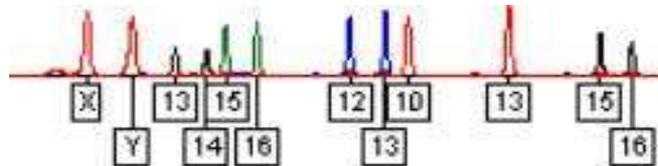


The International Plant & Animal Genome XXII Conference
January 13, 2014
San Diego, CA

Impact of Genomics in Forensic DNA Testing

John M. Butler

National Institute of Standards and Technology



Presentation Outline

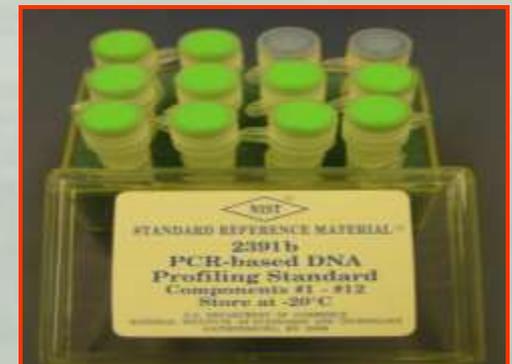
- NIST background and role in forensic science
- Brief history of forensic DNA
- Methods and applications of forensic DNA
- Challenges faced by forensic DNA
- Impact of genomics in forensic DNA
- Thoughts on the near-term future of forensic DNA

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

NIST Today

Major Assets

- ~ 2,900 employees
- ~ 2600 associates and facilities users
- ~ 400 NIST staff on about 1,000 national and international standards committees
- **4 Nobel Prizes in Physics in past 15 years**
(including 2012 to David Wineland for quantum physics)



Major Programs

- **NIST Laboratories**
- Baldrige National Quality Program
- Hollings Manufacturing Extension Partnership
- Technology Innovation Program

Joint NIST/University Institutes:

- JILA
- Joint Quantum Institute
- Institute for Bioscience & Biotechnology Research
- Hollings Marine Laboratory

NIST Reference Materials for Forensic DNA Measurement Assurance



Margaret Kline



DNA quantity measurement calibration

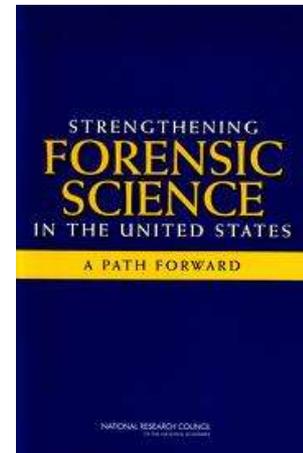


Autosomal and Y-chromosome short tandem repeat (STR) measurement calibration



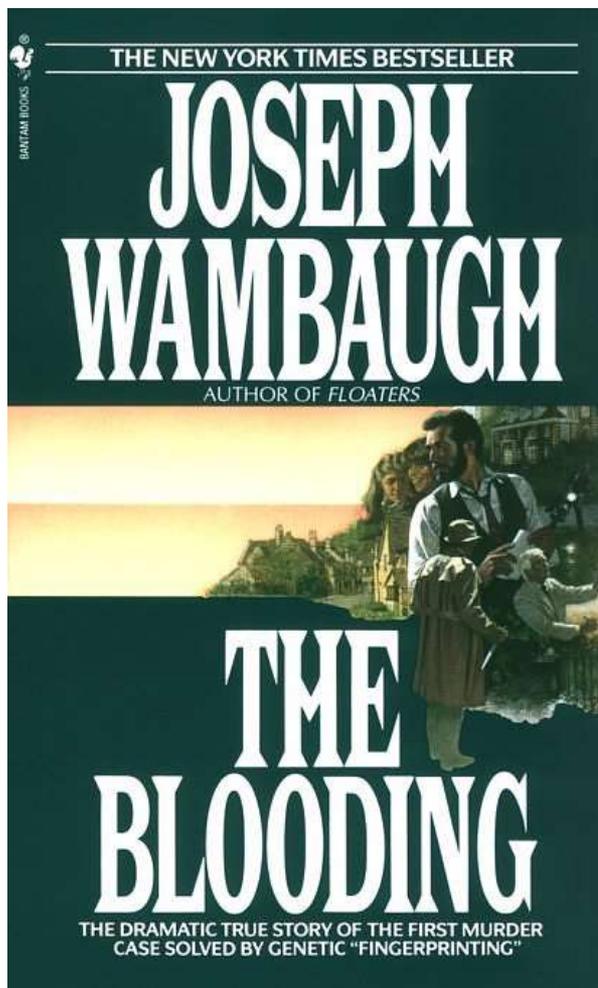
Harry T. Edwards
U.S. Court of Appeals (DC)
Co-Chair, Forensic Science Committee

National Academies Report on Forensic Science



- Released February 18, 2009
- Entitled “Strengthening Forensic Science in the United States: A Path Forward”
- 13 recommendations provided to Congress
- **Recommends establishing a National Institute of Forensic Science (NIFS)**
- NIST and the U.S. Department of Justice announced plans on February 15, 2013 to establish a **National Commission on Forensic Science**

Lessons from the First Case Involving DNA Testing



Describes the first use of DNA (in 1986) to solve a double rape-homicide case in England; about 5,000 men asked to give blood or saliva to compare to crime stains

- Connection of two crimes (1983 and 1986)
- Use of DNA database to screen for perpetrator (DNA only done on 10% with same blood type as perpetrator)
- Exoneration of an innocent suspect
- DNA was an investigative tool – did not solve the case by itself (confession of accomplice)

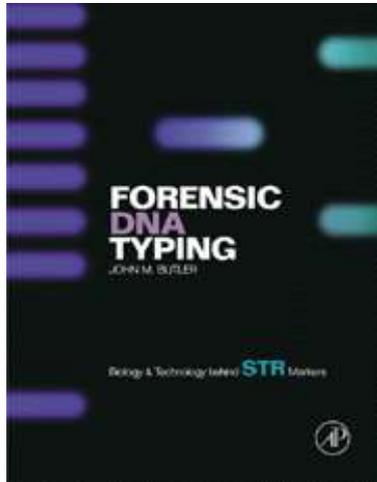
A local baker, Colin Pitchfork, was arrested and his DNA profile matched with the semen from both murders. In 1988 he was sentenced to life for the two murders.

Forensic DNA Typing Textbooks Have Set the Standard for the Field

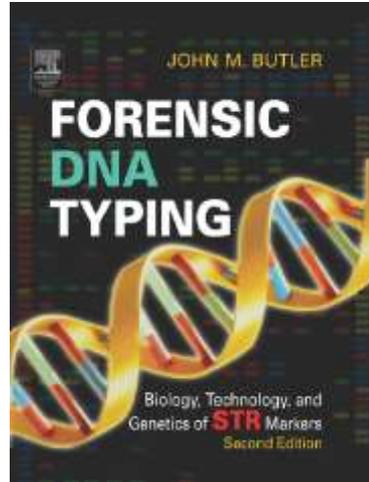
1st Edition

2nd Edition

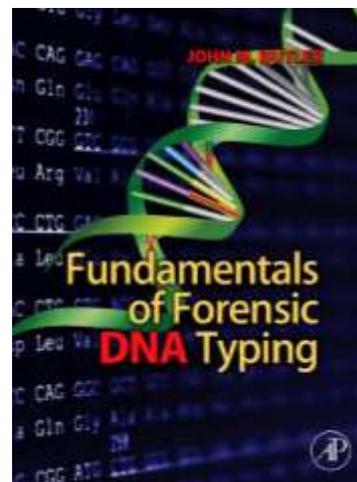
3rd Edition (3 volumes)



