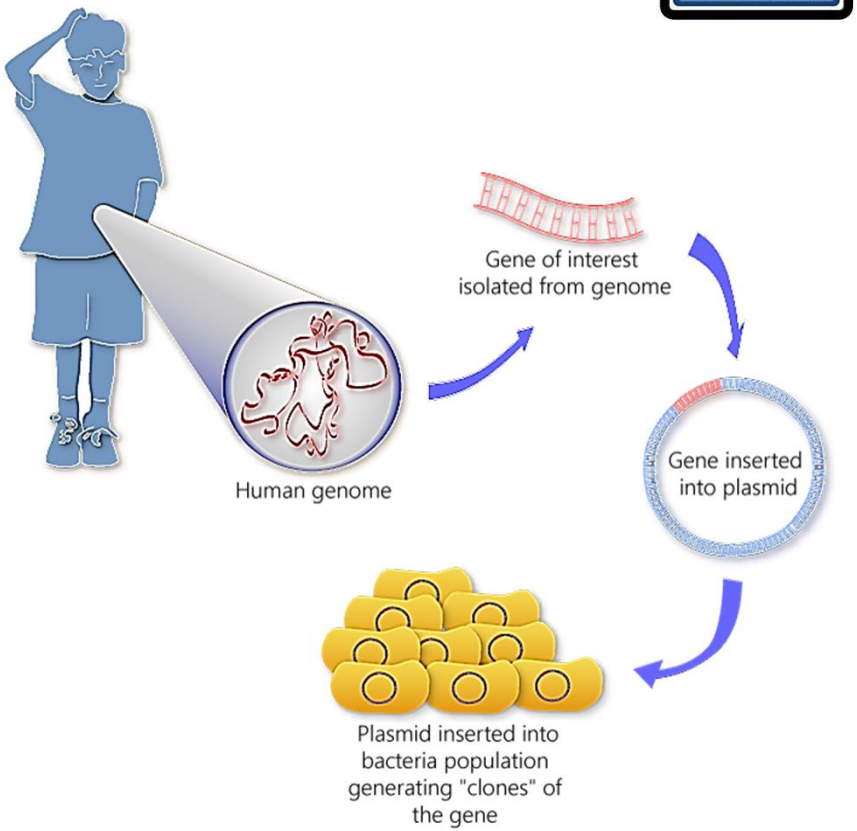
University of Duhok

College of Sciences

Department of Biology

4th Year Class

Lecture4. Gene Cloning



Lecture outlines:

- What is gene cloning?
- Types of cloning technology
- Requirements for Gene Cloning.
- Goals of gene cloning
- Gene cloning steps.
- Importance of DNA cloning

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What is gene cloning?

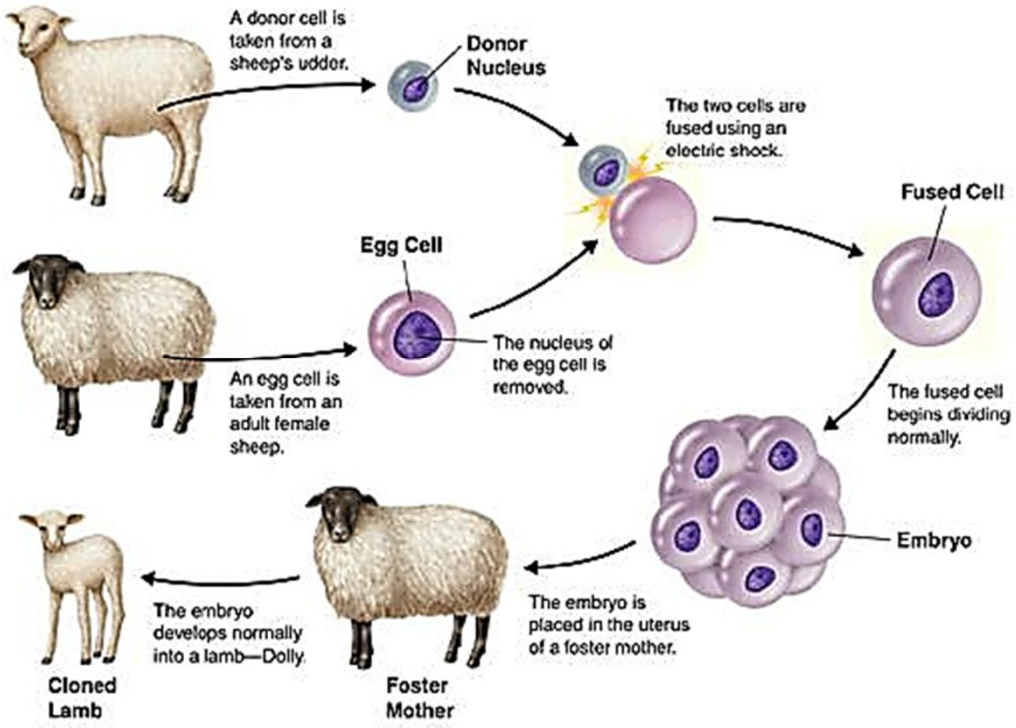
- The production of exact copies of a particular gene or DNA sequence using genetic engineering techniques is called gene cloning.
- In biotechnology, the process of producing multiple identical copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms is referred to as cloning.
- A clone has an exact genetic imprint as that of the original cell, tissue or organism.
- The term “gene cloning,” “DNA cloning,” “molecular cloning,” and “recombinant DNA technology” all refer to same technique.
- In gene (DNA) cloning a particular gene is copied forming “clones”.
- Cloning is one method used for isolation and amplification of gene of interest.

