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Review on cloning vector and expression vector

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Abstract

The advent of recombinant DNA technology has brought a series of dramatic changes in biology. It offered new opportunities for innovations to produce a wide range of therapeutic products. Recombinant DNA technology refers a series of procedures used to produce recombinant DNA (rDNA) molecules. The first step in recombinant DNA technology is to select a piece of DNA to be inserted into a vector. The second step is to cut that piece of DNA with a restriction enzyme and then ligate the DNA insert into the vector with DNA Ligase. And the third step is inserting the combination into host cells. A vector is a small piece of DNA molecule, which is used as a vehicle to artificially carry foreign genetic material into host cell, where it can be replicated and/or expressed. Vectors have three main features: origin of replication, multiple cloning sites and selectable marker. Cloning vector and expression vector are two types of vectors, used in recombinant DNA technology. A cloning vector is a small piece of DNA used to introduce genes into cells while obtaining numerous copies of the insert. Vectors used for cloning include: plasmids, bacteriophages, cosmids, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and human artificial chromosomes (HACs). Expression vectors are mainly plasmids, designed for the transcription and protein expression of the transgene. Since all vectors had been created to meet different purposes, each cloning vector and expression vector should clearly be described.

Keywords: Cloning, expression vector, molecular

Introduction

The advent of recombinant Deoxy Ribonucleic Acid (DNA) technology revolutionized the development in biology, and led to a series of dramatic changes. It offered new opportunities for innovations to produce a wide range of therapeutic products with immediate effects (Li *et al.*, 2017). Recombinant DNA technology is a science by which series of procedures are used to produce recombinant DNA (rDNA) molecule, a piece of DNA that has been created by the combination of at least two strands. It is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure ^[21].

The first step in recombinant DNA technology is to select a piece of DNA to be inserted into a vector. The second step is to cut that piece of DNA with a restriction enzyme and then ligate the DNA insert into the vector with DNA Ligase, and third step is inserting the combination into host cells. A vector is a small piece of DNA molecule, which is used as a vehicle to artificially carry foreign genetic material into host cell, where it can be replicated and/or expressed. A vector containing foreign DNA is termed as recombinant DNA. The purpose of a vector is typically to isolate, multiply, or express the insert in the target cell ^[16].

Useful vectors have three main features: (i) An origin of replication (Ori), which is a specific DNA sequence at which DNA replication is initiated. The essential feature of a vector is that it can replicate autonomously in a host species, usually bacteria. The origin is therefore absolutely essential for the amplification of the vector inside a bacterial host. (ii) Presence of a multiple cloning site (MCS), a region with multiple useful restriction enzyme sites to make a compatible digest of the vector and DNA fragment. (iii) A selectable marker, which is a method of allowing hosts (usually bacteria) containing the vector to be readily identified and purified. The insert contains a selectable marker which allows for identification of recombinant molecules. An antibiotic marker is often used so that a host cell without a vector dies when exposed to a certain antibiotic, and the host with the vector will live because it is resistant ^[13].

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The manipulation of DNA is normally conducted on *E. coli* vectors, which contain elements necessary for their maintenance in *E. coli*. However, vectors having bacterial or viral elements that allow them to be maintained in other organism such as yeast, plant or mammalian cells are called shuttle vectors. Insertion of a vector into the target cell is usually called transformation for bacterial cells, and transfection for eukaryotic cells [6].

Cloning vector and expression vector are two types of vectors, used in recombinant DNA technology. A cloning vector is a small piece of DNA which can be stably maintained within a host cell, and used to introduce genes into cells while obtaining numerous copies of the insert. A vector designed specifically for the transcription and protein expression of the transgene in the target cell is called expression vector, and generally have a promoter sequence that drives expression of the transgene [20]. Hence, both cloning vectors and expression vectors have similarities and differences with each other. Therefore, the objective of this paper is to briefly review cloning vectors and expression vectors.

Cloning Vectors

Gene cloning is a major breakthrough, the important part of which is a cloning vector. The various uses of cloning remain redundant if a suitable cloning vector is not chosen. A cloning vector is a fraction of DNA that can be used to insert a foreign DNA molecule and has the ability to be inserted into a host for cloning purpose [8]. It is capable of self-replication inside the host cell. The purpose of a cloning site is to provide a place for cloning to occur. A selectable marker gene helps identifying successful recombinants after cloning. The cloning vector does not necessarily help to express a protein which the foreign DNA encodes. Thus, the sole purpose of the cloning vector is to carry foreign DNA to the host. Depending on the size and the application of the insert the suitable vector is selected for a particular purpose [14].

Types of Cloning Vectors

Cloning is generally performed using *Escherichia coli*, and cloning vectors in *E. coli* include plasmids, bacteriophages, cosmids, and bacterial artificial chromosomes (BACs). Some DNA, however, cannot be stably maintained in *E. coli*, for example very large DNA fragments. In this case, other organisms such as yeast may be used. Cloning vectors in yeast include yeast artificial chromosomes (YACs). The choice of classic cloning vectors depends on size of the insert and application [3].

Plasmid

Plasmids are extra chromosomal double-stranded DNA sequences found in the cytoplasm of microbes and capable of replication using the host cell's replication machinery. They are generally circular and stable genetic entity that can replicate itself autonomously, independent of the chromosomal DNA of the host organism. They are found widely in many bacteria, for example in *E. coli*, but may also be found in a few eukaryotes, for example in yeast such as *Saccharomyces cerevisiae*. Plasmids usually carry at least one gene, and many of the genes that plasmids carry are beneficial to their host organisms. Although they have separate genes from their hosts, they are not considered to be independent life [4].

Plasmids contain genes that enhance the survival of an organism, either by killing other organisms or by defending the host cell by producing toxins. Multiple plasmids can

coexist in the same cell, each with different functions. In other words, plasmids can only co-occur in a bacterium if they are compatible with each other. They are incompatible if they have the same reproduction strategy in the cell. An incompatible plasmid will be expelled from the bacterial cell [20].

Since plasmids are so small, they usually only contain a few genes that lend beneficial qualities to the organism. These genes are, however, non-essential for the survival of the organism. The number of genes contained and the size of a plasmid varies from organism to organism. Some plasmids contain genes called transfer genes that facilitate the beginning of conjugation. Non-conjugative plasmids cannot start the conjugation process, and they can only be transferred through sexual conjugation with the help of conjugative plasmids. Conjugation is the transfer of genetic material from one bacterial cell to another, either through direct contact or a bridge between the two cells [21].

Plasmids can be eliminated from bacterial cells by a process called curing, which may take place spontaneously or by various curing treatments, such as acridine dyes, ultraviolet (UV) and ionizing radiation, thymine starvation and growth above optimal temperatures that inhibit plasmid replication but do not affect bacterial chromosome replication and cell reproduction [17].

They have three key points: The origin of replication, which is used to indicate where DNA replication is to begin; The selection marker gene, which is used to distinguish cells containing the plasmid from cells that do not contain it; and the cloning site, a site in the plasmid where the DNA is inserted [10].

Based on function there are five main types of plasmids: (i) Fertility plasmids (also known as F-plasmids) contain transfer genes that allow genes to be transferred from one bacterium to another through conjugation. (ii) Resistance plasmids or R plasmids, which contain genes that help a bacterial cell defend against environmental factors such as poisons or antibiotics. Some resistance plasmids can transfer themselves through conjugation. (iii) Virulence Plasmids, inside a bacterium, turn that bacterium into a pathogen. Example, *E. coli* and *Salmonella enterica*. (iv) Degradative plasmids help the host bacterium to digest compounds that are not commonly found in nature, such as camphor, xylene, toluene, and salicylic acid. They are conjugative plasmids. (v) Col plasmids contain genes that make bacteriocins (also known as colicins), which are proteins that kill other bacteria and thus defend the host bacterium. Some col plasmids are conjugative [6, 11, 17].

Use of plasmids as a vector

Plasmids are the standard cloning vectors and the ones most commonly used. Most general plasmids may be used to clone DNA insert of up to 15 kb in size. One of the earliest commonly used cloning vectors is the pBR322 plasmid. Many plasmids have high copy number, for example pUC19 which has a copy number of 500-700 copies per cell and high copy number is useful as it produces greater yield of recombinant plasmid for subsequent manipulation. However low-copy-number plasmids may be preferably used in certain circumstances, for example, when the protein from the cloned gene is toxic to the cells [18].

A plasmid isolated from the bacterial cell at one site by restriction enzyme. The cleavage converts the circular plasmid DNA into a linear DNA molecule. Then the two open ends of linear plasmid are joined to the ends of the foreign DNA to be inserted with the help of enzyme DNA ligase. This

regenerates a circular hybrid or chimeric plasmid, which is transferred to a bacterium wherein it replicates and perpetuates indefinitely^[20].