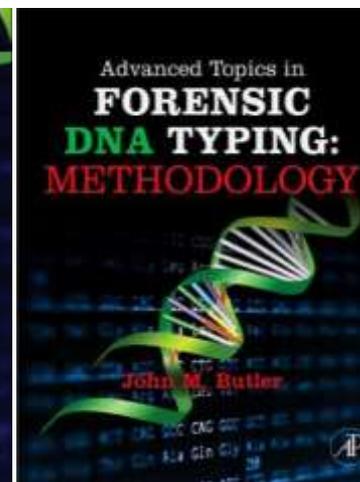
Jan 2001
335 pages



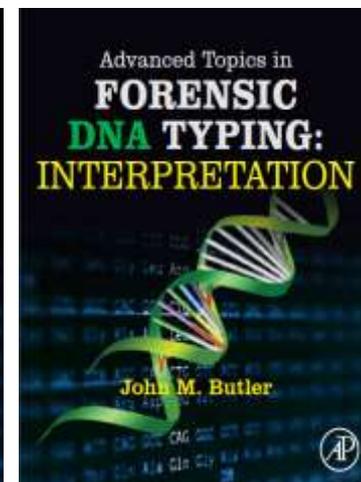
Feb 2005
688 pages



Sept 2009
520 pages



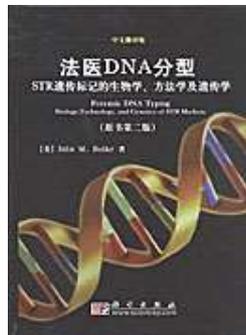
Aug 2011
704 pages



Fall 2014
(being written)
~500 pages

Language Editions

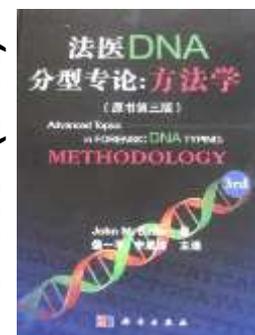
Chinese (2007)



Japanese (2009)

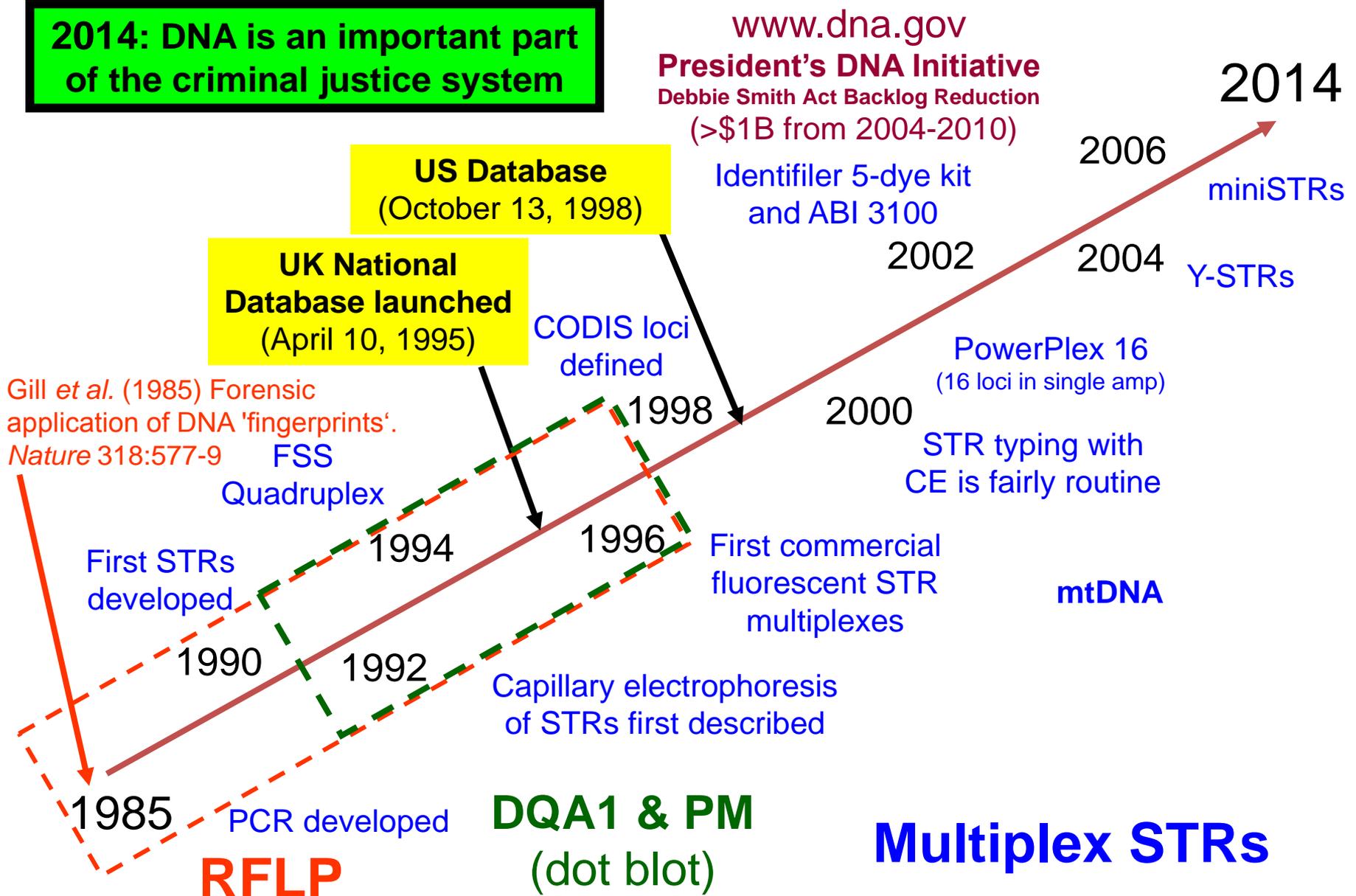


Chinese (2013)



History of Forensic DNA Testing

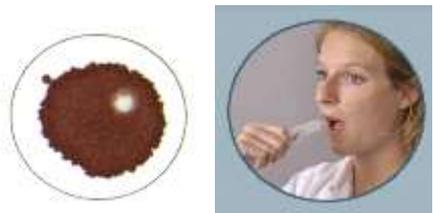
2014: DNA is an important part of the criminal justice system



Stages of Forensic DNA Progression

Stages	Time Frame	Description
Exploration	1985-1995	Beginnings, different methods tried (RFLP and early PCR)
Stabilization	1995-2005	Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards
Growth	2005 - present	Rapid growth of DNA databases, extended applications pursued
<i>Sophistication</i>	<i>The Future</i>	<i>Expanding tools available, confronting privacy concerns</i>

Steps in Forensic DNA Testing

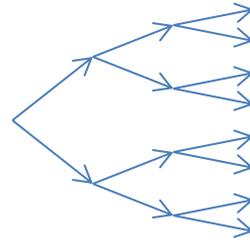


Blood Stain Buccal swab

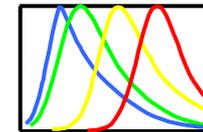
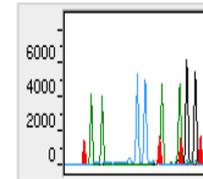
Sample Collection
& Storage



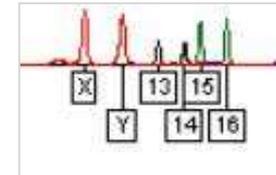
DNA Extraction
& Quantitation



Multiplex PCR
Amplification of
STR Markers



CE with LIF
Detection



Male: 13,14-15,16-...

