pUC Vectors

Introduction:

pUC are obtained by modifying the pBR322 vector. pUC vectors are smaller than pBR322 of being only ~2.7 kb. But comparatively they have a high copy number. A mutation within the origin of replication produces 500 to 600 copies of the plasmid per cell without amplification.

The Nomenclature of pUC Vectors:

1. 'p' indicates the plasmid.

2. 'UC' stands for university of California where it was first developed by J. Messing et al.

We also see many numbers after this like pUC8, pUC18, pUC19 and so on. They are just the series of pUC and have been named just to separate from each other.

The construction of pUC vectors:

1. **Origin of Replication:** It is derived from the origin of replication of pBR322.The ColE1origin of replication of pBR322 has been modified by carrying out a chance mutation so that each transformed E. coli cell has 500-600 copies of the plasmid.

2. **Selectable Marker:** It has an ampicillin resistant gene. The transformed host cells can grow on media having ampicillin whereas non-transformed cells die.

3. lac Z' gene having multiple cloning sites (MCS)

Cloning with pUC8 involves insertional inactivation of the lac Z' gene, with recombinants identified because of their inability to synthesize beta-galactosidase

The lac Z' is incorporated into this vector codes for the enzyme **beta-galactosidase** which acts on a chromogenic (color producing) substrate called **X-gal** (present in bacterial culture media). The expression of lac Z' gene is induced by another compound present in the same media called **Iso-propyl-thiogalactoside (IPTG).**

When the enzyme substrate reaction takes place, then the X- gal is converted from **white to a blue compound.** Now the lac Z' gene itself has MCS. Hence, when the gene of interest has been **introduced into the lac Z'** gene, then it fails to code for beta-galactosidase and thus in this case the substrate (X-gal) is never converted to any other colour(will be white). This type of screening is called blue-white screening.