Significance of plasmids dramatically increased with the advent of recombinant DNA technology as they became the first cloning vectors, and even today they are the most widely used cloning vectors especially in gene cloning in bacteria. They enjoy this status because they have very useful properties that include: small size, which makes the plasmid easy to isolate and manipulate; independent origin of replication, which allows plasmid replication in the cell to proceed independently from direct chromosomal control; multiple copy number, which makes them to be present in the cell in several copies so that amplification of the plasmid DNA becomes easy; presence of selectable markers such as antibiotic resistance genes, which make detection and selection of plasmid-containing clones easier; and cloning site (MCS), also called a polylinker site, which is a stretch of DNA that consists of multiple target sequences for specific restriction enzymes. The plasmid vectors also possess promoter sequences near the MCS for efficient expression of gene of interest^[1].

Plasmids often confer antibiotic resistance to the bacteria, so only bacteria containing the vector will survive treatment with antibiotics. Antibiotic resistance is often used as marker, an example is the beta-lactamase gene which confers resistance to the penicillin group of beta-lactam antibiotics like ampicillin^[7].

One of the first widely used *E. coli* cloning vectors is pBR322 DNA. It is a double-stranded circle molecule with cloning limit of 0.1-10 kb. It was created in 1977 by Z. Bolivar and F. Rodriguez at the University of California. The p stands for "plasmid" and BR for "Bolivar" and "Rodriguez.". pBR322 DNA is 4361 base pairs in length and has two antibiotic resistance genes - the gene *bla* encoding the ampicillin resistance (Amp^R) protein, and the gene *tetA* encoding the tetracycline resistance (Tet^R) protein. It contains the origin of replication of pMB1, and the *rop* gene, which encodes a restrictor of plasmid copy number. The plasmid has unique restriction sites for more than forty restriction enzymes^[20].

The circular sequence is numbered such that 0 is the middle of the unique EcoRI site and the count increases through the Tet^R gene. The Amp^R gene is penicillin beta-lactamase. Promoters P1 and P3 are for the beta-lactamase gene. P3 is the natural promoter, and P1 is artificially created by the ligation of two different DNA fragments to create pBR322. P2 is in the same region as P1, but it is on the opposite strand and initiates transcription in the direction of the tetracycline resistance gene^[2].

Bacteriophage

Bacteriophages are viruses that parasitize bacteria. Bacteriophage means to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacterial culture. They are commonly referred as "phage". Phages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. They occur widely in nature and can readily be isolated from feces and sewage. There are at least 12 distinct groups of bacteriophages that are very diverse structurally and genetically. Examples of phages include: T-even phages such as T2, T4 and T6 that infect *E. coli*; temperate phages such as lambda; RNA phages; spherical phages; and filamentous phages^[6, 18].

All phages contain a head structure, which usually encloses DNA and acts as the protective covering. Some phages have

tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. In some phages, tail is surrounded by a contractile sheath, which contracts during infection of the bacterium. At the end of the tail, phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell^[9].

Depending on the life cycle, phages can be either lytic (virulent) or lysogenic (temperate). Lytic phages multiply in bacteria and kill the cell by lysis at the end of the life cycle. For example, the T-even phages such as T2, T4, and T6 are virulent. Temperate phages establish a persistent infection of the cell without killing it. For example, the Phage Lambda is temperate phage^[4].

Bacteriophages are natural vectors that transduce bacterial DNA from one cell to another. Phage vectors have a natural advantage over plasmids that is they infect cells much more efficiently than plasmid transformed cells, so the yield of clones with phage vectors is usually higher. With phage vectors, clones are not colonies of cells, but plaques formed when a phage clears out a hole in a lawn of bacteria. About one third of bacteriophage genome is non-essential and can be replaced with foreign DNA. There is an upper limit on the amount of DNA that can be packed into a phage (a maximum of 53 kb). The bacteriophages used for cloning are the phage λ and M13 phage.^[17]

Phage λ as Vector

The most extensively studied bacterial virus used for DNA cloning is bacteriophage λ (Lambda). The λ phage virion has a head region containing the viral DNA genome, and a tail which functions in infecting its host, the *E. coli*. Its genome consists of 48.5 kb of DNA. Infection by λ phage requires adsorption of tail fibers on the cell surface, contraction of the tail, and injection of the DNA inside the cell^[20].

