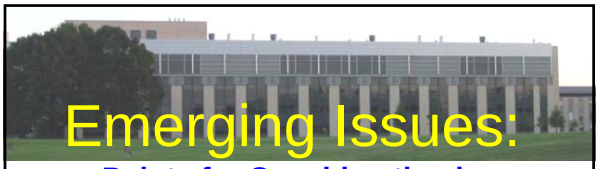


# Emerging Issues:

## Points for Consideration in the Future of Forensic DNA Typing

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

NDAA Training at the National Advocacy Center
Columbia, SC
May 2, 2007

True Identity: DNA
DNA Fingerprinting on the Witness Stand

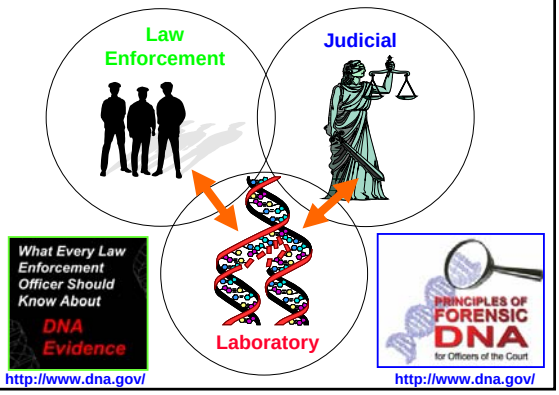
## NIST and NIJ Disclaimer

**Funding: Interagency Agreement 2003-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.

## Interfaces Between Disciplines Are Crucial




http://www.dna.gov/ http://www.dna.gov/

## Information Resources for Defense Attorneys

http://www.nlada.org/Defender/forensics/for_lib/Index/DNA/exhibits/index_html


**Defense Lawyers and Experts are becoming more united and informed**

## Common Defense Attacks

**Compiled from Forensic Bioinformatics website**

forensic bioinformatics
http://www.bioforensics.com

- Contamination
- Statistical Weight of a Match
- Degradation/PCR Inhibition of "True" Perp
- Artifacts (N+4 stutter, etc.)
- **Thresholds Set Too High (missing peaks)**
- Examiner Bias
- **Improper Mixture Interpretation**
- Meaning of a Database Hit
- Protocol Violations

Forensic Bioinformatics
6th Annual Conference
**The Science of DNA Profiling: A National Expert Forum**
August 17 - 19, 2007
Dayton, OH

See http://www.bioforensics.com/conference07/index.html

# NIST Background

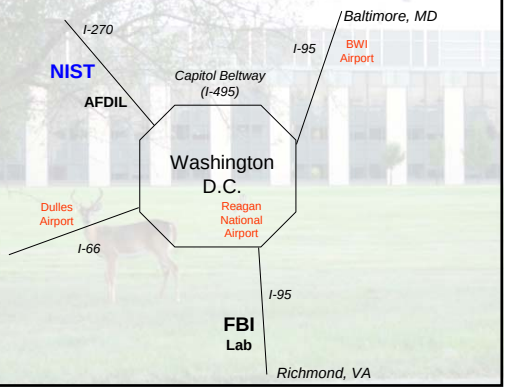
## NIST History and Mission

- 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.**


$573 for 3 jars

**DNA typing standard**

## Location of NIST (near Washington, DC)




## NIST Gaithersburg Campus

Administration (Building 101)

Located in Gaithersburg, Maryland, on approximately 234 hectares (578 acres) just off Interstate 270 about **25 miles northwest of Washington, D.C.**

**http://www.nist.gov**

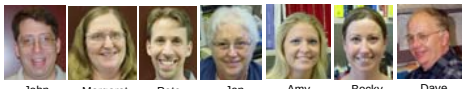
**~2,500 staff**

Advanced Chemical Sciences Laboratory (Building 227)

## Our Team Mission Statement

- The NIST Human Identity Project Team is trying **to lead the way in forensic DNA**… through research that helps bring traceability and technology to the scales of justice.

## NIST Human Identity Project Team



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

**All NIST publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm**


- **26 publications** from Jan-Dec 2006
- **45 presentations** and **10 workshops** to the community from Jan-Dec 2006

**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** http://www.cstl.nist.gov/biotech/strbase/
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

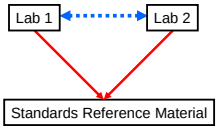
## Standard Reference Materials

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

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


SRM 2391b – CODIS STRs
SRM 2392-I – mtDNA
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation

**Helps meet DAB Std. 9.5 and ISO 17025**




**Calibration with SRMs enables confidence in comparisons of results between laboratories**

## Information Resources

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


**Includes information on**:
- Core STR loci
- Validation
- STR reference list
- NIST publications
- miniSTRs
- Forensic SNPs
- Variant STR alleles
- Population data resources
- Addresses of scientists

*Provides up-to-date information and has been used in court cases to support application of DNA technology*

