Chapter 2

Host–Vector System

EXERCISES

1. Describe the importance of host vector system in rDNA technology.

Ans: The host-vector system is a fundamental component of recombinant DNA (rDNA) technology, playing a crucial role in the manipulation, replication, and expression of foreign genes. Here are several key aspects highlighting the importance of the host-vector system in rDNA technology:

DNA Replication and Maintenance: The vector, often a plasmid or viral genome, contains an origin of replication. This allows the vector to replicate independently within the host organism. This is essential for the production of multiple copies of the recombinant DNA, ensuring that it is passed on to daughter cells during cell division.

Selectable Markers: Vectors often carry selectable markers, such as antibiotic resistance genes. These markers aid in identifying and selecting host cells that have successfully taken up the recombinant DNA. The presence of these markers allows researchers to distinguish between cells that contain the foreign gene and those that do not.

Expression of Recombinant Genes: The host organism provides the cellular machinery for the transcription and translation of the foreign gene. Different host systems, such as bacteria, yeast, or mammalian cells, offer distinct advantages for gene expression. The choice of the host system depends on factors such as the complexity of the protein to be produced and the desired post-translational modifications.

Protein Production: The primary goal of rDNA technology is often to produce proteins encoded by the inserted genes. The host-vector system facilitates the synthesis of these proteins in large quantities. This has numerous applications, including the production of therapeutic proteins, enzymes, and industrial products.

Stability of Recombinant DNA: The host-vector system influences the stability of the inserted DNA within the host cells. The host organism's cellular mechanisms

must maintain the integrity of the foreign gene over successive generations to ensure the long-term stability and functionality of the engineered DNA.

Scalability and Efficiency: Different host-vector systems exhibit varying levels of efficiency and scalability. The selection of an appropriate system depends on factors such as the size of the DNA fragment to be inserted, the desired expression level, and the ease of manipulation. Scalability is particularly important when large quantities of the recombinant protein are required.

Biotechnological Applications: The host-vector system is instrumental in various biotechnological applications, including the production of genetically modified organisms (GMOs), the development of vaccines, and the creation of transgenic animals and plants for agricultural purposes.

In summary, the host-vector system is essential in rDNA technology as it provides the framework for the replication, expression, and maintenance of recombinant DNA constructs. The careful selection of an appropriate host organism and vector ensures the success of genetic engineering experiments and the efficient production of desired gene products.

2. What are the major characteristics of a vector?

Ans: Vectors are carrier molecules, often DNA molecules, used to transport foreign genetic material into a host organism during genetic engineering or recombinant DNA (rDNA) technology. The major characteristics of a vector include:

Origin of Replication (ori): Vectors must contain an origin of replication, which is a specific DNA sequence recognized by the host organism's cellular machinery. This allows the vector to replicate independently within the host cell.

Selectable Markers: Vectors often carry selectable markers such as antibiotic resistance genes. These markers assist in the identification and selection of host cells that have successfully taken up the vector. Cells with the vector can survive in the presence of a specific antibiotic, while cells without the vector cannot.

Multiple Cloning Site (MCS) or Polylinker: Vectors typically have a region that contains multiple restriction enzyme recognition sites. This region is known as the

Multiple Cloning Site or Polylinker, and it allows researchers to easily insert their foreign DNA into the vector using restriction enzymes.

Promoters: Vectors contain promoter sequences that regulate the initiation of transcription. These promoters control the expression of the inserted genes in the vector. Depending on the application, different promoters may be used to achieve specific levels and patterns of gene expression.

Reporter Genes: Some vectors include reporter genes, like beta-galactosidase or green fluorescent protein (GFP), which produce easily detectable products. These genes help researchers assess whether the foreign DNA has been successfully inserted and expressed in the host cells.

Terminator Sequences: Terminator sequences signal the end of transcription. They are important for proper termination of the transcription process, ensuring that the transcribed mRNA is correctly processed.

Plasmid or Viral Origin: Vectors can be plasmids or viral-based. Plasmid vectors are circular DNA molecules that exist independently of the host genome. Viral vectors may integrate into the host genome or remain episomal, depending on the specific vector design.

Size and Capacity: Vectors have limitations on the size of the DNA insert they can carry. The vector's size and capacity influence the size of the DNA fragment that can be cloned and manipulated.

Expression Signals: Vectors often contain sequences that signal the host cell's machinery to transcribe and translate the inserted genes. These signals ensure efficient expression of the foreign DNA.

Compatibility with Host Organism: The vector must be compatible with the host organism. Different hosts have different requirements, and the vector should have features that allow it to function effectively in the chosen host system.

Ease of Manipulation: Vectors should be easily manipulated in the laboratory. This includes the ability to insert, delete, or modify genetic material within the vector.

These characteristics collectively define the functionality and utility of a vector in genetic engineering, allowing researchers to successfully introduce and express foreign genes in a host organism. The choice of a vector depends on the specific requirements of the experiment or application.

3. What is plasmid and what are its different types?

Ans: A plasmid is a small, circular DNA molecule that exists independently of the chromosomal DNA in bacteria and some eukaryotic cells. Plasmids often carry genes that provide selective advantages to the host organism, such as antibiotic resistance or the ability to metabolize certain nutrients. In genetic engineering and recombinant DNA technology, plasmids are commonly used as vectors to introduce foreign genes into host cells.

There are several types of plasmids, each with its own characteristics and functions. Some common types of plasmids include:

Fertility or F Plasmids (F Factor):

- **Function:** These plasmids carry genes responsible for the formation of sex pili (conjugation tubes) and facilitate the transfer of genetic material between bacteria during a process called conjugation.
- **Conjugation:** F plasmids allow the transfer of the entire plasmid from one bacterial cell to another, leading to the rapid spread of genetic information, including antibiotic resistance genes.

Resistance (R) Plasmids:

- **Function:** R plasmids carry genes that provide resistance to antibiotics or other toxic substances. These resistance genes are often used as selectable markers in genetic engineering experiments.
- **Importance:** R plasmids have played a significant role in the spread of antibiotic resistance among bacterial populations.

Col Plasmids (Colicinogenic Plasmids):

- **Function:** Col plasmids carry genes for the production of colicins, which are toxic proteins that can kill other bacteria. Colicins provide a competitive advantage to the host bacterium in environments with limited resources.
- **Role in Antibacterial Defense:** Bacteria harboring col plasmids can release colicins to inhibit the growth of competing bacterial strains.

Degradative Plasmids:

- **Function:** These plasmids carry genes encoding enzymes that enable the host bacterium to break down and utilize unusual substances, such as hydrocarbons or certain organic compounds, as a source of carbon and energy.
- **Applications:** Degradative plasmids have applications in bioremediation, where they can be used to enhance the ability of bacteria to degrade environmental pollutants.

Virulence Plasmids:

- **Function:** Virulence plasmids carry genes that contribute to the pathogenicity of certain bacteria. These genes may encode toxins, adhesins, or other factors that enhance the bacterium's ability to cause disease in a host organism.
- **Pathogenicity Islands:** Some virulence plasmids are associated with pathogenicity islands, which are large genomic regions that contribute to the virulence of bacteria.

Expression Vectors:

- **Function:** Expression vectors are modified plasmids designed for the efficient expression of foreign genes in host cells. They contain additional elements such as promoters, enhancers, and transcription termination signals to regulate gene expression.
- **Applications:** Expression vectors are commonly used in biotechnology to produce recombinant proteins for various purposes, including research, medicine, and industrial processes.

These are just a few examples of the diverse types of plasmids, each serving specific functions in bacterial cells. In genetic engineering, plasmids are extensively used as versatile and customizable vectors for the introduction and expression of foreign genes in various host organisms.

4. Discuss the strategy applied for the development of (pBR322) plasmid cloning vectors.

Ans: pBR322 is a well-known plasmid cloning vector that was developed in the early 1970s by Herbert W. Boyer, Stanley Cohen, and their colleagues. It was a pioneering achievement in the field of recombinant DNA technology. The development of pBR322 involved a strategic design to create a versatile plasmid that could be used for cloning, gene expression, and the manipulation of foreign DNA. Here are the key strategies applied for the development of pBR322:

Introduction of Multiple Cloning Sites (MCS) or Polylinker:

- **Strategy:** A crucial aspect of pBR322 was the incorporation of a Multiple Cloning Site (MCS) or Polylinker region. This region contained multiple unique restriction enzyme recognition sites, allowing researchers to easily insert foreign DNA into the plasmid using restriction enzymes.
- **Advantage:** The MCS facilitated the efficient cloning of DNA fragments at multiple sites, providing flexibility and versatility in genetic manipulation.

