Bharathidasan University
Tiruchirappalli
Tamil Nadu - India

Programme : M.Sc Biotechnology
Course Title : Genetic Engineering
Course code :22BTCC6

Unit -2
Cloning Vectors

Dr.M.Manickavasagam
Associate Professor
Department of Biotechnology

Plasmid Vector

Cloning vectors

- **Cloning Vectors**
- Cloning vectors are essential tools in molecular biology used to carry foreign genetic material into a host cell for replication or expression.
- These vectors facilitate the cloning process, enabling the insertion, replication, and sometimes expression of specific genes in a host organism.
- Cloning vectors are modified plasmids, viruses, or artificial chromosomes that contain the necessary components to insert and replicate foreign DNA.
- The concept of cloning vectors is fundamental in recombinant DNA technology, gene therapy, genetic engineering, and molecular diagnostics.

Cloning vectors

- **Key Features of Cloning Vectors**
- Cloning vectors must possess certain essential characteristics for successful cloning:

1. **Origin of Replication (ori):**
    1. The origin of replication is a sequence of DNA that allows the vector to replicate independently within the host cell. This ensures that the foreign DNA it carries is replicated along with the vector when the host cell divides.
    2. Different types of vectors may have origins of replication specific to certain hosts, e.g., bacterial or eukaryotic origins.

2. **Selectable Marker Genes:**
    1. Selectable markers enable researchers to easily identify and select cells that have successfully taken up the vector containing the foreign DNA. These markers typically confer resistance to antibiotics or other selective agents.
    2. Common examples include the **ampicillin resistance gene (bla)** or **kanamycin resistance gene (kan)**.

Cloning vectors

3. Cloning Site (Multiple Cloning Site or MCS):

1. The MCS, also known as a polylinker, contains a series of restriction enzyme sites that allow for the insertion of foreign DNA. This region is engineered to accommodate a variety of DNA fragments, enabling flexibility in cloning.
2. It is usually located between the promoter and the origin of replication.

4.Promoter:

1. A promoter is a DNA sequence that initiates transcription of the cloned gene. In some cloning vectors, the promoter is necessary for the expression of the inserted gene, especially when the goal is to produce proteins in the host cell.
2. Promoters are specific to the host system (bacterial, yeast, mammalian, etc.).

5.Incorporation of the Gene of Interest:

1. Cloning vectors allow the insertion of a gene or DNA fragment of interest using restriction enzyme-based ligation, where the foreign DNA is inserted into the vector at the MCS.

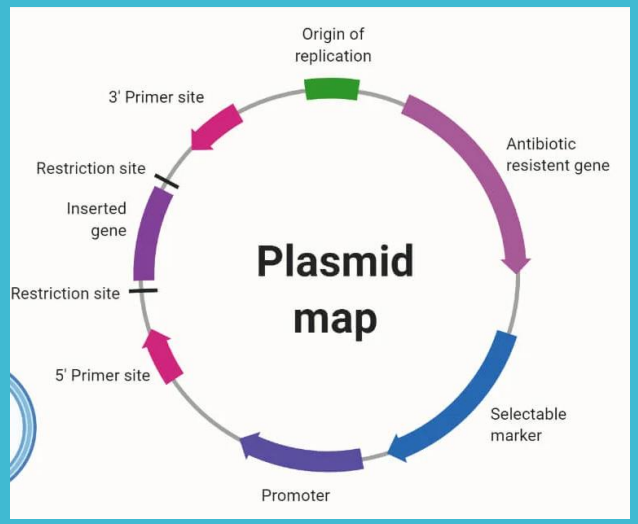
Types of Cloning vectors

1. Plasmid Vectors:
2. Bacteriophage Vectors
3. Cosmid Vectors:
4. Bacterial Artificial Chromosomes (BACs):
5. Yeast Artificial Chromosomes (YACs):
6. Expression Vectors:
7. Viral Vectors:

Plasmid Vector

- **Definition of Plasmid Vectors**
- A plasmid vector is a small, circular DNA molecule commonly used in molecular biology and genetic engineering for cloning, gene expression, and the introduction of foreign genes into host cells.
- These vectors are typically derived from naturally occurring plasmids, which are extrachromosomal DNA elements found in bacteria, and in some eukaryotes.
- Unlike chromosomal DNA, plasmids are independent of the chromosomal DNA and replicate autonomously within the cell.

