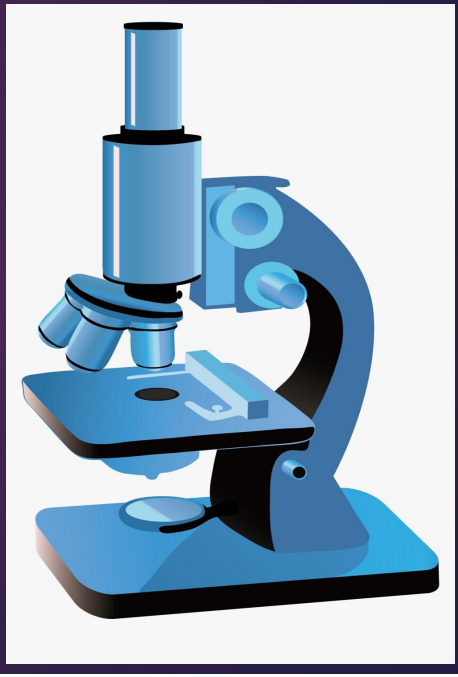
Biological method of research

PCR and DNA diagnostic



BY :- SAKHI INGOLE .

KARMSHIL KUMAR

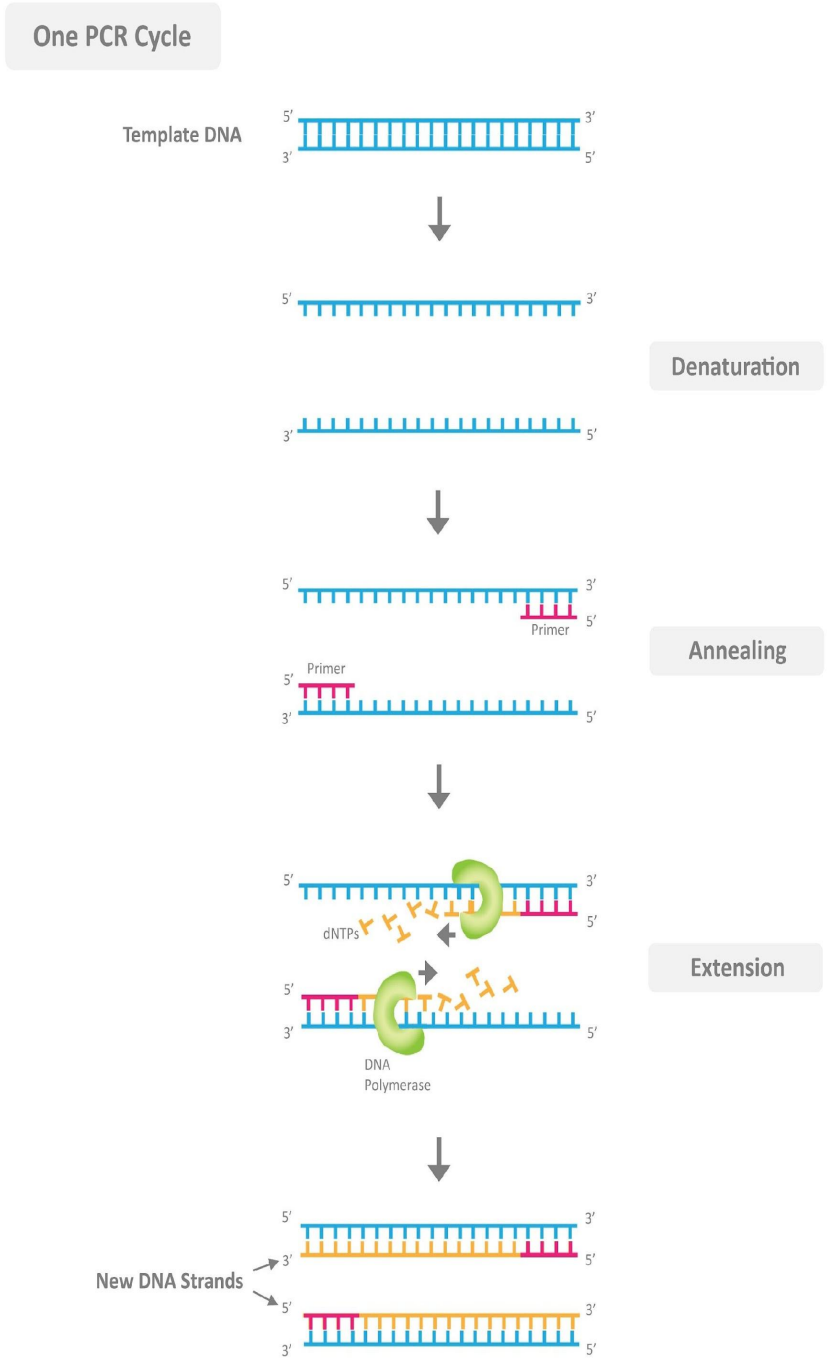
What are biological techniques?

