

11.3.2 Cutting of DNA at Specific Locations

Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme. Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode) (Figure 11.3). The process is repeated with the vector DNA also.

The joining of DNA involves several processes. After having cut the source DNA as well as the vector DNA with a specific restriction enzyme, the cut out 'gene of interest' from the source DNA and the cut vector with space are mixed and ligase is added. This results in the preparation of recombinant DNA.

11.3.3 Amplification of Gene of Interest using PCR

PCR stands for **Polymerase Chain Reaction**. In this reaction, multiple copies of the gene (or DNA) of interest is synthesised *in vitro* using two

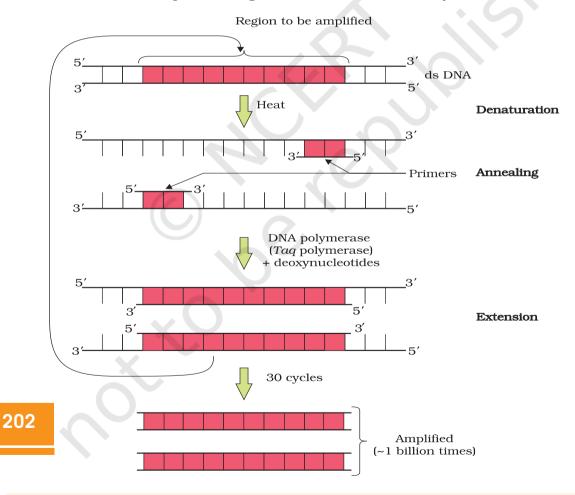


Figure 11.6 Polymerase chain reaction (PCR) : Each cycle has three steps: (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers