Overview of Forensic DNA Typing, Challenges and It's Future

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EDUCATION

- **D.Sc:** Biological Sciences, Tokyo Institute of Technology (TIT).
- **MPS:** Food and Nutrition Planning, UPLB.
- **M.Sc:** Biochemistry, UAF.
- Postgraduate diploma in biochemistry and bioengineering (TIT).

PROFESSIONAL EXPERIENCE

Assistant Professor of Forensic Biology: (2006-)
Department of Natural Sciences, Fayetteville State University.

FORENSIC SCIENTIST: (2001-2006). Indianapolis Marion County Forensic Services Agency (IMCFSA), Indianapolis, IN.

- **Key role in solving homicides, rapes, robberies and burglaries cases for criminal prosecution.**
- **Testified as expert witness.**

SENIOR SCIENTIST: (2000-2001). ReliaGene Technologies Inc.
New Orleans, LA.

- Major role in the development of the forensic DNA database by analyzing about ***26,000*** samples. DNA database is extremely important in a situation where prior suspicion of the offender's involvement in the crime is not available. Cold hits are often useful in identifying offenders in cases without an eyewitness or suspect and can match DNA from a number of crime scenes, linking multiple crimes to a single perpetrator. These CODIS matches are leading to arrests and convictions, making the country safer and better place to live.

PROFESSIONAL EXPERIENCE (Contd')

POSTDOCTORAL FELLOW: Thomas Jefferson University (1998-2000).

RESEARCH FELLOW: East Carolina University (1996-1997).

RESEARCH FELLOW: Center of Excellence, (1996-1996).

UNESCO RESEARCH FELLOW: Tokyo Institute of Technology, (1990-91)

TEACHING EXPERIENCE (1983-86). Teacher In-charge for "Introductory Biochemistry", Barani Agriculture University.

Guest Lecturer for "**Forensic Biology**" at University of Indianapolis.

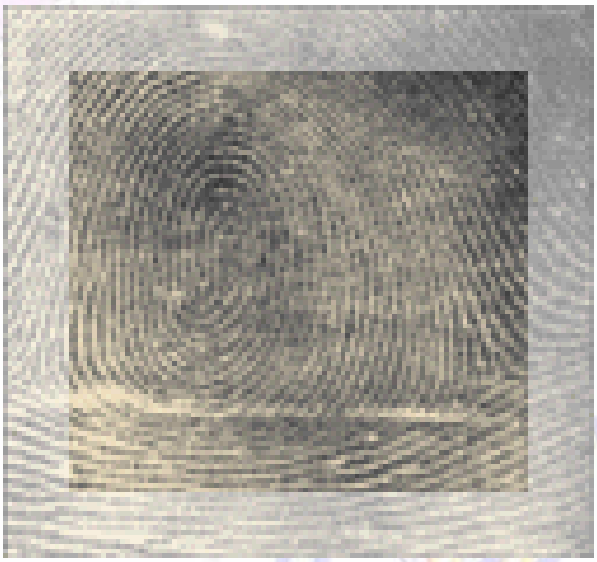
PUBLICATIONS:

- **Lodhi KM,** Mehemt H. Ozdener, Rass M. Shayiq. The upstream open reading frame mediates constitutive effects on translation of cytochrome P-450c27 from the seventh in-frame AUG codon in Rat liver. ***J. Biol. Chem.***278, 40647-40657 (2003).
- Seki T, Naruse M, Naruse K, Katafuchi T, **Lodhi KM,** Yoshimoto T, Hagiwara H, Demura H, Hirose S. Gene _expression of endothelial type isoform of nitric oxide synthase in various tissues of stroke-prone spontaneously hypertensive rats.
- ***Hypertens Res***; 2(1):43-49 (1997).
- **Lodhi, K. M.,** H. Sakaguchi, S. Hirose, S. Shibabe, and H. Hagiwara. Perichondrial localization of ETA receptor in rat tracheal and xiphoid cartilage
- and fetal rat epiphysis. ***Am. J. Physiol. 268*** (Cell Physiol. 37): C496-C502, 1995.
- **Lodhi, K. M.,** H. Sakaguchi, S. Hirose, and H. Hagiwara. Localization and characterization of a novel receptor for endothelin in the gills of the rainbow trout.
- ***J. Biochem. 118***, 376-379 (1995).
- Hagiwara, H., H. Sakaguchi, **K. M. Lodhi,** K. Suda, and S. Hirose. Subtype switching of natriuretic peptide receptors in rat chondrocytes during *in vitro* culture. ***J. Biochem. 116,*** 606-609 (1994).
- Hagiwara, H., T. Nagasawa, T. Yamamoto, **K. M. Lodhi,** T. Ito, N. Takemura, and S.Hirose,: Immunochemical characterization and localization of endothelin ETB receptor. ***Am. J. Physiol.264*** (Regulatory Integrative Comp. Physiol. 33): R777-783, 1993.
- Hagiwara, H., T. Nagasawa, **K. M. Lodhi,** M. Kozuka, T. Ito, and S. Hirose. Affinity chromatographic purification of bovine lung endothelin receptor using biotinylated endothelin and Avidin-Agarose. ***J. Chrom. 597*** (1992) 331-334.
- Kozuka, M., T. Ito, S. Hirose, **K. M. Lodhi,** and H. Hagiwara. Purification and characterization of bovine lung endothelin receptor. ***J. Biol. Chem. 266***: 16892-16896,1991.

