DNA Fingerprinting and Its Application in Paternity Testing

By

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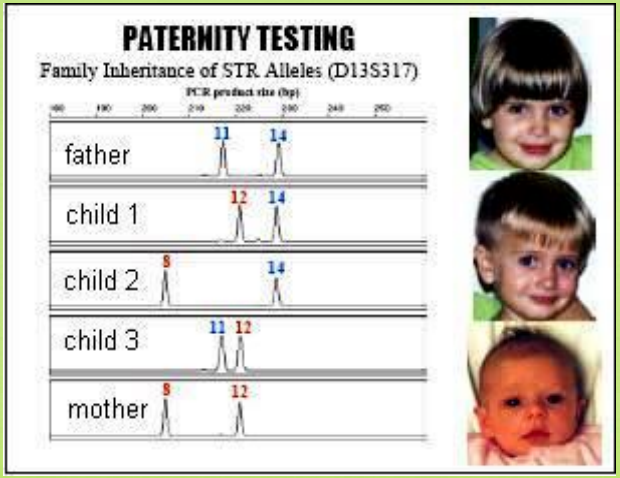
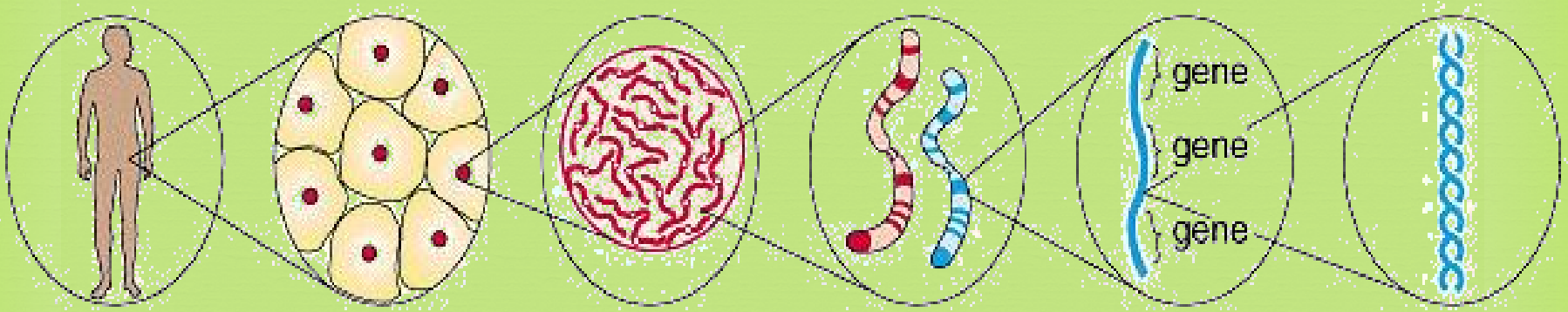


DNA basics



Organism (human)

A human body is made up of trillions of cells.

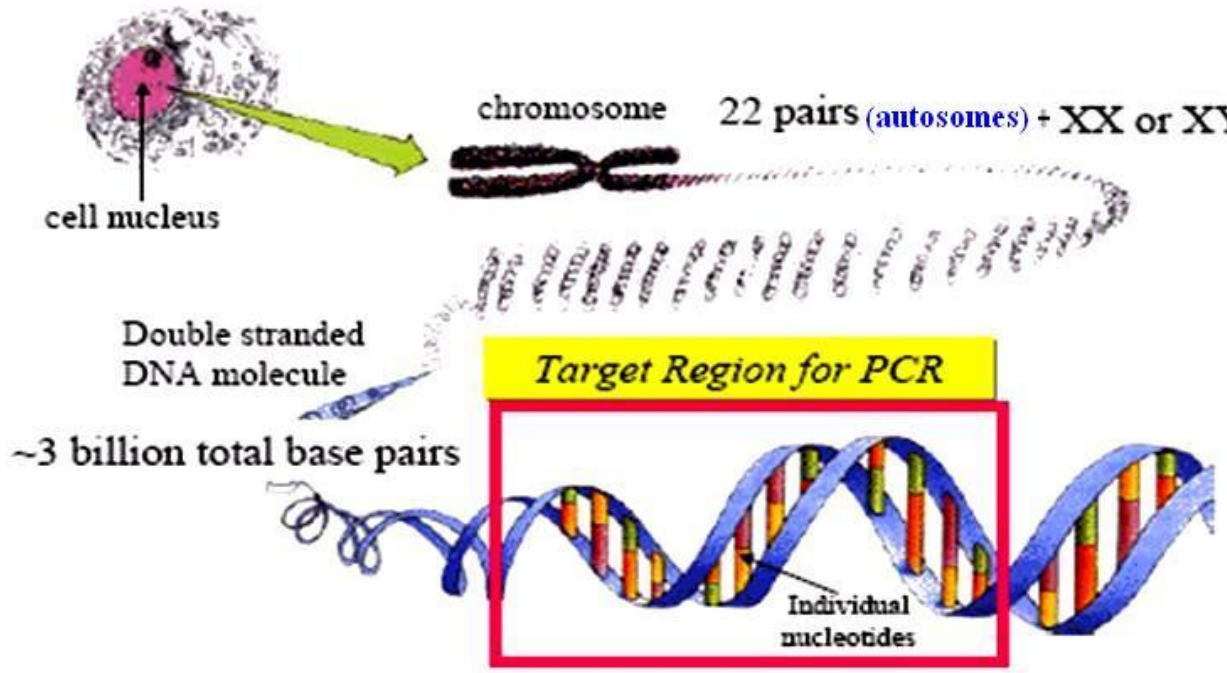
Each cell nucleus contains an identical complement of chromosomes.

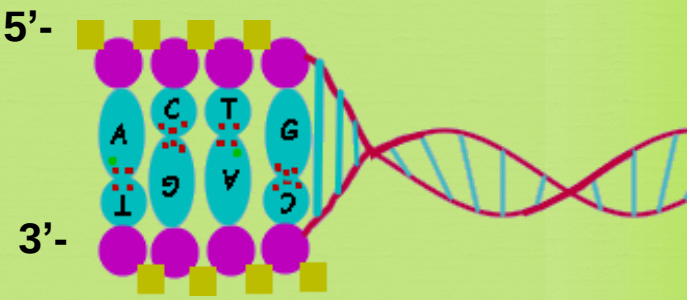
One specific chromosome pair

Each chromosome is one long DNA molecule and genes are functional regions of this DNA.

DNA is a double helix.

DNA in the Cell

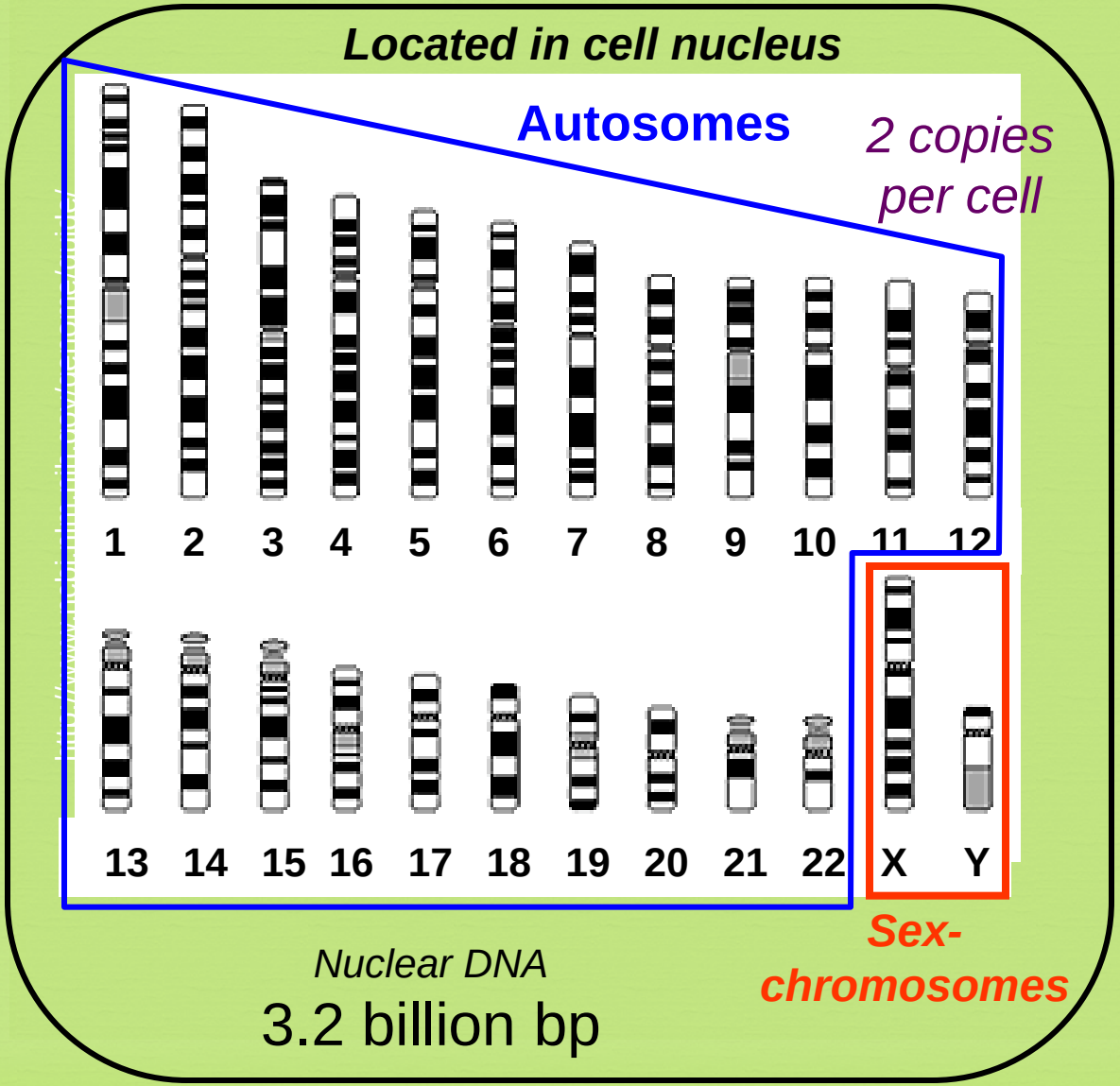




The main building units are nucleotides.Each is composed of **Phosphate** molecule, Deoxyribose **Sugar** molecule and one of 4 nitrogenous **Bases** (A,T, C or G) linked with hydrogen bonds.