Plasmid Vector



- **Key Characteristics of Plasmid Vectors:**

1. **Small Size:** Plasmids are typically small (1–200 kb), which makes them convenient to manipulate in the lab. Their size enables easy isolation and transfer of genetic material.

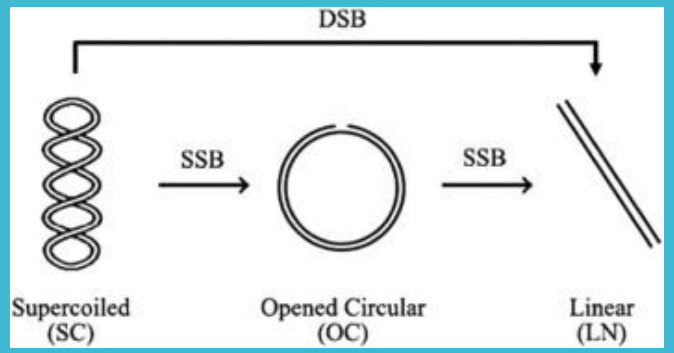
2. **Circular Structure:** Plasmid vectors are usually circular in shape, which contributes to their stability and ease of replication within a host cell.

3. **Autonomous Replication:** Plasmids contain an origin of replication (ORI), a sequence of DNA that allows them to replicate independently of the host cell's chromosomal DNA. This allows for high copy numbers of the plasmid within the host cell.

4. **Selectable Markers:** Plasmid vectors often carry selectable marker genes, such as antibiotic resistance genes (e.g., ampicillin or tetracycline resistance). These genes allow for the identification and selection of successfully transformed cells in which the plasmid has been introduced.

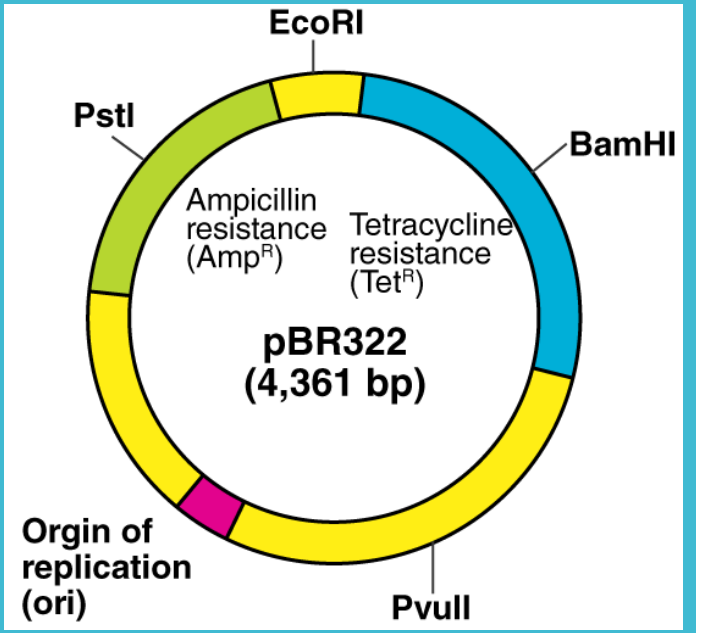
5. **Multiple Cloning Sites (MCS):** Plasmids typically include a region with multiple unique restriction enzyme sites, known as a multiple cloning site (MCS), which facilitates the insertion of foreign DNA. The MCS is placed near a promoter or another regulatory sequence for the expression of the inserted gene.