Data Interpretation ,
Review & Reporting



GeneAmp 9700
Thermal Cycler



ABI 3500
Genetic Analyzer
capillary electrophoresis



GeneMapper ID-X
software

Steps in Forensic DNA Testing



- Sample Collection/Storage
- Extraction
- Quantitation
- **PCR Amplification**
- Separation/Detection
- Interpretation
- Comparison
- Reporting
- Profile Storage

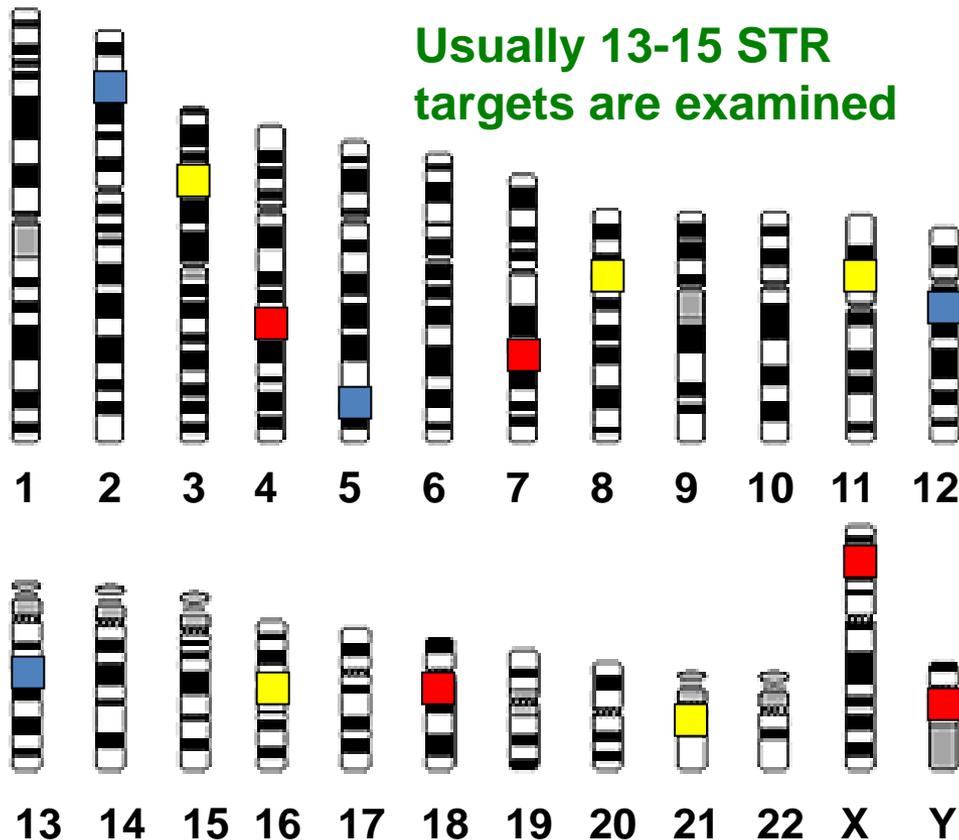
Genome research has benefited information on marker sets, PCR amplification, and improved detection methods

The NIST Applied Genetics Group has research efforts impacting all of these steps

A DNA profile comes from only a small part of the human genome

Human Genome

23 Pairs of Chromosomes (~3 billion bp)

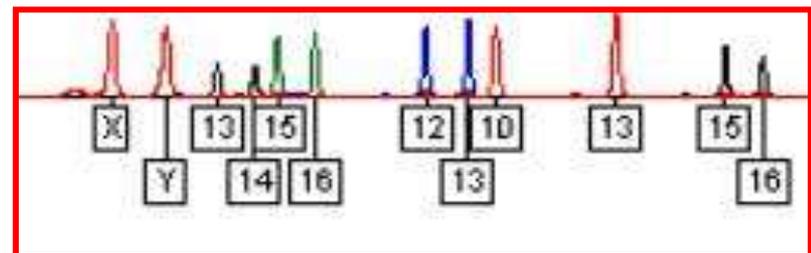


Unique regions of the human genome are targeted

These regions consist of a few hundred base pairs

The regions are copied by the **polymerase chain reaction (PCR)** – billions of exact copies are made

