

DNA PROFILING

DNA FINGERPRINTING: THEORATICAL AND PRACTICAL ASPECTS

What is DNA Profiling?

A technique used by scientists to distinguish between individuals of the same species using only samples of their DNA

Who Invented it?

- The process of DNA fingerprinting was invented by Alec Jeffreys at the University of Leicester in 1985.
- He was knighted in 1994.



Biological materials used for DNA profiling

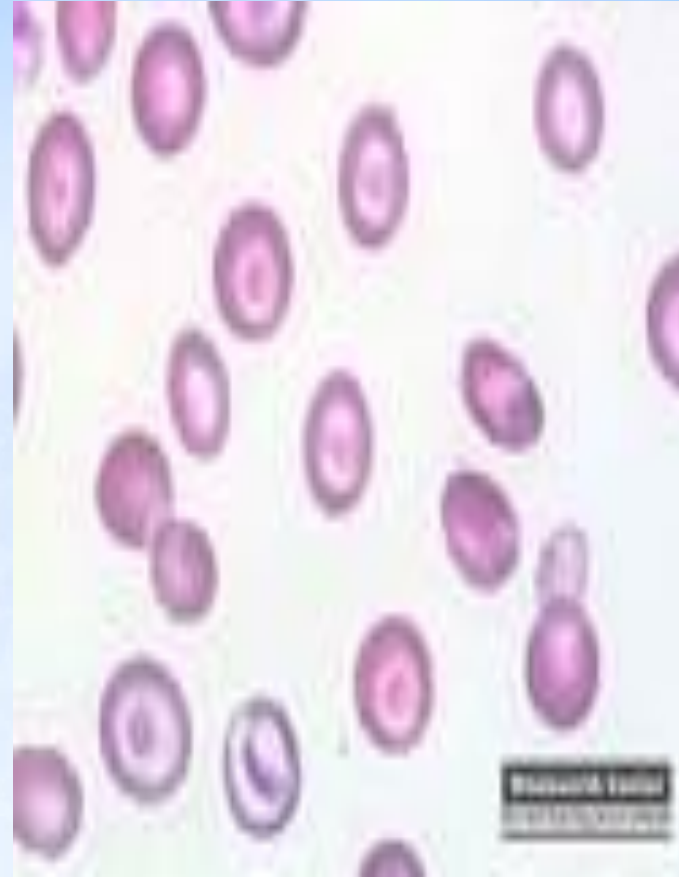
- **Blood**
- **Hair**
- **Saliva**
- **Semen**
- **Body tissue cells**
- **DNA samples have been obtained from vaginal cells transferred to the outside of a condom during sexual intercourse.**



Stages of DNA Profiling

- **Stage 1:**
**Cells are broken down
to release DNA**

**If only a small amount of
DNA is available it can
be amplified using the
*polymerase chain
reaction (PCR)***



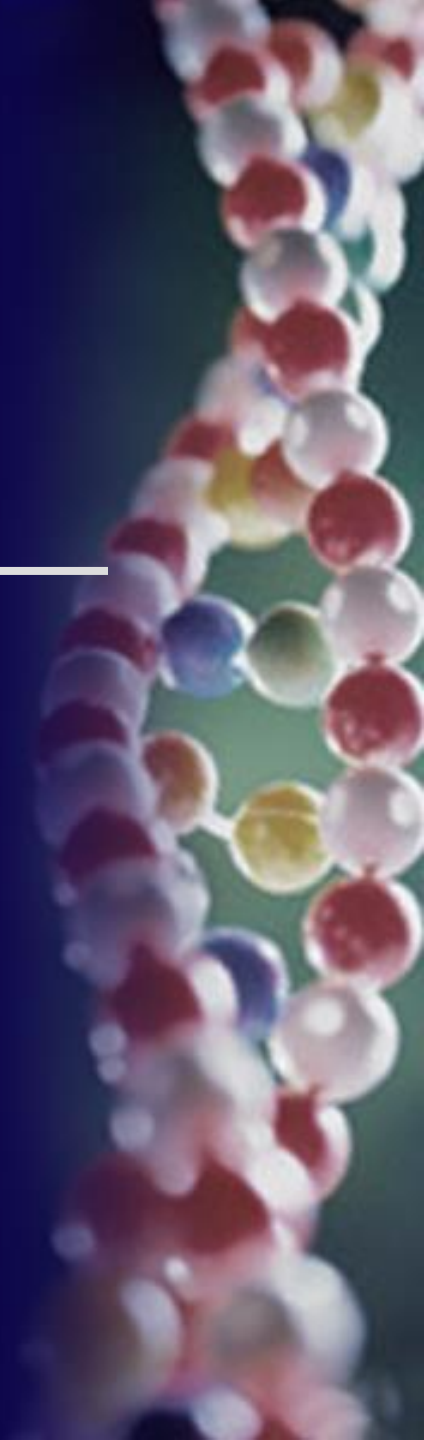
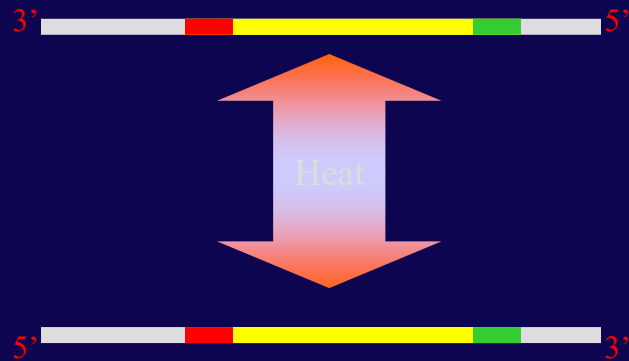
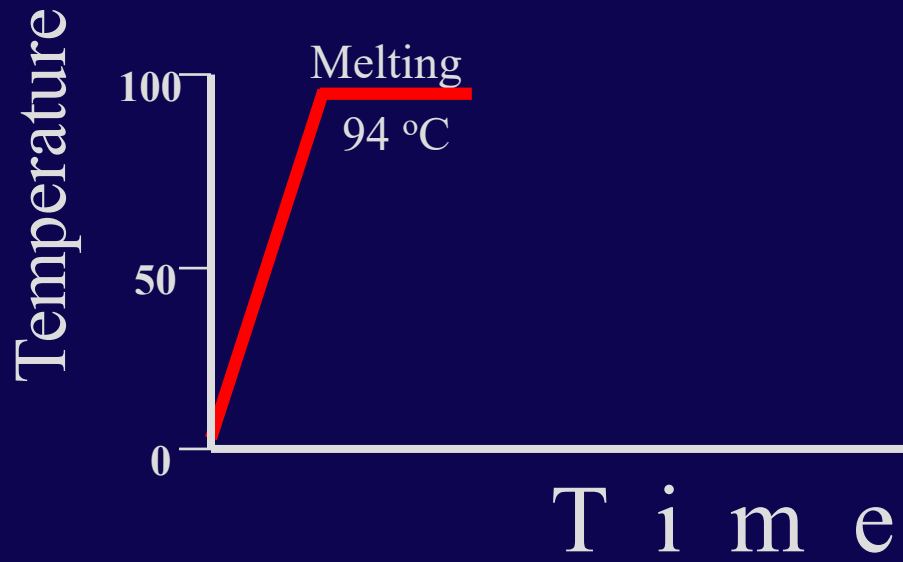
Polymerase Chain Reaction

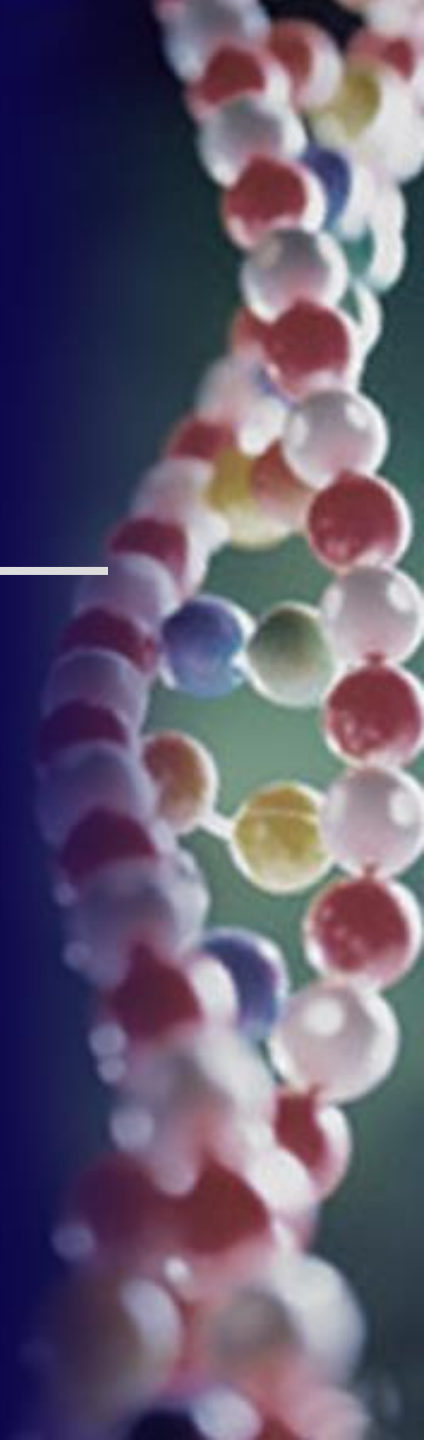
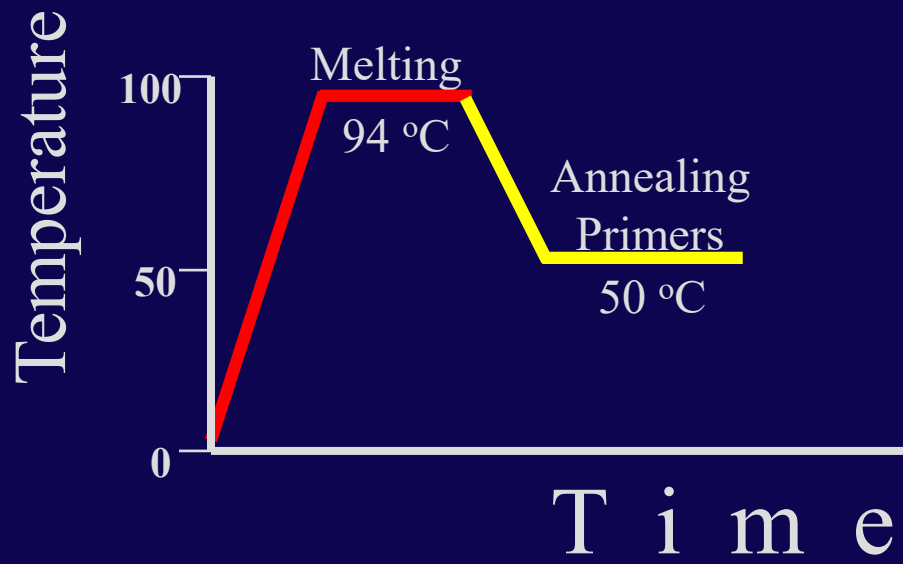
- It is an in vitro technique used for reproducing DNA fragments.
- The Polymerase Chain Reaction (PCR) was not a discovery, but rather an invention
- PCR uses a special DNA polymerase to make many copies of a short length of DNA (100 - 10,000 bp) that is defined by primers
- Kary Mullis was the inventor of PCR
- PCR is so important that Mullis was awarded the 1993 Nobel Prize in Chemistry

