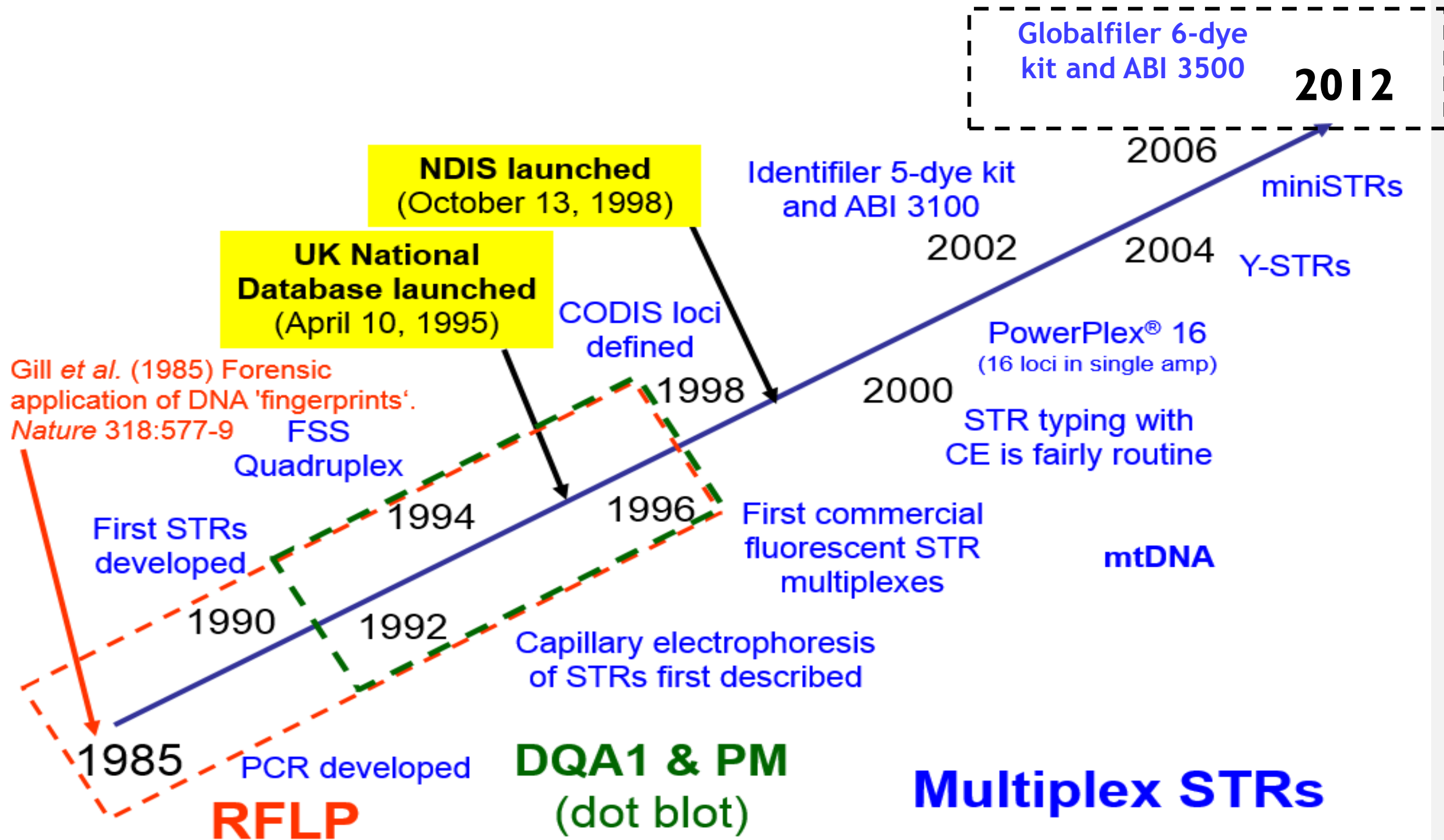


TECHNOLOGY & INSTRUMENTATION IN DNA FINGERPRINTING

Gargi Deshmukh
Scientist In-charge
Genome Foundation

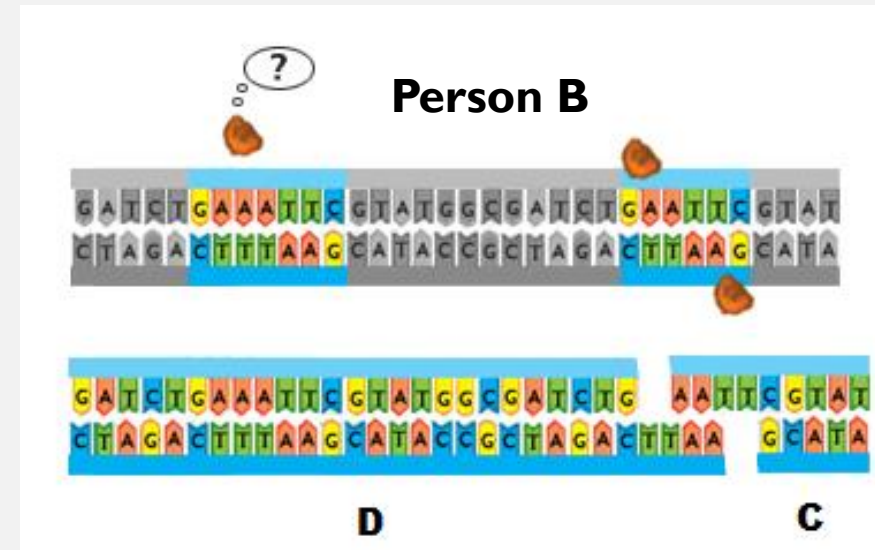
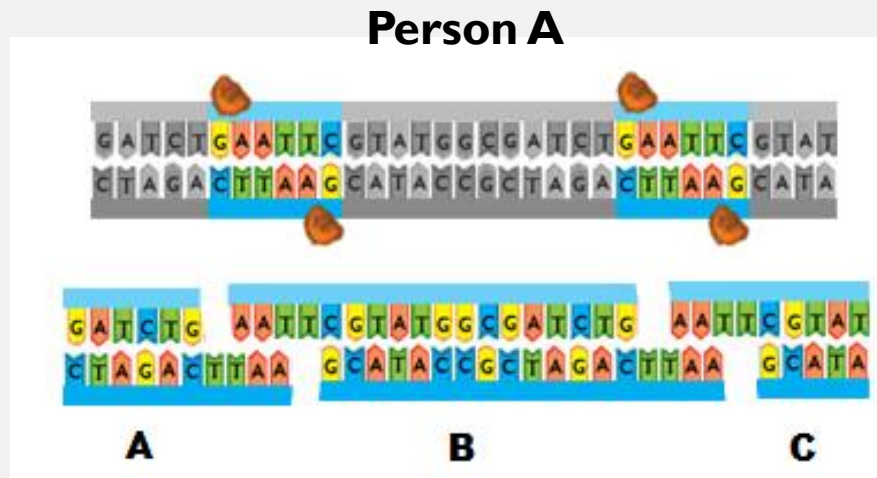
Year	<i>Forensic DNA Science & Application</i>	<i>Parallel Developments in Biotechnology</i>	<i>Microsoft Corporation Chronology</i>
1985	Alec Jeffreys develops multi-locus RFLP probes	PCR process first described	First version of Windows shipped
1986	DNA testing goes public with Cellmark and Lifecodes in United States	automated DNA sequencing with 4-colors first described	Microsoft goes public
1988	FBI begins DNA casework with single locus RFLP probes		
1989	TWGDAM established; <i>NY v. Castro</i> case raises issues over quality assurance of laboratories	DNA detection by gel silver-staining, slot blot, and reverse dot blots first described	
1990	Population statistics used with RFLP methods are questioned; PCR methods start with DQA1	Human Genome Project begins with goal to map all human genes	Windows 3.0 released (quality problems); exceeds \$1 billion in sales
1991	fluorescent STR markers first described; Chelex extraction		Windows 3.1 released