Types of cloning technology

- There are different types of cloning technologies used for various purposes besides producing the genetic copy of an organism. Basically the cloning technology can be divided into three types:

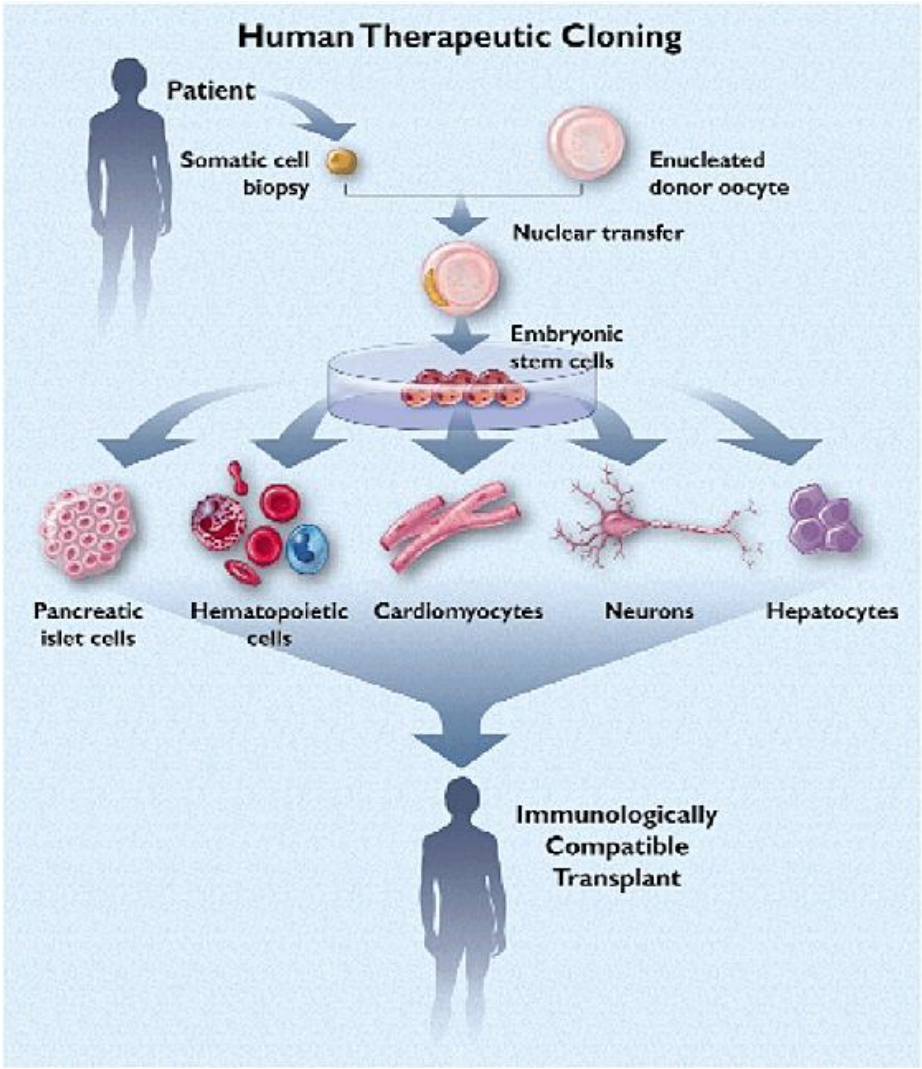
1. Reproductive cloning:

- Is a technology used to generate a twin of an animal that is genetically same as another currently or previously existing animal.
- The best example for reproductive cloning is Dolly, the first cloned sheep.



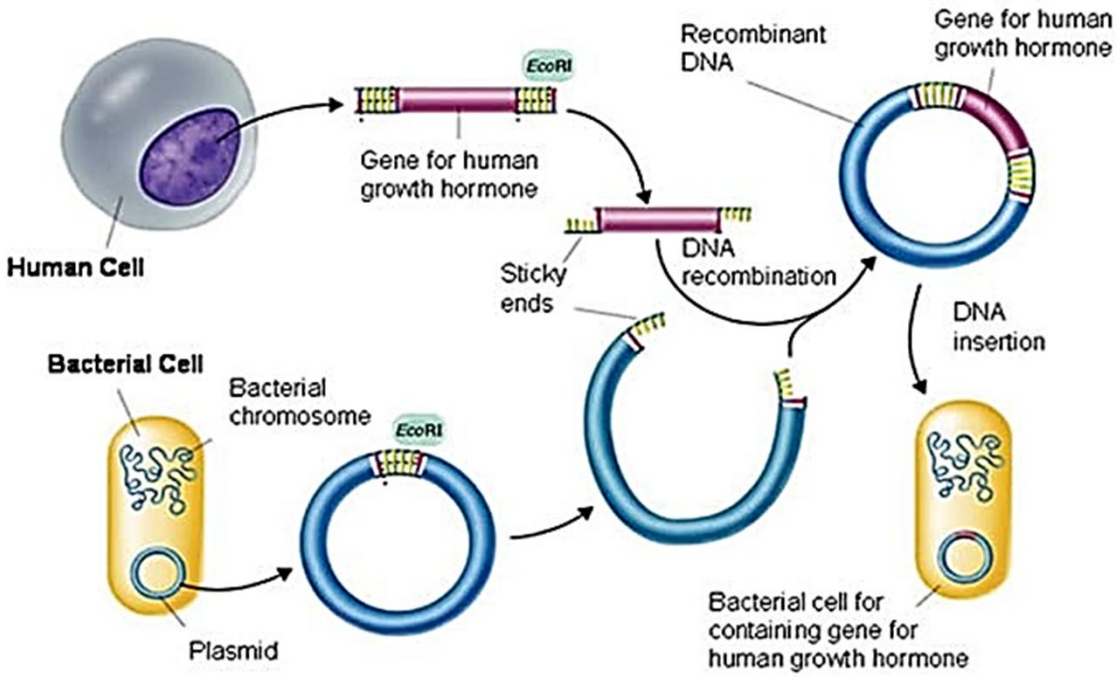
2. Therapeutic cloning:

- Is also known as “embryo cloning,” is production of human embryos for use in research and treatment of diseases. The aim of this technique is not human cloning, but rather to harvest stem cells that are used for research studies and to treat diseases.