6. **Reporter Genes:** Many plasmids contain reporter genes, such as GFP (Green Fluorescent Protein) or lacZ (β-galactosidase), which allow for easy monitoring of gene expression and cloning efficiency.



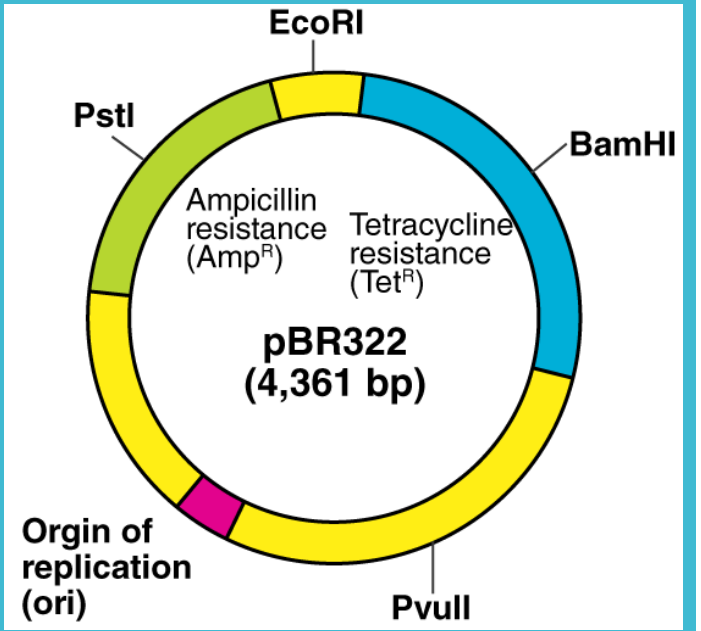
- Most plasmids exist as double-stranded circular DNA molecules
- Plasmids occur widely in nature and are found in most bacterial species. They are circular super coiled double stranded structure.
- If both strands of DNA are intact circles the molecules are described as covalently closed circles or CCC DNA
- If only one strand is intact, then the molecules are described as open circles or OC DNA.
- When isolated from cells, covalently closed circles often have a deficiency of turns in the double helix, such that they have a super coiled configuration
- Not all plasmids exist as circular molecules. Linear plasmids have been found in a variety of bacteria, e.g. *Streptomyces* sp
- To prevent nuclease digestion, the ends of linear plasmids need to be protected
- They vary in size from few thousand base pairs to hundred kilo base pairs , typically 2-5kb. Most important property of plasmid is antibiotic resistance

pBR322 Plasmid Vector



- pBR322 is one of the most widely used plasmid vectors in molecular biology, particularly for cloning, gene expression, and recombinant DNA technology. It was developed in 1977 by Bolivar and Rodriguez and is considered one of the first vectors engineered specifically for the purpose of cloning foreign DNA into bacteria.

pBR322 Plasmid Vector



- **Structure and Components of pBR322**
- The pBR322 plasmid is a circular, double-stranded DNA molecule, with several key features that make it suitable for use as a cloning vector. Below are the notable structural components of pBR322:

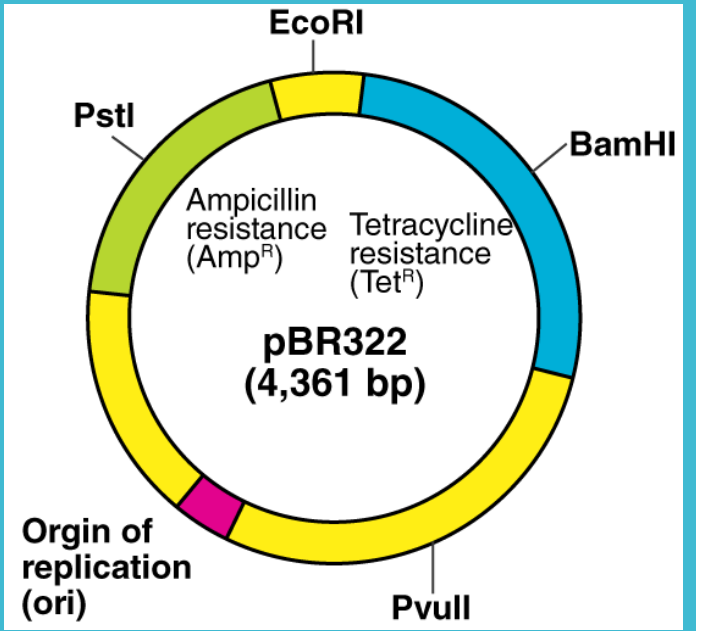
1. Origin of Replication (ori)