Year	<i>Forensic DNA Science & Application</i>	<i>Parallel Developments in Biotechnology</i>	<i>Microsoft Corporation Chronology</i>
1992	NRC I Report; FBI starts casework with PCR-DQA1	capillary arrays first described	
1993	first STR kit available; sex-typing (amelogenin) developed	first STR results with CE	
1994	Congress authorizes money for upgrading state forensic labs; "DNA wars" declared over; FBI starts casework with PCR-PM	Hitachi FMBIO and Molecular Dynamics gel scanners; first DNA results on microchip CE	
1995	O.J. Simpson saga makes public more aware of DNA; DNA Advisory Board setup; UK DNA Database established; FBI starts using D1S80/amelogenin	ABI 310 Genetic Analyzer and TaqGold DNA polymerase introduced	Windows 95 released
1996	NRC II Report; FBI starts mtDNA testing; first multiplex STR kits become available	STR results with MALDI-TOF and GeneChip mtDNA results demonstrated	
1997	13 core STR loci defined; Y-chromosome STRs described		Internet Explorer begins overtaking Netscape

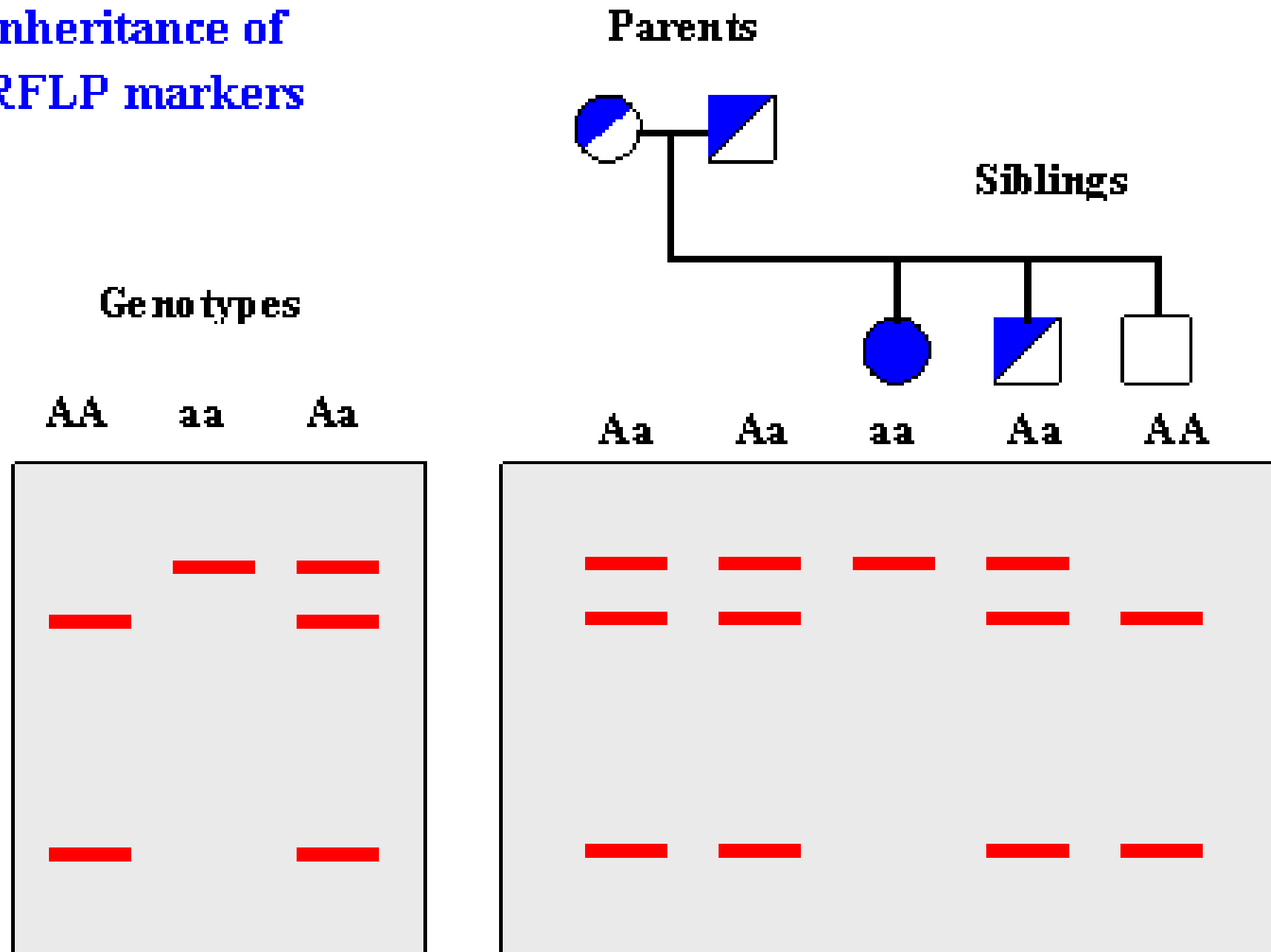


RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

- **Restriction fragment length polymorphism (RFLP)**
 - Nucleotide sequence variations in a region of DNA that generates fragment length differences according to the presence or absence of restriction enzyme recognition sites.



