Forensic DNA Typing: Application to Mass Disaster Investigations, Paternity Testing and Human Identification

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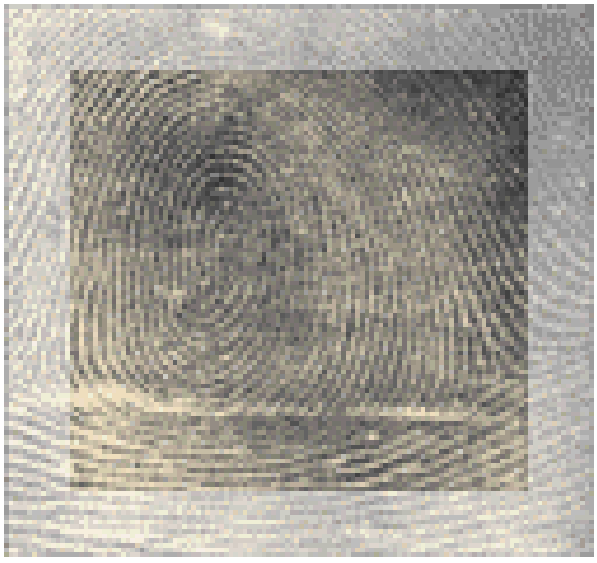
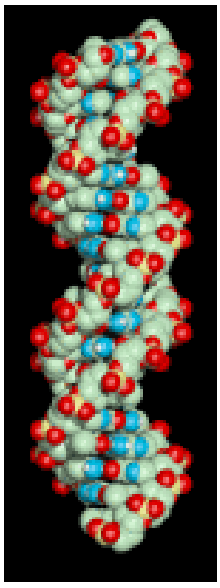
Methods for Human Identification



Fingerprints have been used since 1901



DNA since 1986

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

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

Typically, we are not looking at genes – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

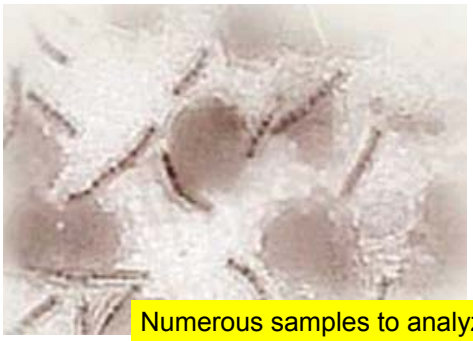
Applications for Human Identity Testing

- 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

As DNA analysis has shown its usefulness, the number of samples gathered for testing purposes has gone up dramatically…

Anthrax Detection

- Bacterial samples grown in culture
- If turbid after 6 hours, they are plated out and a fluorescent antibody test is done (3 hr)
- DNA detection with TaqMan assay (2-2.5 hr)
  - –5 probes performed in duplicate
  - –**RAPID** PCR cycling
- If positive for PCR, then redone at 24 hours

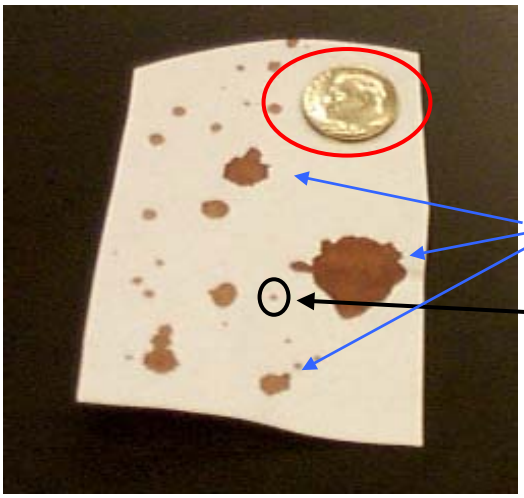


Ruggedized
Advanced
Pathogen
Identification
Device

Numerous samples to analyze; public pressure to get results quickly; accuracy better be good…

Sources of Biological Evidence

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

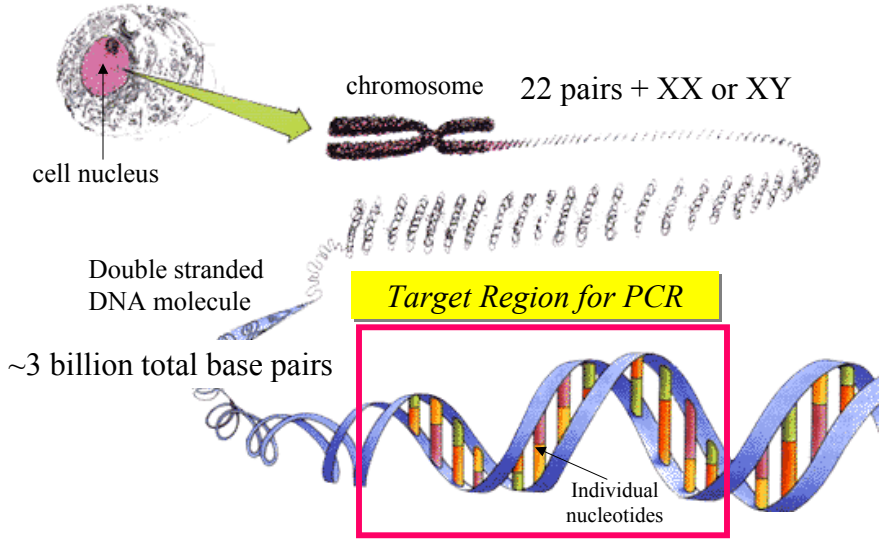


Blood sample

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

Biology

DNA in the Cell



What Type of Genetic Variation?

- Length Variation
  - short tandem repeats (STRs)

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

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

GCTAGTCGATGCTC**(G/A)**GCGTATGCTGTAGC

Basic Concepts

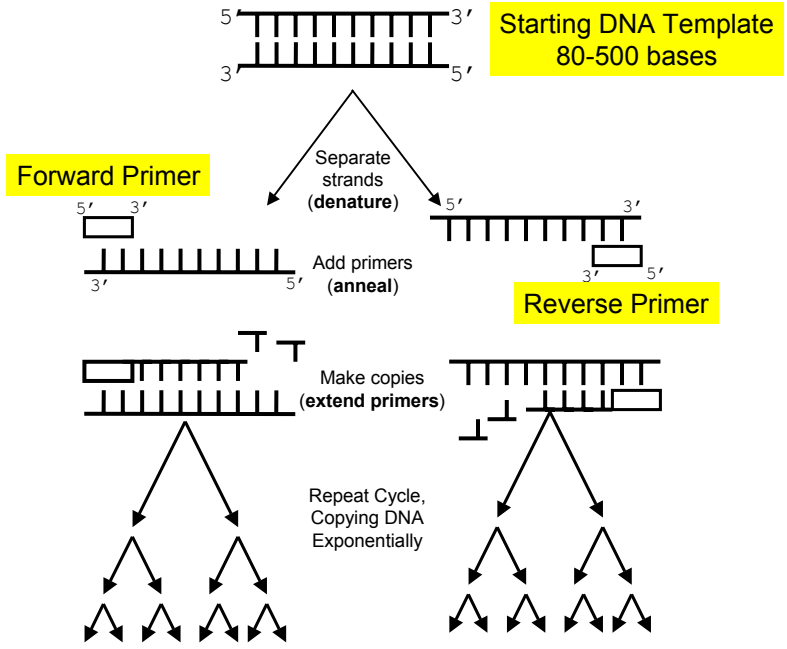
PCR polymerase chain reaction – method of amplifying a specific region of the genome – go from 1 to over a billion copies in about 2 hours

