MEDICAL ACADEMY NAMED AFTER S. I. GEORGIEVSKY OF VERNADSKY UNIVERSITY

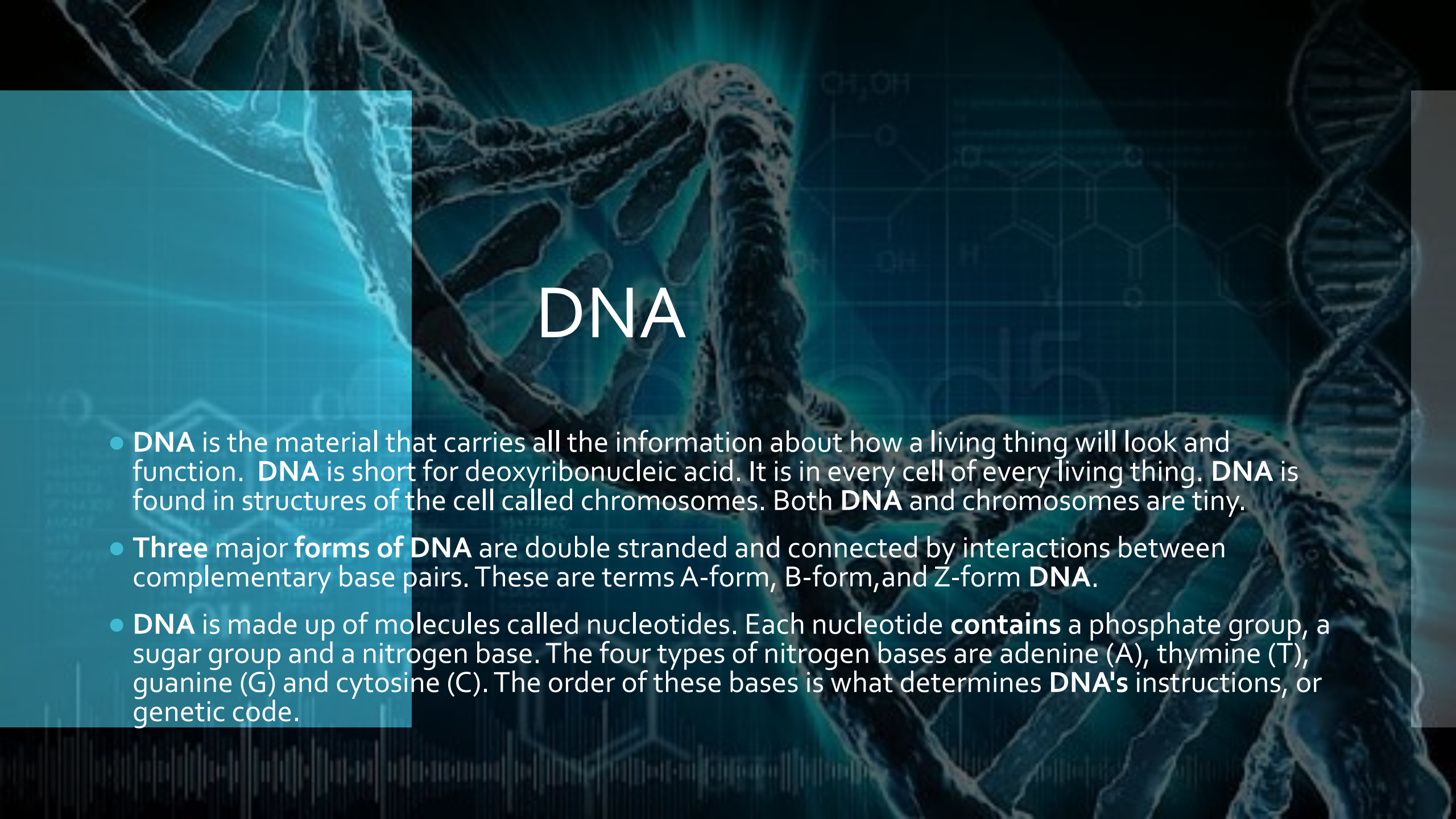
- Name: SUSHANT KUMAR MAURYA

GROUP: L A 2 203(2)

TOPIC: BIO CHEMICAL METHOD. PCR AND DNA DIAGONSTICS

BIO CHEMICAL TEACHNIQUES

- **Biochemistry techniques** are Protein Purification, perfusion, Homogenization, Differential Centrifugation, Purification of LDH, Purification of LDH , LDH Enzyme assays, Protein assays, Characterization of LDH, Western blotting, Gel filtration chromatography, Protein crystallography, PCR, Ligation and transformation.
- **Biochemical** methods are applied to the main chemical compounds of genetics—notably **DNA**, RNA, and protein. ... Special **techniques** (e.g., chromatography and electrophoresis) are used to separate the components of proteins so that inherited differences in their structures can be revealed.