Inheritance of RFLP markers



- DNA from several individual was digested (cut) with a restriction enzyme.
- Detected by southern blot analysis.
- Different banding patterns were seen for different individuals. 1984
- **Sir Alec Jeffreys developed and used multi-locus RFLPs to catch colin pitchfork.**

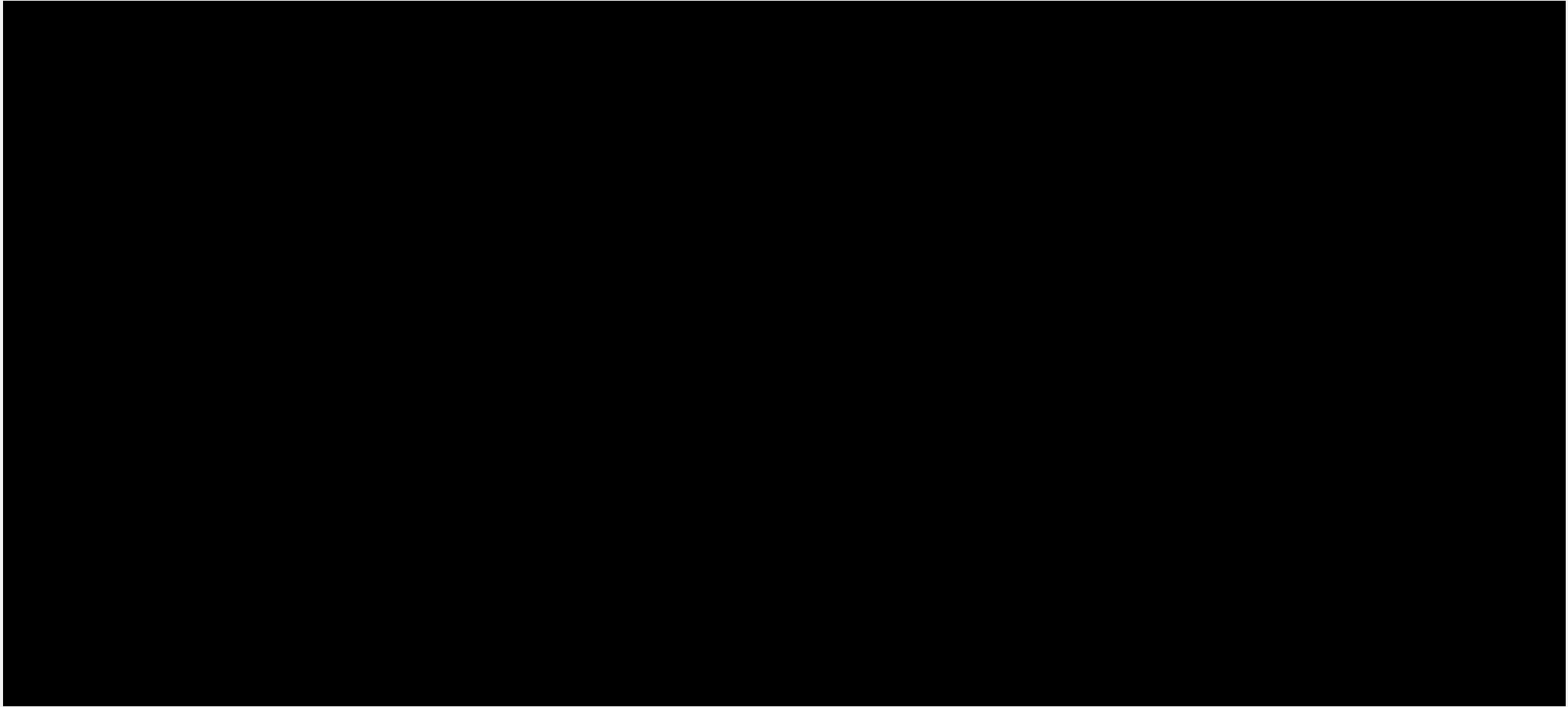


RFLP ANALYSIS

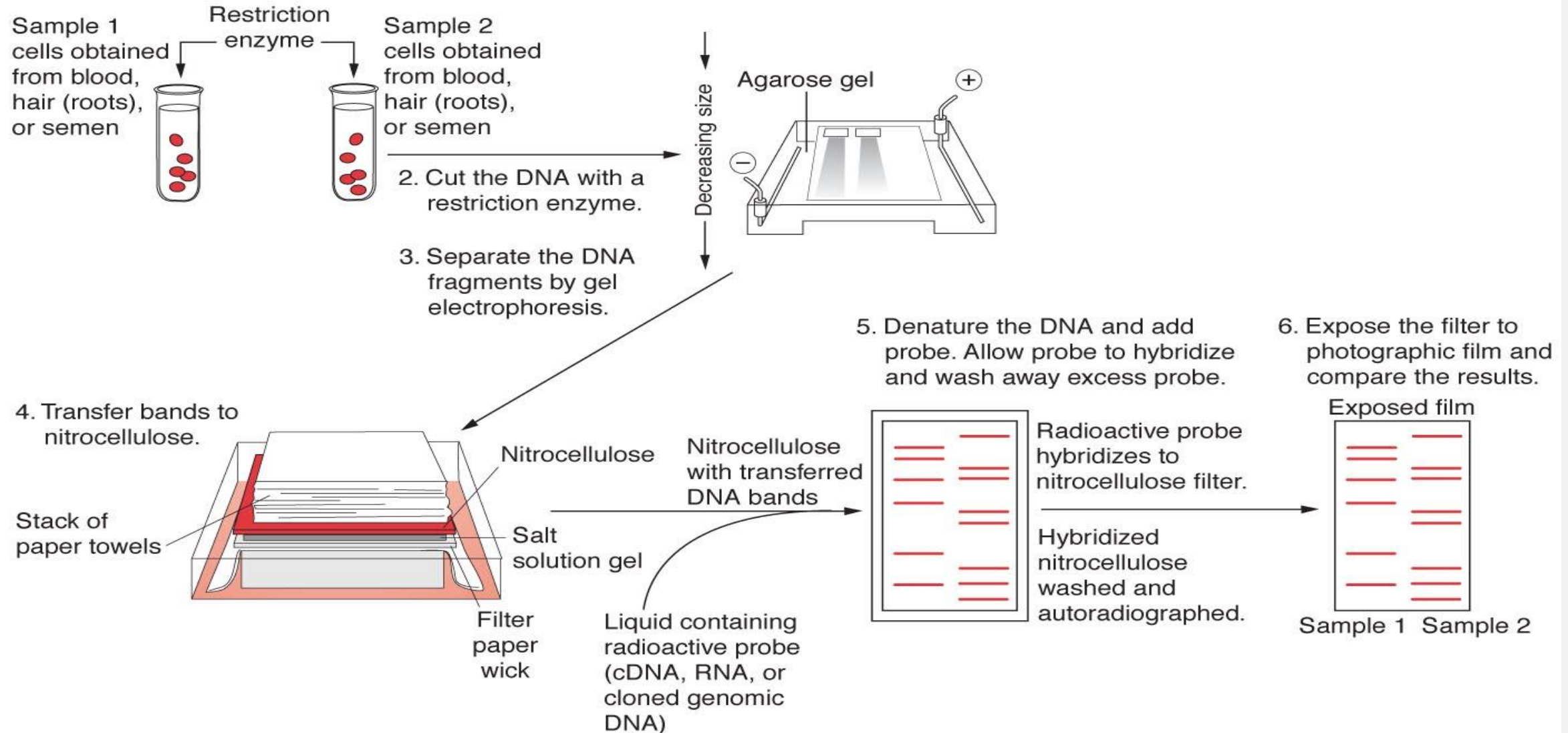
- **RFLP analysis**
 - Treat DNA with restriction enzyme
 - Restriction enzyme cuts DNA at restriction sites
 - Use several restriction enzymes in sequence or combined
 - Use agarose gel electrophoresis to separate the pieces