## Review Scenarios Used for this DNA Course

## Science Principles You Should Have Learned from Scenario #1

- **Scenario 1**: Burglary case with blood evidence (single source DNA sample) – match to suspect
- A DNA profile can be developed from biological evidence such as blood
- DNA requires comparison to a reference sample (in this case, a suspect found through police investigation)
- DNA is only part of an investigation but can link a perpetrator to a crime scene
- Weight of the DNA match can be assessed with random match probability (based on theoretical calculations from allele frequencies)

## Science Principles You Should Have Learned from Scenario #2

- **Scenario 2**: Sexual assault with vaginal swab evidence (mixture DNA sample) – differential extraction used to separate female and male cells and DNA database hit used to identify perpetrator who is then resampled to confirm hit
- Sexual assault evidence contains a mixture of female DNA from the victim's epithelial cells and male DNA from the perpetrator's sperm cells – DNA from intact sperm cells can be isolated from a greater abundance of female cells
- DNA databases, such as CODIS, enable links between crimes committed by repeat/serial offenders
- Testing a confirmatory sample from identified suspect (database hit to offender) provides quality assurance with the match made – **and the evidence used in court**

## Science Principles You Should Have Learned from Scenario #3

- **Scenario 3**: Homicide case with blood evidence (mixture DNA sample) – police investigation leads to a suspect who matches partial profile present in the mixture
- DNA mixtures and statistics involved when components cannot be separated – much lower numbers than what is produced with single source match probabilities
- Victim's profile can be subtracted from a mixture to help identify the alleles present (and thus the DNA profile) in the other component of a two-person mixture
- Partial profiles result from degraded DNA (only 11 of 13 tested loci worked) – usually the larger sized loci fail in a DNA profile

## Science Principles You Should Have Learned from Scenario #4

- **Scenario 4**: Rape/homicide case involving multiple pieces of evidence including human hair (requiring mtDNA), bite mark (female-male mixture requiring Y-STRs), and non-human DNA – police investigation produces three suspects that are related
- Relatives reduce match probabilities (which are typically calculated assuming unrelated individuals)
- Other DNA information can be used for specific purposes – such as Y-STRs to isolate male DNA in female-male mixtures and mtDNA to recover information from low amounts of DNA present in hairs
- Non-human DNA, such as cat hair, can aid efforts to connect a crime scene to a suspect (these specialty DNA tests will likely be outsourced)

## Questions on Science Principles from the Four Scenarios?

## Emerging Trends/Issues

**Things that are coming…**

**(or in development as possibilities)**

## Forensic Science Journals


## 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.

## *Analytical Chemistry* Application Review

**June 15, 2005 issue of *Analytical Chemistry***

**Forensic Science**

**T. A. Brettell***

*Office of Forensic Sciences, New Jersey State Police, New Jersey Forensic Science and Technology Complex, 1200 Negron Road, Horizon Center, Hamilton, New Jersey 08691*

**J. M. Butler**

*National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311*

**R. Saferstein**

*Box 1334, Mount Laurel, New Jersey 08054*

Review Contents
- Forensic DNA Analysis
  - Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material
  - Short Tandem Repeats
  - Single-Nucleotide Polymorphisms
  - Y-STR Typing, Gender Identification, and X-Chromosome Analysis
  - Mitochondrial DNA Typing
  - Nonhuman DNA Typing Systems and Microbial Forensics
  - DNA Databases
  - Interpretation and Statistical Weight of DNA Typing Results
  - General Reviews

**250 articles referenced** covering forensic DNA analysis during 2003-2004

## *Analytical Chemistry* Review Article
(**Will be published** June 15, 2007)

Forensic Science

T. A. Brettell
*Department of Chemical and Physical Sciences, Cedar Crest College, 100 College Dr. Allentown, Pennsylvania 18104-6196*

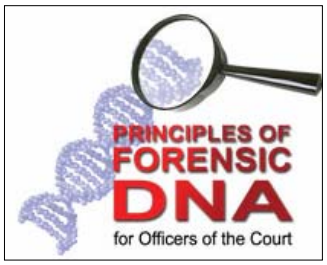
J. M. Butler
*Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311*

J. R. Almirall
*Department of Chemistry and Biochemistry and International Forensic Research Institute, Florida International University, University Park, Miami, Florida 33199*

**Describes 181 forensic DNA articles** published in 2005 and 2006 (**560 references** covering DNA, trace evidence, drugs and poisons)

## DNA Training for Officers of the Court

PRESIDENT'S DNA INITIATIVE *Advancing Justice Through DNA Technology*




- CD-ROM available from the U.S. National Institute of Justice (http://www.ncjrs.gov)
- On-line training available at http://www.DNA.gov