Locus region of the genome being examined

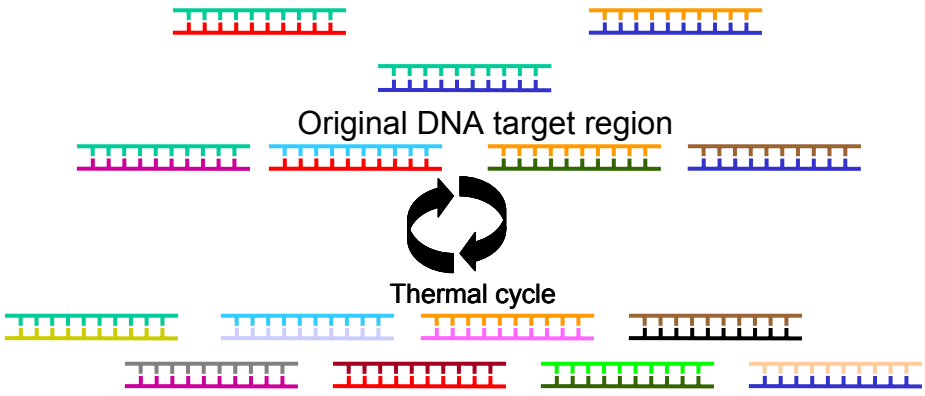
Allele the state of the genetic variation being examined
(STRs = number of repeat units)
(SNPs = base sequence at the site)

Chromosomes are paired so…
Homozygous – Alleles are identical on each chromosome
Heterozygous - Alleles differ on each on each chromosome

PCR Process



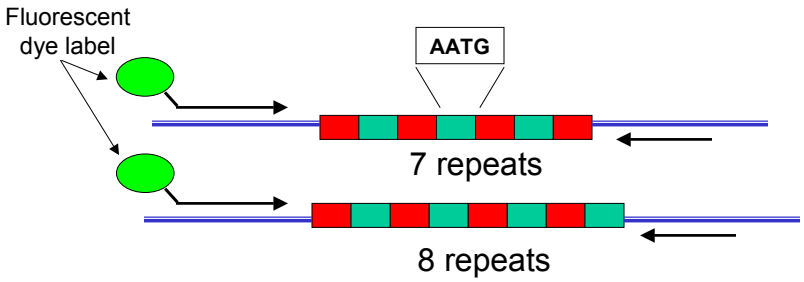
PCR Copies DNA Exponentially through Multiple Thermal Cycles



2^{30}

In 30 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created

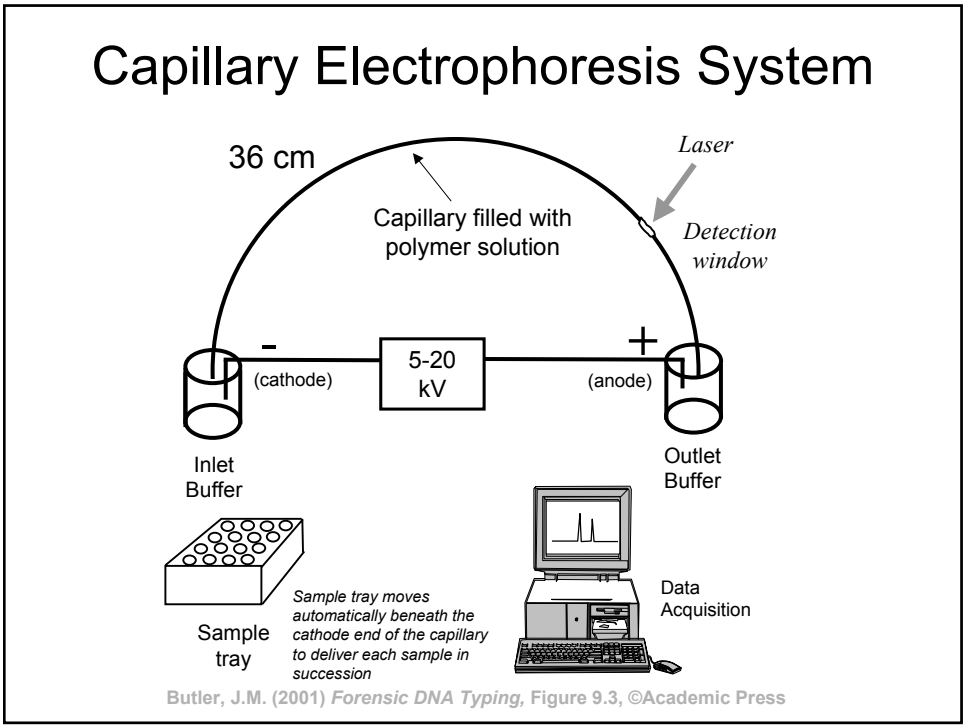
Short Tandem Repeats (STRs)

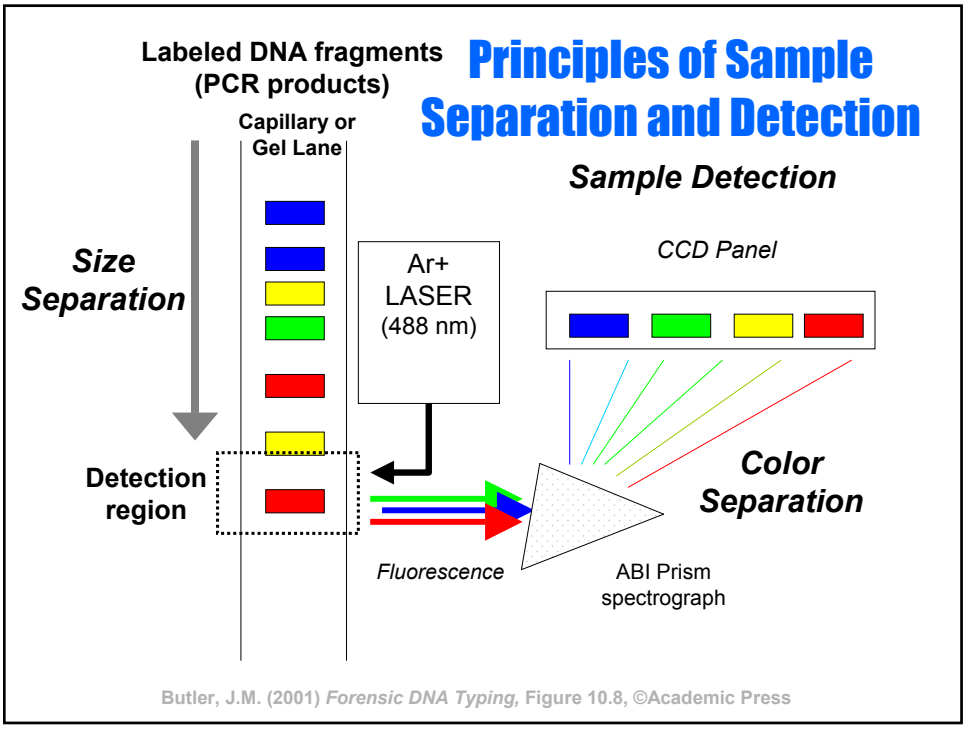


the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

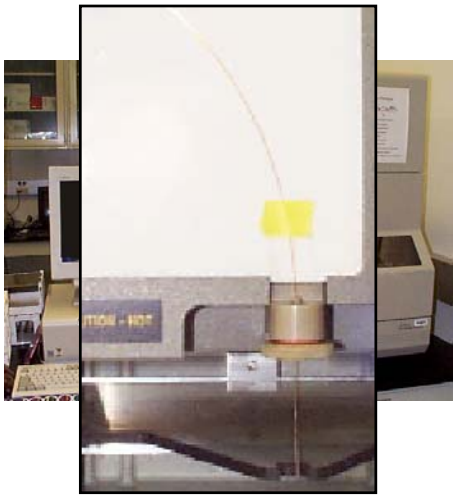
Heterozygote = alleles differ and can be resolved from one another


