Genetic Engineering / Recombinant DNA technology

Genetic engineering is a broad term referring to manipulation of an organisms' nucleic acid. Organisms whose genes have been artificially altered for a desired affect is often called genetically modified organism (GMO). Recombinant DNA technology (rDNA) is technology that is used to cut a known DNA sequence from one organism and introduce it into another organism thereby altering the genotype (hence the phenotype) of the recipient. The process of introducing the foreign gene into another organism (or vector) is also called cloning. Sometimes these two terms are used synonymously.

Basically, these techniques are used to achieve the following:
Study the arrangement, expression and regulation of genes
Modification of genes to obtain a changed protein product
Modification of gene expression either to enhance or suppress a particular product
Making multiple copies of a nucleic acid segment artificially
Introduction of genes from organism to another, thus creating a transgenic organism
Creation of organism with desirable or altered characteristics

The sum total of all genes in an organism makes up its genome. Genes are the segment of nucleic acids that code for a specific polypeptide. Genes are made up of nucleotide sequences where a combination of three nucleotides (codon) code for one amino acid. Genes are transcribed into mRNA that are then translated into polypeptide sequences. The regulation of genes is achieved by operon.

Chromosomal DNA is not the only genetic material, some bacteria posses extrachromosomal genetic elements called plasmids. Plasmid are not necessary for survival but may confer some advantage to the cell harbouring it. Plasmids are circular DNA molecules that can replicate independently. Plasmids contain the requisite genetic machinery, such as replication origin, which permits their autonomous propagation in a bacterial host or in yeast. A bacterial cell may posses single or multiple copies of the same plasmid. Some plasmids are present in one or a few copies per cell and replicate once per cell division as does the bacterial chromosome; their replication is said to be under stringent control. The plasmids used in molecular cloning, however, are under relaxed control; they are normally present in 10 to as many as 700 copies per cell. Since plasmids are small stretches of DNA sequences they are easy to handle in vitro and therefore are suitable vectors in genetic engineering. Foreign DNA sequences can be introduced into bacteria, yeast, viruses, plant and animal cells.

The genes are identified by various methods and once identified, it is convenient to maintain a gene library. A gene library is a population of organisms, each of which carries a DNA molecule that was inserted into a cloning vector. Ideally, all of the cloned DNA molecules represent the entire genome of the organism. A gene library is also called gene bank.

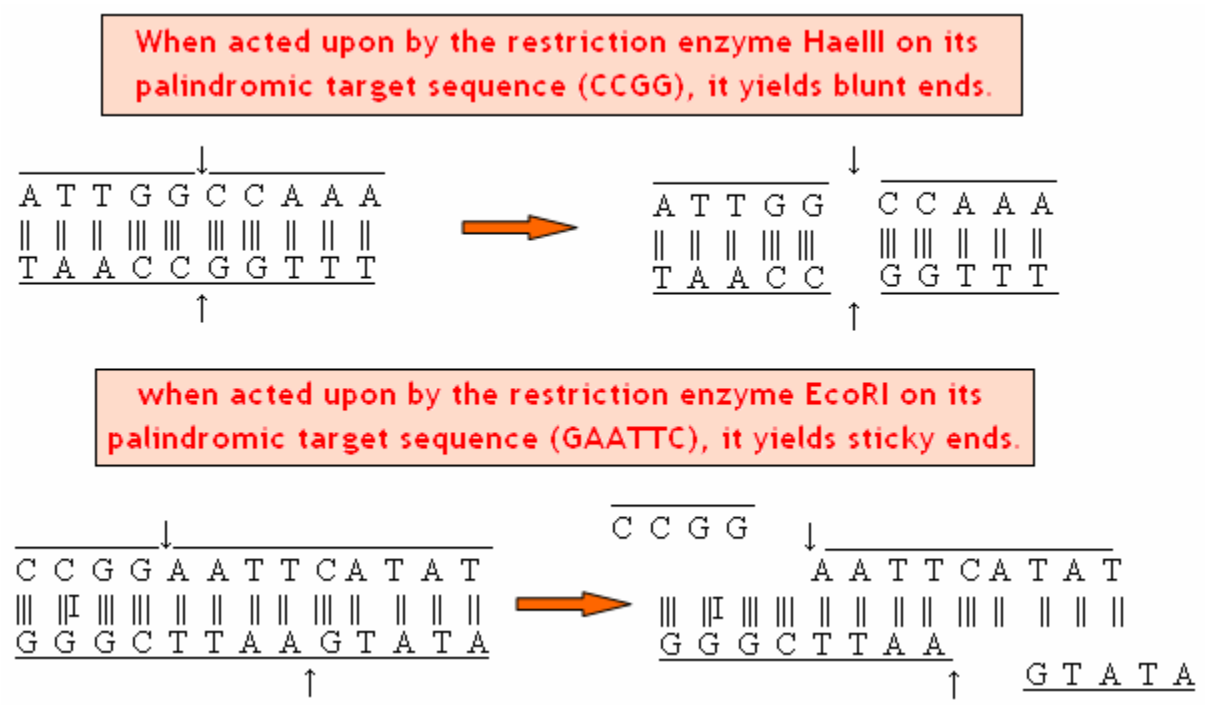
Isolation of the gene (DNA sequence)
The technique involved in recombinant DNA technology is to slice (cut) the desired DNA segment and introduce it into a vector (e.g., plasmid). This is achieved using a specific bacterial enzyme called restriction enzymes or restriction endonucleases. These enzymes function as endonuclease, which can cleave a DNA sequence at a specific site. These enzymes are named with three letters based on the species where it was isolated. For example EcoRI is isolated from E. coli.

