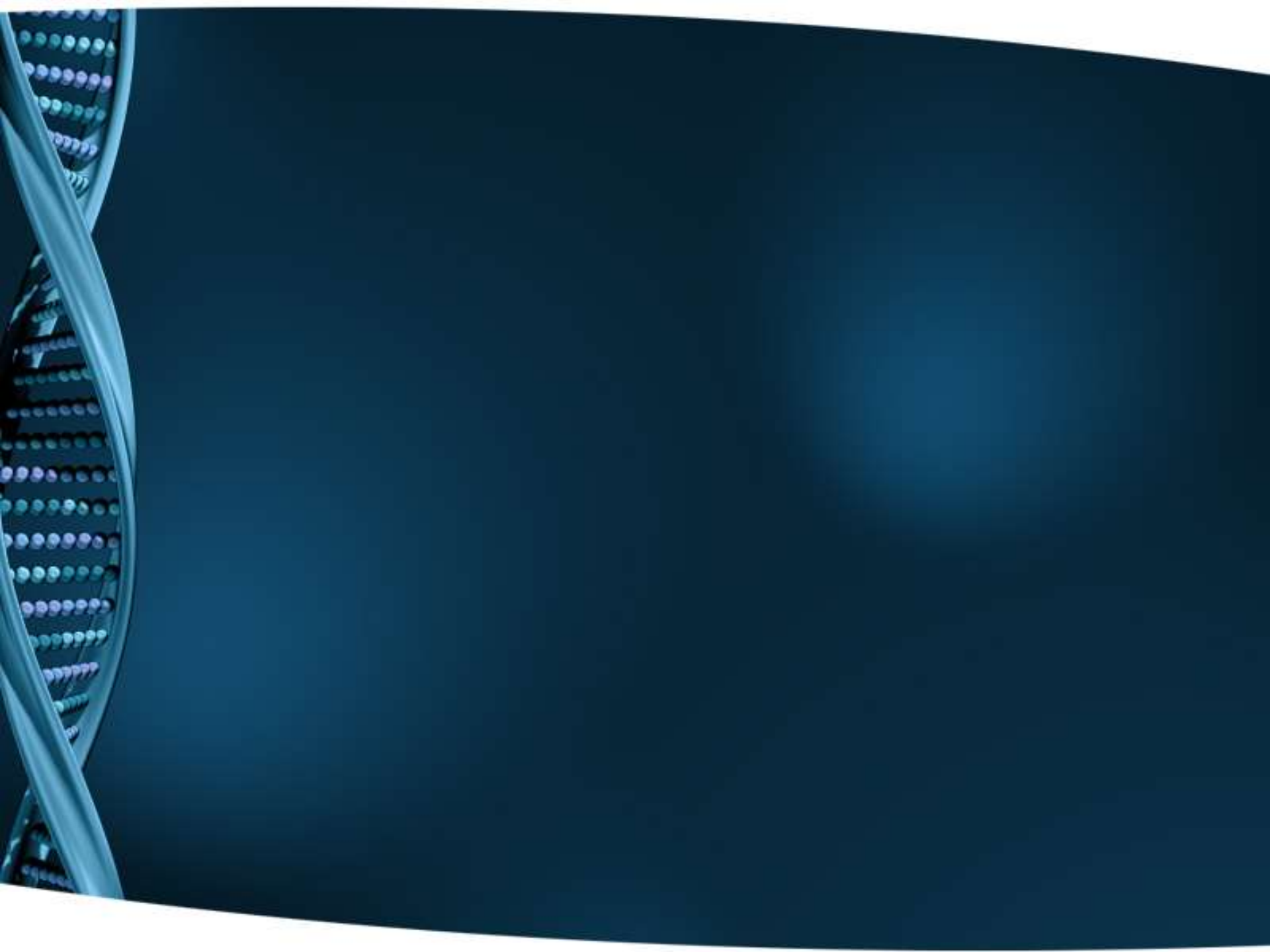

DNA AMPLIFICATION
USING
PCR TECHNOLOGY

Polymerase Chain Reaction
PCR

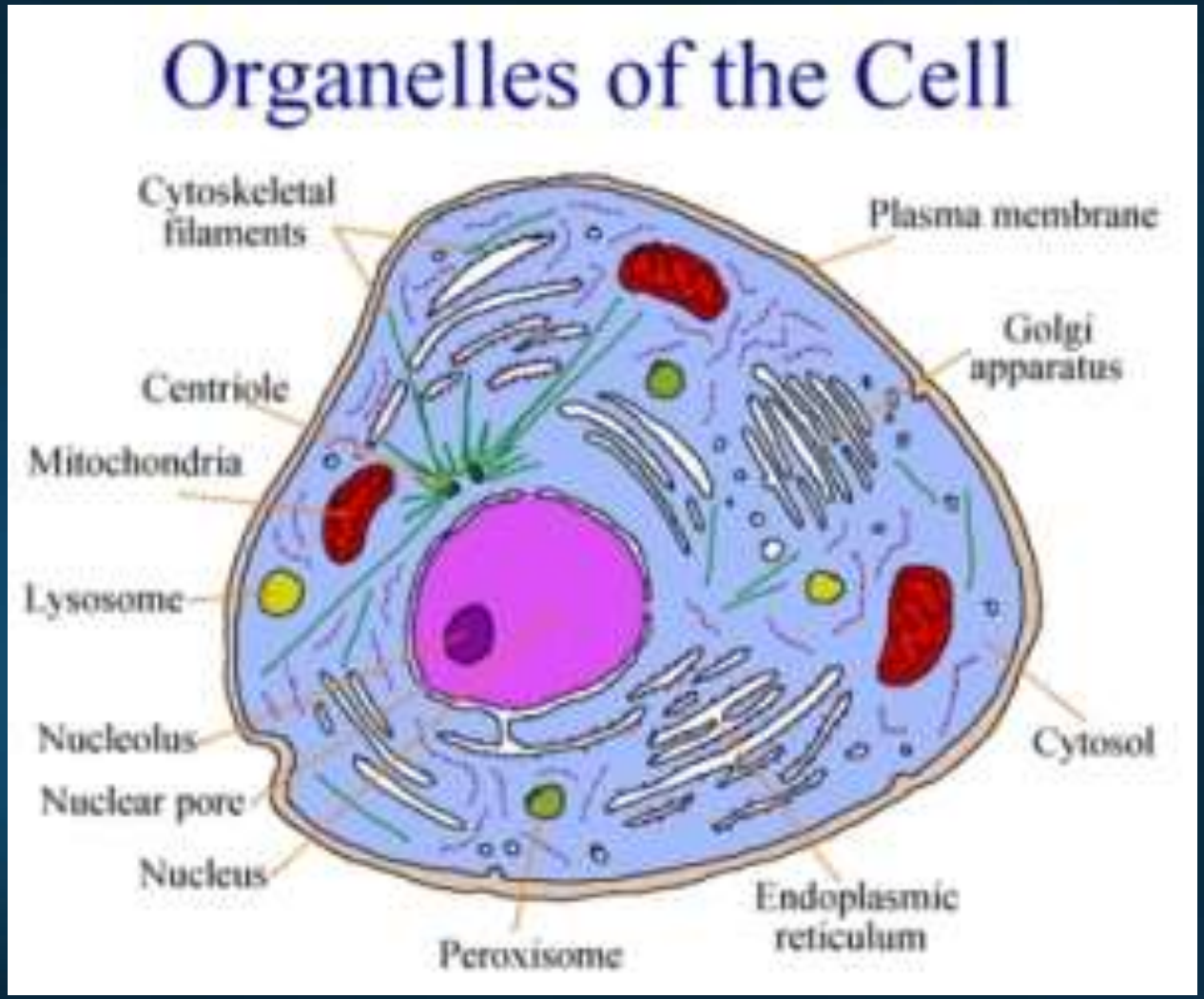
- PCR is an enzymatic process in which a specific region of DNA is replicated over and over again to yield many copies of a particular sequence .
- This molecular process involves heating and cooling samples in a precise thermal cycling pattern over about 30 cycles .
- During each cycle , a copy of the target DNA sequence is generated for every molecule containing the target sequence .