- Biological techniques are methods or procedures that are used to study living things. They include experimental and computational methods, approaches, protocols and told for biological researches.

- *Research methods in biological sciences are as numerous and varied as the diversity of questions asked and the phenomenon studied.*
- *They include the following:-*
- *1) experimental research.*
- *2) observational research.*
- *3) survey, questionnaire, and interviews.*
- *4) biographical and archival research.*
- *5) biological educational research.*

PCR technique.

- *PCR is a technique that takes specific sequence of DNA of small amount and amplifies it to be used for future testing.*
- *It is an in vitro technique.*



Principles of PCR

- 1) purpose
- 2) condition
- 3) components.

Purpose

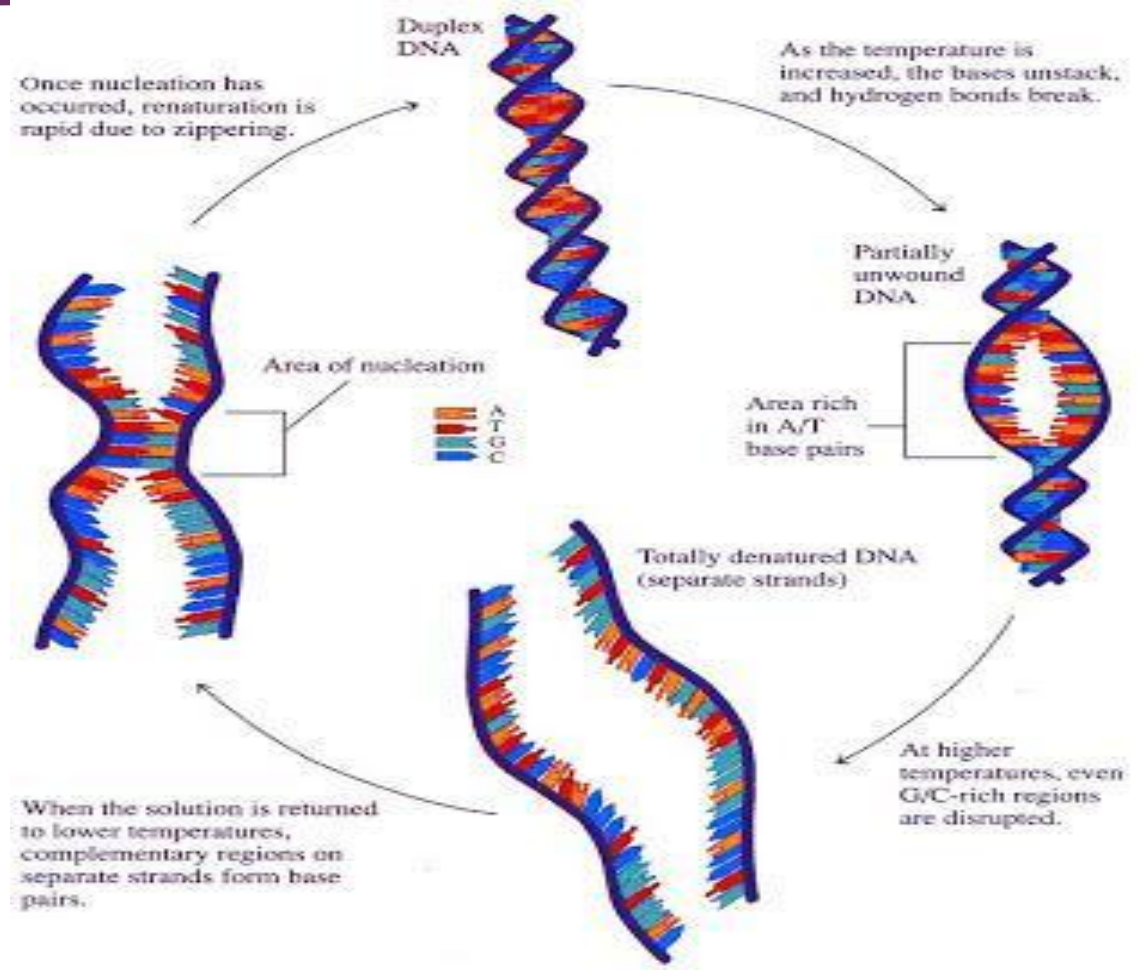
- *To amplify a lot of double stranded DNA molecules(fragments) with same (identical) size and sequence by enzymatic method and cycling condition.*

