Recombinant DNA molecules containing one fragment can subsequently be isolated from a culture of a single colony of the Ecoli.

Treatment of the isolated recombinant DNA with the previously used restriction enzyme releases the fragment from the plasmid-

The fragment can be separated from the plasmid by gel electrophoresis, such as agarose or polyacrylamide gel electrophoresis (PAGE).

Agarose is a cross linked polysaccharide.
(We will describe its structure later.)

Polyacrylamide is a cross linked polymer of acrylamide and N,N' -methylenebisacrylamide as shown below-

When acrylamide is polymerized in water, a gel is formed.

In electrophoresis, molecules move through a medium in response to an electric field. As shown in Fig T2.2, solutions of DNA molecules are loaded on top of a gel in separate wells.

Being charged, the DNA molecules will move through the porous matrix of the gel toward the positively charged anode (anions move toward the anode).

In agarose and PAGE, the mobility of the molecules, i.e., the rate of movement, depends on their size due to the sieving effect of the gel. The gel is more porous to small molecules than large molecules.

Therefore the shortest DNA molecule will have the greatest mobility and will move farthest down the gel as below-

The DNA fragment can be isolated from a slice of the gel.

Consider now the dideoxy method for sequencing DNA.

The dideoxy sequencing method uses the synthetic 2',3'-dideoxynucleoside triphosphates and the enzyme involved in DNA replication.

The structure of a 2',3'-dideoxynucleoside triphosphate is indicated in the handout. Note that both the 3' and the 2' carbon atoms of ribose do not have an OH group.

When DNA is replicated, one strand acts as a template for the synthesis of the complementary strand. An enzyme called DNA polymerase catalyzes the sequential addition of nucleotides to the 3' end of the growing chain as shown in Fig 23.5.

Each nucleotide added is complementary to the nucleotide in the template strand, i.e., A is added if T is in the template, G is added if C is in the template etc.

The reaction uses the deoxynucleoside triphosphates and leads to the formation of a 5'-3' phosphodiester bond between the terminal 3' OH group and the α-phosphate group of the nucleotide being added.

In DNA sequencing, described in Fig T20.1, a single strand of DNA containing the segment of the unknown sequence is obtained from a double stranded recombinant DNA propagated in bacteria infected with a phage.

The single stranded DNA is mixed with an oligonucleotide (short chain of nucleotides) complementary to the known sequence of the plasmid flanking the unknown segment.

The oligonucleotide binds to the known sequence by base pairing and acts as a primer for the synthesis of a new strand of DNA using its 3' OH group.

Four reaction solutions are prepared. All four solutions contain the single strand of DNA and bound oligonucleotide primer, a mixture of 2'-deoxyribonucleoside triphosphates, an α-^{32}P labeled 2'-deoxynucleoside triphosphate, and the DNA polymerase.

^{32}P is the radioactive isotope of phosphorus. Incorporation of this isotopically labeled nucleotide into the synthesized DNA fragments allows one to later determine the position of the fragment on a gel by autoradiography.

In addition to the above components, each reaction solution contains one of the four 2',3'-dideoxynucleoside triphosphates, at a concentration about 1/10 that of the 2'-deoxynucleoside triphosphates.

When a 2',3'-dideoxynucleotide is added to the chain, chain growth is terminated because there is no 3'-OH group to which a subsequent nucleotide can be attached.

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