PCR Application

1.	Research Areas	DNA sequencing , mutation analysis genetic identification and adjustment of DNA sequence & protein structure.
2.	Clinical diagnoses	Bacteria ,Virus , Parasite ,Human hereditary diseases .
3.	Immunology	HLA locus typing , immune body gene mapping .
4.	Human genome project	construction of physical map .
5.	Forensic	specimen analysis .
6.	Oncology	GIT cancers , lung cancer thyroid cancer and leukemia
7.	Biology	diagnosis of animal hereditary disease and detection of plant pathogens.

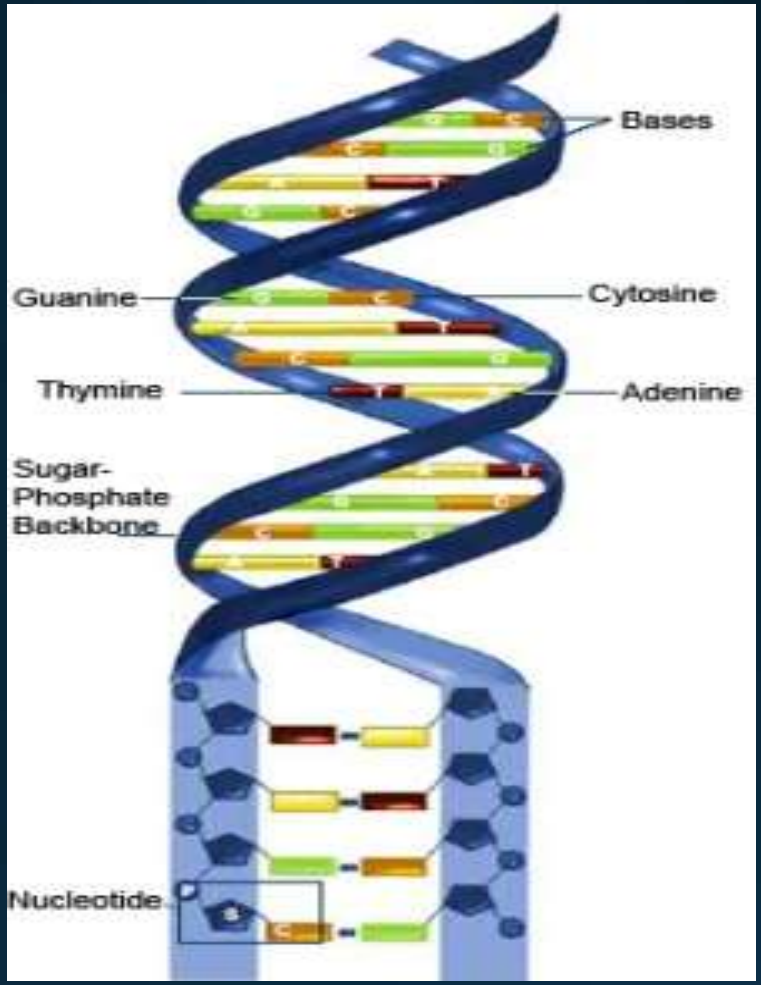


Organelles of the cell



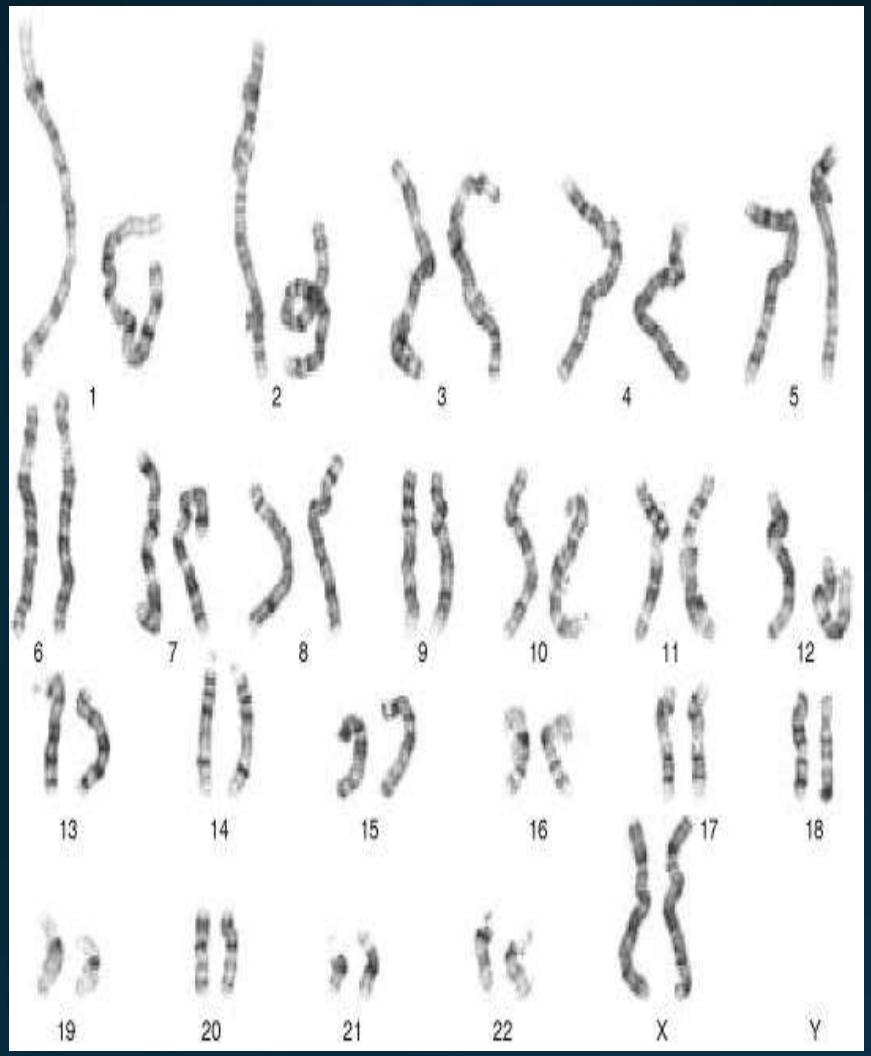
DNA
(Deoxyribose Nucleic Acid)

- In eukaryotes individual DNA molecules are found in the chromosomes of the nucleus and in mitochondria.
- They are large polymers, with a linear backbone of alternating sugar and phosphate residues.
- Nucleotide, is the basic repeat unit of a DNA strand .



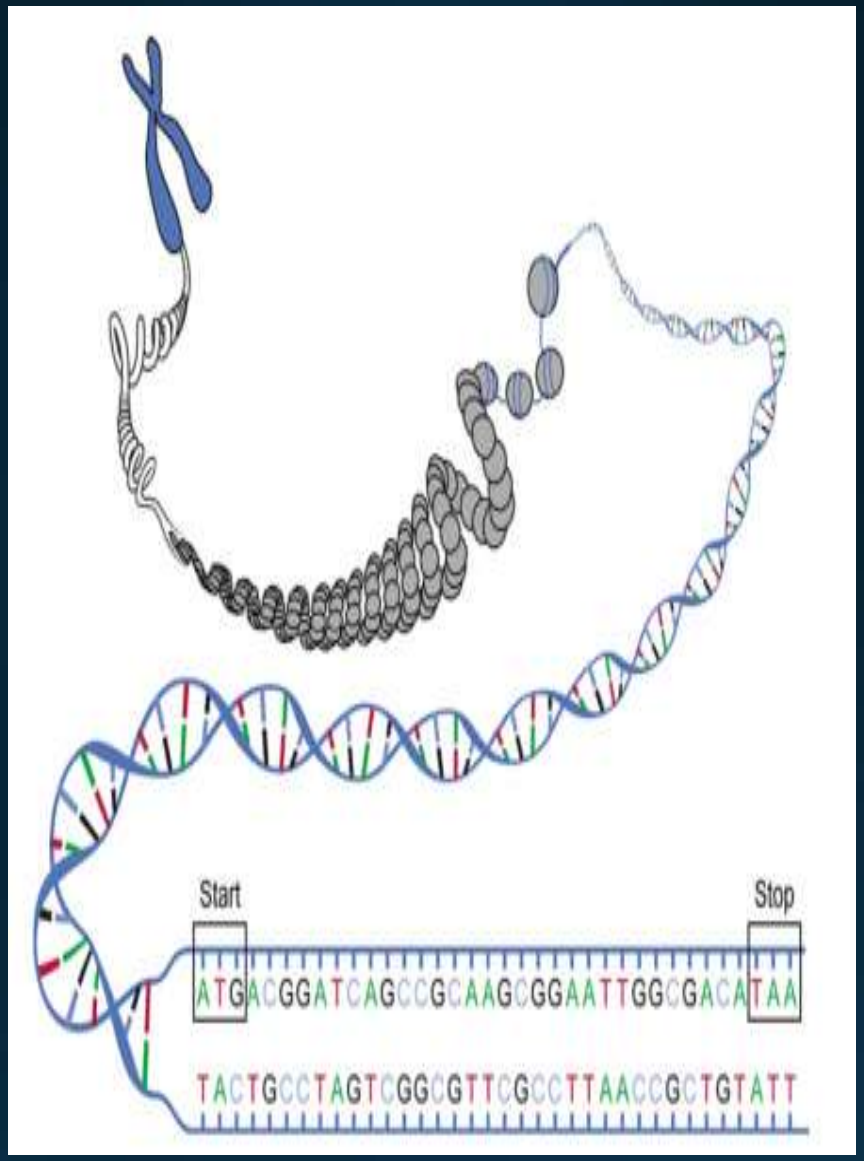
Chromosomes

- Chromosomes are the instruction set for the design, building, and maintenance of each individual.
- Every copy of the human genome has two copies of 23 chromosomes. This means we have 2 x 23 = 46 chromosomes total.
- chromosomes 1-22 are called autosomes.
- Chromosomes X and Y are called sex chromosomes.



Genes

- A gene is a distinct stretch of DNA that determines something about who you are. it is a region of DNA that controls a discrete hereditary characteristic.
- Genes can be located on chromosomes in the nucleus or on mitochondrial DNA.
- Every person has two copies of each gene, one inherited from each parent.
- Most genes are the same in all people, but a small number of genes (less than 1 percent of the total) are slightly different between people.
- Alleles are forms of the same gene with small differences in their sequence of DNA nucleotides. These small differences contribute to each person's unique physical features.



Steps Of DNA Amplification Techniques

- Selection of the representative sample .
- DNA extraction.
- DNA quantification.
- PCR Amplification.
- DNA analysis.

