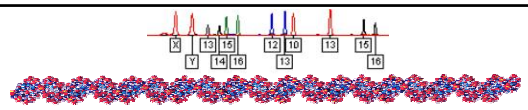


DNA and THE LAW

Brian Zubel, J.D.
and
John M. Butler, Ph.D.
National Institute of Standards and Technology

Frankenmuth, MI
May 4, 2007

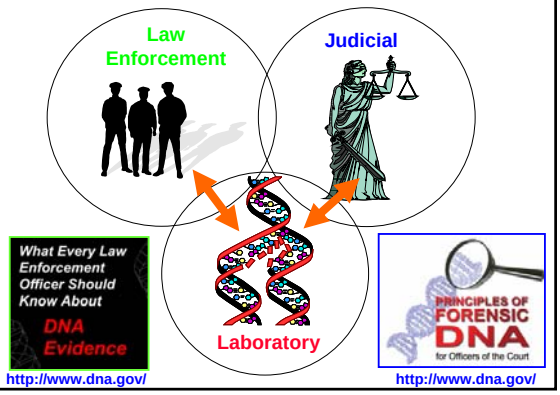
NIST and NIJ Disclaimer

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

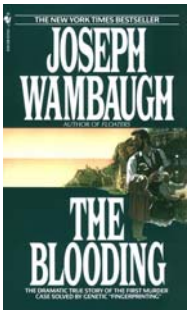
Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

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



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.

Impact of Forensic DNA Testing

Guilt Innocence

Colin Pitchfork

Kirk Bloodsworth

Josiah Sutton

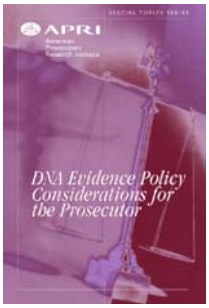
Roger Coleman

Innocence Project

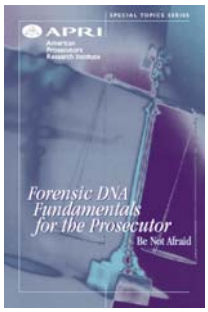
Some DNA Resources for Lawyers

APRI DNA Resources

http://www.ndaa-apri.org/pdf/dna_evidence_policy_considerations_2004.pdf



http://www.ndaa-apri.org/pdf/forensic_dna_fundamentals.pdf



The Silent Witness

APRI American Prosecutors Research Institute

http://www.ndaa-apri.org/apri/programs/dna/newsletter.html

2006	
Volume 10, Number 3	Low Copy Number DNA: Reality vs. Jury Expectations
Volume 10, Number 2	Catching Criminals by Investigating Profiles with Allelic Similarities
Volume 10, Number 1	Admissibility of DNA Evidence: Applying Crawford v. Washington
2005	
Volume 9, Number 4	Forensic Palynology and Plant DNA: the Evidence that Sticks
Volume 9, Number 3	Evolution of DNA Evidence for Crime Solving - A Judicial and Legislative History
Volume 9, Number 2	Biological Evidence: Maintaining Integrity of DNA Samples Through National Standards
Volume 9, Number 1	Evolution of the Quality Assurance Documents for DNA Laboratories
2004	
Volume 8, Number 2	Houston, We Have A Problem . . . Do You?
2003	
Volume 8, Number 2	Keeping a Conviction Secure
Volume 8, Number 1	Meeting Defense Challenges to DNA Evidence
Volume 7, Number 3	Discovery Issues
2002	
Volume 7, Number 2	Preparing for Defense Experts
Volume 7, Number 1	From John Doe to Known Offender: DNA Profile Arrest Warrants

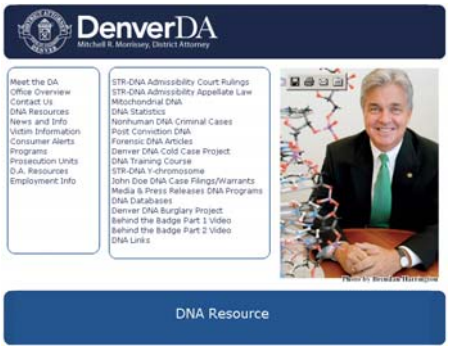
David Kaye's Writings

http://homepages.law.asu.edu/~kayed/

SCIENCE IN THE LAW: STANDARDS, STATISTICS AND RESEARCH ISSUES;
SCIENCE IN THE LAW: SOCIAL AND BEHAVIORAL SCIENCE ISSUES;
SCIENCE IN THE LAW: FORENSIC SCIENCE ISSUES (Faigman, D., David H. Kaye, Michael J. Saks and Joseph Sanders) (St. Paul: West Publishing Co. 2002)

