TECHNOLOGY & INSTRUMENTATION IN DNA FINGERPRINTING

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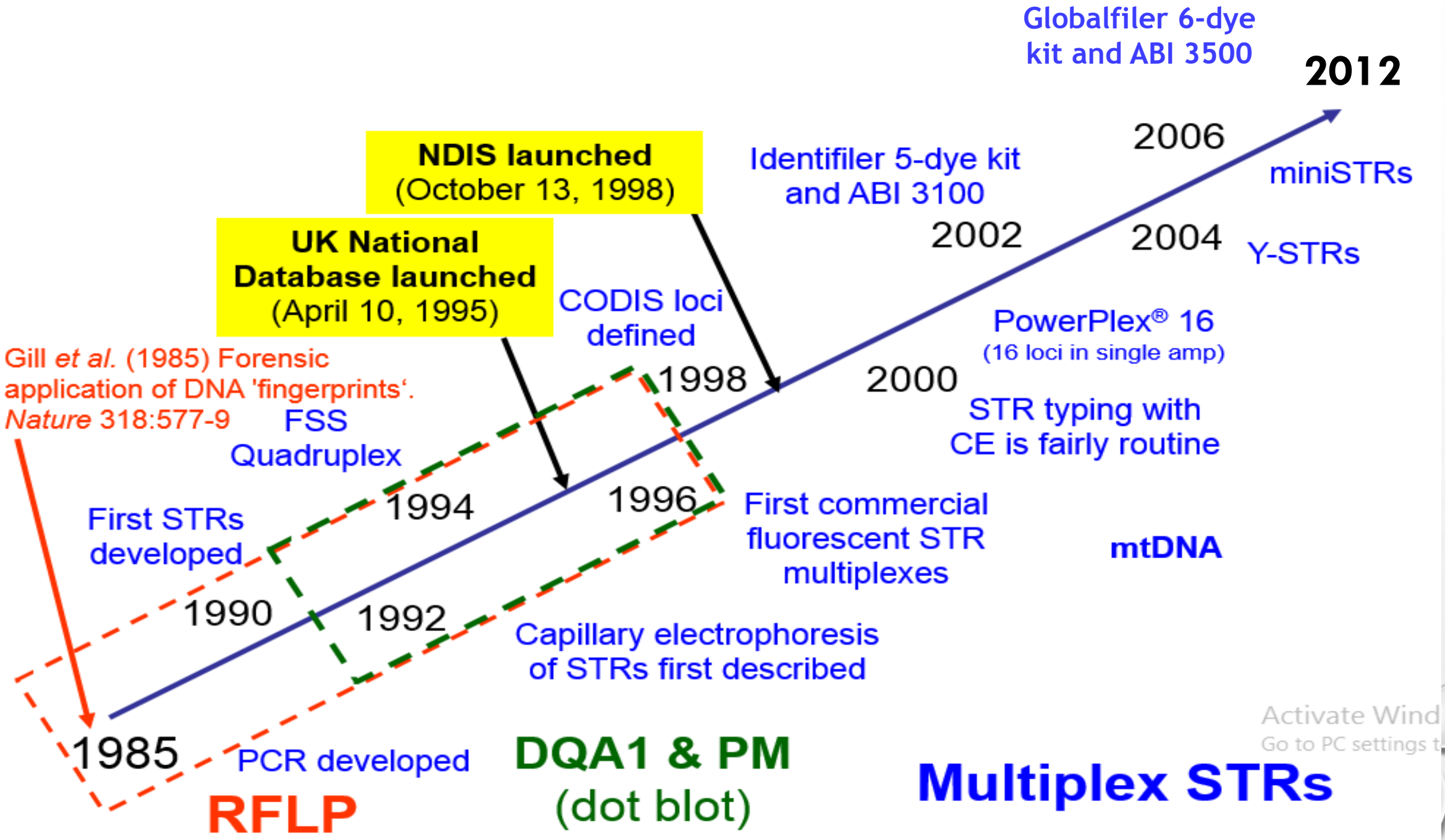
SCIENTIST IN-CHARGE

GENOME FOUNDATION

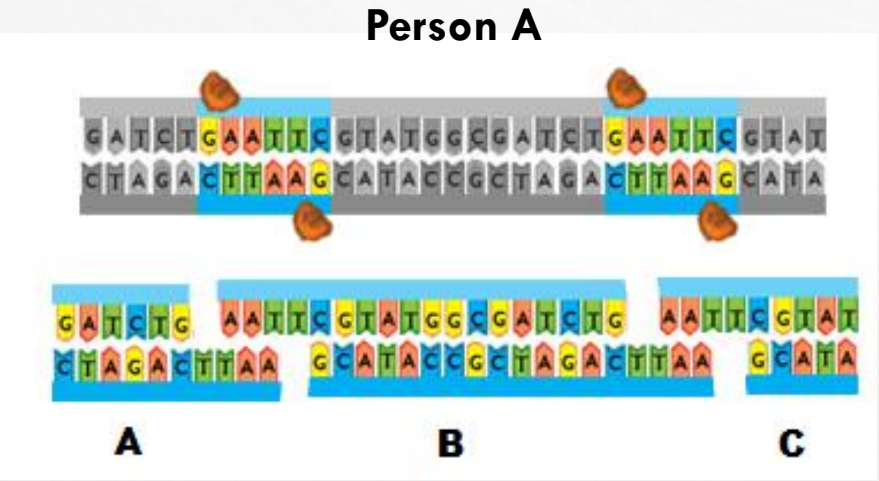
Year	*Forensic DNA Science & Application*	*Parallel Developments in Biotechnology*	*Microsoft Corporation Chronology*
1985	Alec Jeffreys develops multi-locus RFLP probes	PCR process first described	First version of Windows shipped
1986	DNA testing goes public with Cellmark and Lifecodes in United States	automated DNA sequencing with 4-colors first described	Microsoft goes public
1988	FBI begins DNA casework with single locus RFLP probes		
1989	TWGDAM established; *NY v. Castro* 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