 - Gel is chemically treated or heated to denature the DNA
 - Allows the binding of a single-stranded probe

- Southern Blot Technique
 - Transfer DNA fragments from gel to nitrocellulose or nylon membrane
 - Membrane incubated with a probe
 - Short strand of complementary DNA with a radioactive or fluorescent tag
 - Targeted area on the DNA fragment is called a locus
 - Expose X-ray (photo) film to membrane to obtain permanent record of results

SOUTHERN BLOTTING ANIMATION



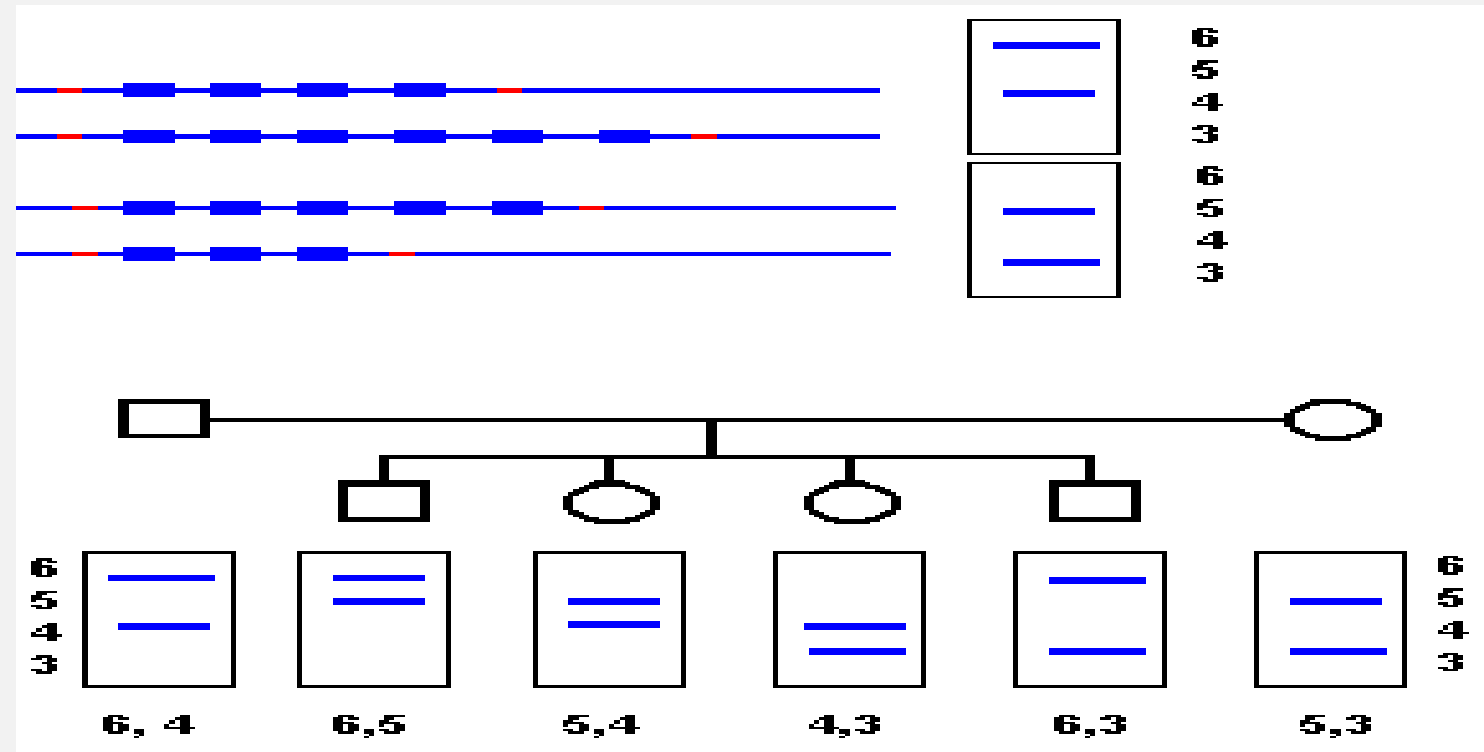
1. Isolate chromosomal DNA from a sample of cells.



