Chapter

Genetic Engineering: Altering the Threads of Life

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Abstract

Over the past 30 years, the field of genetic engineering has grown in a spectacular manner. The methods involved in genetic engineering which were earlier considered cumbersome and involved sophisticated instrumentation have now became a common drill within the laboratories throughout the world. This rising technology is now involved in almost every aspect of biological research. Its application includes medical diagnosis, paternity disputes, forensic analysis, genome sequencing, etc. In the recent years, this technology has attained a large-scale attention, and now the commercial products developed using genetic engineering are known worldwide. The technique of genetic engineering is solely based on genetic information, which is encoded by the DNA in the form of genes. Through genetic engineering the genes can be introduced or manipulated within the host to develop products of value and importance, for treatment of genetic disorders, and to achieve other goals. The present chapter explains the techniques involved in genetic engineering and rDNA technology and its importance in revolutionizing different fields. The objective of this chapter is to highlight the basic principle and methodology involved in genetic engineering and its role in human welfare.

Keywords: genetic engineering, DNA, gene library, plasmid, vector

1. Introduction

Genetic engineering refers to the process of altering the genetic code of any living organisms by transferring the genes from one organism to the chromosome of another in such a way that its biosynthetic properties get modified. The manipulation of genetic material or genes is carried out using selective breeding or through molecular biological techniques [1]. This process alters the biological capabilities of an organism, and it can be utilized for the industrial production of desired proteins, enzymes, antibiotics, agricultural products, etc. [2, 3]. Genetic engineering allows us to develop crops with agronomically beneficial changes like resistance to pests and harsh environment and enhanced productivity with lesser ripening time [4]. Apart from other agricultural applications, genetic engineering can also be advantageous in curing human disease [5].

Genetic engineering can also be referred to as the mechanism of changing the level of protein expression. In a condition where a large amount of protein is required for the purpose of purification, its level of expression can be altered by changing its promoter [6, 7]. Hence, the term "genetic engineering" can also be

referred to as "protein engineering" since the biochemical properties of a protein are changed through gene mutation or in vivo alteration of genes [8].

The technique of genetic engineering has evolved through our context of understanding genetics.

The methodology of manipulation of genetic material was developed in the 1970s. In vitro, the DNA was altered in a test tube and later introduced within the living cell thus altering the life process of the organism [9].

The current chapter debriefs about the outline of the genetic engineering process and its application in various fields.

2. Steps involved in genetic engineering

In a broad perspective, manipulating the DNA is done by isolating it from the cells and cleaving it using sequence-specific restriction endonuclease. Further, the two independently isolated DNA from the microbial cells are mixed and sealed using DNA ligase. Lastly, the DNA is introduced into the cells, which are grown and identified based on the altered properties of hybrid DNA [10].

For example, DNA contains a gene that is responsible for providing antibiotic resistance to the microbial cell "A," isolated and introduced into a vector (plasmid), and then transferred into bacteria "B" which gains antibiotic resistance and is a transformed bacteria.

2.1 Isolation of desired DNA fragment or gene of interest

The first important step in genetic engineering is to acquire the gene of interest which can be obtained by the methods or sources mentioned as follows:

2.1.1 Production of DNA fragments by restriction digestion

The desired DNA fragment carrying the gene of interest is cleaved from the whole DNA using restriction enzymes. These enzymes are the key and an important base of genetic engineering. There are two types of restriction enzymes known till date, i.e., exonucleases and endonuclease [11].

Exonucleases cleave the dsDNA from the terminals, whereas endonucleases cleave the dsDNA at specific nucleotide sequence present amid the center. Different varieties of endonucleases with different cleavage sites have been identified and used in the process of genetic engineering. Certain restriction enzymes like EcoRI produce single-stranded self-complementary fragment with sticky ends, whereas enzymes like Hpa I produce double-stranded noncohesive fragments [8].

Many a times there exists a certain probability that the cleavage site of restriction enzyme is available within our gene of interest, and thus the gene will not remain whole after the restriction digestion [12]. This problem can be overcome by employing hydrodynamic forces to breakdown the DNA. Sonication and homogenization are the common methods employed for the fragmentation of DNA. The DNA fragment acquired by this method is purely random, and also no sticky ends or cohesive ends are generated. Later the DNA fragments are checked for size and purity using agarose gel electrophoresis.