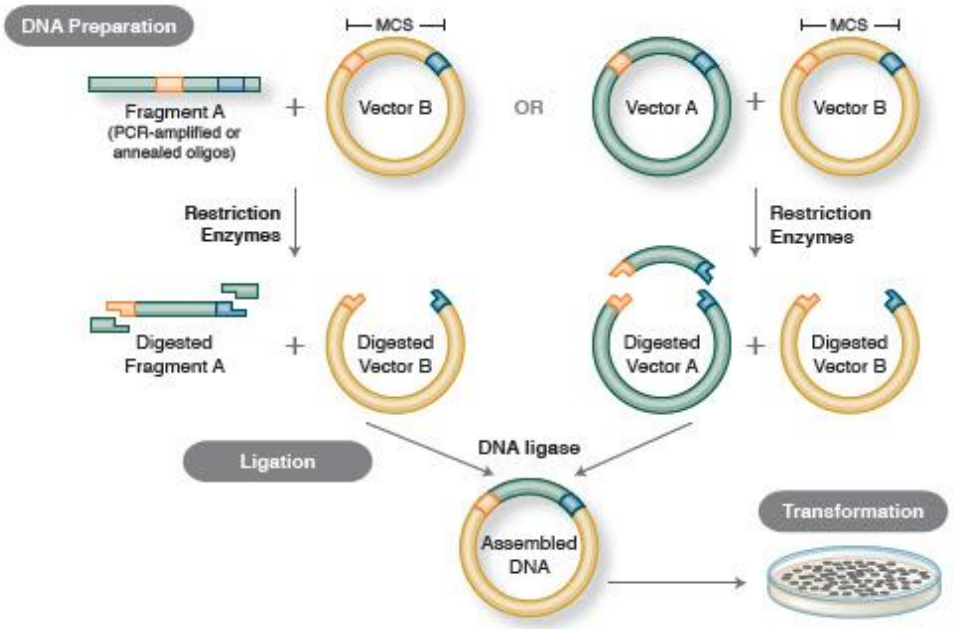
3. DNA cloning or Recombinant DNA technology:

- Is the most widely used cloning technique in biotechnology that makes many identical copies of a piece of DNA, such as a gene.



Requirements for Gene Cloning

1. **DNA fragment:** containing the desired genes to be cloned.
2. **Restriction enzymes:** to Cut of DNA and a vector.
3. **ligase enzymes:** to join DNA and a vector.



4. **Vectors:** to carry, maintain and replicate cloned gene in host cell.
5. **Host cell**: in which recombinant DNA can replicate.

