

Genotyping Technologies

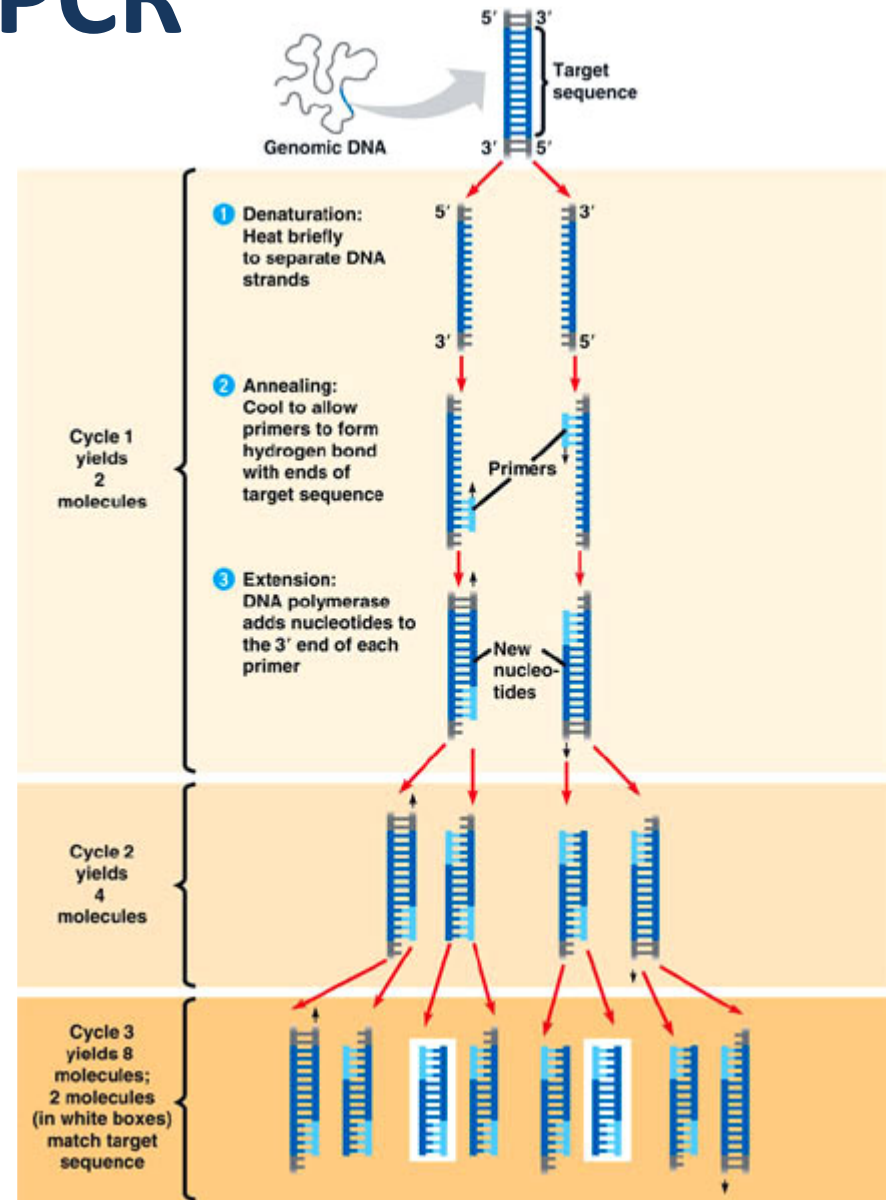
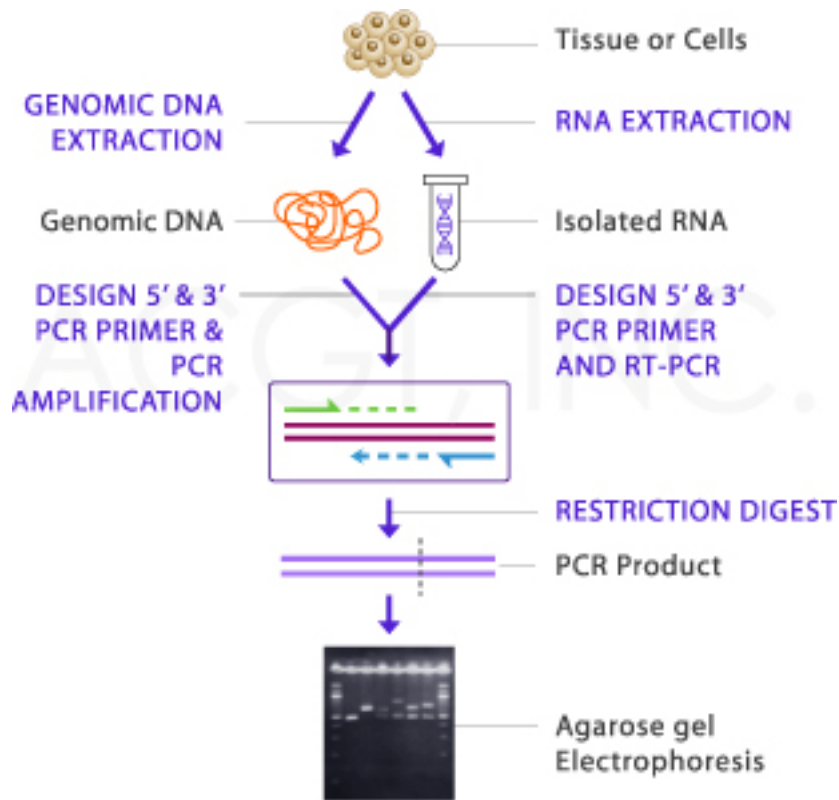
Michelle Amaral, PhD