DNA

- **DNA** is the material that carries all the information about how a living thing will look and function. **DNA** is short for deoxyribonucleic acid. It is in every cell of every living thing. **DNA** is found in structures of the cell called chromosomes. Both **DNA** and chromosomes are tiny.
- **Three** major **forms of DNA** are double stranded and connected by interactions between complementary base pairs. These are terms A-form, B-form,and Z-form **DNA**.
- **DNA** is made up of molecules called nucleotides. Each nucleotide **contains** a phosphate group, a sugar group and a nitrogen base. The four types of nitrogen bases are adenine (A), thymine (T), guanine (G) and cytosine (C). The order of these bases is what determines **DNA's** instructions, or genetic code.

DNA Diagnostic Systems

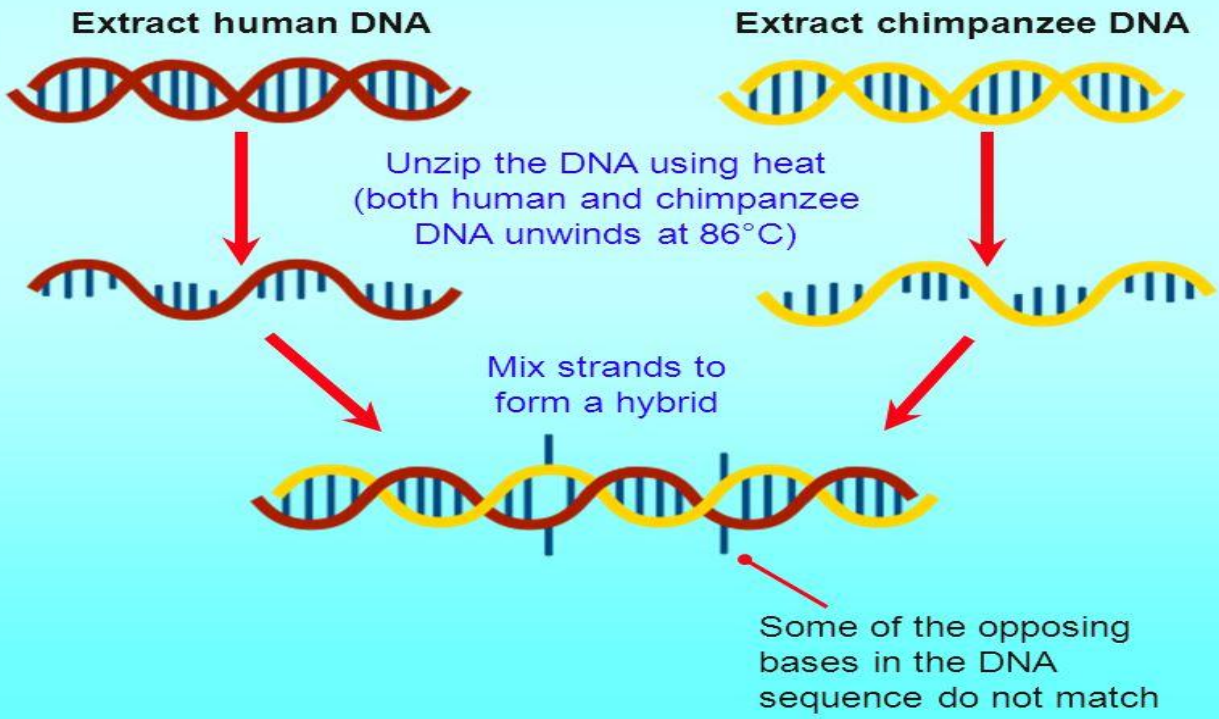
- DNA Diagnostic Systems include:
- DNA Hybridization
- DNA Sequencing
- PCR
- Restriction endonuclease analysis
- RAPD (random amplified polymorphic DNA)
- DNA fingerprinting

DNA Hybridization

- One way to reconstruct the evolutionary history of a species is using **DNA hybridization**.
  - In this technique, the DNA from different species is 'unzipped' and recombined to form **hybrid DNA**.
  - Heat can be used to separate the hybridized strands. The amount of heat required to do this is a measure of how similar the two DNA strands are (% bonding).
- **EXAMPLE**:
  - The relationships among the **New World vultures** and **storks** has been determined on the basis of DNA hybridization.