1. The plasmid contains a replication origin, **ori**, derived from *Escherichia coli* (E. coli) *ColE1* plasmid, which allows the plasmid to replicate independently of the bacterial chromosome once it is inside the bacterial cell.
2. This ensures that the plasmid is maintained in multiple copies per bacterial cell, making it a good vector for amplifying the inserted DNA.

2. Selectable Markers

1. pBR322 contains two **antibiotic resistance genes**:
    1. **Ampicillin resistance gene (bla)**: Confers resistance to the antibiotic ampicillin. This allows for the selection of bacteria that carry the plasmid, as only those that have the plasmid can survive in the presence of ampicillin.
    2. **Tetracycline resistance gene (tet)**: Confers resistance to tetracycline, which can also be used as a selectable marker.
2. These markers help in selecting bacterial cells that have successfully incorporated the plasmid.

pBR322 Plasmid Vector



- **Structure and Components of pBR322**

4.Multiple Cloning Site (MCS)

1. The multiple cloning site (MCS) is a short region in the plasmid that contains multiple unique restriction sites in close proximity. This is a critical feature for cloning because it allows for the insertion of a gene of interest in a region where the vector's function is not disrupted.
2. pBR322's MCS is strategically located between the **Ampicillin** and **Tetracycline** resistance genes, so the insertion of foreign DNA at these sites can be monitored by changes in antibiotic resistance patterns.

5.Gene Disruption for Selection

1. The presence of **two selectable markers** (ampicillin and tetracycline resistance) allows for **blue/white screening** or direct selection based on loss of resistance to one of the antibiotics.
2. If a foreign DNA fragment is inserted into the tetracycline resistance gene, it disrupts the function of the gene, making the transformed cell sensitive to tetracycline but still resistant to ampicillin. This disruption allows for easy identification of recombinant plasmids.

pBR322 Plasmid Vector

- **Applications of pBR322**
- The pBR322 vector has been used in various applications in molecular biology, such as:

1. **Cloning of DNA**
    1. pBR322 is commonly used as a cloning vector, where a gene of interest is inserted into the plasmid and then introduced into bacterial cells. The plasmid allows for easy replication of the gene in the bacterial host, enabling researchers to produce large quantities of the gene or protein encoded by it.

2. **Gene Expression Studies**
    1. Researchers use pBR322 to express genes in bacteria. Though pBR322 itself does not have an inducible promoter for high-level expression, it provides a platform for generating recombinant constructs, which can then be modified further for specific gene expression systems.

3. **Protein Production**
    1. In recombinant protein production, pBR322 is sometimes used to clone a gene that is later expressed in bacterial systems (often *E. coli*). The plasmid can also be adapted with additional regulatory sequences (like promoters) for the controlled expression of the recombinant protein.

