RT PCR

Reverse transcriptase PCR

Principle of RT-PCR

- RT-PCR, is a type of PCR technique that enzymatically amplifies the RNA in vitro.
- RT-PCR combines the reverse transcription process with the conventional PCR process.
- The sample RNA is first converted to double-stranded DNA (complementary DNA) by reverse transcriptase enzyme in the reverse transcription process.
- The cDNA can then be thermally broken down into two single-stranded DNA templates. In these ssDNA templates, primers can anneal to their complementary sequences based on the nucleic acid hybridization principle. DNA polymerase then elongates the primer by sequentially adding the nucleotides to the 3' end and generates a dsDNA following the principle of DNA replication.
- These three processes, denaturation, annealing, and elongation, are repeated in a cyclic manner regulating the reaction temperature and resulting in millions of copies of the cDNA.
- Enzymes required for RT PCR

1. Nucleic Acid Sample (Sample RNA)
2. Reverse Transcriptase Enzyme
3. DNA Polymerase Enzyme
4. Primers (Oligo (dT) primers, random primers, and sequence-specific primers)

5. Deoxynucleotide Triphosphates
6. PCR Buffers and Other Chemicals
7. Thermocycler (PCR Machine).

Applications of RT-PCR

- Study Gene Expression
- Identification of Unknown Species
- Infectious Disease Diagnosis
- Gene Insertion and Gene Therapy Study
- Study Mutation and Cancer Cells
- Tools of Genetic Engineering and Viral Study