**http://www.dna.gov/training/otc/**

## Principles of Forensic DNA for Officers of the Court

1. **Introduction**
2. **Biology of DNA**
3. **Practical Issues Specific to DNA Evidence**
4. **Forensic DNA Laboratory**
5. **Assuring Quality in DNA Testing**
6. **Understanding a Forensic DNA Lab Report**
7. **Statistics and Population Genetics**
8. **Mitochondrial DNA & Y-STR Analysis**
9. **Forensic DNA Databases**
10. **Collection of DNA Evidence**
11. **Pretrial DNA Evidence Issues**
12. **Victim Issues**
13. **Trial Presentation**
14. **Postconviction DNA Cases**
15. **Emerging Trends**

**http://www.dna.gov/training/otc/**

## Content of Section 15 "Emerging Trends" from *Officers of the Court*

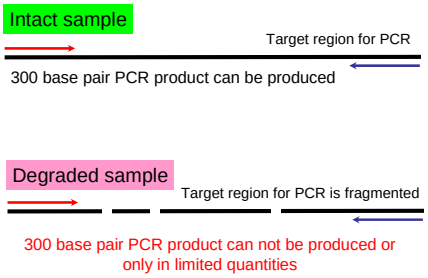
- Topic 1 :: **Single Nucleotide Polymorphisms (SNPs)**
- Topic 2 :: Automation
  - Microarrays (Chip Technology)
  - **Portable DNA Typing Laboratory**
  - Low Copy Number DNA Analysis
- Topic 3 :: Microbial Forensics and DNA Testing
- Topic 4 :: Other **Non-human Forensic DNA Analysis**
- Topic 5 :: **DNA Typing and Physical Appearance**
  - Biogeographical Ancestry
  - Approximate Age Determination

**http://www.dna.gov/training/otc/**

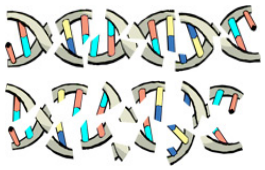
## DNA Degradation

- What causes DNA degradation?
  - Heat, humidity, long term exposure to the elements
  - DNA breaks down into small fragments; smaller than the targeted PCR product size
- Mass disasters (aviation, WTC)
- Aged samples (missing persons, remains of soldiers, ancient DNA)

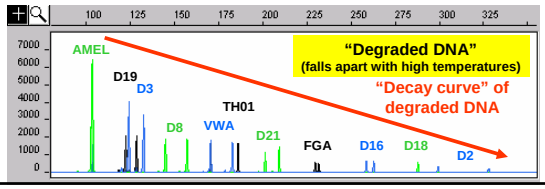
## DNA Degradation



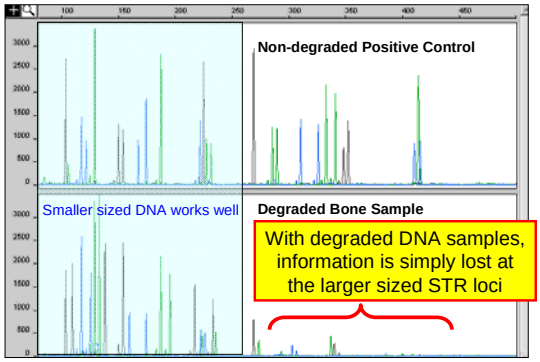
## Degraded DNA



Larger segments of DNA cannot be recovered when DNA molecules have fragmented into small pieces (caused by heat, water, or bacteria)




## DNA Degradation Means Less Loci Work




## Impact of Degraded DNA Samples

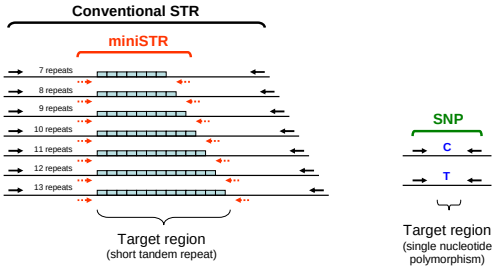
- Comparison to a phone number (string of 13 numbers)

  001-301-975-4049

- If you only had "4049"…this information would be of limited value since it is not as specific (and could match other phone numbers from different area codes)
- DNA profiles are essentially a string of numbers – **if the DNA is damaged, then the string of numbers is shorter and less informative…**

  ----------4049 or ----301-9-------

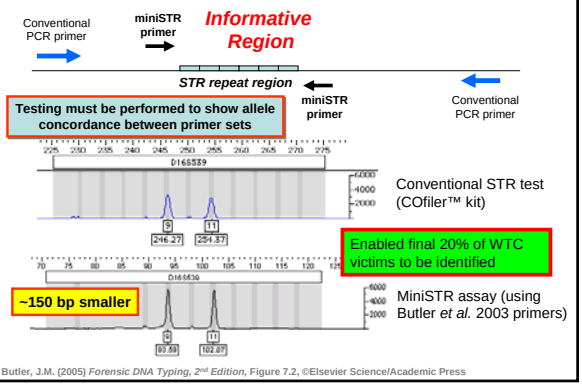
## Comparison of STRs and SNPs




**Larger target region** (miniSTR targets same region)
**More possible variants than SNPs**
**Only need a moderate number of STR markers**
**Range of sizes examined** (e.g., 28 bp spread if 4 bp/repeat)