UAB Heflin Center

Summary

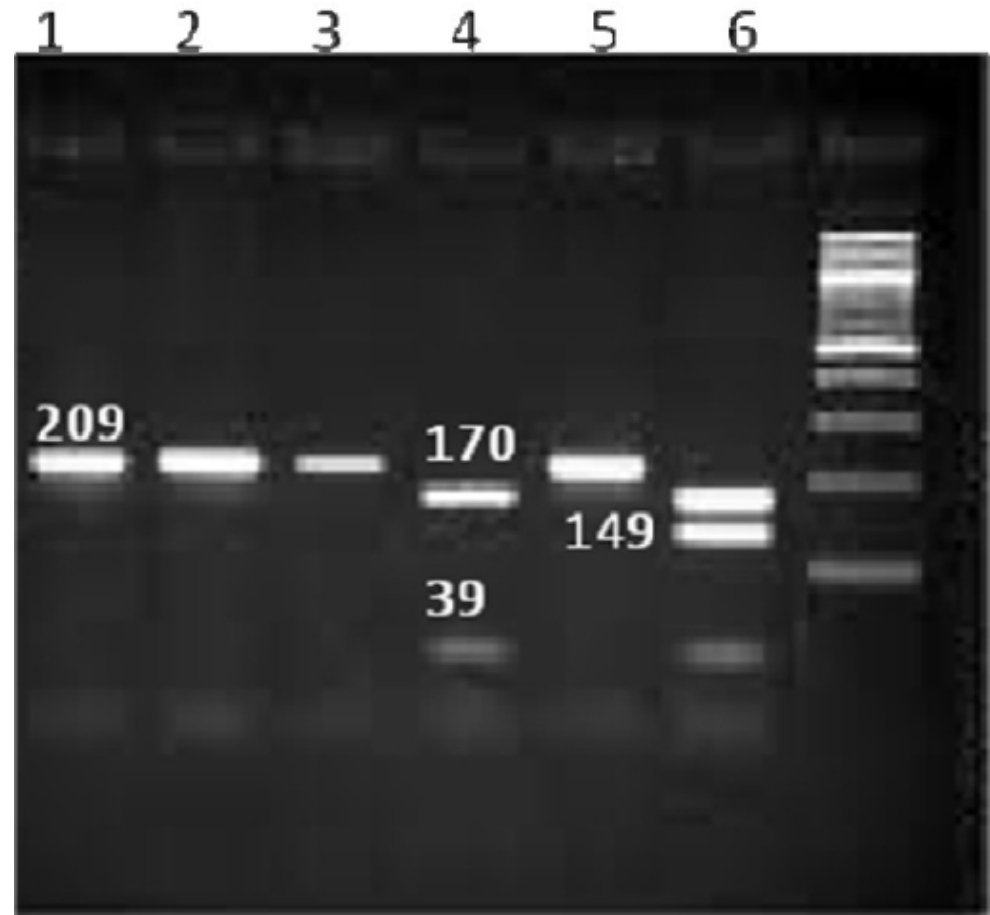
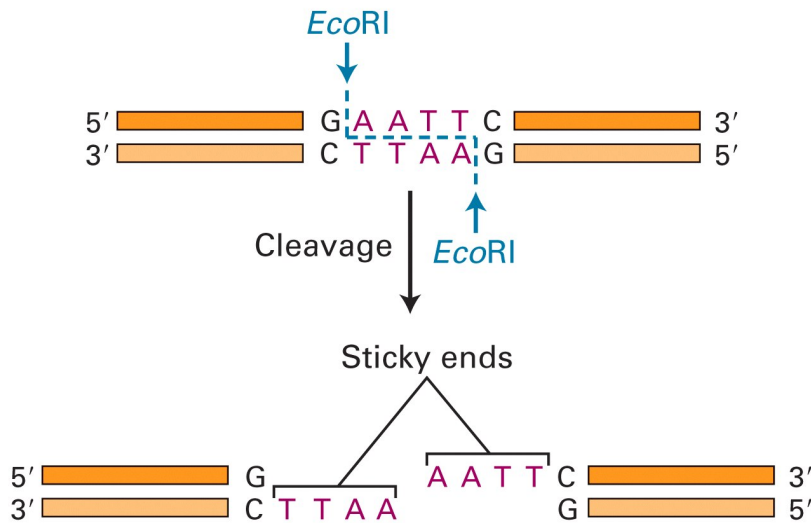
- PCR
- Sanger sequencing
- RFLP
- Microarrays

