QUESTION UNDER CHALLENGE

Main steps in the formation of Recombinant DNA are given below. Arrange these steps in a correct sequence.

A Insertion of recombinant DNA into the host cell.
B Cutting of DNA at specific location by restriction enzyme.
C Isolation of desired DNA fragment.
D Amplification of gene of interest using PCR.

Choose the correct answer from the options given below:

(1) C A B D
(2) C B D A
(3) B D A C
(4) B C D A

Answer given in NTA provisional answer key of NEET 2023 is C B D A

But the correct answer should be B C D A

EXPLANATION WHY NTA ANSWER IS WRONG

As question has mentioned **Isolation of desired DNA fragment** and the same has been mentioned in **para 11.3; page 201; edition 2021 of XII NCERT** (A copy of NCERT page is attached herewith for ready reference). Following NCERT text clearly reveals that

Recombinant DNA technology involves several steps in specific sequence such as

A Isolation of DNA

B Fragmentation of DNA by restriction endonucleases

C Isolation of a desired DNA fragment

D Ligation of the DNA fragment into a vector

E Transferring the recombinant DNA into the host

F Culturing the host cells in a medium at large scale and extraction of the desired product.

Hence the correct answer following NCERT should be B C D A instead of C B D A as has been mentioned in key released by NTA.

The NCERT para 11.3 page 201 edition 2021 states that:

the bacterium through pores in its cell wall. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heat shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

This is not the only way to introduce alien DNA into host cells. In a method known as **micro-injection**, recombinant DNA is directly injected into the nucleus of an animal cell. In another method, suitable for plants, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as **biolistics** or **gene gun**. And the last method uses 'disarmed pathogen' vectors, which when allowed to infect the cell, transfer the recombinant DNA into the host.

Now that we have learnt about the tools for constructing recombinant DNA, let us discuss the processes facilitating recombinant DNA technology.

11.3 Processes of Recombinant DNA Technology

Recombinant DNA technology involves several steps in specific sequence such as isolation of DNA, fragmentation of DNA by restriction endonucleases, isolation of a desired DNA fragment, ligation of the DNA fragment into a vector, transferring the recombinant DNA into the host, culturing the host cells in a medium at large scale and extraction of the desired product. Let us examine each of these steps in some details.

11.3.1 Isolation of the Genetic Material (DNA)

Recall that nucleic acid is the genetic material of all organisms without exception. In majority of organisms this is deoxyribonucleic acid or DNA. In order to cut the DNA with restriction enzymes, it needs to be in pure form, free from other macro-molecules. Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells), **chitinase** (fungus). You know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as collection of fine threads in the suspension (Figure 11.5).

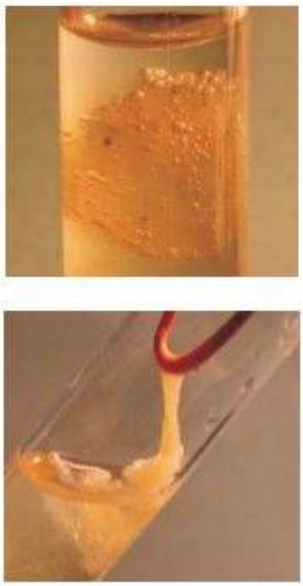

Figure 11.5 DNA that separates out can be removed by spooling