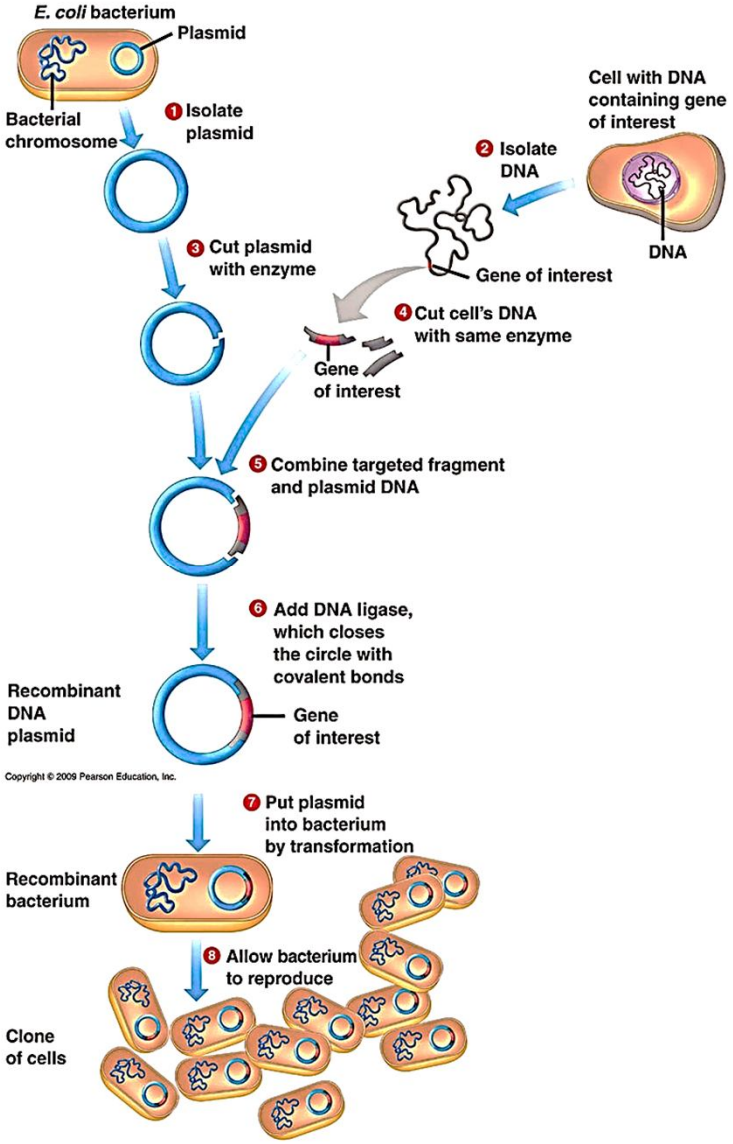
Goals of gene cloning

- To isolate and characterize a gene.
- To make desired alterations in one or more isolated genes.
- To return altered genes to living cells.
- Artificially synthesize new gene.
- Alternating the genome of an organism.
- Understanding the hereditary diseases and their cure.
- Improving human genome.

Steps in Gene Cloning

The basic 7 steps involved in gene cloning are:

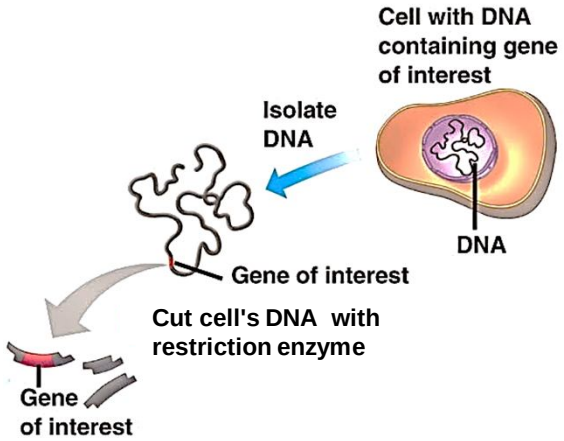
1. Isolation of DNA fragments (gene of interest) to be cloned.
2. Insertion of isolated DNA into a suitable vector to form recombinant DNA.
3. Introduction of recombinant DNA into a suitable organism known as host.
4. Selection of transformed host cells and identification of the clone containing the gene of interest.
5. Multiplication/Expression of the introduced gene in the host.
6. Isolation of multiple gene copies/Protein expressed by the gene.
7. Purification of the isolated gene copy/protein.



Steps in Gene Cloning

1. Isolation of the DNA fragment or gene

- The target DNA or gene to be cloned must be first isolated. A gene of interest is a fragment of gene whose product (a protein, enzyme or a hormone) interests us. For example, gene encoding for the hormone insulin.
- The desired gene may be isolated by using restriction endonuclease (RE) enzyme, which cut DNA at specific recognition nucleotide sequences known as restriction sites towards the inner region (hence endonuclease) producing blunt or sticky ends or the fragment of interest may be amplified by PCR.



- Sometimes, reverse transcriptase enzyme may also be used which synthesizes complementary DNA strand of the desired gene using its mRNA.