Genotyping With PCR



Genotyping With PCR (cont'd)

- Restriction enzymes
- Electrophoresis

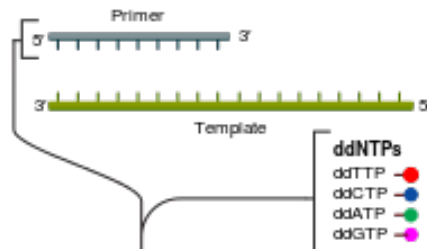


Genotyping With PCR (cont'd)

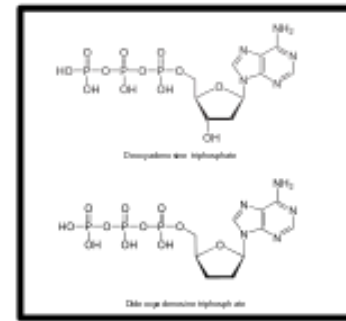
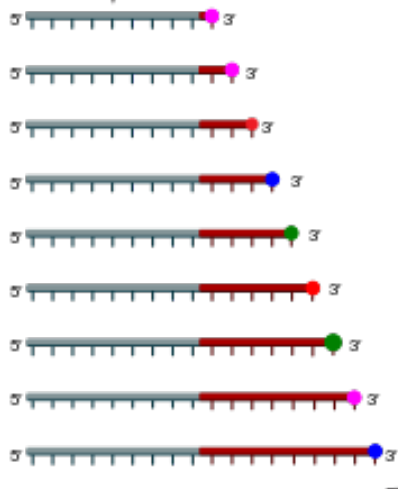
- Sanger DNA sequencing

① Reaction mixture

- ▶ Primer and DNA template
- ▶ DNA polymerase
- ▶ ddNTPs with flouochromes
- ▶ dNTPs (dATP, dCTP, dGTP, and dTTP)



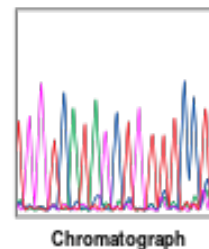
② Primer elongation and chain termination