Human Genome

23 Pairs of Chromosomes + mtDNA



Located in mitochondria (multiple copies in cell cytoplasm)

100s of copies per cell

DNA - Unique, Yet the Same

Of the 3 billion DNA bases, about 0.3% is different among individuals: ~1 million bases.

DNA fingerprinting

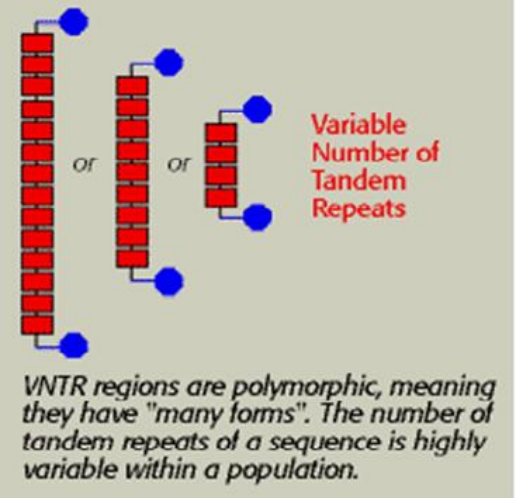
- **DNA has revolutionized older blood grouping and serum proteins systems -DNA fingerprint (Sir Alec Jeffreys).**
- **Any organism can be identified by examination of DNA sequences unique to that species.**
- **75% of human DNA is non-coding that contains hypervariable repetitive sequences e.g. Short Tandem Repeats (STRs). Genes and other associated regulatory sequences represent only 25% (30-35.000 genes).**

DNA Polymorphism

- Most individuals genome sequences are very similar.

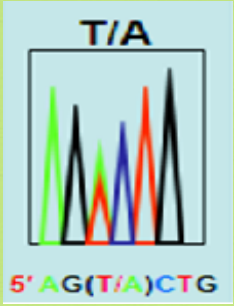
- Difference in nucleotide sequences giving alternative forms of genetic locus is called ***Sequence polymorphism e.g. point mutation or SNPs***.

- Difference in number of tandem repeats units e.g. STRs; is called ***Length polymorphism.***



Allele T **5'-ATCCATGCAT-3'**

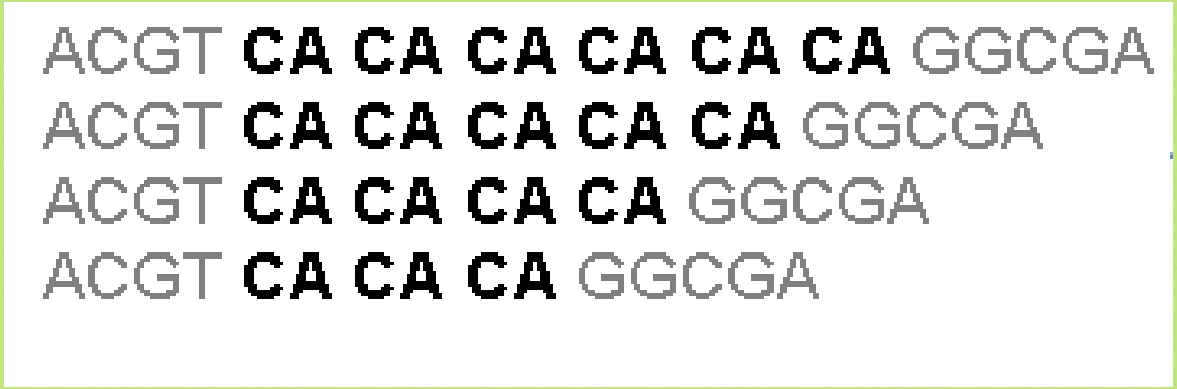
Allele A **5'-ATCCAAGCAT-3'**



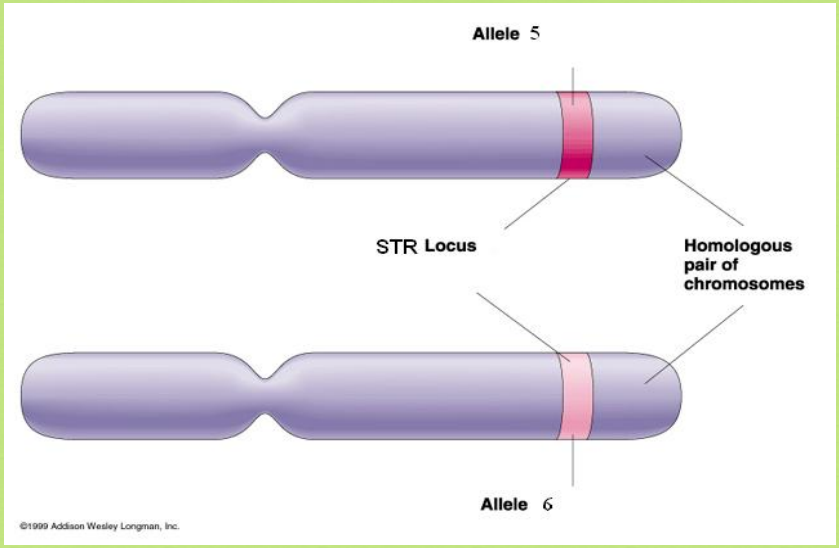
Allele

A variant of a gene or marker. In the context of microsatellite markers, two alleles will differ by the number of repeats present. For example, these are 4 different allele variants for a dinucleotide microsatellite marker.

Allele 1
Allele 2
Allele 3
Allele 4



Genotype What alleles an individual has for a particular marker or gene at a given locus.



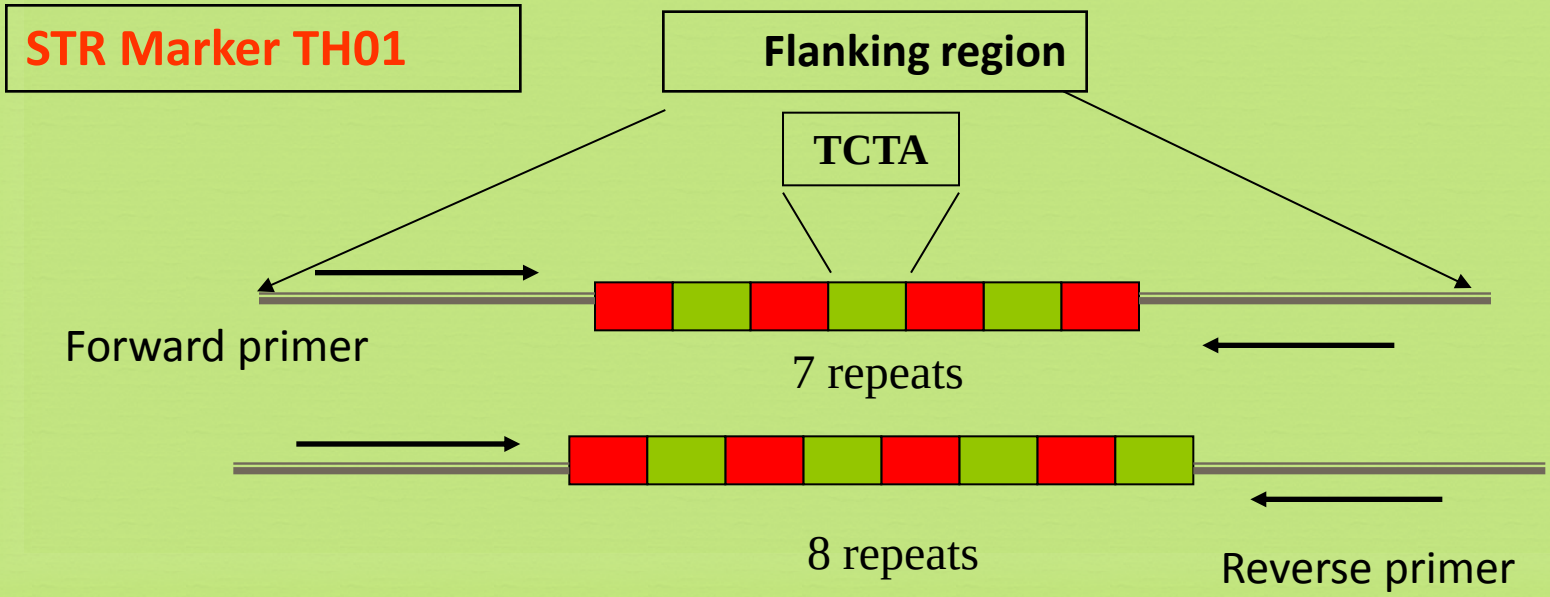
Homozygous- Both alleles for a marker/gene at a specific locus are identical.

Heterozygous- Both alleles for a marker/gene at a specific locus are different.

The genotype of a group of analysed loci (markers) is called ***DNA profile***.