DNA EXTRACTION

Aim & fundamentals of extraction

- Efficient extraction .
- Extracting enough amount of DNA.

Any extraction method involve three steps:

- Disruption of cellular membranes .
- Protein denaturation.
- Separation of DNA from the denatured protein and other cellular components.


DNA QUANTIFICATION

DNA QUANTIFICATION

- To produce the best quantity results in a shortest time , we must add the correct amount of DNA to a PCR.
- PCR work optimally with a DNA range of (0.05-0.125) ng/ul .
- Liquid blood contains 5000-10 000 nucleated blood cell per milliliter.
- Each nucleated cell contains approximately 6 pg of DNA , and 15 nucleated cells are enough for obtaining a good amount of DNA

DNA QUNTIFICATION METHODS

- Ultraviolet spectrophotometery.
- Agarose gel electrophoreses .
- Real time PCR

DNA AMPLIFICATION USING PCR

Components in PCR

- PCR buffer.
- Magnesium chloride.
- Dntps (A,T,C,G).
- Taq DNA polymerase
- Primers .
- Template DNA

- 10-50 mM
- 1.2-2.5 mM
- 200 uM each

- 0.5-5 UM

- 0.1-1.0 uM
- 1-10 ng genomic DNA

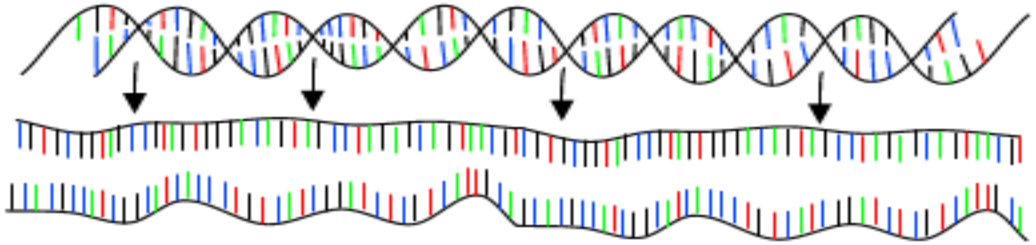
PCR STEPS

There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

1. **Denaturation** at 94°C :
   During the denaturation, the double strand open to single stranded DNA, all enzymatic reactions stop.
2. **Annealing** at 54°C :
   The primers are annealed to the single stranded DNA template. The polymerase can attach and starts copying the template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer, that it does not break anymore.
3. **extension** at 72°C :
   This is the ideal working temperature for the polymerase. The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, bases are added complementary to the template).

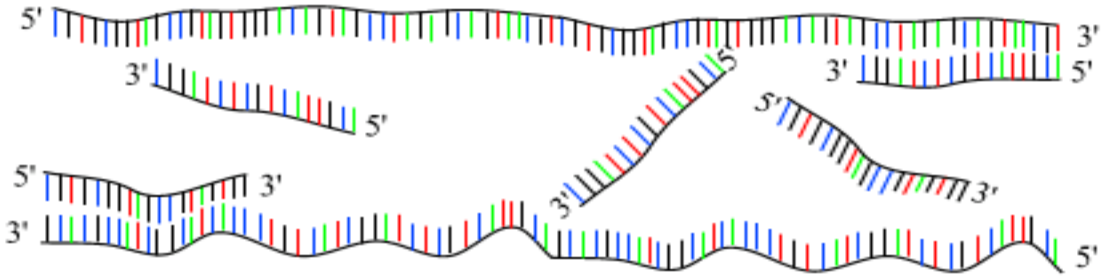
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

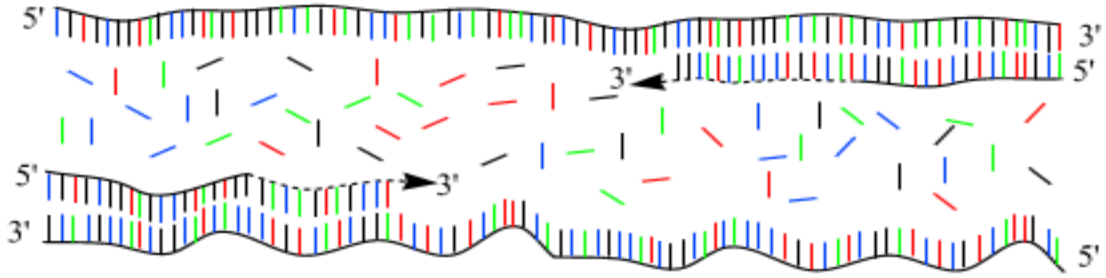
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

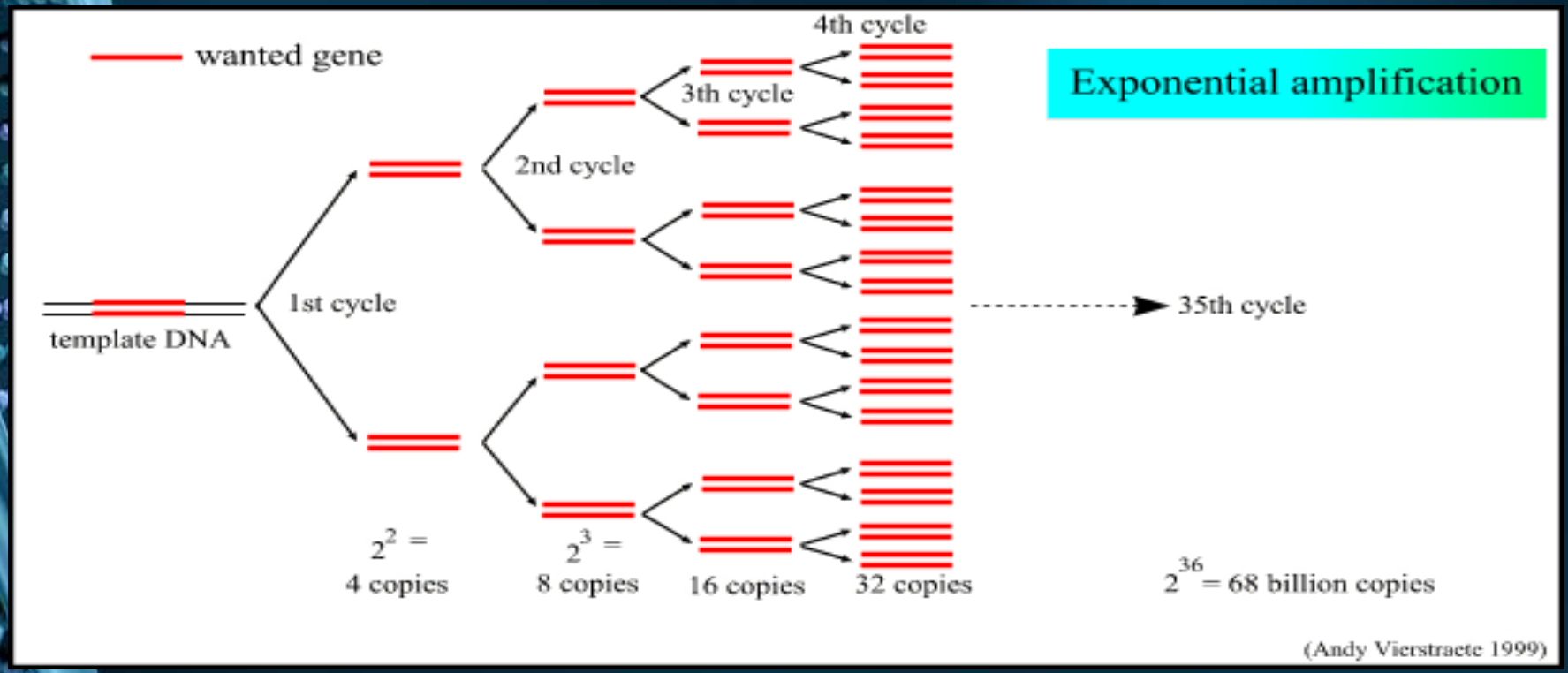
2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