Selectable Markers for Antibiotic Resistance:

- **Strategy:** pBR322 carries two selectable markers for antibiotic resistance—ampicillin resistance (amp^r) and tetracycline resistance (tet^r). The presence of these markers allows for the selection of bacterial cells that have successfully taken up the plasmid.
- **Advantage:** Researchers can use antibiotic selection to identify and isolate bacteria containing the recombinant plasmid, making it a powerful tool for cloning experiments.

Separation of Cloning and Expression Regions:

- **Strategy:** pBR322 was designed with distinct regions for cloning and expression. The cloning region contained the MCS, while the expression region had regulatory elements for transcription and translation.
- **Advantage:** This separation allowed researchers to clone genes of interest in the cloning region and then move them to the expression region if desired. It provided flexibility in controlling gene expression.

Compact Design and Small Size:

- **Strategy:** pBR322 was engineered to be relatively small in size. It is a low-copy-number plasmid, meaning that there are fewer copies of the plasmid within each host cell. This design choice helped minimize the burden on the host cell and facilitated the maintenance of cloned genes.
- **Advantage:** The small size made pBR322 easy to manipulate in the laboratory and allowed for efficient replication and maintenance in bacterial cells.

Use of Replication Origins:

- **Strategy:** pBR322 contains two origins of replication—ori^pMB1 and ori^pSC101. These origins allow the plasmid to replicate independently of the bacterial chromosomal DNA.
- **Advantage:** The presence of multiple replication origins contributed to the stability and efficient replication of the plasmid in bacterial cells.

Integration of Convenient Restriction Sites for Analysis:

- Strategy: The pBR322 plasmid was designed with convenient restriction enzyme sites for analysis and manipulation. These sites allowed researchers to digest and analyze the plasmid using restriction enzymes.
- Advantage: The inclusion of these sites facilitated the cloning process and enabled researchers to verify the successful construction of recombinant plasmids.

Standardization and Wide Adoption:

- **Strategy:** The pBR322 plasmid was intentionally designed to be widely adopted and used as a standard cloning vector. The strategy involved sharing

the plasmid with the scientific community, contributing to the rapid growth of recombinant DNA technology.

- **Advantage:** Standardization allowed researchers worldwide to use a common vector system, promoting collaboration and the exchange of genetic material and methodologies.

The strategic design of pBR322 made it a foundational tool in genetic engineering and paved the way for subsequent developments in recombinant DNA technology. Its principles influenced the design of many other plasmid vectors used in molecular biology and biotechnology.

5. Briefly describe the structure of lambda bacteriophage and also discuss the role of lambda phage based vectors.

Ans: Structure of Lambda Bacteriophage:

The lambda bacteriophage, or simply lambda phage, is a virus that infects Escherichia coli (E. coli) bacteria. It has a complex structure consisting of a head, a tail, and tail fibers. Here is a brief overview of the structure:

1. **Head (Capsid):** The head is an icosahedral structure that contains the viral DNA. It is composed of protein subunits and serves to protect the viral genome during the infection process.
2. Tail: The tail extends from the head and is involved in the attachment of the phage to the bacterial cell surface and the injection of viral DNA into the host cell.
3. **Tail Fibers:** Tail fibers extend from the tail and are responsible for recognizing and binding to specific receptors on the surface of the bacterial cell. This interaction is crucial for the initial attachment of the lambda phage to its host.
4. **Baseplate:** The baseplate is located at the end of the tail and contains proteins that facilitate the attachment of the phage to the bacterial cell wall. It also plays a role in the injection of viral DNA into the host.
5. **Linear Double-Stranded DNA Genome:** The lambda phage genome is a linear double-stranded DNA molecule. It is relatively large, consisting of about 48,502 base pairs.

