Improving the Success Rate of the Analysis of Compromised DNA Evidence

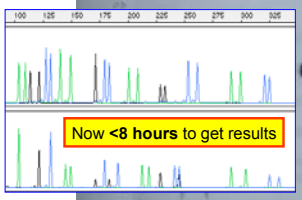
CSI:
Compromised Sample Improvements

John M. Butler
National Institute of Standards and Technology

The NIJ Conference 2006
Washington, DC
July 18, 2006

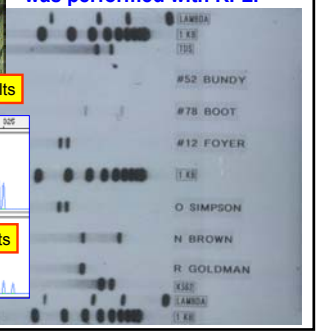
Progress Since 1995…

Almost **8 weeks** needed to get results

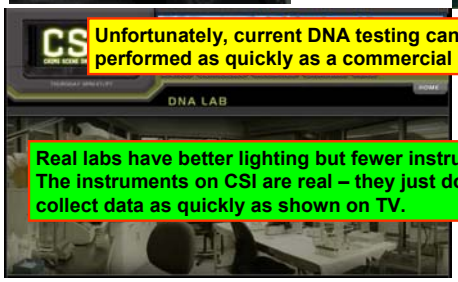


Now **<8 hours** to get results

O.J. Simpson DNA testing was performed with RFLP



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



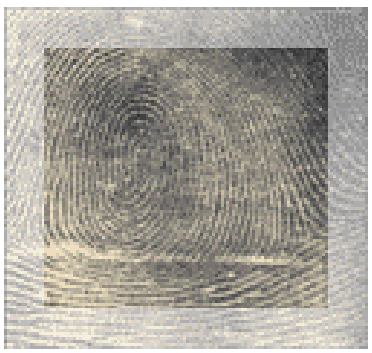
Real labs have better lighting but fewer instruments. The instruments on CSI are real – they just do not collect data as quickly as shown on TV.

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

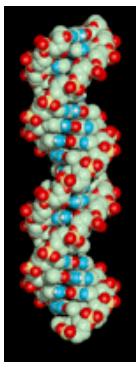
Current Areas of NIST Research Effort

- **Resources for "Challenging Samples"**
- **Standard Reference Materials** (SRM 2391 DNA Profiling Standard)
- **Information on New Loci** (SNPs, Y-Chromosome, new STRs)
- **Standard Information Resources** (STRBase website, training materials/review articles, validation standardization)
- **Allele Sequencing and Interlaboratory Studies** (Real-time qPCR, mixture interpretation)

Methods for Human Identification



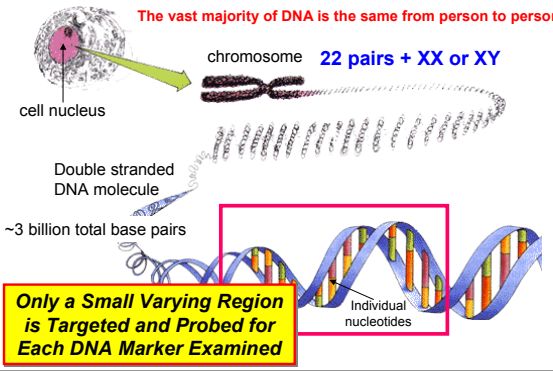
Fingerprints have been used since 1901



DNA since 1986

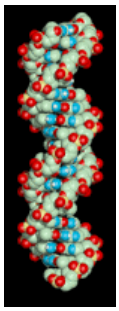
DNA in the Cell

The vast majority of DNA is the same from person to person



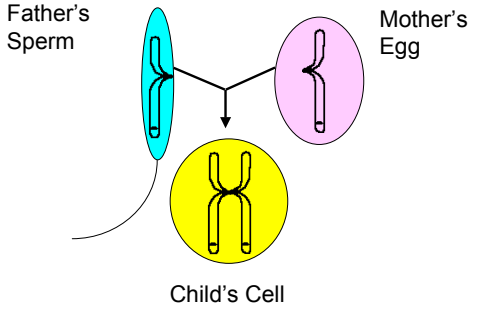
Only a Small Varying Region is Targeted and Probed for Each DNA Marker Examined

Characteristics of DNA

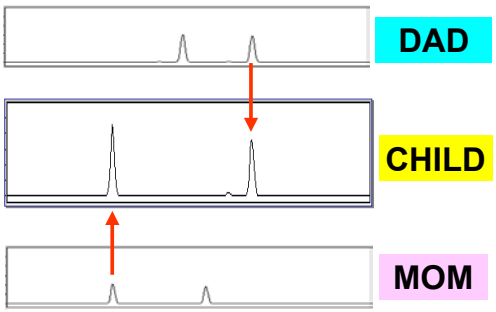


- Each person has a unique DNA profile (except identical twins).
- Each person's DNA is the same in every cell.
- An individual's DNA profile remains the same throughout life.
- Half of your DNA comes from your mother and half from your father.

Our DNA Comes from our Parents



Inheritance Pattern of DNA Profiles



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)

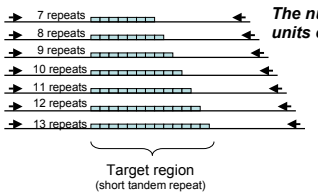
Current standard DNA tests **DO NOT look at genes** – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTG**GATAGATAGATAGATAGATAGATAGATAGATAGATAGA**
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

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



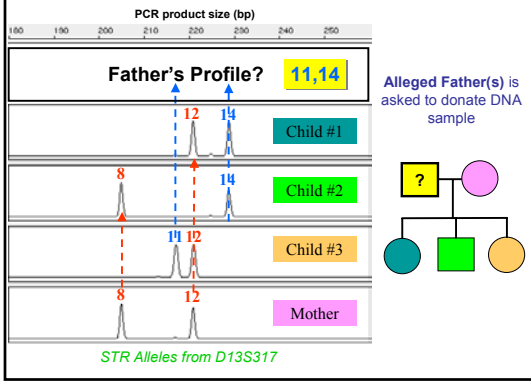
The number of consecutive repeat units can vary between people

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

Identification of Information

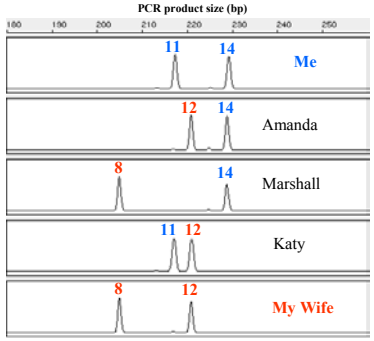
Printed Information	Genetic Information
Library	**Body**
Book	**Cell**
Chapter	**Nucleus**
Page Number	**Chromosome**
Line on Page	**Locus (part of chromosome)**
Word	**Short DNA sequence**
Letter	**DNA nucleotides**

PATERNITY TESTING

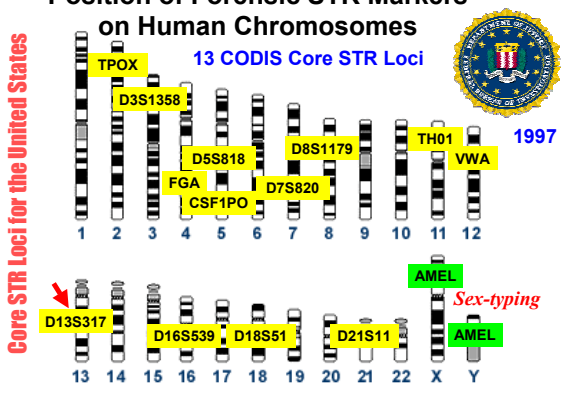


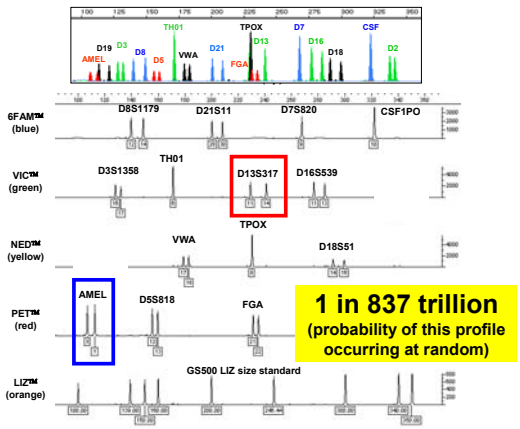
PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)

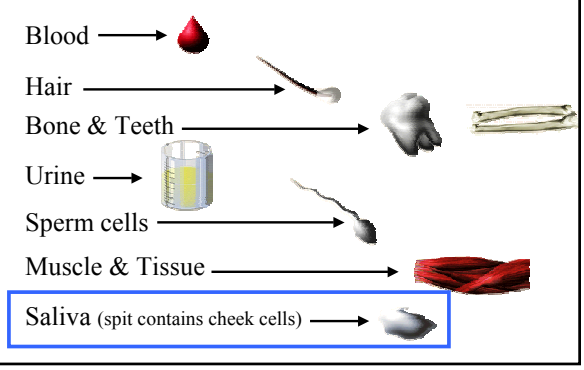


Position of Forensic STR Markers on Human Chromosomes





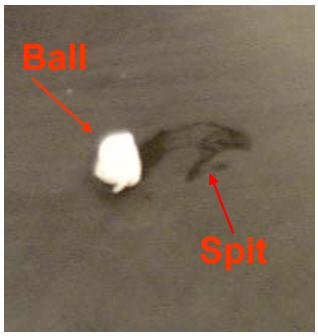
Where can you find DNA?