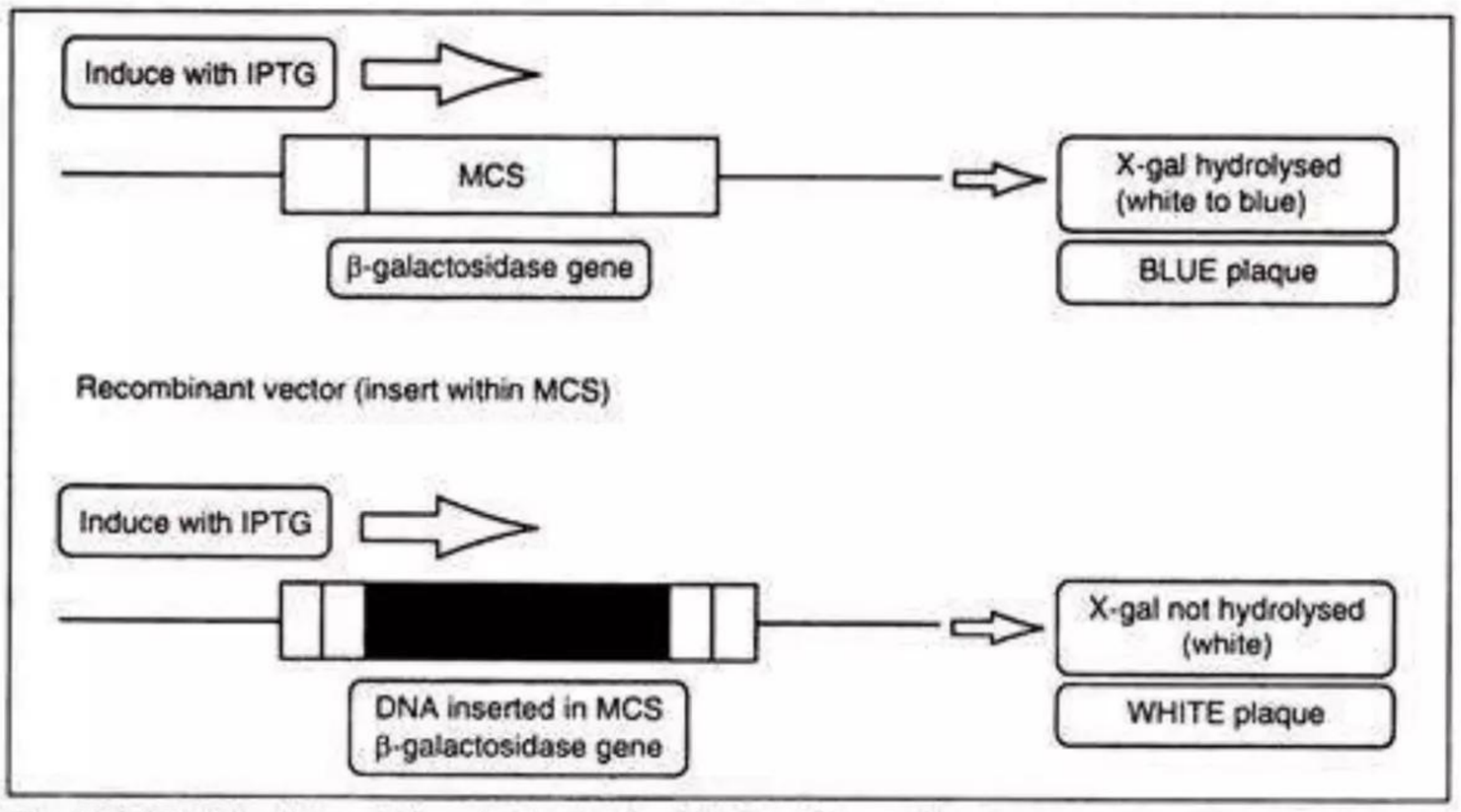

Fig. 4.17: Principle of blue-white selection for the detection of recombinant vectors

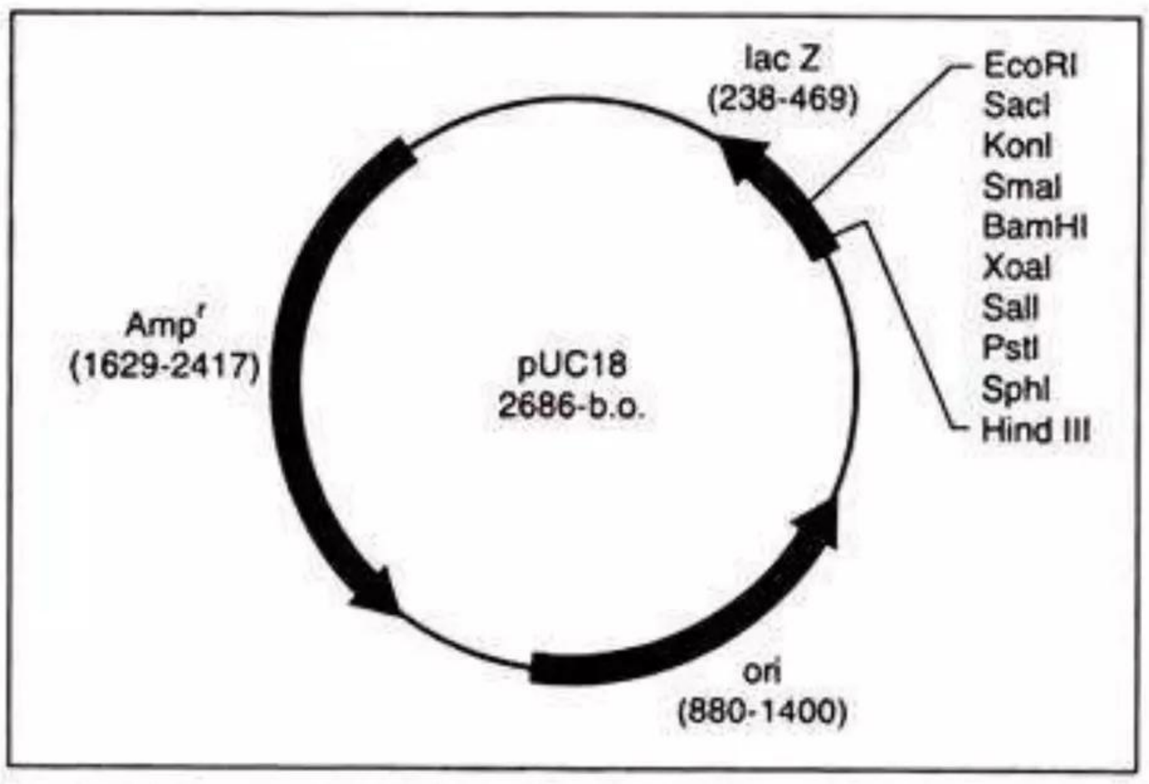

Fig. 4.18: Diagram sketch of pUC18. It differs from the pUC19 only in the region of polylinker site in the arrangement of restriction sites

Uses of pUC Vectors:

- pUC vectors can be used both as cloning vector and expression vector. When used as an expression vector its sequences are slightly modified to meet necessary requirements.

Advantages of pUC Vectors:

The pUC vectors offer following major advantages over pBR322 vectors:

- High copy number of 500-600 copies per cell.
- Easy and single step selection.
- The unique restriction sites used for cloning are clustered within the MCS. This allows cloning of a DNA fragment having two different sticky ends.

Disadvantages of pUC:

- It cannot accommodate a gene of interest larger than 15kb.