The copied fragments are labeled with fluorescent dyes for detection purposes

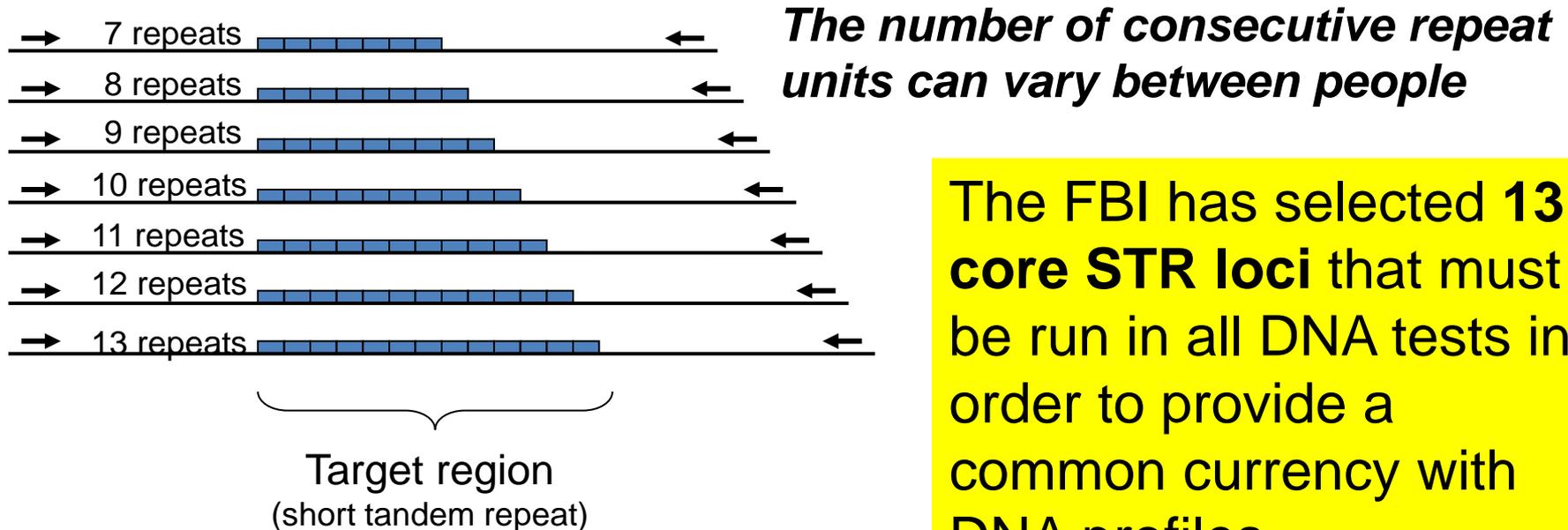


Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

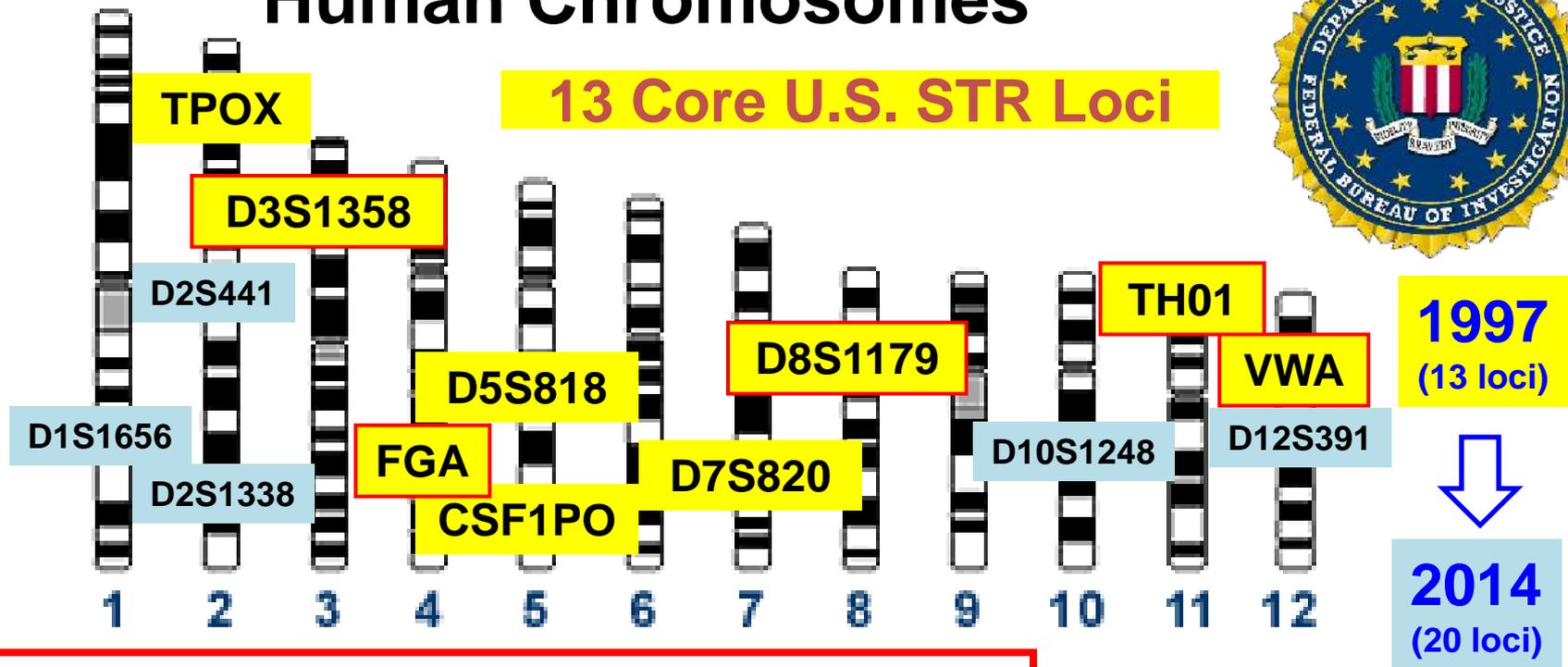
TCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTGGATAGATAGATAGATAGATAGATAGATAGATAGATA
GATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGATACA
TGCTTACAGATGCACAC

= 12 GATA repeats (“12” is all that is reported)

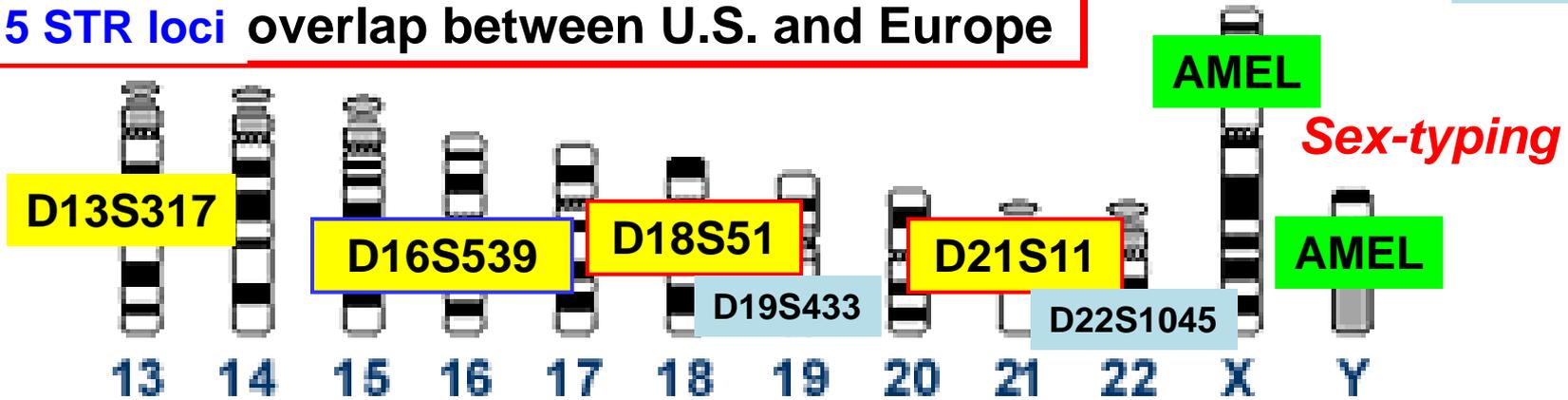


The FBI has selected **13 core STR loci** that must be run in all DNA tests in order to provide a common currency with DNA profiles

Position of Forensic STR Markers on Human Chromosomes



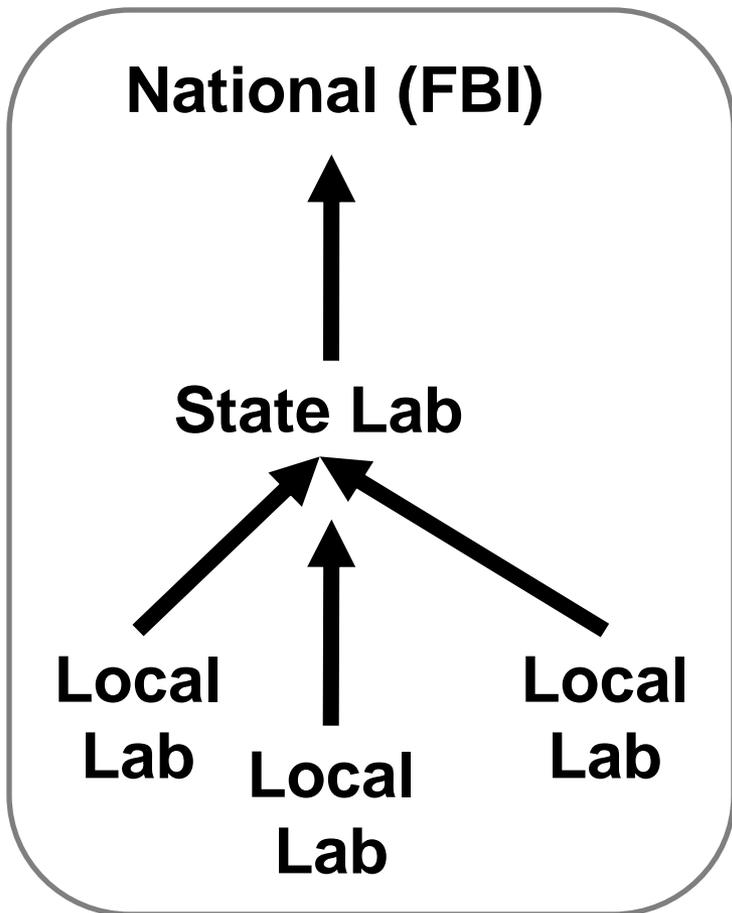
15 STR loci overlap between U.S. and Europe