DNA Hybridization Method

- DNA is isolated from blood samples from each species:
  - Greater similarity in the DNA base sequences = stronger attraction between the two strands and harder to separate.
  - A crude measure of DNA relatedness can be achieved by measuring how hard it is to separate the hybrid DNA.
  - This is done by finding the temperature at which it unzips into single strands again (in this case it would be 83.6°C).



Access melting profile of hybridized DNA

ds DNA bound to the column

⇩

The mixture is heated

⇩

The mixture is heated in small steps

⇩

Each step – column washed

⇩

Sequence melt – ss DNA washed off

⇩

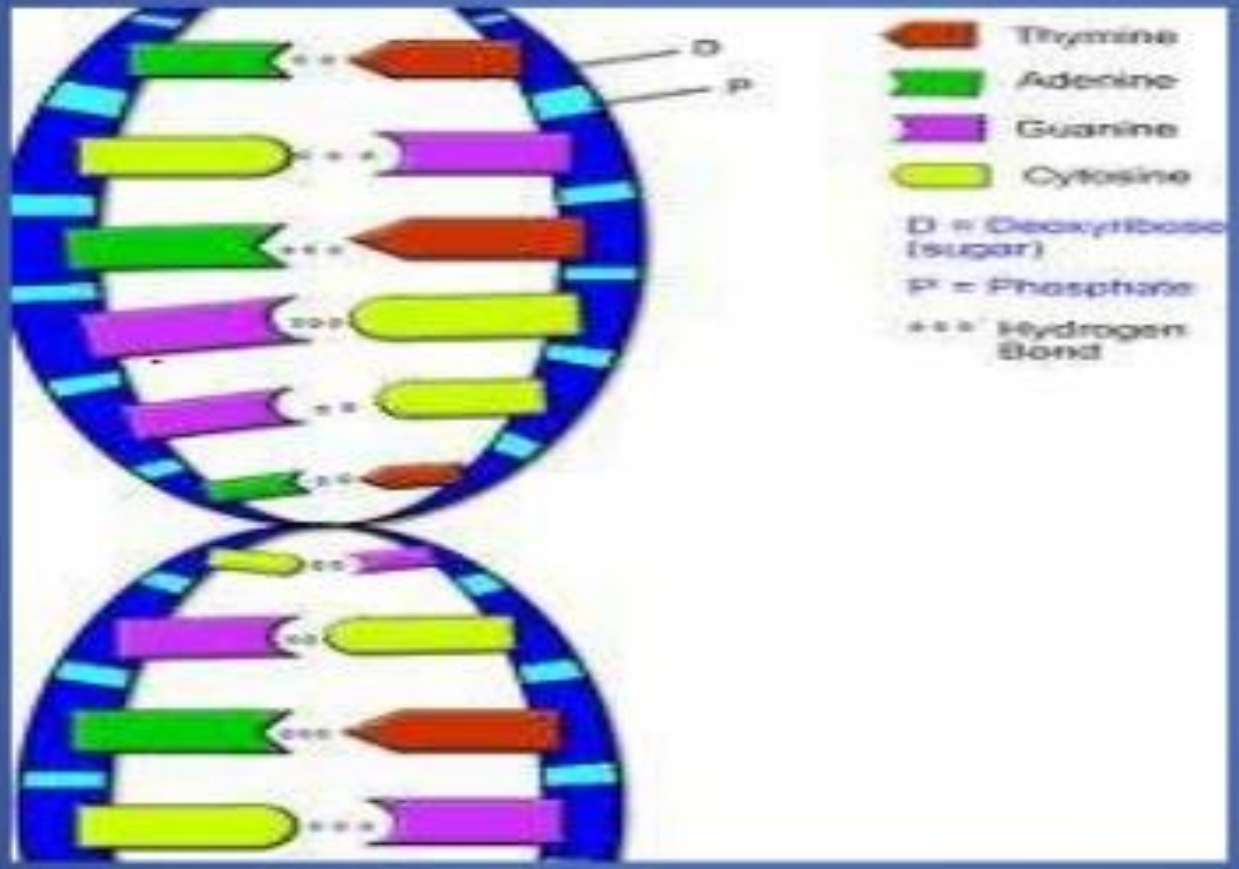
Temperatures at which labelled DNA comes of the column reflects the amount of similarity between sequences

⇩

Combined results – Determine the degree of genetic similarity between organisms

What is DNA Sequencing?