2.1.2 Genomic library

It comprises an entire genome of an organism that has been developed using molecular cloning methodology. The DNA of the organism is stored in population

of identical vectors. In prokaryotes the genes coding for proteins are continuous, while in eukaryotes, exons (the coding region) are interrupted with introns (non-coding region). Thus, developing genomic library for eukaryotic organism remains challenging.

For construction of genomic library, the DNA from the organism is isolated and digested using restriction enzyme to get fragments of DNA of specific sizes. These fragments are later inserted into the vector using DNA ligase, and then the vector is introduced into a host organism which can be *E. coli* or yeast. The *E. coli* is a preferred host for protein production due to its rapid growth and ability to express proteins at high levels. It is also utilized in storing DNA sequences from other organisms. The genetically engineered *E. coli* is used for different studies of medical and pharmaceutical importance. Similarly, the yeast with altered genome has enhanced ability for the production of alcohol in brewery industry. The DNA fragment is later retrieved from the host cell for the purpose of analysis or study.

2.1.3 cDNA library

This library comprises mRNA purified from a cell, tissue, or entire organism which has been changed back to dsDNA using reverse transcriptase. The cDNA/complimentary DNA fragments are inserted into the host cell. A cDNA library comprises fragments of complimentary DNA which constitute certain portion of genome of the organism.

2.2 Insertion of gene into a suitable vector

A vector is a DNA molecule that has the ability to replicate inside the host to which the desired gene has integrated for cloning. The vectors include plasmids, cosmids, bacteriophages, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC), etc.

2.2.1 The vector system

They are important cloning vehicles, within which the fragment of gene of interest is inserted and transferred into a suitable host system [11]. Mostly circular DNA that has small size and is of bacterial origin is utilized as vector. The vector should possess certain characteristics to make it suitable to be used for the process of genetic engineering:

- The vector DNA should possess the ability to infect the host organism and replicate within it.

- It must possess cleavage site for specific restriction enzyme through which a foreign gene can be inserted.

- The ability of the vector DNA to replicate within the host should not be compromised after the insertion of a foreign gene.

In order to promote cloning within vector, it must possess certain important sites:

- **Ori site**—The process of replication initiates from this sequence. The presence of this site is necessary for independent replication of plasmid within the host cell. This sequence is also responsible for controlling the copy

number of the gene of interest. If a large number of copies of DNA of interest are required, it must be cloned in a vector containing ori site supporting high copy number.

- **Cloning site**—It is site that is recognized by the restriction enzyme (preferable one) so that the gene of interest maybe inserted after digestion of the plasmid. The presence of more than a single cloning site may complicate the process of gene cloning as multiple fragments will be generated. The insertion of gene of interest is done at a restriction site present among two antibiotic resistance genes. For example, in the vector pBR322, a foreign piece of DNA can be joined at BamH I site of tetracycline resistance. Due to this, the recombinant might lose tetracycline resistance as its sequence is altered by insertion of foreign DNA, and thus the plasmids will lose tetracycline resistance. The transformants can still be distinguished from non-transformed ones by plating them on ampicillin medium. The transformed ones can grow on ampicillin medium but when transferred to a medium containing tetracycline, they are unable to grow, whereas the non-transformants can still grow on tetracycline medium as they still possess intact tetracycline-resistant genes.

- **Selectable marker**—Within a vector a selectable marker plays a key role in the identification of transformed cells from the non-transformed ones. Usually a selectable marker provides resistance towards antibiotics which are used for selection of transformants from non-transformants. These markers can provide resistance towards antibiotics like ampicillin, tetracycline, etc.

2.2.2 Plasmids

These are extrachromosomal DNA that possess the ability to replicate independently within the host cell. Plasmids are circular in shape and impart special properties to the organism possessing it as it may code for antibiotic resistance, bactericide production, etc. [13].

The plasmids can be classified majorly into two categories:

The first type is self-transmissible, i.e., the ones that possess the ability to promote conjugation and are transferred quickly amid the bacterial population. These kinds are not generally employed as vectors.