Once it gets inside the bacterial cell, λ DNA results in the formation of a double-stranded circular DNA. After that the λ DNA can undergo one of two alternative pathways, lytic or lysogenic cycle. However, the decision of the phage to enter either lytic or lysogenic cycle is controlled by two regulatory genes; *cl* and *cro* which are mutually antagonistic. In the lytic state, the *cro* gene dominates, causing the repression of *cl*. Whereas, in the lysogenic state, the *cl* dominates, and suppresses transcription of some λ genes including *cro*. Normally, the lysogenic state is favored and the phage genome is replicated along the host chromosomal DNA^[6].

Genes that are not involved in lytic pathway and that are involved in lysogenic pathway are not essential for the use of the λ phage as a cloning vector. So, these genes may be removed from the viral DNA and are replaced with other DNA fragment of interest. The recombinant λ phages are able to transform *E. coli* cells at high efficiency. It is achieved by developing an in vitro packaging system which mimicked the way in which wild-type λ DNA is packaged in a protein coat, resulting in high infection efficiency. In this way two important cloning vectors have been developed by modifying the lambda phage genome, replacement λ vectors and insertion λ vectors^[9].

Replacement λ vectors

The λ genome contains some genes at the central segment that are required for the lysogenic cycle, but not for lytic function. Therefore, replacement of λ vectors can be prepared by replacing this segment with a DNA segment of interest. Examples: EMBL3 and EMBL4^[3].

Insertion λ vectors

Insertion vectors are prepared by modification of the λ genome to permit insertional cloning into the *cl* gene. Examples include: Example: λ gt10^[9].

Filamentous phages

Filamentous phages are not lytic. They coexist with the infected cells for several generations and are convenient for cloning genes which produce toxic products. Among the filamentous phages, M13 has been well characterized and its genomes has been sequenced. Phage M13 is widely used in nucleotide sequencing and site-directed mutagenesis since its genome can exist either in a single-stranded form inside a phage coat or as a double stranded replicative form within the infected cell. The vectors derived from M13 include M13mp18 and M13mp19. These vectors are used for obtaining single-stranded copies of the large size cloned DNA. The genome of M13 is 6.4 kb^[17].

Cosmids are plasmids capable of incorporating the bacteriophage λ DNA segment, which contains cohesive terminal sites (*cos* sites). They were first developed in 1978 by Barbara Hohn and John Collins. The first part of their name, "*cos*" comes from the fact that cosmid contains the cohesive ends, or *cos* site of phage λ . The last part of their name "mid" indicates that cosmid carry a plasmid origin of replication. Cosmids are 4 to 6 kb in size and are specifically designed for cloning of large DNA fragments (25 to 45 kb)^[14].

Cosmids contain a drug resistance marker (such as the ampicillin resistance gene), a plasmid origin of replication (*ori*), a fragment carrying the ligated cohesive ends (*cos*) of phage λ , and one or more unique restriction sites for cloning. Presence of *Cos sites* permit *in vitro* packaging of cosmid DNA into Lambda particles. Thus, have some advantages of lambda as cloning vehicle: strong selection for cloning of large inserts; infection process rather than transformation for entry of chimeric DNA into *E. coli* host; and maintain cosmids as phage particles in solution^[11].

In order to clone foreign DNA into cosmid vector, cosmid DNA is first made to linearize by cutting it with appropriate restriction enzyme. The resultant phages are then infected into a suitable *E. coli* host. Inside a cell, two *cos* ends are ligated by the host ligase, resulting in a circular molecule which can be propagated as a plasmid, and a drug resistance marker is expressed. Therefore, selection of transformants is made on the basis of antibiotic resistance and formation of bacterial colonies (rather than plaque) that contain the recombinant cosmids^[17].

Cosmids have high transformation efficiency and are capable of producing a large number of clones from a small quantity of DNA, larger DNA can be cloned than what is possible with phage or plasmid. But, they cannot accept more than 50 kb of the insert. Example of cosmidvector is super COS1, which has 7.9 kb size and 30-50 kb cloning limit^[21].

Bacterial artificial chromosomes (BACs)

Bacterial artificial chromosomes (BACs) are engineered version fertility plasmid (or F-plasmid), used for transforming and cloning in bacteria, usually *E. coli*. They are created in the early 1990s as an alternative to yeast artificial chromosomes (YACs). BACs are not real artificial chromosomes, but modified bacterial F factors. They are capable of accommodating large sequences (up to 350) without any risk of rearrangement, but the eukaryotic DNA inserts with repetitive sequences are structurally unstable in

BACs often resulting in deletion or rearrangement. BACs are present in low copy number, only 1 per cell, which prevents crossing over between repeated sequences in the insert DNA^[20].