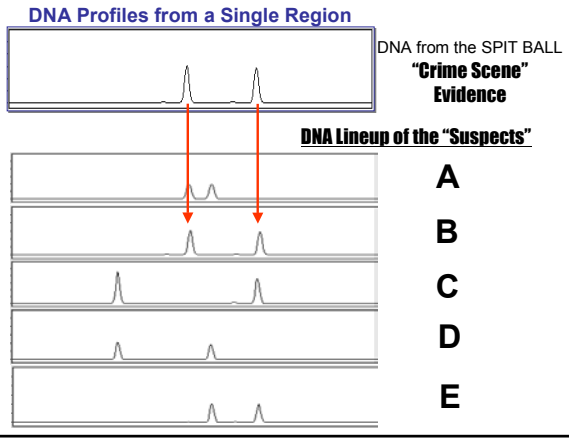


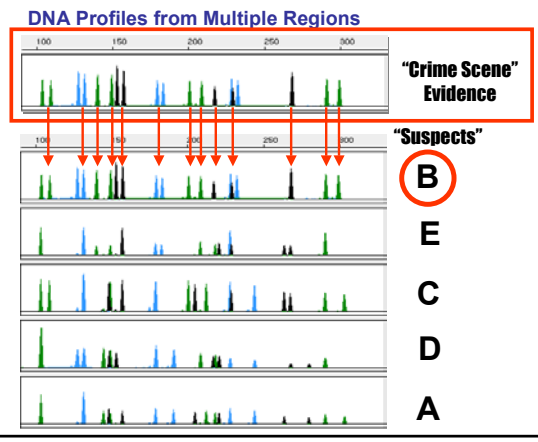
Using DNA to Solve a Case

- A **spit ball** was shot from the back of the classroom and hit the teacher in the eye
- It could have come from any one of five different students
- DNA was obtained from the saliva on the spit ball and used to produce a DNA profile
- Each of the 5 students ("suspects") were asked to give blood in order to obtain a DNA profile for comparison purposes to the spit ball ("crime scene evidence")

Close up of Evidence



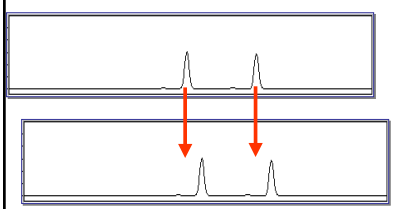




Conclusion of Case

- Student B was brought to justice and was asked to clean the teacher's glasses (& apologize)

What if the measurement of the DNA profile had not been performed correctly?



Failure to match the Perpetrator could result in the guilty going free…

Correct Measurements Helps Identify the Guilty and Free the Innocent

Impact of Forensic DNA Testing

Guilt

Colin Pitchfork

Roger Coleman

Innocence

Kirk Bloodsworth

Josiah Sutton

Innocence Project

Applications of Human Identity Testing

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

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

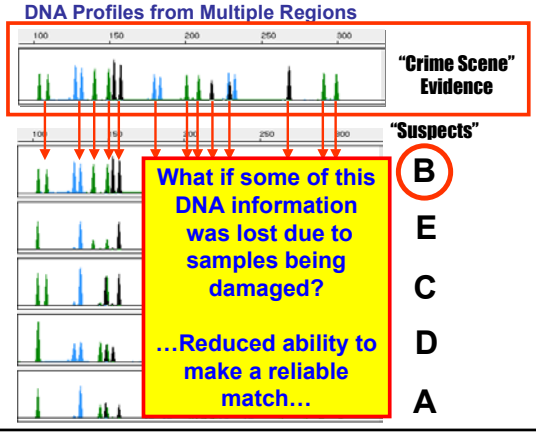
DNA Testing Requires a Reference Sample

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)

DNA Profiles from Multiple Regions



What if some of this DNA information was lost due to samples being damaged?