Steps in Gene Cloning

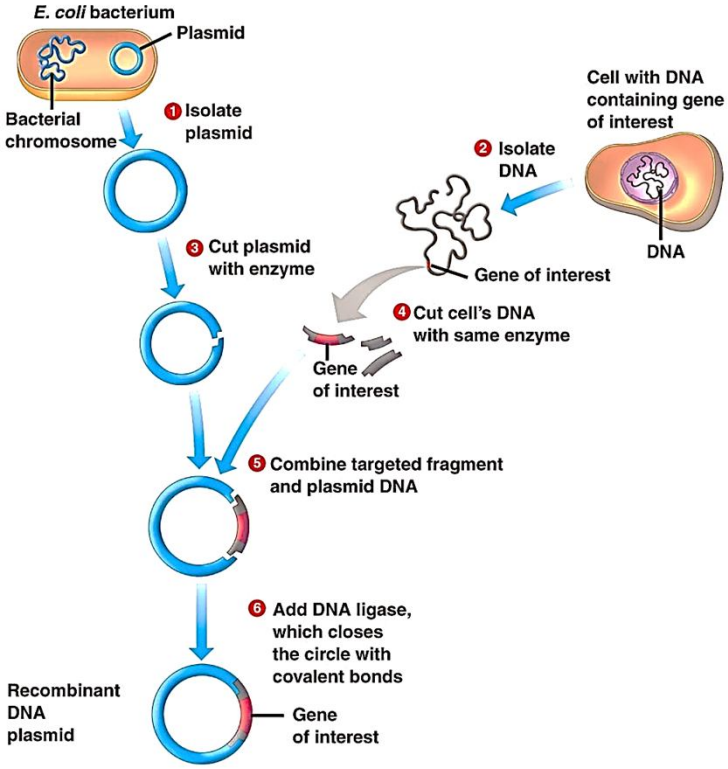
2. Selection of suitable cloning vector

- The vector is a carrier molecule which can carry the gene of interest (GI) into a host, replicate there along with the GI making its multiple copies.
- The cloning vectors are limited to the size of insert that they can carry. Depending on the size and the application of the insert the suitable vector is selected.
- The different types of vectors available for cloning **are plasmids, bacteriophages, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs)** and **mammalian artificial chromosomes (MACs).**
- However, the most commonly used cloning vectors include plasmids and bacteriophages (phage λ) beside all the other available vectors.

Steps in Gene Cloning

3. Formation of Recombinant DNA

- The plasmid vector is cut open by the same RE enzyme used for isolation of DNA fragment.
- The mixture of DNA fragment (gene of interest) and plasmid vector are mixed together.
- In the presence of DNA ligase, base pairing of donor DNA fragment and plasmid vector occurs.
- The resulting DNA molecule is a hybrid of two DNA molecules the GI and the vector. In the terminology of genetics this intermixing of different DNA strands is called recombination.

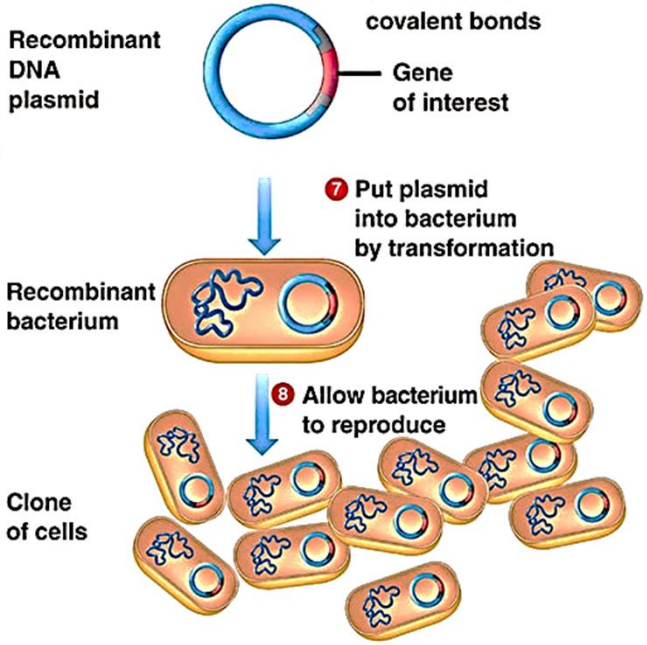


- Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the **recombinant DNA technology**.

Steps in Gene Cloning

4. Transformation of recombinant vector into suitable host

- The recombinant vector is transformed into suitable host cell mostly, a bacterial cell.
- This is done either for one or both of the following reasons:
  - To replicate the recombinant DNA molecule in order to get the multiple copies of the GI.
  - To allow the expression of the GI such that it produces its needed protein product.
- Some bacteria are naturally transformable; they take up the recombinant vector automatically.
- For example: *Bacillus*, *Haemophillus*, *Helicobacter pylori*, which are naturally competent.