- DNA Sequencing is finding the order of nucleotides in a fragment of DNA



- These 4 bases are cytosine, guanine, adenine, or thymine.

DNA sequencing

- Technique used to identify sequence of bases
- The nucleotides are separated from each other in the order that they are found in strand of DNA.
- Nucleotides appear as dark bands
- The sequence in the first segment of DNA reads **CTGA,** second segment **TACG**
- This is how DNA fingerprint is

PCR TECHNIQUES

Polymerase chain reaction (PCR), a technique used to make numerous copies of a specific segment of DNA quickly and accurately. The polymerase chain reaction enables investigators to obtain the large quantities of DNA that are required for various experiments and procedures in molecular biology, forensic analysis, evolutionary biology, and medical diagnostics.

PCR was developed in 1983 by Kary B. Mullis, an American biochemist who won the Nobel Prize for Chemistry in 1993 for his invention. Before the development of PCR, the methods used to amplify, or generate copies of, recombinant DNA fragments were time-consuming and labour-intensive. In contrast, a machine designed to carry out PCR reactions can complete many rounds of replication, producing billions of copies of a DNA fragment, in only a few hours.

Definition

Polymerase Chain Reaction (PCR):
A procedure to amplify a specific DNA region

- Yields millions of copies of the target region
- Makes enough DNA for further molecular work
- Is the first step in preparing DNA for:
  - Sequencing
  - Restriction digestion
  - Bacterial cloning