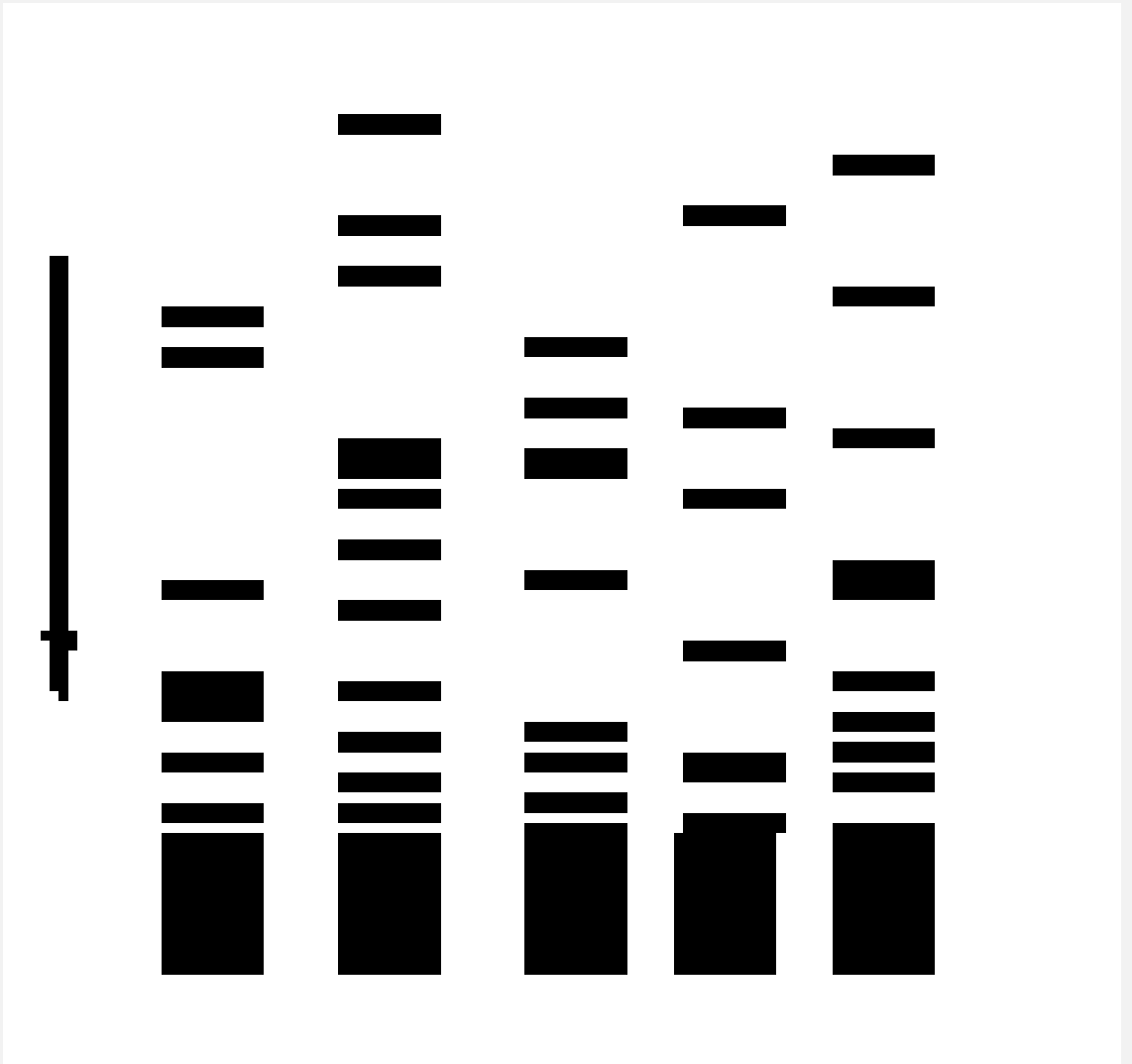
- More than 30% of the human genome contains repetitive DNA nucleotide sequences.
- These repeat sequences or tandem repeats vary in number between individuals and can be inherited from both parents
- **Variable Number Tandem Repeats - VNTRs**
 - 15 to 35 nucleotide bases per repeat
 - Up to 1000 repeats
 - Results in DNA segments of thousands of bases in length

- By digesting genomic DNA with restriction enzymes that cuts outside of these repeats it is possible to obtain different multiples of repeats that can be detected by hybridizing a DNA probe that detects (hybridizes to) the single repeat.

Number of repeats influences the length of the cut fragments after exposure to the restriction enzymes



- VNTR sequence segments occur at many loci in the human genome. These loci are dispersed among the chromosomes. Multi-locus VNTR probes yield patterns like bar codes.