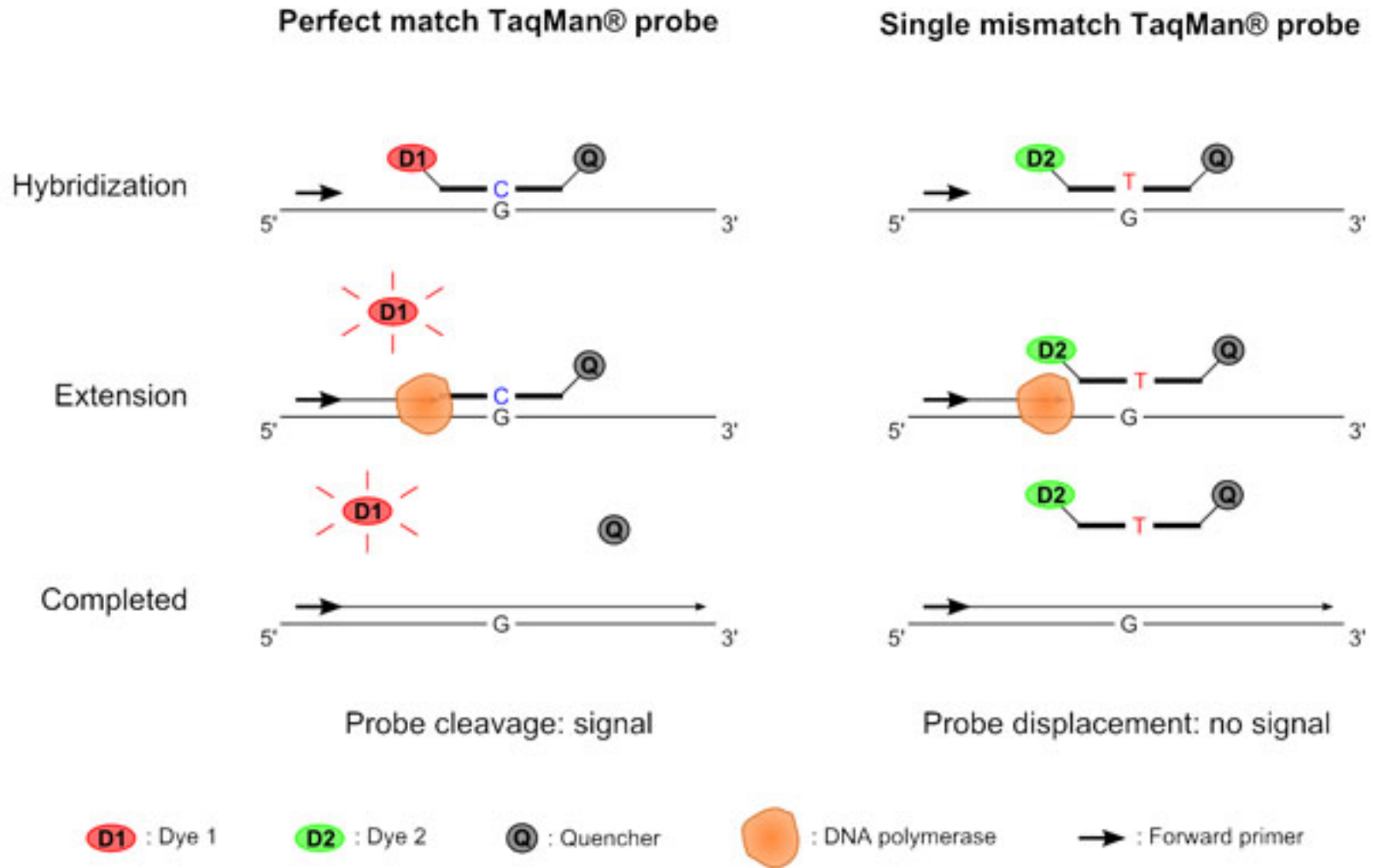
③ Capillary gel electrophoresis separation of DNA fragments