Short Tandem Repeats (STRs)

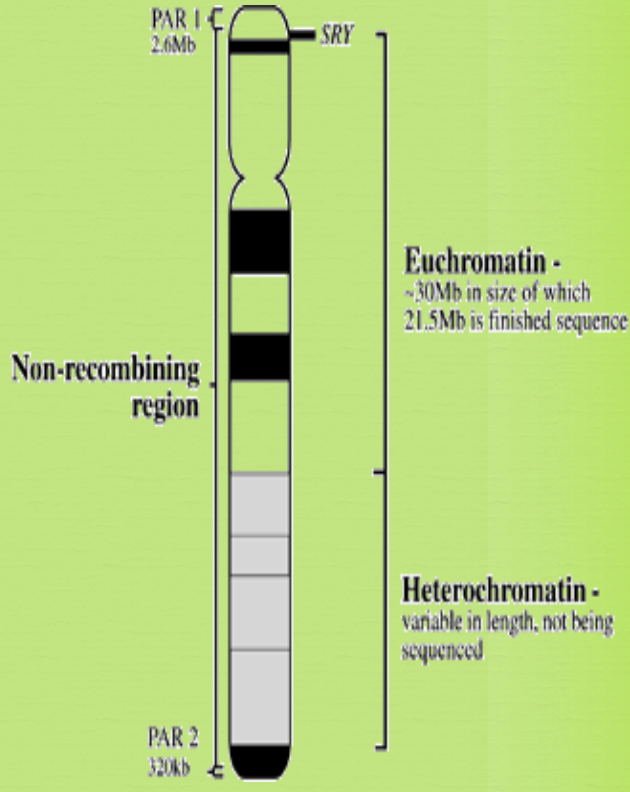
- **Most commonly used nowadays because of very high discrimination power.**
- **Forensic STR analysis looks at the length of up to 24 areas of DNA simultaneously.**
- **Short sequence core repeat unit (2-6 bp).**
- **Located in the nuclear DNA -either on autosomal or sex chromosomes- introns or between genes e.g. TH01 & D3S1358.**
- **Short size array length (100-400bp), ideal for degraded samples.**



Y Chromosome markers

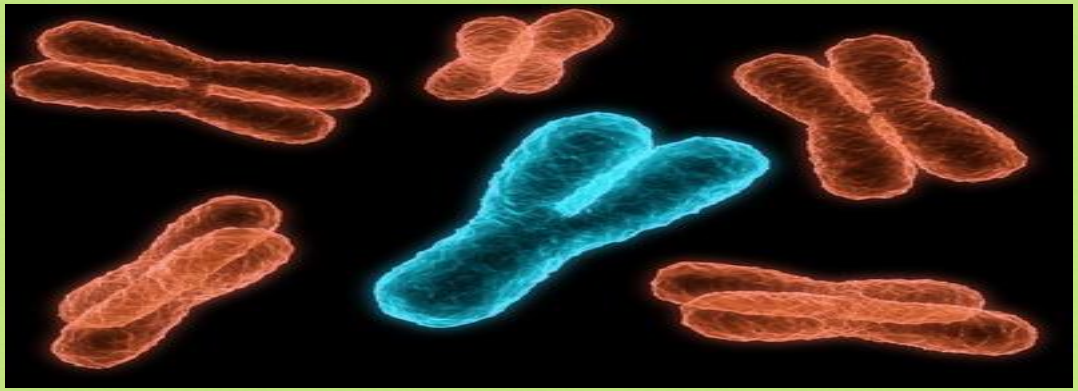
- One of the smallest in the genome. About 95% of this sequence, termed as the non recombining region (NRY); full of repetitive sequences (STRs, SNPs).

- Present only in males, inherited from the father as it is to his sons.



* Ideogram of the Y-chromosome showing the locations of pseudoautosomal regions (PAR), the testis determining gene, SRYand the long arm heterochromatin (Hurles and Jobing, 2001).

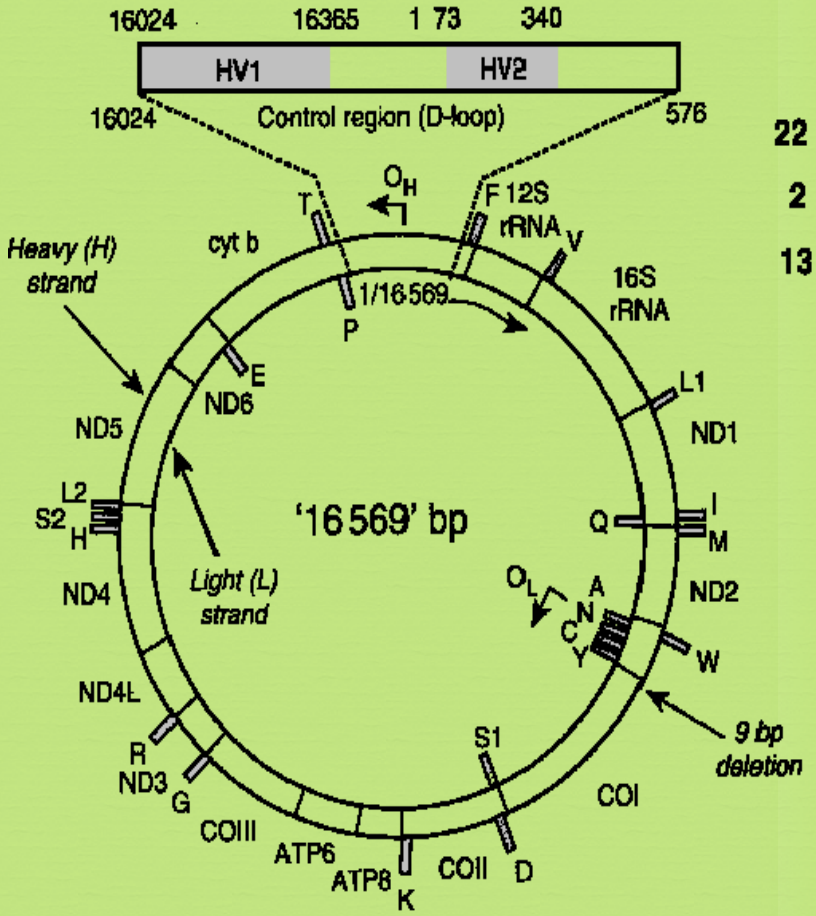
Y Chromosome Testing



- **Up to 17-23 loci are available.**
- **Detects male component of a mixture.**
- **Important for detecting the semen donor in sexual assault mixtures.**
- **Used in motherless cases of paternity testing, for exclusion and for paternal lineage analysis in missing persons & mass disasters.**
- **Less discriminating than standard DNA testing among unrelated men.**

Mitochondrial DNA

- Mitochondria contain an extra-chromosomal circular genome .
- Maternally inherited & passed to all children.
- very valuable in forensic community, trace maternal lineage in missing persons investigations (e.g. maternity testing).
- High copy number justifies its use in degraded and difficult samples e.g. hair shafts and bone remains.
- Most variation in D-loop (non coding control region), Contains HVI & HVII regions. Detected by sequencing



* Circular mtDNA genome, Butler, 2005.

How is paternity testing performed?

Samples Obtained from Father, mother, child trio

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 Child's Sample Genotype to Parents Sample Results → If match occurs, paternity statistics. Population database * → Generation of Case Report with Probability of paternity or PI*

Sources of Biological Evidence

- **Blood(except RBC)**
- **Semen**
- **Saliva**
- **Urine**
- **Hair**
- **Teeth**
- **Bone**
- **Tissue**

Locard's Principle of Exchange

Anytime there is contact between two

surfaces, there will be a mutual exchange of matter across the contact boundary

Other Possible items for DNA Testing:

1. cigarette butts

2. gloves, bandanas, masks, caps
   general clothing

3. condoms (inside vs. outside)

4. stains on furniture, pillows, sheets

5. hair clips, lipsticks

6. letters, envelopes, and stamps

DNA Extraction

• Any source of nucleated cells can be a substrate for DNA extraction.

• **Aims of extraction**: enough DNA for profiling, reasonable purity to avoid PCR inhibition.

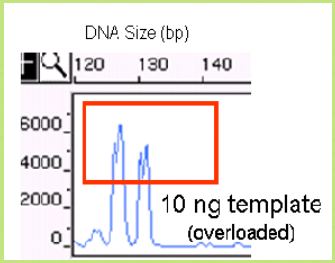
• **Choice of method** depends on : sample type & quantity, speed, successful extraction from forensic samples without PCR inhibitors, cost, avoiding hazardous chemicals e.g. phenol& chloroform.

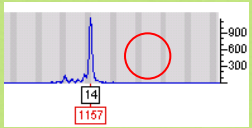
• **Methods of extraction:**

- Manual e.g. Chelex resin, silica based DNA extraction, phenol chloroform …. etc.
- Kits e.g. Qiagen kits and FTA paper

DNA Quantification

• Adding correct amount of DNA to PCR reaction is mandatory to obtain clear profile (not overloaded or with allele drop-outs).





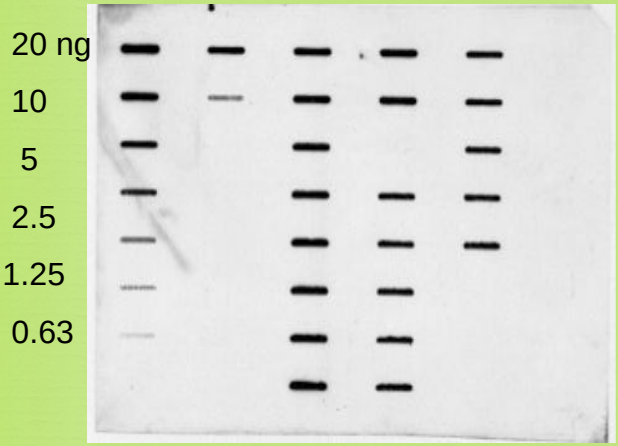
• **Many methods are in use**:

- *UV spectrophotometry* (UV 260/280 – not sensitive, not human or DNA specific).

- *Fluorescence spectrophotometry* (not human specific, sensitive).