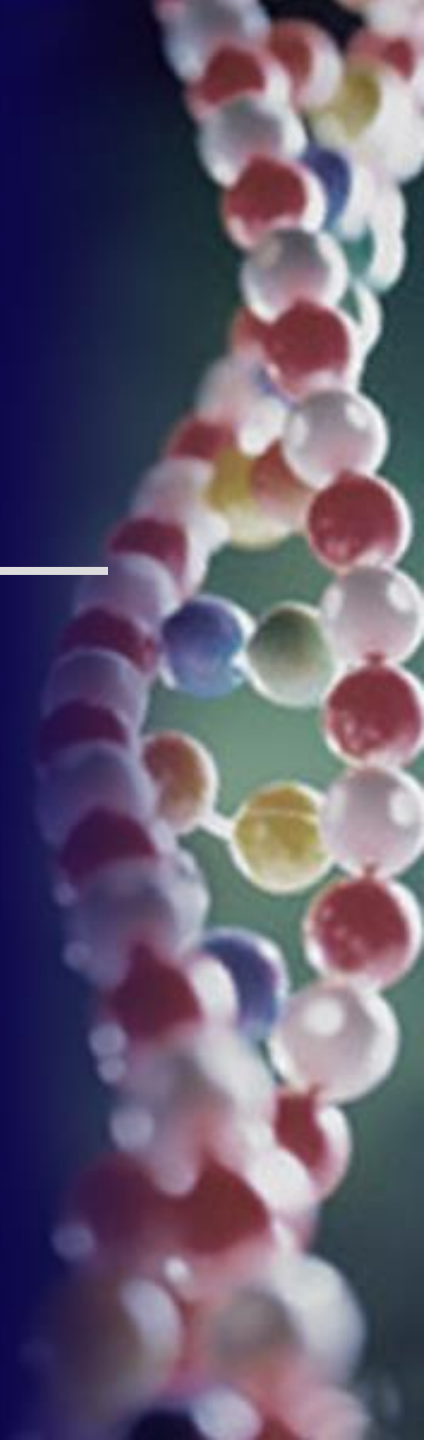
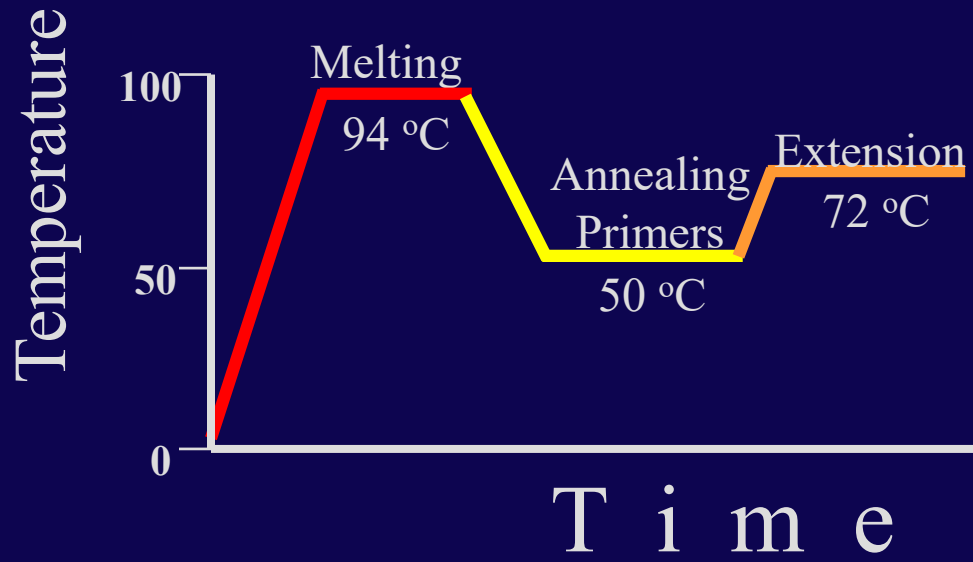


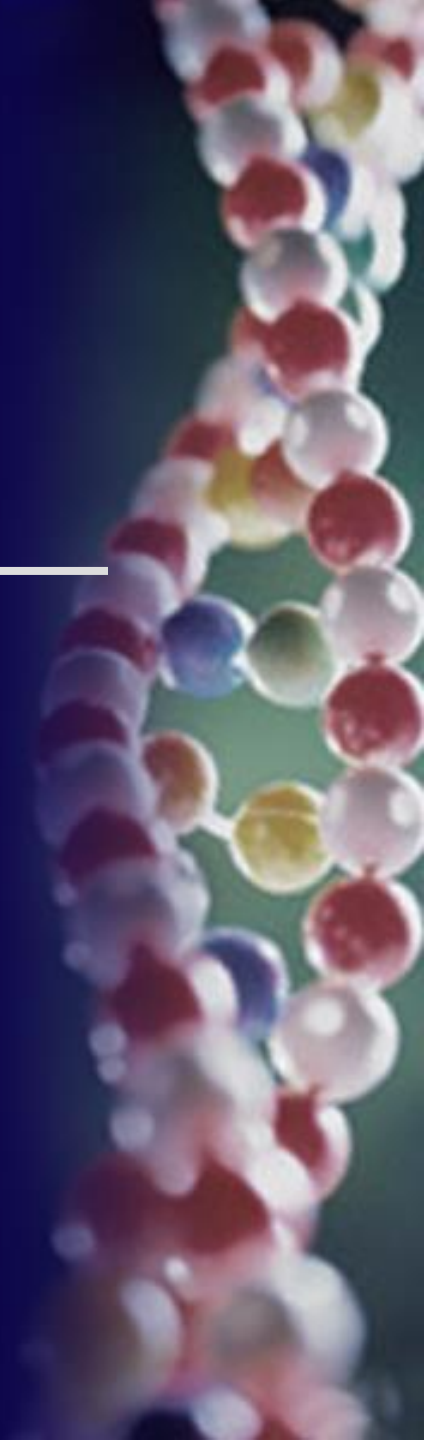
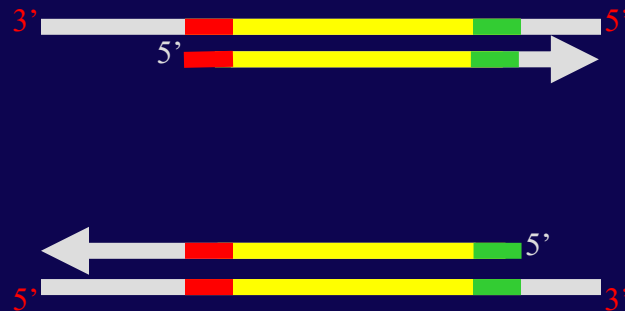
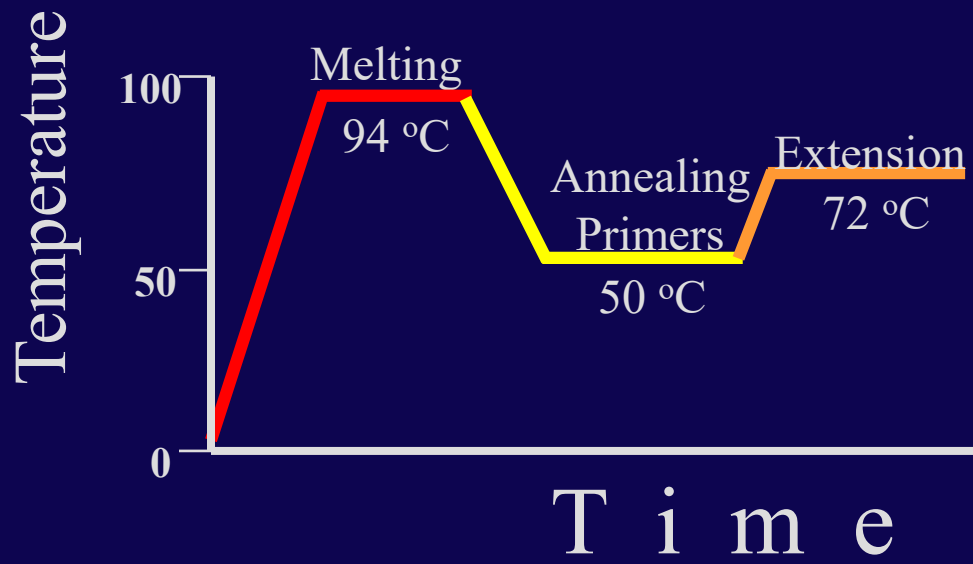