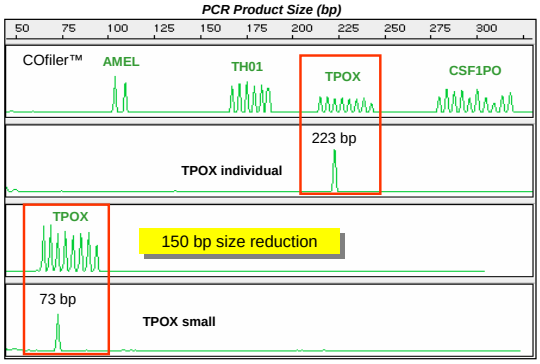
**Smaller target region**
**Fewer possible variants**
**Need more SNP markers**
**Constant size examined**

## A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples




Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition,* Figure 7.2, ©Elsevier Science/Academic Press

## STR Amplicon Size Reduction with New PCR Primers



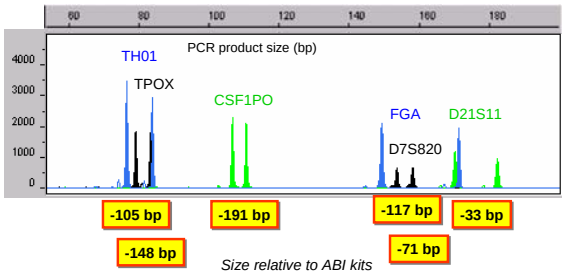

*New primer sets are intended to aid with typing degraded DNA samples as well as future microchip CE and mass spectrometry applications…*

*J. Forensic Sci.* Sept 2003 issue

*J. Forensic Sci.*, September 2003, Vol. 48, No. 5
Paper ID JFS2003043_485
Available online at: www.astm.org

John M. Butler,[1] Ph.D.; Yin Shen,[2,3] Ph.D.; and Bruce R. McCord Ph.D.[2]

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




*Size relative to ABI kits*

## Direct Comparisons of SNPs and STRs on Degraded DNA Templates

- World Trade Center DNA Investigation
  - A panel of 70 SNPs run on >15,000 bone extracts by Orchid Cellmark; no additional identifications made
  - **Reduced size STR markers (miniSTRs) aided last 20% of WTC DNA identifications**
- EDNAP Degraded DNA Study
  - Organized by Lindsey Dixon and Peter Gill (UK FSS); involved 9 labs testing artificially degraded blood and saliva stains
  - **miniSTRs outperformed SNPs**

## EDNAP Exercise on Degraded DNA

**miniSTRs out-performed SNPs**

ELSEVIER
Forensic Science International
Forensic Science International 164 (2006) 33–44
www.elsevier.com/locate/forsciint

Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise

L.A. Dixon[a,*], A.E. Dobbins[a], H.K. Pulker[a], J.M. Butler[b], P.M. Vallone[b], M.D. Coble[b], W. Parson[c], B. Berger[c], P. Grubwieser[c], H.S. Mogensen[d], N. Morling[d], K. Nielsen[d], J.J. Sanchez[d], E. Petkovski[e], A. Carracedo[f], P. Sanchez-Diz[f], E. Ramos-Luis[f], M. Brión[f], J.A. Irwin[g], R.S. Just[g], O. Loreille[g], T.J. Parsons[g], D. Syndercombe-Court[h], H. Schmitter[i], B. Stradmann-Bellinghausen[j], K. Bender[j], P. Gill[a]

[a] The Forensic Science Service, Research and Development, Trident Court, Birmingham, UK
[b] National Institute of Standards and Technology, Gaithersburg, MD, USA
[c] Institute of Legal Medicine, Innsbruck Medical University, Austria
[d] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

Dixon *et al.* (2006) Analysis of artificially degraded DNA using STRs and SNPs--results of a collaborative European (EDNAP) exercise. *Forensic Sci. Int.* 164: 33-44.

**Conducted in the Fall of 2004**

**MiniSTR primer mixes and allelic ladders were provided by NIST**

## European Labs Have Adopted the NIST-Developed NC miniSTRs

*FSI* (2006) **156(2):** 242-244

Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill[a,*], Lyn Fereday[b], Niels Morling[c], Peter M. Schneider[d]

[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
[d] Institute of Legal Medicine, University of Cologne, Germany

Received 25 May 2005; accepted 26 May 2005

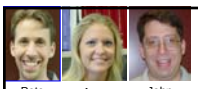
**…recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.**

**(D14 has been replaced with D2S441 from NC02)**

## Single Nucleotide Polymorphisms (SNPs)

**http://www.dna.gov/training/otc/**

- *OTC* Statement: "While the future utility of SNPs is uncertain, **it seems unlikely that this method will replace the standard set of STRs** used for routine DNA analysis due to the limited variation of SNPs and difficulties with mixed sample interpretation."
- Butler, J.M., Coble, M.D., Vallone, P.M. (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. *Forensic Science, Medicine and Pathology, in press*.



