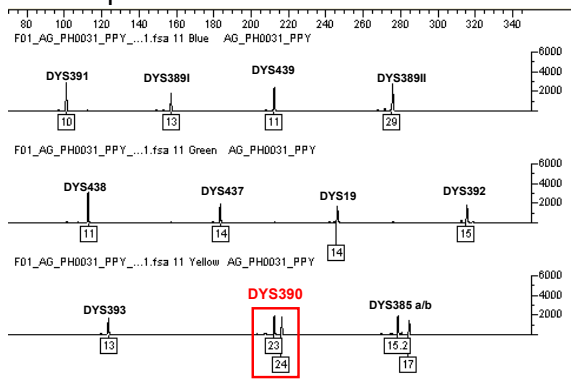
Events that impact Y-STR interpretation

PowerPlex Y Population Study



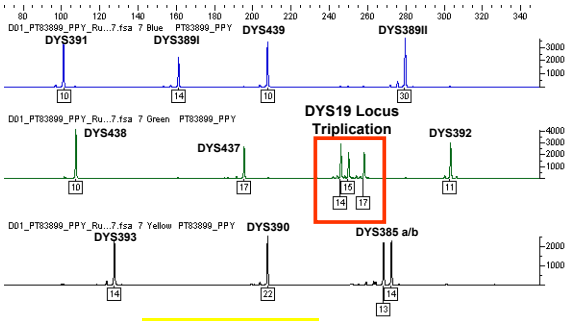
Duplications were noted in this PowerPlex Y population study but not understood or explained…

Duplication Observed at DYS390

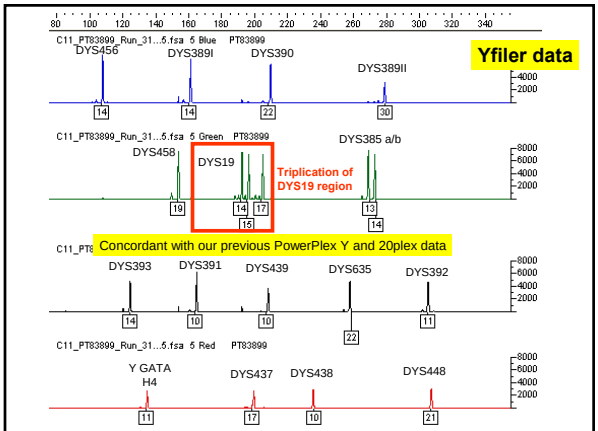


PowerPlex Y results on sample from Ann Marie Gross (MN BCA)