U.S. National DNA Database

National DNA Index System (NDIS)

CODIS Levels



- CODIS = Combined DNA Index System
- **190 public labs (government)**
 - 136 local
 - 54 state (FBI, DoD, DC, Puerto Rico)
- About **a dozen private labs** contribute data that must be reviewed and approved by public labs prior to upload

Current U.S. National DNA Database

As of November 2013, almost **13 million samples**

- 10,692,400 offender DNA profiles
- 1,711,100 arrestee DNA profiles
- 527,400 forensic profiles

As of Sept 2013,
China had 417 labs
and >18 million STR
profiles in their DNA
database

- Has produced **228,500 hits** to help solve cases
- <http://www.fbi.gov/about-us/lab/biometric-analysis/codis/ndis-statistics>

Growth of DNA Databases

- Expanded laws now enable more offenders to be included (28 states and federal government collect from arrestees)
 - Has contributed to sample backlogs
- Have benefited from significant federal funding since 2004 (>\$1 billion for backlog reduction)
- **Have effectively locked technology with core STR markers used** to generate DNA profiles that now number in the millions

U.S. Supreme Court
decision (June 2013)
in *Maryland v King*

Applications for DNA Testing

- **Crime solving** – matching suspect with evidence...
- **Accident victims** – after airplane crashes...
- **Soldiers in war** – who is the “unknown” soldier...
- **Paternity testing** – who is the father...
- **Immigration testing** – are two people related...
- **Missing persons investigations** – whose remains...
- **Convicted felons databases** – cases solved...

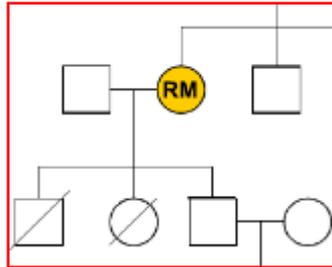
Involves generation of DNA profiles usually with the same core STR (short tandem repeat**) markers and then **MATCHING TO REFERENCE SAMPLE****

DNA Analysis Requires Comparisons

(Unknowns are compared to references samples)

Kinship (Indirect) Reference

Biological relatives of victims



Several family reference samples often required to make an identification

Direct Reference

Personal effects of victims



or



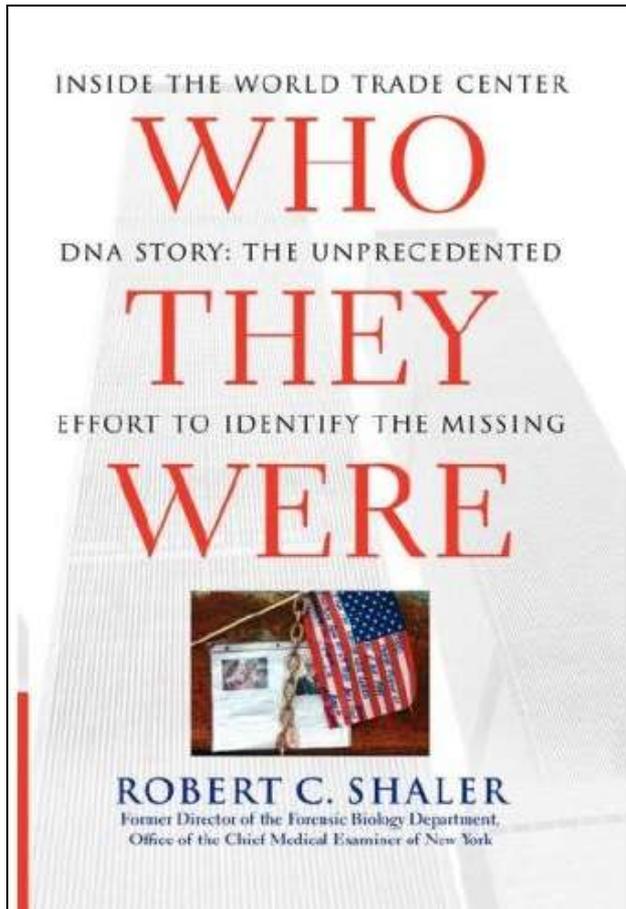
Reference Sample

Unknown Sample

- DNA results from human remains (unknown sample) are compared against DNA results from samples of known origin (reference samples)
- If a match occurs, statistical analysis is performed and a report is issued to the appropriate individual



World Trade Center Victims Remains Identified with DNA



Free Press (2005)

Statistics (July 26, 2004)

Total Reported Missing:	2,749
Number of Remains:	19,915
Number Identified:	1,560 (5 pending)
Whole Bodies Recovered:	239

WTC MFISys Statistics (4.30.2004)

52,528 STR profiles (including miniSTR data)
31,155 mtDNA sequences
10,799 SNP profiles

The Largest Forensic Case in History...

POLICY FORUM

EPIDEMIOLOGY

Science (2005) 310: 1122-1123

DNA Identifications After the 9/11 World Trade Center Attack

Leslie G. Biesecker, Joan E. Bailey-Wilson, Jack Ballantyne, Howard Baum,
Frederick R. Bieber, Charles Brenner, Bruce Budowle, John M. Butler,
George Carmody, P. Michael Conneally, Barry Duceman, Arthur Eisenberg,
Lisa Forman, Kenneth K. Kidd, Benoît Leclair, Steven Niezgoda, Thomas J. Parsons,
Elizabeth Pugh, Robert Shaler, Stephen T. Sherry, Amanda Sozer, Anne Walsh

**Human genomics researchers who contributed significantly
to the WTC Kinship and Data Analysis Panel (KADAP) efforts**

The Los Angeles “Grim Sleeper” Case

DNA Familial Searching’s Biggest Success Story

Ballistics on bullets recovered from multiple victims’ bodies matched

DNA evidence recovered and matched crime scene-to-crime scene (July 2003 & Jan 2007)
(“Grim Sleeper” DNA profile)

CA database and national **database**
search yielded no match

First familial search of CA database (Oct 2008)
yields no result

Christopher Franklin arrested on weapons charge in early 2009 and had his DNA sample put on the CA DNA database

Second familial search of CA database (June 2010)
Grim Sleeper profile matches C. Franklin’s profile with one allele at all 15 loci & follow-up Y-STR test matches

Investigators develop **C. Franklin’s father** as potential suspect due to age and proximity to crime scenes

Detectives follow L. Franklin and collect napkin and left-over pizza slice for DNA comparison