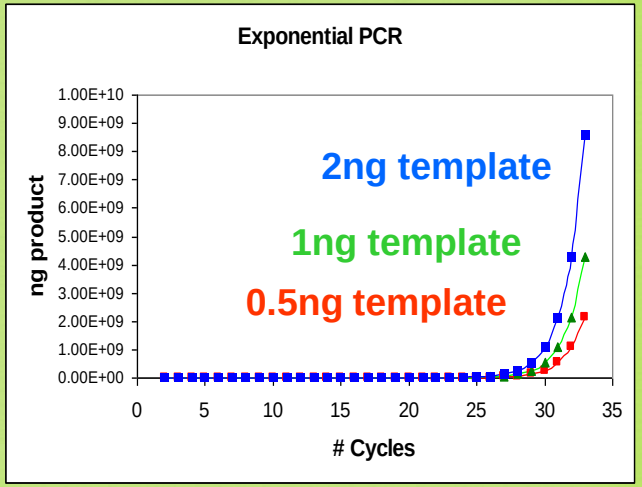
- *Hybridization* (Human specific, sensitive, poor dynamic range).

- *Real time PCR* (human specific, very sensitive, good dynamic range).

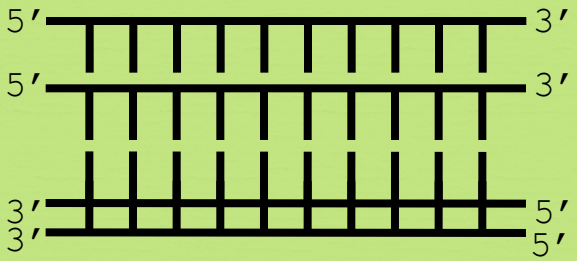


Hybridization

Rt PCR



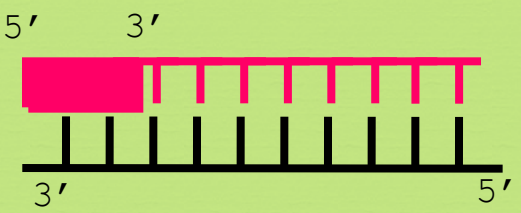
DNA amplification with the Polymerase Chain Reaction (PCR)



Starting DNA Template

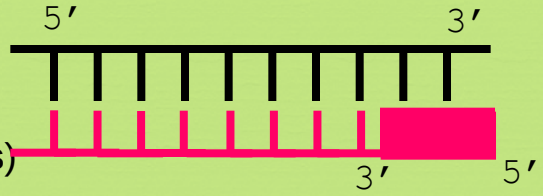
Separate strands (**denature**)

Forward primer



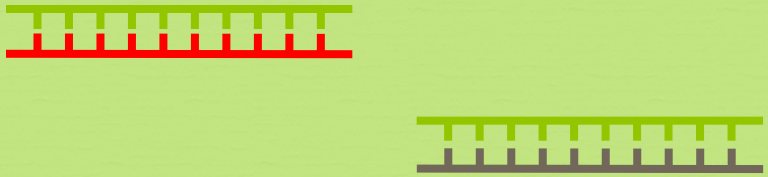
Make copies (**extend primers**)

Add primers (**anneal**)



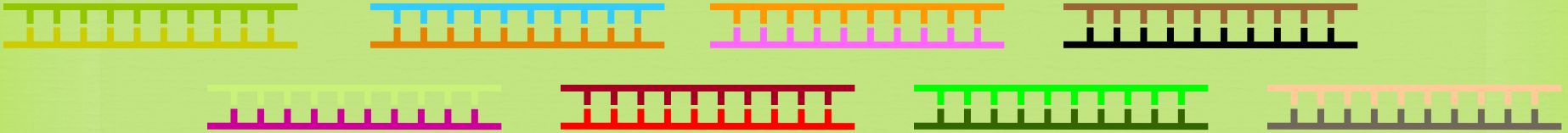
Reverse primer

PCR Copies DNA Exponentially through Multiple Thermal Cycles

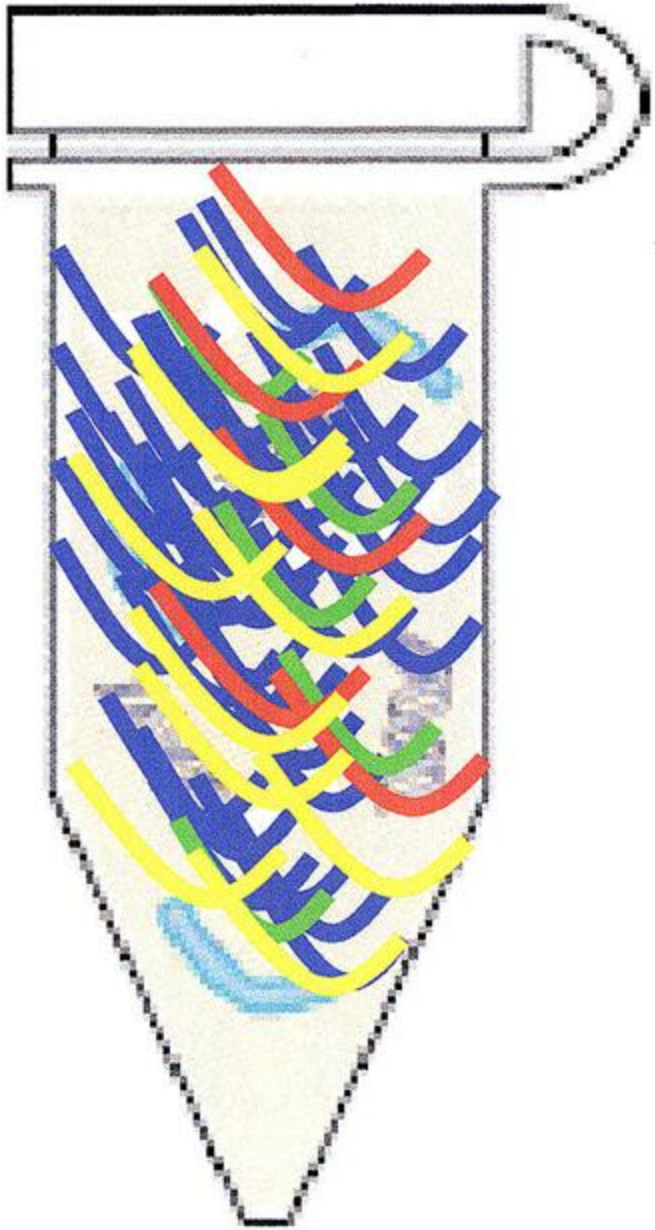


Original DNA target region

Thermal cycle



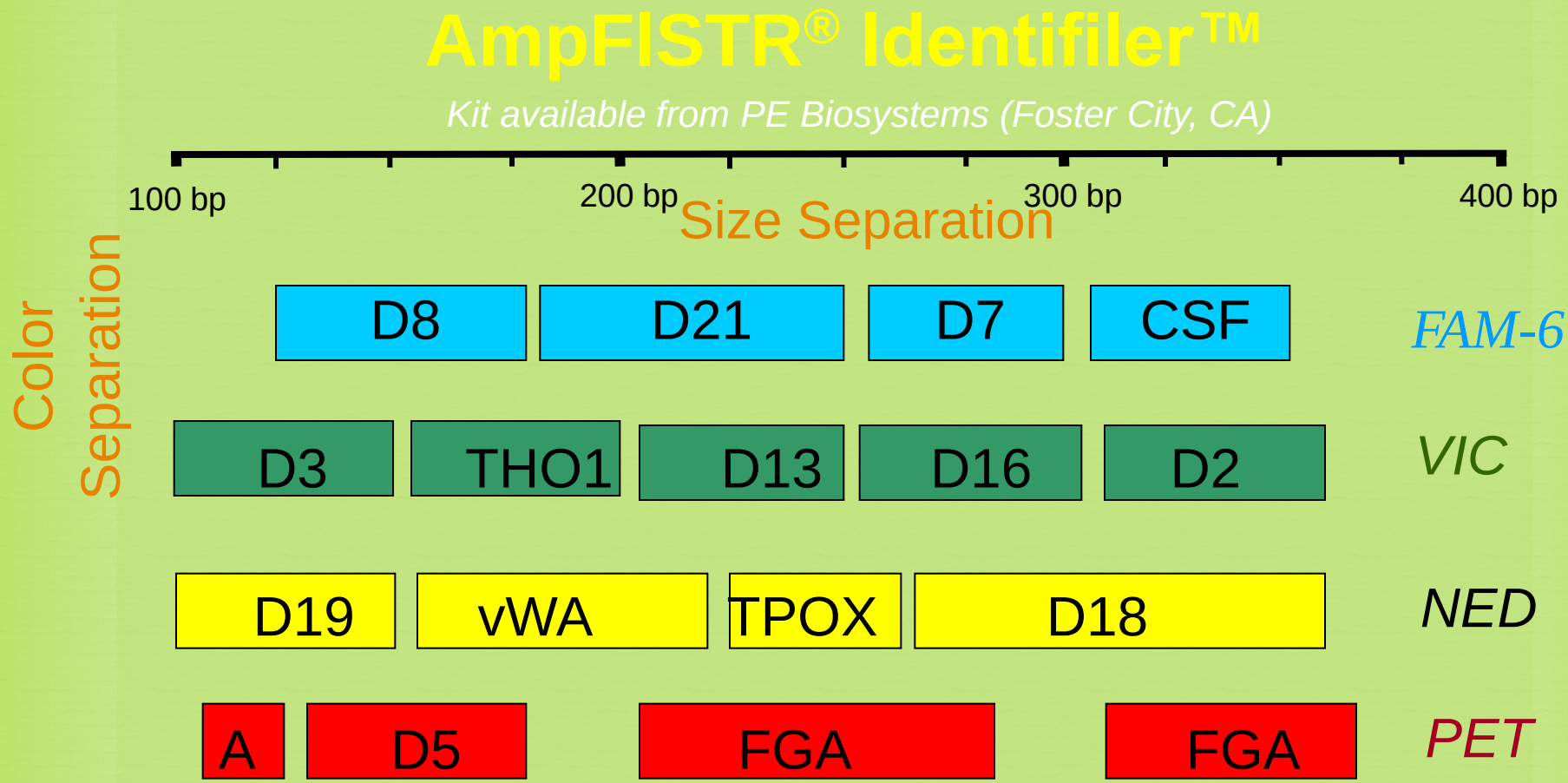
In 32 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created