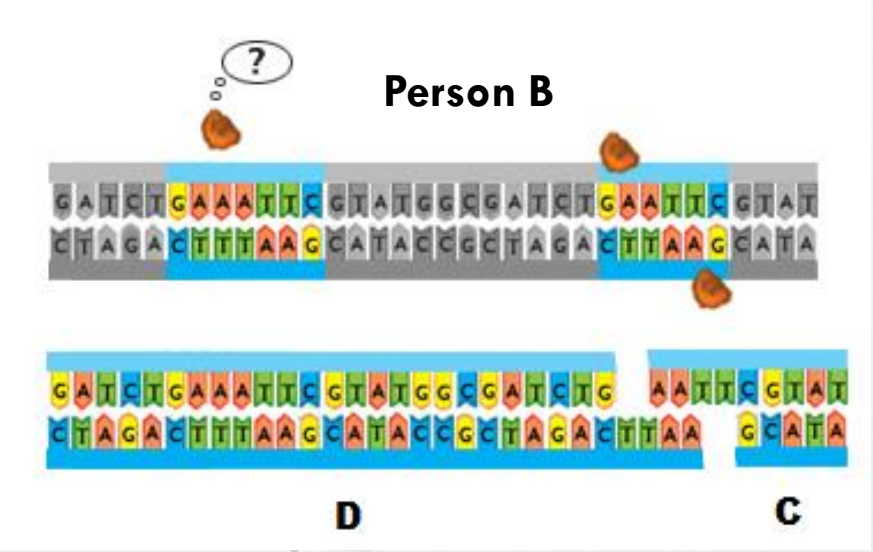
Year	*Forensic DNA Science & Application*	*Parallel Developments in Biotechnology*	*Microsoft Corporation Chronology*
1992	NRC I Report; FBI starts casework with PCR-DQA1	capillary arrays first described	
1993	first STR kit available; sex-typing (amelogenin) developed	first STR results with CE	
1994	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


Globalfiler 6-dye kit and ABI 3500
2012
2006
miniSTRs
Identifiler 5-dye kit and ABI 3100
NDIS launched (October 13, 1998)
UK National Database launched (April 10, 1995)
2002
2004
Y-STRs
CODIS loci defined
PowerPlex® 16 (16 loci in single amp)
Gill et al. (1985) Forensic application of DNA 'fingerprints'. Nature 318:577-9
1998
2000
FSS Quadruplex
STR typing with CE is fairly routine
1994
1996
First STRs developed
First commercial fluorescent STR multiplexes
mtDNA
1990
1992
Capillary electrophoresis of STRs first described
1985
PCR developed
DQA1 & PM (dot blot)
RFLP
Multiplex STRs

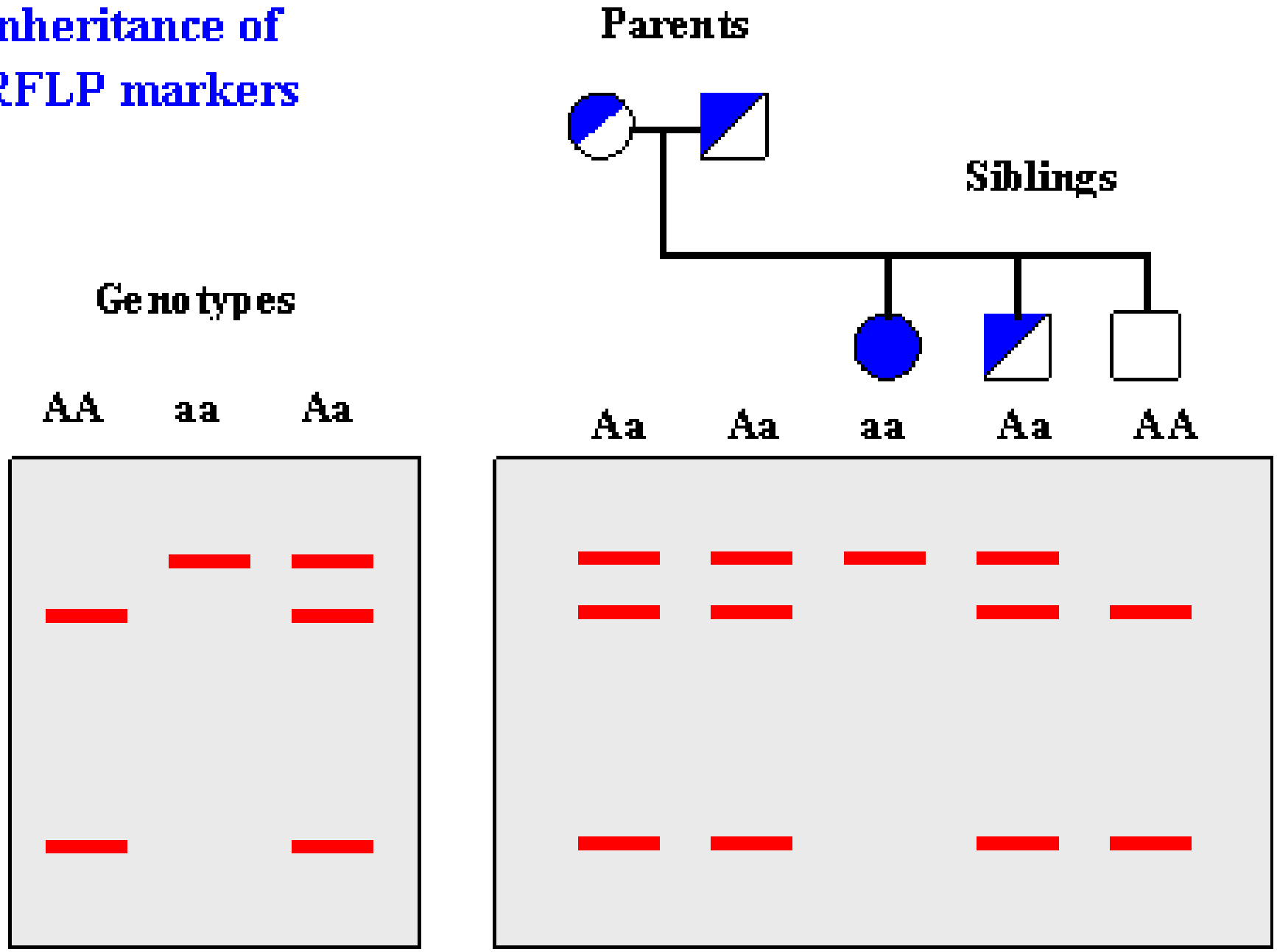
RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

