Advances in the Biochemistry of DNA Fingerprinting Analysis

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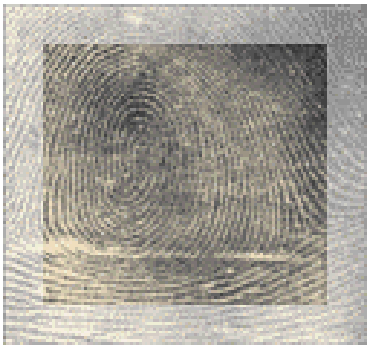
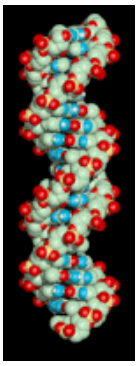
National Institute of Standards and Technology
Biotechnology Division

March 14, 2005
American Chemical Society Meeting

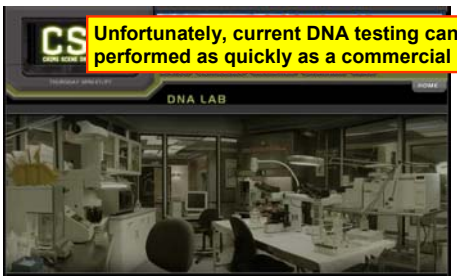
Methods for Human Identification



Fingerprints have been used since 1901



DNA since 1986



Unfortunately, current DNA testing cannot be performed as quickly as a commercial break…

Justice for All Act of 2004

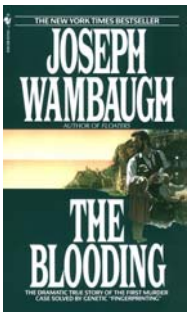
www.dna.gov

Presentation Outline

- Where has the field come in the past 20 years
- Current DNA typing methods and chemistry
- Comments on the future of forensic DNA

STR Typing

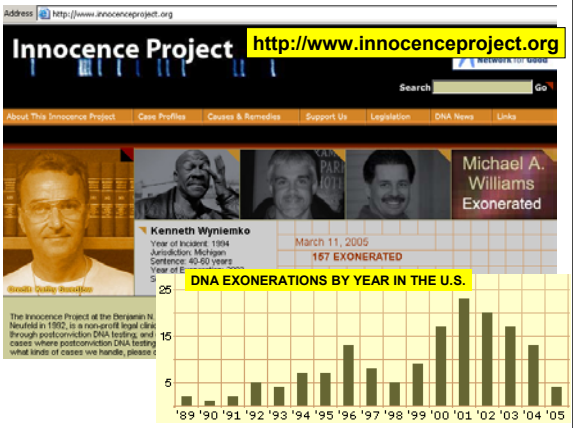
Lessons from the First Case Involving DNA Testing



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.



Uses for DNA Typing

- 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…**
- Inheritance claims – **who gets the money…**

All uses involve accurate measurement of DNA profiles and PATTERN MATCHING

Forensic DNA Testing

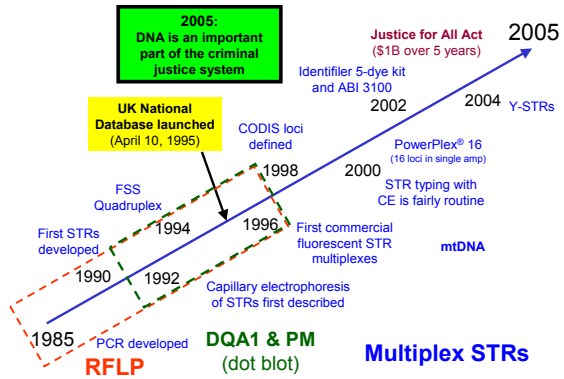
The genome of **each individual is unique** (with the exception of identical twins)

Probe subsets of genetic variation in order to differentiate between individuals (statistical probabilities of a random match are used)

DNA typing must be **performed efficiently and reproducibly** (information must hold up in court)

Current standard DNA tests **DO NOT look at genes** – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