Multiplex PCR

- Over **20** 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

Example of Forensic STR Multiplex Kit

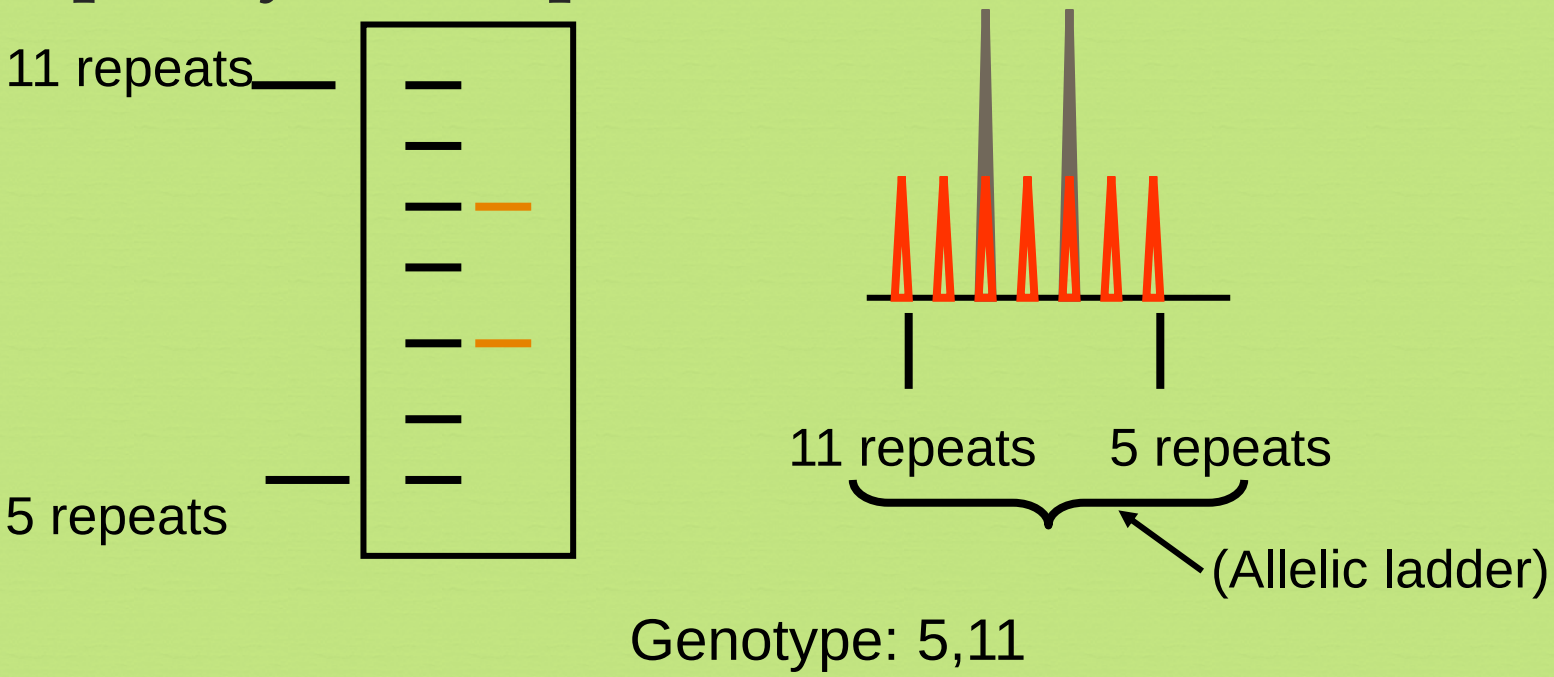


15 STRs amplified along with sex-typing marker amelogenin in a single PCR reaction.

LIZ-internal lane standard

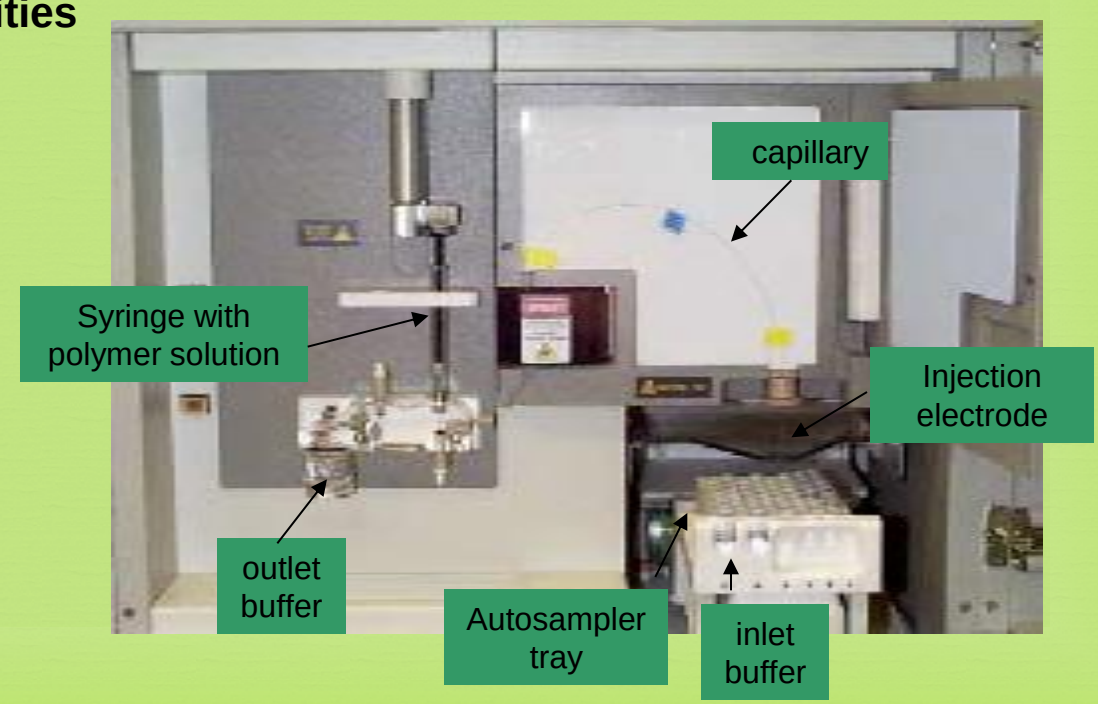
Analysis of Short Tandem Repeat Polymorphisms by electrophoresis

- **STR genotypes are analyzed using gel or capillary electrophoresis.**

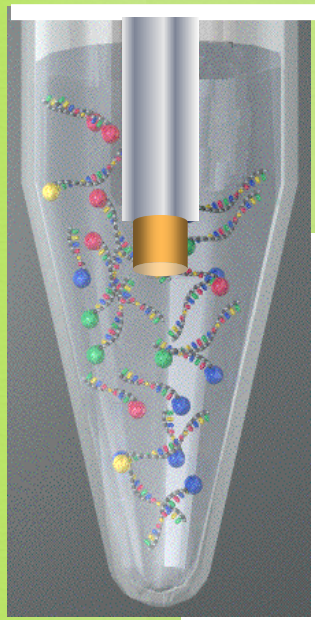


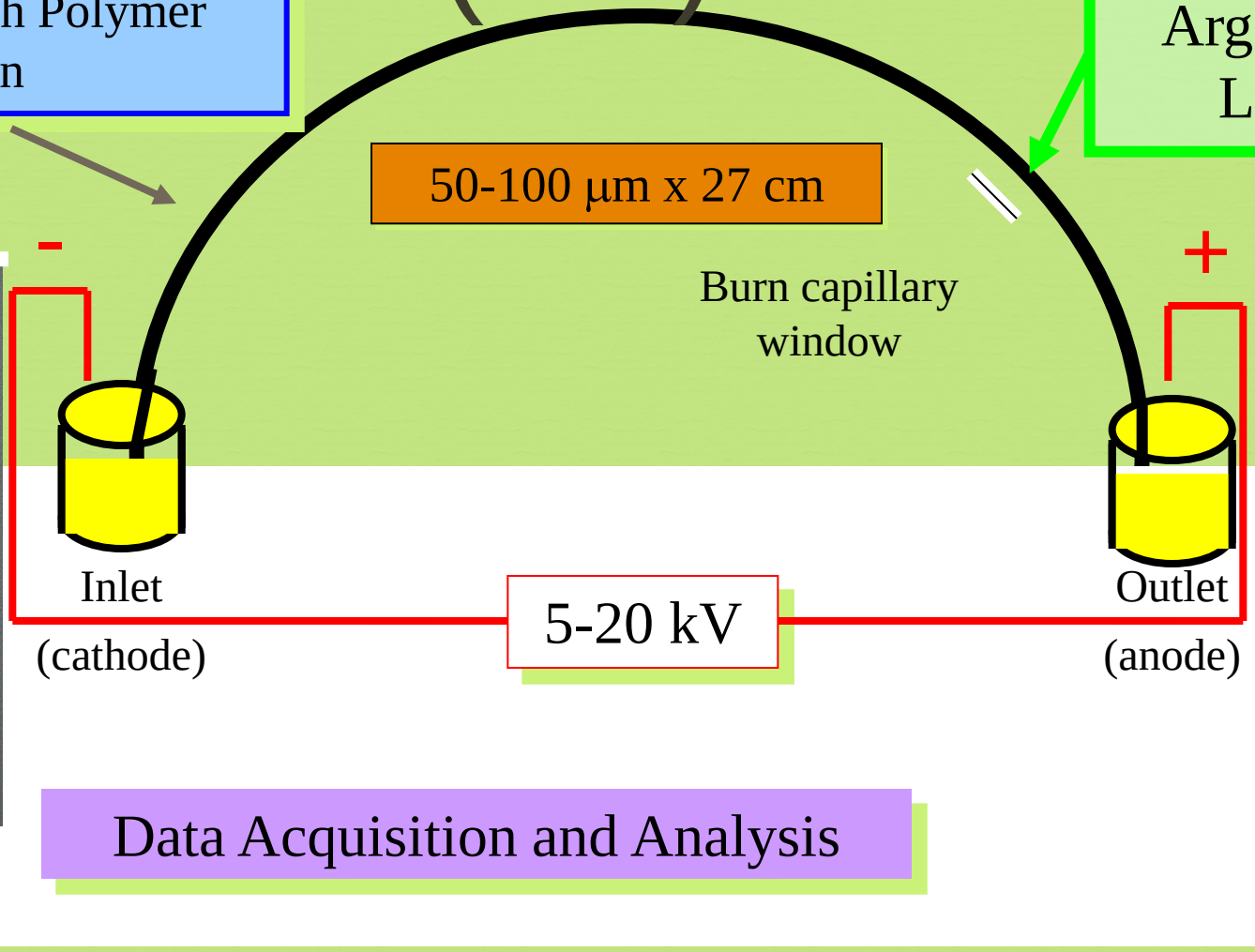
Capillary electrophoresis with multi-color detection capabilities