Role of Lambda Phage-Based Vectors:

Lambda phage-based vectors are widely used in genetic engineering and molecular biology for the cloning and manipulation of DNA fragments. Lambda vectors, such as Lambda gt11 and Lambda ZAP, have specific features that make them valuable tools for these applications. Here are some key aspects of the role of lambda phage-based vectors:

1. **Large Insert Capacity:** Lambda vectors have a large insert capacity compared to plasmid vectors. Lambda gt11, for example, can accommodate DNA inserts of up to 23 kilobases, making it suitable for cloning larger fragments of DNA.
2. **Cloning Libraries:** Lambda phage vectors are often used to construct genomic or cDNA libraries. These libraries contain a collection of DNA fragments representing an organism's entire genome or specific mRNA transcripts.
3. **In Vivo Packaging:** Lambda vectors utilize the natural ability of the lambda phage to package DNA into phage particles in vivo. The packaging process allows for the creation of a recombinant phage particle containing the foreign DNA insert.
4. **Infection of E. coli Hosts:** Lambda vectors can infect E. coli bacterial cells, and the recombinant phage particles deliver the cloned DNA into the host cells.
5. **Lambda Phage Integration:** Lambda vectors can be engineered to allow the integration of foreign DNA into the E. coli chromosome. This integration is reversible, providing a stable environment for the cloned DNA.
6. **Selectable Markers:** Lambda vectors typically carry selectable markers, such as antibiotic resistance genes, to facilitate the identification and selection of bacterial cells that have taken up the recombinant phage.
7. **Expression Libraries:** Lambda vectors can be used to construct expression libraries, allowing for the expression of proteins encoded by the cloned genes. This is particularly useful for studying gene function and producing specific gene products.
8. **Screening and Isolation:** Lambda phage-based vectors simplify the screening and isolation of specific DNA fragments of interest from large

libraries. Techniques such as plaque hybridization and polymerase chain reaction (PCR) are often employed for this purpose.

In summary, lambda phage-based vectors offer advantages in terms of insert capacity, cloning efficiency, and the ability to create genomic libraries. These vectors have played a significant role in advancing molecular biology research, allowing scientists to study and manipulate large DNA fragments with ease.

6. Discuss the M13 based vectors and its application.

Ans: M13-based vectors are DNA vectors derived from the filamentous bacteriophage M13. These vectors have been widely used in molecular biology and genetic engineering for various applications. Here, I'll discuss M13-based vectors and their key features and applications:

Features of M13-Based Vectors:

Single-Stranded DNA (ssDNA) Genome:

- M13 phage carries a single-stranded circular DNA genome. This ssDNA can be used as a template for various applications, such as sequencing and as a template for complementary DNA (cDNA) synthesis.

Cloning Site (Polylinker):

M13-based vectors contain a polylinker or multiple cloning site (MCS) where foreign DNA can be inserted. The polylinker typically contains multiple unique restriction enzyme recognition sites, facilitating the insertion of DNA fragments.

Selectable Markers:

M13 vectors often carry selectable markers, such as antibiotic resistance genes. These markers aid in the identification and selection of bacterial cells containing the recombinant phage.

In Vivo Packaging:

M13 vectors use the in vivo packaging mechanism of the M13 phage to generate recombinant phage particles containing the cloned DNA. This process simplifies the creation of DNA libraries and facilitates the recovery of cloned DNA.

Applications of M13-Based Vectors:

DNA Sequencing:

M13-based vectors have been historically used in DNA sequencing. The single-stranded DNA of the phage serves as a template for sequencing reactions. The resulting sequences are complementary to the original single-stranded DNA.

Phage Display Technology:

M13 phage is extensively used in phage display technology. In this technique, foreign peptides or proteins are displayed on the surface of M13 phage particles. This allows for the screening and selection of specific binding peptides or antibodies from large libraries.

Antibody Engineering:

M13 phage display is employed in antibody engineering to generate libraries of antibody fragments. This enables the isolation of antibodies with specific binding properties for therapeutic or diagnostic applications.

cDNA Cloning:

M13 vectors are used in cDNA cloning, where the single-stranded DNA of the phage can be reverse transcribed into double-stranded cDNA. The resulting cDNA can then be cloned into the M13 vector.

Site-Directed Mutagenesis:

M13 vectors are employed in site-directed mutagenesis studies. Specific mutations can be introduced into the DNA sequence using M13-based vectors to study the functional significance of particular regions or residues.

