Brief History of Forensic DNA Typing

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multilocus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database
What does DNA mean?
Deoxyribonucleic Acid

Where can DNA be found?

Cell Types
- Blood
- Muscle
- Bone
- Tooth Pulp
- Hair Roots
- Saliva
- Sweat
- Semen
- Tissue

Items
- Chewing Gum
- Stamps & Envelopes
- Stains
- Washed Stains
- Door Knobs
- Tooth brushes
- Hair Brushes
- Sanitary Pads
DNA in the cell

cell nucleus

chromosome

Double stranded DNA molecule

Target Region for PCR

 Individual nucleotides
DNA Inheritance Patterns are used in:
criminal paternity
abandoned baby
missing persons
unidentified bodies
Sources of biological evidence

- Blood
- Semen
- Saliva
- Urine
- Hair
- Teeth
- Bone
- Tissue
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
On the victim (female)

- Vaginal swabs
- External genitalia swabs
- Rectal swabs
- Lick/kiss/bite areas
- Fingernail swabs
- Wood’s lamp positive areas
On the victim (male)

- Penile swabs
- Rectal swabs
- Lick/kiss/bite areas
- Fingernail swabs
- Wood’s lamp positive areas
60% of kits are negative for semen

• Absence of sperm does not counter indicate sexual assault

• Reasons:
  – vasectomy
  – perpetrator failing to ejaculate
    • 34% of rapist show evidence of sexual dysfunction at the time of rape
  – time between exam and incident
  – false report
**Steps in DNA Processing**

**Biology**

1. DNA Extraction
2. DNA Quantitation
3. PCR Amplification of Multiple STR markers

**Technology**

1. Separation and Detection of PCR Products (STR Alleles)
2. Sample Genotype Determination

**Genetics**

1. Comparison of Sample Genotype to Other Sample Results
2. Generation of Case Report with Probability of Random Match

*If match occurs, comparison of DNA profile to population databases*
Methods used for analysis of DNA

PCR (Polymerase Chain Reaction)

- Sequence Variation and Tandem Repeat Variation
- Small sample required
- Used in CODIS
Repeat Variation

TGCAT TGCAT TGCAT TGCAT TGCAT = 5 Repeats
TGCAT TGCAT TGCAT TGCAT TGCAT = 4 Repeats

MAY CONSISTS OF 2-7 NUCLEOTIDE REPEATS

MOST STR’s HAVE 7-10 ALLELES PER LOCUS

OFTEN WITH 2 OR 3 ALLELES THAT ARE COMMON
STR
Short Tandem Repeat

DNA Profile = 4, 6

DNA Profile = 5, 7
Four Main Steps

1) Isolation
2) Amplification - 3 steps
   a) Denaturation
   b) Annealing
   c) Extension
3) Analysis / Interpretation
4) Statistics
Isolation of DNA

Blood
Hair Roots
Saliva
Sweat
Tissue

Chemical
DNA
Differential Isolation of DNA

Semen stain

Epithelial DNA

Sperm DNA
Amplification
(making copies)

Solution
Salt
Primers
Taq
Bases

DNA
Amplification - The Cycles

1 Cycle

2 Cycles

3 Cycles

4 Cycles

5 Cycles

30 Cycles
Amplification - Denaturation

Step One

Heat
Amplification - Annealing

Step Two

Flourescent or Color Tag

Primer

A G T C G A
T C A G C T
C A G C T

20
Amplification - Extension

Step Three

Tag

Primer

Taq Polymerase
DNA Amplification with the Polymerase Chain Reaction (PCR)

Starting DNA Template

Separate strands (denature)

Forward primer

Make copies (extend primers) (anneal)

Reverse primer
In 32 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
ABI Prism 310 Genetic Analyzer

Syringe with polymer solution
Outlet buffer
Injection electrode
Autosampler tray
Inlet buffer
Capillary
Close-up of ABI Prism 310 Sample Loading Area

See Technology section for more information on CE
DNA Separation Mechanism

- Size based separation due to interaction of DNA molecules with entangled polymer strands
- Polymers are not cross-linked (as in slab gels)
- "Gel" is not attached to the capillary wall
- Pumpable -- can be replaced after each run
- Polymer length and concentration determine the separation characteristics
Human Identity Testing with Multiplex STRs

AmpFlSTR® SGM Plus™ kit

Simultaneous Analysis of 10 STRs and Gender ID

Two different individuals

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

DNA Size (base pairs)
STR genotyping is performed by comparison of sample data to allelic ladders.

