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Programme : M.Sc Biotechnology
Course Title : Genetic Engineering
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Unit -4
Cloning Strategies

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Steps in Gene cloning

Gene cloning Steps

Gene cloning is the process of making identical copies of a gene or DNA fragment. It involves several key steps, which are summarized below:

1. Isolation of DNA

•Source DNA: The first step is to isolate the DNA that contains the gene of interest. This is usually done from a specific organism, such as human, bacteria, or plants.

•Extraction: The DNA is extracted from cells using a combination of chemical and mechanical techniques.

2. Cutting DNA with Restriction Enzymes

•Restriction Enzymes: These are molecular scissors that cut DNA at specific sequences. The gene of interest is excised from the source DNA using these enzymes.

•Create Sticky or Blunt Ends: The restriction enzymes may generate "sticky" ends (single-stranded overhangs) or blunt ends, which are needed for ligation.

Gene cloning Steps

3. Inserting the Gene into a Vector

•**Vectors**: A vector is a DNA molecule used to carry the gene of interest into a host cell. Common vectors include plasmids (circular DNA molecules) or viruses.

•**Ligation**: The gene of interest is inserted into the vector using another enzyme called **DNA ligase**, which seals the gene into the vector's DNA.

4. Transformation of Host Cells

•**Transformation**: The recombinant vector (with the gene inserted) is introduced into a host cell, usually a bacterium (like *E. coli*), yeast, or mammalian cells.

•This can be done through methods like **heat shock**, **electroporation**, or **viral transfection**.

•The host cell now carries the recombinant DNA and is ready to replicate it.

Gene cloning Steps

5. Selection of Transformed Cells

•**Selection Markers**: Most vectors contain a selectable marker gene, such as an antibiotic resistance gene. Only the cells that successfully took up the vector will survive when exposed to the corresponding antibiotic.

•**Screening**: Additional methods like colony PCR, restriction digestion, or sequencing can confirm that the correct gene is inserted into the host cells.

6. Cloning and Expression of the Gene

•**Gene Expression**: In some cases, the cloned gene may be expressed in the host cells, meaning that it is transcribed and translated into the corresponding protein. This step often involves adding specific inducers or conditions to stimulate gene expression.

•**Harvesting Protein**: If the goal is to produce a protein, the host cells are cultured in large quantities, and the protein is purified for further use.

Gene cloning steps

7. Analysis of Clones

•Verification: To ensure the gene was successfully cloned, the presence of the insert is verified using techniques like PCR, restriction enzyme digestion, or DNA sequencing.

•Characterization: The final product (either the cloned gene or the protein) can be further characterized to confirm its identity and functionality.

8. Amplification of Clones

•The host cells containing the cloned gene can be cultured in large quantities to amplify the gene or protein for further research, industrial applications, or therapeutic use.

These steps form the core of gene cloning, allowing scientists to produce large quantities of genes or proteins for study or use in biotechnology applications.

Thank you