Condition

- 1) denaturation of ds DNA technique.
- 2) Annealing of primers.
- 3) extension of ds DNA.

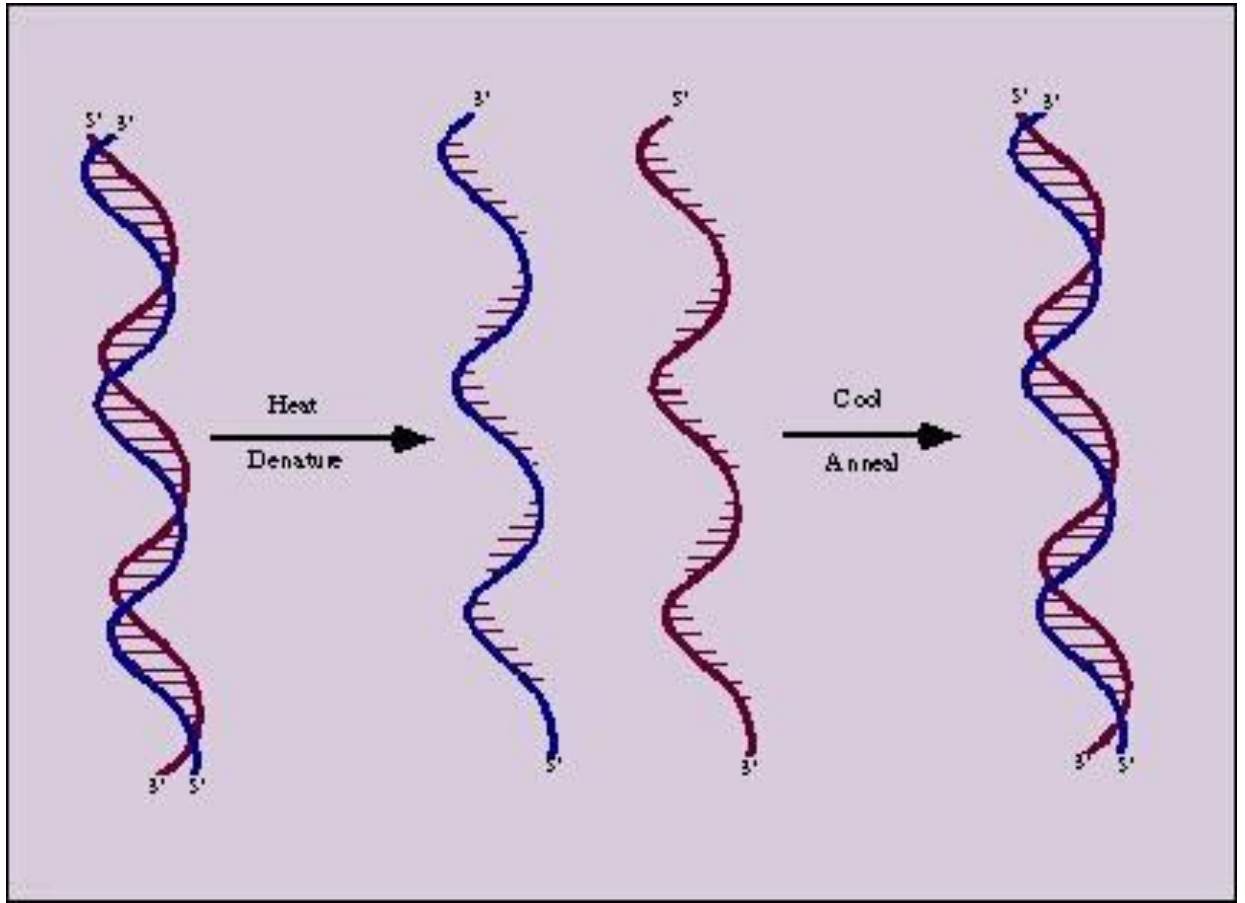
1) Denaturation

- *If we heat up a tube of DNA dissolved in water, the energy of the heat can pull the tube strands of DNA apart.*
- *This process is called denaturation.*



2) Annealing

- *Annealing is the process of heating and cooling two single-stranded oligonucleotides with complimentary sequence.*
- *Heat breaks all hydrogen bonds, and cooling allows new bonds, to form between sequences.*



3) extension

- *When the temperature is raised and the strand of DNA is made by the Taq polymerase enzyme.*

Basic requirements for PCR technique.

- *1) DNA sequence of target region must be known.*
- *2) primers : typically 20-30 bases in size. These can be readily produced by commercial companies. Can also be prepared using a DNA synthesizer.*
- *3) thermo-stable DNA polymerase – e.g Taq polymerase which is not inactivated by heating to 95C.*
- *4) DNA thermal cycler : it is a machine which can be programmed to carry out heating and cooling of sample over a number of cycles.*

Three aspects of PCR

- Specificity
- Efficiency
- Fidelity.

Things to try if PCR does not work

- *1) if no product (of correct) size produced:*
  - *check the DNA quality.*
  - *reduce the annealing temperature.*
  - *increase magnesium concentration.*
  - *add dimethylsulphoxide (DMSO) to assay (at around 10%)*
  - *use different thermo-stable enzymes.*
  - *throw out primers – make new stocks.*
- *2) If extra spurious product bands present:*
  - *increase annealing temperature.*
  - *reduce magnesium concentration.*
  - *try different enzymes.*
  - *reduce number of cycles.*

DNA diagnostic

- *Diagnosis of disease due to pathogens or due to inherent genetic defects if necessary for appropriate treatment.*

- *Traditional diagnostic methods for parasite infection include microscopic examination, in vitro culture, and detection of ab in serum.*