Locus Triplication at DYS19

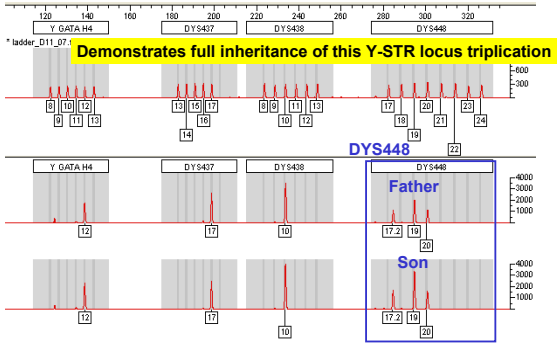


PowerPlex Y data



DYS448 Triplication

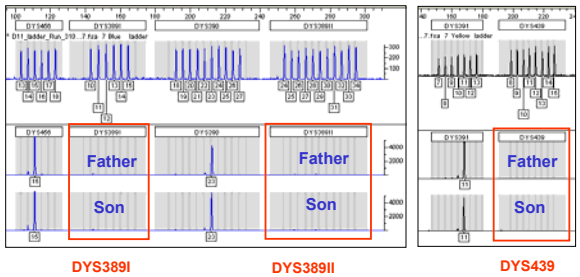
Seen in Both Father and Son



DYS389I, DYS389II, DYS439 Deletions

Seen in Both Father and Son

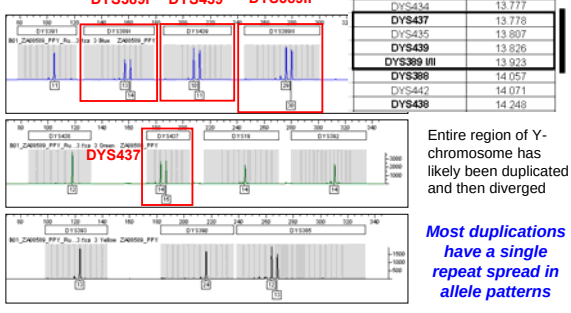
Yfiler data



Full inheritance of these Y-STR locus deletions

Duplication at Multiple Loci with Single-Source Sample

PowerPlex Y data



Y-chromosome mapping

q-arm

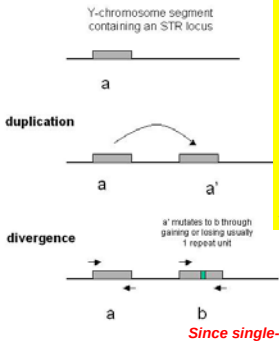
Y STR Marker	Position (Mb)
DYS391	13.413
DYS635 (C4)	13.690
DYS434	13.777
DYS437	13.778
DYS435	13.807
DYS439	13.826
DYS389 I/II	13.923
DYS388	14.057
DYS442	14.071
DYS438	14.248

Entire region of Y-chromosome has likely been duplicated and then diverged

Most duplications have a single repeat spread in allele patterns

Butler et al. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation *J. Forensic Sci.* 50(4): 853-859

Duplication and Divergence Model



Locus	# dup*	>1 repeat
DYS19	23	2
DYS389I	5	0
DYS389II	9	2
DYS390	1	0
DYS391	3	1
DYS392	0	0
DYS393	3	0
DYS385a/b	17	0

*from www.yhrd.org, literature, and our work

92% have single repeat difference

Since single-step mutations are most common, then single repeat spacing in duplicated alleles is expected

Butler et al. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation *J. Forensic Sci.* 50(4): 853-859

Deciphering between a Mixture of Multiple Males and Locus Duplication

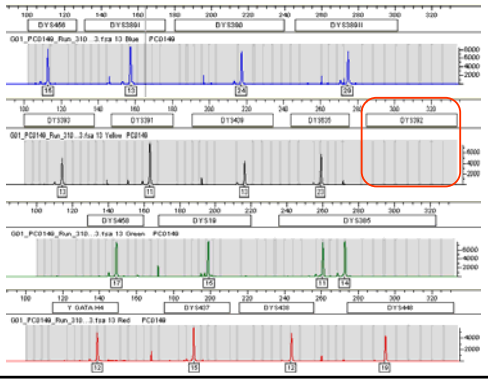
- ***Note the number of loci containing >1 allele (other than multi-copy DYS385)***
- ***Consider relative position on the Y-chromosome if multiple loci have two alleles***
- ***See if repeat spread is >1 repeat unit***
- ***Examine DYS385 for presence of >2 alleles***

Locus duplication along the Y-chromosome is in many ways analogous to heteroplasmy in mitochondrial DNA, which depending on the circumstances can provide greater strength to a match between two DNA samples.

Butler et al. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation *J. Forensic Sci.* 50(4): 853-859

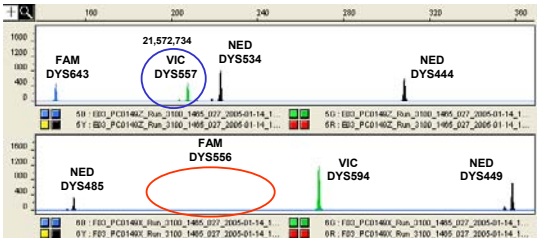
Sample PC0149 with Yfiler

DYS392 is deleted



PC0149 with Additional Y-STRs

One of the closest available loci fails



DYS556 ~32,000 bp away from DYS392 is **missing**

DYS557 ~600,000 bp away from DYS392 is present

Practical Information on Y Deletions

- If DYS458 is deleted in Yfiler, then your sample is likely to lack an Amelogenin Y amplicon as DYS458 and AMEL Y are 1.13 Mb apart on the short arm of the human Y-chromosome
  - Chang *et al.* (2007) *Forensic Sci. Int.* 166: 115-120
- Many Y-chromosomes are more complicated than originally thought!