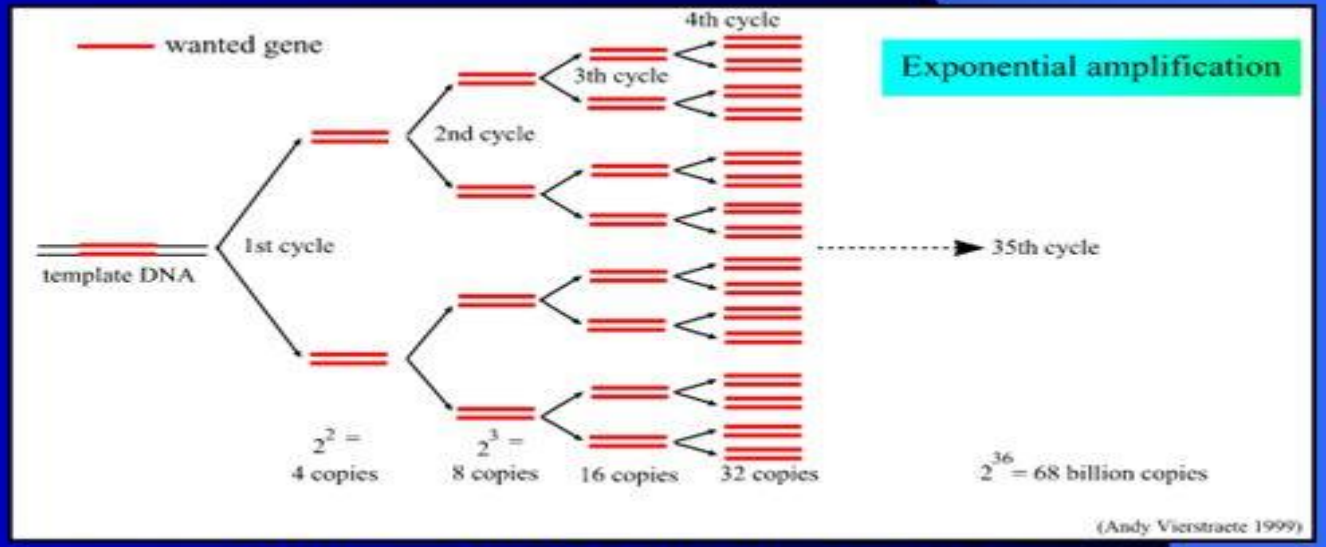
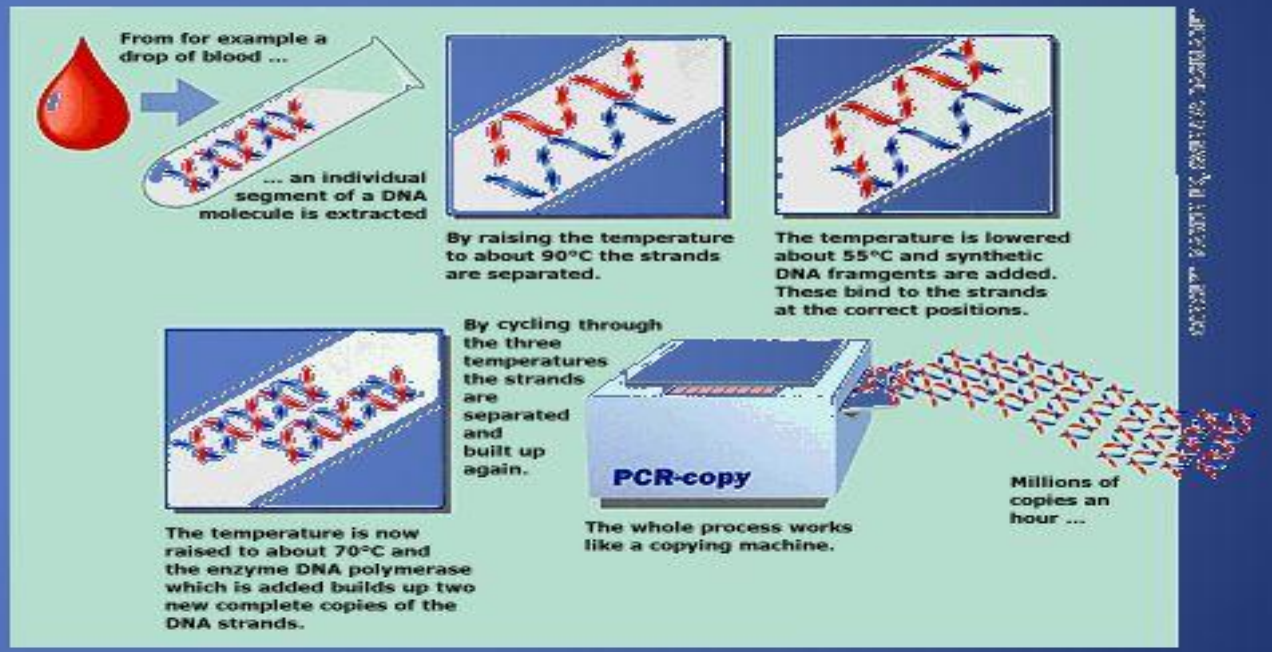