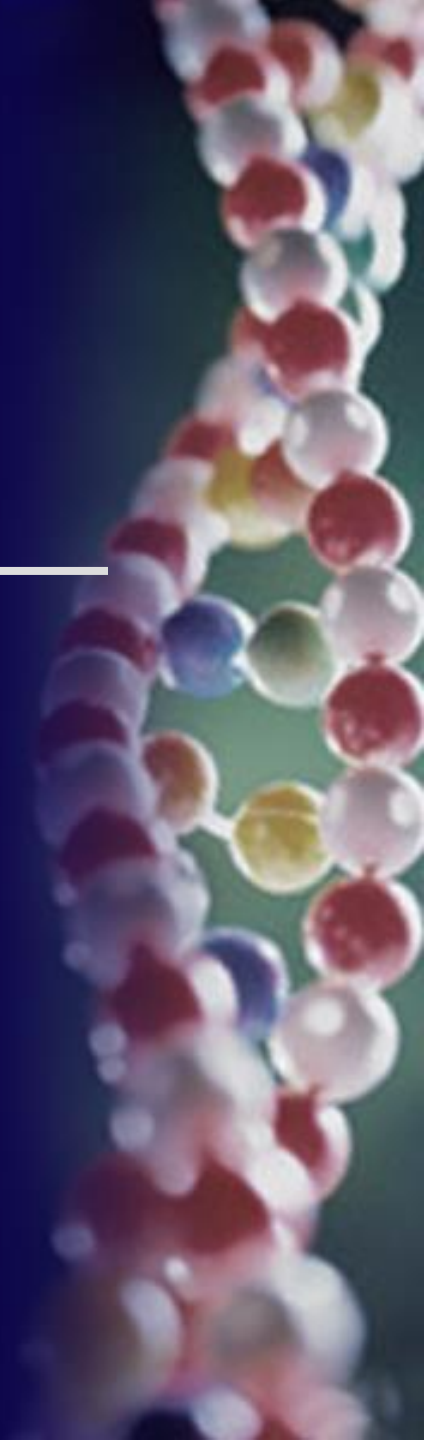
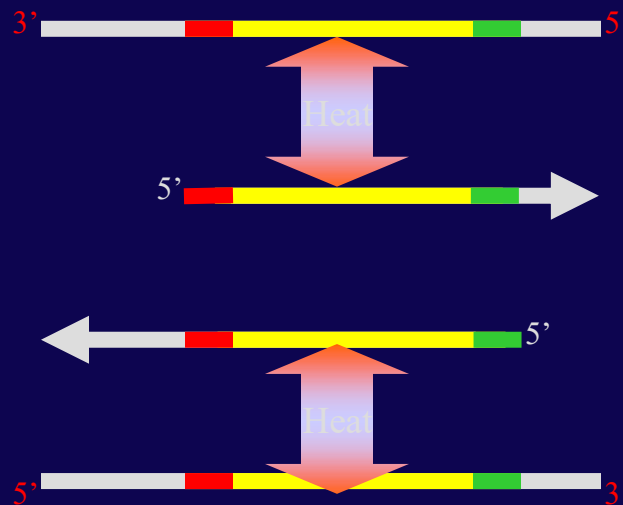
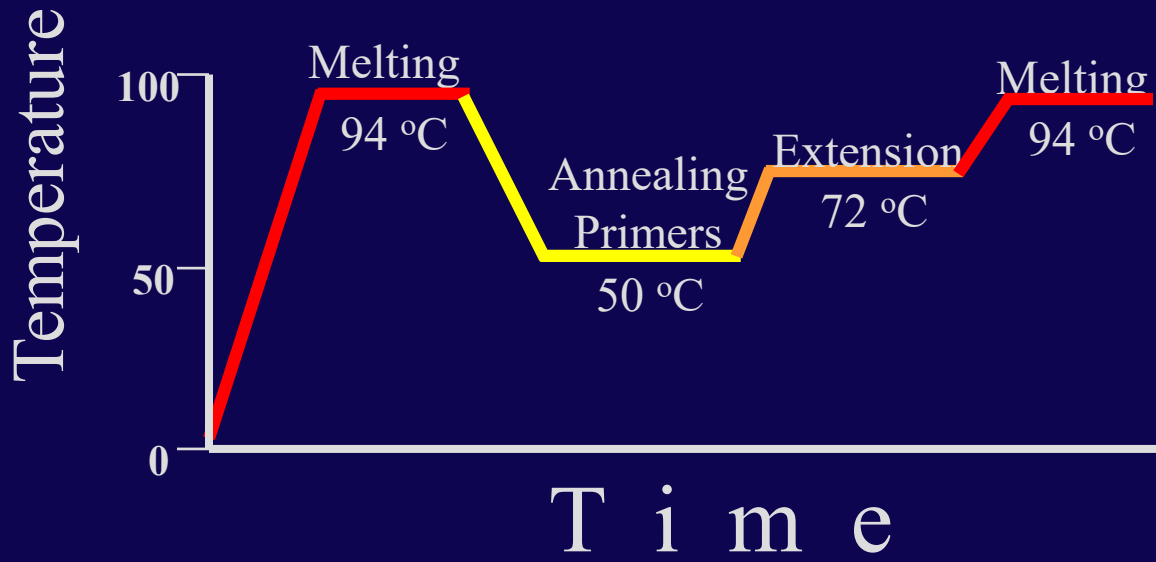
Pcr.exe

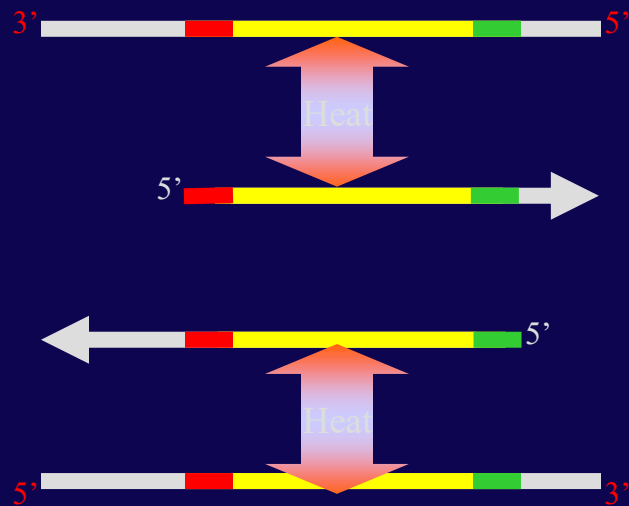
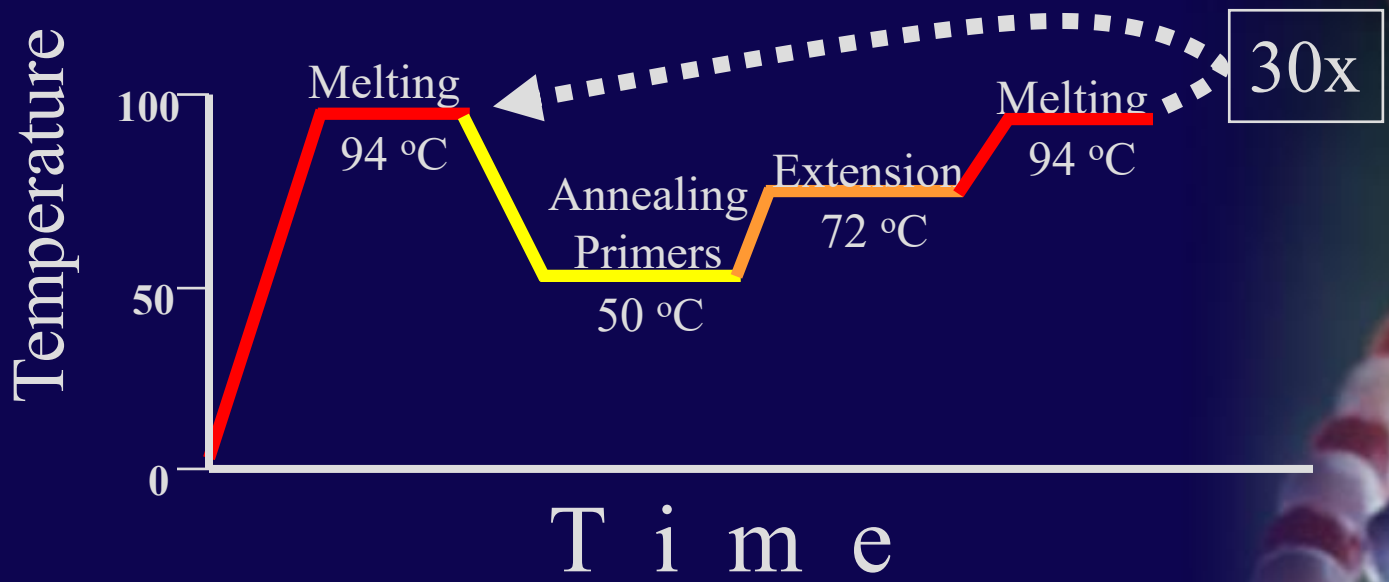