- **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.

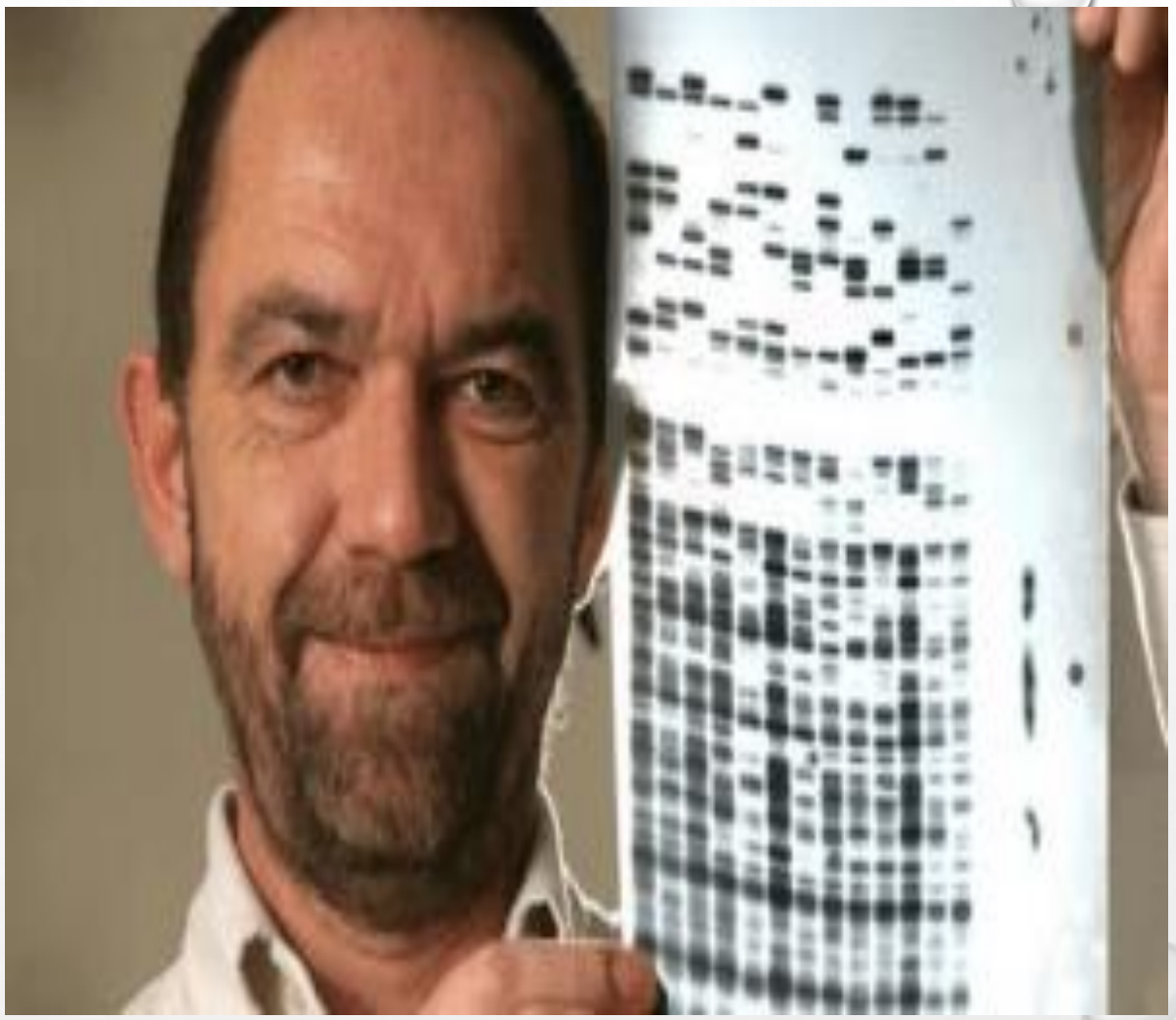




Inheritance of RFLP markers



- 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.**

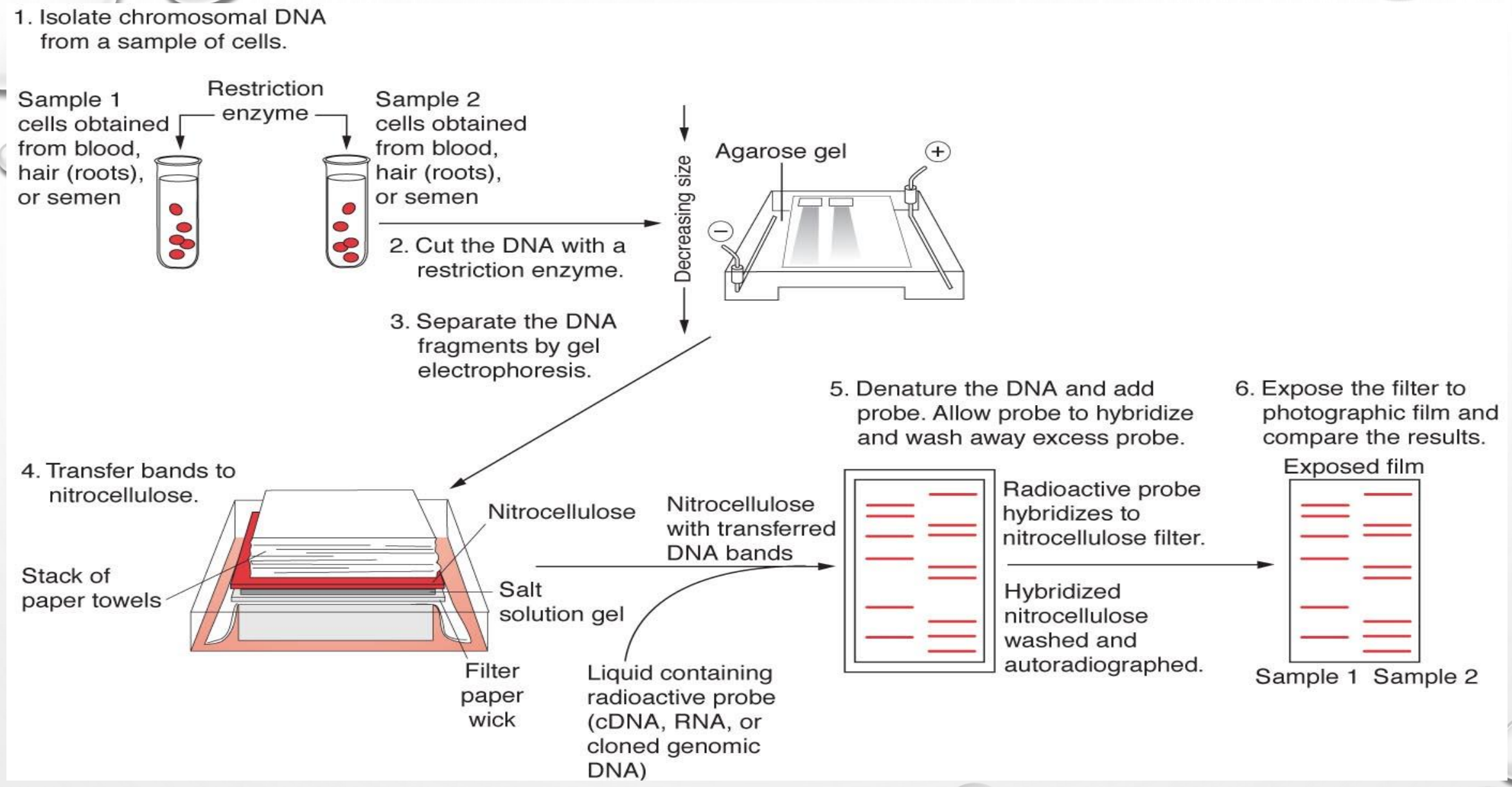


RFLP ANALYSIS

- **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

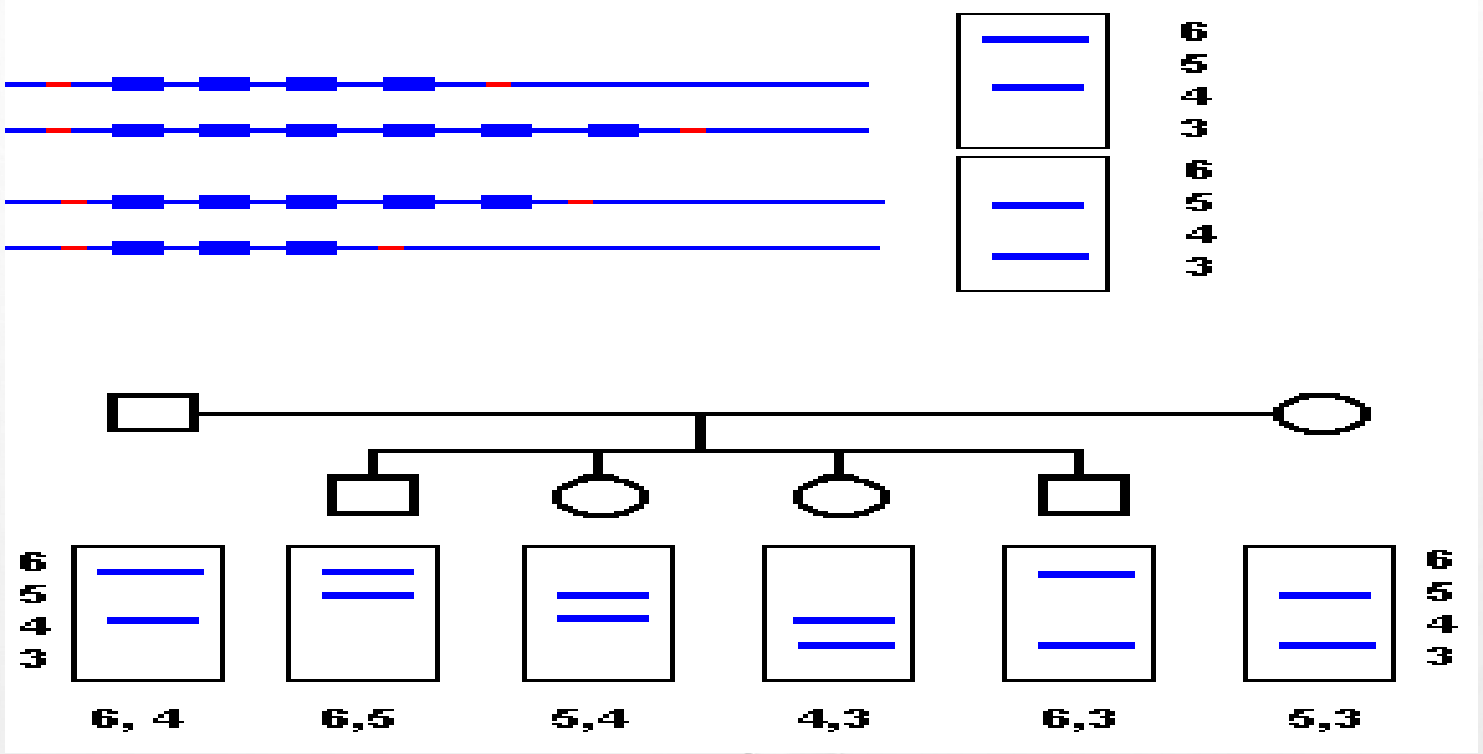

1. Isolate chromosomal DNA from a sample of cells.
Sample 1 cells obtained from blood, hair (roots), or semen
Restriction enzyme