Capillary Electrophoresis System
36 cm
Capillary filled with polymer solution
Laser
Detection window
-
(cathode)
5-20 kV
+
(anode)
Inlet Buffer
Outlet Buffer
Sample tray
Sample tray moves automatically beneath the cathode end of the capillary to deliver each sample in succession
Data Acquisition
Butler, J.M. (2001) Forensic DNA Typing, Figure 9.3, ©Academic Press


Principles of Sample Separation and Detection
Labeled DNA fragments (PCR products)
Capillary or Gel Lane
Sample Detection
Size Separation
Ar+ LASER (488 nm)
CCD Panel
Detection region
Color Separation
Fluorescence
ABI Prism spectrograph
Butler, J.M. (2001) Forensic DNA Typing, Figure 10.8, ©Academic Press

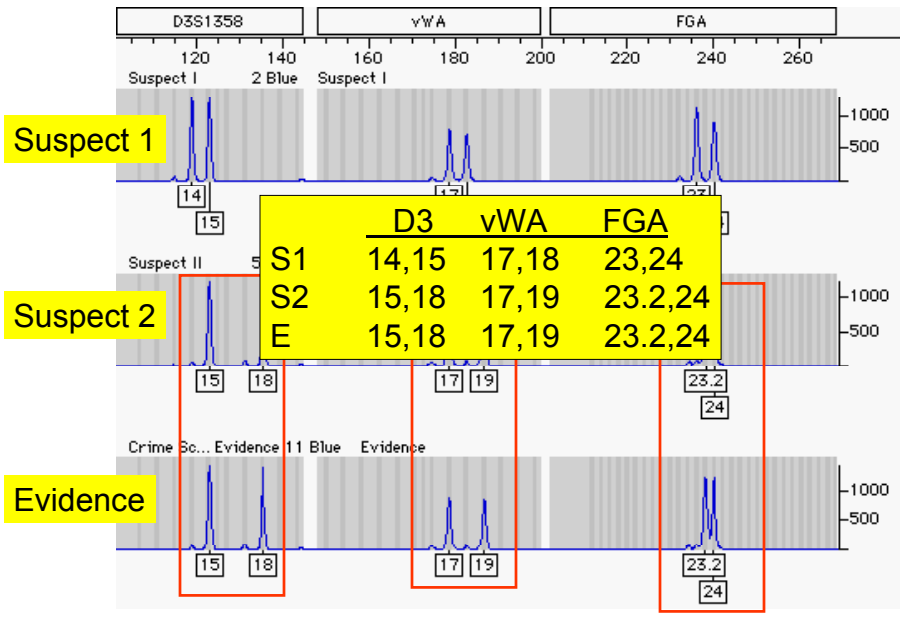
Capillary Electrophoresis Instrumentation

ABI 310
single capillary

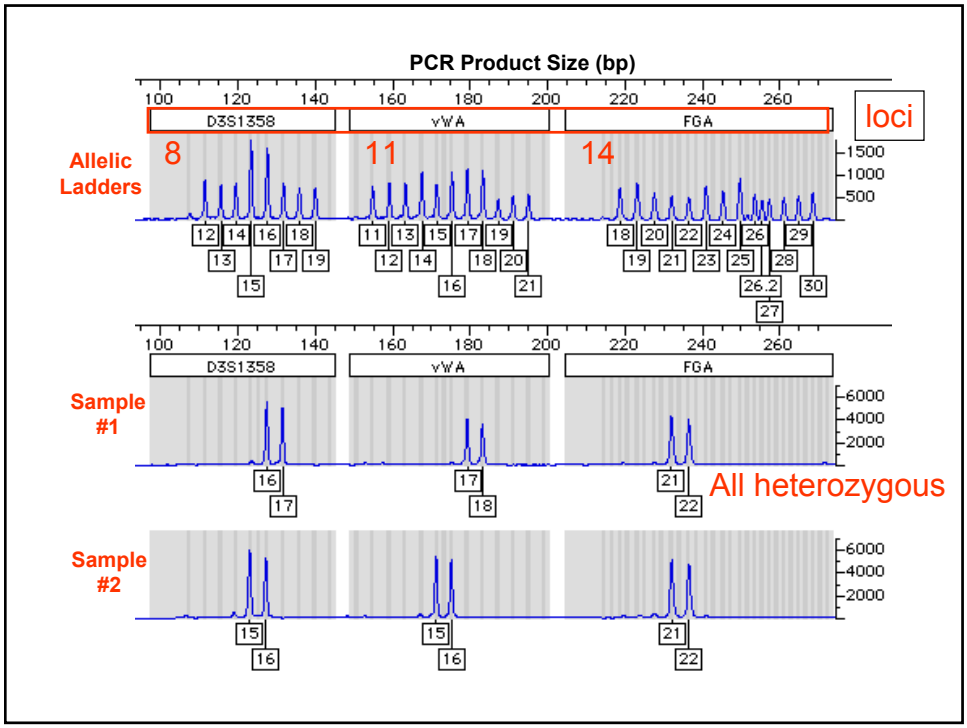


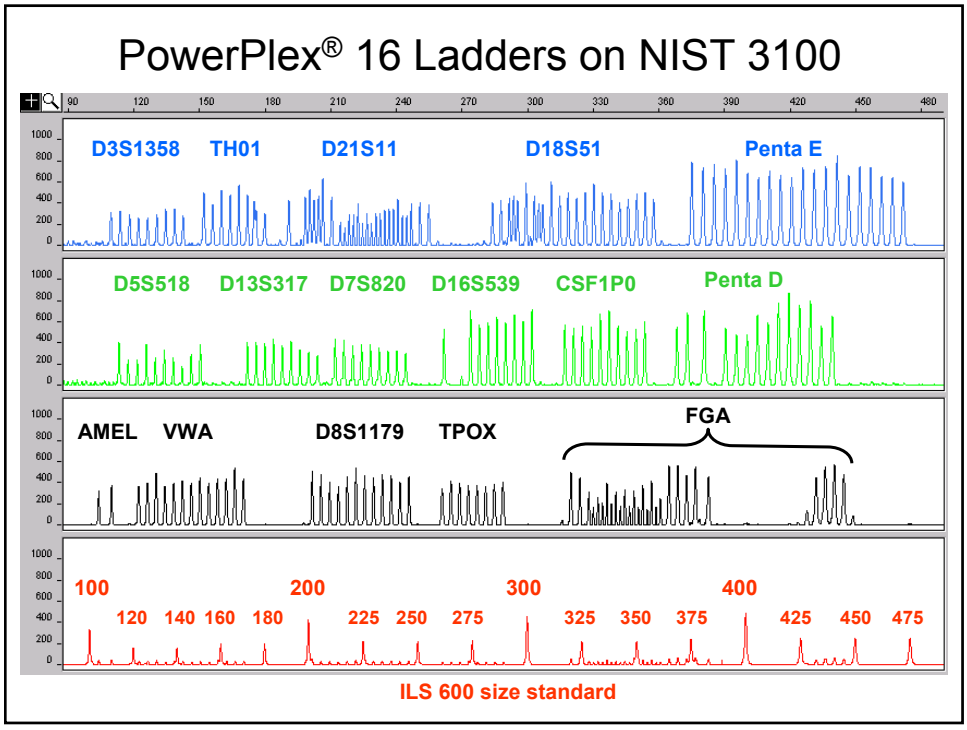
ABI 3100
16-capillary array

Crime Scene - Two Suspects



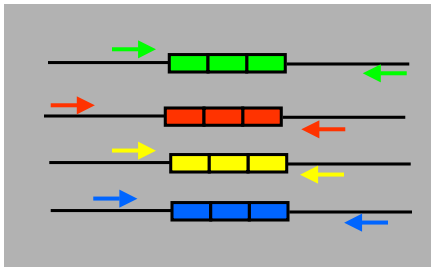
	D3	vWA	FGA
S1	14,15	17,18	23,24
S2	15,18	17,19	23.2,24
E	15,18	17,19	23.2,24


