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Subject BIOLOGY

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RECOMBINANT DNA TECHNOLOGY

- Inserting foreign DNA into a cloning vector

Restriction enzymes are a useful tool for analysing Recombinant DNA

After ligating a particular DNA sequence into a cloning vector, it is necessary to check that the correct fragment has been taken up. 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.