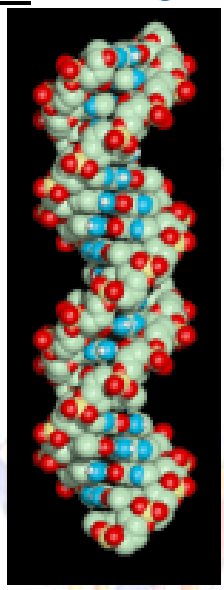
Forensic Science

Forensic science is a means to unravel the story. Eyewitnesses, victims and even suspects can forget or can change their stories. The physical evidence never forgets and cannot change. It is the forensic scientist's job to use, analyze and interpret the physical evidence. They are objective and advocates for the evidence. The criminal investigation may start at the crime scene and end in a courtroom, but an essential part of the process is the forensic laboratory.

Human Identification



Fingerprints have been used since 1901



DNA since 1986

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

Human Identity Testing

- Forensic cases - **matching suspect with evidence**
- Paternity testing - **identifying father**
- Historical investigations
- Missing persons investigations
- Mass disasters - **putting pieces back together**
- Military DNA “dog tag”
- Convicted felon DNA databases

Purpose of DNA Typing

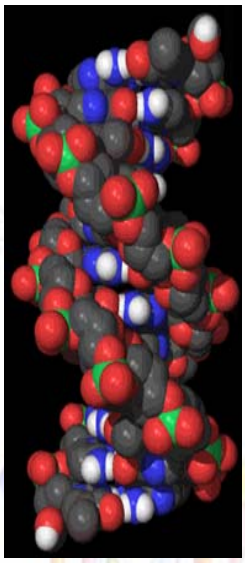
- Fact that each individual's DNA is unique (with the exception of identical twins).
- Approximately 99.5% of the DNA code is the same for all people.
- Other 0.5% that is of interest to the forensic scientists.

Possible Results of Forensic DNA Testing:

- Inclusion
- Exclusion
- Inconclusive

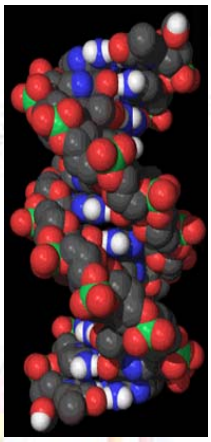
Types of cases involving biological fluids:

Rapes, homicides, child molestation, sodomy, aggravated assaults, hit & run, burglaries, robberies, kidnappings.



Biological Screening - typical types of evidence:

1. Hospital samples
2. Clothing
3. Bedding
4. Weapons
5. Hair
6. Cigarette butts
7. Envelopes/stamps
8. Drinking cups
9. Chewing gum



Forensic Biology

- Screening
- DNA Profiling

Forensic Biological Screening

The use of scientific methods to locate and identify body fluid stains on items of evidence in criminal cases.

Sources of Biological Evidence

- Blood
- Semen
- Saliva
- Urine
- Body tissue
- Teeth
- Bone
- FN Scrapings
- Skin cells
- Hair

Blood stain

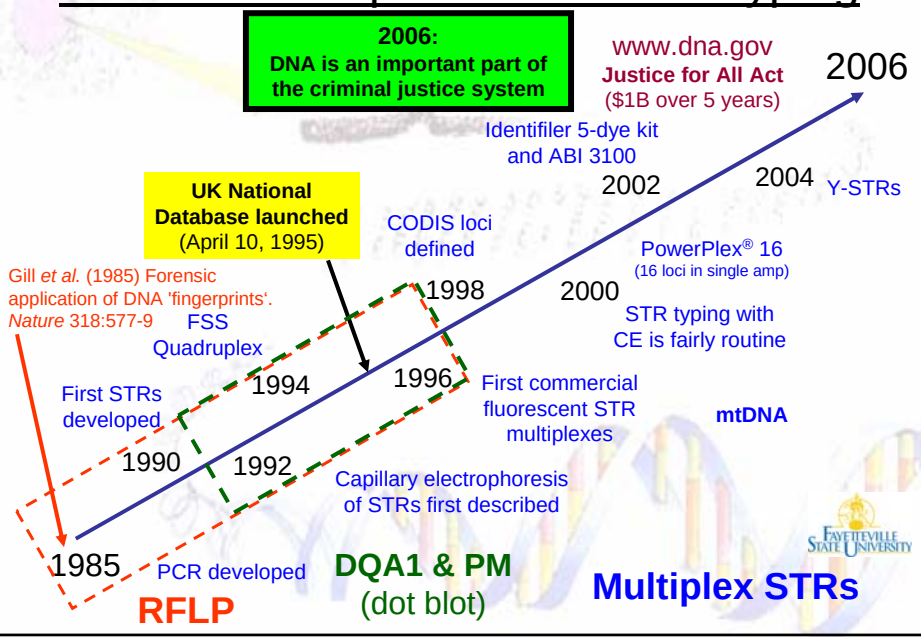
Only a very small amount of blood is needed to obtain a DNA profile

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

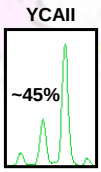
Forensic DNA Analysis

The use of scientific methods to decipher and identify deoxyribonucleic acid (DNA – the human genetic code) from bodily fluid stains in criminal cases.

Historical Perspective on DNA Typing

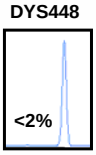


Types of STR Repeat Units



High stutter

Low stutter



Requires size based DNA separation to resolve different alleles from one another

- **Di**nucleotide (CA)(CA)(CA)(CA)
- **Tri**nucleotide (GCC)(GCC)(GCC)
- **Tetra**nucleotide (AATG)(AATG)(AATG)
- **Penta**nucleotide (AGAAA)(AGAAA)
- **Hexa**nucleotide (AGTACA)(AGTACA)

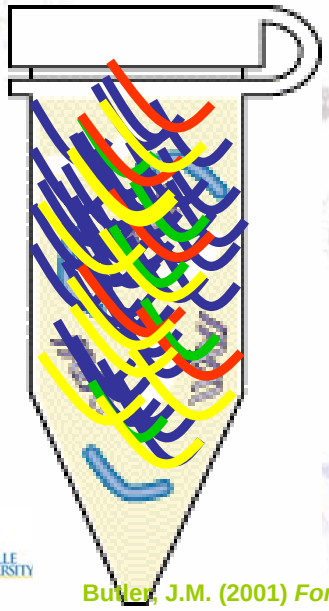
Short tandem repeat (STR) = microsatellite = simple sequence repeat (SSR)

Categories for STR Markers

Category	Example Repeat Structure	13 CODIS Loci
Simple repeats – contain units of identical length and sequence	**(GATA)(GATA)(GATA)**	TPOX, CSF1PO, D5S818, D13S317, D16S539
Simple repeats with non-consensus alleles (e.g., TH01 9.3)	**(GATA)(GAT-)(GATA)**	TH01, D18S51, D7S820
Compound repeats – comprise two or more adjacent simple repeats	**(GATA)(GATA)(GACA)**	VWA, FGA, D3S1358, D8S1179
Complex repeats – contain several repeat blocks of variable unit length	**(GATA)(GACA)(CA)(CATA)**	D21S11

These categories were first described by **Urquhart *et al.* (1994) *Int. J. Legal Med.* 107:13-20**

Advantages for STR Markers

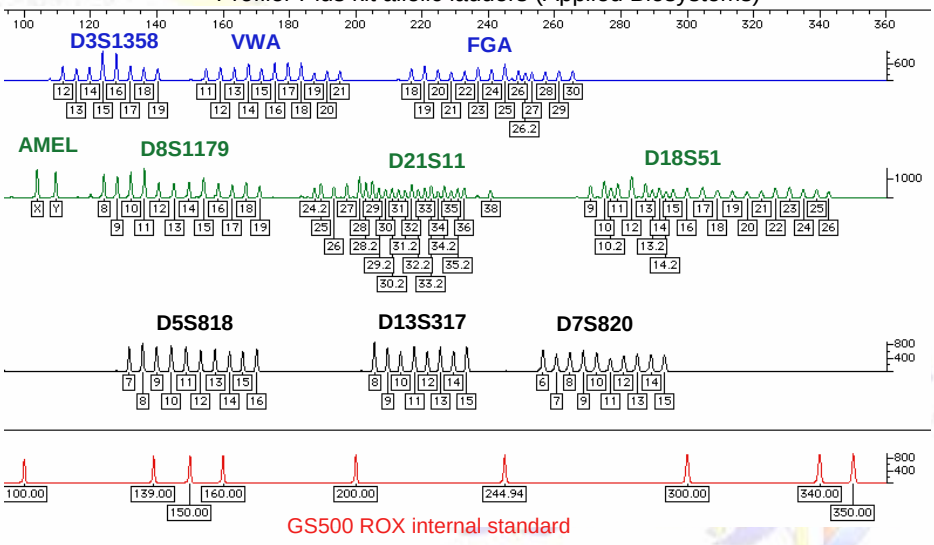


- Over 16 Markers Can Be Copied at Once
- Sensitivities to levels less than 1 ng of DNA
- Ability to Handle Mixtures and Degraded Samples
- Different Fluorescent Dyes Used to Distinguish STR Alleles with Overlapping Size Ranges

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

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

Profiler Plus kit allelic ladders (Applied Biosystems)