Sample 2 cells obtained from blood, hair (roots), or semen
2. Cut the DNA with a restriction enzyme.
3. Separate the DNA fragments by gel electrophoresis.
Decreasing size
Agarose gel
4. Transfer bands to nitrocellulose.
Nitrocellulose
Stack of paper towels
Salt solution gel
Filter paper wick
Nitrocellulose with transferred DNA bands
Liquid containing radioactive probe (cDNA, RNA, or cloned genomic DNA)
5. Denature the DNA and add probe. Allow probe to hybridize and wash away excess probe.
Radioactive probe hybridizes to nitrocellulose filter.
Hybridized nitrocellulose washed and autoradiographed.
6. Expose the filter to photographic film and compare the results.
Exposed film
Sample 1 Sample 2

- 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

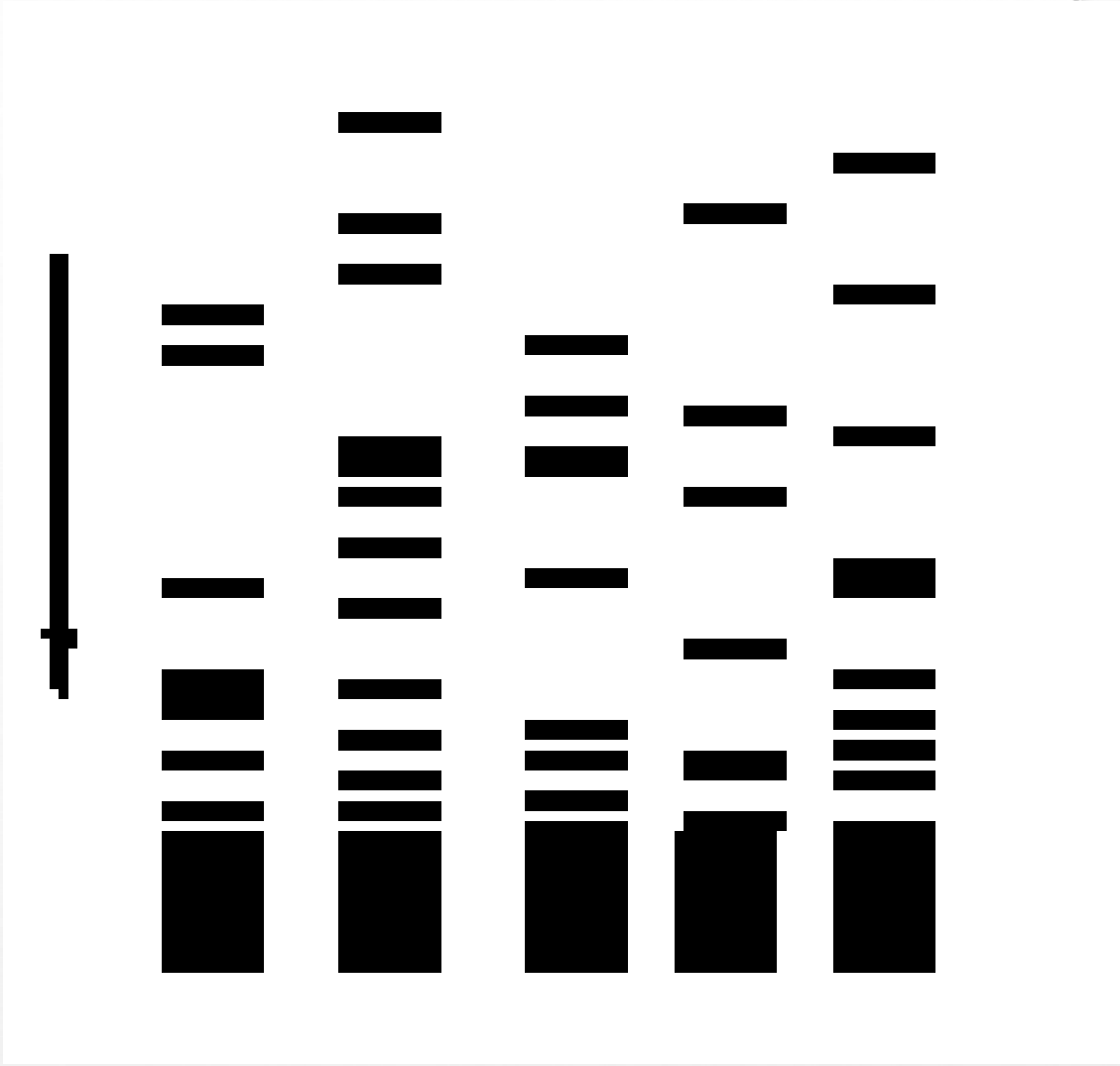
- **Variable Number Tandem Repeats - VNTRs**
  - 15 to 35 nucleotide bases per repeat
  - Up to 1000 repeats
  - Results in DNA segments of thousands of bases in length