Historical Perspective on DNA Typing



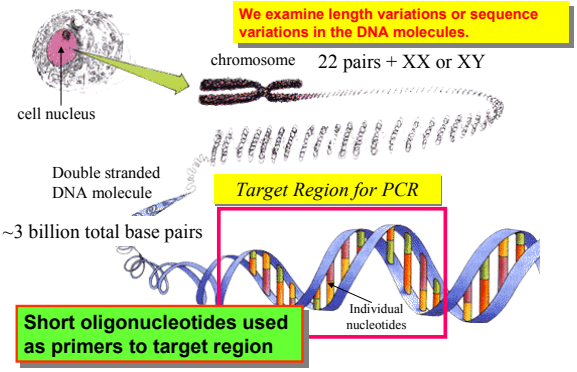
Sources of Biological Evidence

- **Blood**
- **Semen**
- **Saliva**
- **Urine**
- **Hair**
- **Teeth**
- **Bone**
- **Tissue**

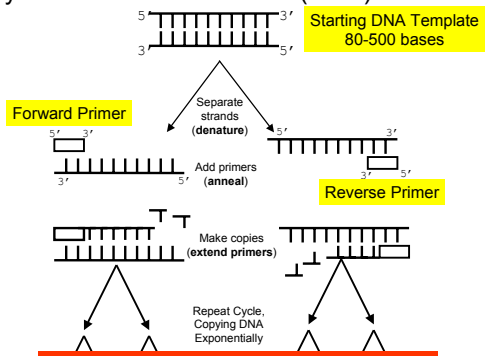
Blood stain

Only a very small amount of blood is needed to obtain a DNA profile

DNA in the Cell



Polymerase Chain Reaction (PCR) Process



In 32 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created

What Type of Genetic Variation?

More alleles are possible

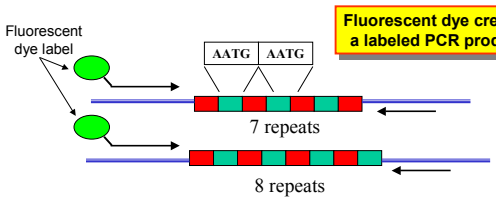
- Length Variation
  - short tandem repeats (STRs)

CTAGTCGT**(GATA)(GATA)(GATA)**GCGATCGT

- Sequence Variation
  - single nucleotide polymorphisms (SNPs)
  - insertions/deletions

GCTAGTCGATGCTC**(G/A)**GCGTATGCTGTAGC

Short Tandem Repeats (STRs)



the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

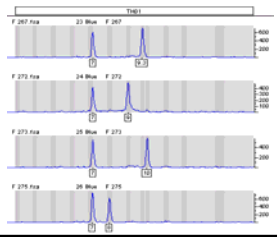
Homozygote = both alleles are the same length
Heterozygote = alleles differ and can be resolved from one another

Primer positions define PCR product size

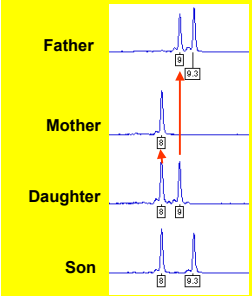
Variation of STRs Among Individuals

An allelic ladder, which is a mixture of common alleles, is used to convert DNA size to STR repeat number