PCR Product Size (bp)
D3S1358
vWA
FGA
loci
Allelic Ladders
8
11
14
Sample #1
Sample #2
All heterozygous


PowerPlex® 16 Ladders on NIST 3100
D3S1358 TH01 D21S11 D18S51 Penta E
D5S518 D13S317 D7S820 D16S539 CSF1P0 Penta D
AMEL VWA D8S1179 TPOX FGA
ILS 600 size standard

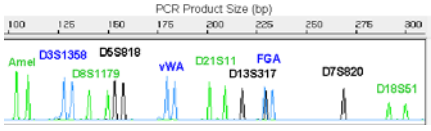
Multiplex PCR

(Parallel Sample Processing)



Multiple primers target more than one site on the DNA strand

Commercial kits are available for targeting and simultaneously amplifying 15 STR markers



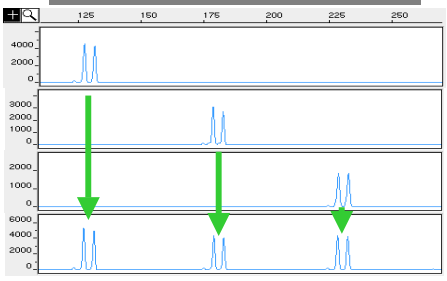
Spectrally distinguishable fluorescent dyes are used as labels

Advantages of Multiplex PCR

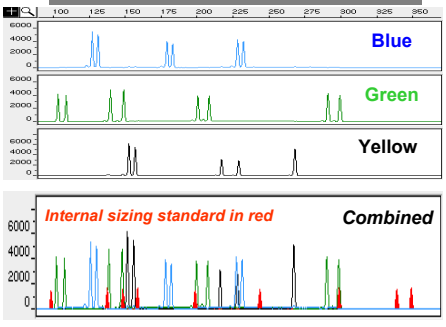
- –Increases information obtained per unit time (increases power of discrimination)
- –Reduces labor to obtain results
- –Reduces template required (smaller sample consumed)

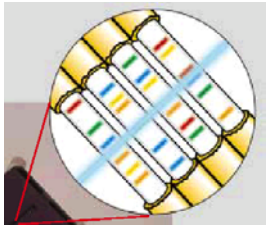
Methods for Parallel Sample Processing

Multiplex by Size



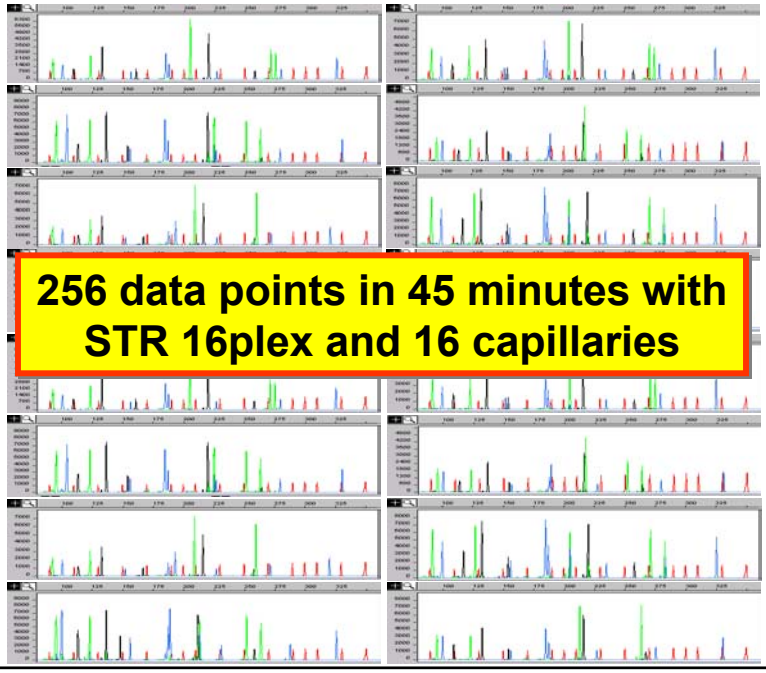
Multiplex by Dye Color





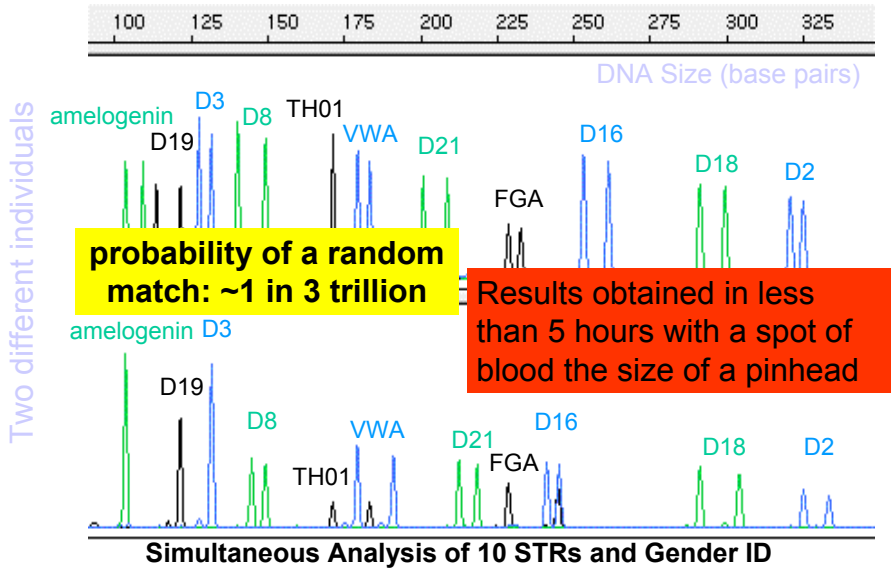
Multiplex by Number of Capillaries

High-Throughput STR Typing on the ABI 3100 (16-capillary array)



Human Identity Testing with Multiplex STRs

AmpFlSTR® SGM Plus™ kit



Simultaneous Analysis of 10 STRs and Gender ID

Product Rule

For heterozygous loci

$$P = 2pq$$

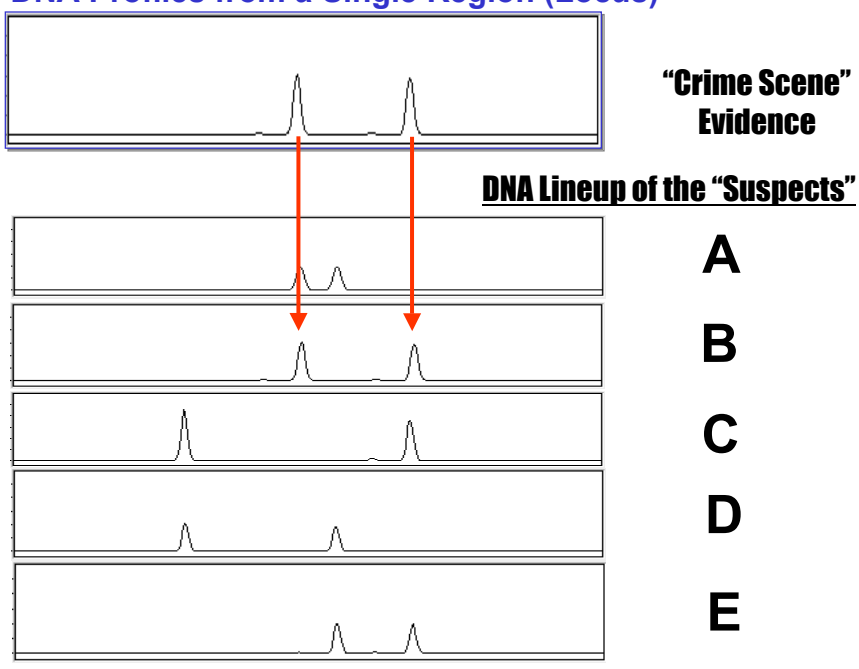
P = probability; p and q are frequencies of allele in a given population