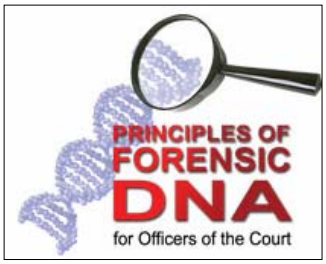
DenverDA DNA Resources

http://www.denverda.org/DNA/DNA_INDEX.htm



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

PRESIDENT'S DNA INITIATIVE

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**

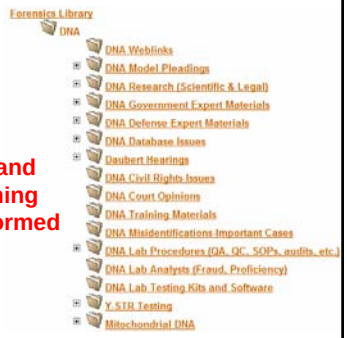
http://www.dna.gov/training/otc/

Information Resources for Defense Attorneys

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

NATIONAL LEGAL AID & DEFENDER ASSOCIATION

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

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

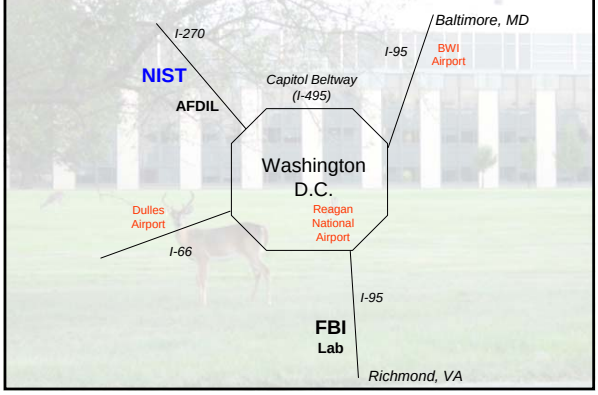
NIST History and Mission

- 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

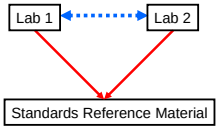
Standard Reference Materials

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

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

Helps meet DAB Std. 9.5 and ISO 17025

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



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

Lab Procedures

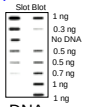
Steps in DNA Analysis

Usually 1-2 day process (a minimum of ~5 hours)

Steps Involved
- Collection
- Specimen Storage
- Extraction
- Quantitation
- Multiplex PCR
- STR Typing
- Interpretation of Results
- Database (Storage & Searching)
- Calculation of Match Probability

Blood Stain Buccal swab
Sample Collection & Storage

DNA Extraction



DNA Quantitation

Biology

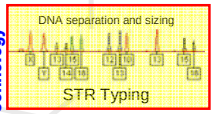
Multiplex PCR Amplification

Genetics

If a match occurs, comparison of DNA profile to population allele frequencies to generate a case report with probability of a random match to an unrelated individual

DNA Database Search

Technology



Male: 13,14-15,16-12,13-10,13-15,16

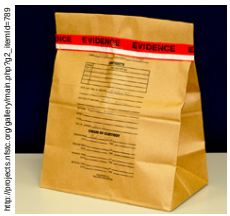
Interpretation of Results

Crime Scene Collection of Evidence

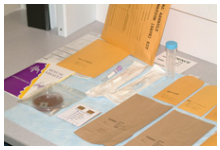
http://projects.nfstc.org/gallery/main.php?g2_itemId=626

- Police officers and crime scene investigators respond to the scene of a crime to collect biological evidence to be used in forensic DNA testing
- Investigators must be careful not to contaminate the evidence with their own DNA

DNA Evidence Received in the Lab



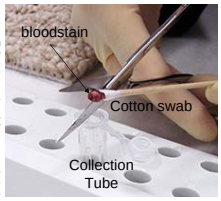
http://projects.nfstc.org/gallery/main.php?g2_itemId=789



http://projects.nfstc.org/gallery/main.php?g2_itemId=749

- Evidentiary samples (commonly in the form of cotton swabs) are brought or shipped to the DNA laboratory after collection from the crime scene or victim
- Sexual assault evidence collection kits provide swabs and bags for clothing collections from the victim

DNA Collection



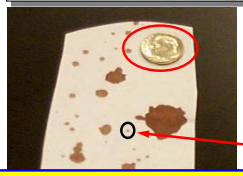
http://projects.nfstc.org/gallery/main.php?g2_itemId=663

http://projects.nfstc.org/gallery/main.php?g2_itemId=668

- Cotton swabs are commonly used to collect biological material from bloodstains or semen from sexual assault victims
- The amount of DNA needed has decreased dramatically in the past decade due to sensitivity of the PCR process (which makes millions of copies of targeted regions)

Sources of Biological Evidence

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



Blood Sample

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

best results with >100 cells, but DNA profiles can be recovered from as little as a single cell

DNA Reference Sample from Suspect

http://projects.nfstc.org/gallery/main.php?g2_itemId=658

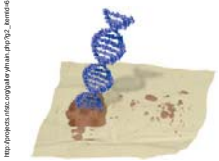
http://shop.armorforensics.com/mm5/graphics/00000002/products/4-4980b_sml.jpg

- Blood samples may be collected but require a phlebotomist to draw blood
- Easier to collect a buccal swab from the inside of an individual's mouth, which scrapes off some cheek cells

Buccal Swab DNA Collection

- The inside of the check is scrubbed to collect cells
- Less invasive than drawing blood
- Swab must be dried before storing and shipping to lab to avoid mold and bacterial growth

DNA Extraction



- DNA is extracted from proteins that protect it in the nucleus of a cell
- Chemicals are added to digest the protecting proteins and produce "naked" DNA molecules
- The final solution looks like a tube of water

DNA Quantitation

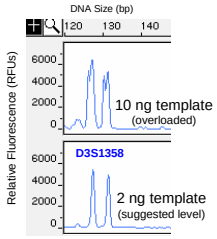
ABI 7500: an instrument used to perform "real-time quantitative PCR"

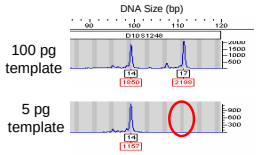
- DNA quantitation is important to determine how much human DNA (as opposed to bacterial DNA) is present in a sample
- A commonly used DNA quantitation kit is called Quantifiler (sold by Applied Biosystems)

Impact of DNA Amount into PCR

Reason that DNA Quantitation is Important Prior to Multiplex Amplification

- Too much DNA
  - Off-scale peaks
  - Split peaks (+/-A)
  - Locus-to-locus imbalance
- Too little DNA
  - Heterozygote peak imbalance
  - Allele drop-out
  - Locus-to-locus imbalance