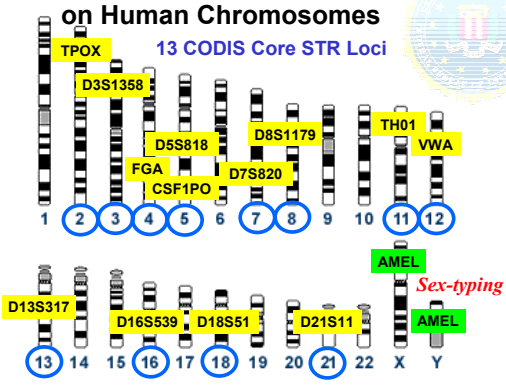
Unrelated Individuals

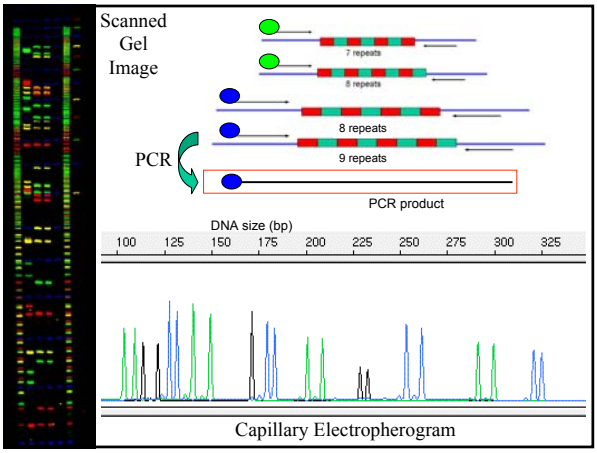


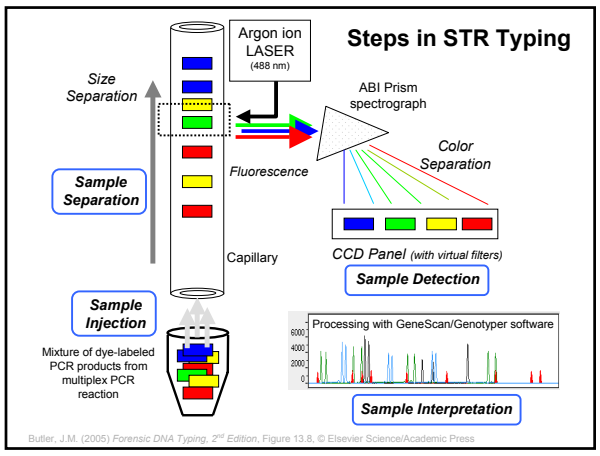
Familial Relationships Can Be Tracked

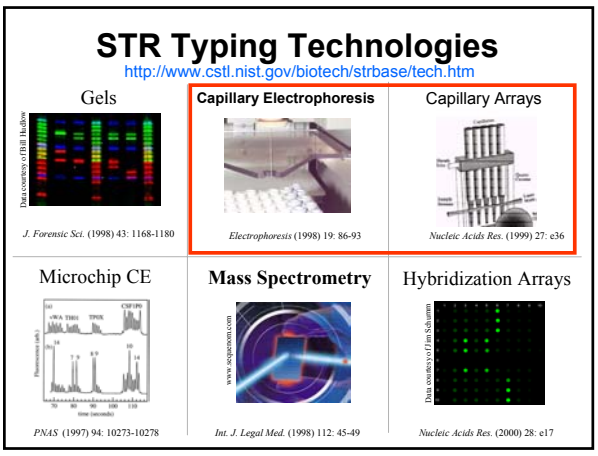


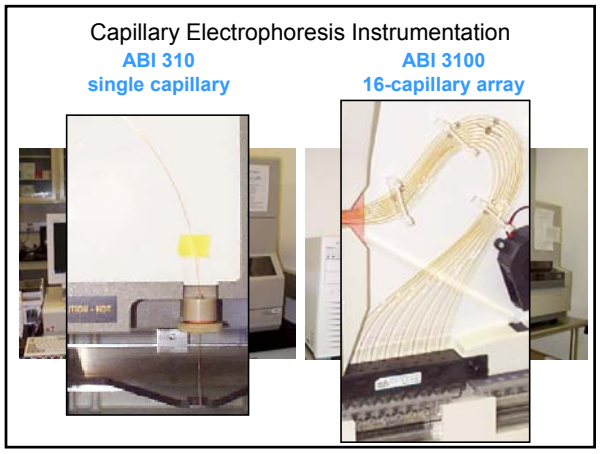
Position of Forensic STR Markers on Human Chromosomes

