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Chapter 11 Biotechnology Principles and Processes

Sometimes it is also necessary to ensure that the foreign DNA sequence is in a certain orientation relative to sequences present in the cloning vector.

- Checking the size of the insert
- Checking the orientation of the insert
- Determining pattern of restriction sites within insert DNA

DNA fractionation

Separation of DNA fragments in order to isolate and analyse DNA cut by restriction enzymes

Electrophoresis

Electrophoresis is a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. When charged molecules are placed in an electric field, they migrate toward either the positive or negative pole according to their charge.

DNA is electrophoresed through the agarose gel from the cathode (negative) to the anode(positive) when a voltage is applied, due to the net negative charge carried on DNA.

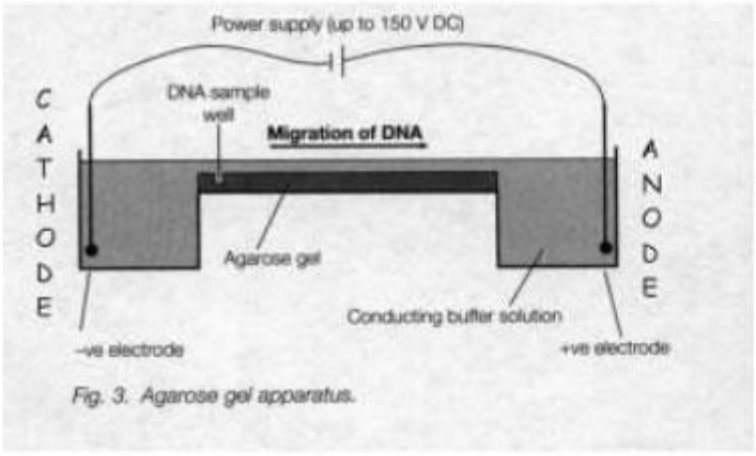
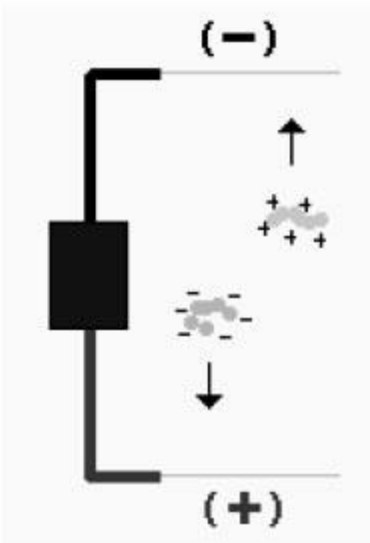


Fig. 3. Agarose gel apparatus.



When the DNA has been electrophoresed, the gel is stained in a solution containing the chemical **ethidium bromide**. This compound binds tightly to DNA and fluoresces strongly under UV light - allowing the visualisation and detection of the DNA.

Recombinant DNA technology :-

Recombinant DNA: Plasmids, cloning

What is DNA cloning?

DNA cloning is the isolation of a fragment or fragments of DNA from an organism and placing in a VECTOR that replicates independently of chromosomal DNA. The RECOMBINANT DNA is propagated in a host organism; the resulting CLONES are a set of genetically identical organisms which contain the recombinant DNA

Three main purposes for cloning DNA

1) DNA sequencing
2) Protein production
3) Engineering animals/plants/proteins

Cloning and Expression Vectors

Isolated DNA is cloned into VECTORS for long term storage, propagation of the DNA and for production of protein from gene(s) encoded in the DNA