…Reduced ability to make a reliable match…

Impact of Degraded DNA Samples

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

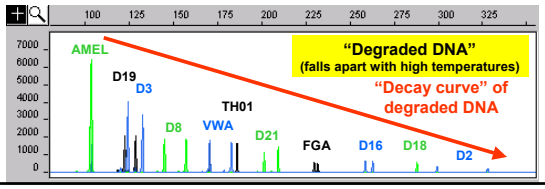
001-301-975-4049

- 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…**

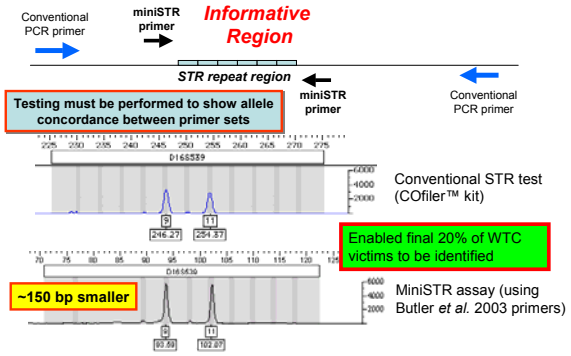
----------4049 or ----301-9-------

Degraded DNA

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



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



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

Timeline for miniSTRs

and Demonstrating the Value of Using Reduced Size Amplicons for Degraded DNA

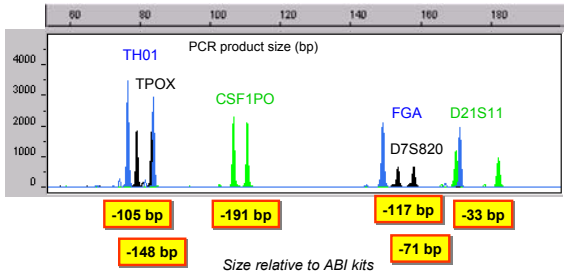
- 1994 – FSS finds that smaller STR loci work best with burned bone and tissue from Branch Davidian fire
- 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- 2004 – Work at NIST with **non-CODIS (NC) miniSTRs**
- 2006 – Applied Biosystems to release a 9plex miniSTR kit

http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm

J. Forensic Sci. Sept 2003 issue

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

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



The International Commission on Missing Persons (ICMP) is Now Using miniSTRs

100s of bones are tested each week with miniSTRs to help in the re-association of remains

Miniplex 02
D21S11, D13S317, D7S820, CSF1PO, vWA and D8S1179

(Tom Parsons, personal communication)

EDNAP Exercise on Degraded DNA

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Forensic Science International xxx (2005) xxx–xxx

Forensic Science International
www.elsevier.com/locate/forsciint

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

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

MiniSTR primer mixes and allelic ladders were provided by NIST

Recent Article Advocating miniSTRs

They recommend that miniSTRs "be adopted as the way forward to increase both the robustness and sensitivity of analysis."

ELSEVIER
Forensic Science International 156 (2006) 242–244
Science International
www.elsevier.com/locate/forsciint

Short communication

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

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

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

They recommend that European laboratories adopt three new mini-STR loci, namely: D10S1248, D14S1434 and D22S1045. (D14 now replaced by D2S441)

Identifying Victims of Mass Disasters

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Chapter 24

POLICY FORUM
EPIDEMIOLOGY

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, Benoit Leclair, Steven Niezgoda, Thomas J. Parsons, Elizabeth Pugh, Robert Shaler, Stephen T. Sherry, Amanda Sozer, Anne Walsh

Science (2005) 310: 1122-1123

Highly Degraded DNA Was Obtained from the Human Remains Recovered

Largest Forensic Case in History
~20,000 bone fragments were processed
>6,000 family reference samples and personal effects samples were analyzed

Progress is Being Made…

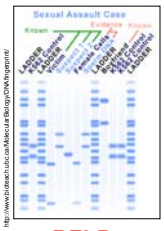
The Past

The Present

The Future

The DNA Field Moves Forward…

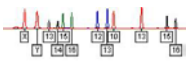
The Past



RFLP

500 – 25,000 bp

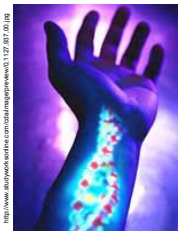
The Present



STRs

100 - 500 bp

The Future



miniSTRs

50 - 150 bp

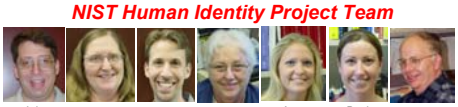
Comprised Sample Improvements (CSI) Conclusions

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

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between the National Institute of Justice and the NIST Office of Law Enforcement Standards

NIST Human Identity Project Team



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

Past and Present Collaborators (also funded by NIJ):
Bruce McCord and students (FL Int. U.) for miniSTR work
Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA LINEAR ARRAYs
Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
Artie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones
Murray Brilliant (U. AZ) for phenotype markers
Ken Kidd (Yale U.) for SNP typing population samples

Thank you for your attention…

Questions?

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