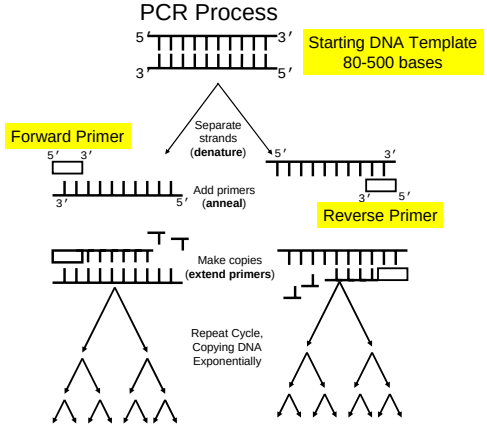


Stochastic effect when amplifying low levels of DNA produces allele dropout

PCR Amplification

- PCR = polymerase chain reaction
- Process that copies a particular region of DNA using two "primers" (short pieces of DNA)
- Each strand of DNA is used as a template to create a replicate that permits a doubling of the number of target molecules with each cycle of heating and cooling

PCR Process

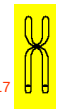


PCR Amplification (Thermal Cycling)

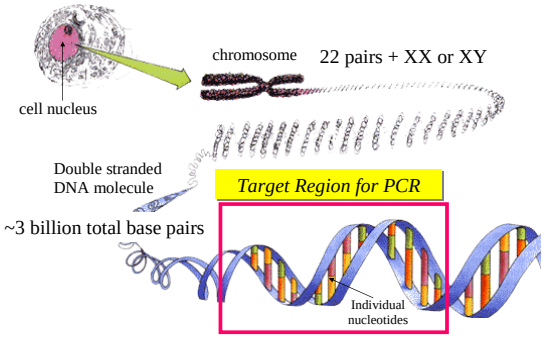
- The polymerase chain reaction (PCR) copies sections of DNA through heating and cooling the sample
- Each DNA strand is copied with each temperature cycle
- A thermal cycler heats and cools DNA samples (usually 28 cycles)

Identification of Information

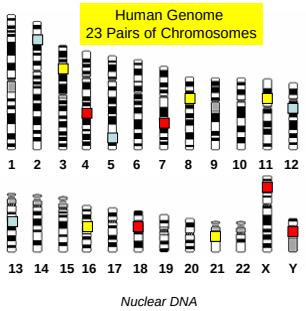
Printed Information	Genetic Information
Library	**Body**
Book	**Cell**
Chapter	**Nucleus**
Page Number	**Chromosome** D13S317
Line on Page	**Locus (part of chromosome)**
Word	**Short DNA sequence**
Letter	**DNA nucleotides**



DNA in the Cell



What is a DNA Profile?



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**

The copied fragments now contain fluorescent dyes for detection

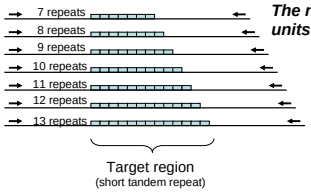
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 2.3, ©Elsevier Science/Academic Press

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTGGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

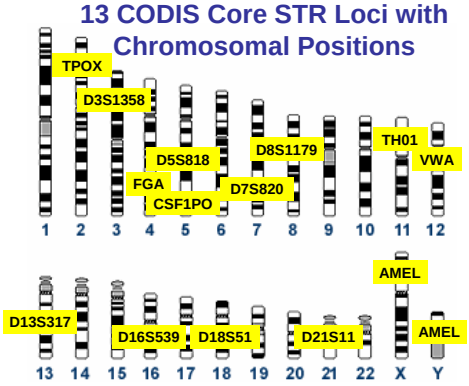
= 12 GATA repeats ("12" is all that is reported)



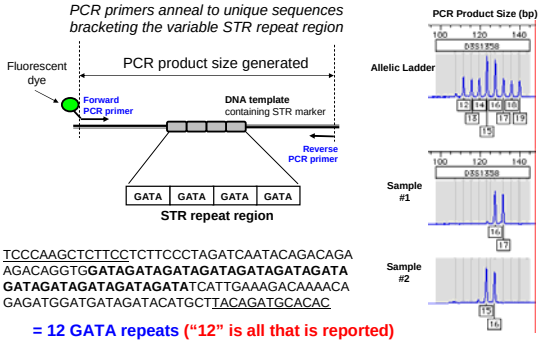
The number of consecutive repeat units can vary between people

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

13 CODIS Core STR Loci with Chromosomal Positions



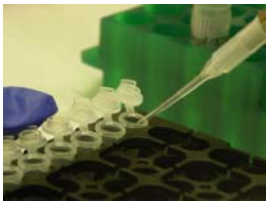
Short Tandem Repeat (STR) Markers

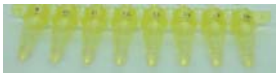


TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGA
AGACAGGTG**GATAGATAGATAGATAGATAGATAGATA**
GATAGATAGATAGATAGATATCATTGAAAGACAAAACA
GAGATGGATGATAGATACATGCTTACAGATGCACAC

= 12 GATA repeats ("12" is all that is reported)

DNA Reaction Setup





Strip of 8 tubes containing **~25 µL** of solution

- DNA sample is added (about 1 ng based on DNA quantitation performed) – **10 µL**
- PCR primers and other reaction chemicals from an STR typing kit are added – **15 µL**

STR Typing Kit

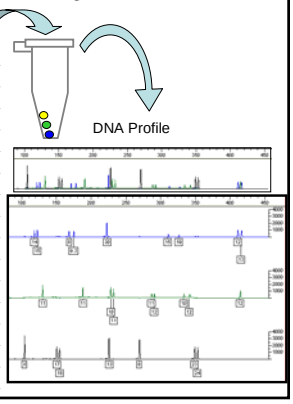
- Kit Components:
  - Primer mix
  - PCR Reaction Buffer and Building Blocks
  - DNA Polymerase (Taq Gold)
- Most expensive reagent
- Common kits used:
  - Identifiler (Applied Biosystems)
  - Profiler Plus/COfiler (Applied Biosystems)
  - PowerPlex 16 (Promega)

What is in an STR Typing Kit?