Each restriction enzymes cleaves DNA strand at a specific site called recognition sequence or restriction site. For example, Eco RI recognizes the sequence GAATTC and cleaves it between G and A (G↓A).

Enzyme	Source	Target sequence
BamHI	Bacillus amyloliquefaciens	G↓GATCC
EcoRI	Escherichia coli	G↓AATTC
HindIII	Haemophilus influenzae	A↓AGCTT
TaqI	Thermus aquaticus	T↓CGA

Sometimes, the restriction sequence occurs on both the strands but in reverse direction. Such a segment of DNA with identical sequences but opposite in direction is called a palindrome. A palindrome site is a sequence of base pairs in double stranded DNA that reads the same backwards and forward across the double strand.

When a restriction enzyme acts on palindrome, it cleaves both the strands of DNA molecule. While some enzymes cut the two strands symmetrically, others cut them asymmetrically. AluI, EcoRV and HaeIII generates cuts the sequence asymmetrically, hence they produce blunt ends when they act on their restriction sites. Only those enzymes that cut the DNA asymmetrically are useful in rDNA technology. When such enzymes cleave DNA, they leave single stranded “sticky ends” on both strands. Same restriction enzymes are used to cleave the DNA molecule to be transferred and the vector. The circular structure of the plasmid is broken by the restriction enzyme, this process leaves a “sticky end” at either strand. The strand of DNA to be transferred must have two restriction sites; one on either side of the DNA segment of interest. When it is acted upon by restriction enzyme, it generates two sticky ends, one at either side of the segment. Since these sticky ends are generated by the same enzyme, they are complementary and hence are cohesive.