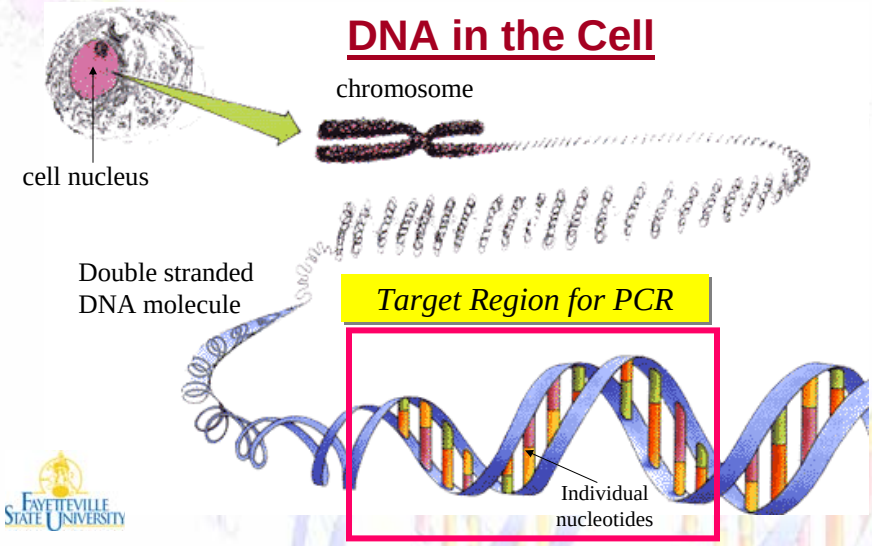


STR (Short Tandem Repeat) Testing

- **AmpFLSTR® Profiler Plus®** - PCR-based test that amplifies ***nine*** tetranucleotide STR loci and the Amelogenin locus. The STR loci amplified are as follows: D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, and vWA. (2.4×10^{-11}) *
- **AmpFLSTR® COfiler®** - PCR-based test when combined with Profiler Plus has the ability to generate information for all ***13 core STR loci required by the CODIS database***. The COfiler kit amplifies the ***four*** remaining STR loci (CSF1P0, D16S539, TH01, and TP0X) as well as two STR loci D3S1358 and D7S820 and Amelogenin, also found in the Profiler Plus kit. (2.0×10^{-7}) *
- **PowerPlex® 16 BIO System** – PCR-based test that amplifies the ***thirteen CODIS loci*** and the Amelogenin locus plus Penta E and Penta D.
- **AmpFLSTR® Identifiler®** - PCR-based test that amplifies ***15 STR loci*** plus the Amelogenin gender-determining marker. Amplifies the thirteen CODIS loci plus D2S1338 and D19S433. (7.2×10^{-19}) *
  - Random Match Probability with Author's Profile*

DNA Profiling

DNA in the Cell



DNA Use in Forensic Cases

- Most are rape cases (2 out of 3)
- Looking for match between evidence and suspect
- Must compare victim's DNA profile

Challenges

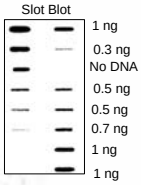
- Mixtures must be resolved
- DNA is often degraded
- Inhibitors to PCR are often present

Steps in DNA Analysis

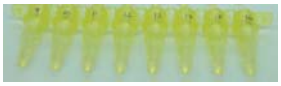
- Collection
- Specimen Storage
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database
  Storage & Searching

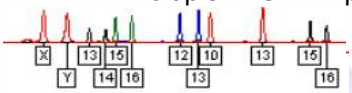
Sample Collection & Storage


DNA Extraction



DNA Quantitation


Multiplex PCR Amplification

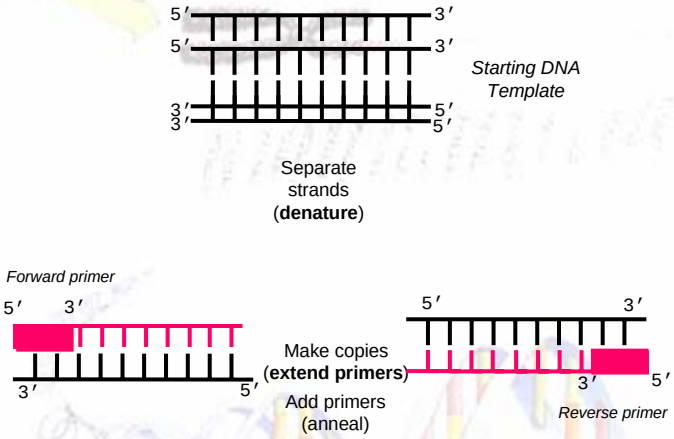


STR TYPING

Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results

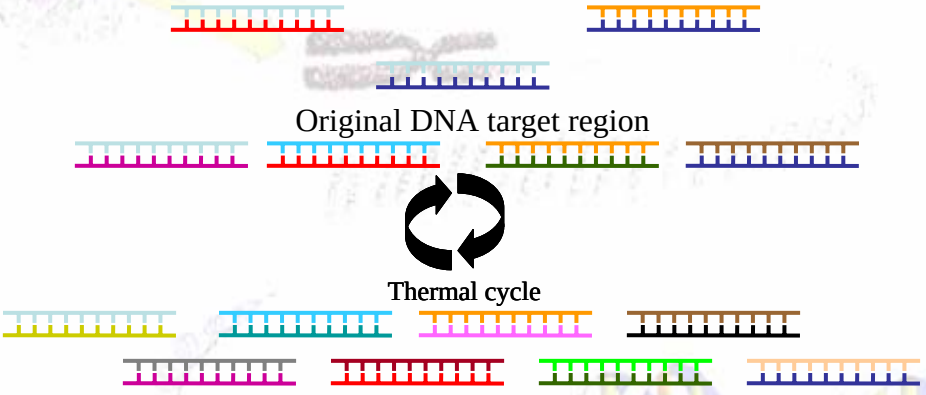

DNA Database

DNA Amplification with the Polymerase Chain Reaction (PCR)