ABI Prism 310 Genetic Analyzer

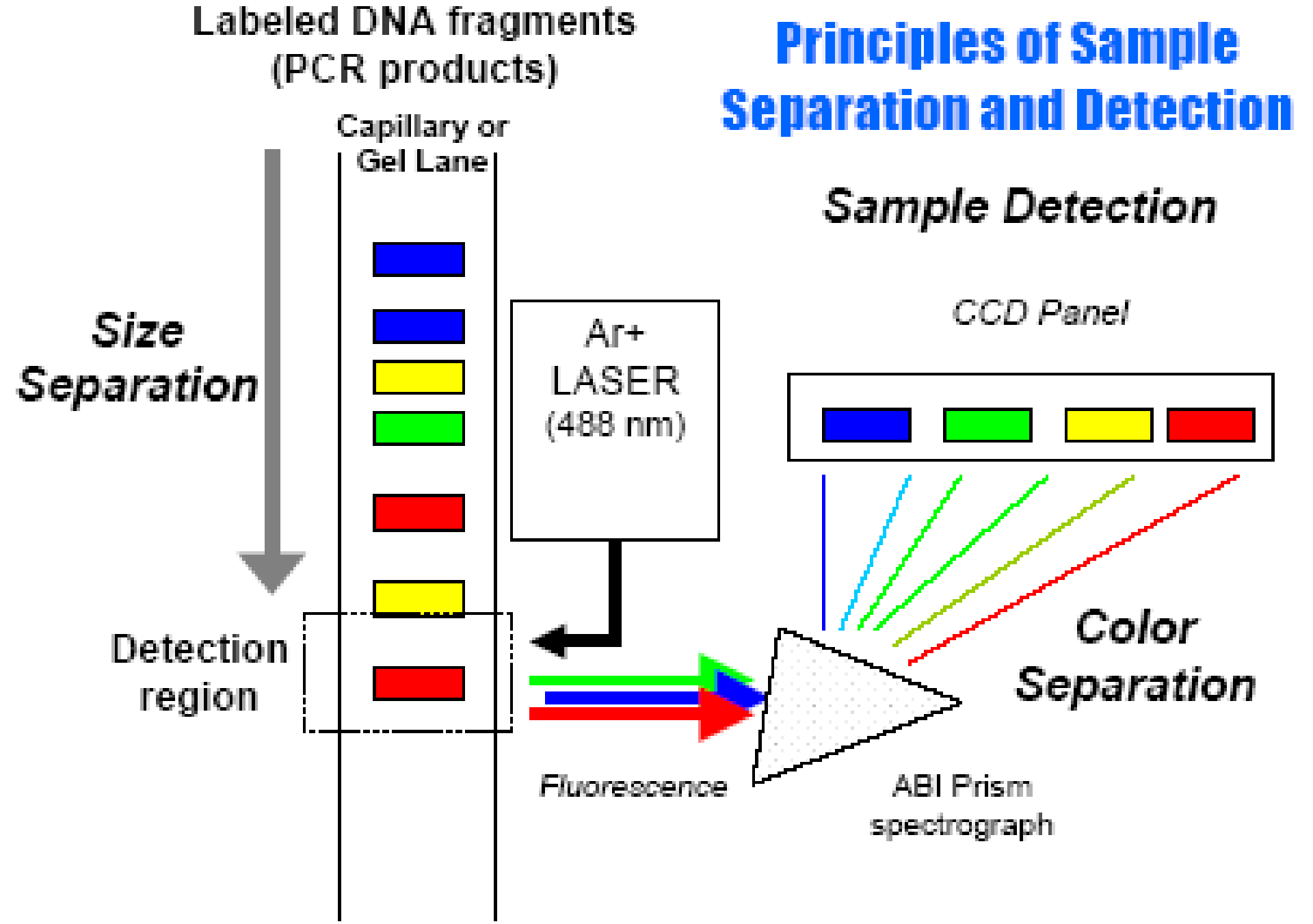


Capillary Electrophoresis (CE)



