

You may have realised that normally, unless one cuts the vector and the source DNA with the same restriction enzyme, the recombinant vector molecule cannot be created.

Separation and isolation of DNA fragments : The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as **gel electrophoresis**. Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix. Nowadays the most commonly used matrix is agarose which is a natural polymer extracted from sea weeds. The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves. *Look at the Figure 11.3 and guess at which end of the gel the sample was loaded.*

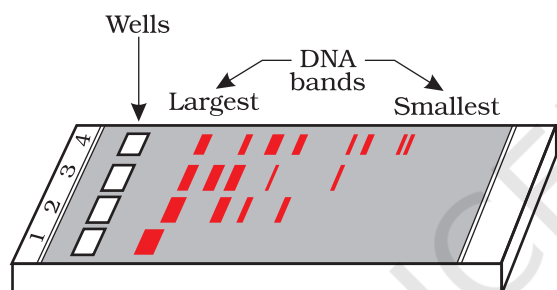


Figure 11.3 A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation (you cannot see pure DNA fragments in the visible light and without staining). You can see bright orange coloured bands of DNA in an ethidium bromide stained gel exposed to UV light (Figure 11.3). The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

11.2.2 Cloning Vectors

You know that plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA. Bacteriophages because of their high number per cell, have very high copy numbers of their genome within the bacterial cells. Some plasmids may have only one or two copies per cell whereas others may have 15-100 copies per cell. Their numbers can go even higher. If we are able to link an alien piece of DNA with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage. Vectors used at present, are engineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non-recombinants.