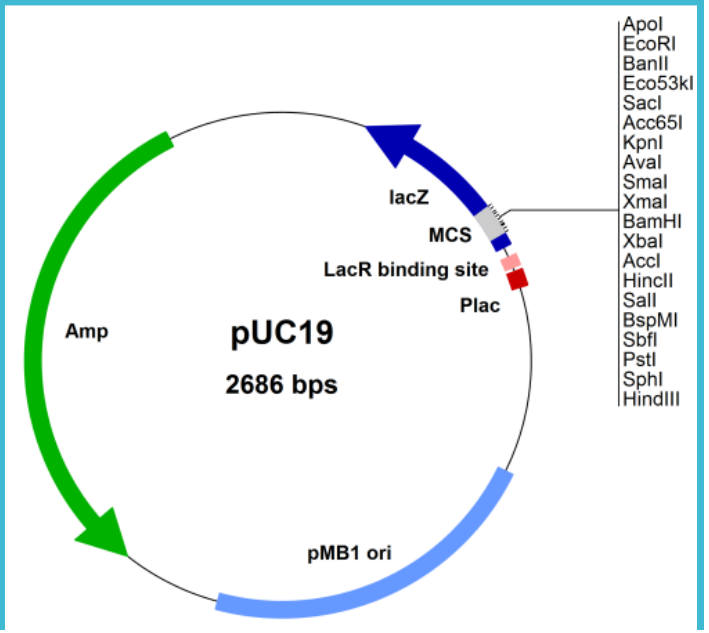
4. **Genetic Engineering and Synthetic Biology**
    1. Due to its versatility and ease of manipulation, pBR322 has been used in the design and construction of more complex plasmids, which have been engineered to express multiple genes, harbor different selectable markers, and contain specialized replication origins.

- .

pBR322 Plasmid Vector

- **Advantages of pBR322**
- **High Copy Number**: The ColE1 origin of replication allows pBR322 to replicate in high copy numbers inside *E. coli*, which is beneficial for amplifying cloned genes.
- **Selectable Markers**: The presence of both ampicillin and tetracycline resistance genes enables efficient selection of transformed cells.
- **Ease of Cloning**: The availability of multiple restriction enzyme sites and the MCS make it convenient for cloning foreign DNA into pBR322.
- **Small Size**: The relatively small size (~4,361 base pairs) of pBR322 makes it easy to manipulate and transform into bacterial cells.
- **Limitations of pBR322**
- **No High-Level Expression System**: pBR322 does not have a strong promoter for high-level expression of inserted genes, so it is not ideal for large-scale protein production unless further modified.
- **Limited Number of Cloning Sites**: While it contains multiple cloning sites, modern cloning vectors may have even more restriction enzyme sites and other advanced features like fusion tags for easier protein purification.

pUC19 Plasmid Vector



- The **pUC19 vector** is a widely used plasmid vector in molecular biology, primarily for cloning, gene expression, and gene manipulation in bacteria. It was developed from the **pUC (plasmid Universal Cloning)** series and remains one of the most common vectors for cloning and sequencing DNA. Below is a detailed account of its structure, features, applications, and uses.

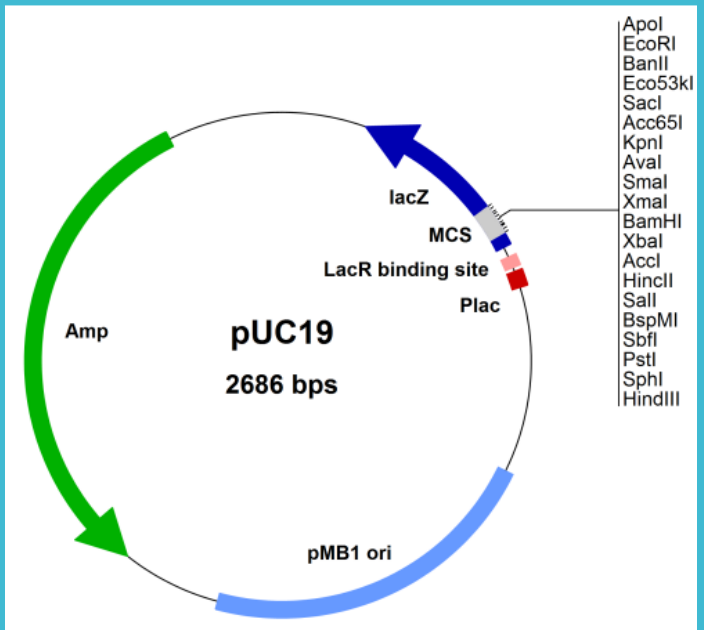
- **1. Vector Background and Origin**

- **pUC19** was derived from the **pBR322** plasmid, which was one of the first cloning vectors.