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

PCR Copies DNA Exponentially through Multiple Thermal Cycles



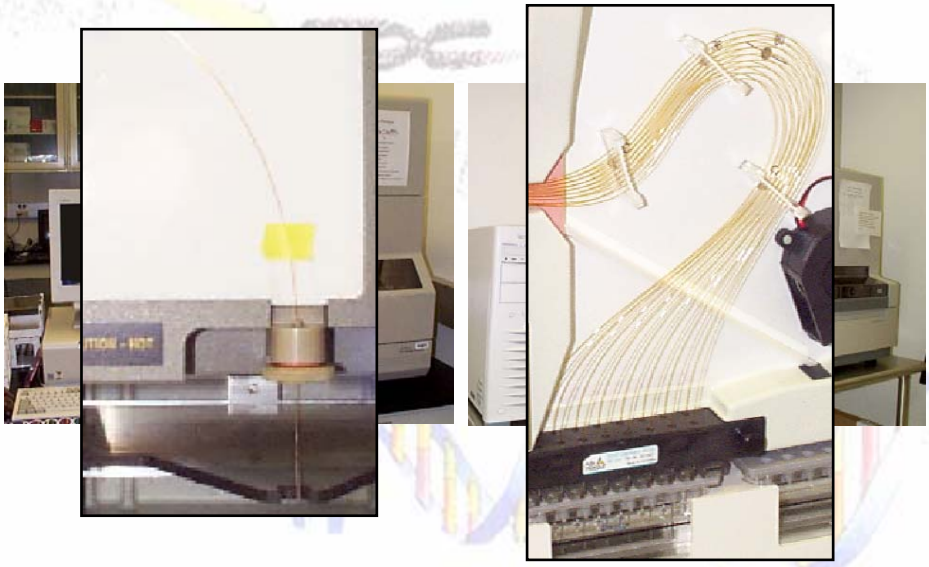
In 32 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created

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

Capillary Electrophoresis Instrumentation

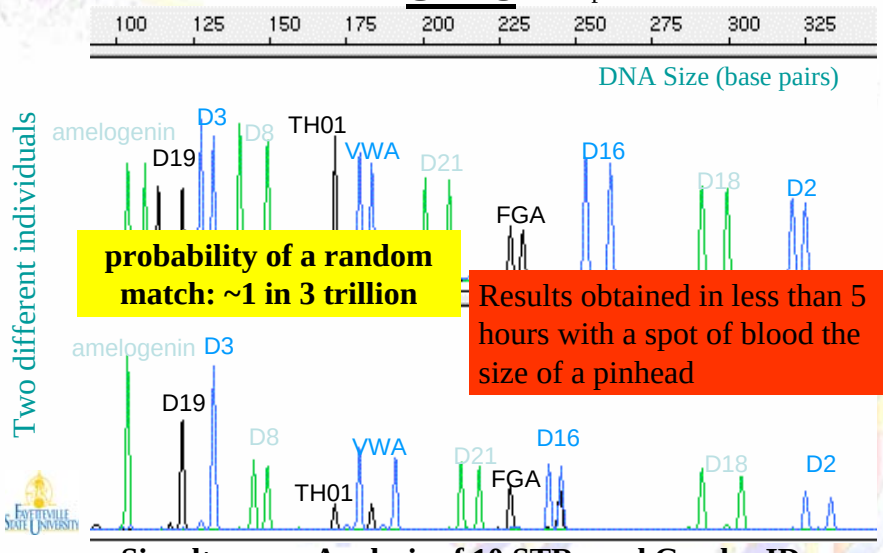
ABI 310
single capillary

ABI 3100
16-capillary array



Human Identity Testing with Multiplex STRs

AmpFlSTR® SGM Plus™ kit



probability of a random match: ~1 in 3 trillion

Results obtained in less than 5 hours with a spot of blood the size of a pinhead

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

Simultaneous Analysis of 10 STRs and Gender ID

Mixtures: Issues and Challenges

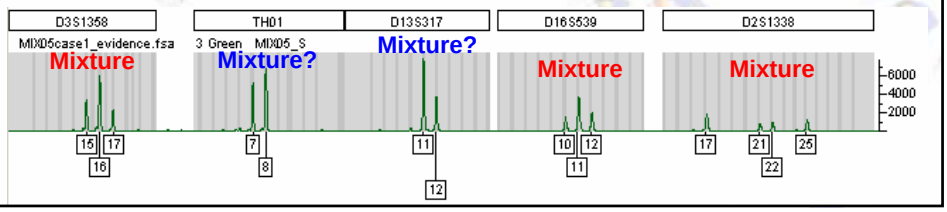
From J.M. Butler (2005) Forensic DNA Typing, 2nd Edition, p. 154

- 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.
- Differential extraction can help distinguish male and female components of many sexual assault mixtures.

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.



Steps in the interpretation of mixtures

(Clayton *et al.* *Forensic Sci. Int.* 1998; 91:55-70)

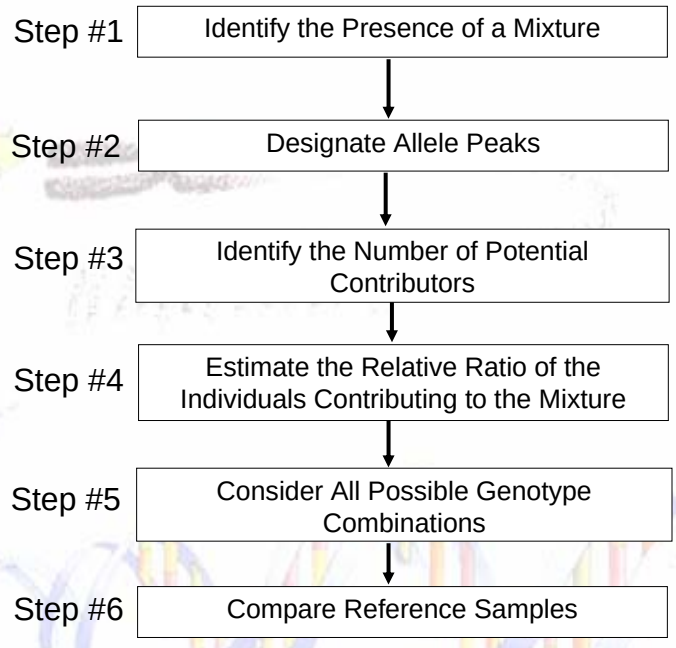


Figure 7.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Step #1: Is a Mixture Present in an Evidentiary Sample?