Pete Vallone | Amy Decker | John Butler

## NIST Work with SNP Loci

- U.S. population frequencies with 70 autosomal SNPs
  - Vallone *et al.* (2005) *Forensic Sci. Int.* 149: 279-286
- U.S. population information with 50 Y-SNPs
  - Vallone *et al.* (2004) *J. Forensic Sci.* 49: 723-732
- Construction of 12plex autosomal SNP assay
  - Vallone *et al.* (2005) *Progress in Forensic Genetics 11*

>1,000 samples examined from 10 populations

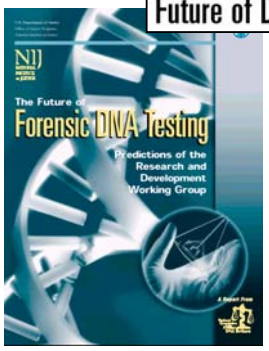
- Creation of Forensic SNP Information website on STRBase
  - see Gill et al. *Science&Justice* 44(1): 51-53

**http://www.cstl.nist.gov/biotech/strbase/SNP.htm**

## Status of Genetic Marker Systems Used in Forensic DNA Testing

- **STRs** – widely used in national databases today
- **miniSTRs** – used in research and WTC; new MiniFiler kit just being released
- **mtDNA** – used in specialty labs for highly degraded specimens
- **Y-STRs** – growing use due to kits now available
- **SNPs** – research; **likely to be limited in use**

## National Commission on the Future of DNA Evidence




http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

- Report published in Nov 2000
- Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles

## Why SNPs Will Likely Not Replace STRs…

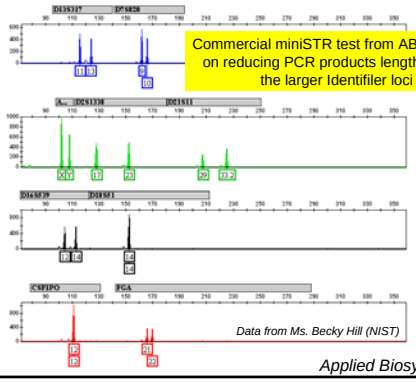
- Large databases containing STR information (would need to replace data on existing samples with new DNA markers)
- Mixture detection and interpretation benefits from marker systems with many alleles (SNPs only have two alleles and three genotype possibilities)
- Degraded DNA can be successfully analyzed in many cases by miniSTRs (thus removing the primary motivation for using SNPs…)


**miniSTRs improve the success rate and recovery of information from compromised DNA evidence**

## Approaches for "challenging" samples: perspectives for the future

- Limited sample material (highly degraded DNA)
  - **mtDNA** (in use for this purpose since mid-1990s due to high copy number per cell)
    Chapter 10 in *Forensic DNA Typing*, 2nd Edition
- Mixed male-female DNA
  - **Y-chromosome STRs**
    http://www.cstl.nist.gov/biotech/strbase/y_strs.htm
- Degraded DNA
  - **miniSTRs** http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
  - SNPs (?) http://www.cstl.nist.gov/biotech/strbase/SNP.htm

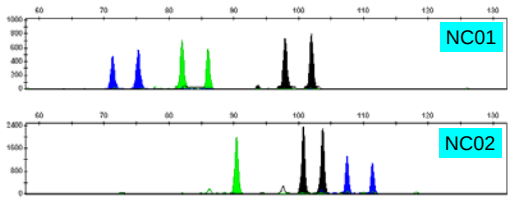
## A MiniFiler Kit Profile




Commercial miniSTR test from ABI focusing on reducing PCR products length for 8 of the larger Identifiler loci

*Applied Biosystems Kit*

## Further work with miniSTRs




We are currently developing tests
for 26 additional miniSTR loci
Potential utility for missing persons work, kinship analysis, paternity testing, mutation rates etc

*Data from Ms. Becky Hill (NIST)*

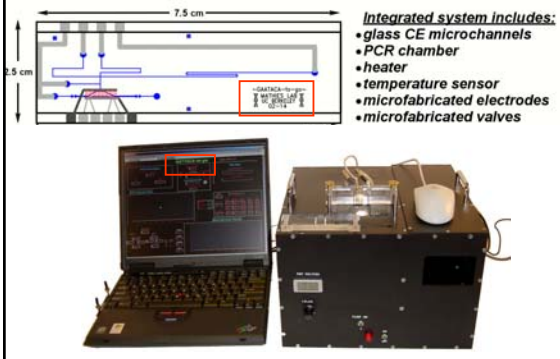
## Comprised Sample Improvements (CSI) Conclusions

- **Analysis of shorter regions of DNA benefits recovery of information from degraded specimens**
- **miniSTRs are now viewed as the primary way forward and a commercial kit is under development**
- **SNPs**, while theoretically beneficial due to small possible amplicons, are limited due to poor abilities to handle mixtures and the need for large multiplexes to improve powers of discrimination
- **mtDNA** due to higher copy number per cell than nuclear DNA will continue to be used where limited samples are recovered (e.g., hair shafts and bone fragments)