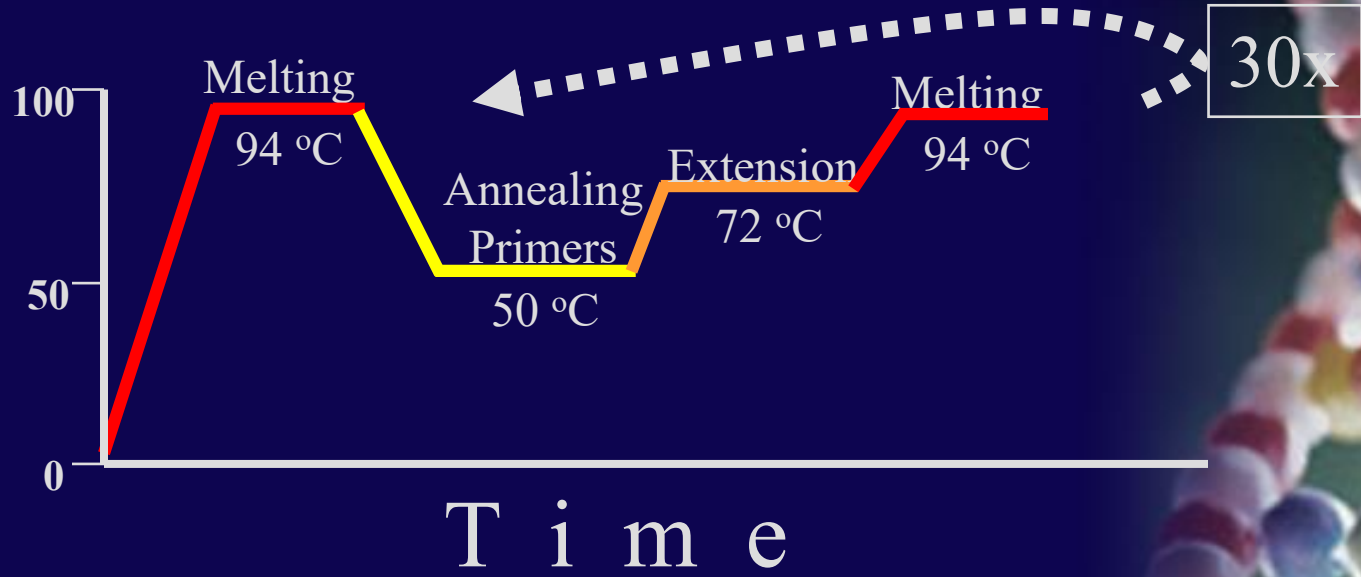








Temperature

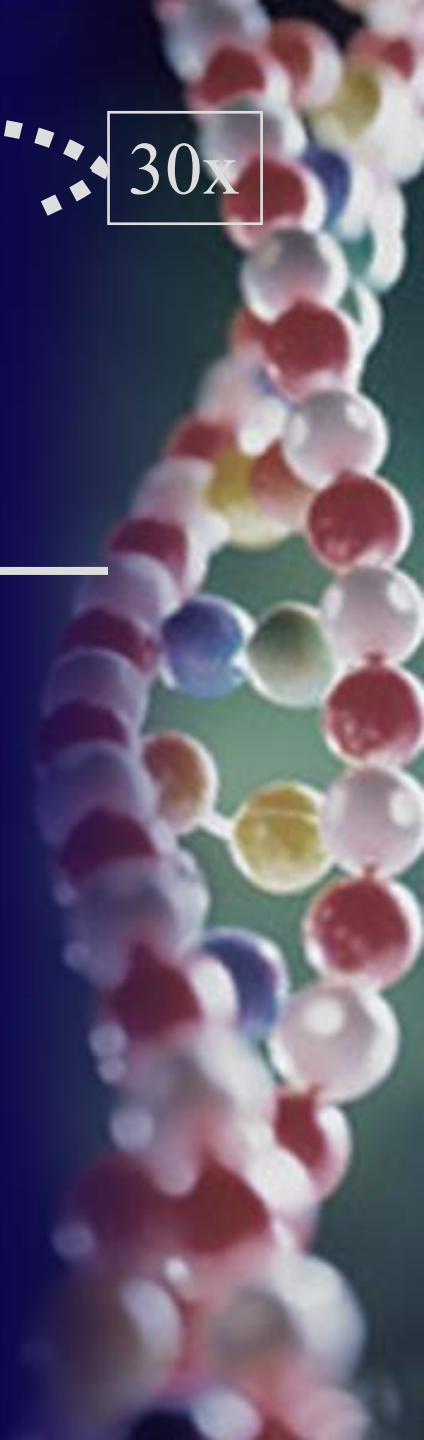


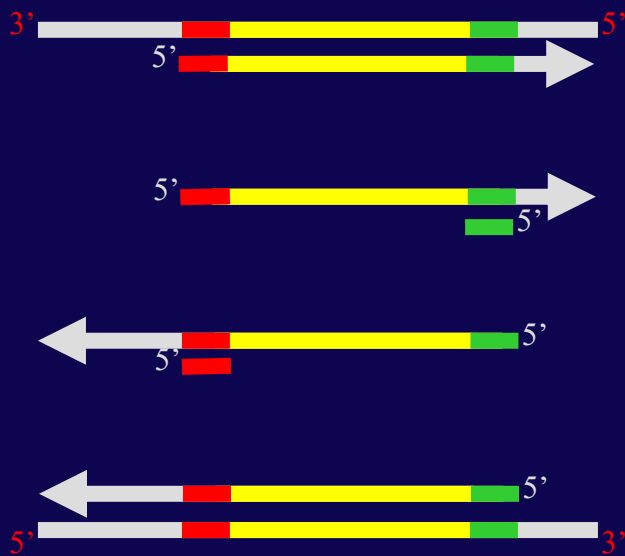
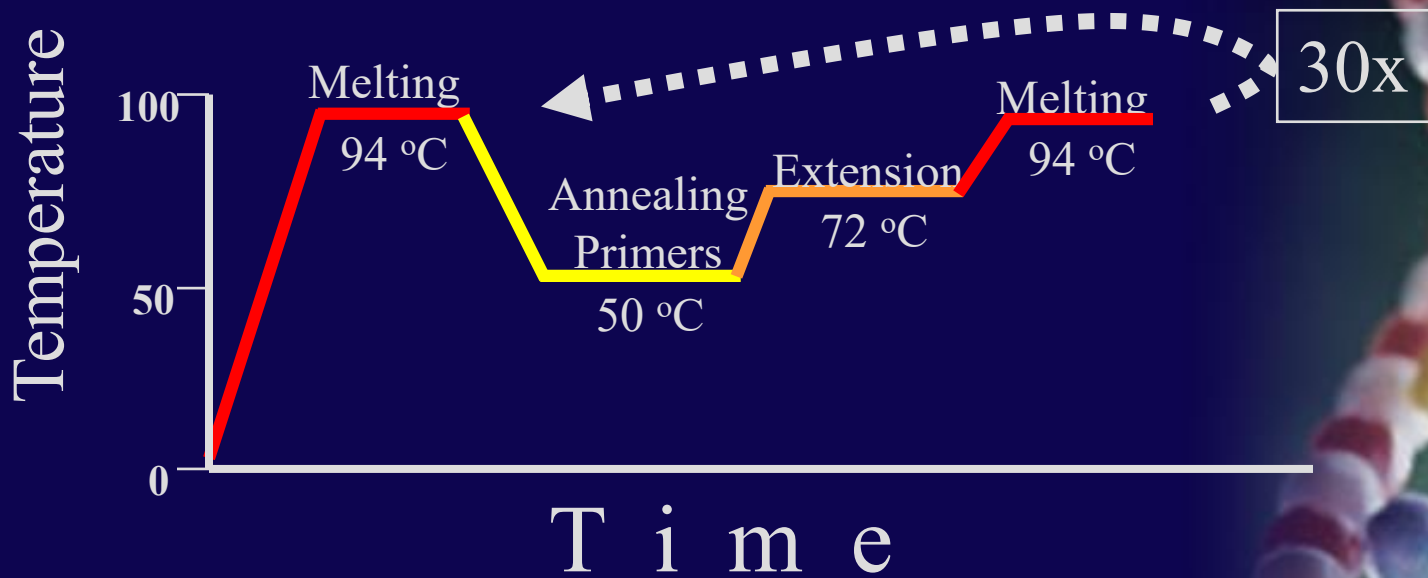
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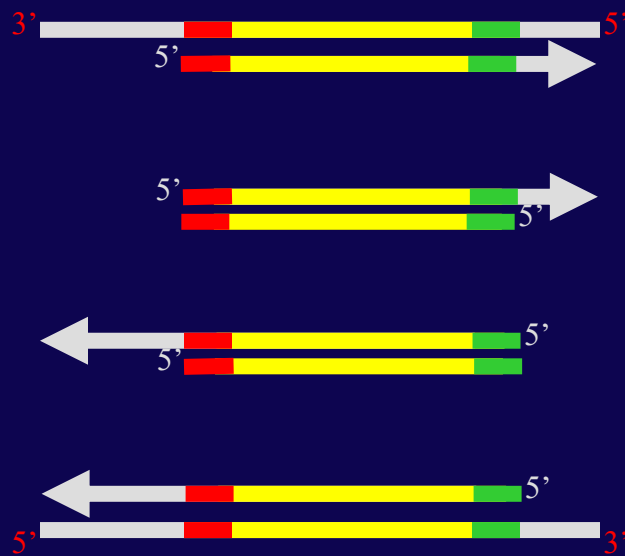
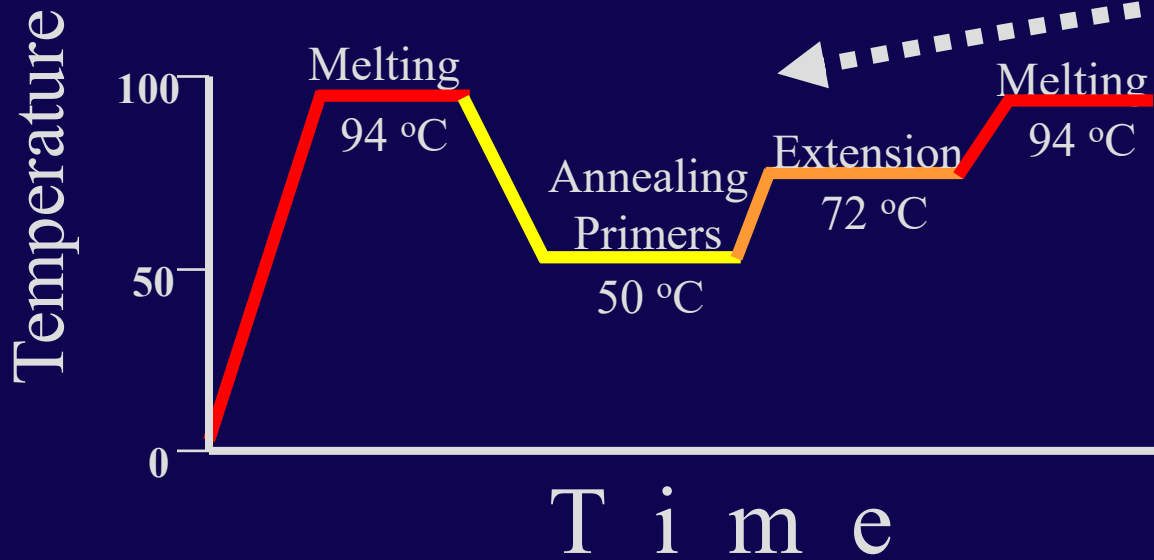
5' ———>

<—— 5'

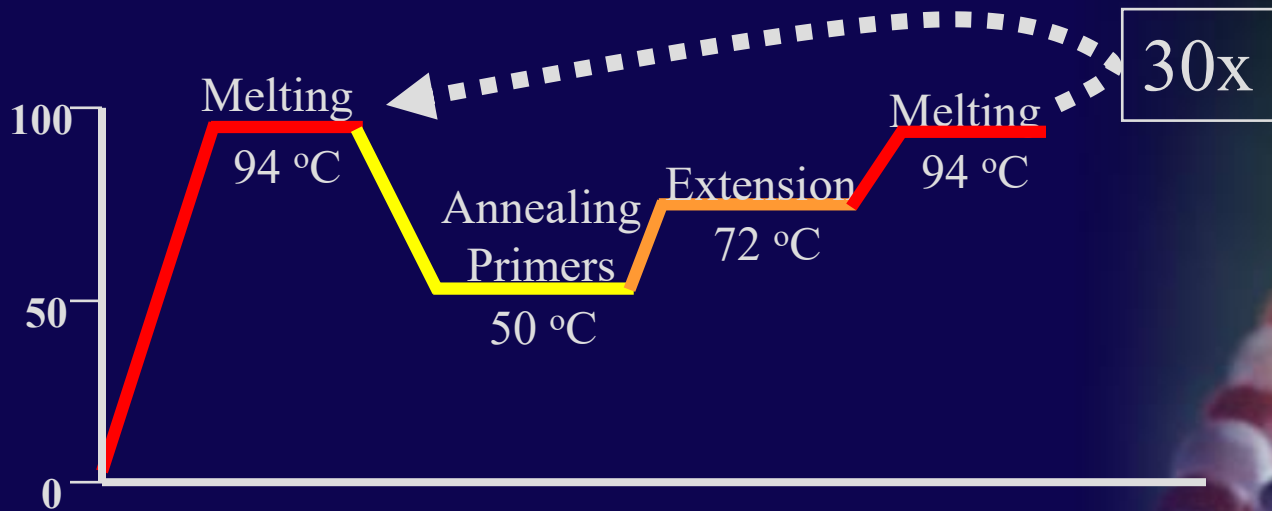
5' ——— 3'



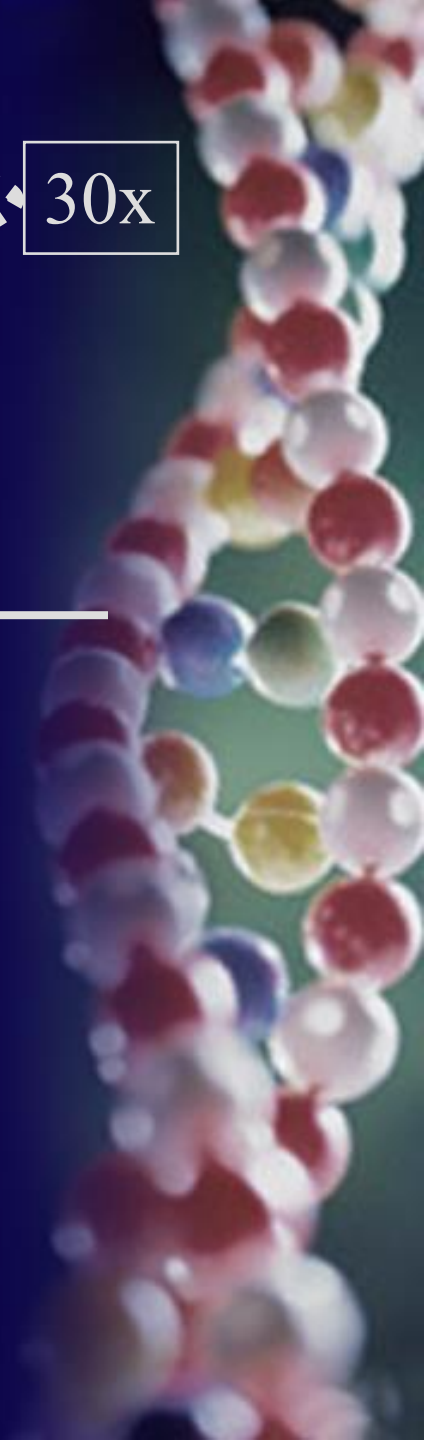
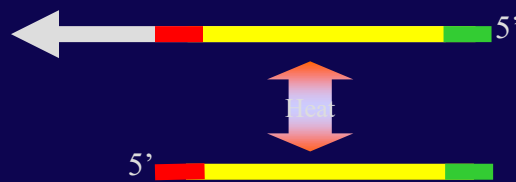
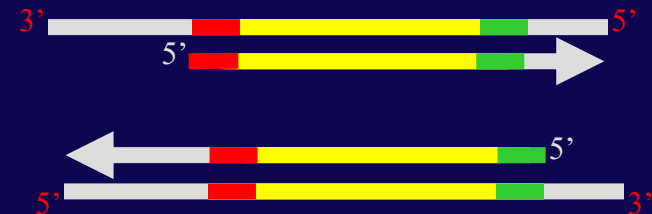