Y-STR Summary

- Mutation rates are similar to autosomal STRs (~0.2%) – based on father-son studies
- Variant alleles are observed as in autosomal STRs due to flanking region mutations, etc.
- Regions of the Y-chromosome can be duplicated or deleted causing Y-STRs to be duplicated or deleted
- Careful primer design is important to avoid X-chromosome homology or Y-chromosome duplications

Topics in Forensic DNA Analysis & Interpretation

Relationship Testing & Parentage Statistics

Indiana DNA Training Workshop

Indianapolis, IN
March 28, 2011

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

Presentation Outline

- Elements of relationship testing
- Parentage testing & kinship analysis
- NIST efforts to aid kinship analysis
  - **http://www.cstl.nist.gov/strbase/kinship.htm**

Topics in Forensic DNA Analysis & Interpretation

Questions

Indiana DNA Training Workshop

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Submitted Question #1

What threshold would be recommended for STRs and Y-STRs?

- **Whatever YOUR validation data shows!**

Submitted Question #2

What is the best way to calculate those thresholds?

- **Evaluate YOUR validation data…**
- **Will discuss several approaches in the validation portion of workshop**

Submitted Question #3

How would you evaluate thresholds that were first evaluated at a central lab then compared to site specific studies?

- **Ideally, each instrument should be evaluated to establish an analytical and stochastic threshold for this instrument**

Submitted Question #4

How would you evaluate a mixture study?

- Think about what questions you are trying to answer by conducting the study (e.g., minor component allele detection and ability to reliably separate a major from a minor)
- Prepare samples with mixtures focusing on the mixture ratio ranges you want to test (consider the allele combinations as well)

Submitted Question #5

How would you set a cut-off for qPCR kits?

- **Remember that qPCR results with low amounts of DNA are subject to stochastic effects**
  - thus, you could fail to get a qPCR result yet have sufficient amplifiable DNA to obtain a full STR profile
- **Replication of results is ideal**
  - Discussed in my forthcoming book *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 3.3

Replicate testing aids confidence in DNA quantitation values of 'zero'

- A study by the Institute of Legal Medicine in Munich, Germany attempted to correlate the DNA quantitation values with STR performance (Kremser et al. 2009). A set of 3,068 casework samples that had been extracted using the Qiagen EZ1 robot was tested twice with the Quantifiler qPCR kit and the results averaged. Based on the internal positive control (IPC) during qPCR showing no increase in cycle threshold values, it was assumed that potential PCR inhibitors had been removed during DNA extraction. STR amplification was then performed with the NanoplexQS kit from Biotype (Dresden, Germany) followed by the SEfiler kit (Applied Biosystems) to confirm allele calls on all positive results. Based on the average of the two Quantifiler results, samples were divided into four groups: Group 1 (0 pg/µL to 5 pg/µL), Group 2 (5 pg/µL to10 pg/µL), Group 3 (10 pg/µL to 30 pg/µL), and Group 4 (>30 pg/µL).

Kremser, A., et al. (2009). Quantifiler Human DNA Quantification Kit (Applied Biosystems) as a screening kit for DNA profiling. *Forensic Science International: Genetics Supplement Series, 2,* 106-107.

J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 3.3

3,068 casework samples

↓ EZ1 DNA extraction (no inhibitors seen)

DNA quantitation

↓ **Quantifiler** (performed twice and results averaged)

STR amplification

↓ **Nanoplex^{QS}** and **SEfiler** (with up to 500 pg DNA added)

	Group 1 0-5 pg/µL 1564 samples	**Group 2** 5-10 pg/µL 279 samples	**Group 3** 10-30 pg/µL 371 samples	**Group 4** >30 pg/µL 854 samples
No results	96%	67%	26%	3%
Full profile	**3%**	**23%**	**67%**	**96%**
Partial profile	1%	10%	7%	1%

Kremser, A., et al. (2009). Quantifiler Human DNA Quantification Kit (Applied Biosystems) as a screening kit for DNA profiling. *Forensic Science International: Genetics Supplement Series, 2,* 106-107.

J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 3.3

Summary of Results

- **Generally, STR typing results correlated with the amount of DNA.** Full profiles were observed 96% of the time when >30 pg/µL were reported. Likewise, **no STR results were obtained 96 % of the time when DNA quantities in the range of 0 pg/µL to 5 pg/uL were reported.** However, **full or partial DNA profiles were observed 4 % of the time when essentially the DNA quantity was zero** (Group 1, 0 pg/µL to 5 pg/µL obtained). The 1564 samples in Group 1 were explored further by examining the individual Quantifiler results.

Kremser, A., et al. (2009). Quantifiler Human DNA Quantification Kit (Applied Biosystems) as a screening kit for DNA profiling. *Forensic Science International: Genetics Supplement Series, 2,* 106-107.