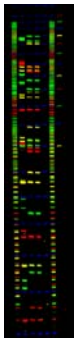
- Primer mix
  - containing fluorescently labeled oligonucleotides used to target specific regions of the human genome
  - Applied Biosystems has not published their primer sequences
  - PowerPlex 16, which amplifies 16 genomic sites, contains 32 PCR primers

PCR Primers in an STR Kit

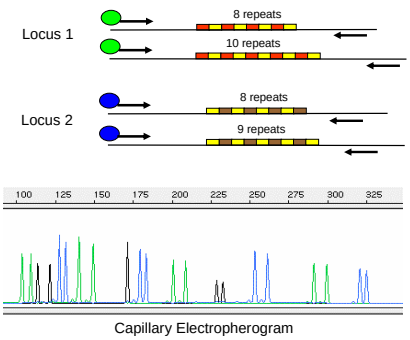
Locus	Dye	Promega PP16 Primer Sequences
D3S1358-F		ACTGCAGTCCAATCTGGGT
D3S1358-R	FL	ATGAAATCAACAGAGGCTTGC
TH01-F	FL	GTGATTCCCATTGGCCTGTTC
TH01-R		ATTCCTGTGGGCTGAAAAGCTC
D21S11-F		ATATGTGAGTCAATTCCCCAAG
D21S11-R	FL	TGTATTAGTCAATGTTCTCCAGAGAC
D18S51-F	FL	TTCTTGAGCCCAGAAGGTTA
D18S51-R		ATTCTACCAGCAACAACACAAATAAAC
PentaE-F		ATTACCAACATGAAAGGGTACCAATA
PentaE-R	FL	TGGGTTATTAATTGAGAAAACTCCTTACAATTT
D5S818-F		GGTGATTTTCCTCTTTGGTATCC
D5S818-R	JOE	AGCCACAGTTTACAACATTTGTATCT
D13S317-F		ATTACAGAAGTCTGGGATGTGGAGGA
D13S317-R	JOE	GGCAGCCCAAAAAGACAGA
D7S820-F	JOE	ATGTTGGTCAGGCTGACTATG
D7S820-R		GATTCCACATTTATCCTCATTGAC
D16S539-F		GGGGGTCTAAGAGCTTGTAAAAAG
D16S539-R	JOE	GTTTGTGTGTGCATCTGTAAGCATGTATC
CSF1PO-F	JOE	CCGGAGGTAAAGGTGTCTTAAAGT
CSF1PO-R		ATTTCCTGTGTCAGACCCTGTT
PentaD-F	JOE	GAAGGTCGAAGCTGAAGTG
PentaD-R		ATTAGAATTCTTTAATCTGGACACAAG
AMEL-F	TMR	CCCTGGGCTCTGTAAAGAA
AMEL-R		ATCAGAGCTTAAACTGGGAAGCTG
vWA-F		GCCCTAGTGGATGATAAGAATAATCAGTATGTG
vWA-R	TMR	GGACAGATGATAAATACATAGGATGGATGG
D8S1179-F	TMR	ATTGCAACTTATATGTATTTTTGTATTTCATG
D8S1179-R		ACCAAATTGTGTTCATGAGTATAGTTTC
TPOX-F		GCACAGAACAGGCACTTAGG
TPOX-R	TMR	CGCTCAAACGTGAGGTTG
FGA-F	TMR	GGCTGCAGGGCATAACATTA
FGA-R		ATTCTATGACTTTGCGCTTCAGGA



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



Scanned Gel Image

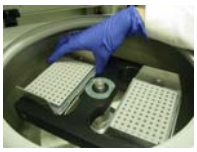


Capillary Electropherogram

Transfer of DNA Samples

- Following PCR, a small portion of the sample is transferred for analysis
- This aliquot of the sample is mixed with a molecular size marker (termed an internal size standard) that permits calibration of sizing measurements

Sample Plates Spun Down via a Centrifuge

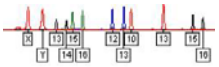


- Sample plates are spun to remove bubbles that would interfere with the injection (loading) process onto the capillary electrophoresis instrument

ABI 3130xl DNA Analysis Instrument

- Import sample names
- Determine run conditions (voltages and times to be used based on laboratory protocols)

Data Collection on ABI 3130xl Instrument



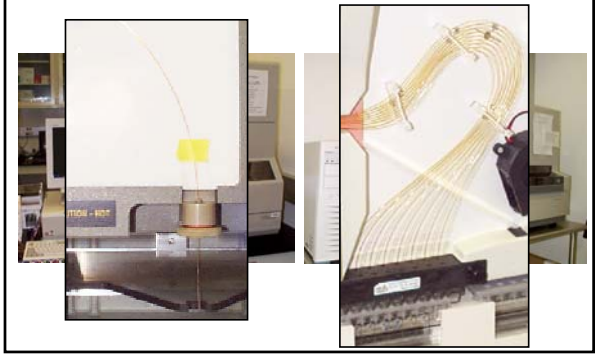
DNA Profile

- Data analysis is performed on an Applied Biosystems (ABI) 3130xl capillary electrophoresis instrument