④ Laser detection of flouochromes and computational sequence analysis



Genotyping: Taqman Assay



Genotyping: Pyrosequencing

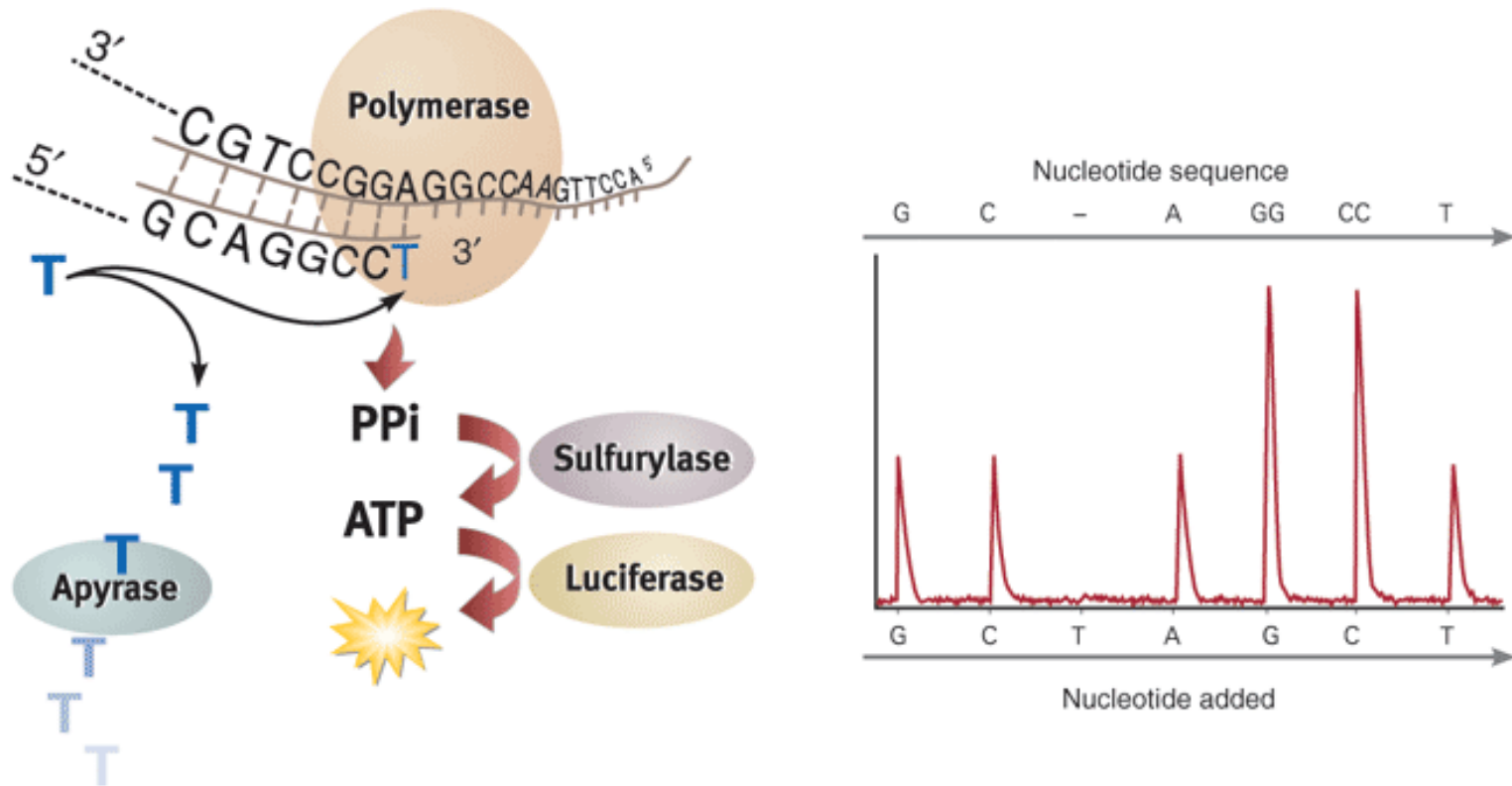
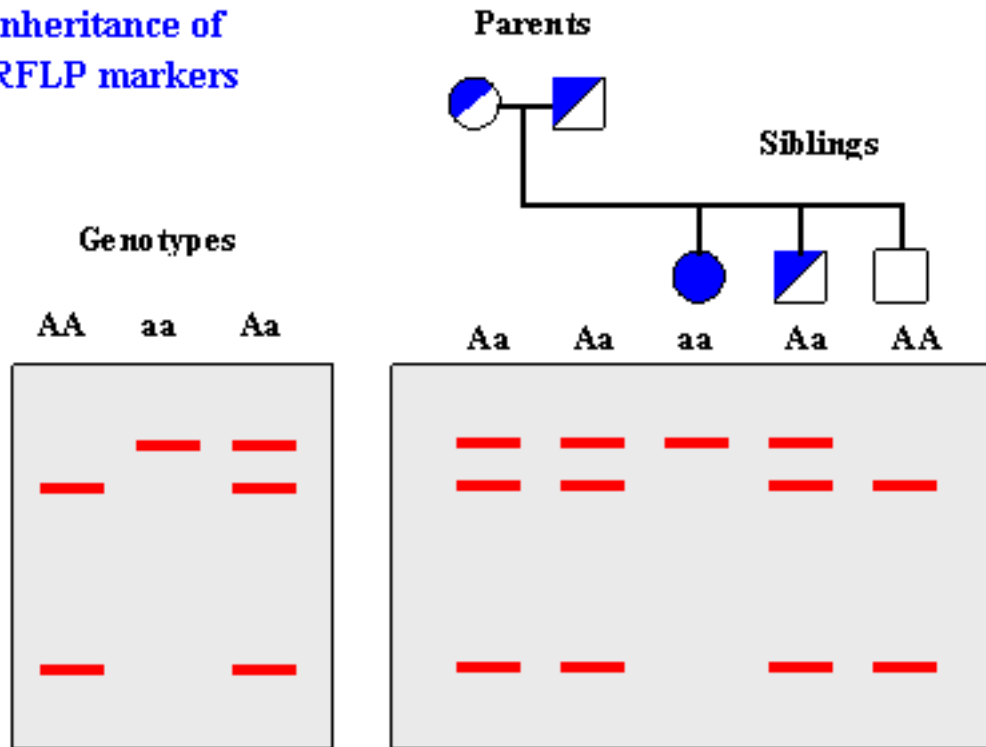


Figure 1 | The principle of Pyrosequencing and the output Pyrogram™. Double peak heights indicate incorporations of two nucleotides in a row.