The second type is the non-self-transmissible, i.e., the ones that cannot regulate their own transfer among bacterial population. These categories of plasmids are extensively used in genetic engineering as vectors [14].

The gene of interest or the foreign gene is inserted within the plasmid DNA vector; for that the closed circular plasmid is cleaved using restriction enzyme to make it linear. A plasmid contains a single cleavage site for a restriction enzyme to avoid multiple digestions. The gene of interest is inserted in the linear plasmid, and then the recombinant is converted to its original circular form [15] (**Figure 1**).

2.2.3 Phage vectors

The viruses that infect the bacteria are termed as bacteriophages. They possess a very simple structure consisting of a genetic material which might be a DNA or RNA surrounded by a protein coat termed as capsid. These phages can also be employed as a vector as large piece of DNA of interest can be incorporated in the genetic material of the phage. Different phages have been developed for the

purpose of genetic engineering containing a single site for the restriction enzymes [16]. These commonly used phage vectors are M13 phage, T4 phage, T7 phage, etc. The DNA of these phage vectors are altered by ligating the alien DNA fragment, and further the phage vector is inserted into the host cell [17]. These cells are later grown in a culture medium containing X-gal. The cells transformed with the recombinants will produce white colonies, and the non-transformants will develop blue colonies.

2.2.4 Cosmids

A cosmid is basically a plasmid with cos site, which contains cos sequences necessary for packaging. Cos sequences are ~200 base pair in length. Cosmids are hybrids comprising phage DNA molecule and plasmid of bacteria. Just like any other vector system, cosmids also require a selectable marker and an ori site. The cosmids lack λ-genes so unlike phages they do not produce plaques. Instead plaques colonies are formed by using selective media according to the marker just as done in the case of plasmids (**Table 1**).

2.3 Introducing vector into host cells

The membrane of the cell prevents the DNA from diffusing in or out. This barrier must be manipulated or altered so that the host cells are able to take up the foreign DNA. This goal can be achieved by different ways.