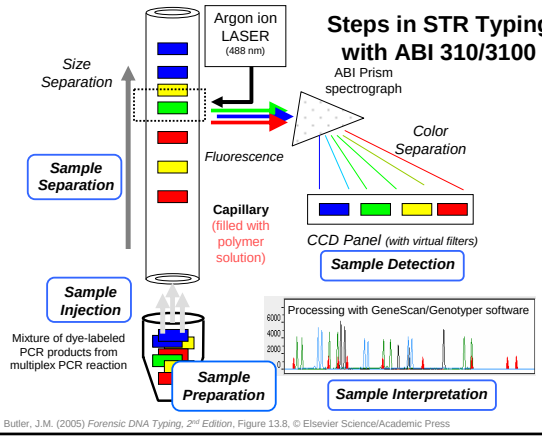
Capillary Electrophoresis Instrumentation

ABI 310
single capillary

ABI 3100
16-capillary array

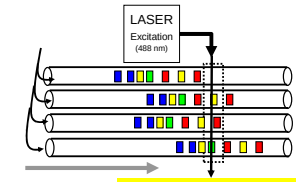


Steps in STR Typing with ABI 310/3100



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

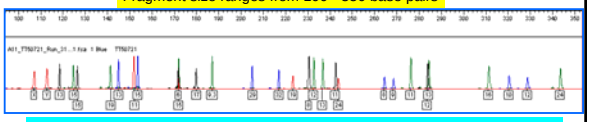
A DNA Profile is Produced by Separating DNA Molecules by Size and Dye Color



The labeled fragments are separated (based on size) and detected on a gel or capillary electrophoresis instrument

~2 hours or less

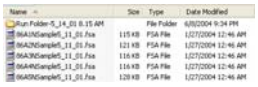
Fragment size ranges from 100 - 350 base pairs



Peaks represent labeled DNA fragments separated by electrophoresis
This 'profile of peaks' is unique for an individual – a DNA type

Data Transfer

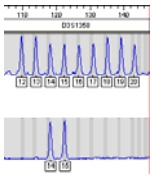
- Following data collection, the data (.fsa) files are typically transferred from the lab computer to one in an office where data analysis is performed



- A USB thumb drive permits rapid and easy transfer of data files

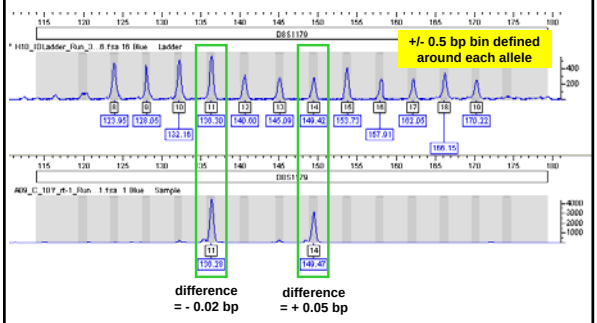
Data Analysis

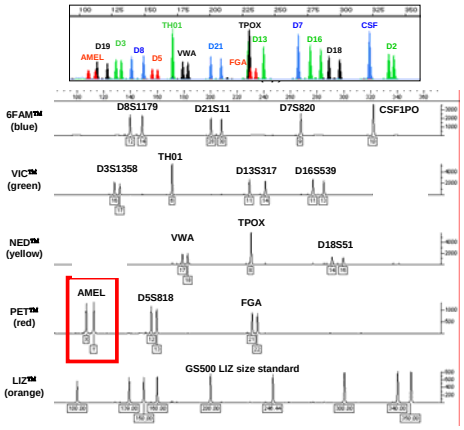
- The analyst carefully reviews the DNA data (electropherogram) and checks software genotype calls and edits out artifacts



- Software designates sample genotypes via comparison to an allelic ladder (mixture of common allele possibilities)

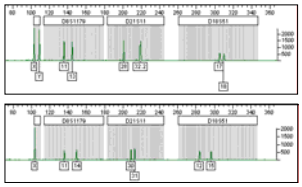
Comparison of Allelic Ladder to Samples to Convert Size into Allele Repeat Number





STR Results

- Individuals will differ from one another in terms of their STR profile
- STR genotype can then be put into an alpha numeric form for search on a DNA database



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

Data is Tabulated

	AMEL	CSF1PO	FGA	TH01	TPOX	VWA	D3S1358	D5S818
Ind(1)	X,Y	11,12	19,21	6,7	8,8	15,18	14,18	10,13

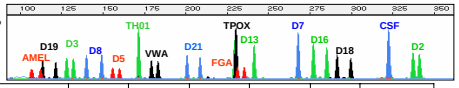
The number of repeats observed for each locus is tabulated

This data format is stored in databases and used for comparisons/matches

Finally a case report is written based on tabulated STR genotype calls

DNA Profile Frequency with all 13 CODIS STR loci

AmpFISTR® Identifiler™ (Applied Biosystems)



What would be entered into a DNA database for searching: 16,17-17,18-21,22-12,14-28,30-14,16-12,13-11,14-9,9-9,11-6,6-8,8-10,10

