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GENE CLONING: CONCEPT & BASIC STEPS

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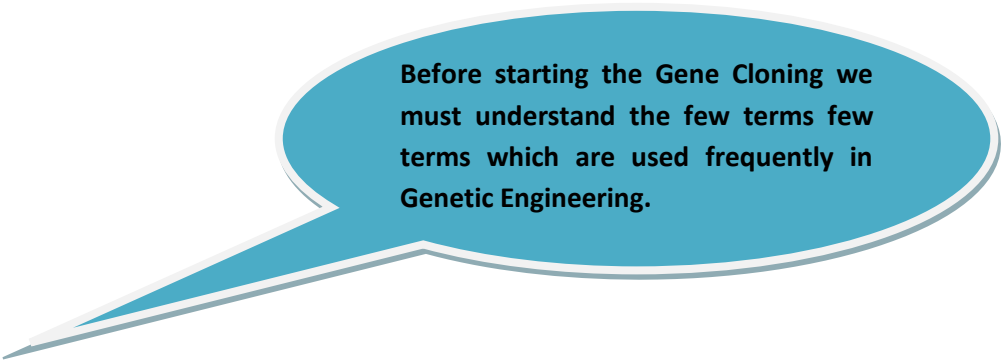
GENE CLONING: CONCEPT & BASIC STEPS



So the very first thing is what is a Gene?

So, a gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Some genes transcribe and later translate into fucntional proteins. However, many genes do not code for proteins.

Now the question comes what is a clone?

The identical copy of any entity is clone, one which is exactly same not similar.

But what is the meaning of clone in Biological aspect?

a) The word "clone" has several different meanings in biology. As a noun, a clone is an identical genetic copy of a piece of deoxyribonucleic acid (DNA), a cell, or a whole organism. Identical twins are clones, as they are two daughter cells produced by mitosis.

b) A propagating population of organisms, either single cell or multicellular, derived from a single progenitor cell is also clones of each other.

How these clones are created

The clones are created by the process of creating an accurate copy of a biological entity (e.g. a DNA sequence, cell, or organism) from the same it was derived is referred to as cloning. Cloning occurs naturally in the plant that reproduces through asexual reproduction (e.g. apomixis) brings about copies of genetically-identical plants. Bacterial cell that divides through binary fission produces clones that are genetically the same as that of the parent cell. Animals capable of parthenogenesis produce clonal offspring. Clones can also be produced through artificial means. Biotechnological methods are employed to produce such clones. Molecular cloning is employed to make the copies of specific gene fragment. Cellular cloning is carried out to produce single-celled organisms with the exact genetic content of the original cell are produced in cell cultures. Reproductive cloning is done to create a multicellular clone

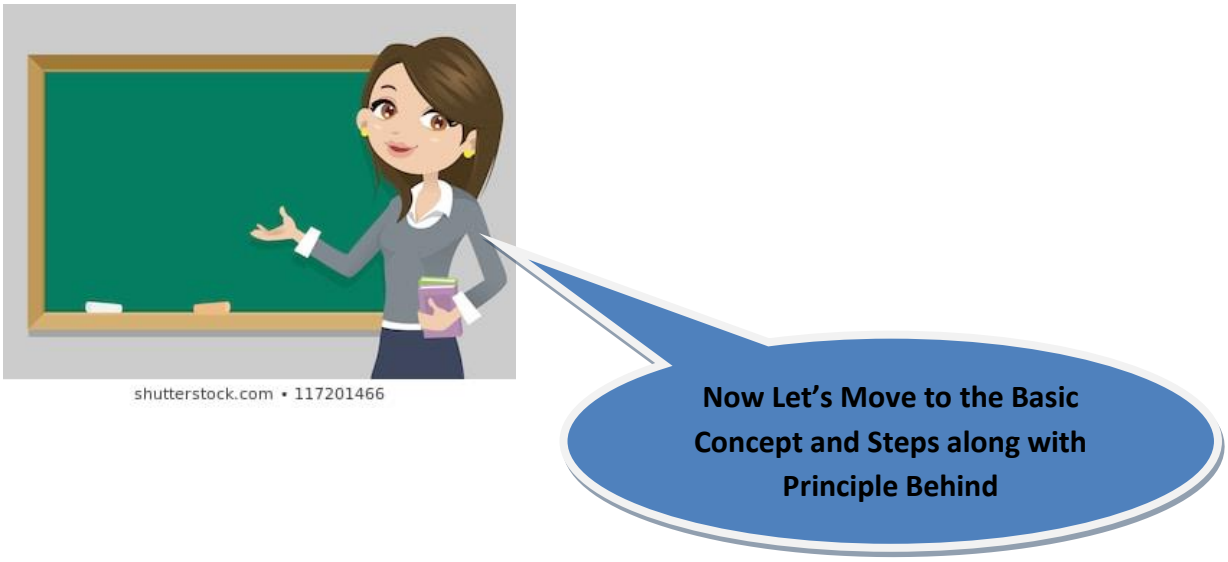
or a whole organism through somatic cell nuclear transfer**. (Word origin: Ancient Greek klṓn ("twig")**