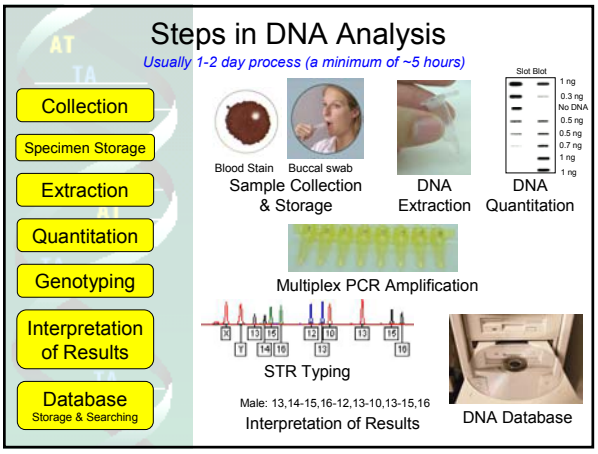


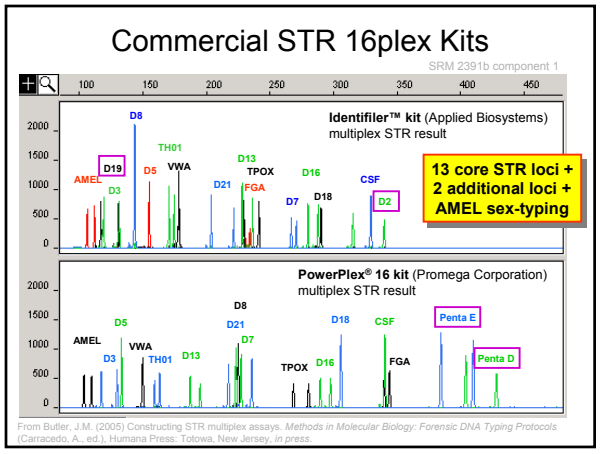


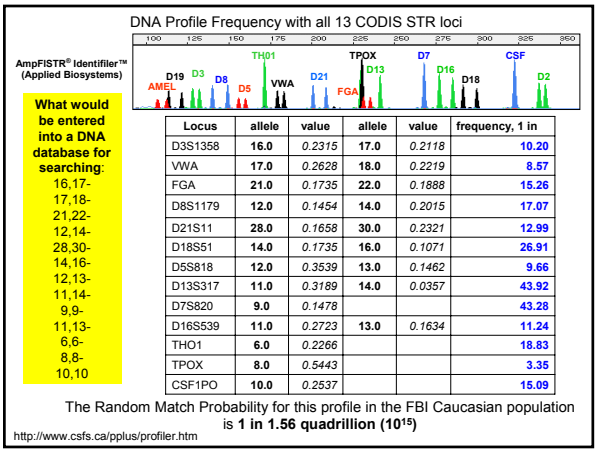

Steps in STR Typing
Argon ion LASER (488 nm)
Size Separation
ABI Prism spectrograph
Color Separation
Fluorescence
Sample Separation
Capillary
CCD Panel (with virtual filters)
Sample Detection
Sample Injection
Mixture of dye-labeled PCR products from multiplex PCR reaction
Processing with GeneScan/Genotyper software
Sample Interpretation
Butler, J.M. (2005) Forensic DNA Typing, 2nd Edition, Figure 13.8, © Elsevier Science/Academic Press


STR Typing Technologies
http://www.cstl.nist.gov/biotech/strbase/tech.htm
Gels
Capillary Electrophoresis
Capillary Arrays
J. Forensic Sci. (1998) 43: 1168-1180
Electrophoresis (1998) 19: 86-93
Nucleic Acids Res. (1999) 27: e36
Microchip CE
Mass Spectrometry
Hybridization Arrays
PNAS (1997) 94: 10273-10278
Int. J. Legal Med. (1998) 112: 45-49
Nucleic Acids Res. (2000) 28: e17


Capillary Electrophoresis Instrumentation
ABI 310
single capillary
ABI 3100
16-capillary array


Steps in DNA Analysis
Usually 1-2 day process (a minimum of ~5 hours)
Collection
Specimen Storage
Extraction
Quantitation
Genotyping
Interpretation of Results
Database
Storage & Searching
Blood Stain
Buccal swab
Sample Collection & Storage
DNA Extraction
Slot Blot
DNA Quantitation
Multiplex PCR Amplification
STR Typing
Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results
DNA Database


Commercial STR 16plex Kits
SRM 2391b component 1
Identifiler™ kit (Applied Biosystems) multiplex STR result
13 core STR loci + 2 additional loci + AMEL sex-typing
PowerPlex® 16 kit (Promega Corporation) multiplex STR result
From Butler, J.M. (2005) Constructing STR multiplex assays. Methods in Molecular Biology: Forensic DNA Typing Protocols (Carracedo, A., ed.), Humana Press: Totowa, New Jersey, in press.