L. Franklin profile matches “Grim Sleeper” profile

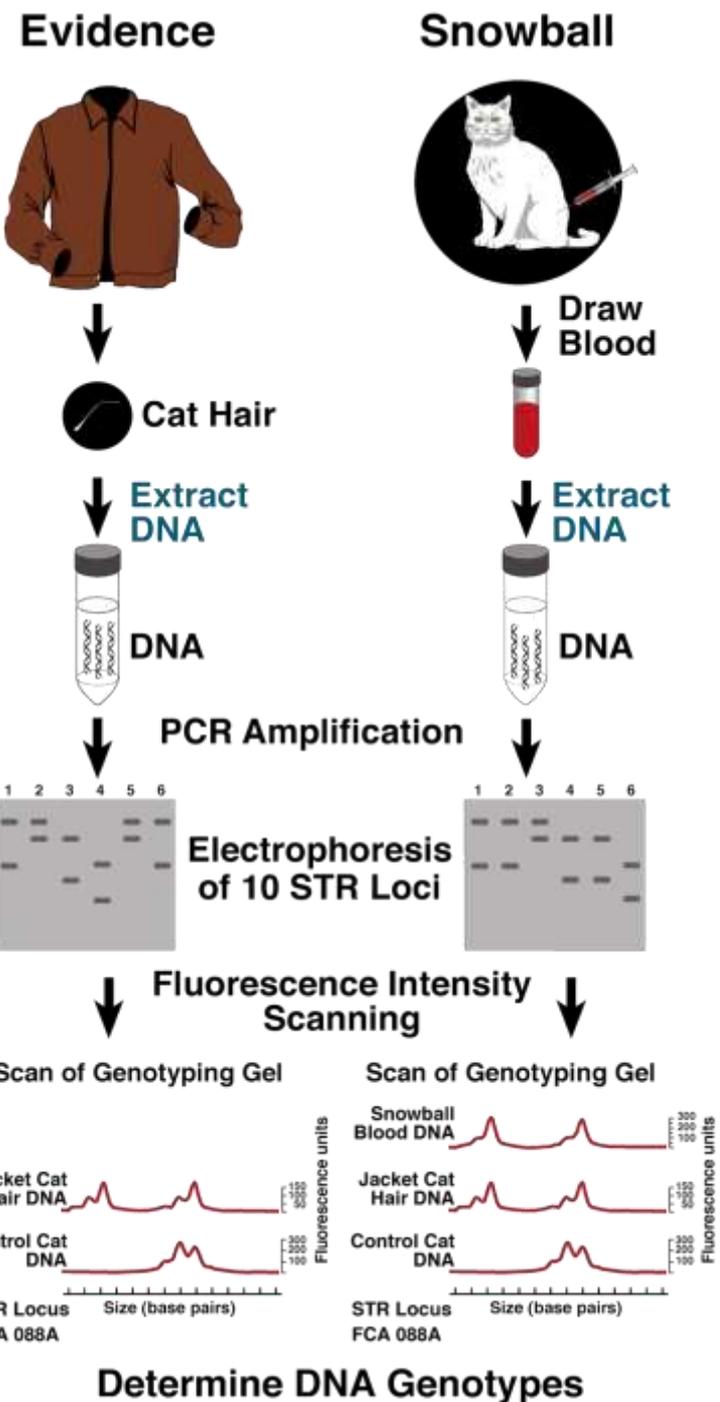
Arrested July 2010
and awaiting trial



Lonnie David Franklin Jr.

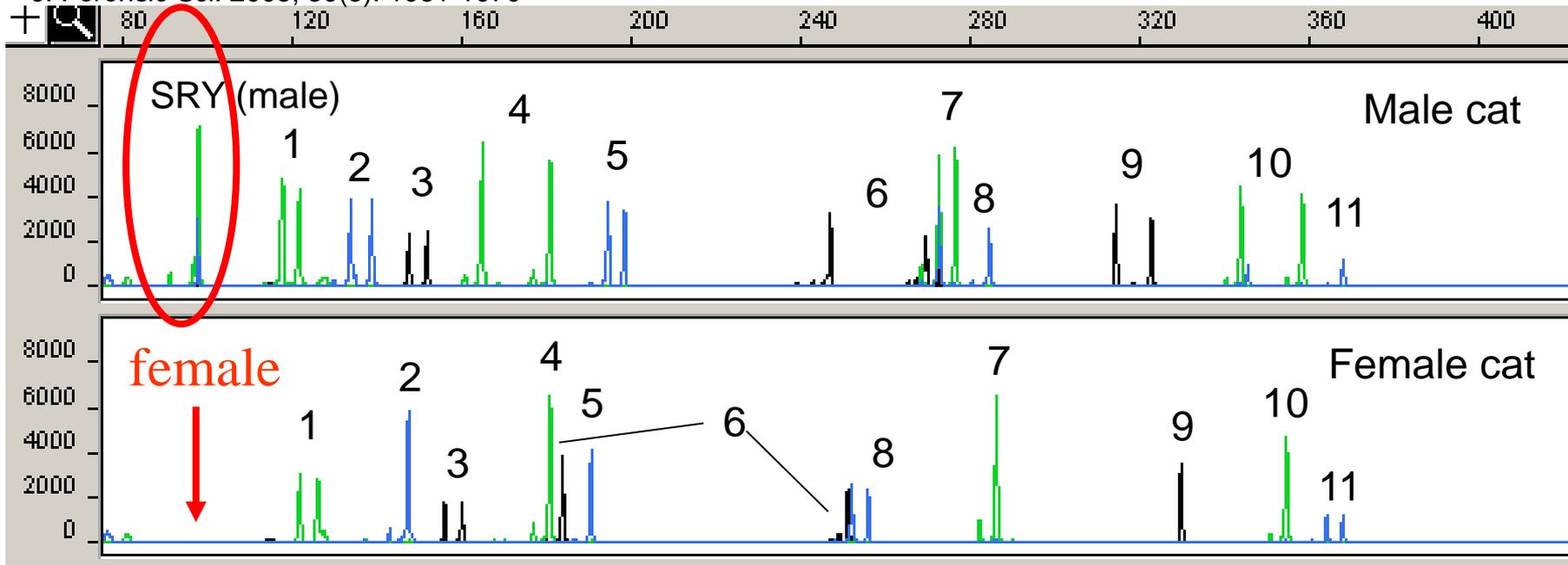
Using Cat DNA Evidence to Catch the Crook

In 1997, a man from Canada was put in prison because hairs from his cat matched those found on evidence at a crime scene...



New DNA Test for Cats Developed at NIST

J. Forensic Sci. 2005; 50(5): 1061-1070



PROFILES IN DNA

MEOWPLEX

The MeowPlex: A New DNA Test Using Tetranucleotide STR Markers for the Identification of Cat Breeds

By John M. Butler¹, Victor A. David², Stephen J. O'Brien², and Marilyn Menotti-Raymond²

¹Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland, and ²Laboratory of Genomic Diversity, National Cancer Institute, National Cancer Research and Development Center, Frederick, Maryland

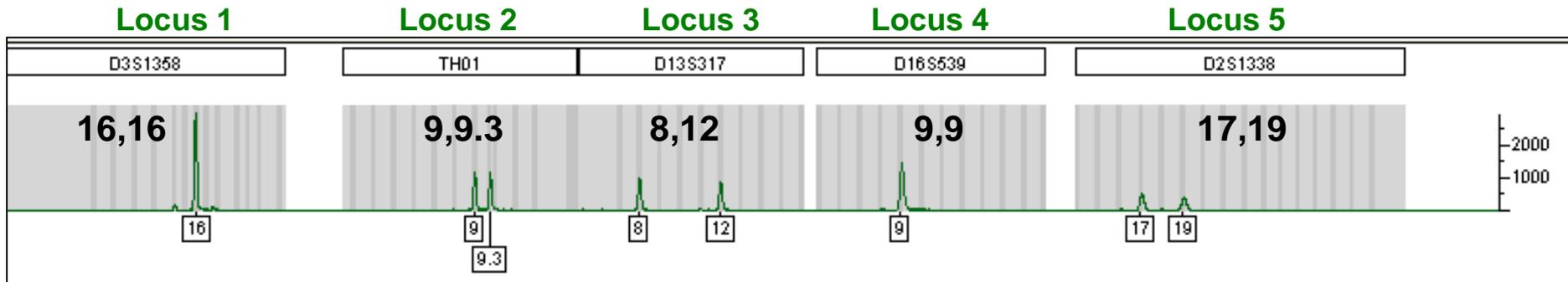


Challenges faced by forensic DNA

- **Limited DNA template**
 - Low quantity and often poor quality due to environmental damage
 - **Multiplex PCR targeting 15-25 STR loci**
- **DNA mixtures from multiple individuals**
 - Sexual assault evidence has both victim and perpetrator DNA mixed
 - **Benefit to multi-allelic STR loci (instead of SNPs)**
- **Court challenges with any new techniques**
 - Extensive validation required
 - **Commercial STR kits typically used**