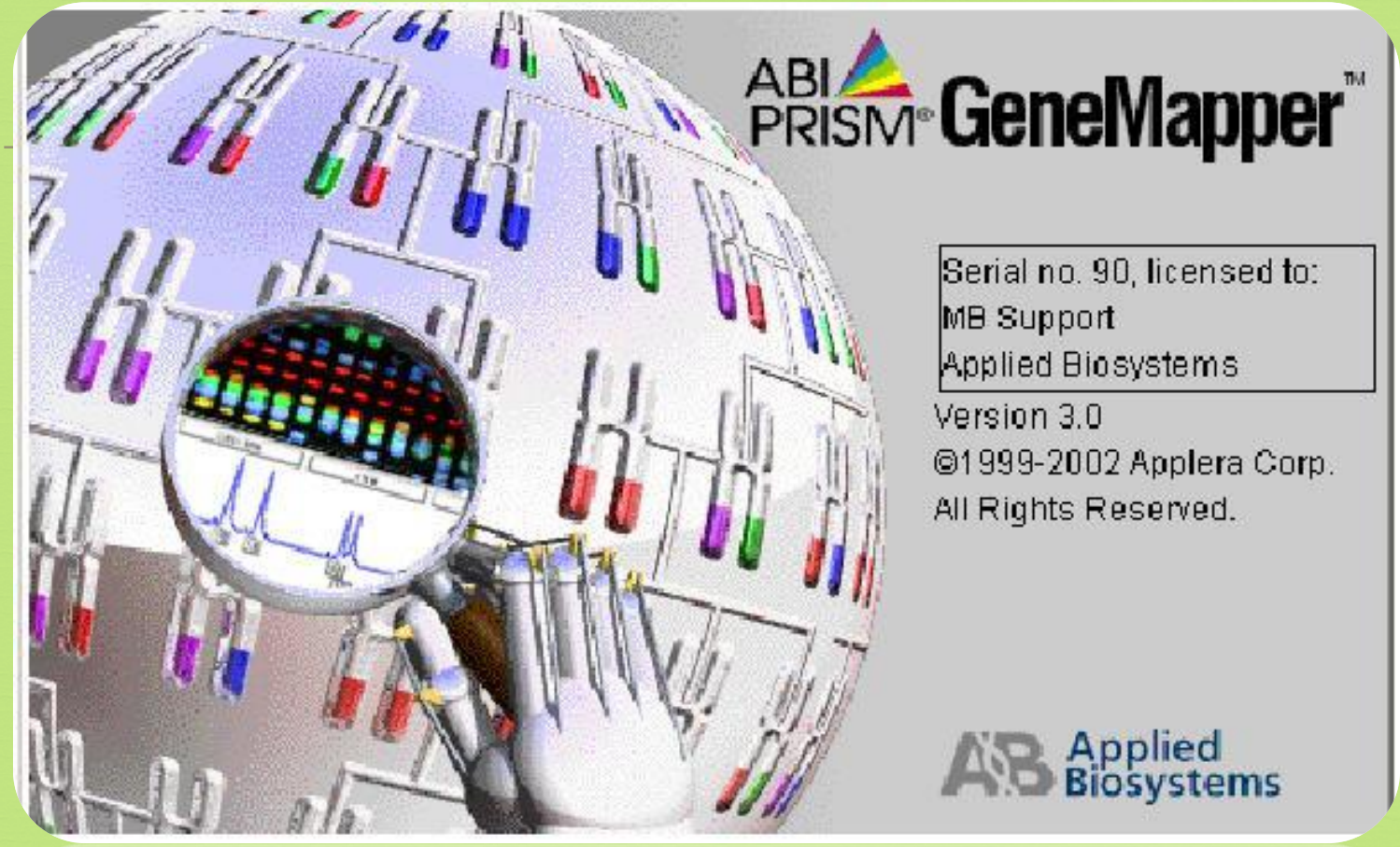


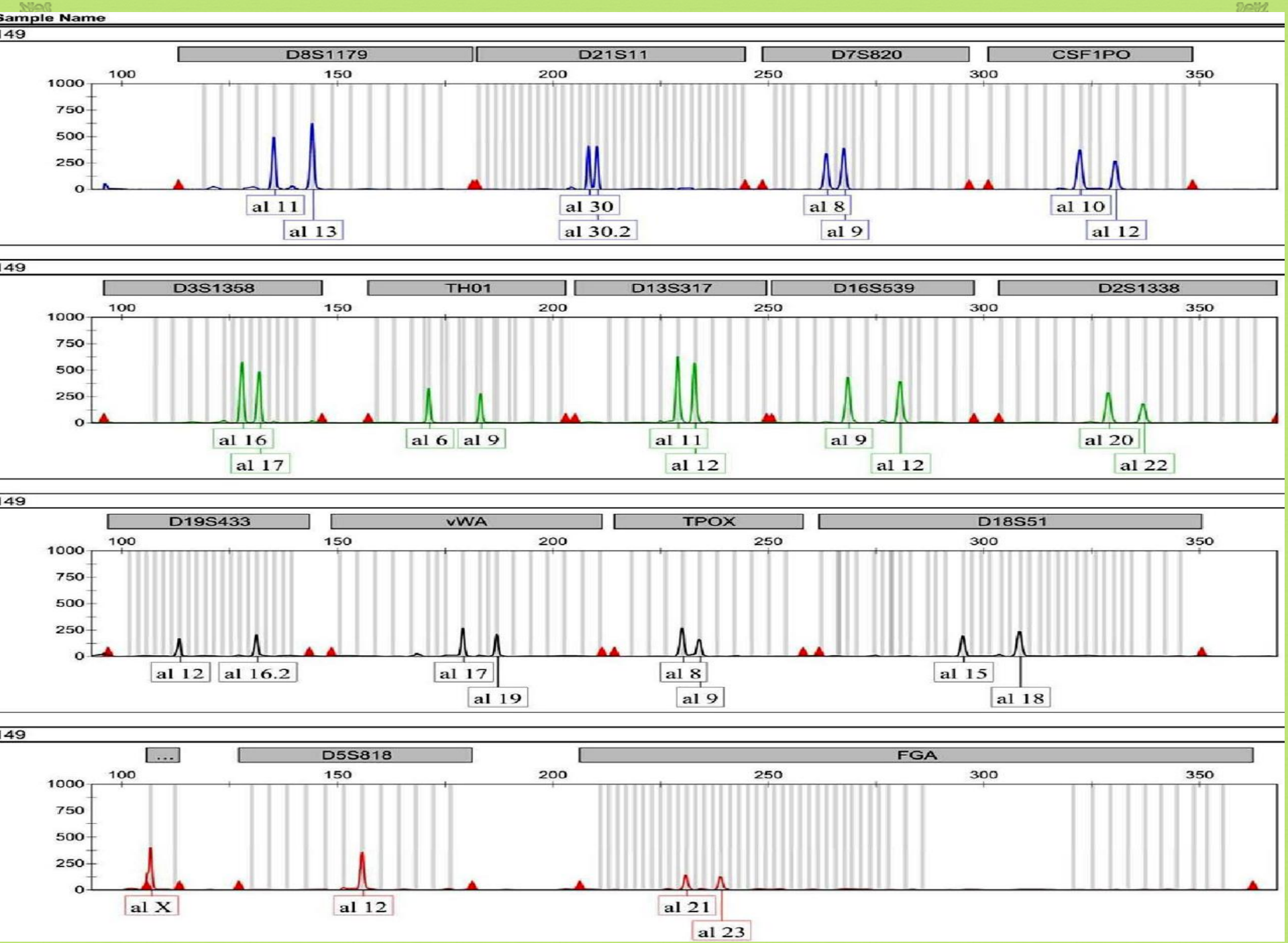
Principles of Sample Separation and Detection



Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.8, ©Academic Press

GeneMapper Software




Sample Name
149
D8S1179
D21S11
D7S820
CSF1PO
100
150
200
250
300
350
1000
750
500
250
0
al 11
al 13
al 30
al 30.2
al 8
al 9
al 10
al 12
149
D3S1358
TH01
D13S317
D16S539
D2S1338
al 16
al 17
al 6
al 9
al 11
al 12
al 9
al 12
al 20
al 22
149
D19S433
vWA
TPOX
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al 19
al 8
al 9
al 15
al 18
149
...
D5S818
FGA
al X
al 12
al 21
al 23

Forensic DNA Paternity Testing

What is paternity?

- **Paternity** means fatherhood. Paternity is established when a laboratory uses genetic fingerprinting to determine whether two individuals have a biological parent-child relationship.
- DNA testing is the standard nowadays, polymerase chain reaction (PCR) and STR (Short Tandem repeats) are currently used.
- Older methods also exist, including ABO blood group typing, enzymes, or human leukocyte antigens (HLA).

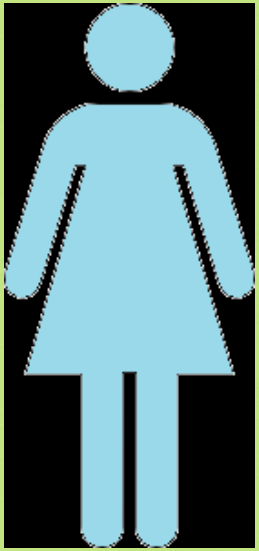
When do we need paternity testing?

- For peace of mind; when a man wants to confirm that a child is his own.
- Sexual crimes resulting in illegal pregnancy.
- Illegal marriage for child support.
- Hidden marriage with inheritance claims of the offspring
- Immigration cases
- Reverse paternity testing in missing person & mass disaster investigations.
- Interchange of infants in maternity hospitals.

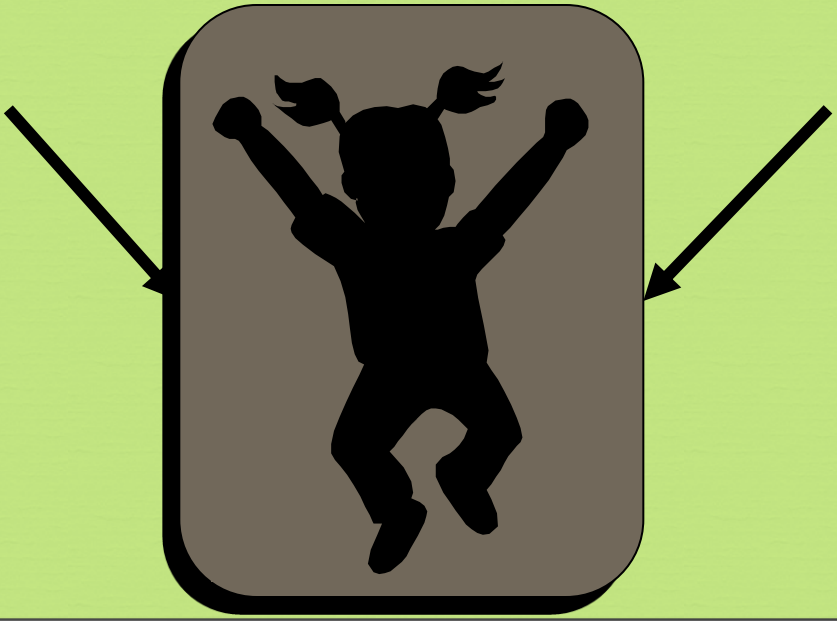
Mendelian inheritance

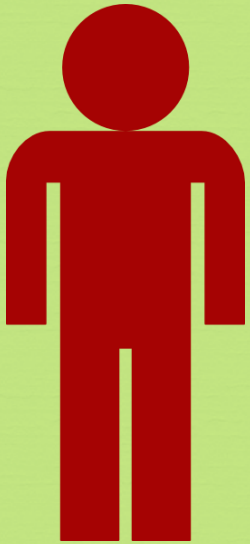
One set of 22 autosomes (plus X)

One set of 22 autosomes (plus X & Y)



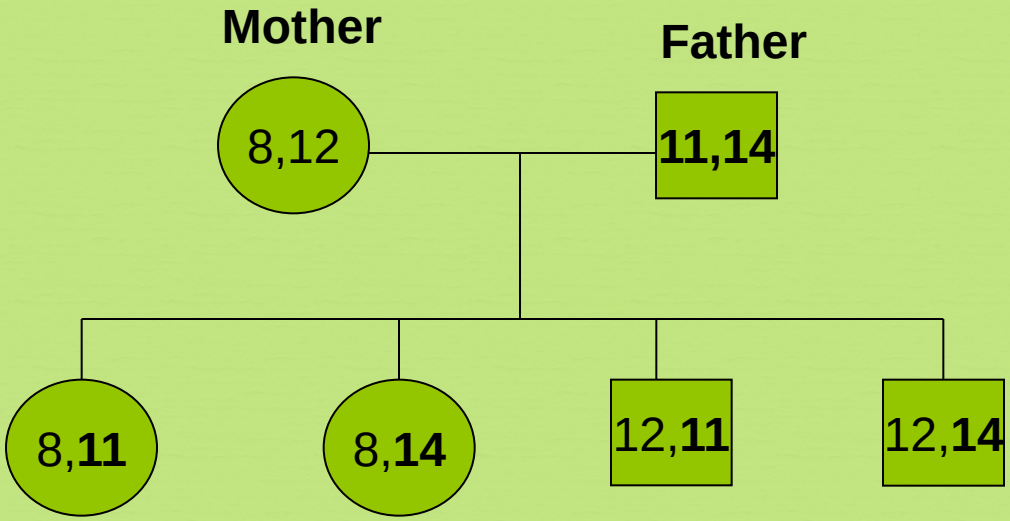
Paternity Testing





Two alleles for each autosomal genetic marker

Mendelian inheritance (con)