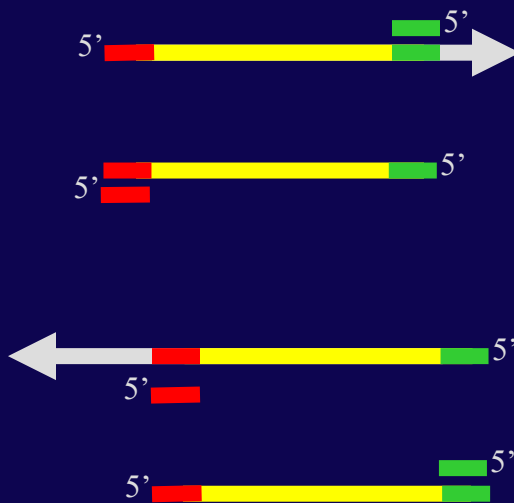
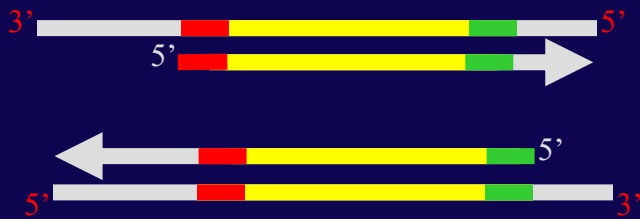
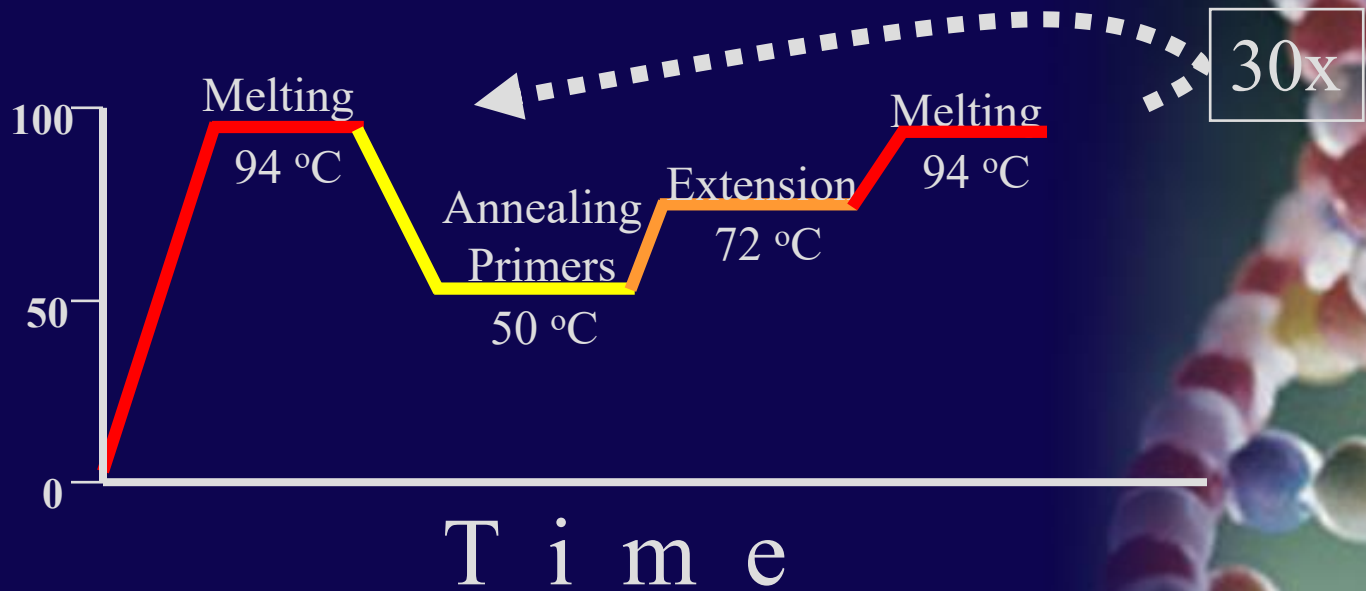
Temperature



Time



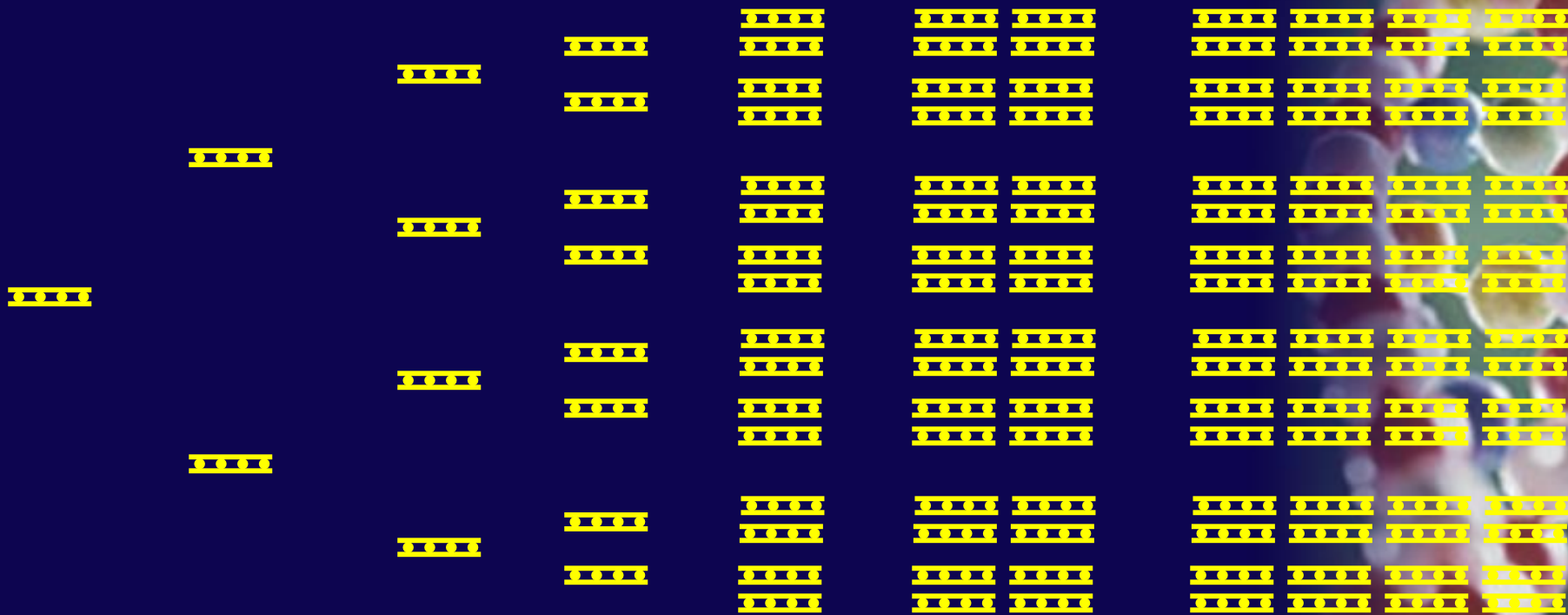
Temperature



DNA Between The Primers Doubles With Each Thermal Cycle

Number

1 2 4 8 16 32 64



0 1 2 3 4 5 6

Cycles

Theoretical Yield Of PCR

$$\text{Theoretical yield} = 2^n \times y$$

Where y = the starting
number of copies and
 n = the number of thermal cycles

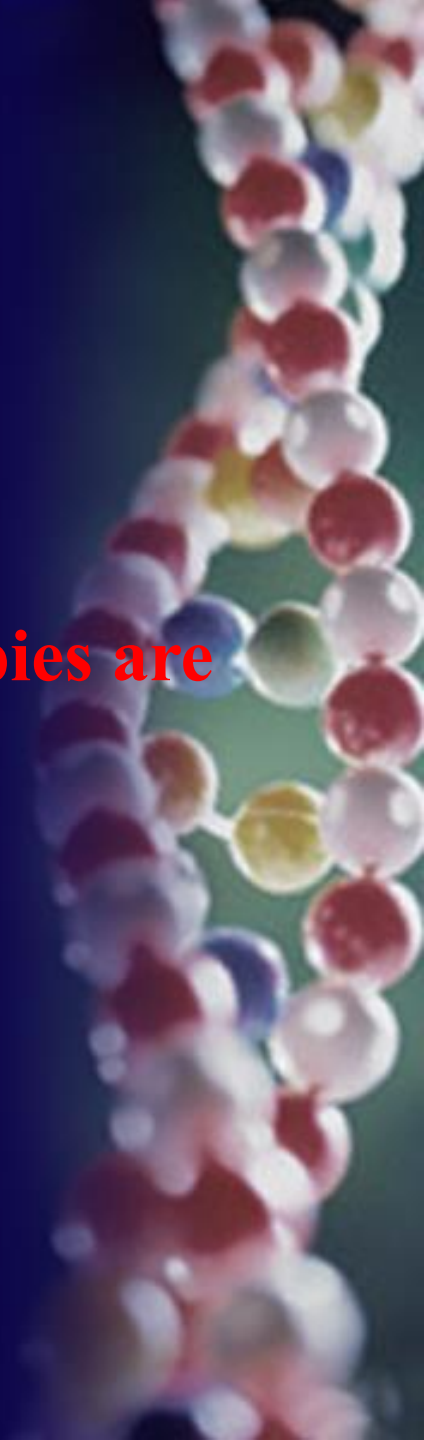
If you start with 100 copies, how many copies are
made in 30 cycles?