- 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

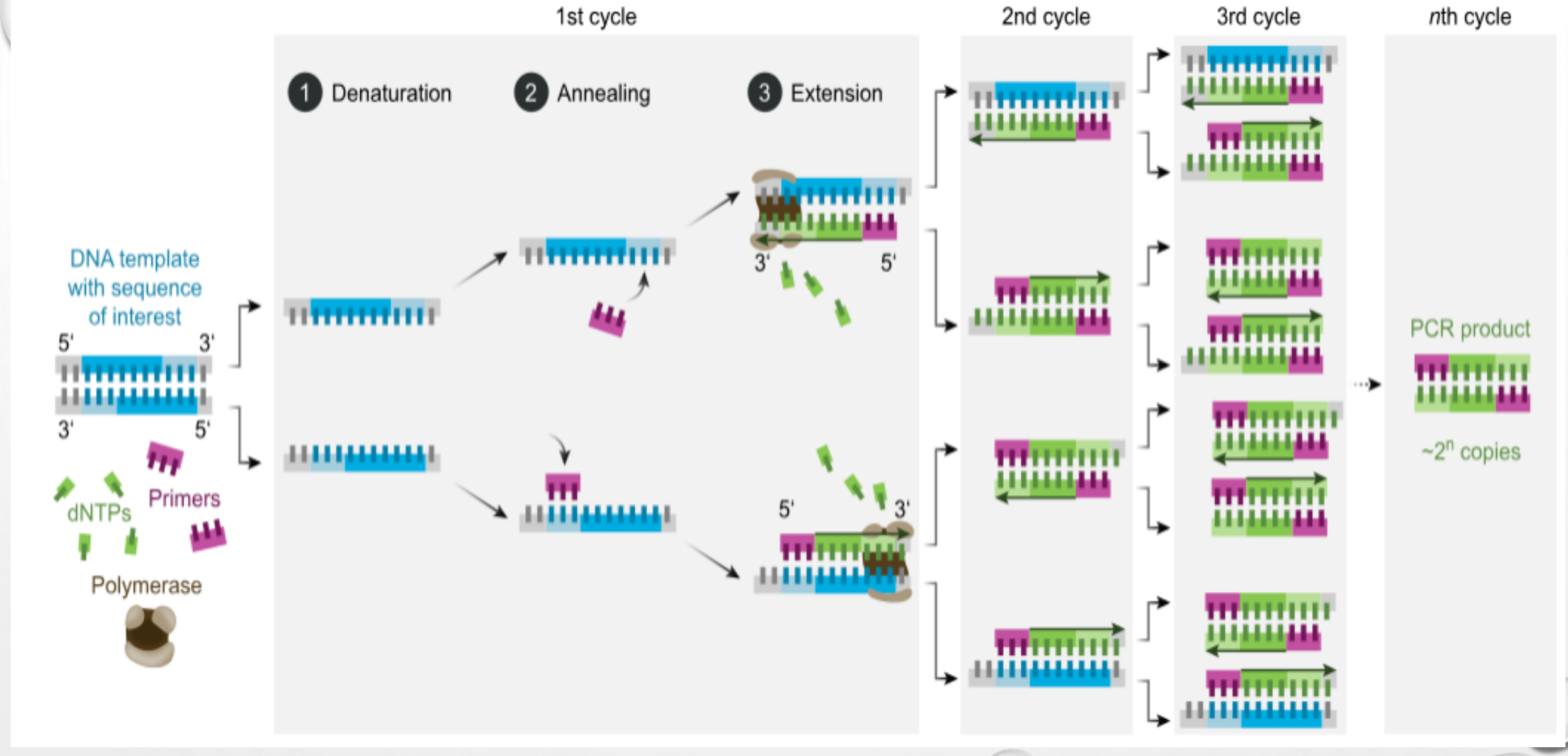
- It is expensive, time, and labor intensive with a low throughput.
- One locus is analyzed at a time.
- Less sensitive – Requires a large sample size.
- It is limited in obtaining DNA from a crime scene since you need enough blood, hair, or semen.
- It requires whole genome analysis.
  - Needs relatively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