- The name **pUC19** refers to **pUC** (plasmid Universal Cloning) and the number **19**, which is simply a designation within the pUC series.

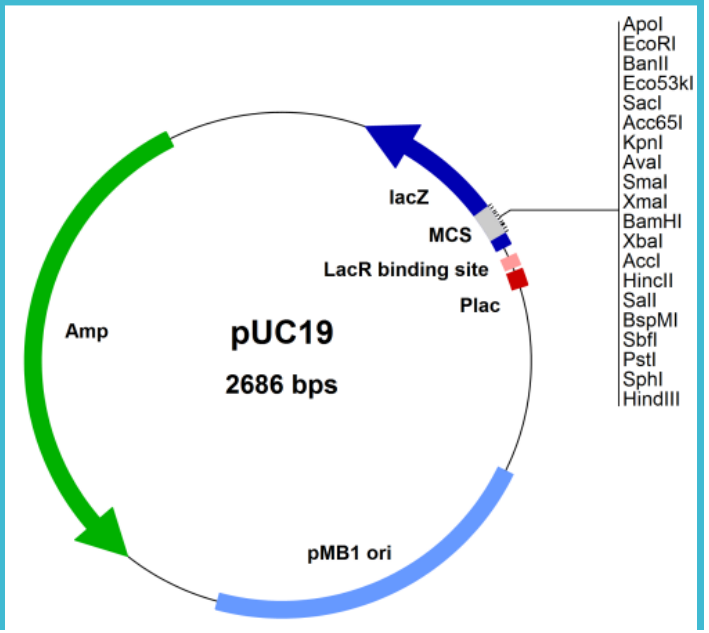
- **Escherichia coli** is the typical host for pUC19, as it allows for efficient transformation and high plasmid replication.

pUC19 Plasmid Vector



- **2. Structure of pUC19**
- The pUC19 vector is a relatively small, circular, double-stranded plasmid. It has the following key features:
- **a. Size**
- pUC19 is **2686 base pairs** (2.7 kb) in length, making it a relatively small vector that is easy to manipulate.
- **b. Origin of Replication**
- It contains the **ColE1 origin of replication**, which allows the plasmid to replicate independently in **E. coli** cells. This origin is widely used in cloning vectors for high plasmid copy numbers in bacteria.
- **c. Multiple Cloning Site (MCS)**
- pUC19 contains a **multiple cloning site (MCS)**, also known as a polylinker, which is a short DNA sequence with several unique restriction enzyme sites. The MCS allows for the insertion of foreign DNA at a specific location.
- This MCS is located within the **lacZ gene**, which encodes **β-galactosidase**. The inclusion of the MCS within this gene plays an important role in screening for successful cloning.

pUC19 Plasmid Vector



- **2. Structure of pUC19**
- **d. LacZα Fragment**
- The **lacZ gene** is split into two parts: **LacZα** and **LacZΩ**.
  - The **LacZα** part of the gene is present in the pUC19 vector.
  - When an insert is ligated into the MCS, it disrupts the LacZα sequence, rendering the β-galactosidase protein nonfunctional.
  - This allows for **blue/white screening**: bacterial colonies containing an insert will be white because β-galactosidase is not functional, while those without an insert will be blue due to the active β-galactosidase enzyme, which metabolizes X-gal to form a blue color.
- **e. Antibiotic Resistance**
- pUC19 contains the **ampicillin resistance gene (bla)**, which allows transformed bacterial cells to survive in the presence of ampicillin. This gene encodes **β-lactamase**, an enzyme that breaks down ampicillin, conferring resistance.