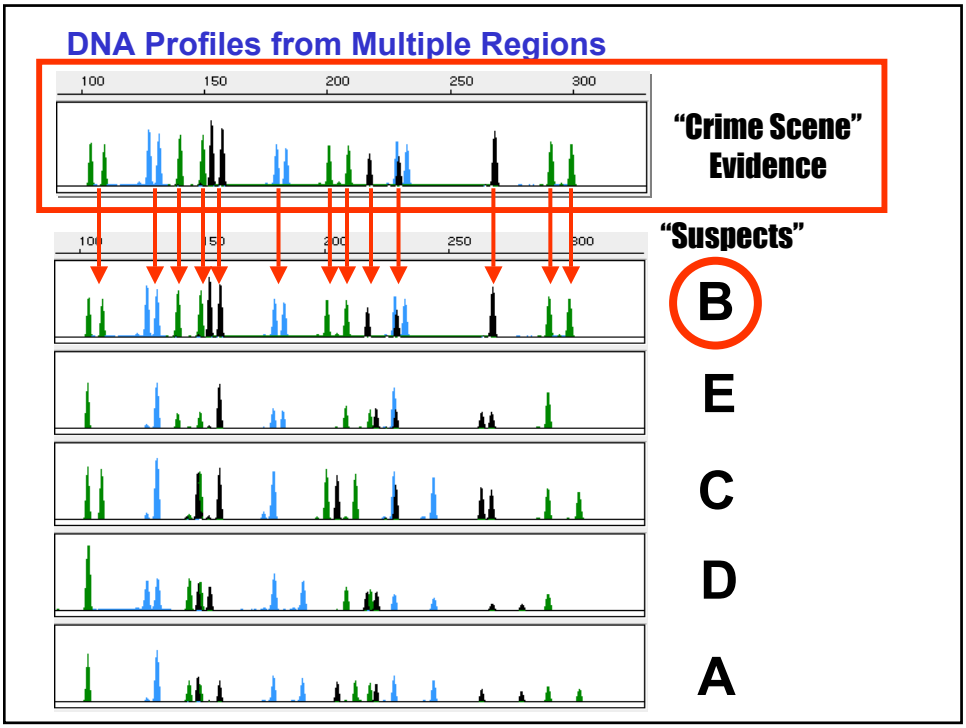
Example: For the locus D3S1358 and individual is 15,18 with frequencies of 0.2825 and 0.1450 respectively

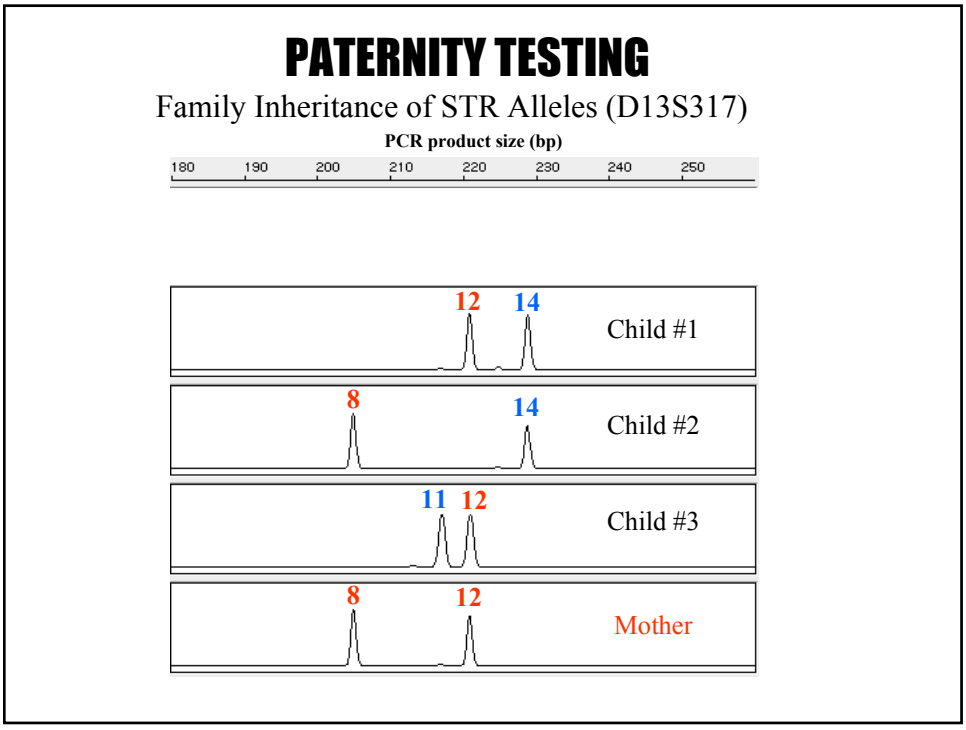
P = 2(0.2825)(0.1450) = 0.0819 or 1 in 12

For 5 loci the
Profile Probability = $(P_1)(P_2)\ldots(P_n)$
= (0.0819)(0.0875)(0.0687)(0.0245)(0.0984)
0.000001187 or 1 in 842,539

DNA Profiles from a Single Region (Locus)

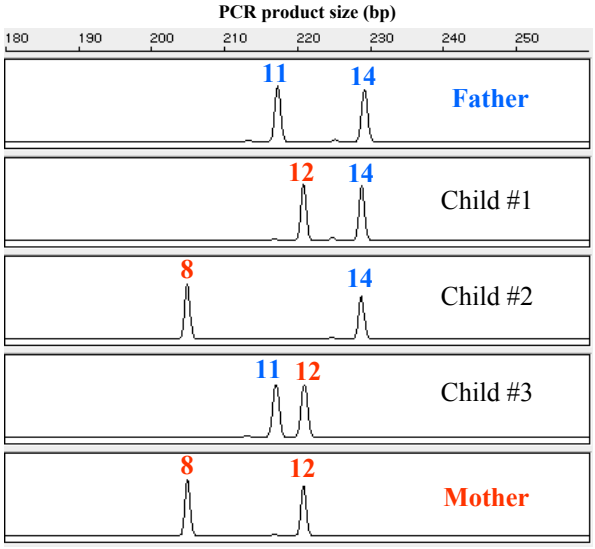



DNA Profiles from Multiple Regions
100
150
200
250
300
"Crime Scene" Evidence
"Suspects"
B
E
C
D
A


PATERNITY TESTING
Family Inheritance of STR Alleles (D13S317)
PCR product size (bp)
180
190
200
210
220
230
240
250
12
14
Child #1
8
14
Child #2
11
12
Child #3
8
12
Mother

PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)