PCR cycles

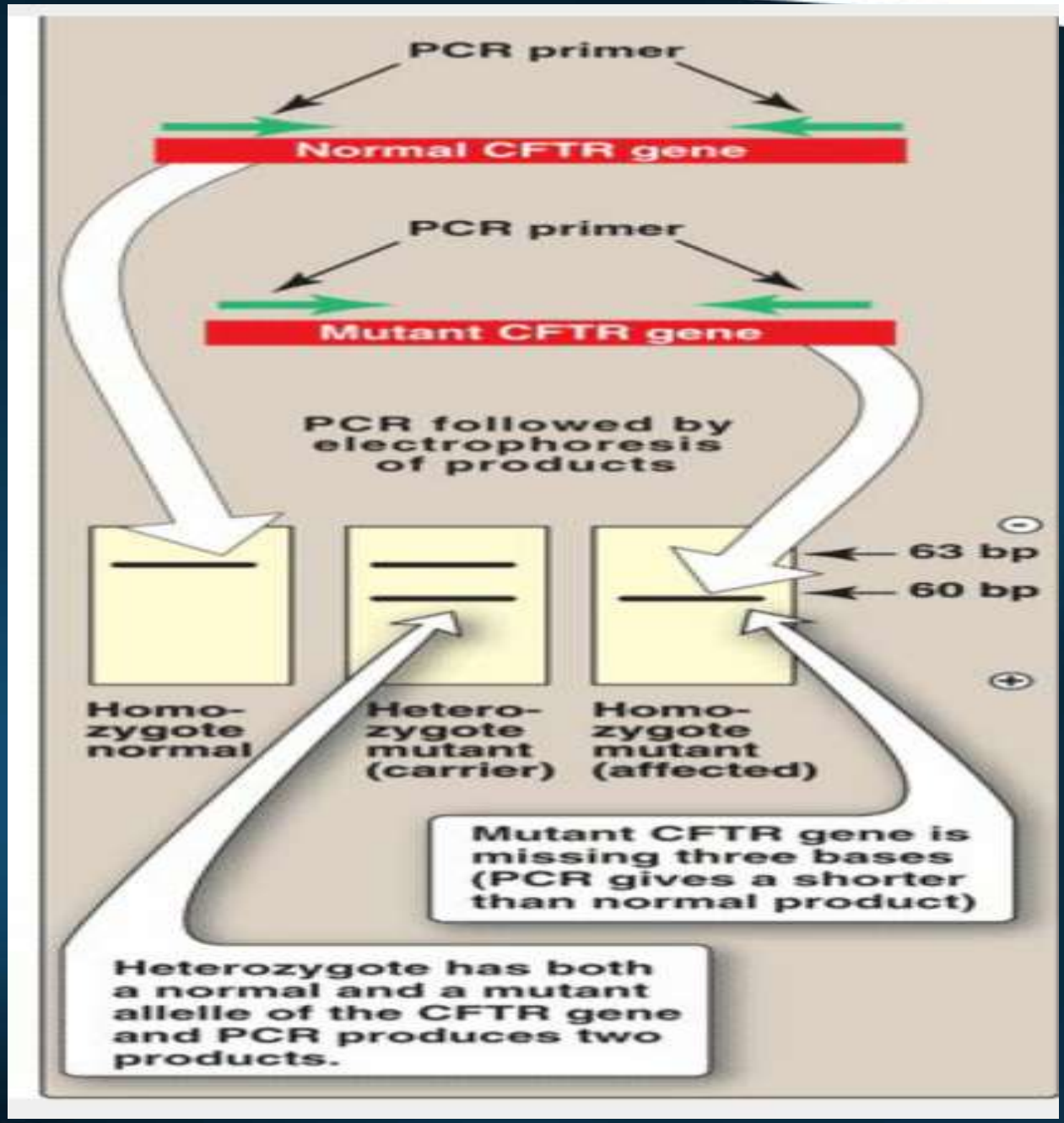
Because both strands are copied during PCR, there is an **exponential** increase of the number of copies of the gene. Suppose there is only one copy of the wanted gene before the cycling starts, after one cycle, there will be 2 copies, after two cycles, there will be 4 copies, three cycles will result in 8 copies and so on.