What is a Recombinant DNA Technology (RDT) and how it is related to Genetic Engineering?

So in simple terms all the techniques involved in the construction, study and use of recombinant DNA molecules is RDT where as Genetic engineering is a term that was first introduced into biological world in the 1970s to describe the emerging field of recombinant DNA technology. Genetic engineering, broadly defined, how we are taking pieces of DNA and combining them with other pieces of DNA by engineering in your own labs. And then collecting what we have engineered and propagating that in number of different organisms that range from bacterial cells to yeast cells, to plants and animals

Few other terms that are related to this area are Recombinant, Recombination and Chimera.

A transformed genetic material that contains a DNA molecule formed by combination of 2 -3 different origins is a recombinant (Chimera)**.** The exchange of DNA sequences between different molecules occurring either naturally or by use of technology is recombination and A recombinant DNA molecule made up of DNA fragments from more than one organism, named after the mythological beast Chimera. The initial product of cloning using embryonic stem cells: an animal made up of a mixture of cells with different genotypes.



Genetic engineering is the process that uses recombinant DNA (rDNA) technology to alter the genetic makeup of an organism. Ingegenoiusly humans influenced genomes

indirectly by controlled breeding and selecting offspring with desired traits. Genetic engineering involves the direct manipulation and alteration of one or more genes. Generally, a gene from another species is added to an organism's genome to confer a desired phenotype.

History behind it

In the mid of the 19th century, Gregor Mendel with set of rules explained the inheritance of biological characteristics. He called each heritable property of an organism is controlled by a factor, which is later called as a gene. The rediscovery of Mendel's laws in 1900 marks the birth of genetics, the science aimed at understanding what these genes are and exactly how they work.

The genes reside on chromosomes was proposed by W. Sutton in 1903 along with experimental backing from T.H. Morgan and his co-workers in 1910 as they developed the techniques for gene mapping, and by 1922 had produced a comprehensive analysis of the relative positions of over 2000 genes on the 4 chromosomes of the fruit fly, *Drosophila melanogaster*. Before experimental proofs of Avery, MacLeod, and McCarty in 1944, and of Hershey and Chase in 1952, hardly anyone believed that deoxyribonucleic acid (DNA) is the genetic material.

Delbrück, Chargaff, Crick, and Monod were among the most influential contributed to the second great age of genetics. In the 14 years between 1952 and 1966, the structure of DNA was elucidated, the genetic code cracked, and the processes of transcription and translation described.

According to DNA folklore, Kary Mullis invented the polymerase chain reaction (PCR) during a drive along the coast of California one evening in 1985.

Principle of Gene Cloning

A fragment of DNA, containing the desired gene to be cloned, is integrated into a suitable vector, to generate a recombinant DNA molecule. The vector acts as a vehicle that transports the gene into a host cell generally a bacterium (Host Cell), although other types of living cell are also in use in which this vector multiplies, producing multiple identical copies not only of itself but also of the gene that it carries. The division in host cells, copies of the recombinant DNA molecule and this passed to the progeny and further vector replication takes place. The clones of identical cells are produced after the large number of cell divisions. Each new cell

in the colony contains one or more copies of the recombinant DNA molecule; the gene integrated in the recombinant molecule is now said to be cloned.

Gene Cloning- Requirements, Principle, Steps, Applications

- The production of accurate copies of a specific gene sequence by the use of genetic engineering techniques is called gene cloning.
- This technique is also known as recombinant DNA technology, gene cloning, and DNA cloning.
- During the extraction of DNA from any organism all of the genes are isolated while in gene cloning only a specific gene is copied to form clones.
- Gene cloning technology is an important method used for specific gene sequence isolation and amplification.