pUC19 Plasmid Vector

- **Key Features and Functions**
- **Cloning and Screening**: The MCS, in combination with the lacZα fragment, enables easy and efficient cloning and screening of recombinant plasmids.
- **High Copy Number**: The ColE1 origin allows for a high number of plasmid copies per cell, which makes it efficient for plasmid isolation and propagation.
- **Blue/White Screening**: The β-galactosidase enzyme system, in combination with the MCS, provides a simple way to differentiate between bacterial colonies with or without an insert.

pUC19 Plasmid Vector

- **4. Applications of pUC19 Vector**
- pUC19 is used for a variety of molecular biology applications:
- **a. Cloning :**pUC19 is commonly used for the **cloning** of foreign DNA fragments into E. coli. The plasmid can accept inserts up to a few kilobases in length. The presence of the MCS allows for the easy insertion of the desired gene or sequence.
- **b. Gene Expression:**Although pUC19 is primarily a cloning vector, it can also be used in gene expression studies, particularly when researchers are interested in producing large amounts of plasmid DNA or expressing small amounts of a gene.
- **c. Blue/White Screening:**The blue/white screening system provided by pUC19 is extensively used in laboratories for selecting recombinant colonies. This system is helpful when the researcher wants to quickly determine which bacterial colonies contain the correct insert.
- **d. Sequencing:**pUC19 can be used as a template for **DNA sequencing**. After cloning a DNA fragment into the vector, sequencing primers can be designed to sequence the insert from the MCS region or vector-specific regions.
- **e. Site-Directed Mutagenesis:**Site-directed mutagenesis protocols often use vectors like pUC19 to introduce specific mutations into cloned genes. The small size of pUC19 and the availability of its MCS make it ideal for such applications.

pUC19 Plasmid Vector

- **5. Advantages of pUC19**

- **High replication rate**: The ColE1 origin provides a high plasmid copy number in E. coli, which is beneficial for cloning and protein production.
- **Efficient transformation**: pUC19 can be easily transformed into E. coli, with high transformation efficiency, making it an ideal vector for molecular cloning.
- **Screening convenience**: The blue/white screening system simplifies the identification of recombinant clones without needing additional selection steps.

- **6. Limitations of pUC19**

- **Insert size limit**: The MCS of pUC19 has a limited size capacity, typically accepting inserts up to 3-5 kb. Larger fragments may require other vectors.
- **No inducible promoter**: pUC19 does not have an inducible promoter, which may limit its use in large-scale protein expression systems.

Plasmid Vector

- **Advantages of Plasmid Vectors**

1. **Ease of Manipulation:** Plasmids are relatively easy to manipulate in the laboratory due to their small size and the availability of many restriction enzymes that cut them at specific sites.

2. **High Copy Number:** Many plasmid vectors replicate to produce high copy numbers within a bacterial host, allowing for large quantities of plasmid DNA to be produced.

3. **Versatility:** Plasmids can be used in a variety of applications ranging from cloning to protein production, making them one of the most versatile tools in molecular biology.

4. **Efficiency:** Plasmids can efficiently introduce foreign genes into host cells. When used for protein production, plasmids can express recombinant proteins at high levels, which is ideal for industrial and research purposes.

5. **Selection and Tracking:** The presence of selectable markers and reporter genes allows for easy identification and monitoring of successful transformation or gene expression.

Plasmid Vector

- **Limitations of Plasmid Vectors**

1. **Size Constraints:** Plasmids have a limited capacity for inserting large DNA fragments. For large genes or genomic libraries, other vectors like BACs or YACs may be required.
2. **Host Range:** Some plasmid vectors are specific to certain host organisms. Using a vector with a broad host range can overcome this limitation.
3. **Transformation Efficiency:** While transformation methods (such as heat shock or electroporation) can be efficient, they still vary depending on the plasmid and host cell type.
4. **Expression in Eukaryotes:** Although plasmids can express genes in eukaryotic cells, some features (like bacterial promoters) may not work efficiently in eukaryotic systems, requiring modifications or the use of specialized expression systems.