- Examine the number of peaks present in a locus
  - More than 2 peaks at a locus (except for tri-allelic patterns at perhaps one of the loci examined)
- Examine relative peak heights
  - Heterozygote peak imbalance <60%
  - Peak at stutter position >15%
- Consider all loci tested

Compare Reference Samples

- If there is a suspect, a laboratory must ultimately decide to include or exclude him…
- If no suspect is available for comparison, does your laboratory still work the case? (Isn't this a primary purpose of the national DNA database?)
- Victim samples can be helpful to eliminate their allele contributions to intimate evidentiary samples and thus help deduce the perpetrator

The Issues

1. **Although the PCR is rapid and efficient, sample loads keep increasing.**

2. **Soon all sexual offenders (and other felons) will be required to submit a sample for testing. Current estimated backlog is 540,000 samples.**

3. **The number of untested rape kits nationwide is estimated to be 180,000 to 500,000.**

4. **What technique could be used to automate the analysis of so many samples?**

National DNA Index System (NDIS)

FBI LABORATORY

CODIS COMBINED DNA INDEX SYSTEM

http://www.fbi.gov/hq/lab/codis/index1.htm

Combined DNA Index System (CODIS

- **Launched in October 1998 and now links all 50 states**
- **Used for linking serial crimes and unsolved cases with repeat offenders**
- **Convicted offender and forensic case samples along with a missing persons index**
- **Requires 13 core STR markers**

Fayetteville State University

Importance of DNA Database:

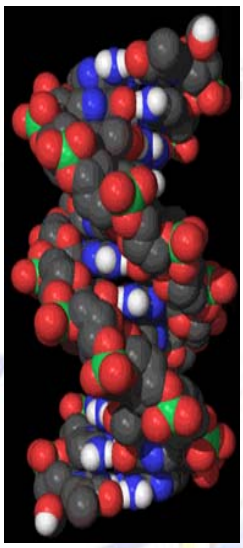
➢**Majority of crimes are committed by repeat offenders.**

➢**To locate suspects in violent crime cases that would otherwise never have been solved.**

➢**Data banks make associations between groups of unsolved cases.**

Fayetteville State University

Combined DNA Index System (CODIS)



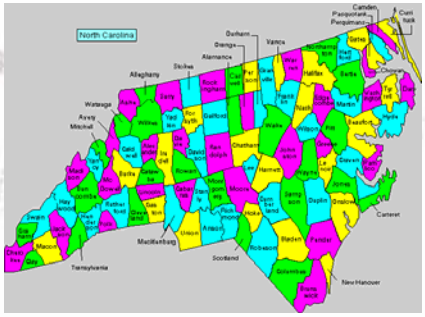
National Level — **NDIS (FBI Laboratory)**

State Level — **State Bureau of Investigation (Raleigh, North Carolina)**

Local Level — **Charlotte-Mecklenburg Police (Crime Laboratory)**

DNA profile for database search:
15-17,16-17,18-21,12-14,14-28,30-14,16-12,13-11, 14-9,9-11,13-6,6-8,8-10 and XY

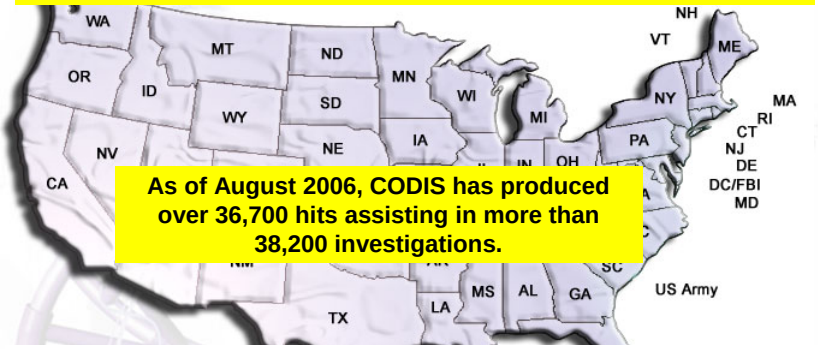
North Carolina



Statistical Information

Offender Profiles	70,613
Forensic Samples	2,389
Number of CODIS Labs	2
NDIS Participating Labs	2
Investigations Aided	437

All 50 states now require convicted offenders to submit a sample for DNA testing purposes



As of August 2006, CODIS has produced over 36,700 hits assisting in more than 38,200 investigations.

