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Recombinant DNA Technology and Molecular Cloning

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Recombinant DNA is any artificially created DNA molecule which brings together DNA sequences that are not usually found together in nature. The production and use of recombinant DNA molecule to produce new genetic combinations that are of value to science, medicine, agriculture, and industry is termed as recombinant DNA technology. The propagation of recombinant DNA molecule inside a particular host cell so that many copies of the same sequence are produced is known as molecular cloning.

The entire procedure for molecular cloning may be classified into the following five steps

1. Identification and isolation of the desired gene or DNA fragment to be cloned
2. Insertion of the isolated gene in a suitable vector
3. Introduction of this vector into a suitable host
4. Selection of the transformed cells
5. Multiplication of the introduced DNA molecule in the host

Two major classes of enzymes are important tools in the preparation of recombinant DNA: restriction endonucleases and DNA ligases.

Restriction endonucleases along with methylases are part of a defense mechanism known as restriction modification system (RM). The RM system is used by bacteria, and perhaps other prokaryotic organisms as a defense mechanism that protects bacterial cells against invasion by foreign DNA molecules such as those contained in viruses. Restriction endonucleases work by cutting the invading DNA into small, nonthreatening pieces, crucial to this protective device is the ability of the nuclease to discriminate between its own DNA and the invading DNA; this is done by methylases, they modify bacterial DNA to protect it from its own restriction endonucleases. There are three kinds of RM system: type I, type II and type III, the name given in order of discovery. Type II restriction endonucleases is the one employed in recombinant DNA technology. The cuts made by these enzymes often leave single DNA strands with sticky ends due to the asymmetry of the cut (made to a double-stranded molecule). Type II restriction endonucleases serve as a powerful tool for manipulating DNA in a controlled way, there are several hundreds of them and a hundred different specific recognition sequences. The recognition sequences are symmetric; the same sequence of four to eight nucleotides is found on both strands, but run in opposite directions. Restriction enzymes

usually cut phosphodiester bonds of both strands in a staggered manner, so that the resulting double-stranded DNA fragments have single-stranded ends, called sticky ends. Blunt end cutting endonucleases are rather rare. The single-stranded short extensions form hydrogen-bonded base pairs with complementary single-stranded stretches on other DNA molecules. These unions are temporary since they are only held by a few hydrogen bonds. These unions can be made permanent by adding the enzyme DNA ligase. DNA ligase joins two pieces of DNA by forming covalent phosphodiester bonds.

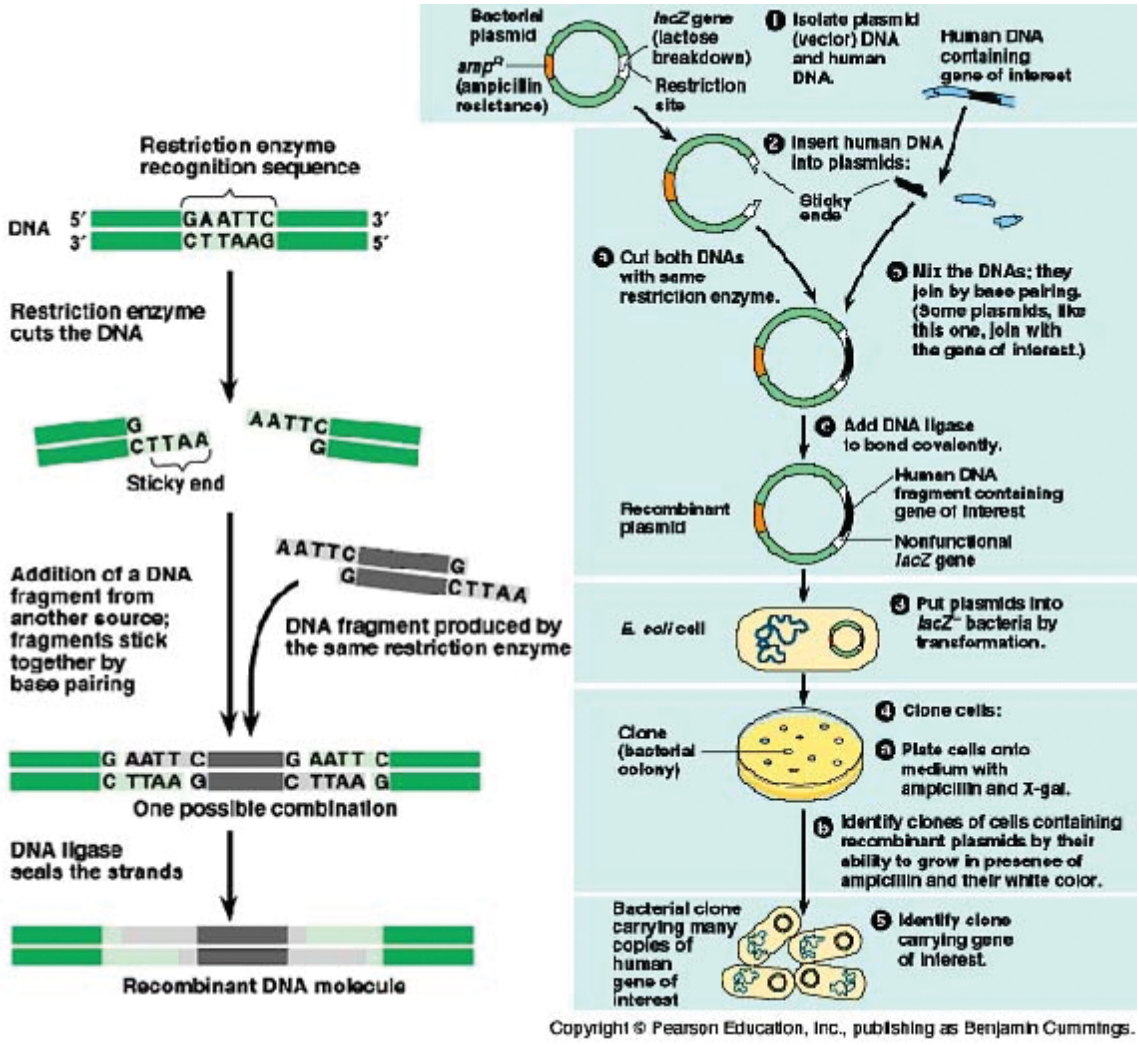
For molecular cloning of DNA molecules of interest they have to be inserted into a DNA molecule that has the capability to replicate autonomously in the host cells. Such DNA molecules are called as cloning vectors. The most commonly used cloning vectors are plasmids, they are covalently closed circular (CCC) extra chromosomal DNA havig the chareteristic sequence of orgin of replication found in most gram negative and gram positive bacteria, and also in some yeasts. A plasmid used for recombinant cloning should ideally posses certain properties like:

- Ability to replicate autonomously in bacterial cells
- Must possess selectable markers
- Should contain a unique spectrum of restriction endonucleases cleavage sites
- Should not be transmissible nor mobilisable
- Molecular weight of the plasmids should be as low as possible, because the transformation efficiency decreases as the size increases
- Should be able to maintain multiple copies per cell.

But naturally occurring plasmids may not possess all the essential properties suitable to be an ideal cloning vector, so they are modified/engineered for incorporating the above mentioned characteristics. Plasmids can normally incorporate foreign inserts of up to 10kb. Other commonly used cloning vectors are phages, and cosmids; they can accommodate 20kb and 45kb, respectively. For cloning of larger fragments of DNA molecules, new generation of artificial chromosomes vectors are employed namely bacterial artificial chromosomes (BAC), yeast artificial chromosome (YAC), and mammalian artificial chromosome (MAC), which can accommodate 300kb, 1000kb, and more than 1000kb, respectively. There is another class of vectors called as expression vectors that is used to produce a recombinant protein from a desired gene. Expression vectors apart from the ability of self replication in the host should have a regulatory region called as promoter for efficient transcription of the recombinant gene resulting in large number of stable mRNA, and thus proteins.

A recombinant vector is produced by first digesting both the DNA molecule of interest and the cloning vector using the same pair of restriction enzymes, the resulting fragments will be complimentary to each other. The DNA fragments thus generated is then ligated together using DNA ligase. For multiplication and propagation of the recombinant vector thus produced, they should be transferred to a suitable host cell; this process is known as transformation/transfection. Foreign DNA cannot be readily sent across the membrane, this is achieved using any of the following methods like,

- Heat shock- the recombinant plasmid is co incubated with calcium chloride treated cells known as competent cells, and a heat shock is given for 1-2 minutes at 42°C, the host cell membranes become permeable to recombinant plasmid which passes into the cell.



Panel on left depicts production of recombinant molecule using restriction enzyme and DNA ligase, panel on right illustrates the various steps involved in molecular cloning

- Electroporation- the host cells are subjected to a high voltage pulse which temporarily disrupts the membrane allows the vector to enter.
- Viruses- since viruses have mechanism to infect susceptible cell and replicate themselves a genetically engineered virus can deliver the desired DNA sequence into the target host cell.
- Gene gun- gold particles coated with foreign DNA segments are fired into the host cell.
- Microinjection- a cell is held in place with a pipette under a microscope and foreign DNA injected directly into the nucleus using fine needle.
- Liposome- vectors can be enclosed in a liposome, which are small membrane bound vesicles; liposome's fuse with the cell membrane or nuclear membrane and deliver the DNA into the cytoplasm/nucleus.

For distinguishing transformed and non transformed cells the cloning vector contains a selectable marker, selectable marker can be for antibiotic resistance, substrate utilization or any other characteristics which can distinguish transformed hosts from untransformed hosts. The transformed cells are selected and further cultured, within the transformed cell the recombinant DNA molecule replicates, producing dozens of identical copies known as clones. Further as the host cell replicate, the recombinant DNA is passed on to all progeny cells, creating a population of identical cells, all carrying the cloned sequence. The cloned recombinant can be recovered from host cell, purified and used for various applications.

A collection of clones that contains all the genetic information in an individual is called a genomic library. When the clone contains a reverse transcribed DNA from m-RNA, it is alled as a cDNA clone. Such clones representing all the reverse transribed m-RNA of a particular organism or tissue, it is called a cDNA library. cDNA clones lack intron sequences, making them the clones of choice for expressing and characterizing the protein product of a gene.

Recombinant DNA research is an exciting and challenging field, which holds great promises. It finds application in various fields of science like;

- Gene isolation/purification/synthesis
- Sequencing/Genomics/Proteomics
- Expression analysis (transcriptional and translational levels)
- Restriction fragment length polymorphisms (RFLPs)
- Gene therapy
- Recombinant Vaccines/ Monoclonal antibodies
- Genetically modified organisms/ Xenotransplantation
- Molecular modeling
- Bioremediation
- Forensics/Bioterrorism detection

In the near future recombinant DNA technologies will play a key role in preventing genetic diseases, producing targeted and personalized medicines, provide effective and less toxic pharmaceuticals. It will also have a great impact on agriculture and livestock through genetically modified organisms that possess traits for increased productivity and disease resistance.

Suggested Reading:

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