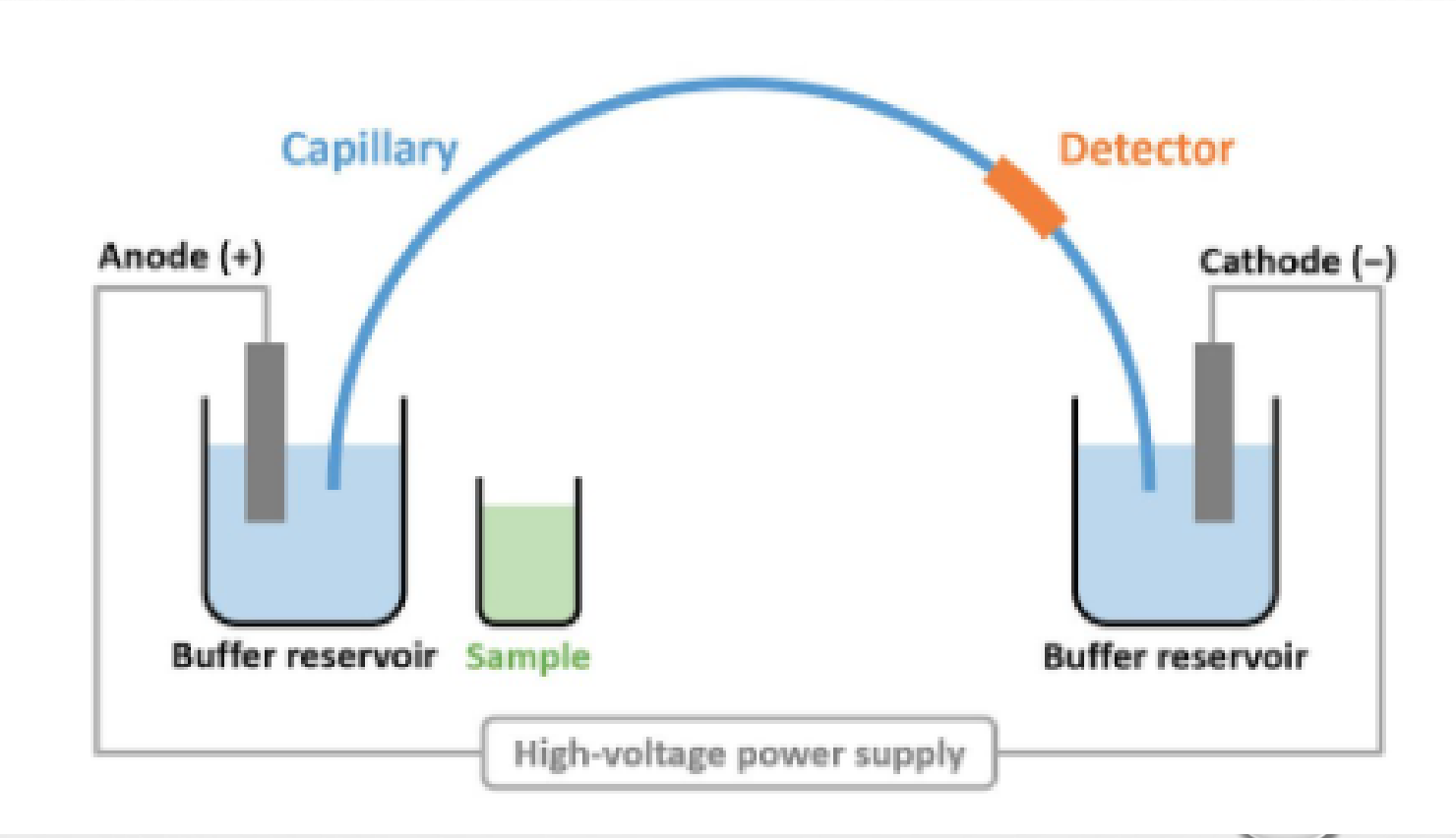
- **PCR** – used to amplify DNA found at crime scene into an amount that can be analyzed
  - DNA produced is identical to the original sample

- **STR Analysis**
    - Use primers to amplify STR's in DNA using PCR


1st cycle
2nd cycle
3rd cycle
nth cycle
1 Denaturation
2 Annealing
3 Extension
DNA template with sequence of interest
5'
3'
3'
5'
dNTPs
Primers
Polymerase
3'
5'
5'
3'
PCR product
~2^n copies

Capillary Electrophoresis

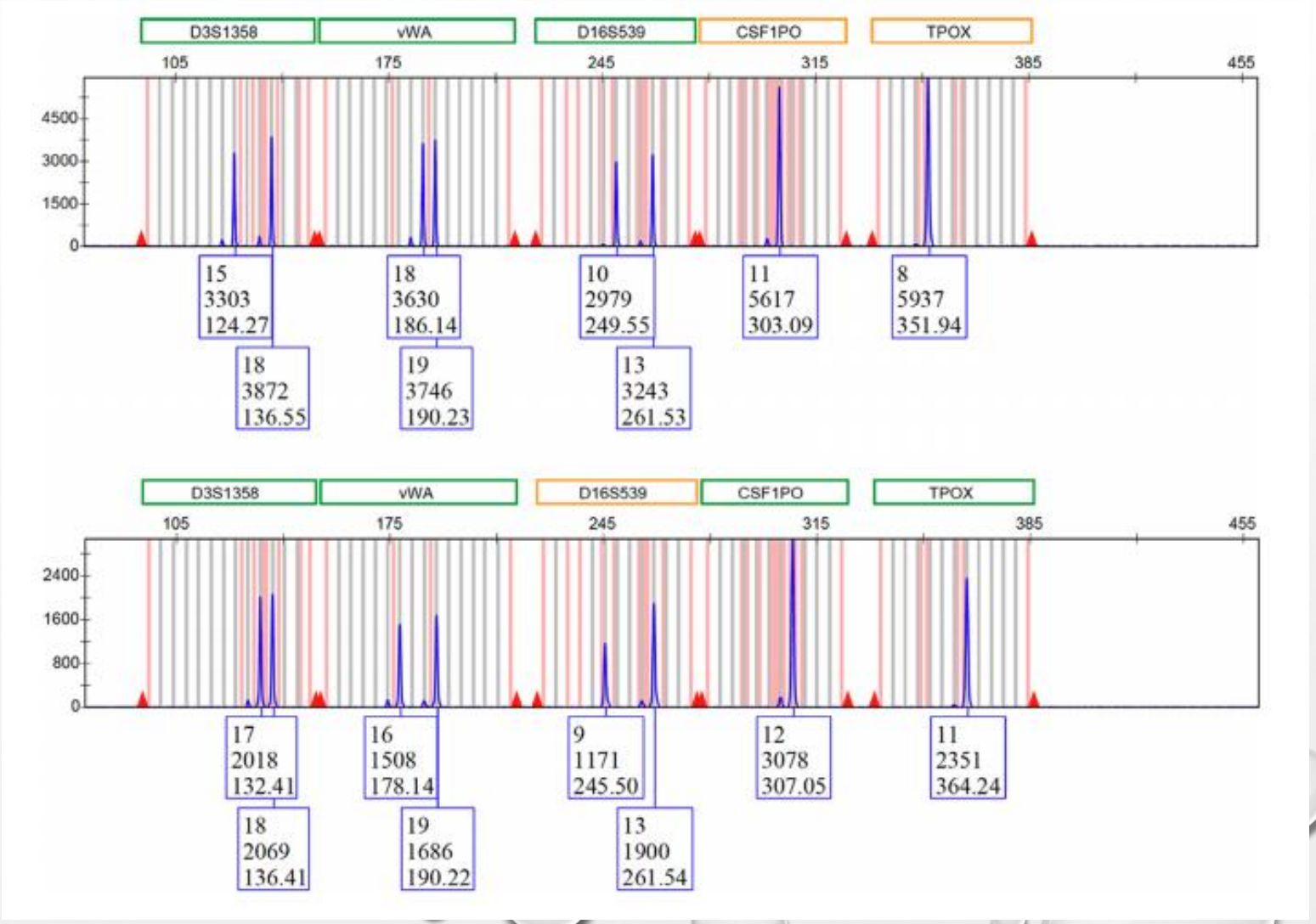
- Tertiary step - to separate and detect the STRs present in a given DNA sample
- The separation occurs on the basis of the size of the STRs

