Progression of DNA Typing Markers

• RFLP
  – multilocus VNTR probes
  – single locus VNTR probes ($^{32}$P and chemiluminescence)

• PCR
  – DQ-alpha (reverse dot blot)
  – PolyMarker (6plex PCR; dots for SNPs)
  – D1S80 (AMP-FLPs)
  – singleplex STRs with silver staining
  – multiplex STRs with fluorescent dyes
Changing Technologies Paradigm Shift: Restriction Fragment-Length Polymorphisms to Short Tandem Repeats

- Five RFLP probes provide almost exclusive identity (~ 1 in $10^9$ individuals)
- RFLP requires a minimum of 25 ng of relatively undegraded DNA (1000 - 20,000 basepairs)
- Short Tandem Repeats (STRs) only require ~ 1 ng DNA that can be partially degenerated
- Discrimination power: 5 RFLP probes equals ~ 12 STR loci
Which Suspect, A or B, cannot be excluded from potential perpetrators of this assault?
Capillary Electrophoresis (CE)

Fill with Polymer Solution

Inlet (cathode)

50-100 μm x 27 cm

DNA Separation occurs in minutes...

5-20 kV

Burn capillary window

Outlet (anode)

Argon Ion Laser

Data Acquisition and Analysis
ABI PRISM® 310 Genetic Analyzer

Automated gel pouring

Automated sample injection

Capillary electrophoresis with multi-color detection capabilities
ABI Prism 310 Genetic Analyzer

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

- Electrode
- Capillary
- Sample Vials
- Autosampler Tray
ABI 310 Result
9.3 allele: 1071 sec
10 allele: 1073 sec

Mass Spec Result
9.3 allele: 203.3 µsec
10 allele: 204.8 µsec

Allele 10
COfiler™ size: 187 bp
MS size: 83 bp
GeneChip® Expression Analysis Process

GeneChip® expression analysis probe array

Each probe cell contains millions of copies of a specific oligonucleotide probe

Biotinylated RNA target from experimental sample

Streptavidin-phycoerythrin conjugate

Image of hybridized probe array
STR Analysis by Hybridization on Microchips

- Discriminates single base pair mismatch
MWG Biotech RoboAmp 4200

Automated PCR Setup and Mass Spec Sample Preparation
Mass Spec Sample Plates

PerSeptive Biosystems
(100 positions)

Bruker
(384 positions)
Time-of-Flight Mass Spectrometry

DNA separations occur in microseconds!

DNA Reaction Products (Size separated and drifting to the detector)

Pulsed Laser Beam

High-Density Sample Array

X-Y sample control

Detector

Drift Region Electric-Field Free

Ion Extractor Acceleration Region

Gels
Bruker BIFLEX III Time-of-Flight Mass Spectrometer

Capable of fully automated data acquisition on 384 or more samples per plate