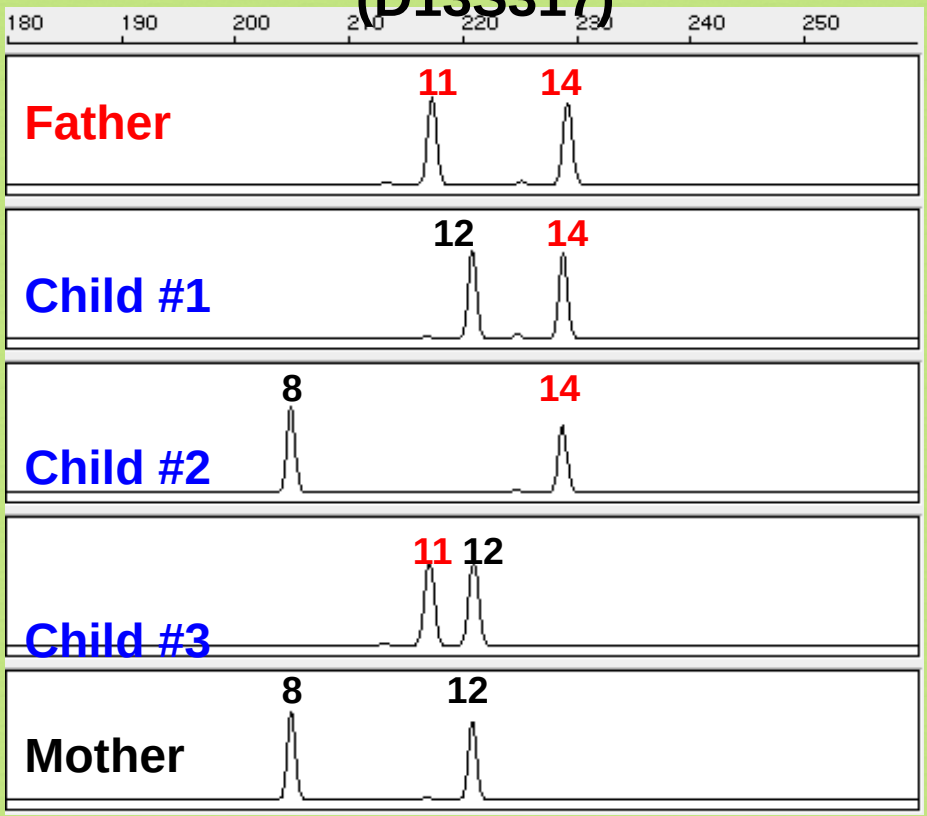


Obligate
Paternal allele

Rules of inheritance

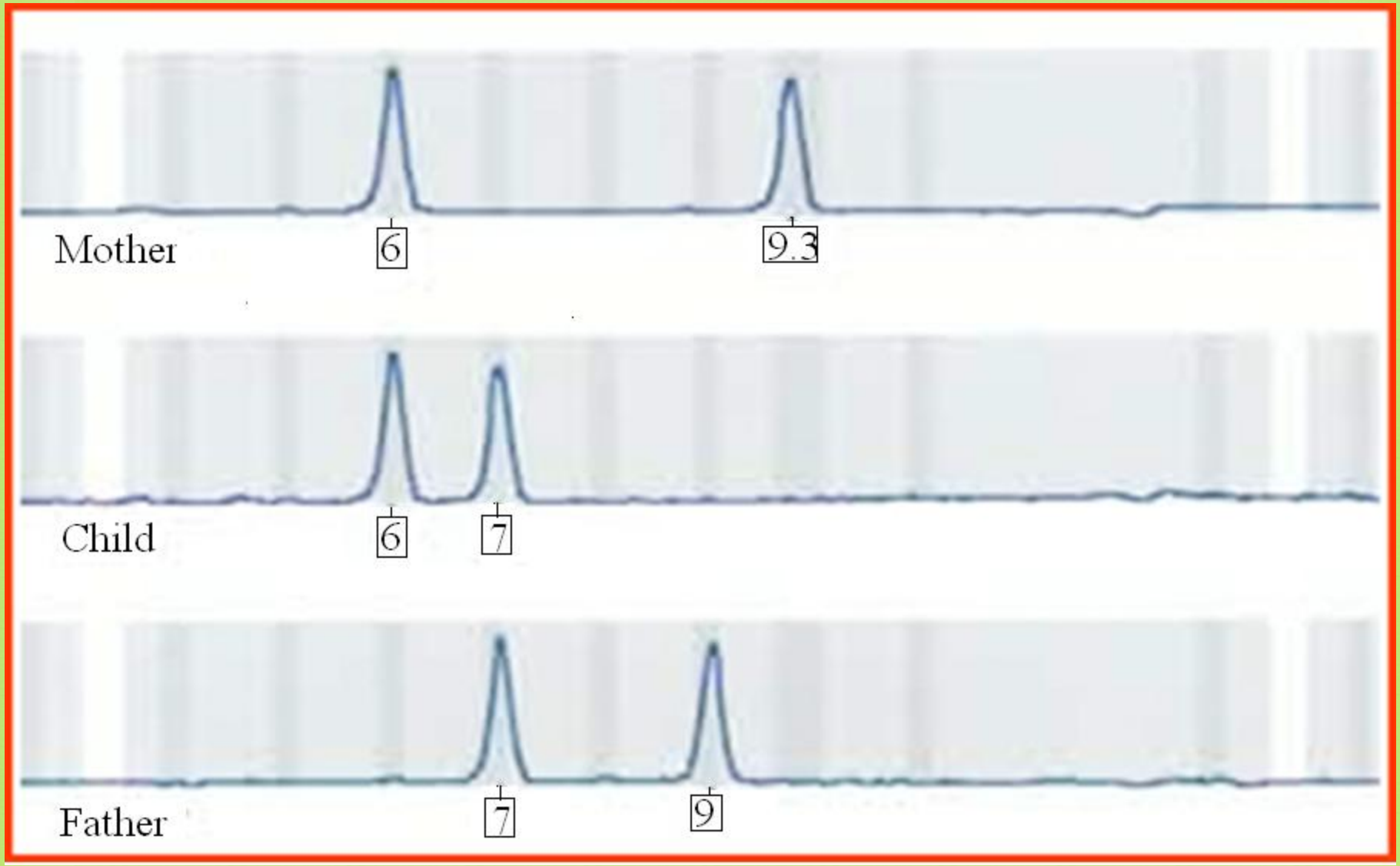
1. **A child has two alleles for each autosomal marker (one from mother and one from father.**
2. **A child will have mother mitochondrial DNA haplotype (baring mutation)**
3. **A male child will have father's Y chromosome haplotype (baring mutation)**

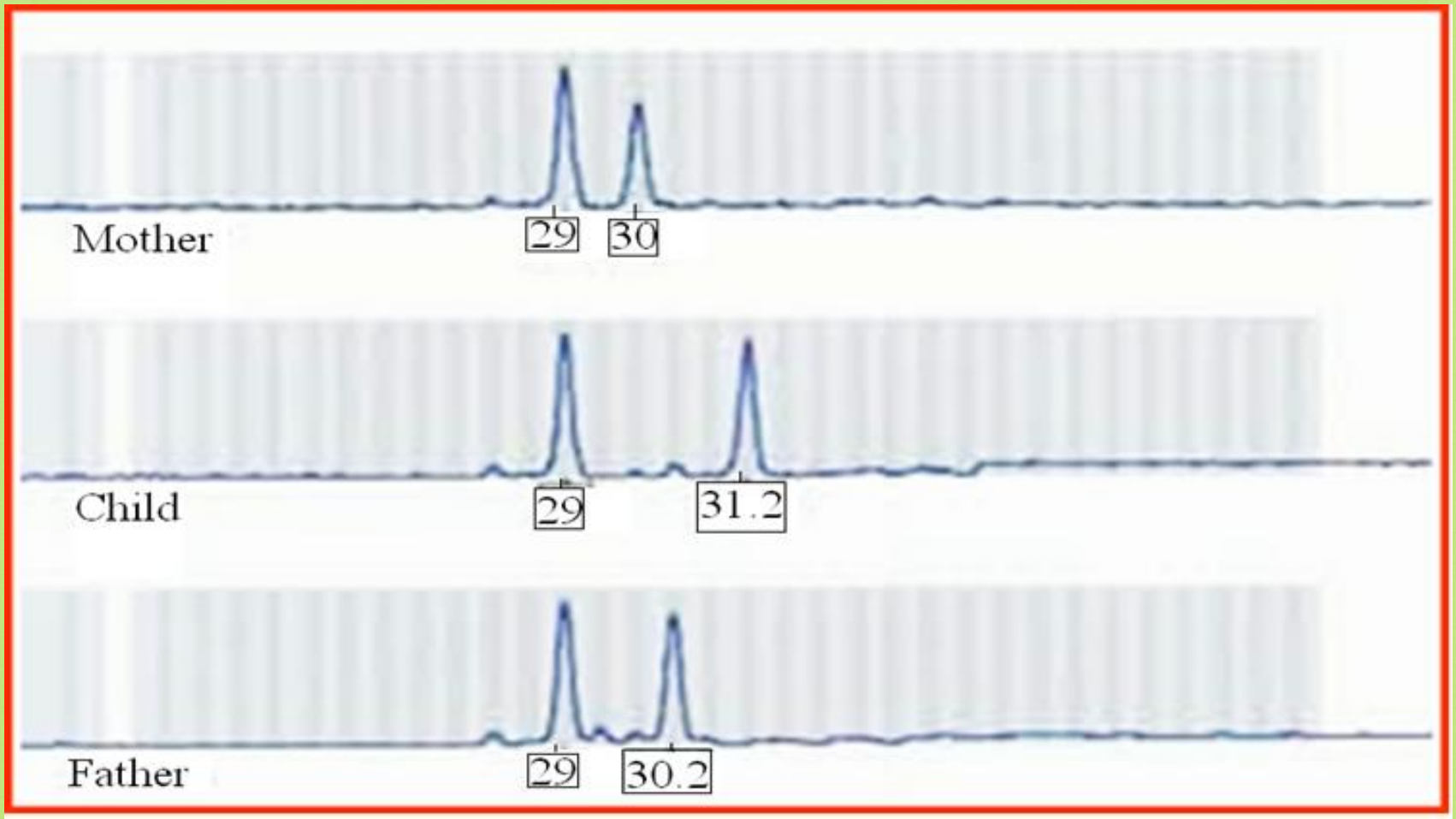
Family Inheritance of STR Alleles (D13S317)



• In a test including samples from the mother, child and alleged father, the probability of paternity is 99.99% or greater when an alleged father's DNA profile matches that of the child for all the genetic markers.

• On the other hand, an alleged father is 100% excluded from paternity if there is a mismatch for three or more genetic markers between the profiles of the child and alleged father.


Mother
6
9.3
Child
6
7
Father
7
9


Mother
29
30
Child
29
31.2
Father
29
30.2

Modern Use Of Y-STR Testing

Matching Y-STR Haplotype Used to Confirm Identity

(along with allele sharing from autosomal STRs)

Uday and Qusay Hussein

Is this man really Sadaam Hussein?


Thank you