## Portable DNA Typing Laboratory

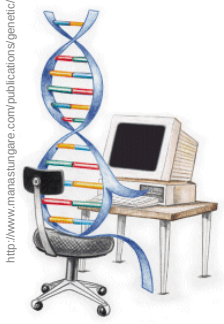
- Network Biosystems
  - http://www.networkbiosystems.com
  - Based on Dan Ehrlich's work at MIT/Whitehead
- Microchip Biotechnologies Inc.
  - http://www.microchipbiotech.com
  - Based on Rich Mathies' work at UC Berkeley

## Portable Genetic Diagnostics Device




From Richard Mathies presentation at 14th International Symposium on Human Identification, Oct 2003

## The Future




- **More Robotics**
- **Expert Systems**
- **Animal & Plant DNA**
- **Physical Characteristics**
- **Ethnicity Estimation**

# Non-Human Forensic DNA Analysis

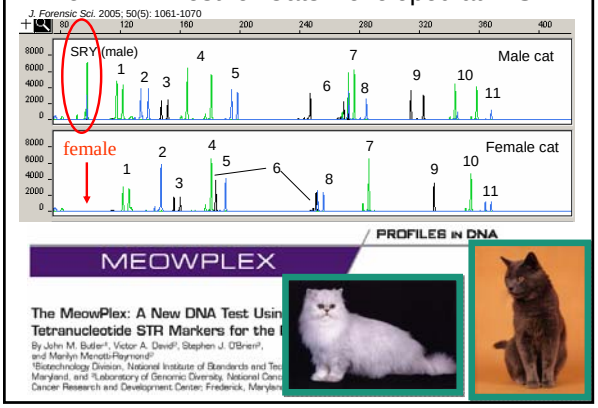
## Non-Human DNA Testing

- Cat DNA
- Dog DNA

**Animal as**
1) Victim (abuse case)
2) Perpetrator (dog bite)
3) Silent witness (crime scene linkage)

- Other uses of non-human DNA
  - Plant DNA – for possible linkage to crime location
  - Marijuana DNA – tracking drug sources

## New DNA Test for Cats Developed at NIST




## Animal DNA Testing

- Most non-human DNA tests are specialty tests that will be rarely used by public labs and thus typically will be performed through outsourcing to a contract lab

- QuestGen Forensics
  - http://www.questgen.biz/
- UC Davis Vet Gen Lab
  - http://www.vgl.ucdavis.edu/

QuestGen FORENSICS
Forensic DNA Analysis of Animal-derived Samples

VGL Veterinary Genetics Laboratory

## Challenges with Presenting Non-Human DNA in Court (or other novel DNA methods)

**Sensabaugh and Kaye (1998) *Jurimetrics* 38: 1-16**

- Novelty of the application
- Validity of the underlying scientific theory
- Validity of any statistical interpretations
- Relevant scientific community to consult in assessing the application may be limited

- **New methods may not have undergone the scientific scrutiny of regular forensic human DNA testing techniques**

# DNA Typing and Physical Appearance

Biogeographical Ancestry
Approximate Age Determination

## Biogeographical Ancestry

- **Shriver, M.D. *et al.* (2003) Skin pigmentation, biogeographical ancestry and admixture mapping. *Hum. Genet.* 112(4):387-99**

- **From abstract:** Ancestry informative markers (AIMs) are genetic loci showing alleles with large frequency differences between populations. AIMs can be used to estimate biogeographical ancestry at the level of the population, subgroup (e.g. cases and controls) and individual…. **This work indicates that it is possible to estimate the individual ancestry of a person based on DNA analysis with a reasonable number of well-defined genetic markers.**

## Biogeographical Ancestry (2)

- Mark Shriver's work on ancestry informative markers has been commercialized through the company **DNAPrint Genomics**
- http://www.dnaprint.com
- http://www.ancestrybydna.com
- Used in Derrick Todd Lee (Louisiana serial killer) case to overcome faulty eyewitness testimony of a Caucasian perpetrator…

## Pigmentation (Skin Color, etc.) Prediction

- **Lamason, R.L. *et al.* (2005) SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* 310:1782-1786**
- **From abstract:** Lighter variations of pigmentation in humans are associated with diminished number, size, and density of melanosomes, the pigmented organelles of melanocytes. **The variant allele is nearly fixed in European populations**, is associated with a substantial reduction in regional heterozygosity, **and correlates with lighter skin pigmentation** in admixed populations, suggesting a key role for the SLC24A5 gene in human pigmentation.