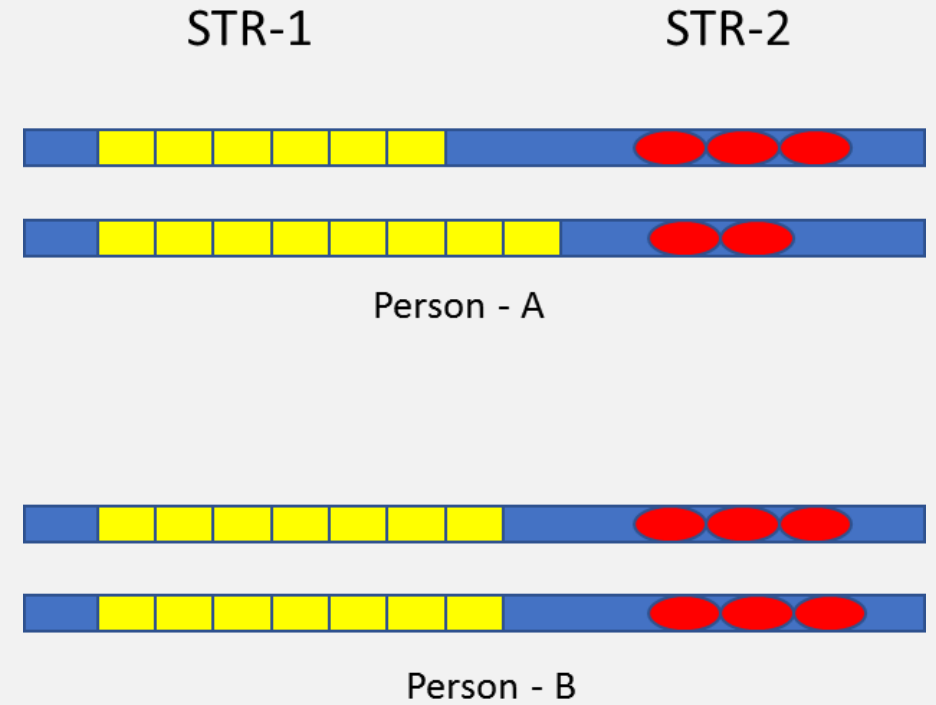
DISADVANTAGES OF RFLP ANALYSIS

- It is expensive, time, and labor intensive with a low throughput.
- One locus is analyzed at a time.
- Less sensitive – Requires a large sample size.
- It is limited in obtaining DNA from a crime scene since you need enough blood, hair, or semen.
- It requires whole genome analysis.
 - Needs relatively recently collected samples as the DNA tends to degrade.

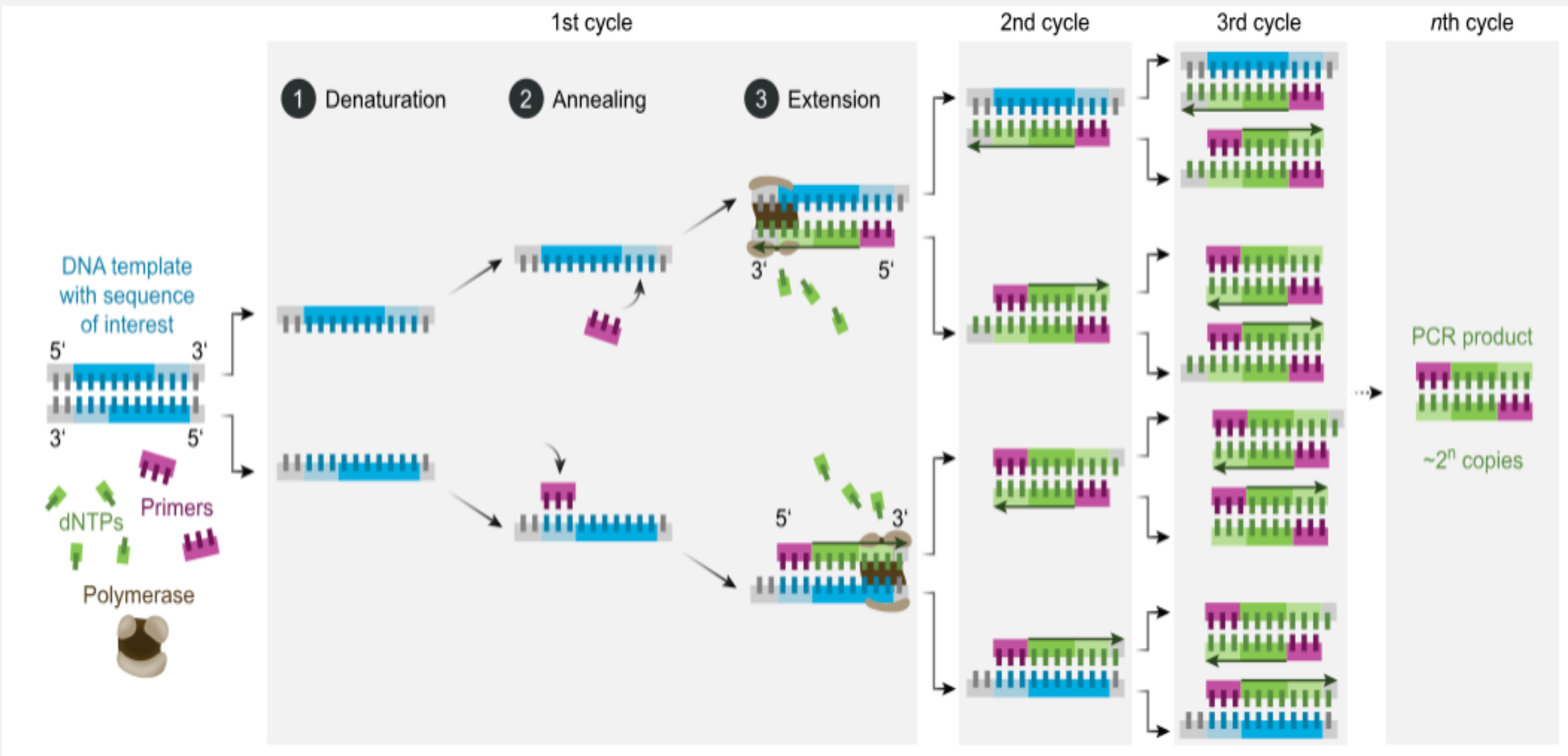
SHORT TANDEM REPEATS (STRS)

- **Short Tandem Repeats - STRs**

- 1-7 nucleotide bases per repeat
- Results in DNA segments of about 500-600 bases in length
- Shorter segments make it less susceptible to degradation
- Homozygote = both alleles are of the same length
- Heterozygote = alleles differ in length and can be resolved from one another

