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
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
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
Steps in DNA Sample Processing

**Biology**
- DNA Extraction
- DNA Quantitation
- PCR Amplification of Multiple STR markers

**Technology**
- Separation and Detection of PCR Products (STR Alleles)
- Sample Genotype Determination

**Genetics**
- Comparison of Sample Genotype to Other Sample Results
- Generation of Case Report with Probability of Random Match

If match occurs, comparison of DNA profile to population databases

Sample Obtained from Crime Scene or Paternity Investigation
Sources of Biological Evidence

- Blood
- Semen
- Saliva
- Urine
- Hair
- Teeth
- Bone
- Tissue
DNA in the Cell

- Cell nucleus
- Chromosome
- Double stranded DNA molecule
- Target Region for PCR
  - Individual nucleotides
DNA Amplification with the Polymerase Chain Reaction (PCR)

- **Starting DNA Template**

1. **Separate strands (denature)**
2. **Add primers (extend primers)**
3. **Make copies (anneal)**

**Forward primer**

**Reverse primer**
PCR Copies DNA Exponentially through Multiple Thermal Cycles

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
Different primer sets produce different PCR product sizes for the same STR 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

- **D3**, **vWA**, **FGA**: 5-FAM (blue)
- **A**, **D8**, **D21**, **D18**: JOE (green)
- **D5**, **D13**, **D7**: NED (yellow)
- **ROX** (red)

Size Separation

Color Separation

GS500-internal lane standard
Available Kits for STR Analysis

- Kits make it easy for labs to just add DNA samples to a pre-made mix
- 13 CODIS core loci
  - Profiler Plus and COfiler (PE Applied Biosystems)
  - PowerPlex 1.1 and 2.1 (Promega Corporation)
- Increased power of discrimination
  - CTT (1994): 1 in 410
  - SGM Plus™ (1999): 1 in 3 trillion
  - PowerPlex ™ 16 (2000): 1 in 2 x 10^{17}
Close-up of ABI Prism 310 Sample Loading Area

See Technology section for more information on CE
Human Identity Testing with Multiplex STRs

Simultaneous Analysis of 10 STRs and Gender ID

Two different individuals

amelogenin  D3  D8  TH01  VWA  D21
D19

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)

AmpFlSTR® SGM Plus™ kit

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STR genotyping is performed by comparison of sample data to allelic ladders.
STR Allele Frequencies

TH01 Marker

- Red: Caucasians (N=427)
- Blue: Blacks (N=414)
- Green: Hispanics (N=414)

Number of repeats

Frequency

*Proc. Int. Sym. Hum. ID (Promega) 1997, p. 34
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
13 CODIS Core STR Loci with Chromosomal Positions

1. TPOX
2. D3S1358
3. D5S818
4. FGA
5. CSF1PO
6. D8S1179
7. D7S820
8. TH01
9. VWA
10. D13S317
11. D16S539
12. D18S51
13. D21S11

AMEL