- *And for genetic diseases, the procedure such as estimation of metabolites (blood and urine) and enzymes assay are used.*
- *These laboratory technique are indirect, not always specific.*

- *DNA being genetic material of living organism, contain the information which contributes to various characteristics features of specific organism.*
- *Thus the presence of disease causing pathogens can be detected by identifying a gene or a set of genes of the organism.*
- *Inherited genetics defect can be diagnosed by identifying the alteration in Gene.*

Methods of DNA assay

- *1) Nucleic acid hybridization :*
  - *radioactive detection system.*
  - *non-radioactive detection system.*
- *DNA probe :*
  - *PCR in use of DNA probe.*
  - *DNA probes and signal amplification.*
- *DNA chip:*
  - *micro-array of Gene probe.*

Questions

- *Aswin – what are biological techniques?*

  What are some research methods in biological sciences?
- *Nidhi – what to do when extra spurious bands are present in PCR?*

  What are some methods of DNA assay?
- *Ekta – what are principles of PCR?*

  Explain: purpose (principle of PCR).
- *Aishwary – what is PCR technique?*

  What is annealing process?
- *Vikram – explain: condition (principle of PCR).*

  What is denaturation?

- *Harishankar – what is extension?*

  What are aspects of PCR?
- *Gracy - What are some basic requirements for PCR technique?*

  What to do if no product of correct size is formed during PCR?
- *Amit – what does traditional method of parasite diagnosis include?*

  What is DNA diagnostic?
- *Teena – how does heating and cooling affects the hydrogen bonds of DNA?*

  How is the presence of disease causing pathogens detected?

- *Keerthana – methods in nucleic acid hybridization.*

  How are genetic disease diagnosed?
- *Haris – how are inherited Gene defects identified?*

  Explain: primers as a requirement for PCR technique.

Thanks for watching!!