$$2^n \times y$$

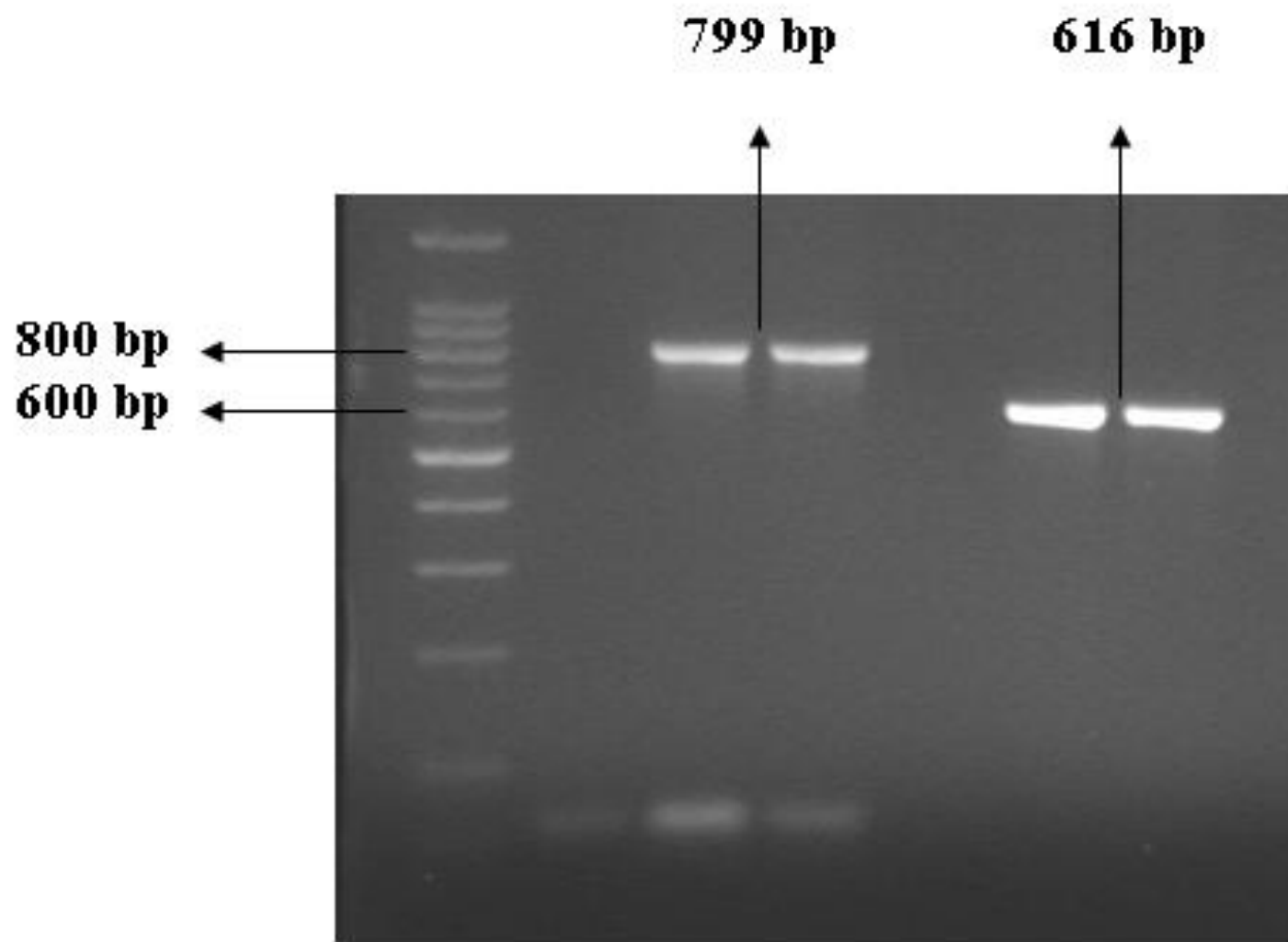
$$= 2^{30} \times 100$$

$$= 1,073,741,824 \times 100$$

$$= 107,374,182,400$$



PCR products





Pcr.exe

Stages of DNA Profiling

- **Step 2:**

The DNA is cut into fragments using **restriction enzymes**.

Each restriction enzyme cuts DNA at a specific base sequence.



Stages of DNA Profiling

- **The sections of DNA that are cut out are called restriction fragments.**
- **This yields thousands of restriction fragments of all different sizes because the base sequences being cut may be far apart (long fragment) or close together (short fragment).**



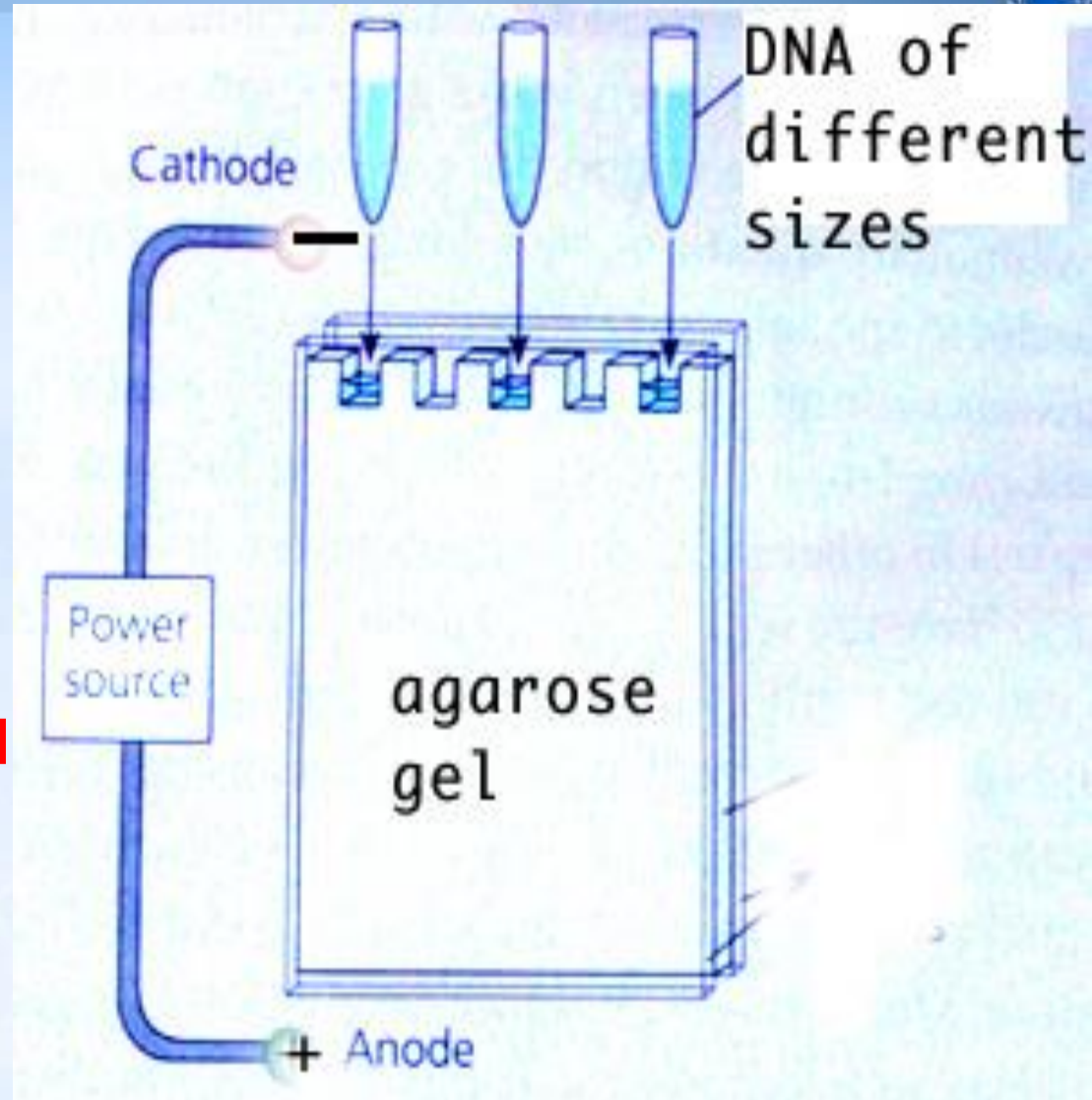
Mode of action of restriction enzymes?

Recombinant DNA.flv

Stages of DNA Profiling

Stage 3:

- Fragments are separated on the basis of size using a process called gel electrophoresis.
- DNA fragments are injected into wells and an electric current is applied along the gel.

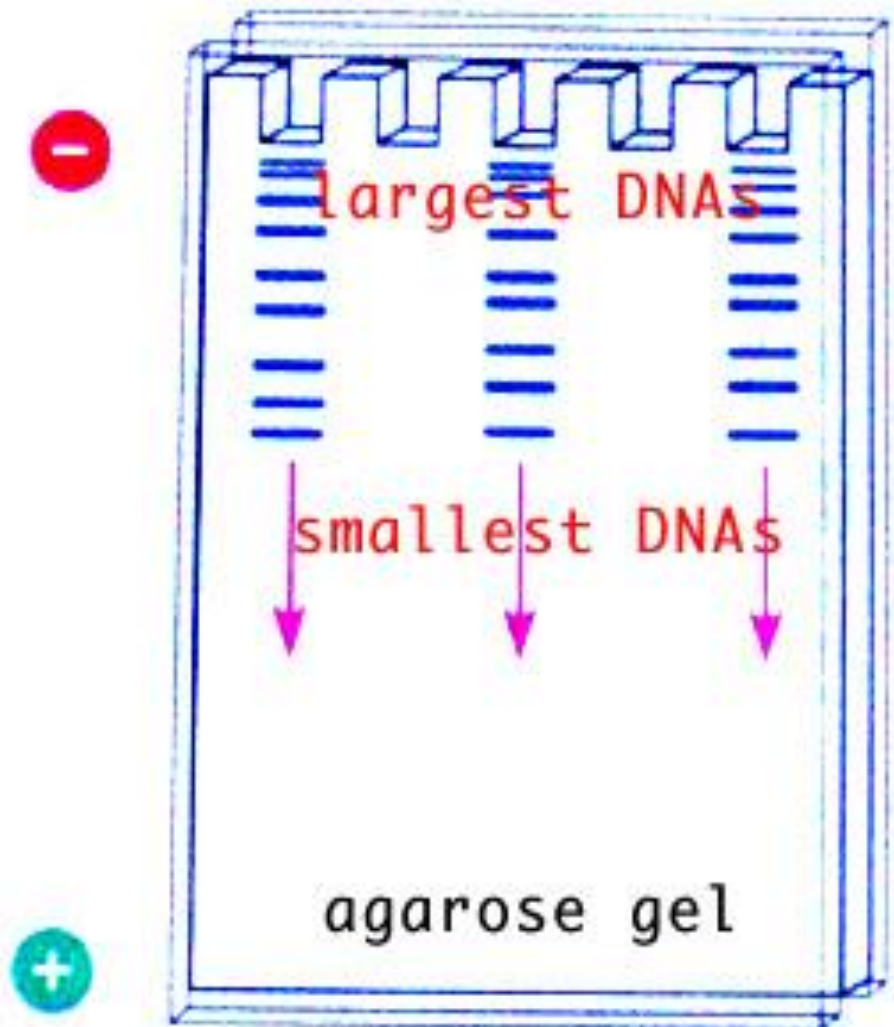


Stages of DNA Profiling

DNA is negatively charged so it is attracted to the positive end of the gel.