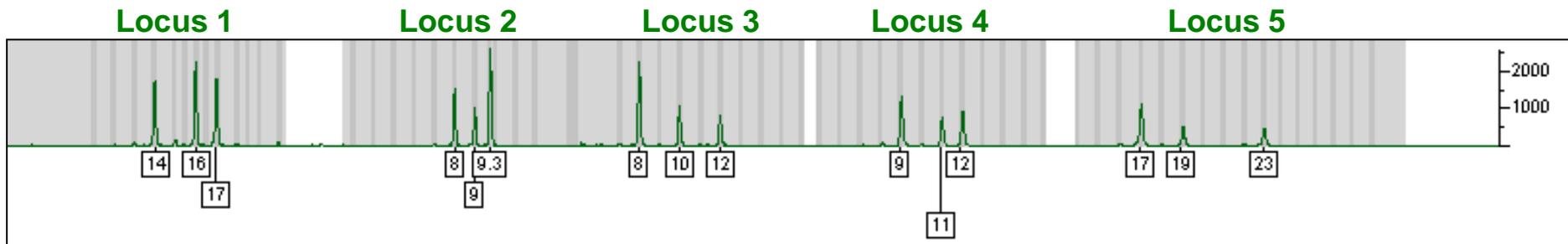
Single Source vs. Mixture Samples

Single Source Sample



One or two peaks observed at each locus (tested DNA region)

Mixture Sample

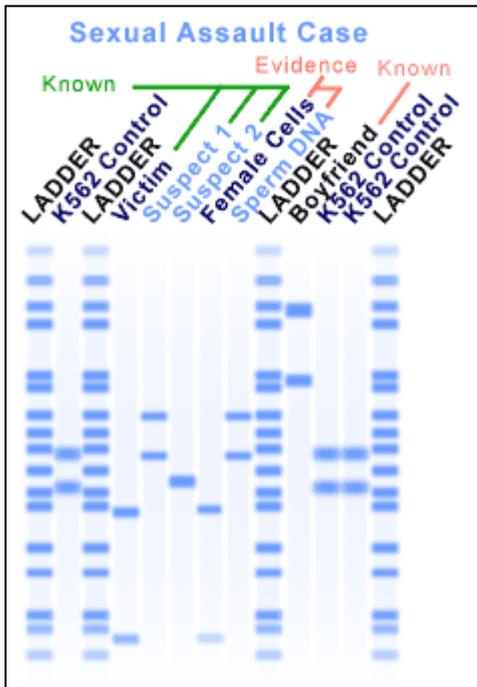


More than two peaks observed at more than two loci (tested DNA regions)

Different possible combinations could have given rise to the particular mixture observed

The DNA Field Moves Forward...

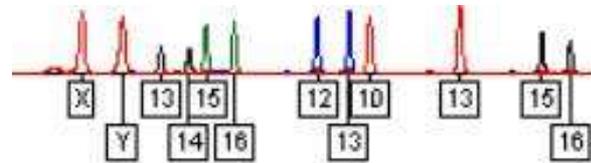
The Past



RFLP

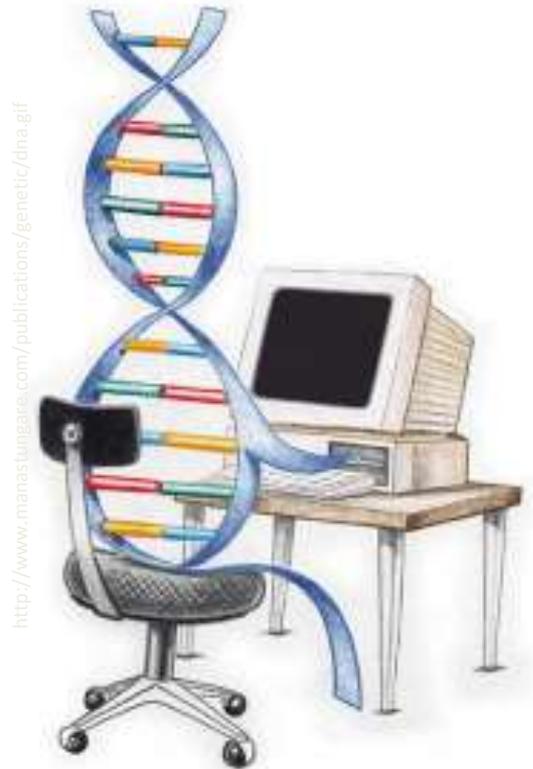
<http://www.bioteach.ubc.ca/MolecularBiology/DNAfingerprint/>

