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Commentary

Procedure Involved in Recombinant DNA Technology

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DESCRIPTION

Recombinant DNA (rDNA) is a form of artificial DNA that's framed through the combination or insertion of one or further DNA strands, thereby combining DNA sequences that would not typically do together. In terms of inheritable modification, recombinant DNA is produced through the addition of applicable DNA into a living organismal genome, similar as the plasmid of bacteria, to code for or alter different traits for a specific purpose, similar as immunity. It differs from inheritable recombination, in that, it doesn't go through processes within the cell or ribosome, but is simply framed.

To make a recombinant organism, the gene of interest must first be separated from the original donator organism. To separate the gene, scientists use restriction enzymes, proteins that can be allowed of as molecular scissors that cut DNA at specific nucleotide sequences. The restriction enzymes cut the DNA on either side of the gene of interest. The DNA fraction containing the gene is also ligated (fused) into a different piece of DNA called a vector. The vector serves as a medium to carry the gene of interest into the host. It frequently includes fresh inheritable information similar as selectable markers and inheritable signals that control when and where it'll be expressed. The vector is also introduced into a single host cell. From this cell, an entire organism, plant or creature is grown.

The organism must be tested to make sure the gene is performing appropriately and the organism is exhibiting the desired particularity. Multiple generations are grown and tested before the crop, medication or sensor is made commercially available.

Seven main steps in recombinant DNA technology

- Isolation of the inheritable material (DNA)
- Cutting of DNA at specific locations
- Isolation of desired DNA fragment
- Amplification of gene of interest using PCR
- Ligation of DNA fraction into a vector
- Insertion of Recombinant DNA into the host cell/organism
- Obtaining or culturing the foreign gene product

1. Isolation of the inheritable material (DNA): Nucleic acid is the inheritable material, which is present in all living organisms. In majority of organisms, this is present in the form of Deoxyribo Nucleic Acid (DNA). DNA must be present into pure form, i.e., free from othermacro-molecules (like proteins, RNA, enzymes,etc.) in order to cut the DNA with restrictor enzymes.

2. Cutting of DNA at specific locations: Restriction enzymes disassemble foreign DNA by cutting it into fractions. This disassembling process is called restriction. Recombinant DNA technology relies on restriction enzymes to produce new combinations of genes. The cell protects its own DNA from disassembly by adding methyl groups in a process called modification. DNA ligase is a actually important enzyme that helps to join DNA strands together *via* covalent bonds.

3. Isolation of desired DNA fragment: Agarose gel electrophoresis can be used to assess the activity of the restriction enzymes. DNA tends to separate out in this process as it goes towards the positive electrode or anode since it is negatively charged. The desired DNA segment is then extracted.

4. Amplification of gene of interest using PCR: A DNA sequence can be copied numerous times using the DNA polymerase *in vitro* enzyme in a process known as Polymerase Chain Reaction (PCR). It is beneficial to increase the number of copies of DNA from one to millions. On thermal cyclers, PCR reactions are carried out utilising the following elements:

(i) DNA template that will be amplified.

(ii) Primers are short oligonucleotides that are chemically produced and complementary to a certain DNA sequence.

(iii) DNA polymerase nucleotides are required for the enzyme to lengthen the primers.

(iv) The cut vector can then be ligated with the amplified DNA fragments that have been produced using PCR.

5. Ligation of DNA fragment into a vector: A source DNA and a vector DNA are needed for this technique. Both of them should be cut using the same endonuclease to produce sticky ends. Then, to create the recombinant DNA/hybrid DNA, both are ligated by combining the vector DNA, the gene of interest,

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and the enzyme DNA ligase. Foreign DNA is not readily accepted by bacterial cells. In order to make them "capable" to take fresh DNA, they are therefore treated. The techniques utilised may include electroporation, thermal shock, and Ca++ ion therapy.

6. Insertion of Recombinant DNA into the host cell/ organisms: Numerous processes can make the recipient cells capable of receiving the DNA before this happens. When E.coli cells are exposed to rDNA containing a gene for resistance to an antibiotic (like ampicillin), the host cells change into ampicillin-resistant cells. In this instance, the ampicillin resistance gene is referred to as a selectable marker. Only converted cells will proliferate and the others will perish when cultivated on agar plates with ampicillin.

7. Obtaining or culturing the foreign gene product: A fragment of alien DNA multiplies when it is inserted into a cloning vector and introduced into a bacterial cell. The final goal is to express a desired protein. Understanding a lot of technical aspects is necessary for the expression of the foreign gene or genes in host cells. Recombinant protein is created when a protein-encoding gene is produced in a heterologous host. In the lab, the cells that contain the desired cloned genes are cultivated on a small scale. Using several separation procedures, the necessary protein is extracted from these cell cultures.