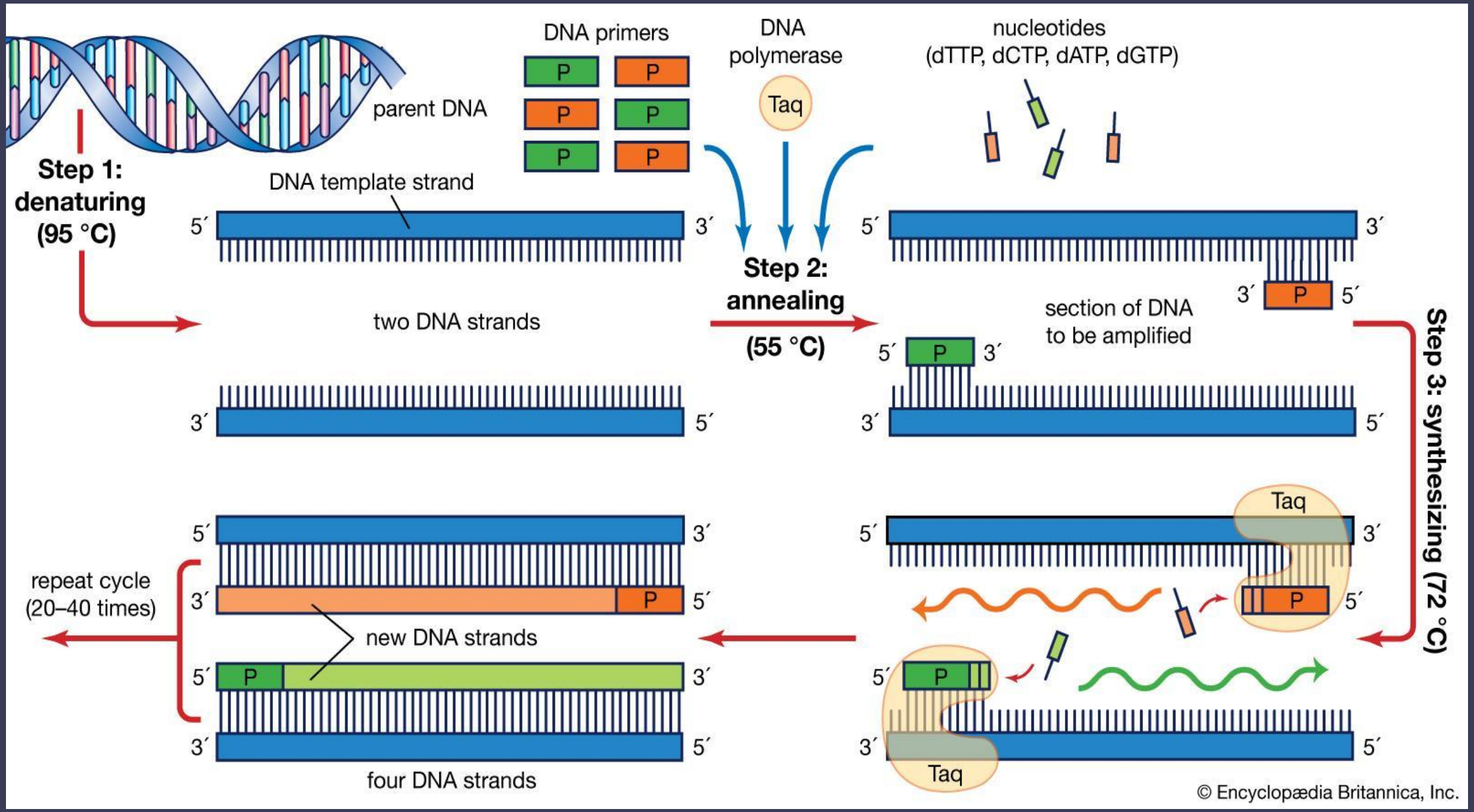