Gene cloning can be achieved by following two different methods:

1. Cell based DNA cloning
2. Cell-free DNA cloning (PCR)

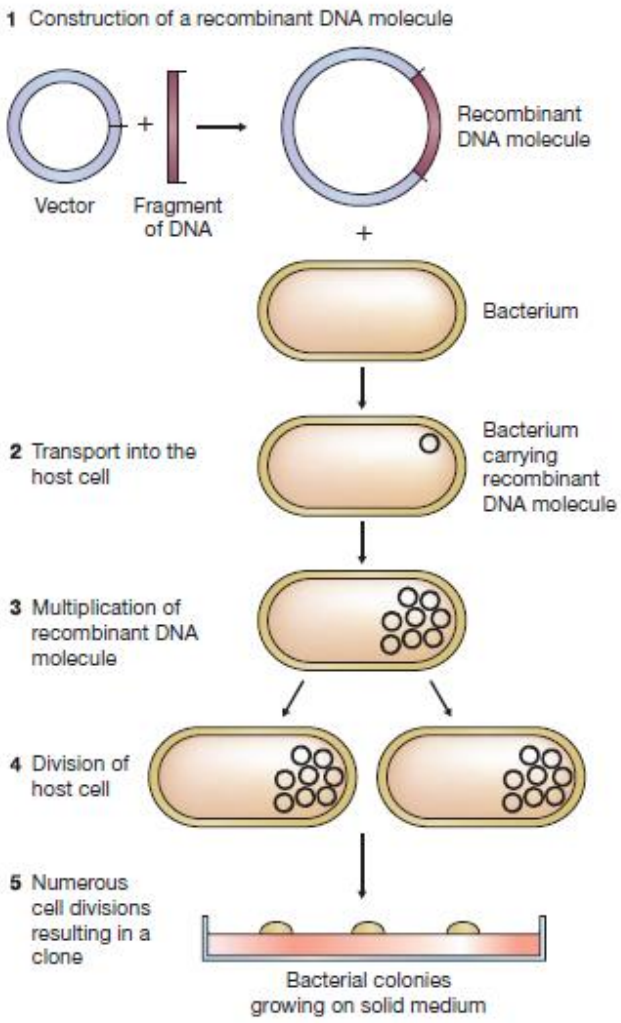
Requirements for Gene Cloning (Cell-based)

1. **DNA fragment** containing the desired genes to be cloned.
2. **Restriction enzymes** and **ligase enzymes**.
3. **Vectors** – to carry, maintain and replicate cloned gene in host cell.
4. **Host cell**– in which recombinant DNA can replicate.

Gene cloning involves following 7 essential steps:

1. Isolation of specific DNA fragment containing gene of interest which is 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 and Expression of the introduced Gene inside host.
6. Isolation of multiple gene copies/Protein expressed by the gene.
7. Purification of the isolated gene copy/protein



A. Isolation of the DNA fragment or gene

- The very first step is isolation of target DNA or gene fragment to be cloned. 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.

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

B. 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.
- Diverse range of vectors available for gene 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.

C. Essential Characteristics of Cloning Vectors

The cloning vectors are carrier of GoI (DNA molecules). These vectors molecules have few common features such as:

- They must be self-replicating inside host cell.
- They must possess a unique restriction site for restriction enzymes.
- Introduction of GoI must not interfere with self replication of the vector.
- They must possess some selectable marker gene such that can be used for later identification of recombinant cell/ transformed cell (usually an antibiotic resistance gene that is absent in the host cell).
- They should be easily isolated from host cell.

D. Formation of Recombinant DNA

- The plasmid vector should cut open by the same restriction enzyme that is used for isolation of GoI form DNA fragment.
- The GoI DNA fragment and plasmid vector should mixed together.

- In the presence of DNA ligase, base pairing of GoI DNA fragment and plasmid vector should take place.
- The resulting DNA molecule is a hybrid of two DNA molecules – the GoI and the vector. In the terminology of genetics this is called **recombination**.
- Therfore, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the **recombinant DNA technology**.