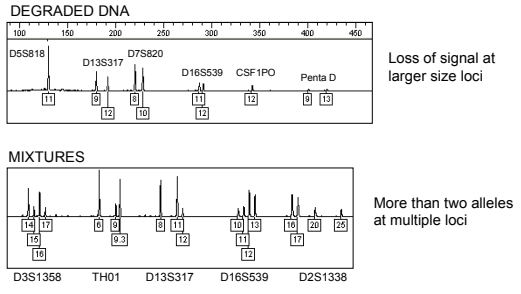

DNA Profile Frequency with all 13 CODIS STR loci
AmpFISTR® Identifiler™ (Applied Biosystems)
What would be entered into a DNA database for searching: 16,17-17,18-21,22-12,14-28,30-14,16-12,13-11,14-9,9-11,13-6,6-8,8-10,10
Locus | allele | value | allele | value | frequency, 1 in
D3S1358 | 16.0 | 0.2315 | 17.0 | 0.2118 | 10.20
VWA | 17.0 | 0.2628 | 18.0 | 0.2219 | 8.57
FGA | 21.0 | 0.1735 | 22.0 | 0.1888 | 15.26
D8S1179 | 12.0 | 0.1454 | 14.0 | 0.2015 | 17.07
D21S11 | 28.0 | 0.1658 | 30.0 | 0.2321 | 12.99
D18S51 | 14.0 | 0.1735 | 16.0 | 0.1071 | 26.91
D5S818 | 12.0 | 0.3539 | 13.0 | 0.1462 | 9.66
D13S317 | 11.0 | 0.3189 | 14.0 | 0.0357 | 43.92
D7S820 | 9.0 | 0.1478 | | | 43.28
D16S539 | 11.0 | 0.2723 | 13.0 | 0.1634 | 11.24
THO1 | 6.0 | 0.2266 | | | 18.83
TPOX | 8.0 | 0.5443 | | | 3.35
CSF1PO | 10.0 | 0.2537 | | | 15.09
The Random Match Probability for this profile in the FBI Caucasian population is 1 in 1.56 quadrillion (10^15)
http://www.csfs.ca/pplus/profiler.htm

Databank vs Casework Data Challenges

- Databank (single source samples)
  - Too much DNA may be added to the PCR reaction resulting in pull-up between dye colors
  - Lots of data to review – often produced by contractors
- Casework (mixtures or low level samples)
  - Often limited DNA material to work with
  - Low copy number samples can result in allele dropout
  - Can produce complicated STR profiles to interpret

Improved computer software for rapid data interpretation is really the biggest need currently

