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Sem-V Sec-B
Life sciences

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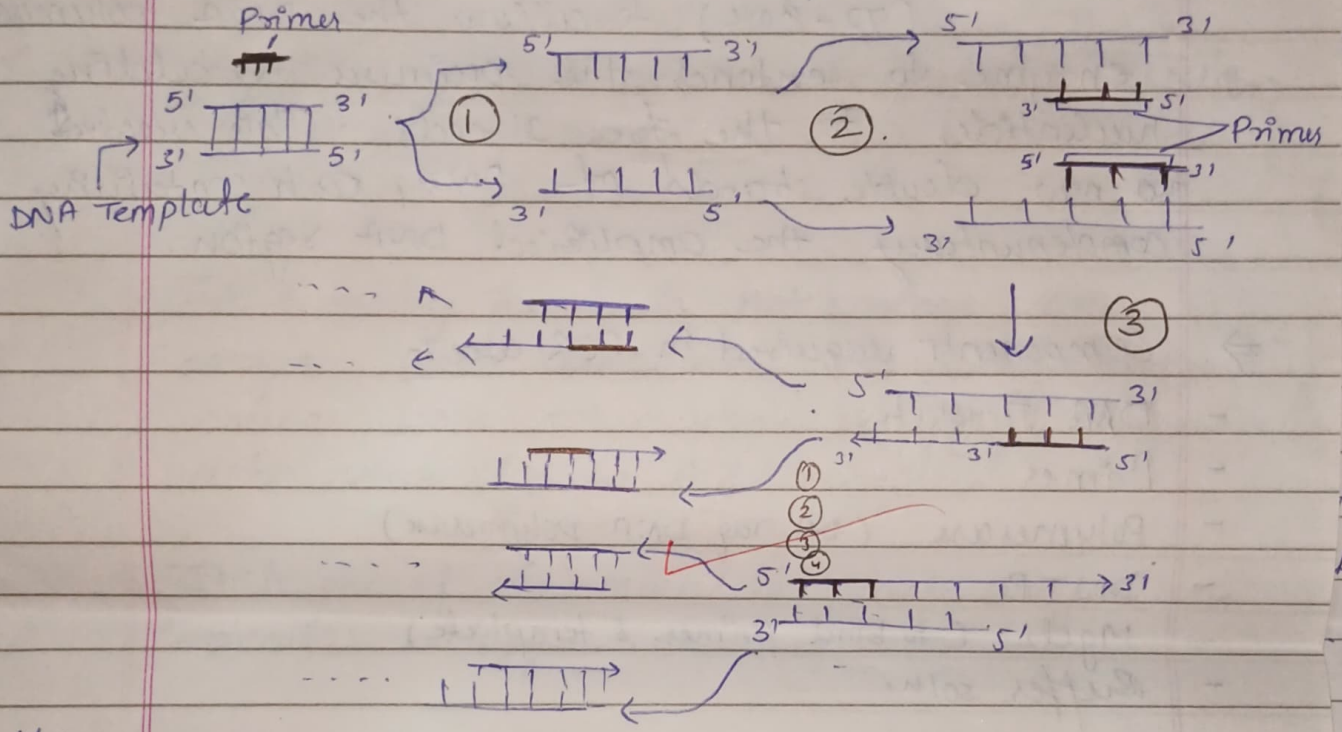
Ans-1(g) (PCR) Polymerase chain Reaction technique is used when any recombinant DNA with desired gene is required in billion copies for commercial use.



Here,

① → Denaturation
② → Annealing
③ → Extension / Elongation.

PCR involves three main steps:

① Denaturation → The double-stranded DNA is heated to a high temp. (94-95°C) to break the hydrogen bonds b/w the two strands. This creates two single strands of DNA.