E. Transformation of recombinant vector into suitable host

- The recombinant generated is transformed into suitable host cell, bacterial cell generally.
- This is done may be for the following reasons:
- To replicate the recombinant DNA molecule in order to get the multiple copies of the GoI.
- To allow the expression of the GoI such that it produces its needed protein product.
- Some bacteria are natural transformers; they do the uptake the recombinant vector automatically. For example: *Bacillus*, *Haemophillus*, *Helicobacter pylori*, which are naturally competent bacterial cells.
- On the other hand some bacteria require the incorporation by artificial methods such as Ca^{++} ion treatment, electroporation, PEG etc.

F. Isolation of Recombinant Cells

- The transformation generates both transformed and non-trans- formed host cells (a mixed population of cells in colony).
- The selection of transformed cells involves filtering the useful ones only.
- The selectable marker gene of plasmid used as vector is used for isolation or selection of recombinant cell from non-recombinant cell.
- For examples, pBR322 most common plasmid vector contains two selectable marker gene (Ampicillin resistant gene and Tetracycline resistant gene). When pst1 RE is

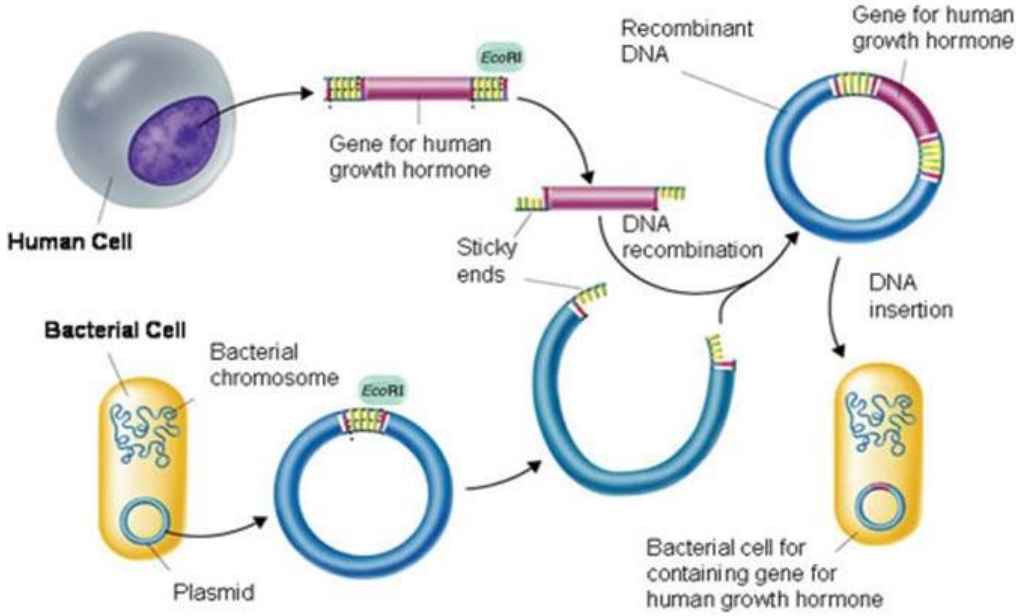
used it knock out Ampicillin resistant gene from the plasmid, so that the transformed cell become suseptable for the presence of Ampicillin.

G. Multiplication of Selected Host Cells

- After selecting and seperationg the transformed host cells by the screening methods; it becomes necessary to provide them optimum parameters to grow and multiply for generation of transformed colonies in pertiplates.
- In order to achieve this transformed host cells are introduced into fresh culture media.
- The host cells divide and re-divide along with the replication of the recombinant DNA carried by them and so generating multiple copies of desired gene.
- If the aim is obtaining numerous copies of GoI, then simply replication of the host cell is allowed.
- For obtaining the product of interest like protein, hormones, secondary metabolites, favourable conditions must be provided so that the GoI inserted in the vector expresses the product of interest.

H. Isolation and Purification of the Product

- The last step involves segregation of the multiplied GoI attached with the vector or to isolate the product of interest encoded by GoI.
- This is carried by purification processes of the isolated gene copy/protein.



Applications of Gene Cloning

- The selected gene (GoI) can be isolated and its nucleotide sequence can be determined
- Control sequences of DNA can be identified and analyzed
- Function of Protein/enzyme/RNA can be investigated and studied.
- Identification of mutants, e.g. gene defects related to specific diseases. Defected cells in organisms can be 'engineered' for specific purposes, e.g. insulin production, insect resistance, hormone production, TAP etc.

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