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Class 12th

Subject BIOLOGY

DATE:- 20.02.21

PCR is a technique for the in vitro amplification of a desired sequence of DNA. PCR allows the generation of a large quantity of DNA product (up to several

• g) from only a few starting copies. it has been shown that PCR can be used to generate a detectable quantity of DNA from only one starting target (or template) molecule.

PCR developed in the mid-1980, has found multiple applications, such as :-

1. Rapid amplification of intact genes or gene fragments
2. Generation of large amounts of DNA for sequencing
3. Generation of probes specific for uncloned genes by selective amplification of a specific segment of cDNA
4. Analysis of mutations for medical applications
5. Detection of minute amounts of DNA for forensic purposes
6. Amplification of chromosomal regions adjacent to genes of known sequence and many more.

Development of PCR won the Nobel prize for Kary Mullis and co-workers.

PCR principle

PCR reaction is a DNA synthesis reaction that depends on the **extension** of primers **annealed** to opposite strands of a dsDNA template that has been denatured (**melted apart**) at temperatures near boiling. By repeating the **melting, annealing** and **extension** steps, several copies of the original template DNA can be generated.

The amount of starting material (target) needed is very small

Not necessary to isolate the desired sequence, because it will be defined by the **primers** that are used in the reaction. The **primers** are oligonucleotides **complementary** to different regions on the 2 strands of DNA template (**flanking** the region to be amplified). The primer acts as a starting point for **DNA** synthesis. The oligo is **extended** from its 3' end by **DNA**

polymerase.