BACs are often used to sequence the genome of organisms in genome projects, for example the Human Genome Project. A short piece of the organism's DNA is amplified as an insert in BACs, and then sequenced. Finally, the sequenced parts are rearranged *in silico*, resulting in the genomic sequence of the organism. A chloramphenicol-antibiotic resistance is used as a marker gene. BACs are preferred for genetic studies, because they accommodate much larger sequences without the risk of rearrangement, and are therefore more stable than other types of cloning vectors (Trent, 2012). Example of BAC is pUvBBAC, which has cloning limit of 35-300 kb and marker gene, chloramphenicol resistant gene and lactose metabolizing gene (*LacZ*).

Yeast artificial chromosome (YAC)

Yeasts, eukaryotic unicellular fungi, contribute a great deal to the study of molecular genetics. They are popular organisms to clone and express DNA in because they are eukaryotes, and can therefore splice out introns. A species of yeast, *Saccharomyces cerevisiae* has been an important model system for biological research because its entire genome has been base sequenced. It is used as a reference to human and other higher eukaryotic genes. This is because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved between yeast and larger eukaryotes, including mammals^[8].

Yeast artificial chromosomes are genetically engineered chromosomes derived from the DNA of the yeast, *Saccharomyces cerevisiae*. They are used as vectors to clone DNA fragments of more than 1 mega base (1Mb) in size. YACs are linear vectors that replicate in yeast cells. They contain telomeres, which are ends of chromosomes involved in the replication and stability of linear DNA; origin of replication sequences necessary for the replication in yeast cells; a yeast centromere, which is a specialized chromosomal region where spindle fibers attach during mitosis; selectable marker for identification in yeast cells; ampicillin resistance gene for selective amplification; and yeast autonomously replication (ARS), which are features required to replicate linear chromosomes in yeast cells. They also contain suitable restriction sites to clone foreign DNA^[18].

A very large DNA fragments whose sizes ranging from 100 kb to 3000 kb can be cloned using YACs. But overall transformation efficiency is low resulting low yield of cloned DNA. YACs have an advantage over BACs in expressing eukaryotic proteins that require post translational modifications. But, YACs are known to produce chimeric effects which make them less stable compared to BACs^[15].

Example of YAC is pYAC3, which is an artificial chromosome having yeast centromere isolated from *Saccharomyces cerevisiae* and ligated to bacterial plasmid. pYAC₃ has size of 11.4kbp with cloning limit, 100-1000 kb. Also, it has a marker similar as for identification of yeast cell^[14].

Human artificial chromosomes (HACs)

Human artificial chromosomes also known as mammalian artificial chromosomes (MACs) are artificially synthesized exogenous minio chromosomes that are utilized for gene transfer or gene delivery into human cells for studying their expression and mammalian chromosomal

function. They are stable throughout many cell divisions, at least in human cells. HACs mimic the normal pattern of gene expression, because they can hold complete genomic loci, including upstream and downstream regulatory elements. They are used extensively in expression studies and determining the function of the human chromosomes [12].

HACs also have the advantages of being mitotically stable in the absence of selection and they have an indefinite cloning capacity (no upper cloning limit), thus allowing for the insertion of all control elements for the correct expression of the transgene. They range in size from 6 to 10 Mb that carry new genes introduced by researchers. However, as a result of their large size, they are difficult to handle and can only be recovered in small quantities. [15]

Expression vectors (or Constructs)

Cloning vectors provide a backbone for the DNA insert to be reproduced and propagated in bacteria; however, these vectors are only useful for storing a genetic sequence. By themselves, they are incapable of allowing for transcription and translation of the gene into a functional protein product (Li *et al.*, 2017). The expression vectors are vectors which act as vehicles for DNA insert and also allow the DNA insert to be expressed efficiently. They are special types of cloning vectors containing the regulatory sequences necessary to allow the transcription and translation of a cloned gene or genes.

These constructs are often derivatives of the plasmid vectors used in the host: Origin of replication, which is a DNA segment recognized by the cellular DNA-replication enzymes; selectable markers, which constitute genes showing resistance to certain antibiotics like ampicillin and tetracycline; multiple cloning sites; a promoter, is a sequence recognized by sigma subunit of RNA polymerase which is required for initiation of transcription of gene of interest; terminator, which is short nucleotide sequences present at the end of a gene where transcription of gene ends; and translation initiation site, such as ribosome binding site and start codon. Ribosome binding site is a short nucleotide sequence recognized by the ribosome as the point at which it should attach to the messenger molecule. The initiation codon of the gene is always a few nucleotides downstream of this [7].