## Approximate Age Determination

- **Alvarez, M. and Ballantyne, J. (2006) The identification of newborns using messenger RNA profiling analysis. *Anal. Biochem.* 357(1):21-34.**
- **From abstract:** In theory, it may be possible to determine patterns of gene expression that are age specific, thereby permitting the distinction among tissue samples originating from individuals of different ages (e.g., newborn, adolescent, middle-age, elderly). **We have discovered two novel isoforms of gamma hemoglobin messenger RNA, designated HBG1n and HBG2n, which exhibit an extremely restricted pattern of gene expression, being confined to newborn individuals.** Multiplex quantitative reverse transcription PCR (qRT-PCR) assays incorporating these novel mRNAs have been designed, tested, and evaluated for their potential forensic use. The results indicate that the assays provide the ability to determine whether a bloodstain originated from a newborn.

## Age of Bloodstain Deposition

- **Anderson, S., Howard, B., Hobbs, G.R., Bishop, C.P. (2005) A method for determining the age of a bloodstain. *Forensic Sci. Int.* 148(1):37-45**
- **From abstract:** If there were independent evidence that the biological sample was deposited at the time of the crime, then its age would reveal when the crime occurred. If the time of the crime were known through another means, then the age of the biological sample could potentially exclude the human source as a suspect. **We have used real-time reverse transcriptase PCR to show that the ratio between different types of RNA (mRNA versus rRNA) changes over time** in a linear fashion when dried human blood from eight individuals was examined over the course of 150 days.

## Determination of Body Fluid Type

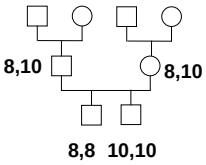
- **Juusola, J. and Ballantyne, J. (2005) Multiplex mRNA profiling for the identification of body fluids. *Forensic Sci. Int.* 152(1):1-12**
- **From abstract:** We report the development of a multiplex reverse transcription-polymerase chain reaction (RT-PCR) method for the **definitive identification of the body fluids that are commonly encountered in forensic casework analysis, namely blood, saliva, semen, and vaginal secretions**. Using selected genes that we have identified as being expressed in a tissue-specific manner we have developed a multiplex RT-PCR assay which is composed of eight body fluid-specific genes and that is optimized for the detection of blood, saliva, semen, and vaginal secretions as single or mixed stains. The genes include beta-spectrin (SPTB) and porphobilinogen deaminase (PBGD) for blood, statherin (STATH) and histatin 3 (HTN3) for saliva, protamine 1 (PRM1) and protamine 2 (PRM2) for semen, and human beta-defensin 1 (HBD-1) and mucin 4 (MUC4) for vaginal secretions.

## Partial Matching/Familial Searching

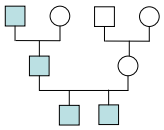
- Current searching software not designed for partial matches
- Need Y-STRs along with autosomal STR information to help sort through false positive matches obtained with single allele sharing hits
- See Bieber *et al.* (2006) Finding criminals through DNA of their relatives. *Science* 312:1315-1316

## Why Y-STRs Are Needed for Familial Searching

**Autosomal STRs**



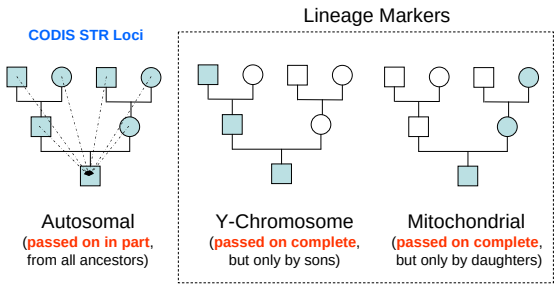

**Y-Chromosome STRs**



**Y-STRs match**

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

## Different Inheritance Patterns




Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition,* Figure 9.1, ©Elsevier Science/Academic Press

# Recent Defense Challenges to DNA

**Forensic Bioinformatics 5th Annual Conference**
**The Science of DNA Profiling: A National Expert Forum**
August 11 - 13, 2006 (Dayton, OH)

http://www.bioforensics.com/conference06/index.html

**Defense Expert Training**

**Friday, August 11**

**Session I: Mitochondrial DNA profiling: Dan Krane, Mitch Holland, Norah Rudin, Bill Shields, Jason Eshleman**

**Session II: Objective characterization of STR testing results: Keith Inman, Carrie Rowland, Simon Ford, Dan Krane, Bill Shields, Thaddeus Tarpey, Jason Gilder**

**Saturday, August 12**

**Session III: DNA profile database statistics: Larry Mueller, Fred Bieber, David Balding, Jason Gilder, Dan Krane**

**Session IV: Laboratory oversight and reform: Michael Saks, Paul Giannelli, Norah Rudin, Dan Krane, Bill Thompson**

**Sunday, August 13**

**Session V: Psychological aspects of DNA evidence: Keith Inman, Bill Thompson, Michael Saks, David Balding, Jay Koehler**