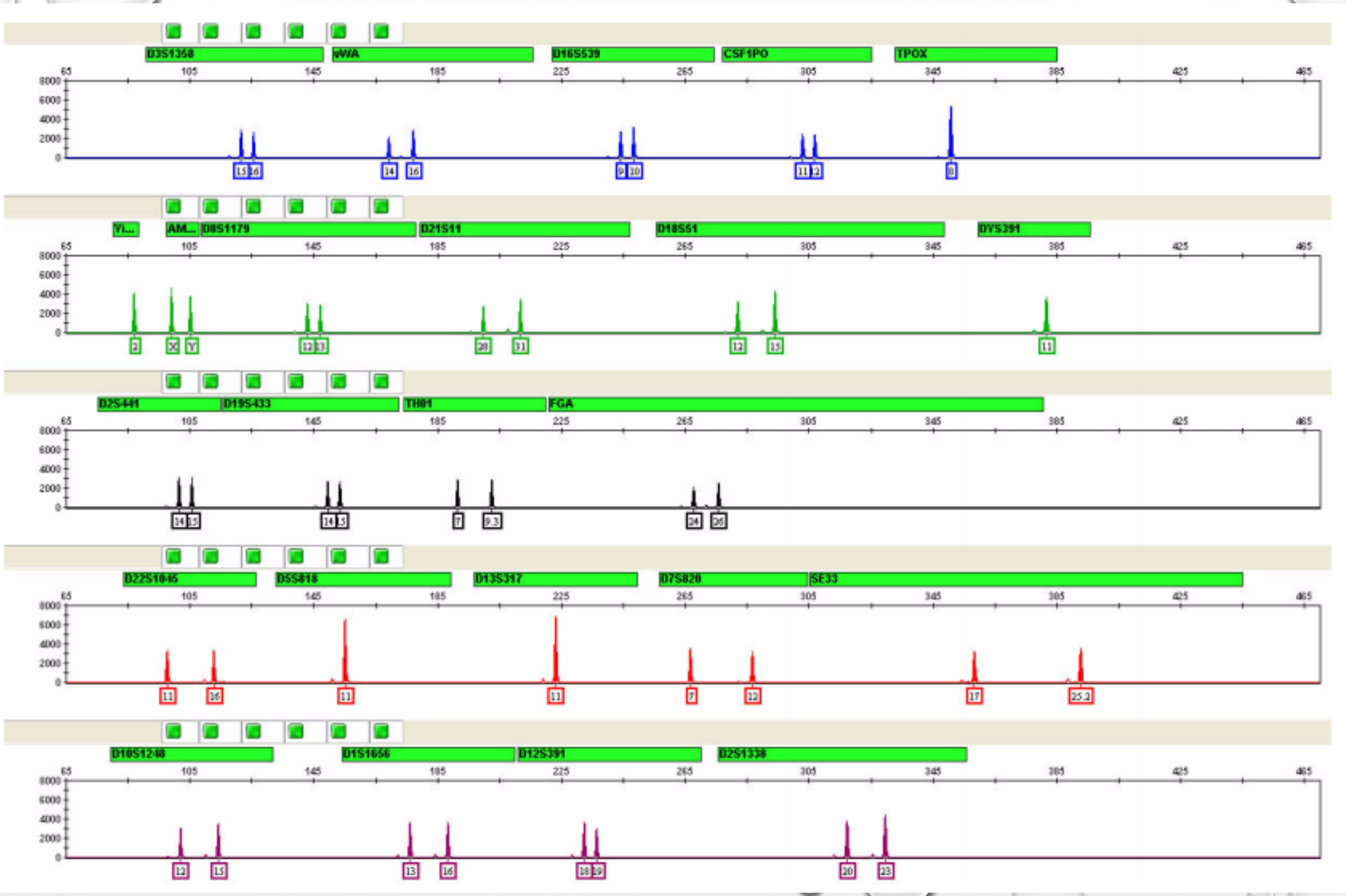


Capillary
Electrophoresis
Genetic Analyzer

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




D3S1358
vWA
D16S539
CSF1PO
TPOX
Yi...
AM...
D8S1179
D21S11
D18S51
DYS391
D2S441
D19S433
TH01
FGA
D22S1045
D5S818
D13S317
D7S820
SE33
D10S1248
D1S1656
D12S391
D2S1338