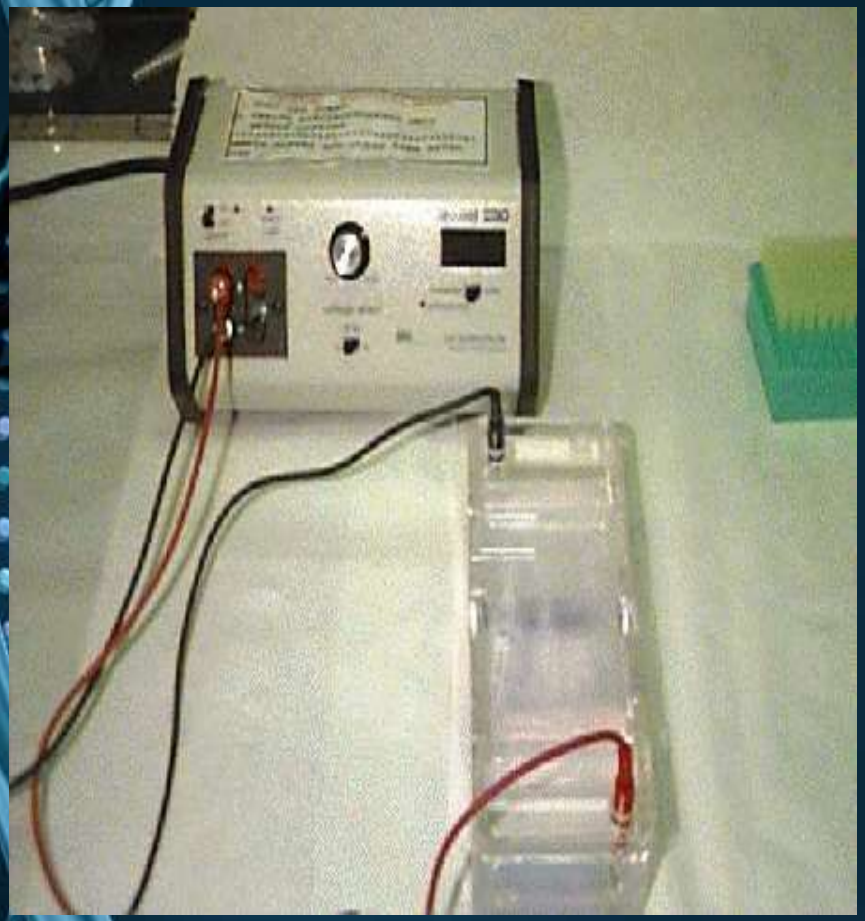


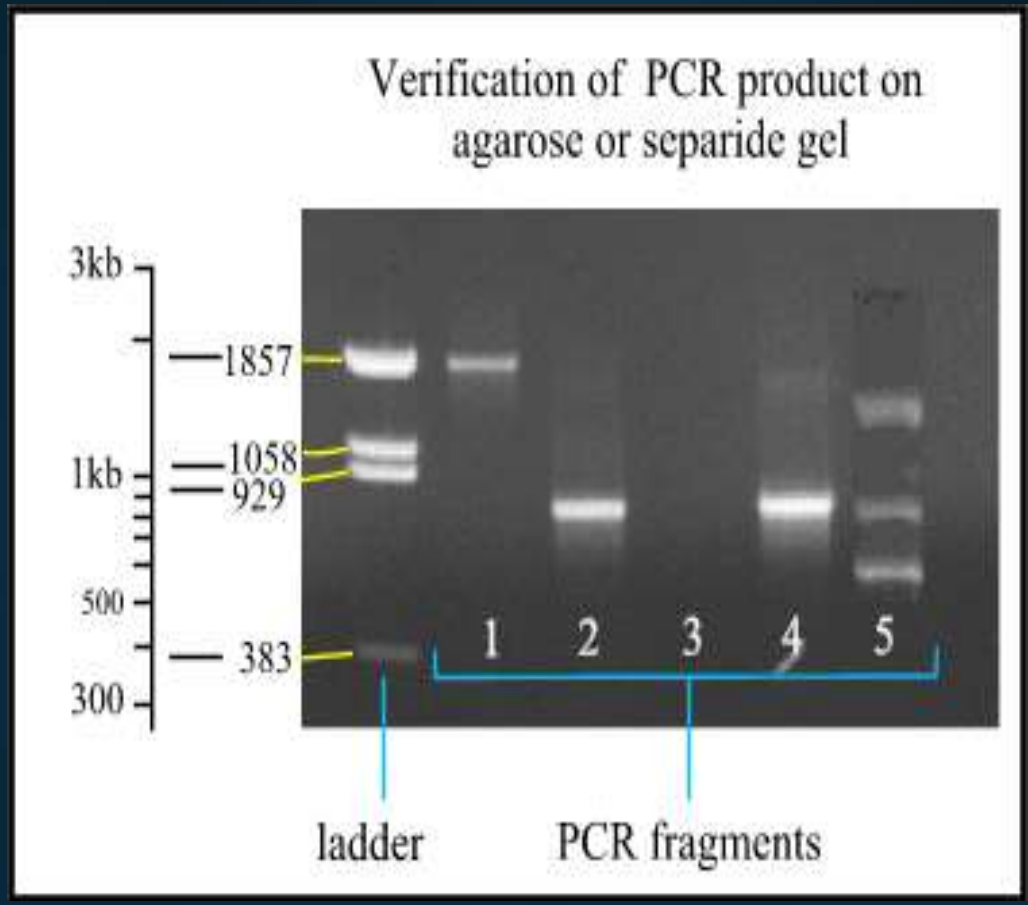

DNA ANALYSIS

DNA ANALYSIS METHODS

- Gel electrophoresis.
- Genetic analyzer.


PCR primer
Normal CFTR gene
PCR primer
Mutant CFTR gene
PCR followed by electrophoresis of products
63 bp
60 bp
Homo-zygote normal
Hetero-zygote mutant (carrier)
Homo-zygote mutant (affected)
Mutant CFTR gene is missing three bases (PCR gives a shorter than normal product)
Heterozygote has both a normal and a mutant allelle of the CFTR gene and PCR produces two products.