## Common Defense Attacks

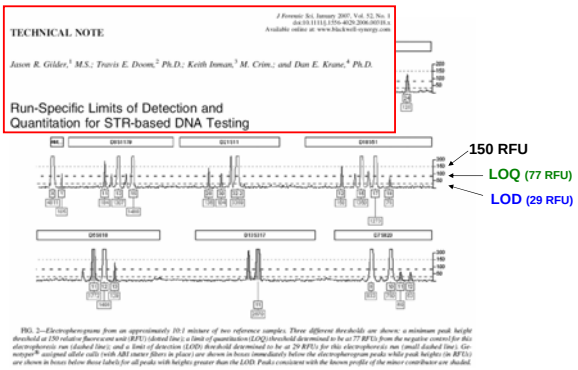
**Compiled from Forensic Bioinformatics website**

forensic bioinformatics
http://www.bioforensics.com

- Contamination
- Statistical Weight of a Match
- Degradation/PCR Inhibition of "True" Perp
- Artifacts (N+4 stutter, etc.)
- **Thresholds Set Too High (missing peaks)**
- Examiner Bias
- **Improper Mixture Interpretation**
- Meaning of a Database Hit
- Protocol Violations

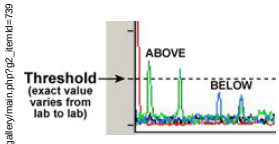
**See http://www.bioforensics.com/conference07/index.html**

## Different Thresholds of Detection Influence Allele Calls




Gilder, J.R., Doom, T.E., Inman, K., Krane, D.E. (2007) Run-specific limits of detection and quantitation for STR-based DNA testing. *J. Forensic Sci.* 52(1): 97-101.

## Detection Thresholds




http://projects.nfstc.org/gallery/main.php?g2_itemId=739

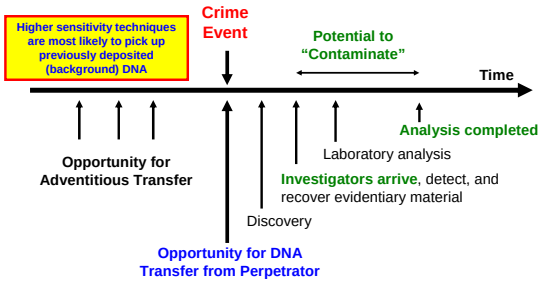
- Thresholds are set to separate signal from noise – in other words, are we confident that a peak is real?
- Signal peak height is measured in relative fluorescence units (RFUs) that are related to the amount of DNA present in the sample loaded onto the analysis instrument

Detection thresholds typically vary from 50 RFU to 200 RFU

## DNA Testing Has Become Extremely Sensitive…

- What does it mean to obtain a DNA match between a suspect and material from a crime scene?
- Is the fact that a DNA profile obtained mean that this information is probative?
- More complicated samples (mixtures) and more items per case being submitted to labs

## Time Line Showing the Potential for DNA Deposition/Transfer




Adapted from Gill, P. (2002) *BioTechniques* 32(2): 366-385, Figure 5

## Checks and Controls on DNA Results

| | |
|---|---|
| Community | FBI DNA Advisory Board's Quality Assurance Standards *(also interlaboratory studies)* |
| Laboratory | ASCLD/LAB Audits and Accreditation |
| Analyst | Proficiency Tests & Continuing Education |
| Method/Instrument | Validation of Performance *(along with traceable standard sample)* |
| Protocol | Standard Operating Procedure is followed |
| Data Sets | Allelic ladders, positive and negative amplification controls, and reagent blanks are used |
| Individual Sample | Internal size standard present in every sample |
| Interpretation of Result | Second review by qualified analyst/supervisor |
| Court Presentation of Evidence | Defense attorneys and experts with power of discovery requests |

## Some Final Thoughts

- "DNA" + "Match" ➔ "Guilty" in the minds of many jurors
- Be careful to state assumptions going into the weight of the evidence particularly for mixtures
- General population (i.e., jury pool) is becoming more informed regarding DNA testing thanks to genetic genealogy and TV shows like CSI
- Low-level DNA recovered from a crime scene may not be relevant to the committed crime

## STRBase

**Short Tandem Repeat DNA Internet Database**

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

| General Information | Forensic Interest Data | Supplemental Info |
|---|---|---|
| •Intro to STRs (downloadable PowerPoint) | •FBI CODIS Core Loci | •Reference List >2500 |
| •STR Fact Sheets | •DAB Standards | •Technology Review |
| •Sequence Information | •NIST SRMs 2391 | •Addresses for Scientists |
| •Multiplex STR Kits | •Published PCR Primers | •Links to Other Web Sites |
| •Variant Allele Reports | •Y-Chromosome STRs | •DNA Quantitation |
| •Training Slides | •Population Data | •mtDNA |
| | •Validation Studies | •New STRs |
| | •miniSTRs | •Forensic SNPs |

*New information is added regularly…*

## Conclusions

- This is an exciting time to be involved in forensic DNA testing
- However, it is a little scary because technology is advancing so rapidly on some fronts
- Thus, training for both the scientific and legal communities is vital to make the most effective use of the wonderful power of DNA technology