Diagram by Andy Vierstraete 1999

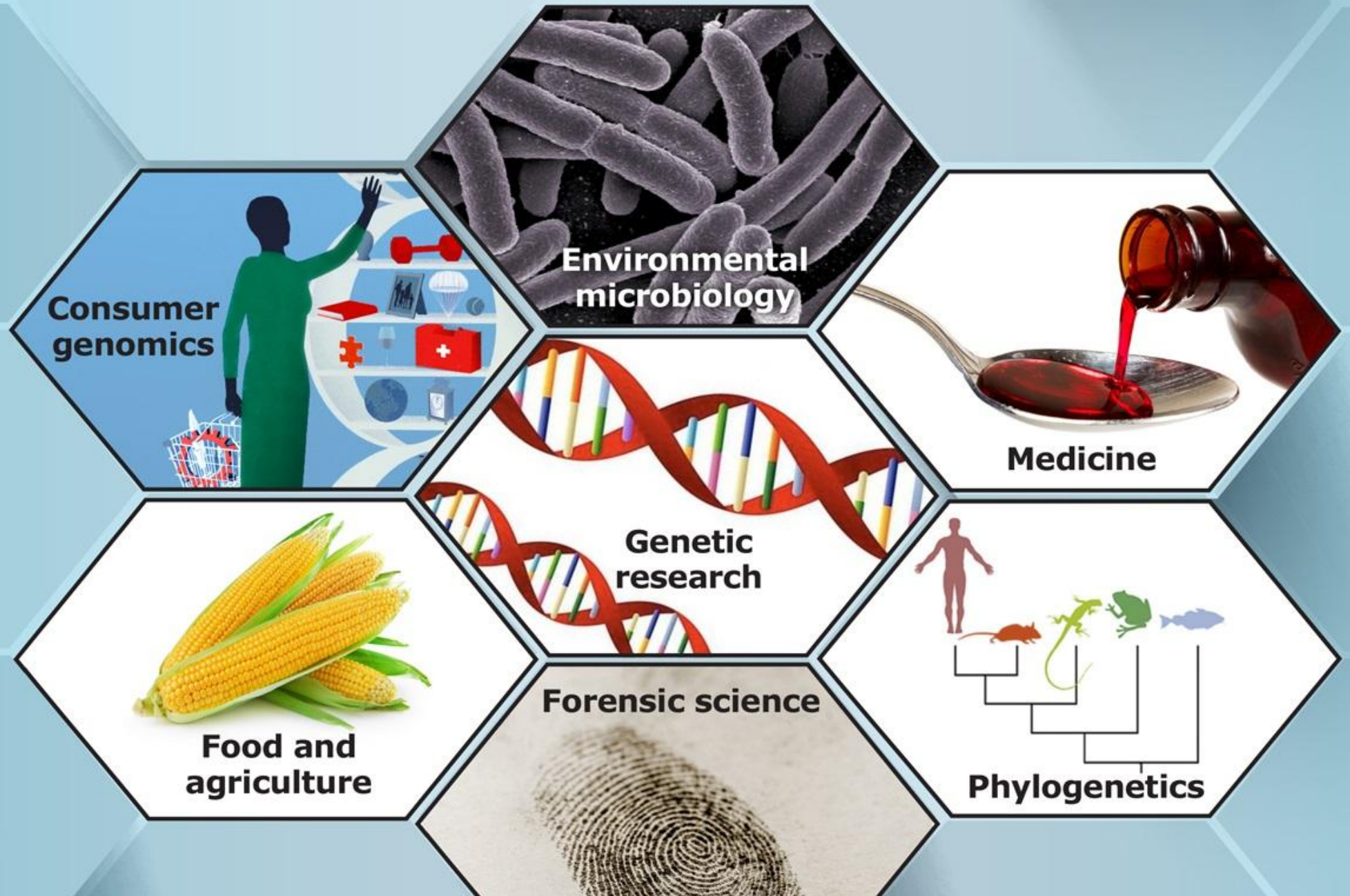
Polymerase Chain Reaction

- How is it used?
  - Medical and biological research for a variety of applications
    - DNA cloning, functional analysis of genes, diagnosis of hereditary diseases, genetic fingerprints (forensic science and paternity testing) and the detection of infectious diseases.




parent DNA
DNA primers
DNA polymerase
Taq
nucleotides
(dTTP, dCTP, dATP, dGTP)
Step 1:
denaturing
(95 °C)
DNA template strand
5′
3′
P
two DNA strands
Step 2:
annealing
(55 °C)
section of DNA
to be amplified
Step 3: synthesizing (72 °C)
repeat cycle
(20–40 times)
new DNA strands
four DNA strands
© Encyclopædia Britannica, Inc.

WHAT IS PCR USED FOR?