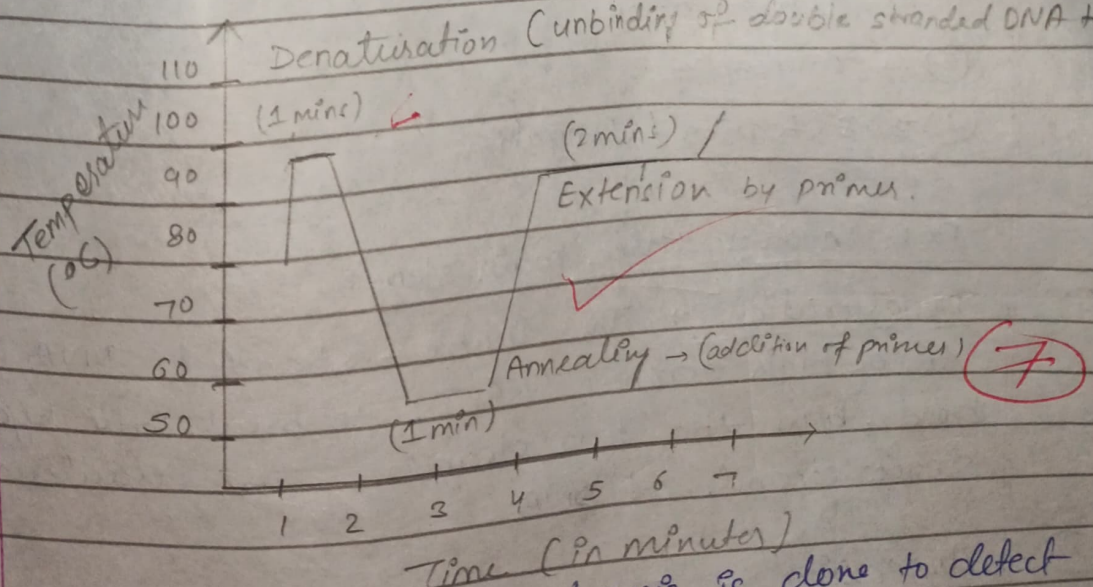
② Annealing → The temp is lowered (50-56°C) to allow two short synthetic DNA fragments called primers to bind with the complimentary sequences on single strands of DNA.

Primers should be of 15-25 base pairs long.

(3) Extension :- The temperature is raised again (72-80°C) to allow the DNA polymerase enzyme to extend the primers by adding nucleotides to the free 3' ends. This creates two new double strands of DNA, each containing complementary the amplified DNA region.

⇒ Components required in PCR are :-

- DNA Template
- Primer
- Polymerase (Taq DNA polymerase)
- DNTPs
- $MgCl_2$ (to bind primer & template)
- Buffer soln.



⇒ At last, Gel electrophoresis is done to detect different DNA fragments acc. to their sizes. DNA is negatively charged so, DNA fragments moves towards anode.

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(b) Thermus aquaticus : It is a source of thermostable DNA polymerase for which PCR (Polymerase Chain Reaction) is possible at high temperature. It is extracted from the bacterium, Thermus aquaticus, made it possible to generate billion copies of DNA in a very short time using a process.

It is mostly found in hot springs. Other enzymes get denatured at high temperature whereas DNA polymerase ie. Taq DNA Polymerase act properly in PCR technique.

It is one of the most important enzyme in molecular biology.

Ans-2 (a) DNA sequencing :- DNA sequencing is a process of determining the nucleic acid sequences - the order of nucleotides in DNA. It is used to determine 4 nucleotide base ie. adenine, guanine, cytosine and thymine.

There are many further types of DNA sequencing:

→ Sanger sequencing
→ Next generation sequencing
→ Pyrosequencing etc.