Gene Cloning and Expression:

M13 vectors can be used for gene cloning and expression in Escherichia coli. The phage carries the foreign DNA into the bacterial cell, allowing for the production of proteins encoded by the cloned genes.

Probe Synthesis for DNA Hybridization:

Single-stranded DNA derived from M13 vectors can be used as probes for DNA hybridization studies, allowing researchers to detect and analyze specific DNA sequences.

In summary, M13-based vectors are versatile tools with applications ranging from DNA sequencing and cloning to phage display and antibody engineering. Their unique features, such as the single-stranded DNA genome and in vivo packaging mechanism, make them valuable in various molecular biology and genetic engineering studies.

7. Differentiate between cosmids and phagemids.

ANS: Cosmids and phagemids are both types of vectors used in molecular biology for cloning and manipulating DNA. Here are the main differences between cosmids and phagemids:

Nature and Origin:

- **Cosmids:** Cosmids are hybrid vectors that combine features of plasmids and bacteriophages (viruses that infect bacteria). They are usually derived from plasmids but contain a phage lambda origin of replication, which allows them to be packaged into lambda phage particles.
- **Phagemids:** Phagemids are also hybrid vectors, but they are primarily based on plasmids. They have features of both plasmids and filamentous bacteriophages. Phagemids contain the origin of replication of a plasmid and some elements of a phage that allow them to be packaged into phage particles.

Replication:

- **Cosmids:** Cosmids replicate like plasmids within the host bacterial cell. They have a plasmid origin of replication, and they can be maintained as extrachromosomal DNA.
- **Phagemids:** Phagemids replicate both as plasmids within the bacterial cell and as phages outside the cell. They can be propagated as plasmids, but they can also be packaged into phage particles and released from the host cell.

Cloning Capacity:

- **Cosmids:** Cosmids have a larger cloning capacity compared to plasmids. They can accommodate larger DNA fragments, typically up to 45-50 kilobases (kb) of foreign DNA.
- **Phagemids:** Phagemids usually have a smaller cloning capacity compared to cosmids. They are generally used for cloning smaller DNA fragments, typically up to 15-20 kb.

Packaging:

- **Cosmids:** Cosmids are not packaged into phage particles naturally. They are introduced into host cells by transformation.
- **Phagemids:** Phagemids can be packaged into phage particles using helper phages. The phage particles can then infect bacterial cells, delivering the phagemid DNA.

Applications:

- **Cosmids:** Cosmids are often used for constructing genomic libraries and large-insert cloning because of their larger cloning capacity.
- **Phagemids:** Phagemids are commonly used in the construction of DNA libraries and for the production of single-stranded DNA for sequencing.

In summary, both cosmids and phagemids are versatile cloning vectors with some similarities, but they differ in terms of their origin, replication mechanisms, cloning capacities, and applications in molecular biology.

8. Why is a vector required for cloning of a gene?

Ans: A vector is required for cloning a gene because it serves as a carrier or vehicle to transport the gene of interest into a host organism, typically a bacterium. The process of cloning involves the insertion of a specific DNA fragment (which could be a gene or other DNA sequence) into a vector, followed by the introduction of the recombinant vector into a host cell. Here are the key reasons why vectors are essential for gene cloning:

DNA Transport:

- Vectors act as vehicles to carry the foreign DNA (gene of interest) into the host cell. The vector serves as a shuttle that facilitates the transfer of the gene from one organism to another.

Replication and Amplification:

- Vectors have their own origin of replication, allowing them to replicate autonomously within the host cell. This ensures that the inserted gene is also replicated along with the vector, resulting in the production of multiple copies of the gene.

Selectable Markers:

- Many vectors carry selectable markers, such as antibiotic resistance genes. These markers help in identifying and selecting cells that have successfully taken up the recombinant vector. For example, if the vector carries a gene for antibiotic resistance, only the transformed cells (those with the vector) will survive in the presence of the antibiotic.

Cloning Sites:

- Vectors contain specific DNA sequences, known as cloning sites or multiple cloning sites (MCS), where the gene of interest can be inserted. These sites are engineered to have unique restriction enzyme recognition sequences, facilitating the insertion of foreign DNA in a controlled and directional manner.

Size Compatibility:

- Vectors come in different sizes, and researchers can choose a vector based on the size of the DNA fragment they want to clone. Plasmids, cosmids, bacteriophages, and other types of vectors offer different cloning capacities to accommodate various sizes of DNA inserts.