J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 3.3

Importance of Replicate Testing to Overcome Stochastic Effects

1564 Samples
with 'Zero' Quantifiler Results (pg/µL)
(Original averaged result was 0-5 pg/µL)

	Result 1 / Result 2 **0,0**	**0,>0**	**>0,>0**
Number of Samples	750	478	336
Positive results	**0%**	7%	27%
Negative results	**100%**	93%	63%

When both Quantifiler results were zero, then all subsequent STR testing failed to obtain a result

Kremser, A., et al. (2009). Quantifiler Human DNA Quantification Kit (Applied Biosystems) as a screening kit for DNA profiling. *Forensic Science International: Genetics Supplement Series, 2,* 106-107.

J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, D.N.A. Box 3.3

Summary of "0" qPCR Results

- When both Quantifiler replicate results were zero, then 100 % of the time subsequent STR typing failed to obtain results (from 750 tested samples). Thus, **when using DNA quantitation in a gatekeeper function for whether or not to proceed with further testing, replicate zero values were important to guarantee no DNA was present**. This same concept of replicate testing aids reliability with low template DNA

Kremser, A., et al. (2009). Quantifiler Human DNA Quantification Kit (Applied Biosystems) as a screening kit for DNA profiling. *Forensic Science International: Genetics Supplement Series, 2,* 106-107.

Submitted Question #6

What would you consider as LCN or low template (LT-DNA) analysis?

- **low amounts of DNA being tested** often with "enhanced interrogation" techniques (such as higher cycle numbers or sample desalting to boost CE injection)
  - **It is not a pre-set DNA quantitation threshold** (e.g., 200 pg) because quantitation does not always match PCR amplification performance
  - **It is not a pre-set cycle number** as each STR kit has a different sensitivity

Submitted Question #7A

If you were switching kits, how would you compare the kits?

- **Cost per sample**
- **Customer support**
- **Concordance**
  - **What loci are included in the kit to provide overlap with legacy data (largest possible, well-performing multiplex in order to obtain as much information as possible from a tested sample)**
  - **If different primer positions caused a significant discordance in results, then this could be a problem (but most primer discordances are rare because of careful design & testing)**

Submitted Question #7B

What criteria would you use (if switching kits)?

- **Performance**
  - **How well the kit performs in YOUR hands**
  - **How robust on challenging samples (can it cope with PCR inhibitors?)**
- **Sensitivity**
  - **Relative kit sensitivity is not an issue in my opinion as the addition of a PCR cycle (following validation experiments) would address this difference**

Submitted Question #8

What is your opinion on subtracting out the victim's profile from an intimate sample (e.g., vaginal swab)? What statistics would you apply?

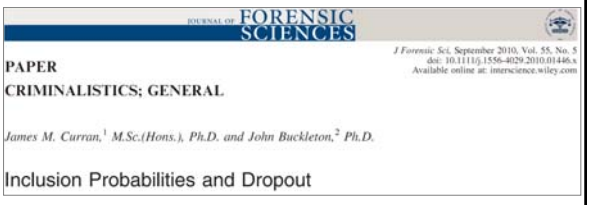
- **Fine to do as long as you clearly document what you have done (assumptions made)**
- **Statistics used would depend on the profile and whether the perpetrator portion of the profile is the major or minor component**

Submitted Question #9

What are your thoughts on recent discussions regarding CPI stat calculations for DNA mixtures regarding using/omitting loci where a suspect (or victim) is not fully represented?

- Curran & Buckleton (JFS Sept 2010 article)
  - If the person is innocent, then CPI is not conservative
- Charles Brenner (AAFS Feb 2011 talk)
  - CPI works fine in simple cases but should not be used in more complex (low level DNA) cases because CPI cannot account for the possibility of allele dropout

Curran and Buckleton
(JFS Sept 2010)


JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, September 2010, Vol. 55, No. 5
doi: 10.1111/j.1556-4029.2010.01446.x
Available online at: interscience.wiley.com

PAPER
CRIMINALISTICS; GENERAL

James M. Curran,[1] M.Sc.(Hons.), Ph.D. and John Buckleton,[2] Ph.D.

Inclusion Probabilities and Dropout

Created 1000 Two-person Mixtures (Budowle *et al.*1999 AfAm freq.).

Created 10,000 "third person" genotypes.

Compared "third person" to mixture data, calculated PI for included loci, ignored discordant alleles.

Curran and Buckleton
(JFS Sept 2010)