The shorter DNA fragments move faster than the longer fragments.

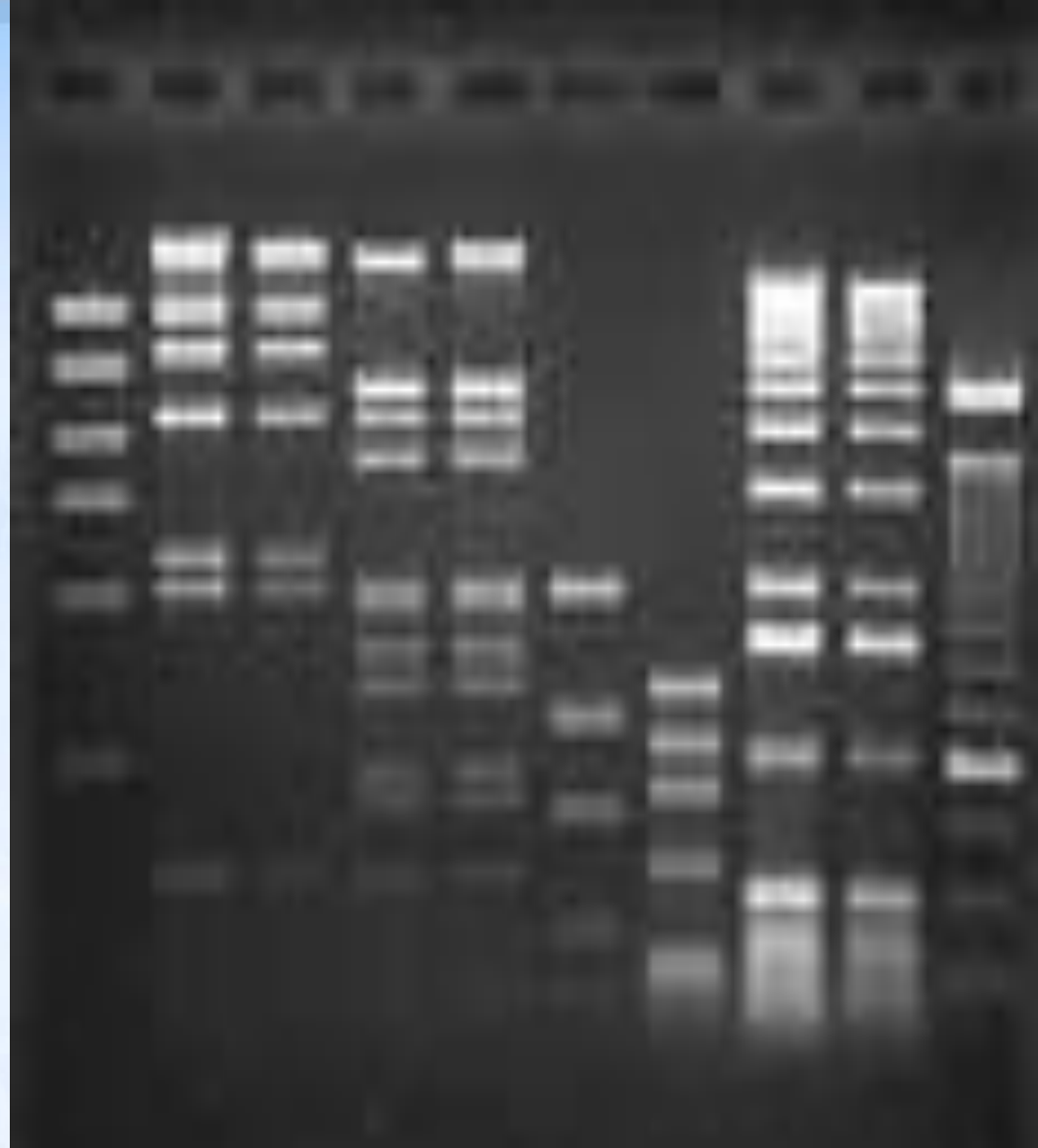
DNA is separated on basis of size.



Stages of DNA Profiling



- **A radioactive material is added which combines with the DNA fragments to produce a fluorescent image.**
- **A photographic copy of the DNA bands is obtained.**



Stages of DNA Profiling

Stage 4:

- **The pattern of fragment distribution is then analysed.**

APPLICATIONS

- **Most notable applications besides profiling include:**
- **DNA fingerprinting**
- **Paternity testing**
- **Maternity testing**
- **Race horse and rare wildlife species identity**
- **General crime cases (burglary, sexual offences)**

- **Forensic DNA profiling may be grouped into:-**

- **1-Autosomal STR typing**
- **2-Y-STR haplotyping**
- **3-Mitochondrial DNA testing**

Steps in DNA Sample Processing

Sample Obtained from
Crime Scene or Paternity
Investigation

DNA
Extraction



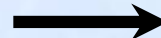
DNA
Quantitation



PCR Amplification
of Multiple STR
markers

Technology

Separation and Detection of
PCR Products
(STR Alleles)



Sample Genotype
Determination

Genetics

Comparison of Sample
Genotype to Other
Sample Results

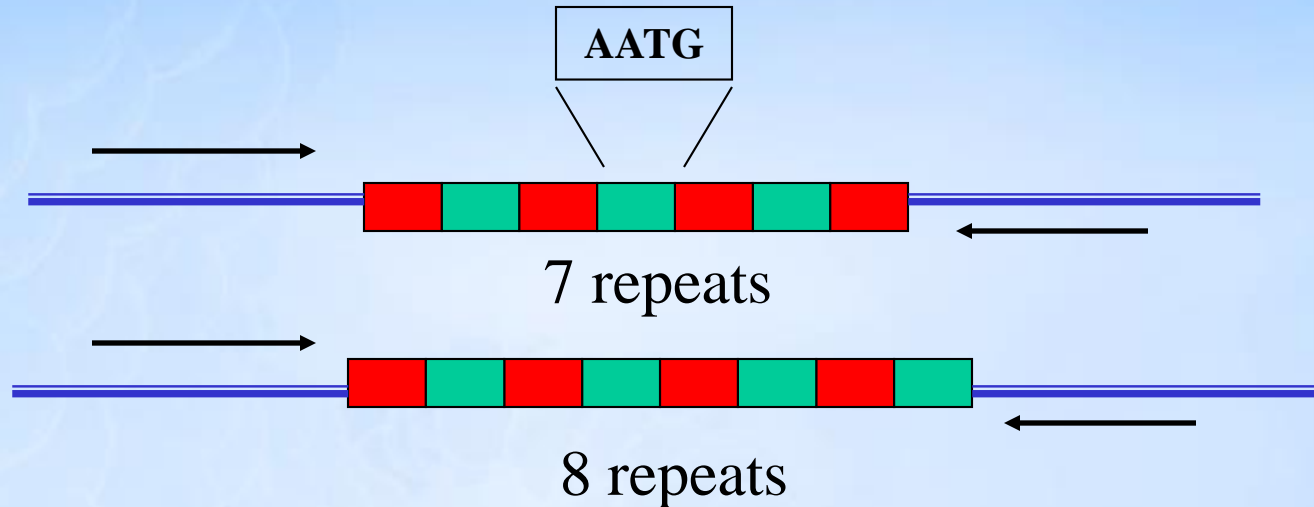
Generation of Case
Report with Probability
of Random Match

If match occurs, comparison
of DNA profile to population
databases

Short Tandem Repeats (STRs)

- The human genome is full of repeated DNA sequences. These repeated sequences come in various sizes and are classified according to the length of the core repeat units, the number of contiguous repeat units, and/or the overall length of the repeat region. DNA regions with short repeat units (usually 2-6 bp in length) are called Short Tandem Repeats (STR).

Short Tandem Repeats (STRs)