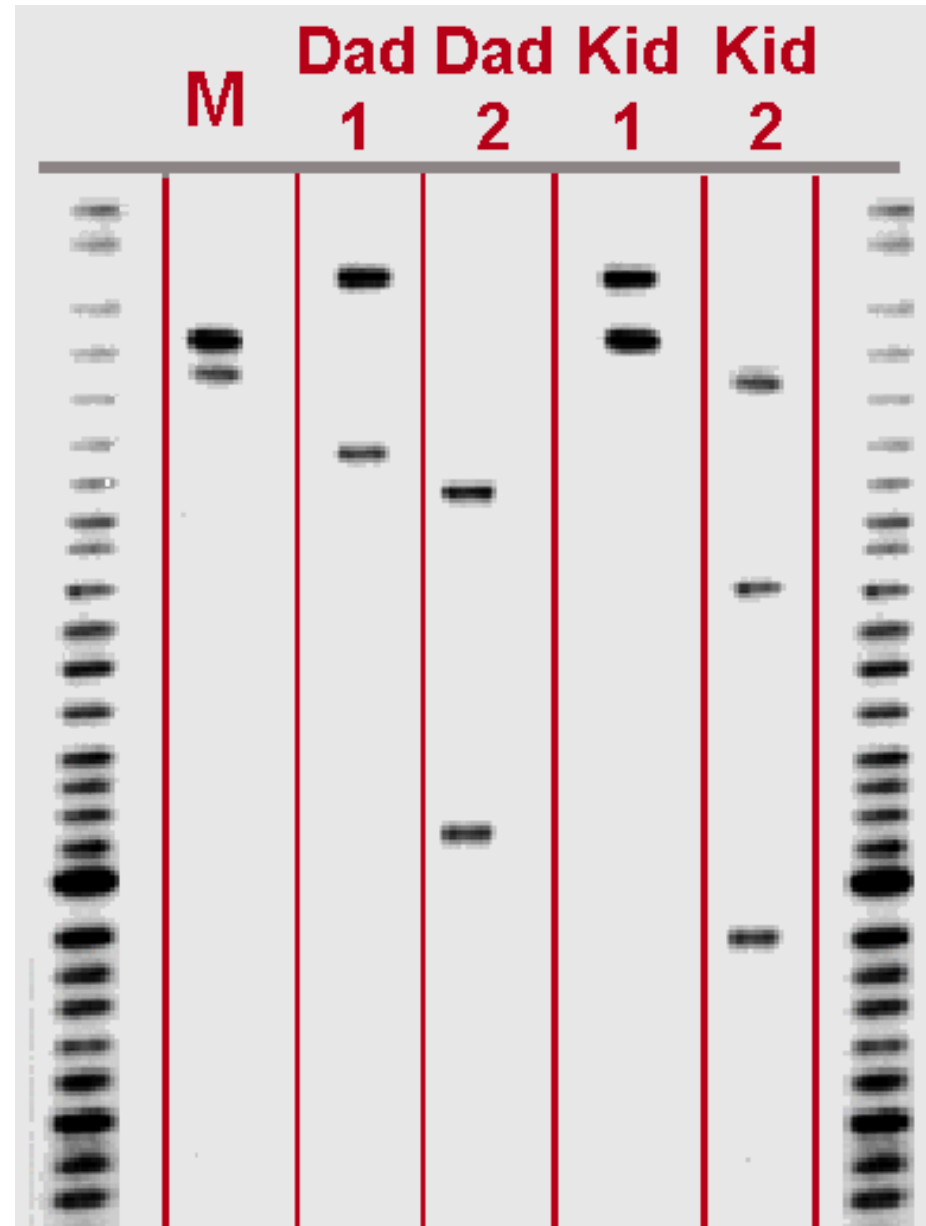
Restriction Fragment Length Polymorphism (RFLP)

Inheritance of RFLP markers



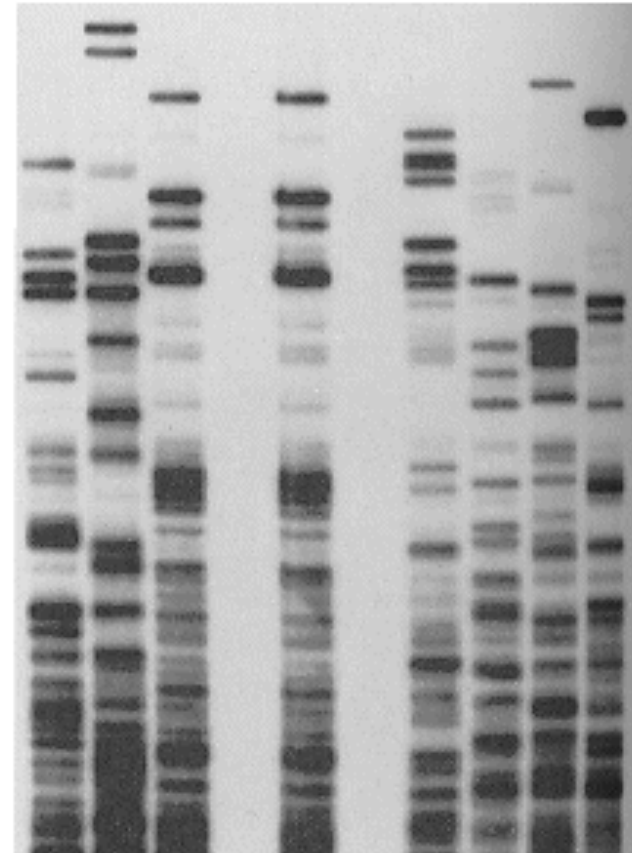
Restriction Fragment Length Polymorphism (RFLP)