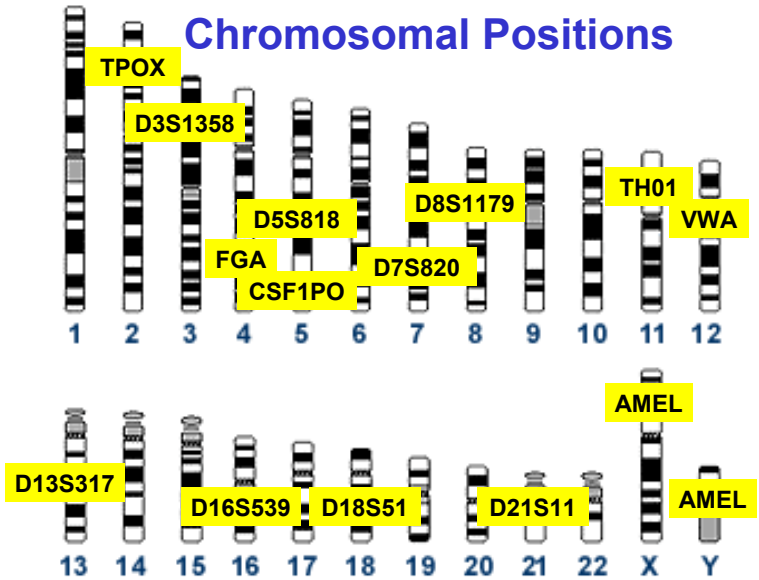
FBI database

CODIS DNA Database

Combined DNA Index System

- Used for linking serial crimes and unsolved cases with repeat offenders
- Convicted offender and forensic case samples
- Launched October 1998 and links all 50 states
- Requires 13 core STR markers
- Current backlog of >750,000 samples ($15M in FY2002 to reduce backlog)

13 CODIS Core STR Loci with Chromosomal Positions



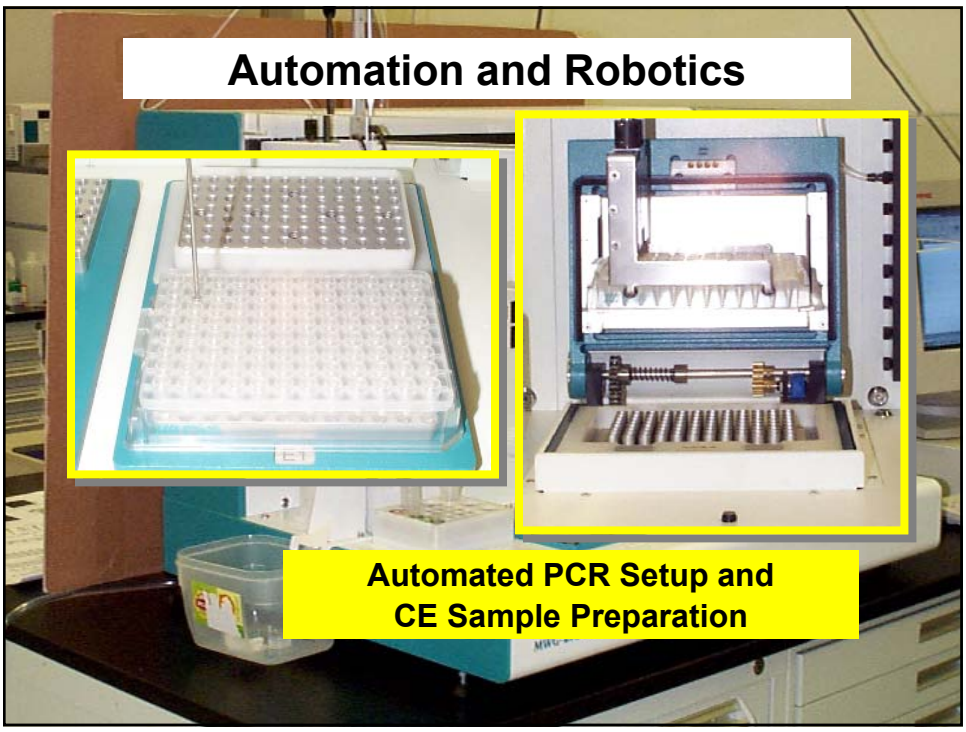
All 50 states now require convicted offenders to submit a sample for DNA testing purposes

7,788 Investigations Aided through April 2003

As of April 2003 the total profile composition of the National DNA Index System (NDIS) is as follows:

Total number of profiles: 1,376,749
Total Forensic profiles: 54,895
Total Convicted Offender Profiles: 1,321,854

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



The Role of NIST Scientists

- Develop DNA standards so that laboratories around the world may compare their results.
- Conduct tests of laboratories around the world to insure accurate results in DNA testing.
- Develop new DNA tests which are more rapid and efficient than those currently used.
- Create useful information databases (STRBase) http://www.cstl.nist.gov/biotech/strbase.
- Evaluation and development of new technologies.

NIST Standard Reference Materials (SRMs)

SRM 2390 - DNA Profiling Standard
Meets RFLP Needs

SRM 2391 - PCR-Based DNA Standard
Cell Lines and Genomics

SRM 2392 - Mitochondrial DNA Standard
Cell Lines and Cloned HV1 Plasmid

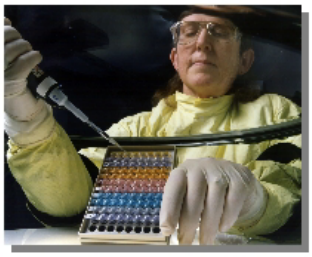
SRM 2393 – mtDNA heteroplasmy

SRM 2395 – Y chromosome DNA standards

NIST

SRM 2391: DNA Profiling Standard for Forensic and Paternity Testing Laboratories

- Human identity testing using Polymerase Chain Reaction (PCR) technologies growing rapidly
- PCR techniques diverse, requiring DNA standards that perform under various amplification and electrophoretic methods
- SRM 2391 certified for genetic locus D1S80; expanding for other loci as needs evolve



DNA Quality Assurance recommendations by the DNA Advisory Board were signed by the FBI director on July 15, 1998.

Beginning October 1, 1998, all federally-funded laboratories that conduct DNA testing must verify procedures using NIST SRMs

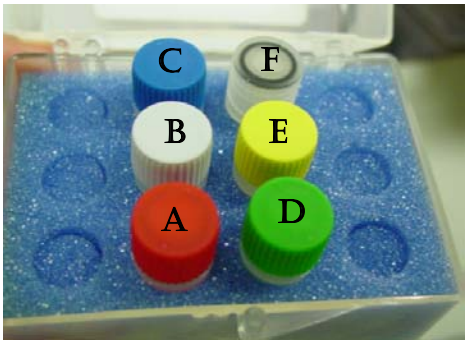
Chemical Science and Technology Laboratory