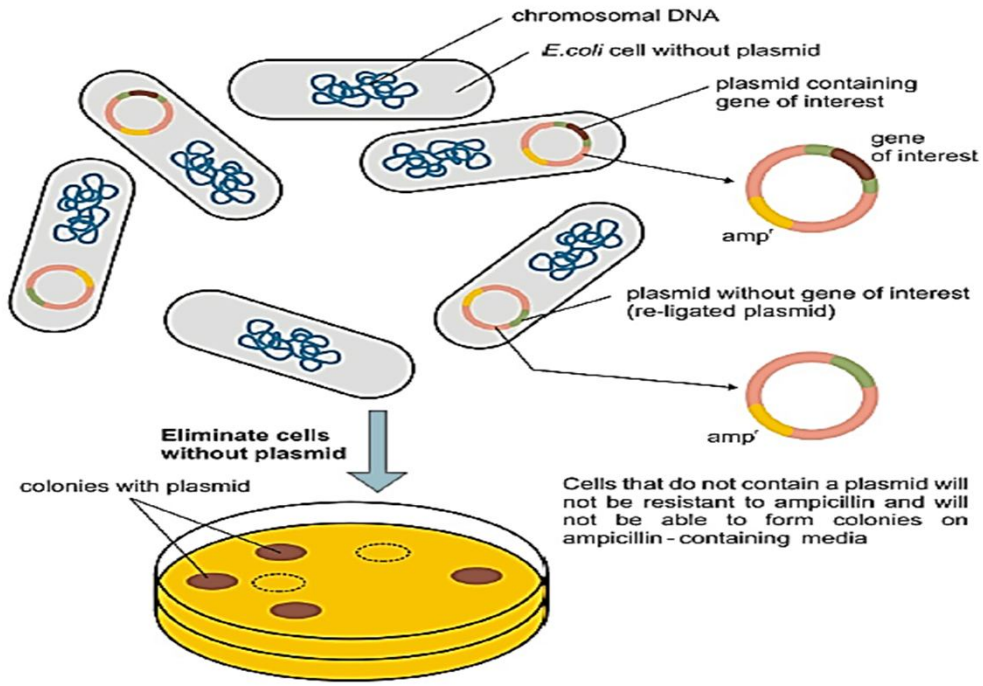


- Some other bacteria, on the other hand require the incorporation by artificial methods such as Ca^{++} ion treatment, electroporation, etc.

Steps in Gene Cloning

5. Selection of Recombinant Cells

- The transformation process generates a mixed population of transformed and non-transformed host cells.
- The selection process involves filtering the transformed host cells only.
- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.

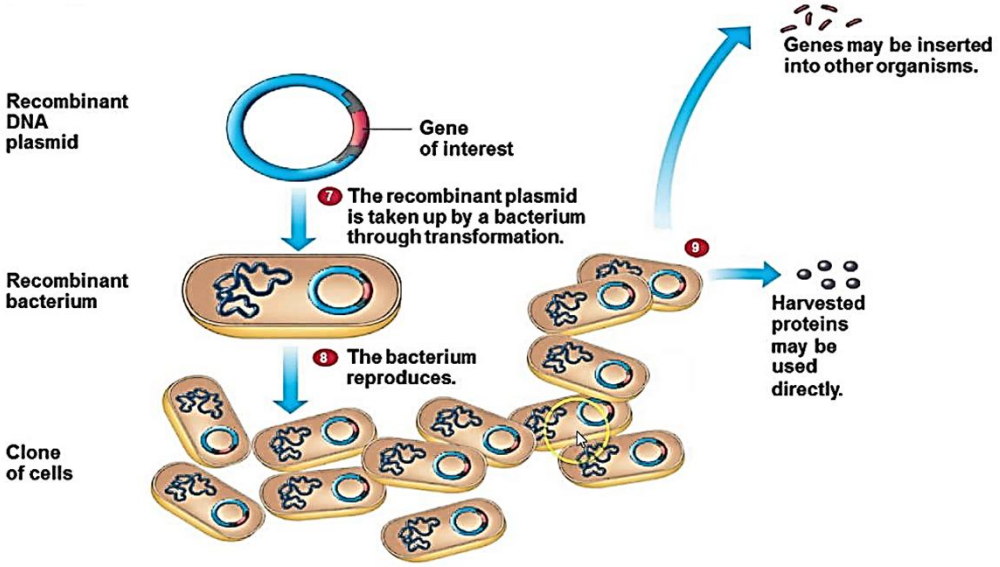


- For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.

Steps in Gene Cloning

6. Multiplication of Selected Host Cells

- Once transformed host cells are separated by the screening process; becomes necessary to provide them optimum parameters to grow and multiply.
- In this step the transformed host cells are introduced into fresh culture media .
- At this stage the host cells divide and re-divide along with the replication of the recombinant DNA carried by them.