Expression Control:

- Some vectors are designed for the controlled expression of the inserted gene. This means that the cloned gene can be regulated in terms of when and how much protein it produces in the host cell.

Phage Display and Protein Production:

- In certain applications, such as phage display or protein expression, vectors are crucial for presenting the gene product (e.g., a protein) on the surface of a phage or for driving the expression of the gene to produce the desired protein.

In summary, vectors are indispensable tools in gene cloning as they provide the means for introducing, replicating, and expressing foreign DNA within a host cell, allowing researchers to study and manipulate specific genes for various purposes, including basic research, genetic engineering, and the production of recombinant proteins.

9. A plasmid capable of getting integrated into host chromosome is called:

(a) Col plasmid

(b) Episome

(c) Ti plasmid

(d) R plasmid

Ans: (b) Episome

10. Why the replication of single copy plasmid called stringent replication?

Ans: The term "stringent replication" refers to the regulation and control of the replication process in plasmids, specifically single-copy plasmids. This mechanism is designed to ensure that the plasmid maintains a low copy number within the host cell. Here are the key features of stringent replication:

Low Copy Number:

- Single-copy plasmids replicate to maintain only a few copies per bacterial cell. This is in contrast to high-copy plasmids that can exist in many copies within a single host cell.

Regulation of Replication:

- Stringent replication involves tight control over the initiation of plasmid replication. The process is regulated in response to cellular conditions, ensuring that the plasmid copy number is kept at a minimum.

Conservation of Cellular Resources:

- By limiting the copy number of the plasmid, the host cell conserves its resources. High-copy plasmids can place a greater burden on the host cell's machinery and energy resources, so stringent replication helps minimize this burden.

Cellular Stress Response:

- Stringent replication is often associated with cellular stress responses. Under adverse conditions, such as nutrient limitation or other stressors, the host cell may downregulate the replication of plasmids, including single-copy plasmids. This response allows the cell to prioritize essential cellular functions over the maintenance of extrachromosomal elements.

Increased Stability:

- Single-copy plasmids are more stable than high-copy plasmids. This stability is advantageous in situations where maintaining a stable genetic element is crucial for the survival of the host cell.

Maintenance of Genetic Information:

- Stringent replication helps ensure the faithful maintenance of the genetic information carried by the plasmid. This is important for the proper inheritance of the plasmid during cell division.

It's important to note that stringent replication is a characteristic associated with certain types of plasmids, especially those that carry essential genes or functions for the host cell. The regulatory mechanisms involved in stringent replication vary among different plasmids, and they are often influenced by environmental cues and the physiological state of the host cell.

11. Identify the incorrect match pair from the following:

(i) Multi copy plasmid	**(a) Stringent replication**
(ii) Col plasmid	**(b) Kills bacteria**
(iii) pBR322	**(c) Plasmid**
(iv) Prophage	**(d)Phage genome inserted into a hostgenome**

Ans: The incorrect match pair is:

(ii) Col plasmid (b) Kills bacteria

Col plasmids (colicinogenic plasmids) typically carry genes for the production of colicins, which are protein toxins lethal to other bacteria. So, the correct match for (ii) should be:

(ii) Col plasmid (b) Produces colicins

12. How can a large size eukaryotic gene insert be cloned?

Ans: Cloning large eukaryotic genes can be challenging due to their size and complexity. However, several techniques and strategies have been developed to overcome these challenges. Here are some common methods used to clone large eukaryotic genes:

Using Bacterial Artificial Chromosomes (BACs) or Yeast Artificial Chromosomes (YACs):

- BACs and YACs are large cloning vectors that can accommodate very large DNA inserts, including entire eukaryotic genes or genomic regions. BACs are used in bacterial hosts, while YACs are utilized in yeast cells. These vectors are capable of carrying DNA fragments ranging from 100 to 300 kilobases (kb) or even more.

Fragmenting the DNA:

- If the eukaryotic gene is too large to be directly cloned, the DNA can be fragmented into smaller, more manageable pieces. These fragments can then

be individually cloned into vectors, and the entire gene can be reconstructed by assembling the cloned fragments.