The Present



STRs

The Future



<http://www.manastungare.com/publications/genetic/dna.gif>

Rapid DNA Efforts



Pete Vallone Erica Butts

Accelerated Nuclear DNA Equipment (ANDE) developed by **NetBio**



<http://ishinews.com/wp-content/uploads/2012/10/Rapid-DNA-Miles-1.58MB.pdf>

RapidHIT 200 developed by **IntegenX**



<http://integenx.com/wp-content/uploads/2010/06/RapidHIT-200.png>

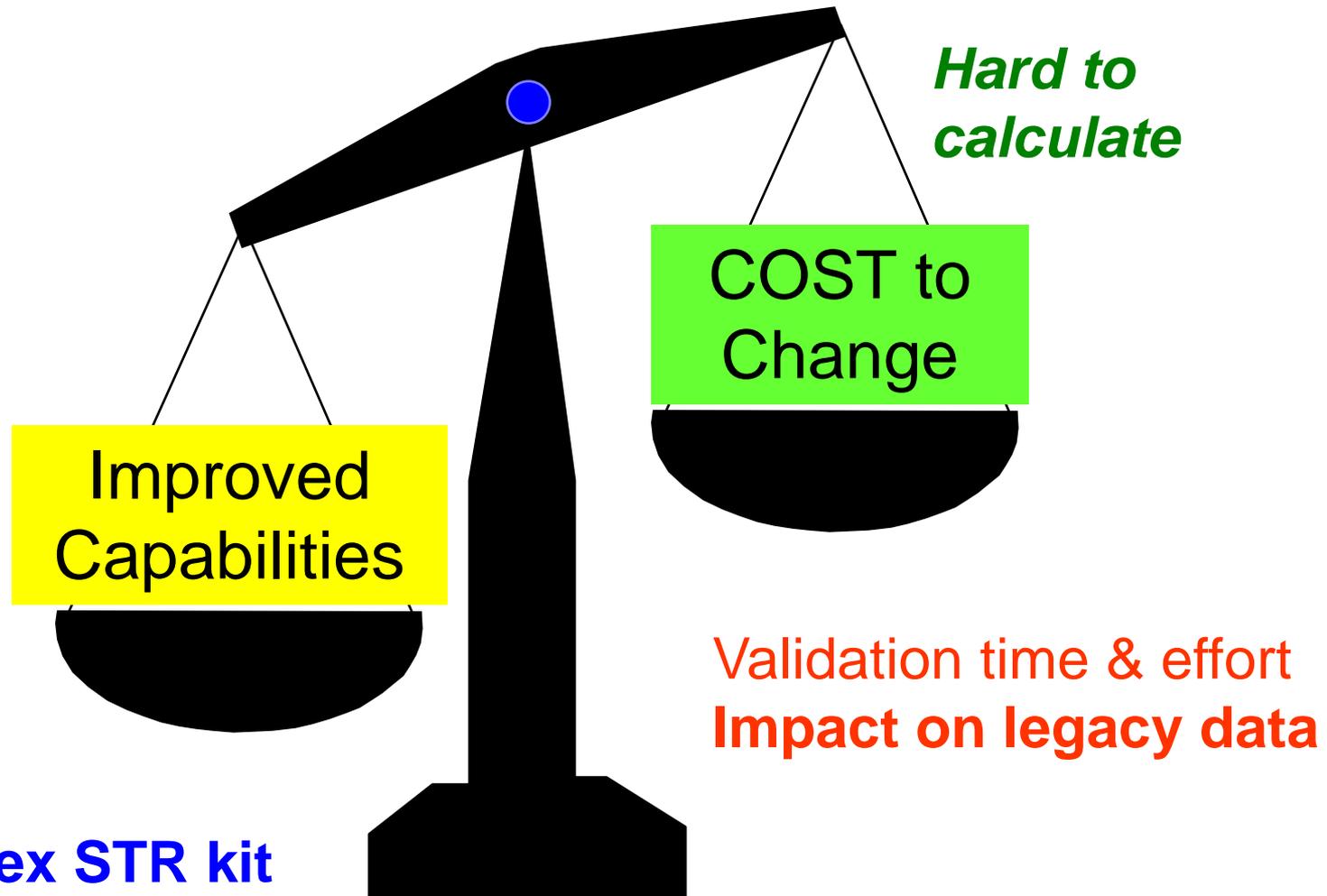
- Evaluating ANDE (NetBio) and IntegenX rapid DNA instruments
 - both instruments are capable of swab in → STR profile out in less than 90 minutes without user intervention
- Exploring rapid DNA techniques including direct PCR and rapid PCR
 - STR profiles generated in <2 hours with standard lab equipment and rapid protocols
 - See ISHI 2012 poster available on STRBase “Rapid DNA Testing Approaches for Reference Samples”

Fastest results swab-to-profile (Identifiler): 57 minutes

Impact of genomics in forensic DNA

- Human genome reference sequence has enabled discovery of numerous genetic markers
 - U.S. core loci in process of being expanded to additional short tandem repeat (STR) loci
- Plant and animal genome information likewise benefits forensic testing with these organisms
- Media attention from the ENCODE project has led to some confusion in court on whether or not STR genetic markers are no longer “junk” DNA
 - *State v. Abernathy* case in Vermont (June 2012)
 - See also Budowle, B. (2013) ENCODE and its first impractical application. *Investigative Genetics*, 4(4)

Decision to Switch/Upgrade to New Technology



New multiplex STR kit
New detection technology
New DNA markers

Next Generation Sequencing

Forensic Applications

- Going in depth **into** STR loci and beyond
 - STRs are useful for legacy (databases)
 - SNPs within STRs identify ‘sub-alleles’
 - Millions of bases of sequence variants (SNPs)
- Opens up new human identity applications: biogeographical ancestry, externally visible traits, complex kinship, **degraded samples, mixtures, other applications**

Applications are currently being addressed
by the forensic genetics community (*Kayser and deKnijff 2011*)

Specific issues with STRs

- Typically comprised of tetra nucleotide repeats
- Range 70 - 450+ bp regions
- Longer STRs can be difficult to assemble based on read length
- Illumina GAIIx (read length 150 bp)
 - Generated 1000-2500 bp amplicons (13 core loci)
 - Problems detecting D21S11 **32.2** and **34.2** alleles
 - Issues detecting long STR alleles in D18S51
 - Custom informatics tools for assembling STRs

Bornman et al., 2012 Biotechniques Rapid Dispatch: 1-6

Next Generation Sequencing

- Challenges
 - Repeating sequences (STRs) and read lengths
 - **Sample amount requirements (10 ng to 5 µg)**
 - **Cost** and **time** per unit of information
 - Data analysis (storage, assembly, interpretation)
 - **Policy, privacy, disease related markers**
 - Validation
 - Standards/reference materials
 - Nomenclature
 - Accuracy of sequence information
 - Errors, platform and bioinformatics-based bias

Next Generation Sequencing Workshop

- Interagency Workshop on the use of Next-Generation DNA Sequencing for Human Identification and Characterization (Jan 31 2012)
- Discussion of forensic applications of NGS (NIST, DoD, FBI, DHS) – materials can be found at:
 - http://www.nist.gov/mml/bmd/genetics/ngs_hid_workshop.cfm
- NIST and other researchers are looking at NGS platforms to characterize forensic markers (mitochondrial, STRs, SNPs)
- Evaluate accuracy, reproducibility, identify initial requirements for a NGS forensic reference material

The Future of Forensic DNA

is Similar to the Olympic Motto of
“Swifter, Higher, Stronger”



Resources

Training

Action

Acknowledgments



- Funding support from the U.S. National Institute of Justice and the FBI Laboratory



- Support from an excellent group of research scientists within the NIST Applied Genetics Group

Thank you for your attention

Contact Information

John M. Butler, Ph.D.

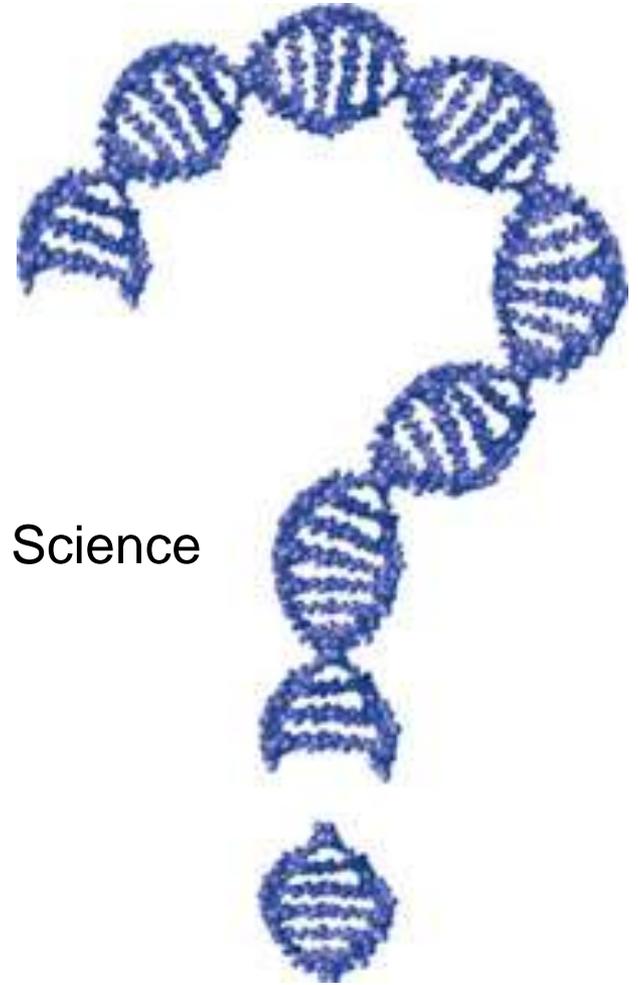
NIST Fellow

Special Assistant to the Director for Forensic Science

john.butler@nist.gov

301-975-4049

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



Our DNA publications and presentations are available at:
<http://www.cstl.nist.gov/strbase/NISTpub.htm>