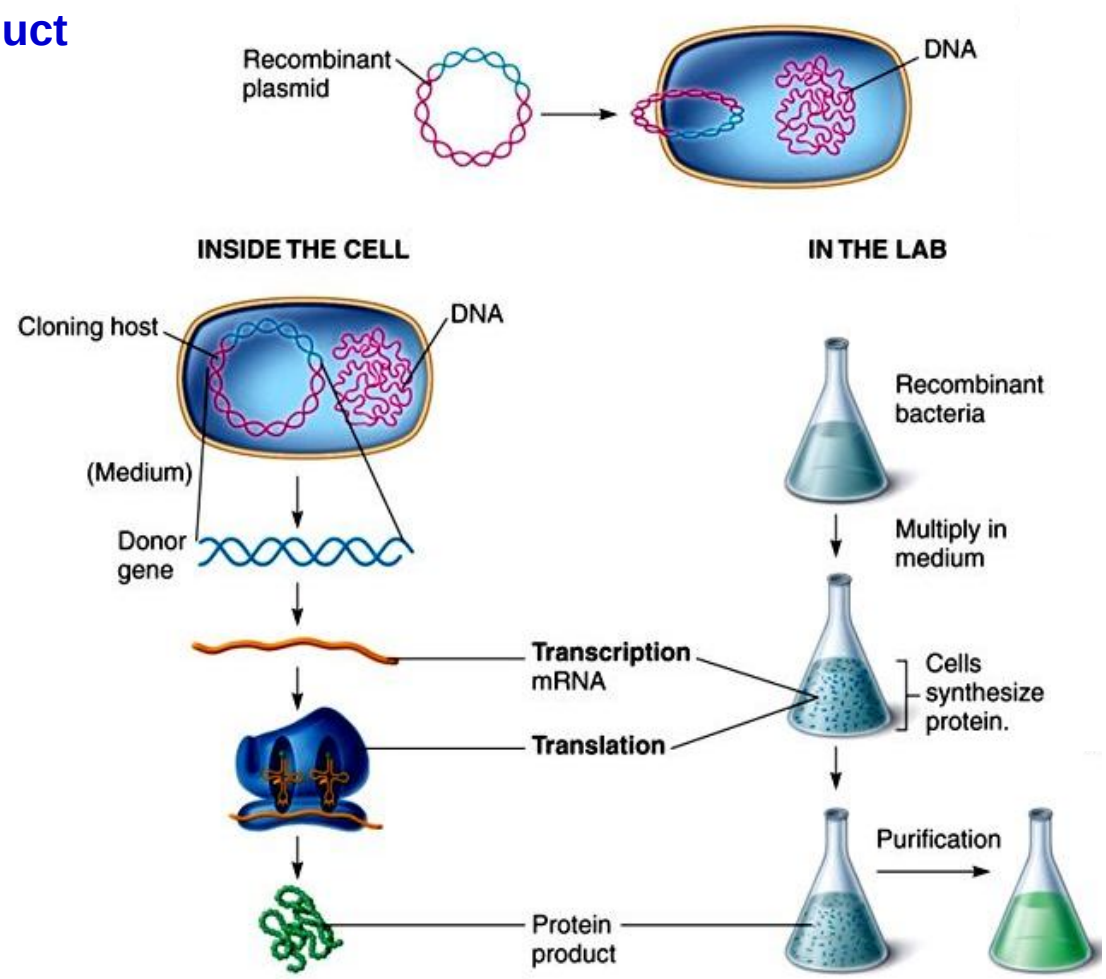
- If the aim is obtaining numerous copies of **GI**, then simply replication of the host cell is allowed.
- But for obtaining the product of interest, favorable conditions must be provided such that the **GI** in the vector expresses the product of interest.

Steps in Gene Cloning

7. Isolation and Purification of the Product

•The next step involves isolation of the multiplied **GI** attached with the vector or of the protein encoded by it.

•This is followed by purification of the isolated gene copy/protein.



Why gene cloning is important?

1. It is the only way or method that can be used to isolate and/or separate a single gene.
2. This will allow the possibility to multiply a single gene into large quantities or amounts, so you can work with and study the gene.
3. It allows the transfer of a single gene into an individual like introducing the insulin gene.
4. It allows overcoming the genetic barriers.