# Plasmid Vector

## What are Plasmids?

Essentially, plasmids are small, circular molecules of DNA that are capable of replicating independently. As such, they do not rely on chromosomal DNA of the organism for replication. Because of this characteristic, they are also referred to as extra-chromosomal DNA.

## Plasmid Vector

A vector refers to any piece of molecule that contains genetic material that can be replicated and expressed when transferred into another cell. Based on this definition, it is possible to see why the words "vector" and "plasmids" are sometimes interchanged. However, this is not to say that all plasmids are vectors.

A suitable vector must have following characteristics:

1. It must have minimum amount of DNA.
2. It must have relaxed replication control.
3. It must have at least two suitable markers for identification.
4. It should be easily isolated from the cell.
5. It should possess a restriction site for one or more restriction enzymes.
6. Insertion of a foreign DNA molecule as one of these restriction sites does not alter its replication property.

| Features | Cloning Vector | Expression Vector |
|---|---|---|
| **Definition** | A cloning vector is a small piece of DNA, which is used to introduce the foreign gene of interest into the host cell. It can be stably maintained within the host organism. | An expression vector is a plasmid that not only introduces a gene of interest into host organism but also aids in the analysis of the gene of interest via relevant protein product expression. |
| **Role** | It is used to obtain multiple copies of the foreign gene of interest. | It is used to obtain or analyse the gene product, which may be RNA or protein, of the inserted gene of interest. |
| **Types** | They can be plasmids, cosmids, and phages, BACs, YACs or MACs. | Only a plasmid can be an expression vector. |
| **Features** | A typical cloning vector consists of an origin of replication, unique restriction sites, reporter gene and an antibiotic resistance site. | An expression vector consists of regulatory elements like enhancers, promoters, termination sequences, transcription initiation site and translation initiation sites, in addition to having all the features of a typical cloning vector. |

# Different types of plasmid vector

**Plasmid pUC18**

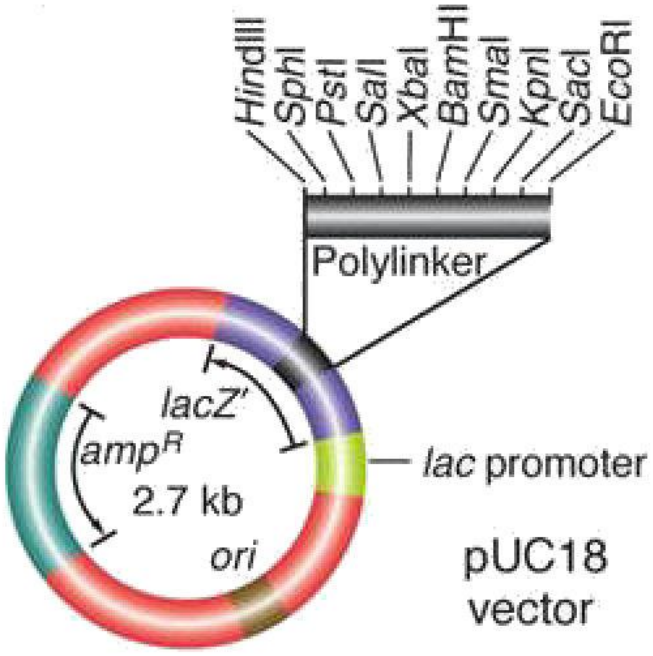
p- stands or plasmid, UC stands for University of California

A plasmid is a circular dsDNA molecule a few hundred or thousand base pairs in circumference. Naturally-occurring plasmids are viruses of bacteria. The artificial plasmid pUC18 has been genetically engineered to include –

(1) a gene for antibiotic resistance to Ampicillin (ampR)

(2) a gene (and its promoter) for the enzyme beta-galactosidase (lacZ).

(3) polylinker region, with a series of unique restriction sites found nowhere else in the plasmid. Digestion with any one of these endonucleases will make a single cut that linearizes the circular plasmid DNA, and allow it to recombine with foreign DNA that has been cut with the same endonuclease.



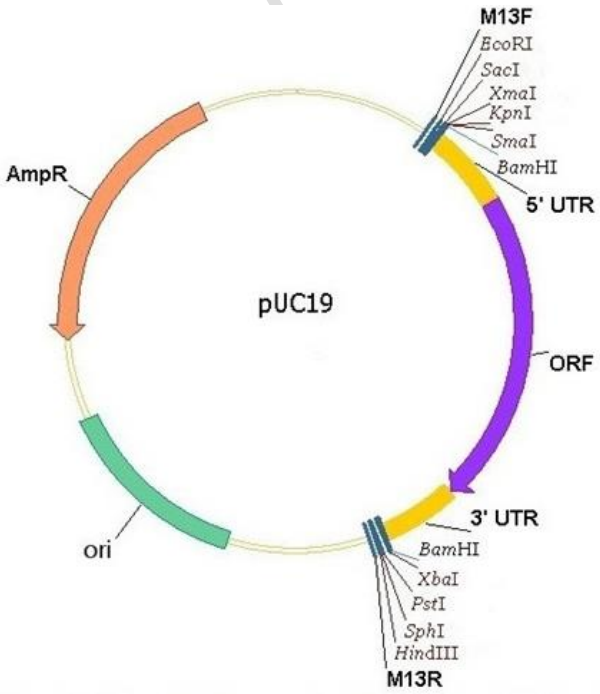
**Plasmid pUC19**

p- stands or plasmid, UC stands for University of California

pUC19 is one of a series of plasmid cloning vectors created by Joachim Messing and co-workers. The designation "pUC" is derived from the classical "p" prefix (denoting "plasmid") and the abbreviation for the University of California, where early work on the plasmid series had been conducted.

