BSB 1163
CELL AND MOLECULAR BIOLOGY

Analysis of DNA

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Polymerase Chain Reaction

PCR is an *in vitro* technique for the amplification of a region of DNA which lies between two regions of known sequence.

PCR – first described in mid 1980's, Mullis Nobel prize in 1993

Requires

- Two specific oligonucleotide primers
- Thermostable DNA polymerase
- dNTP's
- Template DNA
- Sequential cycles of (generally) three steps (temperatures)

- Based on rapid changes in temperature:
  i. Denaturation—double stranded DNA is heated to 94°C to break the hydrogen bonds and separate the DNA strands
  ii. Annealing—cool to 50°C to allow primers to bind to DNA
  iii. Extension—increase the temperature to 72°C to add nucleotides to the 3' end of each primer

PCR types

- Q-PCR
- Allele-specific PCR
- Assembly PCR
- Colony PCR
- Inverse PCR
- Ligation-mediated PCR
- Nested PCR and many more...

- PCR:

  DNA polymerase

  -catalyzes the polymerization of deoxyribonucleotides into a DNA strands

- Reverse transcription PCR (RT-PCR):

  Reverse transcriptase

  -enzymes that transcribes single stranded RNA into complementary double stranded DNA

Application of PCR

- selective amplification of a specific region of DNA for hybridization probes
- Isolation of a DNA sequence for DNA cloning
- Bacterial colonies can be rapidly screened by PCR for correct DNA constructs.
- Genetic fingerprinting.
- Determine evolutionary relationships

Amplification and quantification of DNA

- Forensic analysis
- Gene expression studies :using quantitative PCR methods
- Analysis of ancient DNA

PCR in diagnosis of diseases

- Early diagnosis of malignant diseases such as leukemia (cancer marker)
- Identification of non-cultivatable or slow-growing microorganisms