For a gene to give rise to a protein product, an expression vector must be used that contains the necessary elements for a host cell to transcribe and translate the gene. The most common reason for this is that the promoter may not be recognized by the RNA polymerase of the new host. In the case of a mammalian cell, a standard mammalian expression vector will contain an origin of replication, MCS, and selectable marker. However, the expression vector will also need a promoter found in mammalian cells that can drive the expression of the gene. The coding DNA needs other features to be transcribed and translated, such as the polyadenylation tail that normally appears at the end of transcribed pre-mRNA and a sequence that attracts the ribosome for translation [19].

Once the expression constructs are inside the host cell, the protein encoded by the gene of interest is produced by the transcription and translation, which utilizes the translation machinery and ribosomal complexes of the host organism. They are extensively used as tools which help in the production of mRNAs and, in turn, stable proteins like insulin. However, if the cloned gene is to be expressed across the prokaryotic-eukaryotic boundary, then different mechanisms are used in prokaryotes and eukaryotes for the translation machinery to identify the start codon. For example, expression of a eukaryotic gene in *E. coli* requires addition of

a *Shine-Dalgarno* sequence at a position upstream of the start codon. Vectors that can be propagated in two or more different hosts (both in prokaryotes and eukaryotes) are called shuttle vectors [6, 17].

After the protein product is expressed, it is to be then purified. The purification of a protein poses a challenge since the protein of interest, whose gene is carried on the expression vector, is to be purified independently of the proteins of the host organism. To make the process of purification simpler, the gene of interest carried on the expression vector should always have a 'tag'. This tag can be any marker peptide or histidine (His tag) [6].

Expression vectors have numerous applications in producing peptides and proteins for the pharmaceutical industry such as producing insulin, growth hormone, antibiotics, vaccines, and antibodies. Moreover, expression vectors help in enzyme production in food and garment industries. Not only that, expression vectors are essential in producing transgenic plants such as golden rice, insect resistant plants, herbicide-resistant plants, etc. [15].

The most commonly used expression vector is pET vector system. It is the most powerful system yet developed for the cloning and expression of recombinant proteins in *E. coli*. Target genes are cloned in pET plasmids under control of strong bacteriophage T7 transcription and (optionally) translation signals; expression is induced by providing a source of T7 RNA polymerase in the host cell. They are initially cloned using hosts that do not contain the T7 RNA polymerase gene, thus eliminating plasmid instability due to the production of proteins potentially toxic to the host cell. Once established in a non-expression host, plasmids are then transferred into expression hosts containing a chromosomal copy of the T7 RNA polymerase gene in the genome, and expression is induced by the addition of IPTG. Ampicillin and kanamycin resistance genes are available in pET vectors as selection marker. pET28 and pET32 are the most commonly used pET vectors [1, 15].

Similarities and Differences between Cloning Vector and Expression Vector

Cloning vector and expression vector are two types of vectors used in recombinant DNA technology and genetic engineering. Both of them contain multiple cloning sites, origin of replication and selectable marker [4, 15].

However, cloning vector is a small DNA molecule that carries a foreign DNA fragment into the host cell while expression vector is a type of vector that facilitates the introduction, expression of genes and production of proteins. Furthermore, another significant difference between cloning vector and expression vector is that a cloning vector introduces a foreign DNA fragment into a host while expression vectors express the introduced gene by producing the relevant protein [14, 19, 21]. Besides, a cloning vector consists of an origin of replication, restriction sites, and a selectable marker while the expression vector contains enhancers, promoter region, termination codon, transcription initiation sequence, an origin of replication, restriction sites, and a selectable marker. Plasmids, bacteriophages, cosmids, bacterial artificial chromosomes, yeast artificial chromosome, mammalian artificial chromosomes are examples of cloning vectors. Meanwhile, expression vectors are mostly plasmids [6].

Conclusions and Recommendations

The advent of recombinant DNA technology revolutionized the development in biology, and has offered new

opportunities for the production of therapeutic products. Recombinant DNA technology uses a vector as a tool, which is used as a vehicle to artificially carry genetic material into host cells. Vectors have essential features that include origin of replication, presence of multiple cloning sites and selectable marker. Generally, there are two types of vectors: cloning vectors and expression vectors, both of which have similarities and differences with each other. All vectors had been created for different purposes in molecular biology. Therefore, all useful cloning vectors and expression vectors should clearly be described so as to use them accordingly.

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