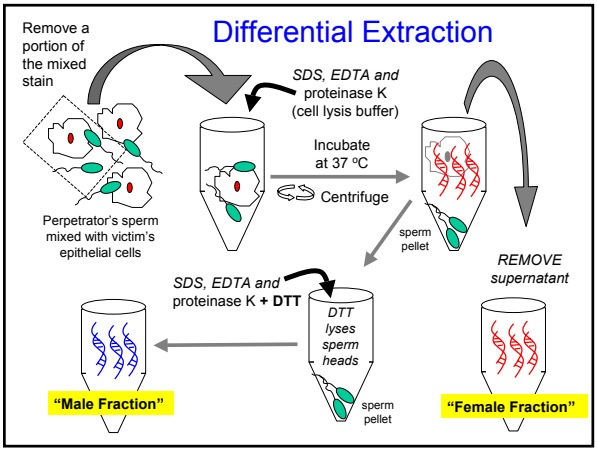
Common Casework Challenges



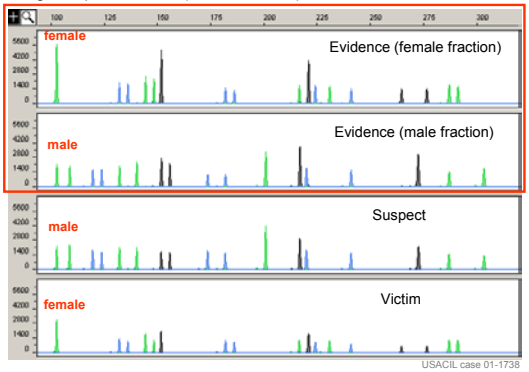
From Butler, J.M. (2004) Short tandem repeat analysis for human identity testing. *Current Protocols in Human Genetics*, John Wiley & Sons, Hoboken, NJ, Unit 14.8, *(Supplement 41)*, pp. 14.8.1-14.8.22

Advantages for STR Markers

- Small product sizes are generally compatible with degraded DNA and PCR enables recovery of information from small amounts of material
- Multiplex amplification with fluorescence detection enables high power of discrimination in a single test
- Commercially available in an easy to use kit format
- Uniform set of core STR loci provide capability for national and international sharing of criminal DNA profiles

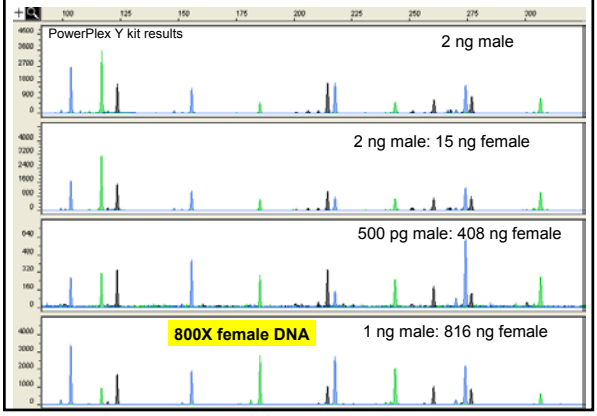


Differential extraction used to separate sperm (male fraction) from vaginal epithelial cells (female fraction)

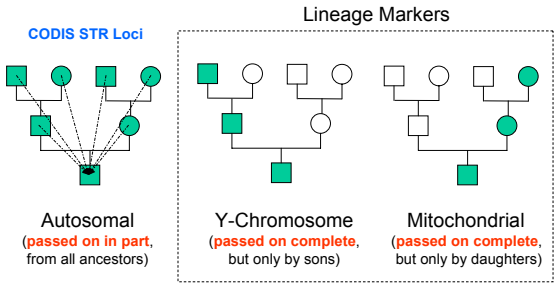


The four samples typically associated with a forensic DNA case…

Male-Specific Amplification in Presence of Excess Female DNA

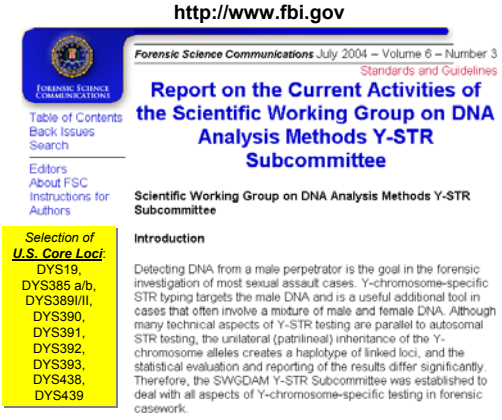


Different Inheritance Patterns



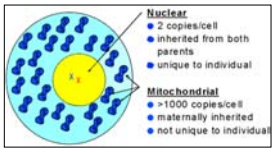
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.1, ©Elsevier Science/Academic Press

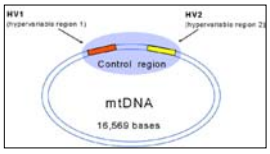
http://www.fbi.gov



Mitochondrial DNA

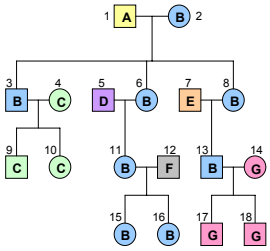
High copy number is the primary advantage for degraded DNA samples