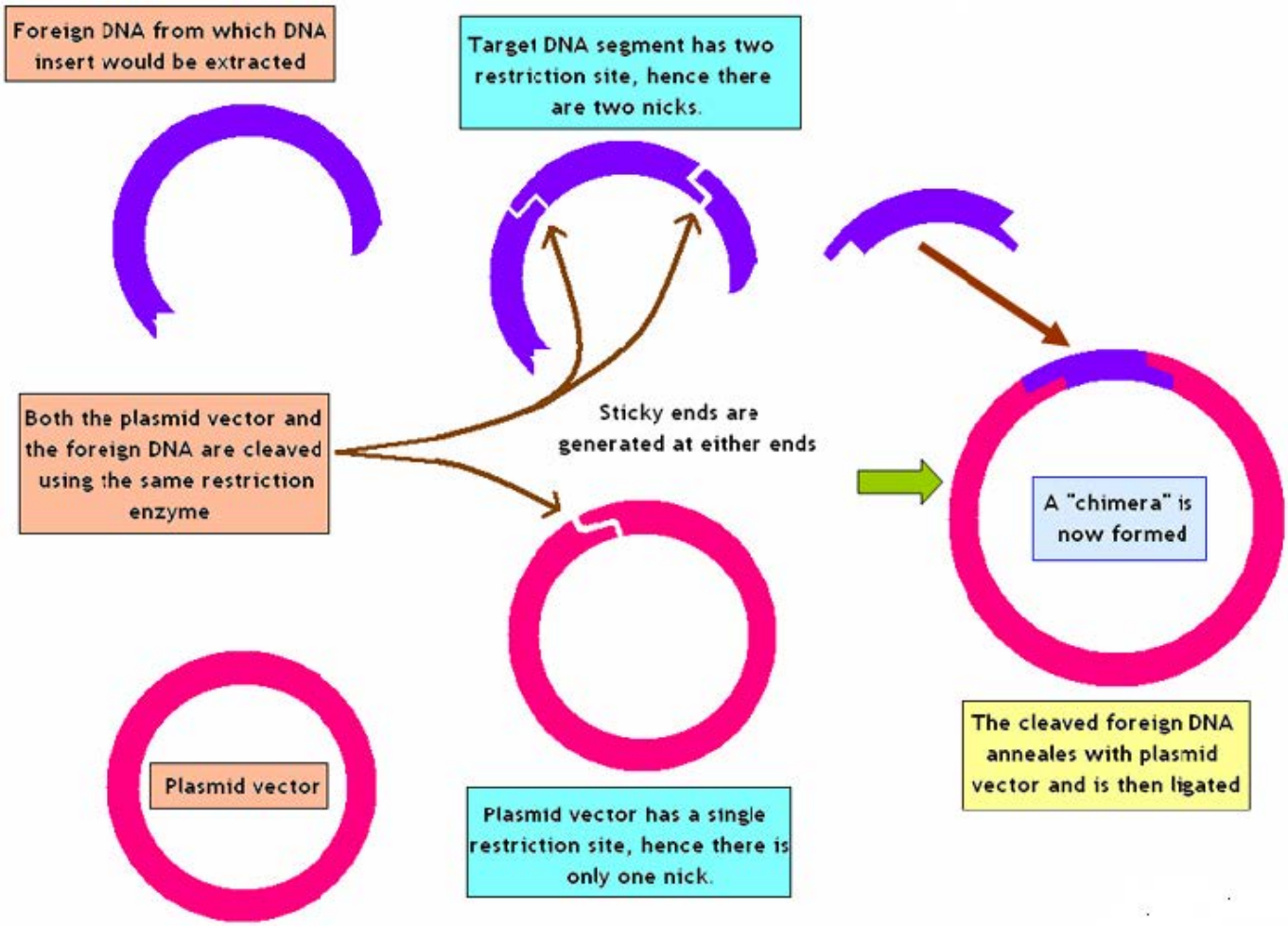


Vectors

- Bacterial plasmid is the most commonly used vector. Plasmids used in genetic engineering are said to be under relaxed control, their replication is totally independent of chromosomal replication. These plasmids may be present in copies of 10-700 per cell. The most popular plasmid is pUC18. Bacterial plasmids can not accept DNA strands larger than 5000 base pairs, hence they are restricted to cloning DNA ≤5000 base pairs.
- Specially developed bacteriophage lambda chromosome can incorporate up to 15-16 kilobases of DNA segment. A central one-third of its genome is normally not required for phage infection and therefore can be replaced by foreign DNA. The chimeric phage DNA can be introduced into the host cells by infecting them with phages.
- Cosmids are recombinant vectors that combine features of both plasmids and bacteriophage chromosome. It can accommodate DNA segments up to 50 kilobases. Since cosmids have no phage DNA, upon introduction into a host cell via phage infection, they reproduce as plasmids.
- Yeast artificial chromosome (YAC) is a specially constructed linear yeast chromosome that can incorporate DNA strands up to 1 million base pairs.