DAB Quality Assurance Standards for Forensic DNA Testing Laboratories

STANDARD 9.5

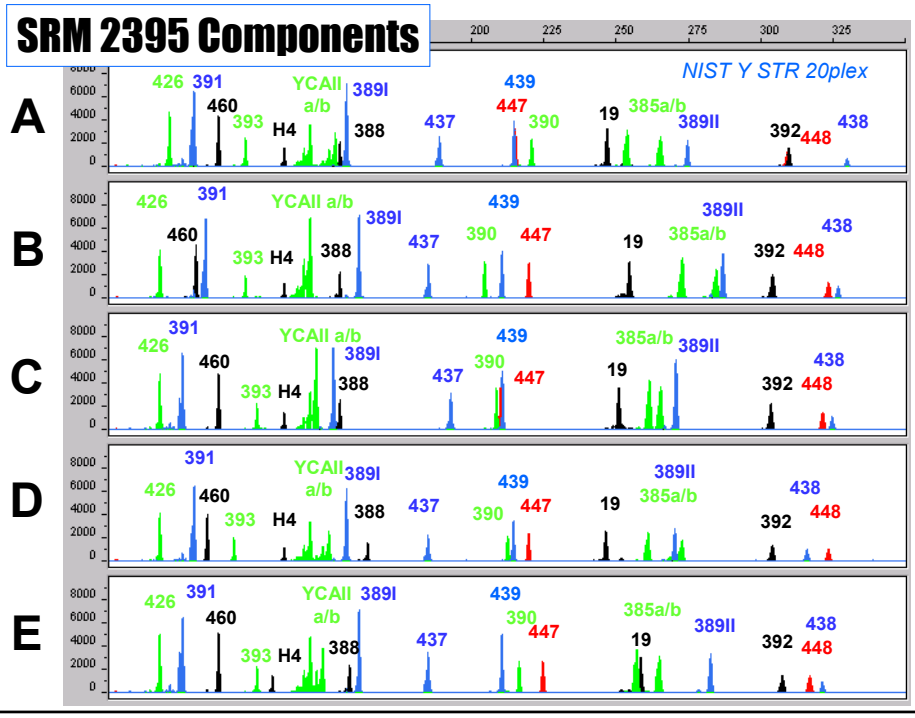
The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

NIST Y Chromosome Standard



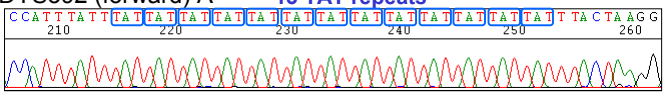
6 genomic DNA samples
5 male and 1 female
Typing Information on 27 Y STRs and 50 Y SNP markers

Available as of 07/2003

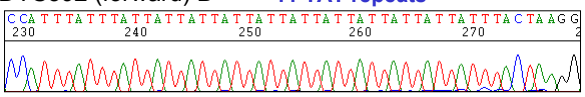


Sequence Determination of Y STR Repeat Region for Each Component

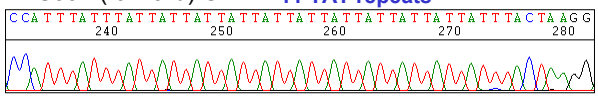
DYS392 (forward) A **13 TAT repeats**



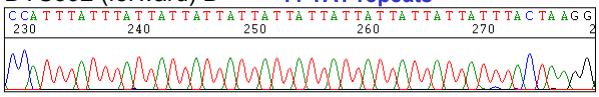
DYS392 (forward) B **11 TAT repeats**



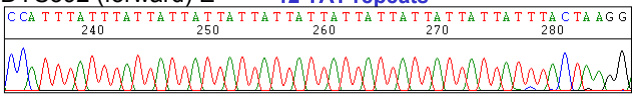
DYS392 (forward) C **11 TAT repeats**



DYS392 (forward) D **11 TAT repeats**



DYS392 (forward) E **12 TAT repeats**



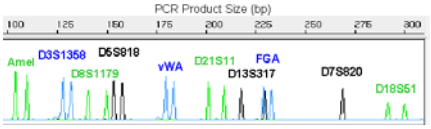
Sequencing Performed

DYS19	DYS388
DYS385 a/b	DYS426
DYS389 I/II	
DYS390	DYS435
DYS391	DYS436
DYS392	DYS437
DYS393	DYS438
	DYS439
DYS447	DYS460 (A7.1)
DYS448	DYS461 (A7.2)
Y-GATA-H4	DYS462

WTC DNA Identifications

World Trade Center Towers
(Sept 11, 2001)

DNA typing being used as only possible method to identify over 2,000 victims of this tragedy



Highly degraded DNA; ~20,000 samples recovered; 3 years to complete…

Wreckage at Ground Zero

Identifying Mass Disaster Victims

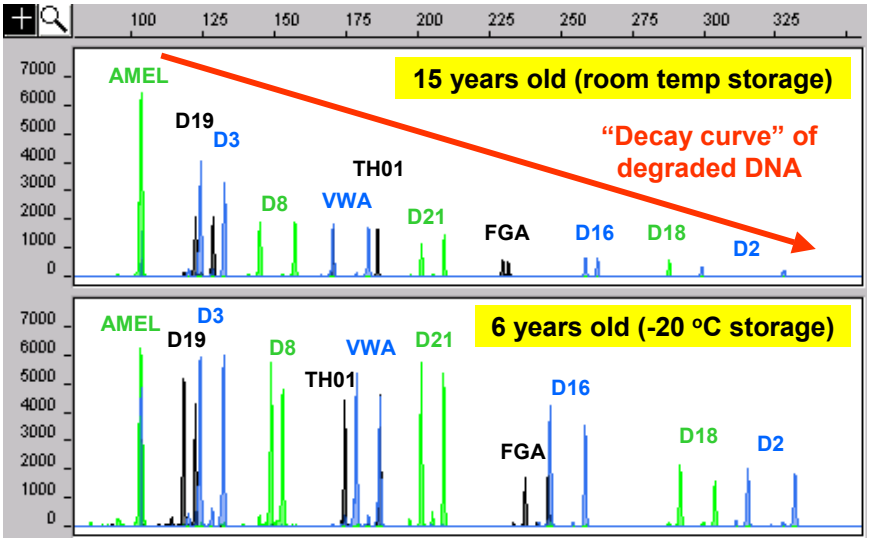
Medical Specimen (Biopsy sample/bloodspot)
Personal Effect (toothbrush/hairbrush)
Kin (close relative)
Remains (bone/ tissue)

DNA profiles obtained are stored in a database and attempts at identification are made

Challenge lies in typing degraded samples

Aged Blood Stain Studies

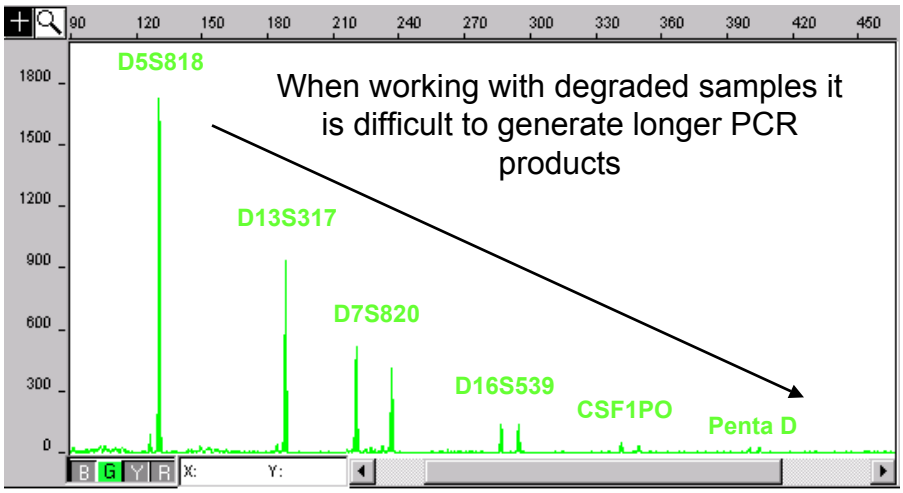
(results from two different DNA samples)



Results with SGM Plus STR kit (Applied Biosystems)

Typing Result on Aged Blood Stain

(15 years at room temperature storage)



86A47N

STR Size Reduction

Through Moving Primer Positions Closer to Repeat

Primer positions define PCR product size
Repeat information is independent of amplicon size