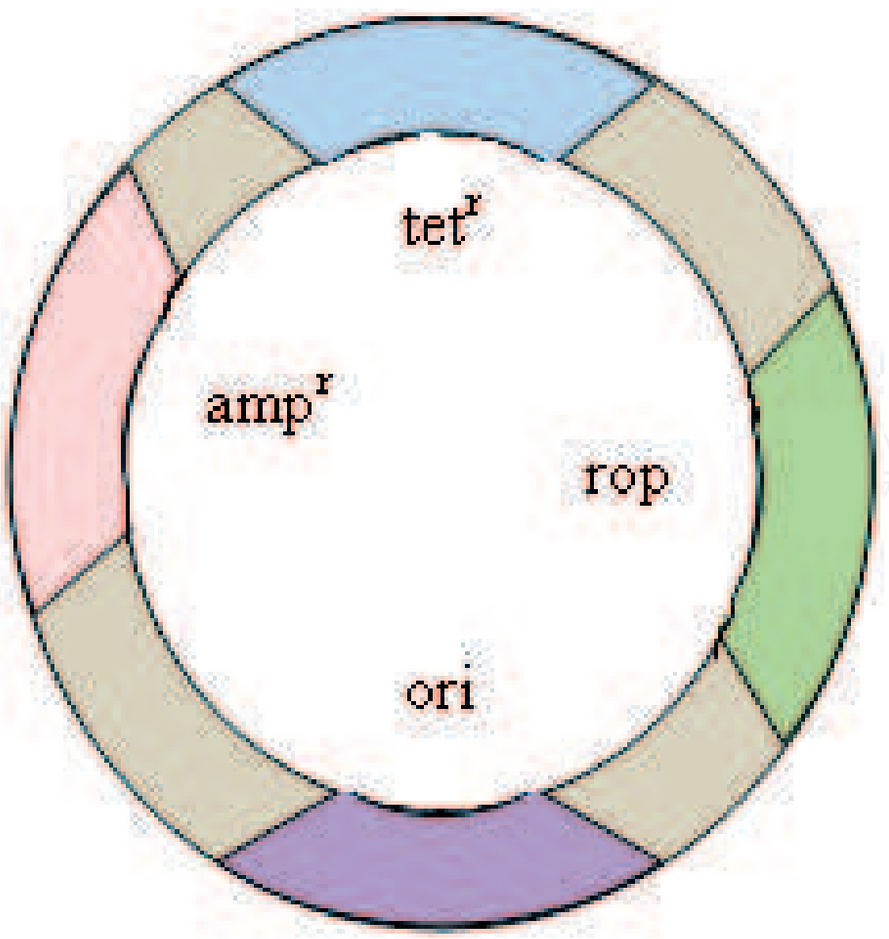

Figure 1.
Plasmid pBR322 exhibiting ori site, ampicillin- and tetracycline-resistant selectable markers.

Enzymes	Specific function
Restriction endonucleases	Cuts the DNA at specific nucleotide sequence
DNA polymerase	Addition of nucleotides at the 3′ end of the DNA
DNA ligase	Seals/joins the two fragments of DNA
Exonucleases	Remove nucleotide from the 3′ terminal of the DNA strand
Alkaline phosphatase	Removes phosphate group from both 5′ and 3′ terminals
Polynucleotide kinase	Adds phosphate group to the 5′-OH end of the DNA to promote ligation

Table 1.
Enzymes involved in genetic engineering and their function.

2.3.1 Transduction

The mechanism of inserting the foreign DNA into host cell using a phage is termed as transduction. The virus can attach themselves on the host and transfer their DNA into the cells. In genetic engineering, this ability of viruses can be utilized purposefully for the transfer of recombinant DNA into the host cells. The recombinant DNA can be transferred to a virus by inserting it within any bacteria and infecting it with the bacteriophage [18]. The virus when multiplying within the cell also intakes the recombinant DNA, and after the completion of lytic cycle, the virus containing recombinant DNA bursts out the cell. This virus now contains recombinant DNA which can be transferred to the host cell by infecting it with the phages.

2.3.2 Transformation

The mechanism by which the bacterial cell intakes DNA from the surrounding environment in which the bacteria is found or within the experimental solution is termed as transformation. This mechanism is carried out by exposing the bacteria to calcium chloride followed by heating the medium. This process will enable the bacteria to take up the recombinant DNA that we have introduced within their surroundings [15].

2.3.3 Electroporation

It is a physical method in which electric pulse is applied across the cell which creates temporary pores in the cell membrane. The DNA is then transferred using these pores within the cells. In electroporation the host cells are introduced into a conductive medium, and an electric pulse at specific voltage is passed through the solution lasting for a few microseconds. The electric current alters the phospholipid layer of the membrane, and pores are generated through which foreign DNA is transferred [14].

2.3.4 Microinjection

It is a common method that is usually employed in transferring the recombinant DNA within any plant cell. In this process the DNA is injected physically in the cells using a gene gun. The microscopic particles of tungsten or gold are coated with the recombinant DNA and are loaded to a gun. The gun comprises of high-pressure helium which ejects out the particles at a very high velocity. The outer

covering of the plant cell, i.e., the cell wall, is easily penetrated using a gene gun. After entering the plant cell, the particles release out the recombinant DNA which now becomes the part of the cell [19].

3. Applications of genetic engineering

3.1 Gene therapy

Gene therapy is the process of correcting defective genes or introducing new genes into the existing cells for the cure and treatment of diseases [20]. Through gene therapy we can correct the root cause of the disease, i.e., the genes. The first approved gene therapy was employed to correct the deficiency of the enzyme adenosine deaminase (ADA) which was carried out on a 4-year-old girl Ashanti DeSilva. This girl suffered from severe combined immunodeficiency (SCID) as the gene coding for ADA was defective causing deoxyadenosine to accumulate and destroy T lymphocytes. After gene therapy, the 4-year-old developed no noteworthy side effects and grew normally into adulthood.

The gene therapy is of two types:

- In germ line therapy, the modified/therapeutic genes are transferred into the germ cells, and the individual's offspring would remain unaffected. In germ line therapy the genes are introduced into sperms and eggs.

- In somatic cell therapy, the modified/therapeutic genes are transferred into the somatic cells due to which only the treated individual will possess the modification. In this therapy the genes are introduced into the bone marrow, blood cells, etc. [6, 7].