- Used for paternity testing



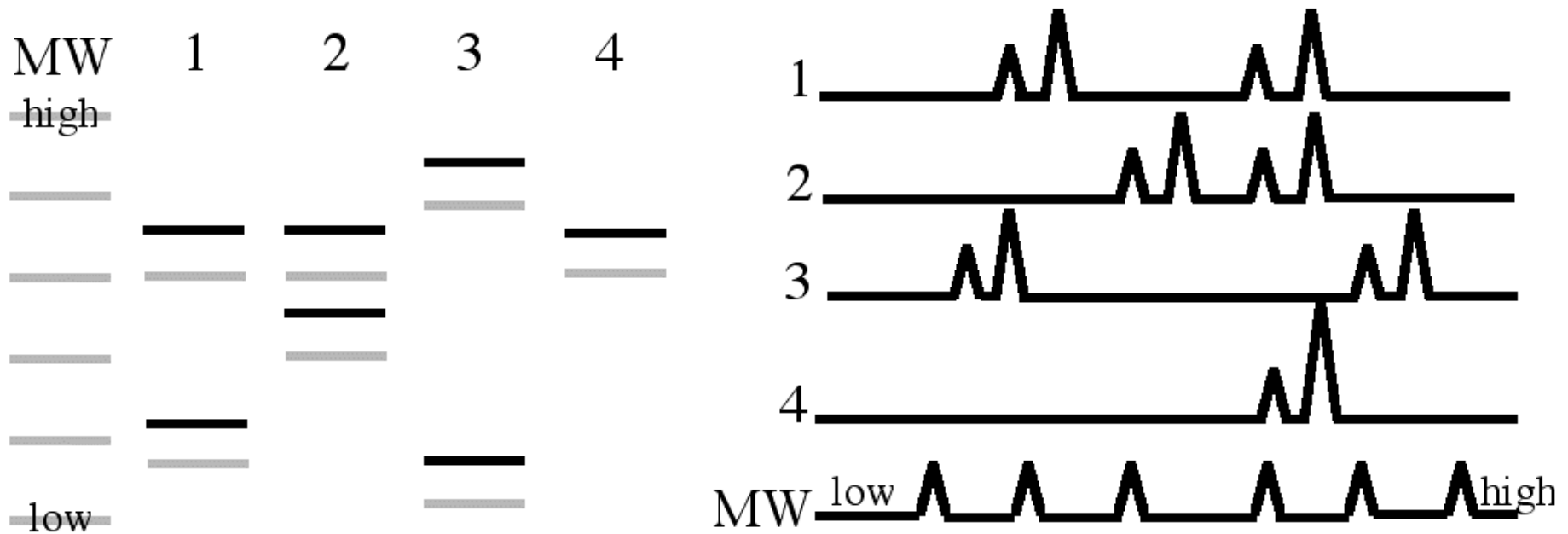
Restriction Fragment Length Polymorphism (RFLP)