Microvariant allele
Multiplex PCR

- Over 10 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
An Example Forensic STR Multiplex Kit

**AmpFISTR® Profiler Plus™**

*Kit available from PE Biosystems (Foster City, CA)*

9 STRs amplified along with sex-typing marker amelogenin in a single PCR reaction.

Size Separation

Color Separation

100 bp 200 bp 300 bp 400 bp

- **5-FAM (blue)**
  - D3
  - vWA
  - FGA

- **JOE (green)**
  - A
  - D8
  - D21
  - D18

- **NED (yellow)**
  - D5
  - D13
  - D7

- **ROX (red)**

**GS500-internal lane standard**
STR Results

The DNA from the evidence stain and the reference sample from the suspect match.

Freq of the match is about

1 in 520,000,000,000 in the Cauc pop

1 in 618,500,000,000 in the AA pop

1 in 532,000,000,000 in the Hispanic pop.
13 CODIS Core STR Loci with Chromosomal Positions

- TPOX
- D3S1358
- D5S818
- FGA
- CSF1PO
- D7S820
- D8S1179
- D13S317
- D16S539
- D18S51
- D21S11
- TH01
- VWA
- AMEL
- AMEL
Pet DNA

• Snowball the cat owned by suspect
  – White cat hairs found on victim’s body
  – Matched cat hairs to those found on victim’s body
  – Meowplex for cats

• LA Times March 16, 2002
  – Ventura County woman sexually assaulted in her home
  – Victim described suspect
  – Police canvassed neighborhood found individual matching suspect
  – Dog hair recovered from his pants matching victim’s dog
Human Identity Testing

- Forensic cases -- matching suspect with evidence
- Paternity testing -- identifying father
- Historical investigations
  - Possible offspring of Thomas Jefferson and Sally Hemmings
- Missing persons investigations
- Mass disasters -- putting pieces back together
  - Tsunami survivors
  - Infants returned to parents using DNA
Human Identity Testing

• **Military DNA “dog tag”**
  – 4 sets of remains from WWI, WWII, Korea and Vietnam
  – All matched to family members
  – Future no more unknown soilders
  – Each soldier has a blood card stored in freezer
  – 4.5 million samples

• **Convicted felon DNA databases**

• **Innocence project**
  – As of 3/8/07, 196 prisoners have been released
  – Thousands have come back as a match
FBI’s CODIS DNA Database

Combined DNA Index System
• Used for linking serial crimes and unsolved cases with repeat offenders
• Launched October 1998
• Links all 50 states
• Requires >4 RFLP markers and/or 13 core STR markers
• Current backlog of >600,000 samples
1) Law requiring collection of convicted offender

2) Databasing of the DNA profile from offenders

3) DNA Typing of evidentiary samples
   a) Unknown suspect cases

4) Search a national database
   a) Compare samples to the database
   b) Compare cases to each other
Over 1 million profiles at national level

National Database

State Database

Local Database

Weekly searches at each level

CODIS
CODIS

Population Index

Casework

Convicted Offender Index
HITS

• Case to case--links 2 or more cases

• aids investigation, one of the cases may be a solved case

• Case to offender

• links case to convicted offender
WHY THE CHANGE?

- **Database Successes**
  - ✔ Virginia is averaging one “cold hit” per week in 2001.
  - ✔ Diversion of resources to other cases.

- **New Data**
  - ✔ Approximately 85% of hits would have been missed if the databank were limited to only violent offenders.
  - ✔ 52% of Florida offenders linked to sexual assaults and homicides by DNA database matches have had prior burglary convictions.
WHY THE CHANGE?

– Citizen Demand / Public Safety

✓ Consider the following US Department of Justice statistics:

➤ The **average** rapist commits 8-12 sexual assaults.

➤ Of 108,580 persons released from prisons in 11 States in 1983, an estimated 62.5% were rearrested for a felony or serious misdemeanor within 3 years

➤ 46.8% were reconvicted, and 41.4% returned to prison or jail.
DNA DATABASE SUCCESSES

✓ Virginia Database Statistics
  ~ Over 1100 DNA database hits
  ~ 85% of hits would have been missed if database limited to only violent offenders.

✓ Florida Database Statistics
  ~ 52% of Florida offenders linked to sexual assaults and homicides by DNA database matches have had prior burglary convictions.

✓ New York Database Statistics
  1999 New York law expands DNA database to include many non-violent felonies (including burglary and drug crimes).
  ~ January 2002 Report “The First 100 Hits.”
    ➔ February 2000 – July 2001
    ➔ 104 crime scenes matched with 102 offenders.