As of March 2006 the profile composition of the National DNA Index System (NDIS) is as follows:

Total number of profiles: 3,676,971
Total Forensic profiles: 148,068
Total Convicted Offender Profiles: 3,528,903

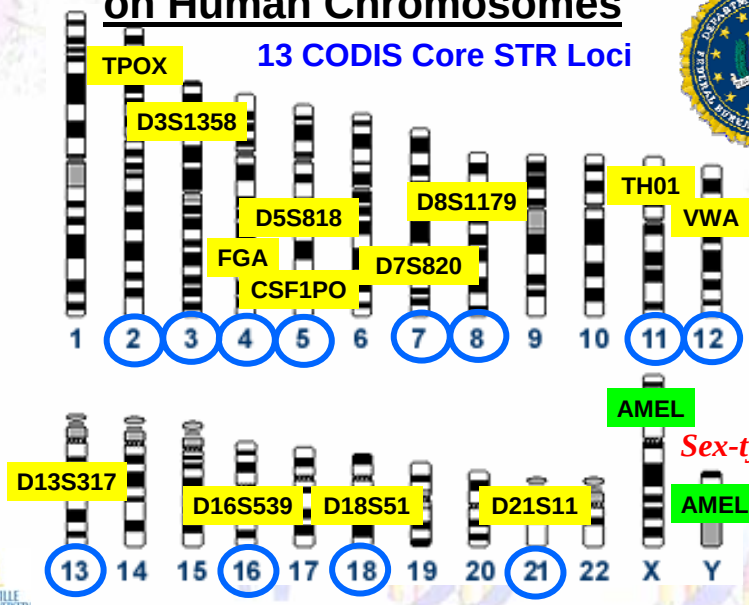
http://www.fbi.gov/hq/lab/codis/clickmap.htm

Position of Forensic STR Markers on Human Chromosomes

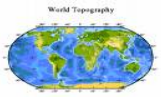
Core STR Loci for the United States

13 CODIS Core STR Loci

1997



International DNA Database



- The first national DNA data bank and so far the most effective were created in the United Kingdom in 1995. In less than five years, more than 500,000 DNA profiles have been entered into database, which has aided more then 50,000 criminal investigations.
- As of August 2006, CODIS has produced over 36,700 hits assisting in more than 38,200 investigations.
- This is a time to expand this CODIS system at to travel across borders. It is important for us to take full advantage for information technology to link and share information globally for our security.

International DNA Database

Before issuing visa for visitors wanting to visit foreign country

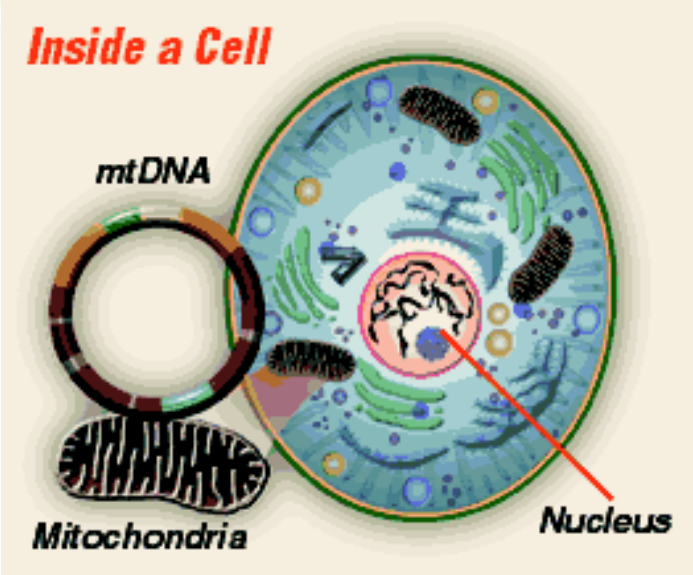
1. DNA samples should be taken and shipped to visiting country for DNA typing.
2. Visa would not be issued until DNA profile was generated and searched against a national/international database.
3. DNA profile would be included in biometric passport for future confirmation of identity.

Value of Y-Chromosome Markers

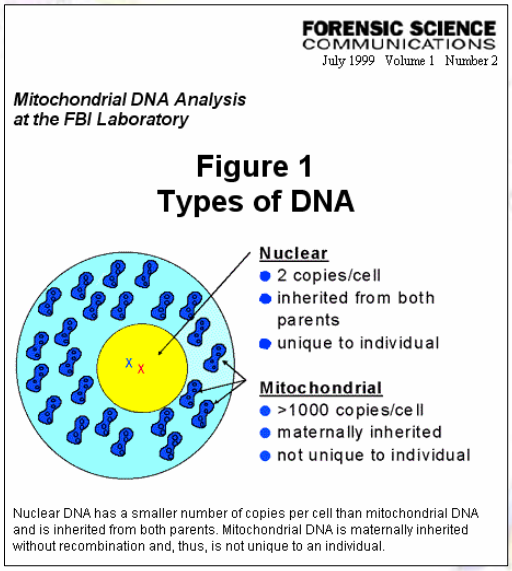
J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition; Table 9.1

Application	Advantage
Forensic casework on sexual assault evidence	**Male-specific amplification** (can avoid differential extraction to separate sperm and epithelial cells)
Paternity testing	Male children can be tied to fathers in motherless paternity cases
Missing persons investigations	**Patrilineal male relatives may be used for reference samples**
Human migration and evolutionary studies	Lack of recombination enables comparison of male individuals separated by large periods of time
Historical and genealogical research	Surnames usually retained by males; can make links where paper trail is limited

Mitochondrial DNA (mtDNA)



Comparison of Nuclear and Mitochondrial DNA



http://www.fbi.gov/hq/lab/fsc/backissu/july1999/dnaf1.htm

Advantages of mtDNA testing:

Higher copy number per cell
Results with highly degraded DNA
Results with limited sample (hair shaft)