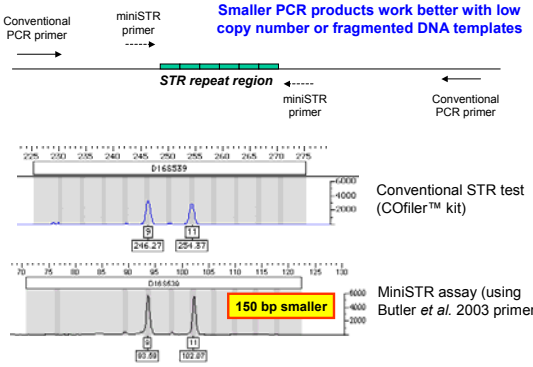
Isenberg et al. (1999) Forensic Sci. Comm.

mtDNA Inheritance Patterns



Maternal relatives have the same mtDNA (barring mutation)

miniSTRs: new tool for degraded DNA

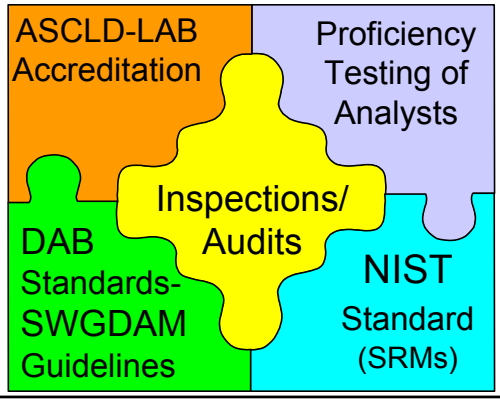


Future Methods Used in DNA Analysis

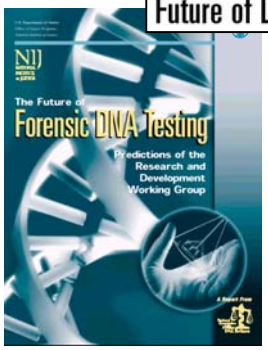
- Improved capabilities for multiplex analysis (parallel processing of genotypes)
- More rapid separation/detection technology (higher throughputs)
- More automated sample processing and data analysis
- Improved sensitivities and resolution
- Less expensive sample analysis

We must maintain accurate and robust methods

Ensuring Accurate Forensic DNA Results



National Commission on the Future of DNA Evidence



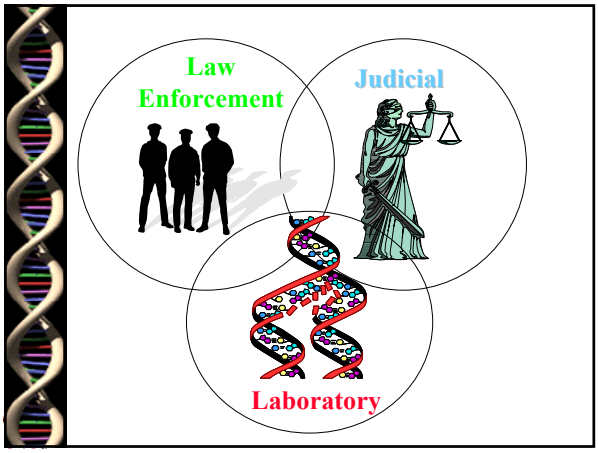
•Report published in Nov 2000

•Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

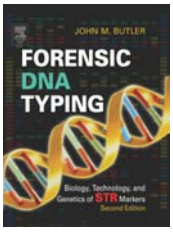
STR typing is here to stay for a few years because of DNA databases

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



If you want to know more…

- *Forensic DNA Typing: Biology and Technology behind STR Markers*
- NIST website: http://www.cstl.nist.gov/biotech/strbase
- John Butler email: john.butler@nist.gov



STRBase

Thank you for your attention…

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

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Marilyn Raymond (NCI)
Mecki Prinz (NYC OCME)
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