Verification of PCR product on agarose or separide gel
3kb
1kb
500
300
1857
1058
929
383
1
2
3
4
5
ladder
PCR fragments

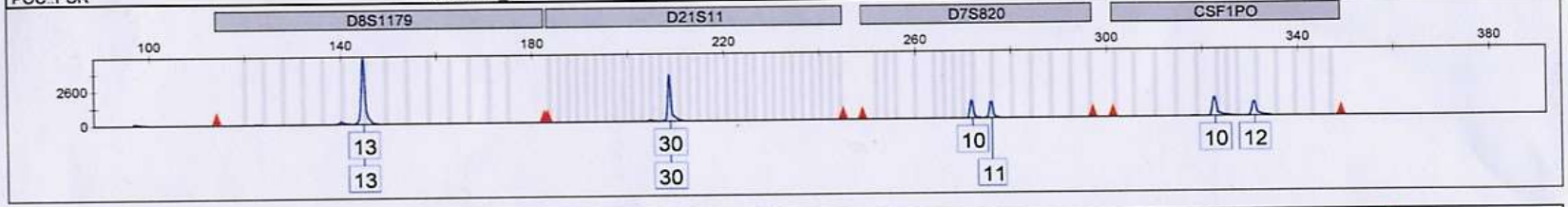
Genetic analyzer (Capillary electrophoresis)

Applied Biosystems

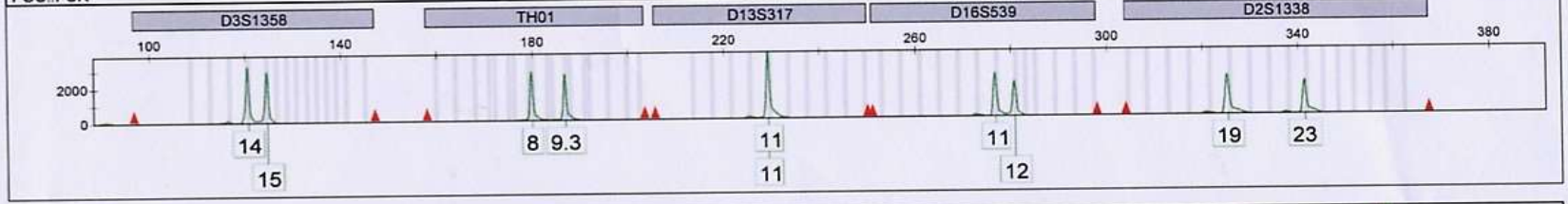
GeneMapper ID v3.2

020309PATERNATY

Sample Name	Panel	SQO	SQ
POC..PCR	Identifiler_v1		



POC..PCR Identifiler_v1



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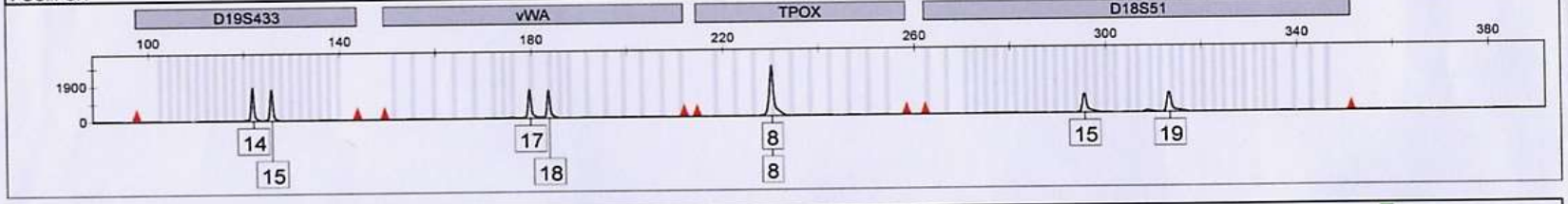

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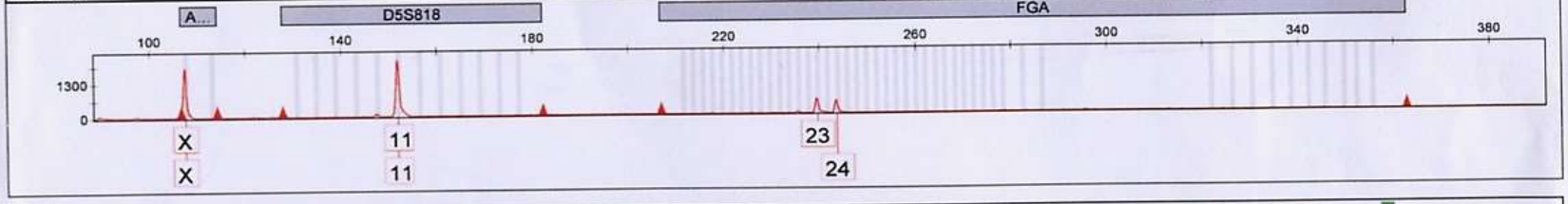

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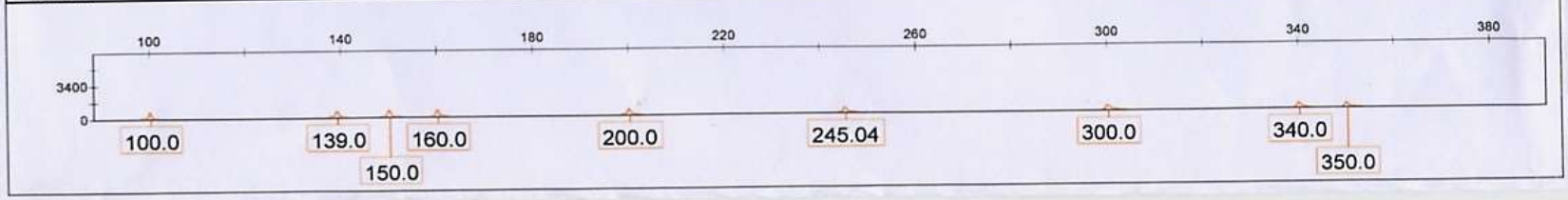
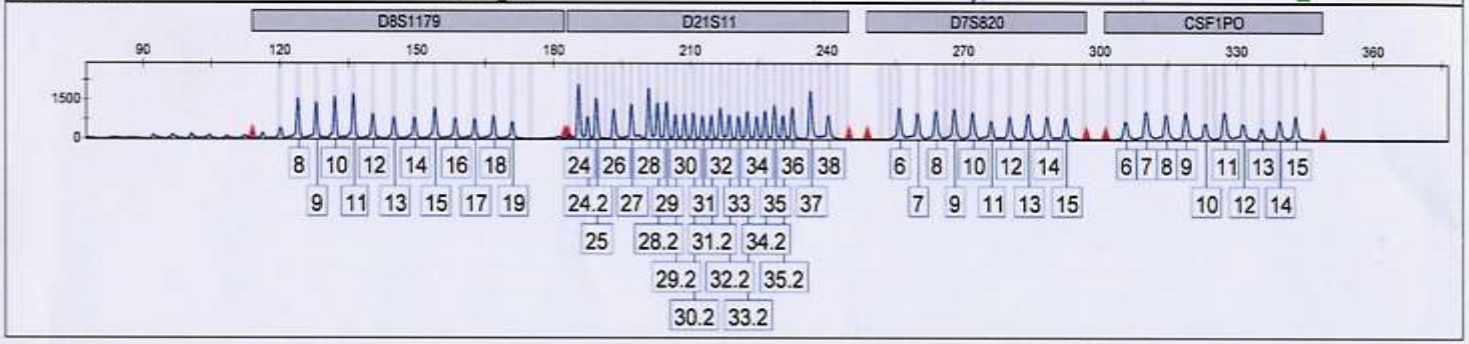