Cloning

The sticky ends are generated by the same enzyme on vector as well as the target DNA are complementary and hence are cohesive.



The sticky ends of the cleaved DNA segment cohere with those of the vector, thus the cut DNA sequence can now be introduced into the plasmid. The cut ends are joined by DNA ligase enzyme and the introduced gene becomes a part of the plasmid. Ligase is an enzyme that covalently joins the sugar-phosphate backbone of bases together. Ligase will join either "sticky" ends or "blunt" ends, but it is more efficient at closing sticky ends. The process of introducing foreign gene into a vector is called as cloning

and the plasmid containing a cloned gene is called chimera. The DNA sequence that has been inserted into the vector is also called an “insert”.

The chimera is then introduced into its host (e.g.,a bacterium) by various methods. Vectors carrying the genes must be incorporated into the living cells so that they can be expressed or replicated. The cells receiving the vector is called the host cell and once the vector is successfully incorporated into the host cell, the host cell is said to be “transformed”.

Foreign DNA cannot be readily sent across the membrane, following are few methods.

- Heat shock: The chimera plasmids are placed in a solution containing cold calcium chloride and normal host bacteria. On heating suddenly to 42°C for 2-5 minutes the host bacterial membranes become permeable to plasmid chimeras, which pass into the cell.
- Electroporation: The host cells are subjected to a high voltage pulse which temporarily disrupts the membrane and allows the vector to enter the cell.
- Viruses: Since viruses have mechanism to infect susceptible cell and replicate themselves, a genetically engineered virus can deliver 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 in held in place with a pipette under a microscope and foreign DNA is injected directly into the nucleus using fine needle.
- Liposome: Vectors can be enclosed in a liposome, which are small membrane bound vesicles. The liposomes fuse with the cell membrane (or nuclear membrane) and deliver the DNA into the cytoplasm/nucleus.

Selection of transformed cells

A pUC18 plasmid containing gene (lacZ’) coding for galactosidase activity is inserted with a foreign DNA. The plasmid also codes for ampicillin resistance. Due to the insertion, the gene gets interrupted and the bacterium transformed with this plasmid lacks galactosidase activity. Bacteria lacking this plasmid as well as those transformed by the chimeric plasmid lack galactosidase activity. When grown on medium containing a chromogenic substrate, bacteria containing chimeric plasmid produce colourless colonies.
Bacteria containing plasmid without the insert produce blue colonies and the bacteria not transformed by plasmid also produce colourless colonies. If ampicillin is also incorporated in the medium, bacteria not transformed with plasmid do not produce colonies. Thus, on this medium the colourless colonies indicate bacteria that have received chimeric plasmid.
Other methods to detect successful transfer of DNA include DNA hybridization and PCR.

Applications of Genetic Engineering

Genetic engineering has wide, applications in modem biotechnology. Since microbial cells have a much higher metabolic rate, genes of desired enzymes could be introduced into plasmid of bacteria.
The bacterial insulin, humulin was prepared by cloning the DNA from chromosome number 11 of human cells in bacteria.
The hormone somatostatin, thymosin alpha-l, as well as Beta-endorphin has been produced by genetically engineered microorganisms.
Subunit vaccines can be prepared by cloning the DNA coding for the antigenic protein present on a pathogen. E.g, Hepatitis B, Foot & mouth disease, malaria etc. Plants can be made to express antigenically important microbial proteins (edible vaccine).
Weissmann and his associates have produced alpha-interferon by recombinant DNA methods.
The enzyme urokinase, which is used to dissolve blood clots, has been produced by genetically engineered microorganisms.
Chimeric monoclonal antibodies with human Fc region can be made using this technology.
This technology has been applied to treat some of the genetic diseases (gene therapy).
AAT (alpha-1 antitrypsin), tissue plasminogen activator, factor VIII, antithrombin, erythropoeitin etc are some of the other proteins produced using this technology.
Transgenic animals with required characteristics have been created by this technique.