Use of Cosmids:

- Cosmids are hybrid vectors that combine features of plasmids and bacteriophages. They can carry larger DNA fragments compared to conventional plasmids. Cosmids are often used for cloning larger eukaryotic genes.

PCR Amplification and Assembly:

- Polymerase Chain Reaction (PCR) can be employed to amplify specific regions of a large eukaryotic gene. Once the gene is divided into smaller, overlapping fragments, these fragments can be individually amplified and then assembled in vitro to reconstruct the entire gene.

Homologous Recombination in Yeast:

- Yeast cells have efficient homologous recombination machinery. Large eukaryotic genes can be cloned into yeast vectors, and then the yeast cells can be induced to undergo homologous recombination to assemble the complete gene.

Phage Lambda Cloning:

- The lambda phage vector system can be used for cloning large eukaryotic genes. The lambda phage can accommodate larger DNA inserts, and the recombination system of the phage can be exploited for gene cloning.

Transposon-Mediated Cloning:

- Transposons are mobile genetic elements that can be used to introduce eukaryotic genes into a host genome. Once integrated, the genes can be cloned along with the surrounding genomic DNA.

Artificial Chromosome Vectors (e.g., HACs):

- Human Artificial Chromosomes (HACs) are designed to carry large DNA fragments, including entire eukaryotic genes. They mimic natural

chromosomes and can be used for cloning and manipulating large genomic DNA.

The choice of method depends on factors such as the size of the gene, the complexity of the genome, and the downstream applications of the cloned DNA. Researchers often use a combination of these techniques to successfully clone and manipulate large eukaryotic genes.

13. Assertion: An ideal vector should have selectable marker.

Reason: Selectable markers are required to screen out transformation.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

Ans: The correct answer is:

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

Explanation:

An ideal vector should indeed have a selectable marker. Selectable markers, such as antibiotic resistance genes, are crucial for screening and selecting transformed cells.

The reason correctly explains the assertion, as selectable markers are required to identify and isolate cells that have successfully taken up the vector during transformation.

14. Assertion: Cosmid is a hybrid vector.

Reason: Cosmid has properties of both plasmids and lambda phage vector.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false

Ans: The correct answer is:

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

Explanation:

A cosmid is indeed a hybrid vector, combining features of plasmids and lambda phage vectors.

The reason correctly explains that a cosmid has properties of both plasmids (such as the ability to replicate autonomously) and lambda phage vectors (such as the ability to be packaged into phage particles for efficient transfer).

SUMMARY

• rDNA technology is a two-component system: a compatible host and a vector combination, where the vector provides essential sequences required for its replication in a compatible host which provides various replication functions.
• The cloning vector should be small in size and have an origin of replication or ori site, unique restriction sites and selectable marker.
• Plasmids are circular, extra-chromosomal double stranded DNA

(dsDNA) capable of autonomous replication.

• The bacteriophages lambda (λ) and M13 are the two most common phages whose genomes have been frequently used to make cloning vectors for E. coli host.

• The bacteriophage lambda, a bacterial virus that infects E. coli, has been widely used as a cloning vector.

• Typical vectors coming out of lambda genome fall into two broad classes, namely 'insertion vectors' and 'replacement vectors'.

• M13 is a filamentous bacteriophage of E. coli having genome consisting 6.4kb long circular DNA packaged in a tubular capsid.

• An example of M13 based vector for E. coli is M13mp18 which facilitates blue/white selection of recombinants.

• Cosmids are a type of hybrid (combination) vector that replicate like a plasmid but can be packaged in vitro into lambda phage coats.

• A typical cosmid has replication functions, unique restriction endonuclease sites, and selective markers contributed by plasmid DNA, combined with a lambda DNA segment that includes the joined cohesive ends (cos sites).

• Phasmids are true combination vectors between phage and plasmid. They are linear duplex DNAs whose ends are lambda segments that contain all the genes required for a lytic infection and the middle segment is linearised plasmid.

• Among eukaryotic host vector system, the most common is the baker's yeast, Saccharomyces cerevisiaes, from which YAC's have been derived through genetic engineering.

• A YAC cloning vector consists of two copies of a yeast telomeric sequence (telomeres are the sequences at the ends of chromosomes), a yeast centromeric sequence, a yeast ARS (an autonomously replicating sequence) and appropriate selectable markers.