3.2 Synthesis of insulin using genetically engineered *E. coli*

In humans, insulin is produced as prohormone and needs to be processed to work as a functional hormone. Before the advent of genetic engineering, the insulin was extracted from the slaughtered cattle. This insulin when injected to the human patients in majority of cases induced allergies. The active human insulin comprises chains A and B linked together by disulfide bridges. The synthesis of insulin using genetic engineering was achieved by Eli Lilly, an American company in 1983 [5]. They isolated the human gene responsible for synthesis of insulin and introduced it into *E. coli* through a vector. The chains from the host cell were extracted, separated, and joined through disulfide bridges.

3.3 In agriculture

The genetic engineering has revolutionized the agriculture sector and resolved the problem of feeding the ever-increasing population to a certain bit. Using genetic engineering different varieties of plants have been developed that possess better agronomic characteristics.

The crops developed through genetic engineering are termed as transgenic or genetically modified crops. The transgenic crops can be produced by transferring the gene of interest within the plant to obtain the desirable traits. The characteristics of plant can also be altered by silencing or removing their own genes.

The important characteristics of transgenic crops include:

- Crops with better ability to tolerate environmental conditions like drought, cold, salinity, etc. For example, the salt-tolerant transgenic maize plant was produced by incorporation of gut D gene from *Escherichia coli* [4]. Crops resistant against insects, herbicides, and other chemicals. For example, Sulphonylurea-resistant tobacco plants are produced by incorporating the mutant acetolactate synthase (ALS) gene from *Arabidopsis* [4].

- Biofortification—The improved crop variety has enhanced nutritional qualities as compared to that of the conventional crops. For example, the golden rice is very rich in vitamin A.

- An altered rate of growth for higher productivity in less time, e.g., genetically modified tomatoes, potatoes, tobacco, etc. [21].

3.4 Production of insect resistant Bt cotton using genetic engineering

The bacteria *Bacillus thuringiensis* synthesize certain proteins with the ability to kill insects like dipterans and lepidopterans. This toxin was termed Bt toxin which remains inactivate, but when engulfed it gets activated due to the alkaline pH of the gut. The insects engulfing it eventually die. Through genetic engineering the genes responsible for production of Bt toxin were isolated and were used in crops like cotton to make it pest resistant. The toxin responsible for killing the pest was coded using *cry* gene. The Bt cotton was resistant against budworms, beetles, mosquitoes, and flies [22].

3.5 Transgenic animals

The animals whose genetic makeup has been altered to express foreign genes are known as transgenic animals [23]. The transgenic animals are important as they enable us to:

- Study the regulation and expression of gene.

- Study genes responsible for causing disease in the humans. Transgenic models allow us to infect them with diseases like cancer, CFTR, etc. and to develop an effective treatment against it [22].

- Ensure safety of treatment that the human receive. Transgenic mice are developed to study the effect of vaccines before using it on humans [24].

- Produce biologically important products like human proteins, protein-enriched milk for human babies, etc. [25].

4. Conclusions

Genetic engineering has become an integral part of the modern era, and its vast applications have touched and evolved almost all important fields necessary for living. This technology has revolutionized medical, agricultural, and pharmaceutical sectors. The genetic engineering involves a group of techniques that are employed for varied purposes based on our requirements. This technology has provided us the cure for many diseases, pest-resistant crops, transgenic organisms and hormones, and other important products. The modern era of biological research remains

incomplete without this technology. Genetic engineering holds the future of modern medicine, agriculture, and pharmaceutical industries. The genome editing tools are gaining much attention in the present era. Genetic engineering is the answer to all the solutions that we are facing today. In order to feed the rising population of the world, it is the tool for enhancing crop productivity. Through genetic engineering the fermentation ability of yeast can be enhanced for increased alcohol production. Similarly all sectors like antibiotic production can also be augmented, and the structure can also be modified for better efficacy. Genetic engineering also opens the gateways for the treatment of diseases that have medically no potential cure available till date. The technique of gene manipulation can provide better longevity of life. This technique is the future of almost all sectors, and with the advent of time it may provide solution to all the present problems.

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