Ideal Characters of Plasmid Vector

- An **ideal plasmid vector** should possess the following key characteristics to be effective for use in molecular cloning, gene expression, or other genetic manipulation tasks:
- **1. Origin of Replication (Ori):** The plasmid must have a functional **origin of replication** to allow for autonomous replication within the host cell. The Ori ensures that the plasmid is copied during cell division, maintaining plasmid copies in each bacterial generation.
- **2. Selectable Marker:** The plasmid should contain a **selectable marker gene**, such as an antibiotic resistance gene (e.g., ampicillin resistance, kanamycin resistance). This allows researchers to identify and select cells that have taken up the plasmid, facilitating the isolation of recombinant bacteria.
- **3. Multiple Cloning Site (MCS) or Polylinker:** The vector should have a **Multiple Cloning Site (MCS)**, also known as a polylinker, which is a short region with several unique restriction enzyme sites. This makes it easier to insert foreign DNA into the plasmid at specific locations.
- **4. Small Size:**A **small plasmid size** facilitates easier transformation into host cells, more efficient replication, and a lower metabolic burden on the host. Small plasmids are also easier to purify.
- **5. High Copy Number:**An ideal plasmid should have a **high copy number** to ensure that large amounts of the plasmid (and any inserted gene) are produced per cell. This is particularly important for large-scale production of recombinant proteins or plasmid DNA for other applications.

Ideal Characters of Plasmid Vector

- **6. Inclusion of Promoters for Expression (if needed):** If the plasmid is intended for gene expression, it should contain a **promoter** that can drive the transcription of the inserted gene. This could be a bacterial, viral, or eukaryotic promoter, depending on the host organism.
- **7. Efficient Transformation:**The plasmid should be designed to enable **efficient transformation** into host cells (such as E. coli or yeast). Features such as small size and high copy number contribute to this characteristic.
- **8. Compatibility with Host Cells:**The plasmid should be compatible with the host organism. For bacterial vectors, this often means being compatible with **common bacterial strains** such as E. coli. In some cases, vectors are designed for specific host strains, depending on the desired experiment.
- **9. Resistance to Host Nucleases:**The plasmid should be resistant to host **nucleases** to avoid degradation after transformation, ensuring that the plasmid remains intact during propagation.
- **10. Convenient Purification Methods:**The plasmid should allow for easy purification, with clear **separation of plasmid DNA** from host genomic DNA, often achieved by including certain sequences that enable the plasmid to be isolated via **alkaline lysis** or other purification methods.

Ideal Characters of Plasmid Vector

- **11. Low Toxicity to Host:**The plasmid vector should ideally not be toxic to the host cells, allowing them to grow and divide efficiently while carrying the plasmid.
- **12. Compatibility with Reporter Systems (if applicable):**In cases where monitoring gene expression or cloning efficiency is important, the vector may include a **reporter gene** (e.g., GFP or lacZ) for convenient screening and assessment.
- **13. Tissue-specific Promoters (if for Eukaryotic Cells):**For eukaryotic systems, a plasmid vector might have **tissue-specific promoters** or inducible promoters, allowing for controlled gene expression in specific cell types or under specific conditions.
- **14. High Stability:**An ideal plasmid should be **stable** in host cells, ensuring that the plasmid is maintained over multiple generations without the need for selection pressure.
- **15. Low Background:**A vector with **low background expression** is preferred, especially in the case of reporter gene assays or expression vectors, to minimize interference from unwanted plasmid-driven activity. By incorporating these features, a plasmid vector becomes an invaluable tool for genetic engineering, cloning, gene expression, and many other molecular biology applications.