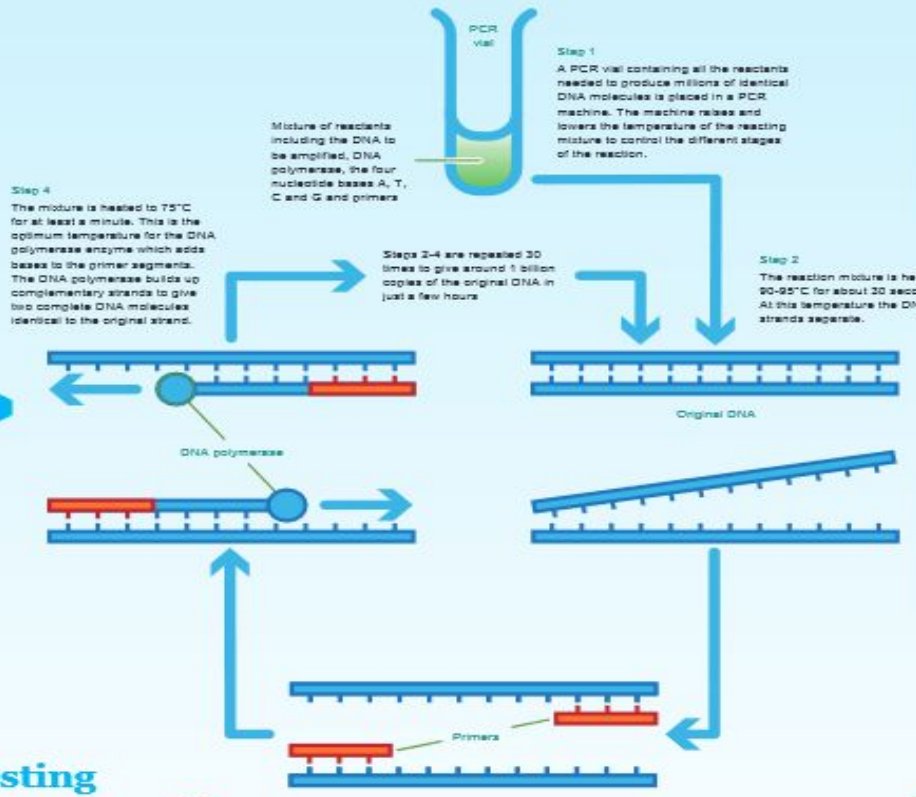


polymerase chain reaction

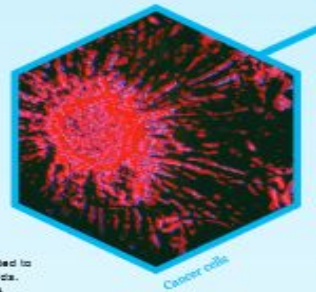
new horizons in medicine

abpi
Bringing medicines to life

The polymerase chain reaction (PCR) has revolutionised molecular biology and DNA technology. Invented in the 1980s by Kary B Mullis, PCR enables us to produce large quantities of DNA from very small samples in a remarkably short time. The process has been refined over the years but the basic principle remains exactly the same. PCR makes it possible for us to analyse tiny samples of DNA and unravel the mysteries of the individual genes.



Cancer warning



Using PCR to amplify DNA, scientists are developing tests to pick up the genetic changes in cancerous cells early in the disease. Using PCR we can already detect bowel cancer from the DNA of cells extracted from the faeces – a rapid, easy way to make an early diagnosis and increase treatment success. PCR is also being used, along with genome sequencing, to track mutations within cancers as the disease progresses. Scientists hope this will lead to more effective targeting of chemotherapy in future.

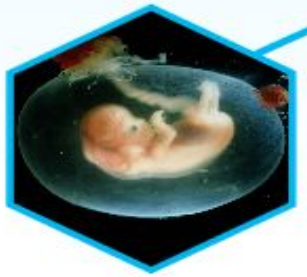
Infection detection

Amplifying the DNA from a single bacterium or virus using PCR can provide a speedy and accurate diagnosis for serious infections, where getting the right treatment quickly can mean the difference between life and death. PCR is already used in the diagnosis of AIDS, viral meningitis, TB and an ever-growing number of other infections. PCR can also be used to amplify the DNA of a pathogen so it can be sequenced. This can enable scientists to pinpoint the source of some serious outbreaks of infection.

Tissue matching

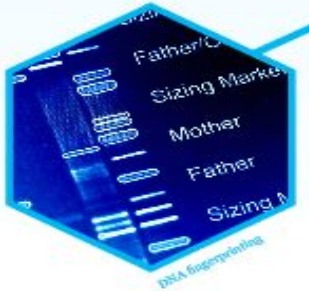
In organ transplants, a close tissue match between the donor and the recipient reduces the chances that the new organ will be rejected. PCR technology is leading to increasingly sophisticated levels of tissue matching at the DNA level – and more successful transplants.

Genetic testing



PCR makes it easier to identify individuals who carry the genes responsible for problems such as cystic fibrosis and muscular dystrophy. It is the key process in prenatal testing of foetuses at risk of carrying severe genetic conditions. PCR is also used for preimplantation testing of IVF embryos at risk of genetic conditions, amplifying the DNA, or even a single gene, from a single cell.

Forensic medicine



PCR is the first stage in DNA profiling – also known as DNA fingerprinting. The ability to amplify the tiniest fragment of DNA found at a crime scene has resulted in amazing developments in identifying and eliminating suspects in crimes including murder and rape, even years after the event. PCR is also used to identify relatives in immigration cases and fathers in paternity cases.

more information: www.abpischools.org.uk

THANK YOU

1.insulin making process in short?

2. hirudin medicinal uses?