Disadvantages of mtDNA testing:

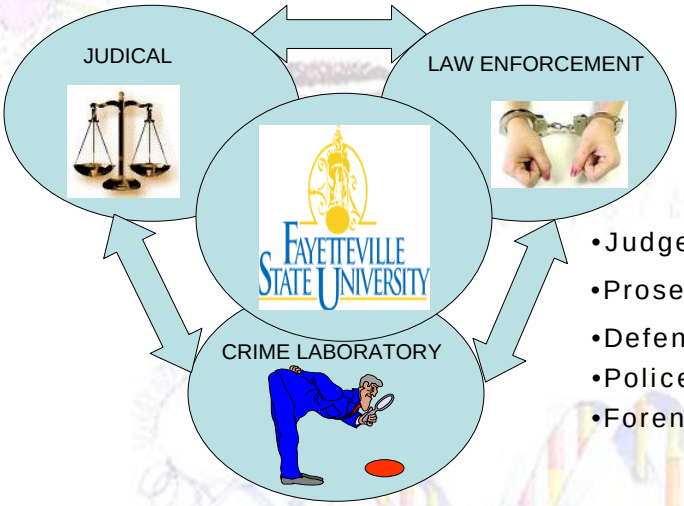
Low power of discrimination
Labor intensive
Expensive

Role of Y-STRs and mtDNA

Compared to Autosomal STRs

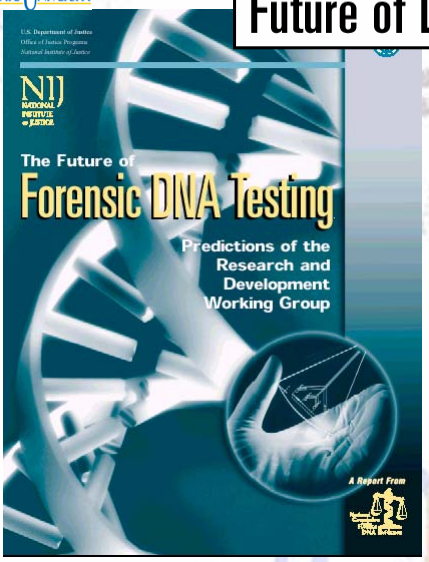
- **Autosomal STRs provide a higher power of discrimination and <u>are the preferred method</u> whenever possible**
- **Due to capabilities for male-specific amplification**, Y-chromosome STRs (**Y-STRs**) can be useful in extreme female-male mixtures (e.g., when differential extraction is not possible such as fingernail scrapings)
- **Due to high copy number**, mitochondrial DNA (**mtDNA**) may be the only source of surviving DNA in highly degraded specimens or low quantity samples such as hair shafts

Training and Continuing Education for Law Enforcement Agencies



- Judges
- Prosecutors
- Defense attorneys
- Police officers
- Forensic Scientists

National Commission on the Future of DNA Evidence

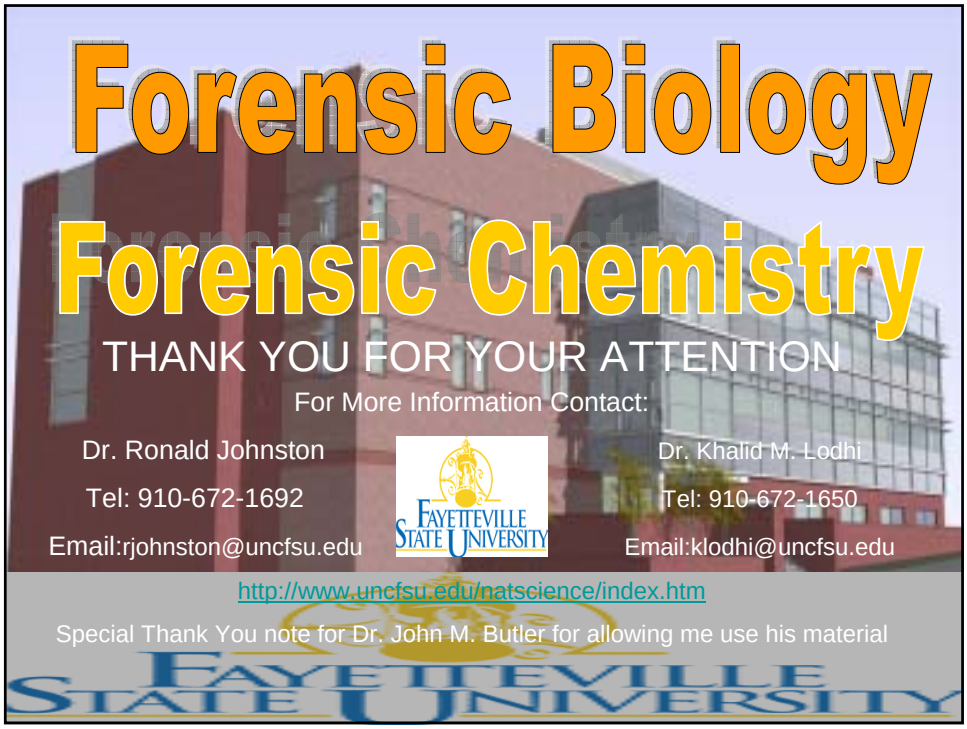


http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

- 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


Forensic Biology
Forensic Chemistry
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Special Thank You note for Dr. John M. Butler for allowing me use his material
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