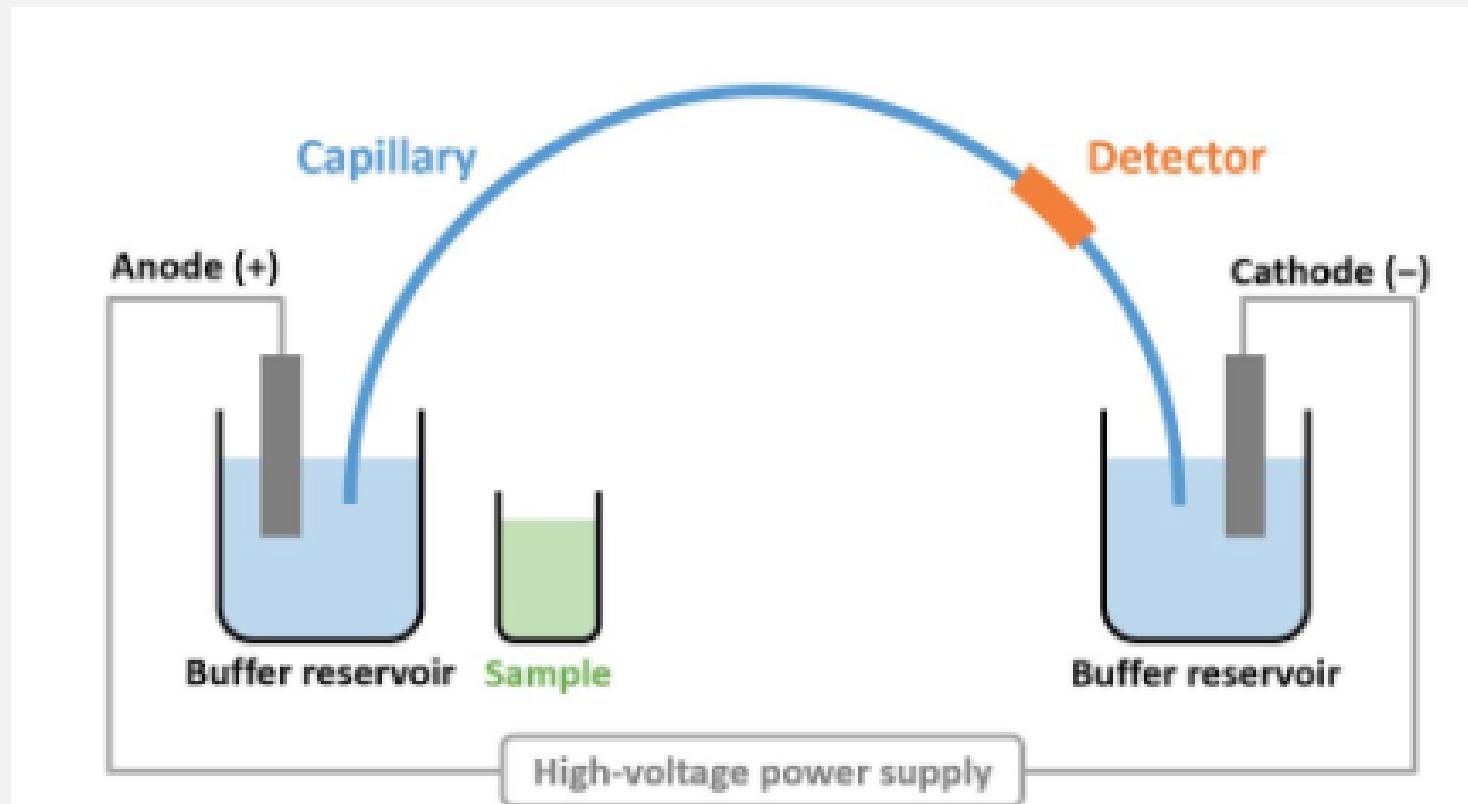


- **PCR** – used to amplify DNA found at crime scene into an amount that can be analyzed
 - DNA produced is identical to the original sample
- **STR Analysis**
 - Use primers to amplify STR's in DNA using PCR



Capillary Electrophoresis

- Tertiary step - to separate and detect the STRs present in a given DNA sample
- The separation occurs on the basis of the size of the STRs

