





**Applications of PCR:**

- Amplification of small amounts of DNA for further analysis by DNA fingerprinting.
- The analysis of ancient DNA from fossils.
- Mapping the human (and other species) genome.
- The isolation of a particular gene of interest from a tissue sample.
- Generation of probes: large amount of probes can be synthesized by this technique.
- Production of DNA for sequencing: Target DNA in clone is amplified using appropriate primers and then its sequence determined. Helpful in conditions where amount of DNA is small.
- Analysis of mutations: Deletions and insertions in a gene can be detected by differences in size of amplified product.
- Diagnosis of monogenic diseases (single gene disorders): For pre-natal diagnosis, PCR is used to amplify DNA from foetal cells obtained from amniotic fluid. PCR has also proved very important in carrier testing.
- Detection of microorganisms: Especially of organisms and viruses that are difficult to culture or take long time to culture or dangerous to culture.
- The PCR has even made it possible to analyze DNA from microscope slides of tissue preserved years before.
- Detection of microbial genes responsible for some aspect of pathogenesis or antibiotic resistance.
- Crucial forensic evidence may often be present in very small quantities, e.g. one human hair, body fluid stain (blood, saliva, semen). PCR can generate sufficient DNA from a single cell.

**Limitations of PCR:** PCR is an extremely sensitive technique but is prone to contamination from extraneous DNA, leading to false positive results. Another potential problem is due to cross-contamination between samples. It is for this reason that sample preparation, running PCR and post-amplification detection must be carried out in separate rooms. Concentration of Mg is very crucial as low  $Mg^{2+}$  leads to low yields (or no yield) and high  $Mg^{2+}$  leads to accumulation of nonspecific products. Non-specific binding of primers and primer-primer dimer formation are other possible reasons for unexpected results. Reagents and equipments are costly, hence can't be afforded by small laboratories.

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