METHODS OF GENE CLONING

BY

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GENE CLONING:

"It is a molecular biological technique wherein exact copies of clones of a particular gene or DNA sequence are produced using the principles of genetic engineering".

Gene cloning:

- The DNA containing the target genes is segregated into fragments with the help of restriction enzymes.
- These fragments are then inserted into the cloning vectors, namely the bacteriophages or bacterial plasmids that transfer the recombinant DNA to appropriate host cells; one such is the bacterium E.coil.
- On the other hand, the complementary DNA is inserted into the naked DNA fragments or vectors that can be taken up by the bacterium directly from its medium

- In gene cloning, the major steps are cutting and joining lengths of DNA using restriction endonuclease and ligase.
- Several steps are involved in gene cloning.
- The following points highlight the seven main steps involved in gene cloning.some of the steps are:

1. Isolation of DNA (gene of interest) fragments to be cloned.
2. Insertion of isolated DNA into a suitable vector to form the recombinant DNA.

3. Introduction of recombinant DNA into a suitable organism known as host and other steps too.

4.Selection of the transformed host cells and identification of the clone containing the gene of interest.

5.Multiplication/ expression of the introduced gene in the host.

6.Isolation of the multiplied gene copies/protein expressed by the introduced gene.

Step1:Isolation of DNA(Gene of interest) fragments to be cloned

1. Before we carryout operation of gene coning we need two basic things in their purified state- the gene of our interest(GI) and the vector.
2. A GI is a fragment of gene whose product(a protein,enzyme or a hormone) interests us.
3. For e.g gene encoding for the hormone insulin.
4. Similarly, the vector is a carrier molecule which can carryour GI into a host, replicate there along with the GI making its multiple copies.
5. In this state the GI can also beexpressed in the host cell producing the product of the gene which is needed by us.

- Step-2: Insertion of isolated DNA into a suitable vector to form a recombinant DNA:

1. Our next step will be to cut both the vector and GI by restriction endonucleases(RE)
2. A {R.E} is an enzyme that cuts the ds or ssDNA at specific recognition nucleotide sequences known as rrestriction sites towards the inner region(hence endonuclease)
3. They are also regarded as molecular scissors as the cut open the DNA strands.
4. After this cutting step we move to pasting.

5. Here the GI is taken and pasted to the cut vector.

6. This procedure also needs an enzyme called as DNA ligase.They are also cosidered as molecular glue.

7.The resulting DNA moleculeis a hybrid of two DNA molecules- our GI and the vector.

8. In the terminology of genetics this intermixing of different DNA strands is called recombination.

9.Hence, this new hybrid DNA molecule is called a recombinant DNA molecule and this technology is called recombinant DNA technology.

Step-3: Introduction of recombinant DNA into a suitable organism called host:

1. When our recobinant DNA is ready we need to introduce it into suitable host(a living system)
2. This is done either for one or both of the following reasons:
3. To replicate the recombinant DNA ,molecule in order to get the multiple copies of our GI.
4. To let our GI get express and produce the protein which is needed by us.
5. Introduction of recombinant DNA into the hoost cell is done by various ways and strictly depends on the size of DNA molecule and nature of GI.

6.Some of the methods followed to carry out this step includes electroporation, microinjection, lipofection etc.

7.When we carry out this process some of the host cells will take up the recombinant DNA and some will not.

8.The host cells which have taken up the recombinant DNA are called transformed cells and the process is called transformation.

Step-4: Selection of the transformed host cells and identification of the clone conatining the GI:

1. The transformation prcess generates a mixed population of transformed and non-transformed host cells.
2. As we are interested only in transformed host cells it becomes necessary to filter them out.
3. This is exactly what is done in the selection process.
4. There are many existing selection strategies some of which include taking the help of reporter genes, colony hybridization technique.

Step-5: Multiplication/Expression of the introduced gene in the host:

1. Once we have purified our transformed host cells by the screening process; it is our job to provide the optimum prarmeters to grow and multiply.
2. In this step the transformed cells are introduced into fresh culture media which provide them roch nourishment followed by an incubation at right temperature.
3. At this stage the host cells divide and re-divide along with the replication of recombinant DNA carried by them.Now at this point we have 2 choices

4.When te aim of the cloning process is to generate a gene library, then our target will be obtaining numerous copies of GI. So with this plan in our mind we will simply go with the reeplication of recombinant DNA and not beyond that.