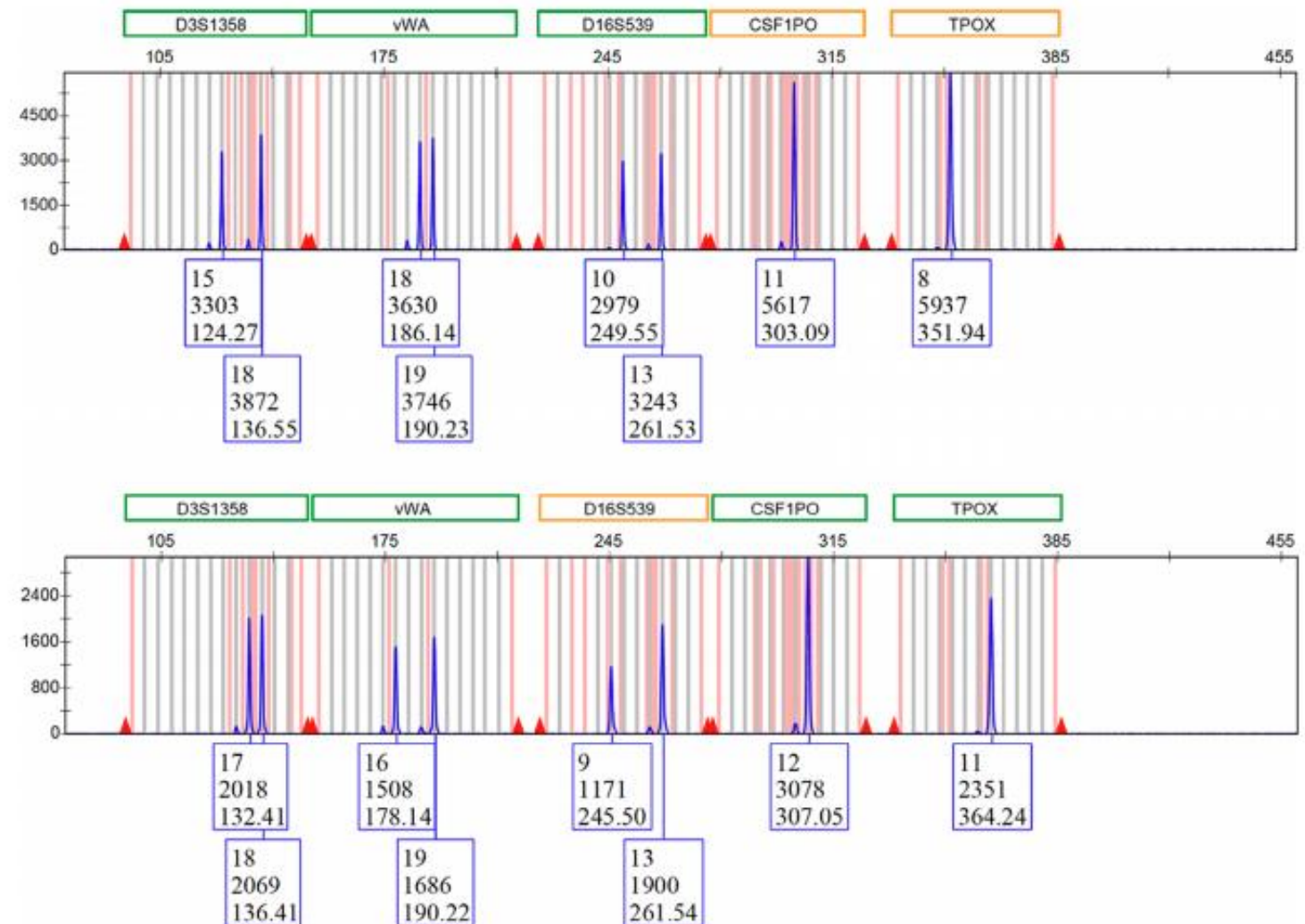


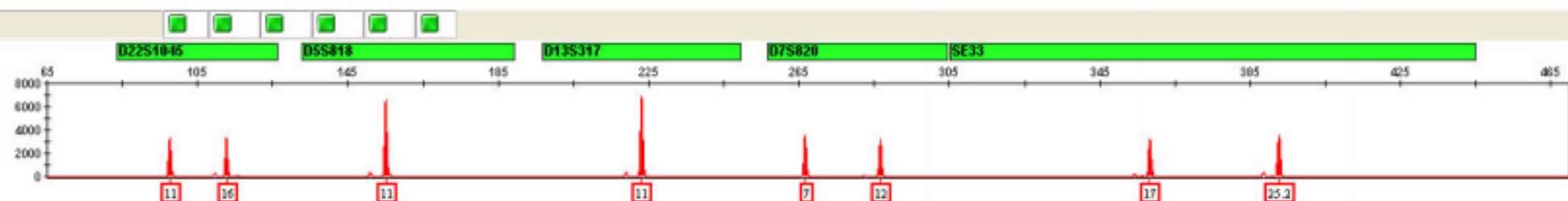
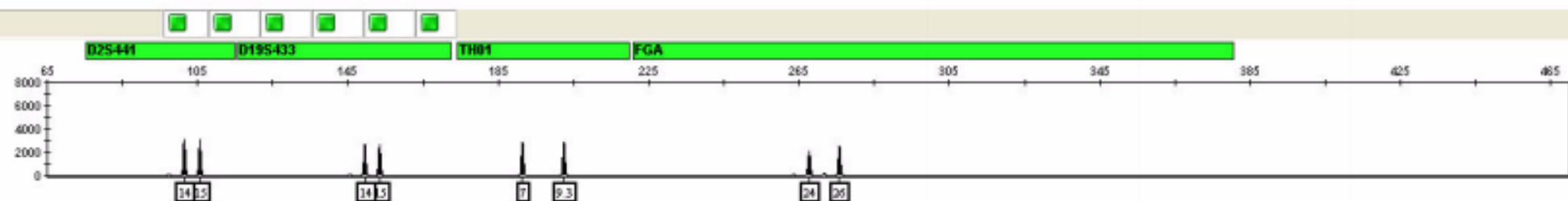
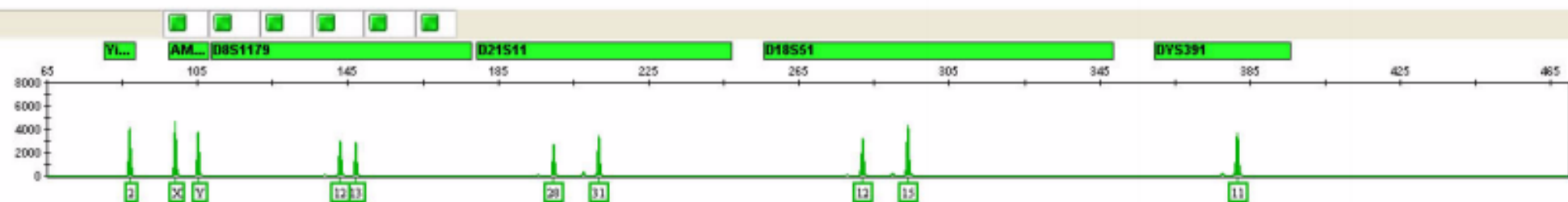
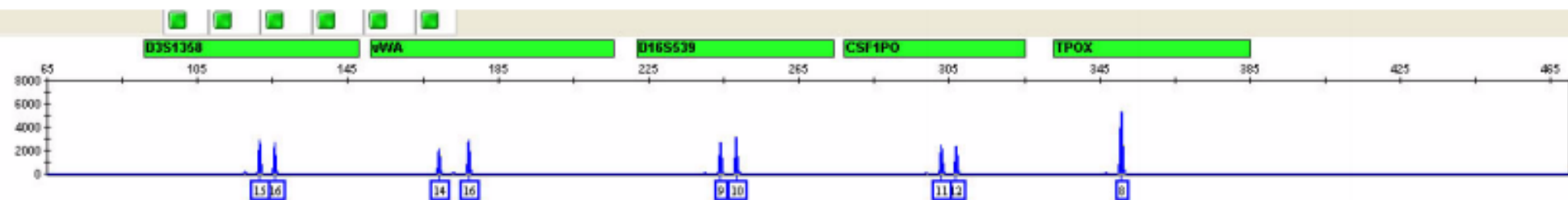


Capillary
Electrophoresis
Genetic Analyzer

VISUALIZATION

- Final step - to visualize the STRs (size & location) in the capillary for any given DNA sample
- The visualization is only possible due to the tagged primers used during PCR







THANK YOU!