Locus	allele	value	allele	value	1 in	Combined
D3S1358	16	*0.2533*	17	*0.2152*	9.17	9.17
VWA	17	*0.2815*	18	*0.2003*	8.87	81
FGA	21	*0.1854*	22	*0.2185*	12.35	1005
D8S1179	12	*0.1854*	14	*0.1656*	16.29	16,364
D21S11	28	*0.1589*	30	*0.2782*	11.31	185,073
D18S51	14	*0.1374*	16	*0.1391*	26.18	4,845,217
D5S818	12	*0.3841*	13	*0.1407*	9.25	44,818,259
D13S317	11	*0.3394*	14	*0.0480*	30.69	1.38 x 10^9
D7S820	9	*0.1772*			31.85	4.38 x 10^{10}
D16S539	9	*0.1126*	11	*0.3212*	13.8	6.05 x 10^{11}
THO1	6	*0.2318*			18.62	1.13 x 10^{13}
TPOX	8	*0.5348*			3.50	3.94 x 10^{13}
CSF1PO	10	*0.2169*			21.28	8.37 x 10^{14}

PRODUCT RULE

The Random Match Probability for this profile in the U.S. Caucasian population is **1 in 837 trillion (10^{12})**

The Same 13 Locus STR Profile in Different Populations

1 in 837 trillion

1 in 0.84 quadrillion (10^{15}) in U.S. Caucasian population (NIST)
1 in 2.46 quadrillion (10^{15}) in U.S. Caucasian population (FBI)*
1 in 1.86 quadrillion (10^{15}) in Canadian Caucasian population*

1 in 16.6 quadrillion (10^{15}) in African American population (NIST)
1 in 17.6 quadrillion (10^{15}) in African American population (FBI)*

1 in 18.0 quadrillion (10^{15}) in U.S. Hispanic population (NIST)

These values are **for unrelated individuals** assuming no population substructure (using only p^2 and 2 pq)

NIST study: Butler, J.M., *et al.* (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.* 48(4):908-911. (http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm)

*http://www.csfs.ca/pplus/profiler.htm

DNA Testing Requires a Reference Sample

A DNA profile by itself is fairly useless because it has no context…

DNA analysis for identity only works by comparison – **you need a reference sample**

Crime Scene Evidence compared to **Suspect(s)** (Forensic Case)
Child compared to **Alleged Father** (Paternity Case)
Victim's Remains compared to **Biological Relative** (Mass Disaster ID)
Soldier's Remains compared to **Direct Reference Sample** (Armed Forces ID)

Steps in DNA Analysis

Steps in DNA Analysis
- Collection
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database (Storage & Searching)

Combined DNA Index System (CODIS)

- Used for linking serial crimes and unsolved cases with repeat offenders
- Convicted offender and forensic case samples
- Launched October 1998
- Requires 13 core STR markers
- Annual Results with NIST SRM required for submission of data to CODIS

No names are associated with DNA profiles uploaded to NDIS
If my profile was entered for searching:
16,17-17,18-21,22-12,14-28,30-14,16-12,13-11,14-9,9-9,11-6,6-8,8-10,10

Samples in State DNA Database

(as of Feb 2007)

Michigan

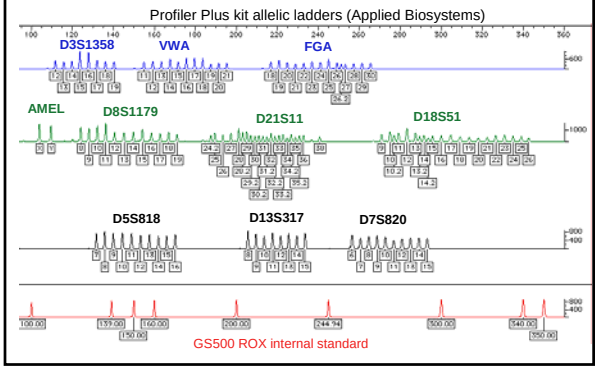
As of February 2007 the profile composition of the National DNA Index System (NDIS):

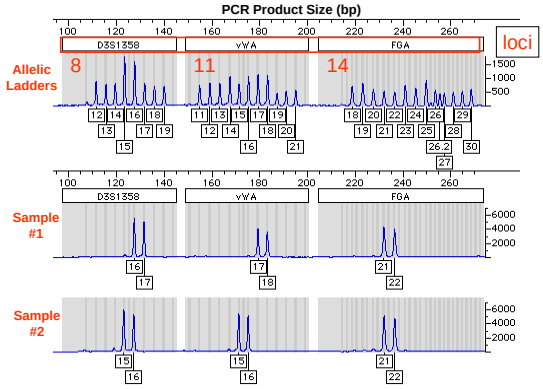
Total number of profiles: 4,398,639
Total Forensic profiles: 167,103
Total Convicted Offender profiles: 4,231,536

Statistical Information	Total
Offender Profiles	202,849
Forensic Samples	5,813
Number of CODIS Labs	4
NDIS Participating Labs	4
Investigations Aided	1,958

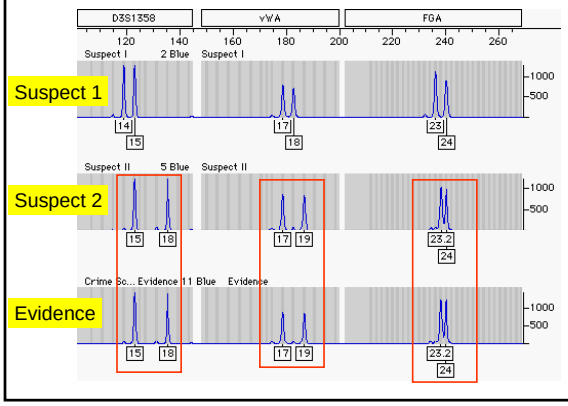
http://www.fbi.gov/hq/lab/codis/mi.htm

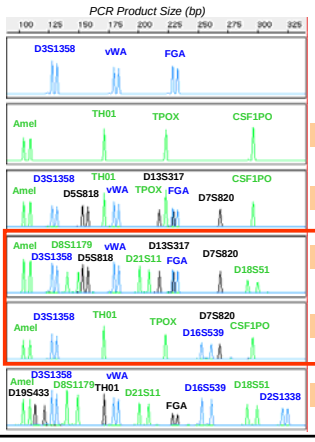
Companies Supply **Allelic Ladders** in STR Kits to **Aid Interlaboratory Consistency**