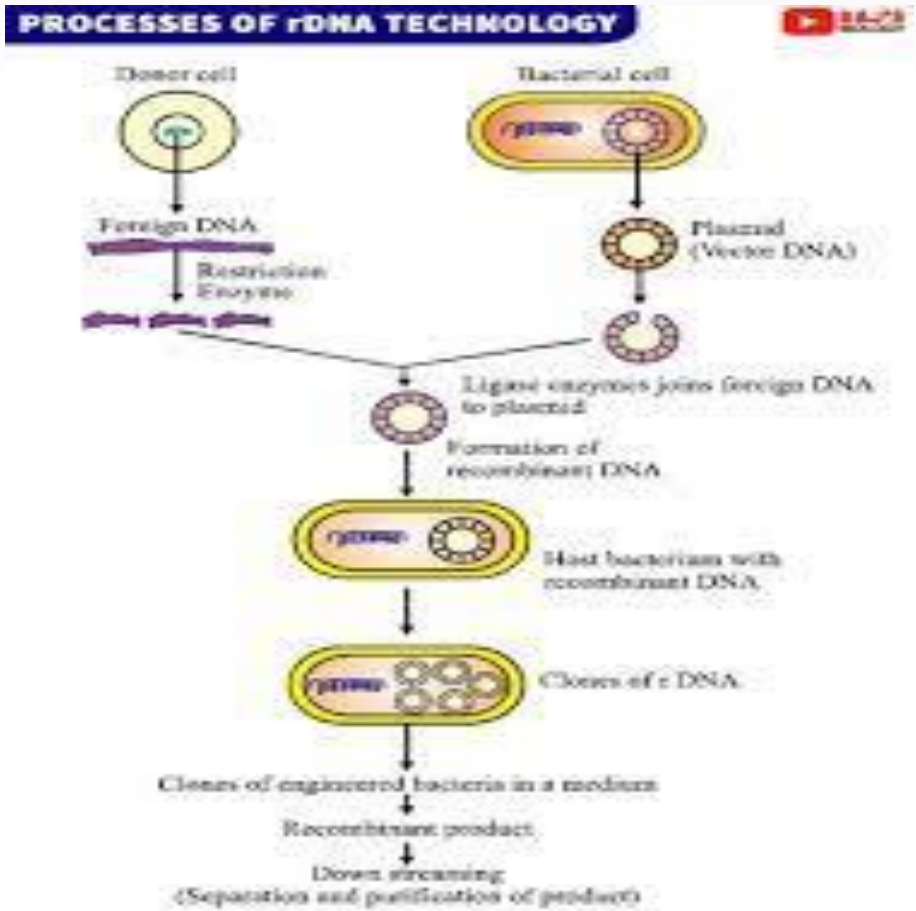
5. If the aim of cloning exp is to obtain the product of GI, then we will go a step ahead where we will provide favourable conditions to the host cells in which the GI sitting in the vector can express our product of interest.(PI)

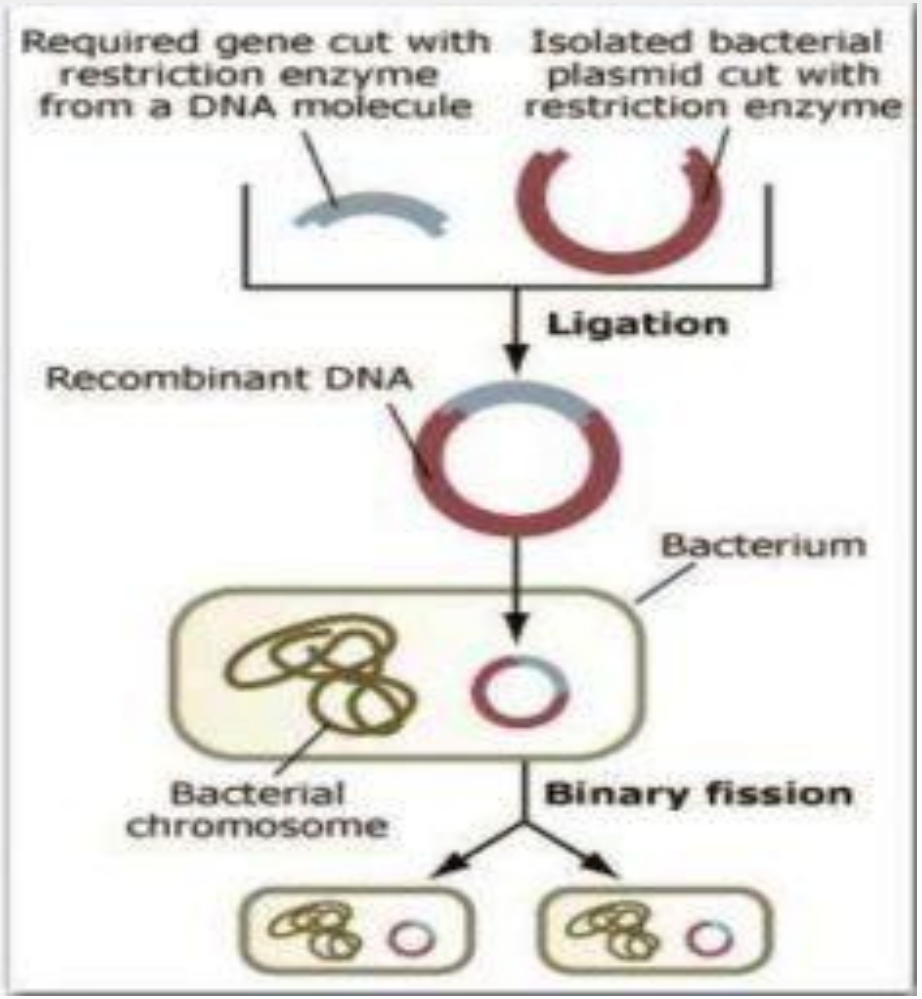
step- 6: Isolation of multiplies gene copies/ protein expressed by the introduced gene:

1. In this step we isolate our multiplied GI which is present attached with the vector or the protein encoded by it.
2. This can be rightly compared with the process of harvesting where we collect the crop from the field.
3. There are many processes of isolation, the selection of which varies from case to case.

Step-7: Purification of the isolated gene copy/protein:

After the harvesting of the isolated gene copy or the protein it is now our job to purify.


PROCESSES OF rDNA TECHNOLOGY
Donor cell
Bacterial cell
Foreign DNA
Restriction Enzyme
Plasmid (Vector DNA)
Ligase enzymes joins foreign DNA to plasmid
Formation of recombinant DNA
Host bacterium with recombinant DNA
Clones of r DNA
Clones of engineered bacteria in a medium
Recombinant product
Down streaming (Separation and purification of product)


Required gene cut with restriction enzyme from a DNA molecule
Isolated bacterial plasmid cut with restriction enzyme
Ligation
Recombinant DNA
Bacterium
Bacterial chromosome
Binary fission