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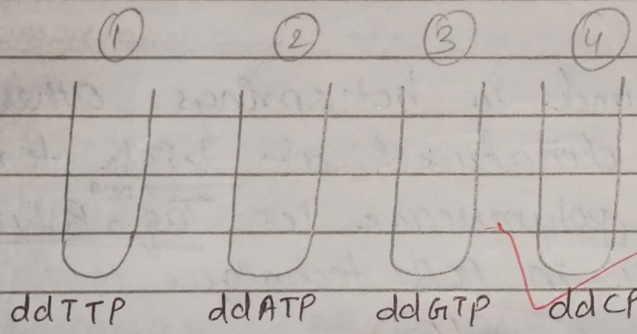
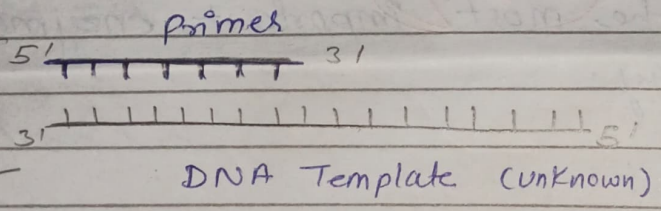
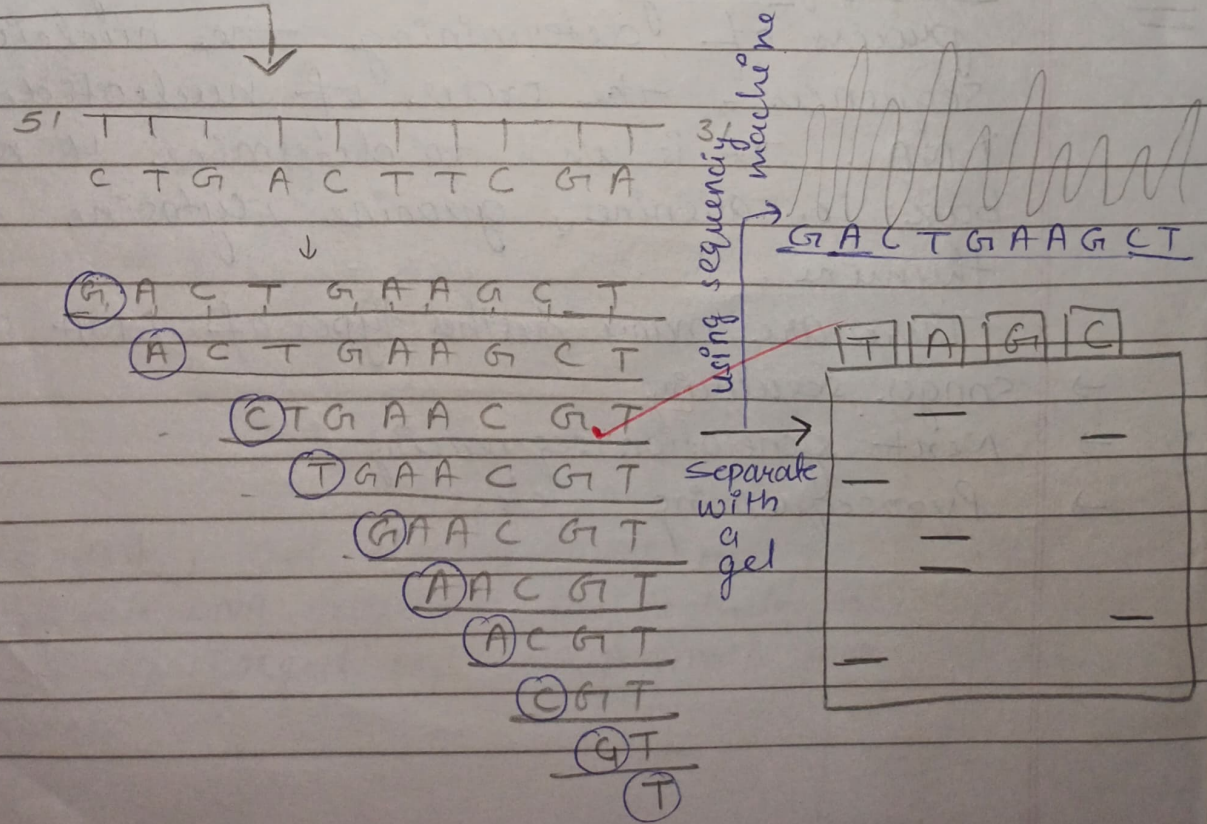
Sanger Sequencing : Sanger Sequencing, also known as chain Termination method or dideoxyribonucleotides method.

- It was developed by Frederick Sanger and his collegues in 1977.



Reach minute contains;
- DNA Template
- Primer
- DNTPs → ~~Fluoroch~~
- ddNTPs (ddTTP, ddCTP...)
- Polymerase
- Buffer.





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~~Component~~ The sanger method is based on the chain termination method.

① First, a primer is hybridised to the template DNA. The reaction mixture also contains DNA polymerase, dNTPs, ddNTPs which are labelled with diff. fluorescent dyes.

② Then, the reach mixture is heated to denature the double-stranded DNA template. The DNA polymerase then begins to extend the primer, add nucleotides to the growing strand in 5' to 3' direction. At random, a ddNTP is incorporated into the growing strand, terminating the chain.

③ The reaction mixture is then electrophoresed on a gel. The DNA fragments are separated by size, with the smallest fragments migrating the furthest. The DNA fragments are then visualised using a fluorescent scanner. The sequence of template DNA is determined by reading the order of the florescently labeled ddNTPs in the DNA fragments.

④ It uses only 900 base pair length DNA fragment or less.

⑤ It is used in Gene sequencing, Mutation detection, Forensic science etc.

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(b) Ingredient of sanger sequencing are:-

1) DNA Template
2) Primer (15-25 base pair long)
3) Deoxyribonucleotidetriphosphates (DNTPs)
   - DATPs
   - DTTPs
   - DCTPs
   - DGTPs
4) ddNTPs → dideoxyribonucleotidetriphosphates
   - ddATPs
   - ddTTPs
   - ddGTPs
   - ddCTPs
5) Buffu solution
6) Gel electrophoresis machine (3)
   ↓
   Polyacrylamide Gel (PAA)
7) DNA Polymerase enzyme
8) $MgCl_2$ (To bind templat and Primer)
9) If done in one test tube (single) then flurochromes dye are required to detect each ddNTPs.