Crime Scene - Two Suspects

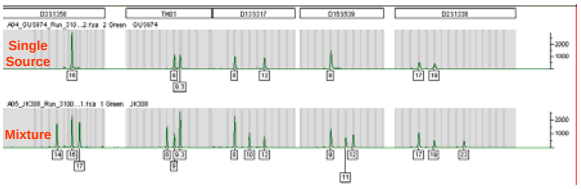




Same DNA sample run with Applied Biosystems STR Kits

	Random Match Probability
Blue	1.0×10^{-3}
Green I	7.8×10^{-4}
Profiler™	9.0×10^{-11}
Profiler Plus™	**2.4×10^{-11}**
COfiler™	**2.0×10^{-7}**
SGM Plus™	4.5×10^{-13}

Mixture vs Single Source Samples



STR Locus	Likely "true" genotype for other component
D3S1358	14,17
TH01	8,9.3
D13S317	8,10
D16S539	11,12
D2S1338	17,23

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

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

Additional Challenges

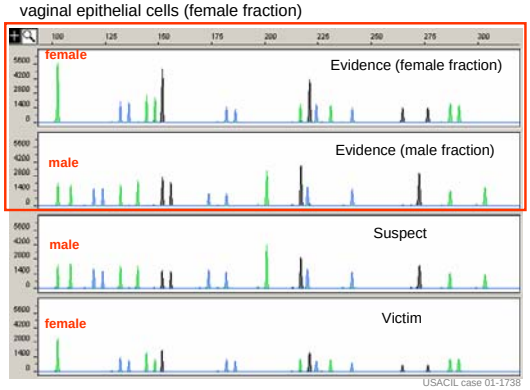
- Multiplex STR amplification require a fairly narrow amount of input DNA to product high quality results
- High-throughput needs for databanking labs
  - Automated software for data review
- An attitude of being (and needing to be) “error-free”
- Separating biological fluids – perpetrator’s sperm from victim’s vaginal epithelial cells
- Mixture components can be difficult to decipher

Mixtures: Issues and Challenges

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

- Mixtures arise when two or more individuals contribute to the sample being tested.
- Mixtures can be challenging to detect and interpret without extensive experience and careful training. **Even more challenging with poor quality data when degraded DNA is present…**
- Differential extraction can help distinguish male and female components of many sexual assault mixtures. **Y-chromosome markers can help here in some cases…**

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



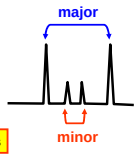
The four samples typically associated with a forensic DNA case…

Principles of Mixture Interpretation

Most mixtures encountered in casework are 2-component mixtures arising from a combination of victim and perpetrator DNA profiles

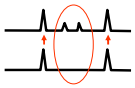
Torres *et al.* (2003) *Forensic Sci. Int.* 134:180-186 examined 1,547 cases from 1997-2000 containing **2,424 typed samples** of which **163 (6.7%) contained a mixed profile** with **only 8 (0.3%) coming from more than two contributors**

95.1% (155/163) were 2-component mixtures



Ratios of the various mixture components stay fairly constant between multiple loci enabling deduction of the profiles for the major and minor components

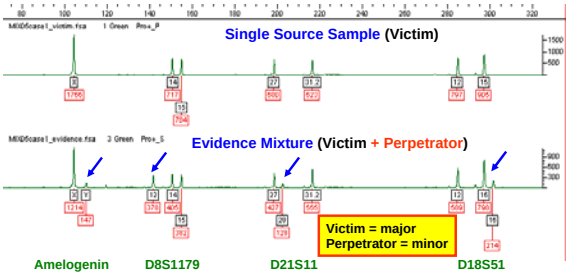
Some mixture interpretation strategies involve using victim (or other reference) alleles to help isolate obligate alleles coming from the unknown portion of the mixture



http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

MIX05 Case #1; Profiler Plus green loci

Example Mixture Data (MIX05 Study-Profiler Plus)



Obligate Alleles (not present in the victim reference)

Y	12	28	16

True “Perpetrator” Profile

X,Y	12,12	28,31.2	15,16

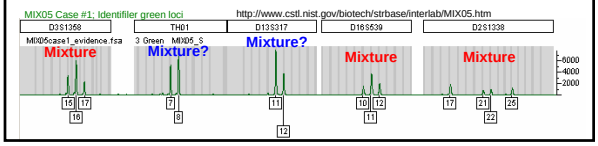
Mixtures: Issues and Challenges

- Artifacts of PCR amplification such as stutter products and heterozygote peak imbalance complicate mixture interpretation
- Thus, only a limited range of mixture component ratios can be solved routinely

Mixtures: Issues and Challenges

From J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 155

- The probability that a mixture will be detected improves with the use of more loci and genetic markers that have a high incidence of heterozygotes.
- The detectability of multiple DNA sources in a single sample relates to the ratio of DNA present from each source, the specific combinations of genotypes, and the total amount of DNA amplified.
- Some mixtures will not be as easily detectable as other mixtures.



Two Parts to Mixture Interpretation

- **Deduction of alleles present in the evidence** (compared to victim and suspect profiles)
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - An ISFG DNA Commission (Peter Gill, Bruce Weir, Charles Brenner, etc.) is evaluating the statistical approaches to mixture interpretation and has made recommendations

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

ISFG Recommendations on Mixture Interpretation

July 13, 2006 issue of *Forensic Science International*

Our discussions have highlighted a significant need for continuing education and research into this area.