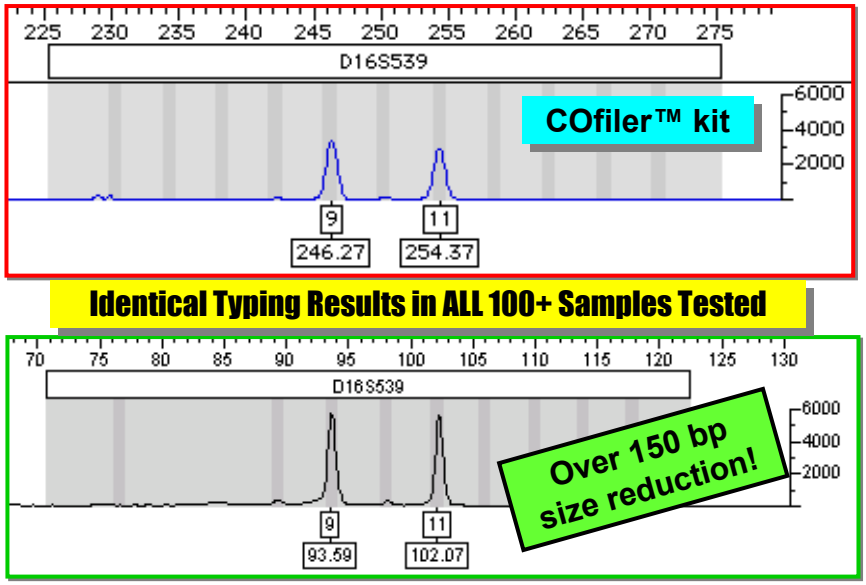
Advantages of Approach:
Size reduction enhances success rate with degraded DNA
Retains same marker information (database compatibility)
Uses highly polymorphic STR loci (high discriminatory power)

Limitation: Lower levels of multiplexing 5-6 plex vs 10-15 plex

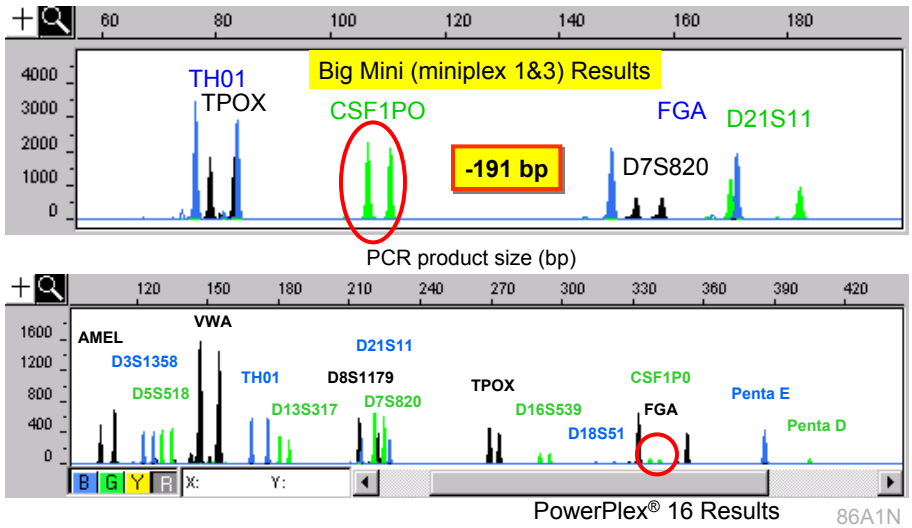
Development of miniSTR Assays

- Primers designed to come as close as possible to the repeat region to generate the smallest possible PCR products
- Equivalent genotypes are obtained when compared with commercial STR kits
- Available as singleplexes or miniplexes (usually one locus per dye color)
- Smaller amplicons offer improved chances of success with degraded DNA samples
- Project begun in November 2001 at the request of Bob Shaler to aid WTC DNA identifications

Development of **miniSTRs** to Aid Testing of Degraded DNA



Comparison between miniSTR and Commercial Kit Results
(Aged Blood Stain Known to Contain Degraded DNA)



Current Status of WTC Samples

28,251 Total Profiles

Personal Effects 4,903

Kin 6,876

Remains 16,472

2795 reported missing – 1511 Identified

Use of miniSTRs has resulted in ~20 additional identifications

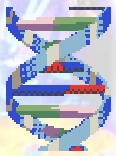
WTC Kinship and Data Analysis Panel

STRBase

Short Tandem Repeat DNA Internet Database

National Institute of Standards and Technology

. . .working with industry to develop and apply technology, measurements and standards



General Information

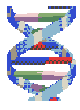
- Intro to STRs (downloadable PowerPoint)
- STR Fact Sheets
- Sequence Information
- Multiplex STR Kits
- Variant Allele Reports

Forensic Interest Data

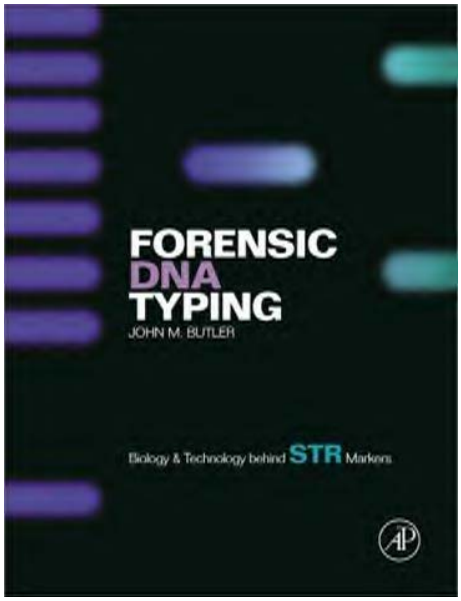
- FBI CODIS Core Loci
- DAB Standards
- NIST SRM 2391
- Published PCR Primers
- Y-Chromosome STRs
- Population Data
- Validation Studies

Supplemental Info

- Reference List
- Technology Review
- Addresses for Scientists
- Links to Other Web Sites



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



FORENSIC DNA TYPING
Biology and Technology
behind STR Markers

John M. Butler

Listed on amazon.com
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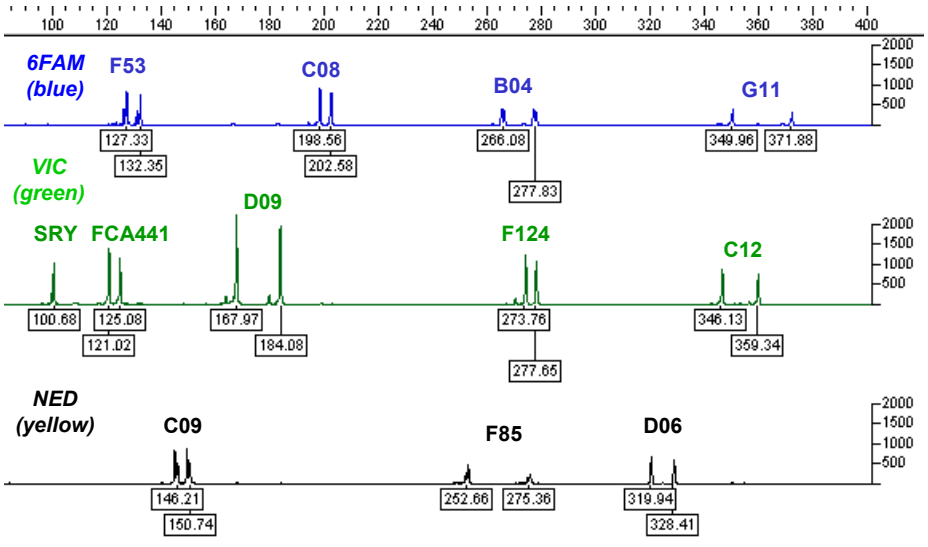
A Harcourt Science and Technology Company

http://www.apnet.com/aps/forensics/for_bio.html

Cat STR 12plex under development at NIST



4 ng male cat DNA; Sample #5; 3/12/02

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