- Use at a crime scene



1 2 3 Blood stain from crime **4 5 6 7**

Microsatellite Markers

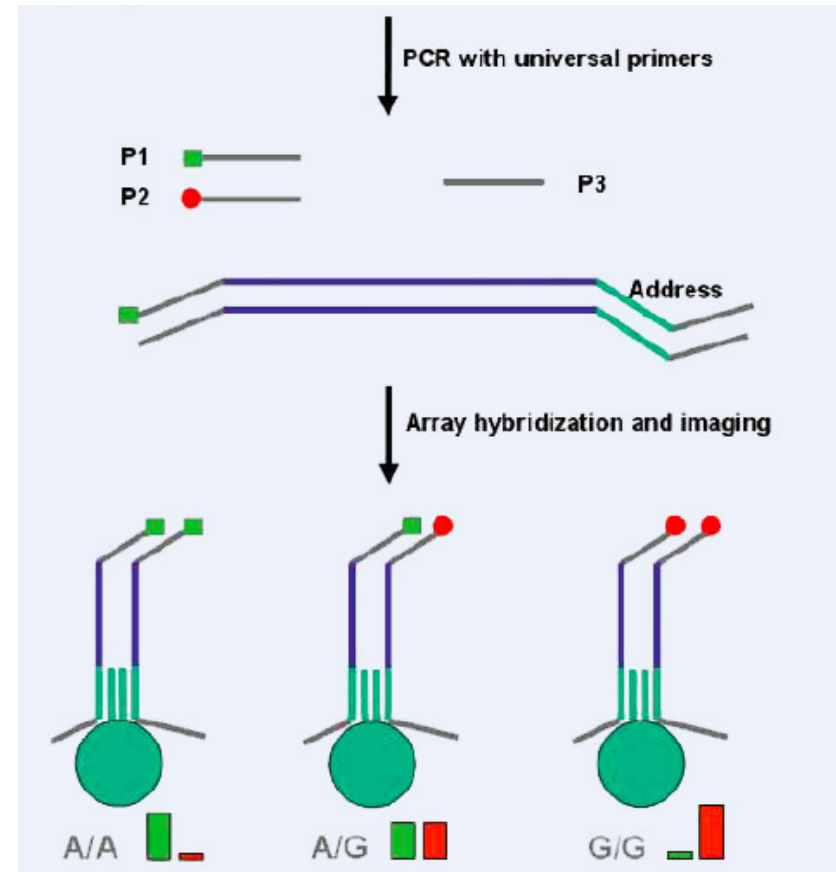
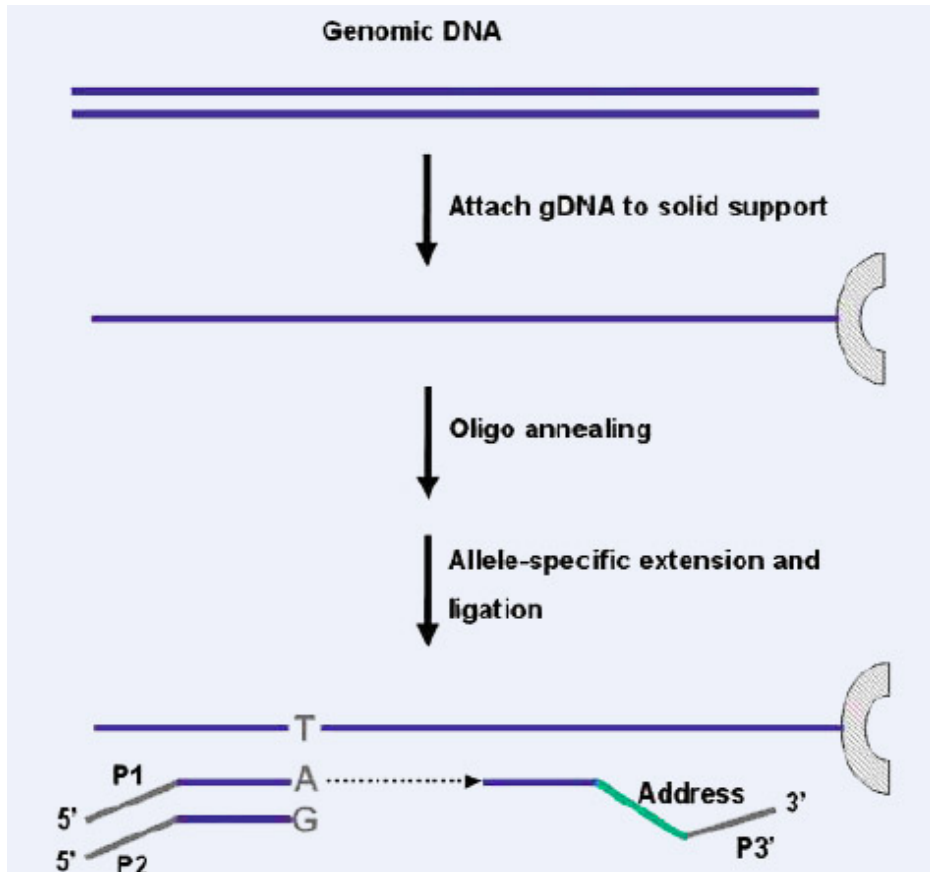


High Throughput Genotyping

- Illumina assays
 - GoldenGate & VeraCode: 48-1,536 SNPs
 - Infinium: 200K to 5M SNPs
- Affymetrix



High Throughput Genotyping



High Throughput Genotyping