ELSEVIER

Forensic Science International 160 (2006) 90–101

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

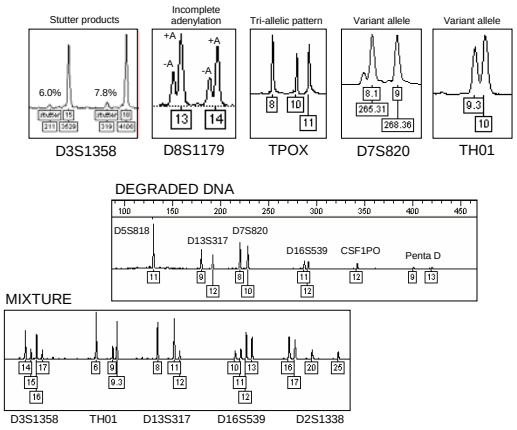
P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

[a] Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK
[b] Forensic Science Group, School of Public Health, University of California, Berkeley, CA 510-330-3911, USA

Abstract

The DNA commission of the International Society of Forensic Genetics (ISFG) was convened at the 21st congress of the International Society for Forensic Genetics held between 13 and 17 September in the Azores, Portugal. The purpose of the group was to agree on guidelines to encourage best practice that can be universally applied to assist with mixture interpretation. In addition the commission was tasked to provide guidance on low copy number (LCN) reporting. Our discussions have highlighted a significant need for continuing education and research into this area. We have attempted to present a consensus from experts but to be practical we do not claim to have conveyed a clear vision in every respect in this difficult subject. For this reason, we propose to allow a period of time for feedback and reflection by the scientific community. Then the DNA commission will meet again to consider further recommendations.

Keywords: STR typing; Biostatistical analysis; Likelihood ratio; Probability of exclusion; Mixtures; ISFG DNA commission



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-------

How Can DNA Mixtures Arise?

- **Two (or more) individuals** contribute to the biological evidence examined in a forensic case (e.g., sexual assault with victim and perpetrator or victim, consensual sexual partner, and perp)

  Victim Reference and Spouse or Boyfriend Reference
- **Contamination** of a single source sample from
  - evidence collection staff
  - laboratory staff handling the sample
  - Low-level DNA in reagents or PCR tubes or pipet tips

Examine Staff Profiles (Elimination Database), Maintain Contamination Log

Reference elimination samples are useful in deciphering both situations

Contamination

- **Systematic**
  - e.g., Contaminated water or PCR buffer
- **Sporadic**
  - e.g., individual PCR tube contamination
- **To reduce risks of contamination**:
  - Careful lab cleanliness
  - Constant monitoring of reagents and consumables
- Contaminants are more likely to show up in the low molecular weight STR loci because they amplify more efficiently (miniSTRs will have a greater chance of detecting contaminating DNA)
- **A negative control can detect systematic contamination but may not detect sporadic contamination**, such as could be found in a single PCR tube

Impact of Contamination on Casework

J Forensic Sci, May 2004, Vol. 49, No. 3
Paper ID JFS2003366
Available online at: www.astm.org

Peter Gill,[1] Ph.D. and Amanda Kirkham,[1] B.Sc.

Development of a Simulation Model to Assess the Impact of Contamination in Casework Using STRs

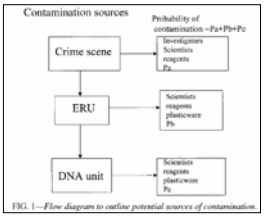


FIG. 1—*Flow diagram to outline potential sources of contamination.*

- **Use negative controls to predict the level of overall contamination in a lab**
- **Conclude that most likely outcome of a contamination event is a false exclusion** …if contaminating DNA is preferentially amplified over original LCN material

Potential Impact of Contamination on Cold Cases or Post-Conviction Testing

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

- While this contamination possibility might only rarely impact a careful forensic DNA laboratory, it can have potential significance on old cases under review including the Innocence Project. For example, if biological evidence from a 20-year old case was handled by ungloved police officers or evidence custodians (prior to knowledge regarding the sensitivity of modern DNA testing), then the true perpetrator's DNA might be masked by contamination from the collecting officer. Thus, when a DNA test is performed, the police officer's or evidence custodian's DNA would be detected rather than the true perpetrator. In the absence of other evidence, the individual in prison might then be falsely declared "innocent" because his DNA profile was not found on the original crime scene evidence. *This scenario emphasizes the importance of considering DNA evidence as an investigative tool within the context of a case rather than the sole absolute proof of guilt or innocence.*

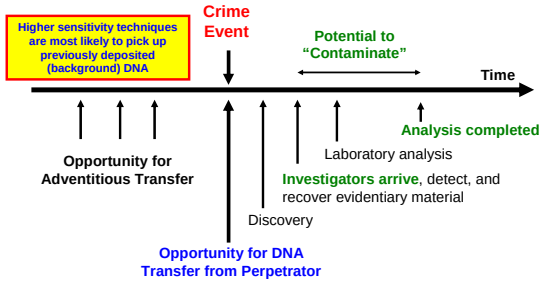
How Are Such Large Numbers Generated with Random Match Probabilities?

- Each allele is sampled multiple times to produce a statistically stable allele frequency
- Using theoretical model from genetics, multiple loci are multiplied together to produce an estimate of the rarity of a particular DNA profile (combination of STR alleles based on individual allele frequencies)
- Remember that relatives will share genetic characteristics and thus have STR profiles that are more similar to one another than unrelated individuals
- We are not looking at every person on the planet nor are we looking at every nucleotide in the suspect's genome

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

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