pUC19 vector is a small, high copy number, E. coli plasmid, 2686 bp in length. It contains identical multiple cloning site (MCS) as pUC18 vector except that it is arranged in opposite orientation.

pUC19 is a small, high-copy number E. coli plasmid cloning vector, of which multiple cloning sites as shown below. The molecule is a small double-stranded circle, 2686 base pairs in length. pUC19 encodes the N-terminal fragment of b-galactosidase (lac Za), which allows for blue/white colony screening (i.e., a-complementation), as well as a pUC origin of replication. Most commercially available competent cells are appropriate for the plasmid, e.g. TOP10, DH5α and TOP10F´, JM109. Selection of the plasmid in E. coli is conferred by the ampicillin resistance gene.



**Plasmid pBR322**

p- Plasmid, B- Boliver, R- Rodriguez, 322- numeral form that distinguished from other developed plasmid

pBR322 is a reconstructed plasmid. It is most widely used plasmid having 4363 base pairs. The structure of this vector is described below –

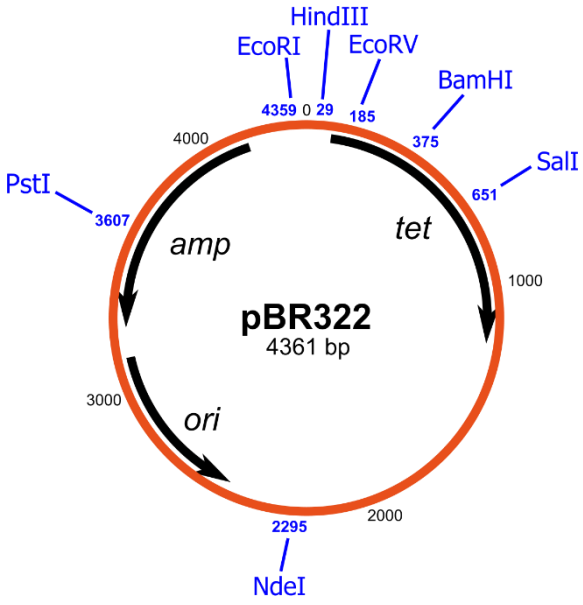
i. **Origin of replication**: it is derived from plasmid pMBI. This plasmid is closely related to naturally occurring plasmid Col EI.

ii. **Marker gene**: Gene *amp*$^r$ (ampicillin resistant) is derived from plasmid RI and gene *tet*$^r$ (tetracycline resistant) from plasmid R6.5. These genes are used as markers.

iii. **Restriction sites:** Insertion of foreign DNA fragment into the plasmid using restriction enzyme *Pst 1* or *pvu 1* places the DNA insert within the marker gene amp$^r$ makes it nonfunctional. Similarly, when restriction enzyme Bam HI or Sal I is used, the DNA insert is placed within the marker gene *tet*$^r$. This process allows an easy selection of a single bacterial cell having recombinant recombinant DNA.

Some useful character of pBR322 –

1. Small size (4363 base pairs) enables easy handling, purification and manipulation
2. Two selectable markers *amp*$^r$ and *tet*$^r$ permits easy selection of rDNA.
3. About 15 copies of them occur per cell which can be increased to 1000 to 3000 when protein synthesis is blocked.
4. It is a cloning vector.



## Bacterial Artificial chromosome (BAC)

A bacterial artificial chromosome (BAC) is shuttle vector created for cloning large sized foreign DNA. It is an engineered DNA molecule used to clone DNA sequences in bacterial cells (for example, E. coli). BACs are often used in connection with DNA sequencing. Segments of an organism's DNA, ranging from 100,000 to about 300,000 base pairs, can be inserted into BACs. The BACs, with their inserted DNA, are then taken up by bacterial cells. As the bacterial cells grow and divide, they amplify the BAC DNA, which can then be isolated and used in sequencing DNA.

First BAC vector was pBAC108L. The vector pBeloBAC11 has 7.4kb and allows selection of recombinant clones by lacZa. It has following modules-

oriS, repE, CM, cosN, lacZ, T7, SP6, parA, parB, parC and loxP

**Importance:**

1. BAC vectors can clone DNA insert of upto 300kb
2. They are stable and more user friendly.
3. BAC vectors are extensively used in the analysis of genomes.
4. The low copy number of BAC vectors per host cell maintain DNA insert in their original form.