Applied Biosystems

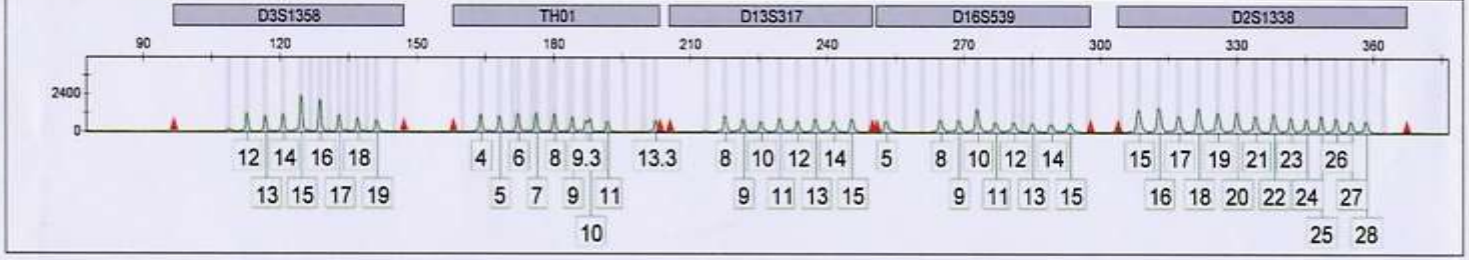
GeneMapper ID v3.2

020309PATERNATY

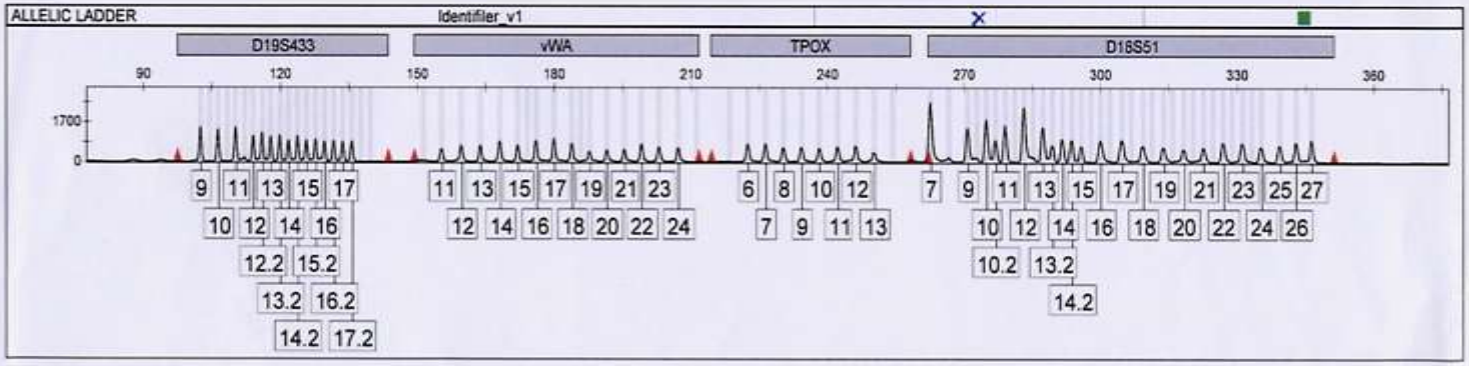
Sample Name	Panel	SQO	SQ
ALLELIC LADDER	Identifiler_v1	X	



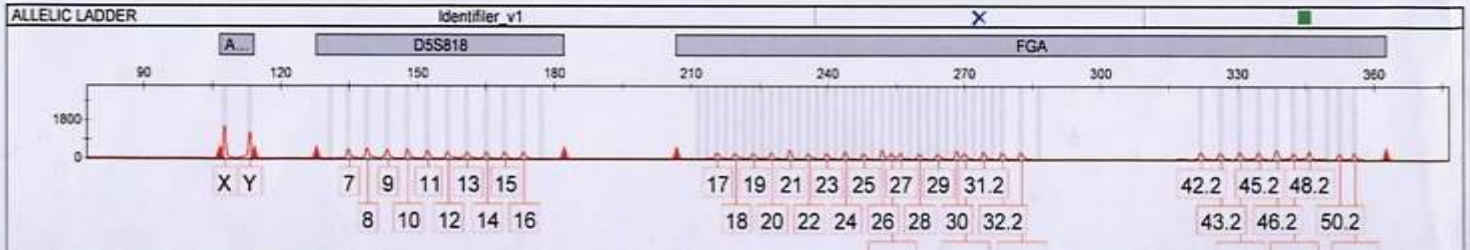
ALLELIC LADDER | Identifiler_v1 | X



ALLELIC LADDER | Identifiler_v1 | X



ALLELIC LADDER | Identifiler_v1 | X



FTA CARDS

- FTA Cards provide a **safe, secure and reliable** eht rof dohtem moor efas dna ,tropsnart ,noitcelloc .AND fo egarots erutarepmet
- When cells are applied to FTA Cards, the cells are lysed and the nucleic acids are immobilized and stabilized within the FTA Card's matrix .
- Genomic DNA stored on FTA Cards at room temperature for **over 11 years** RCP ni ssol on stibihxe detcepxE .(nevorp) ycneiciffe revo si htgnel egarots50 years .
- Amplification or restriction enzyme digestion can be performed directly on the treated paper without the need for extensive extraction procedures .


THANK YOU