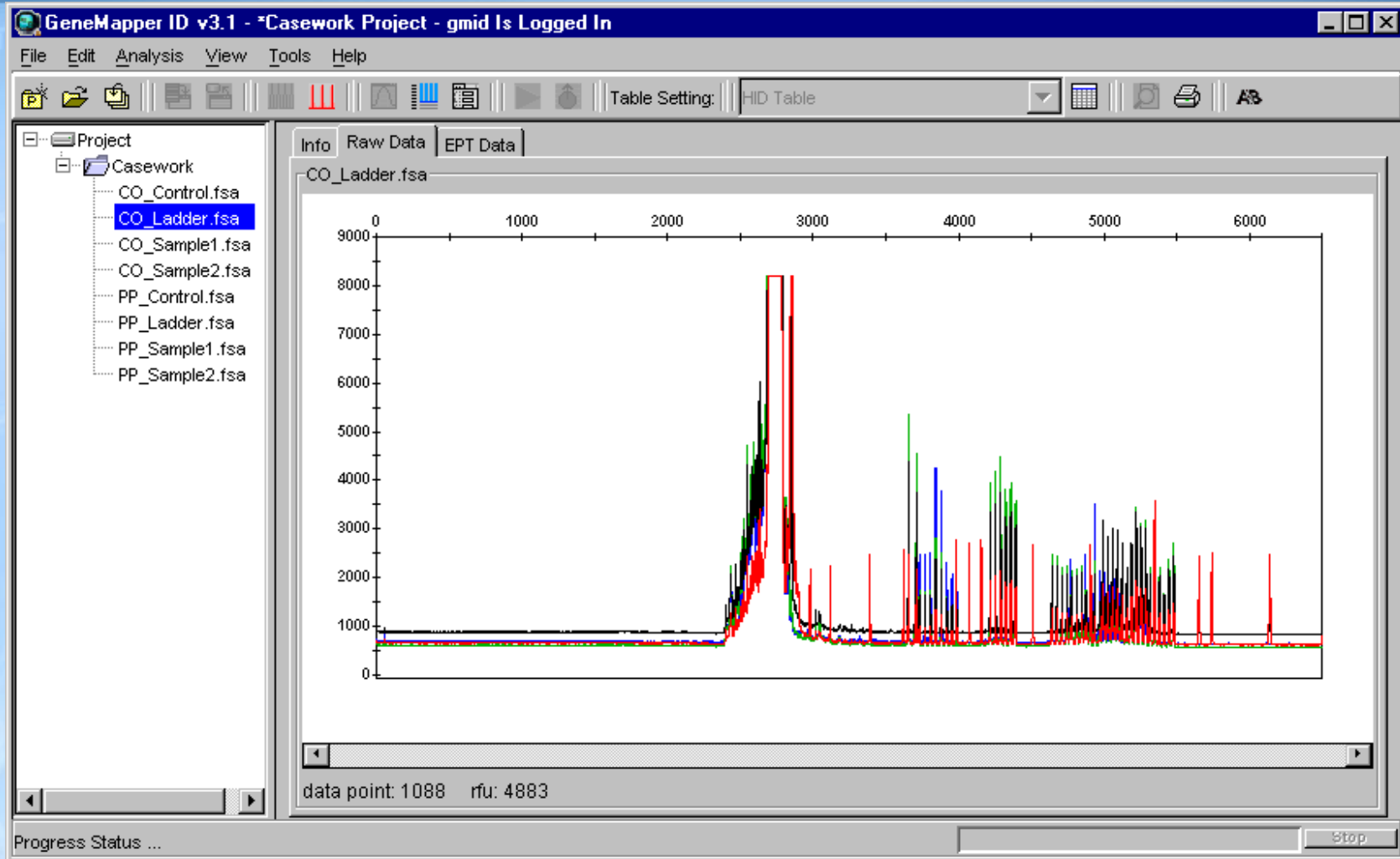


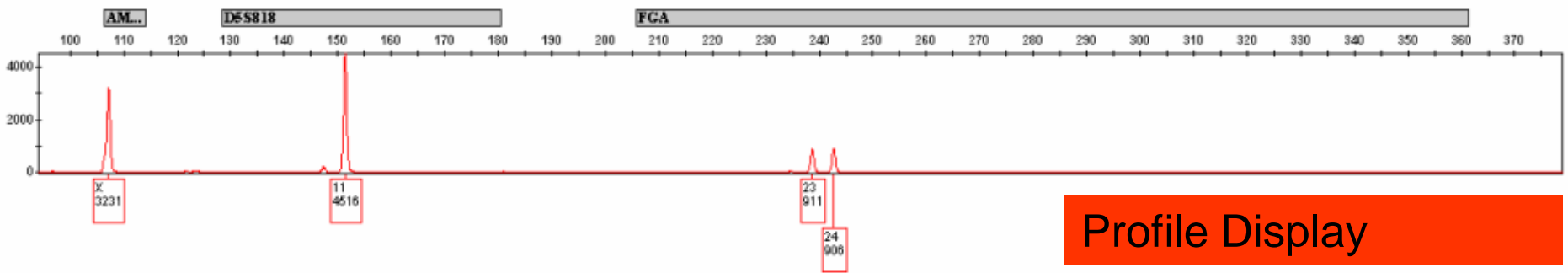
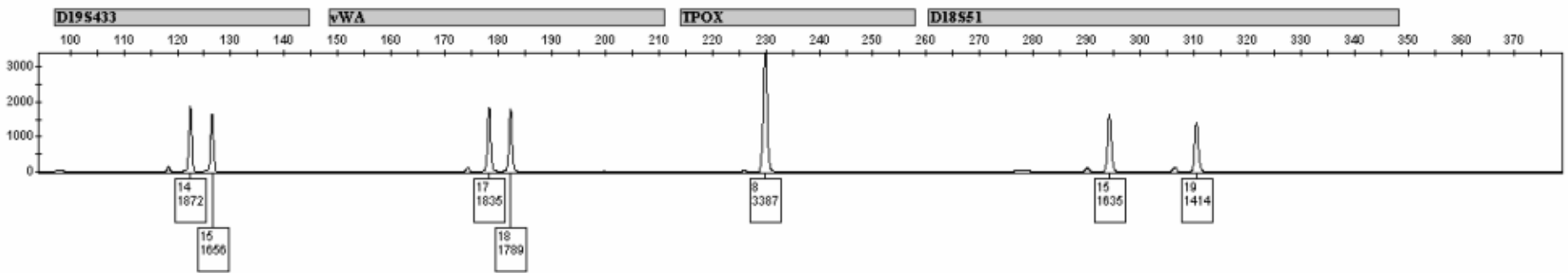
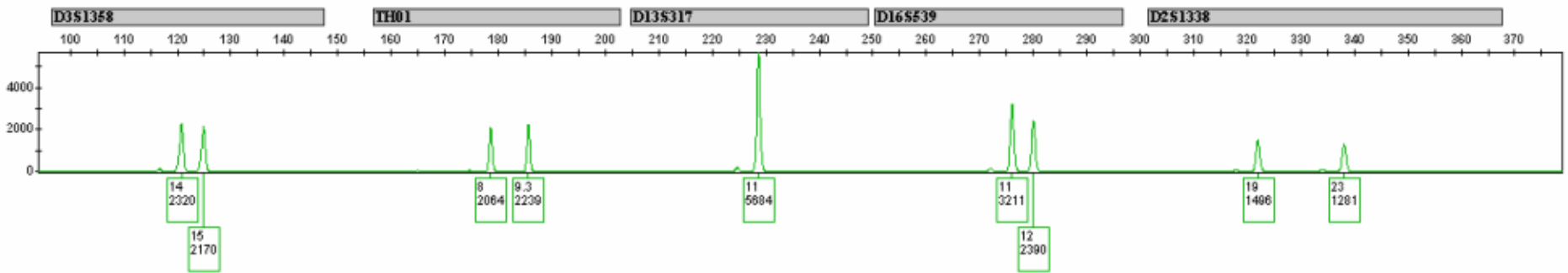
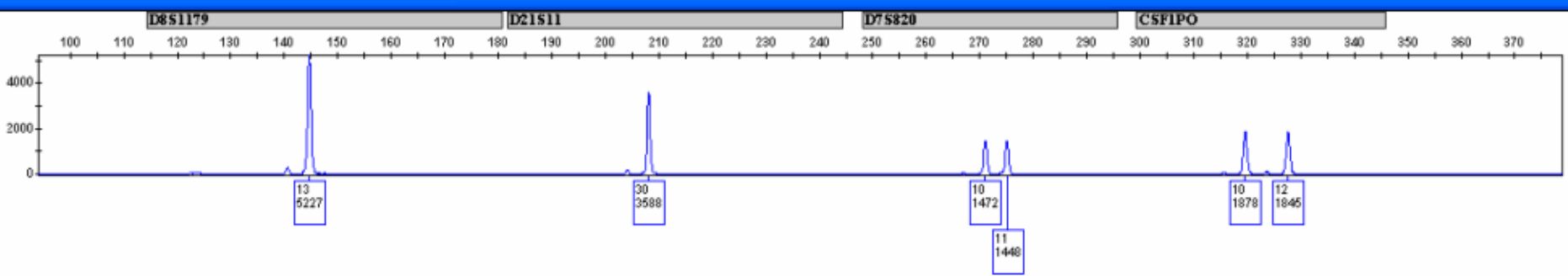
the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

Electropherogram





Profile Display

Item Description Locus	Item 1 Bone sample	Item 2 Reference Sample
D8S1179	12,14	12,14
D21S11	29,30	29,30
D7S820	10,10	10,10
CSF1P0	11,13	11,13
D3S1358	15,18	15,18
TH01	6,9	6,9
D13S317	13,13	13,13
D16S539	12,15	12,15
D2S1338	22,25	22,25
D19S433	14,14	14,14
VWA	18,19	18,19
TPOX	8,9	8,9
D18S51	16,19	16,19
Amelogenin	X,Y	X,Y
D5S818	11,13	11,13
FGA	22,22	22,22

Uses of DNA Profiling

- **DNA profiling is used to solve crimes and medical problems**



Crime

- Forensic science is the use of scientific knowledge in legal situations.
- The DNA profile of each individual is highly specific.
- The chances of two people having exactly the same DNA profile is **30,000 million to 1 (except for identical monozygous twins)**.
- **In case dizygous twin, single nucleotide polymorphism (SNP) technique instead of DNA fingerprinting is used.**

DNA Profiling can solve crimes

- The pattern of the DNA profile is then compared with those of the victim and the suspect.
- If the profile matches the suspect it provides strong evidence that the suspect was present at the crime scene
- If the profile doesn't match the suspect then that suspect may be eliminated from the enquiry.

Example

- **A violent murder occurred.**
- **The forensics team retrieved a blood sample from the crime scene.**
- **They prepared DNA profiles of the blood sample, the victim and a suspect as follows:**

Was the suspect at the crime scene?

Suspects
Profile

=====

=====

Blood sample
from crime
scene

=====

=====

Victims
profile

=====

=====

Solving Medical Problems

DNA profiles can be used to determine **whether a particular person is the parent of a child.**

A Childs **paternity** (father) and **maternity** (mother) can be determined.

This information can be used in:-

- Paternity suits
- Inheritance cases
- Immigration cases

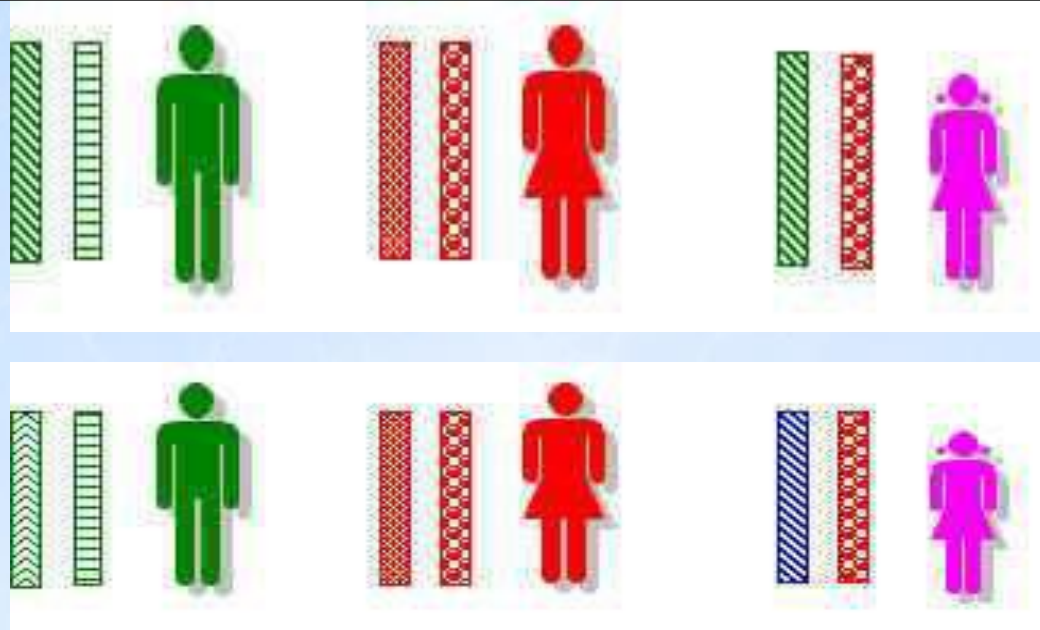
Example: A Paternity Test

- DNA is inherited from our parents, with half coming from the mother, and the other half from the father.
- Paternity test allows the opportunity where the mother of the child is not disputed, to produce a putative DNA profile of the biological father
- **MATCH SCENARIO**
- **No MATCH SCENARIO**
- By comparing the DNA profile of a mother and her child it is possible to identify DNA fragments in the child which are absent from the mother and must therefore have been inherited from the biological father.

PATERNITY TEST

The two possibilities are diagrammed briefly below:

CASE 1: Possibility of Paternity



CASE 2: Exclusion of Paternity

Famous cases

- In 2002 Elizabeth Hurley used DNA profiling to prove that Steve Bing was the father of her child Damien

