A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

Plasmid as Cloning Vector

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Vector

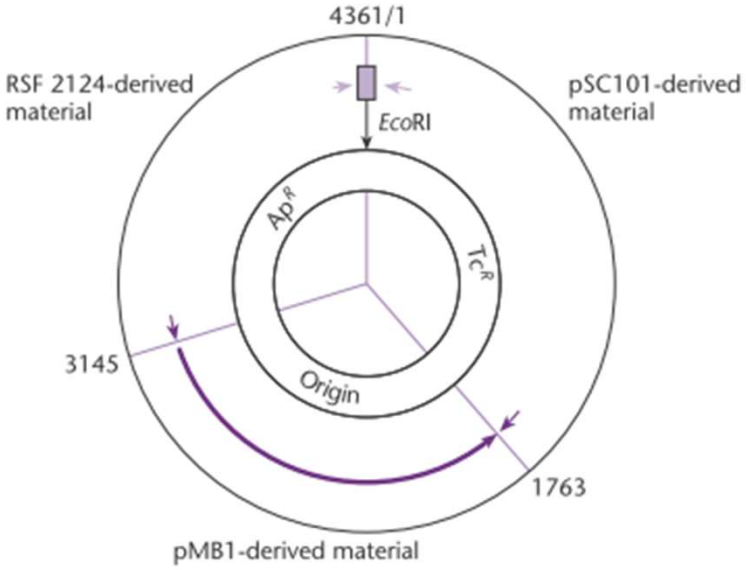
- Vector is an agent which carry DNA into a cell.
- Naturally plasmids often lack several important features that are required for a high quality cloning vector. These features are:
- 1. plasmid should be of small size
- 2. should contain unique restriction sites for multiple RE
- 3. should have one selectable genetic markers

pSC101 plasmid

- Earliest used plasmid vector
- Contains *ori* module for replication in *E.coli*
- Contains tetracycline resistant gene
- Contains single recognition site for *Eco*RI RE (outside tet^r gene)
- *Hin*dIII, *Bam*HI and *Sal*I sites are present within the tet^r gene
- Insertion of DNA into the *Eco*RI site leaves tet^r gene intact and functional
- Cloning of gene in tet^r gene leads to inactivation of this gene which is unable to grow on teteracycline containing media and leads to detection of transformed cell cumbersome
- Cloning in of gene/DNA fragment outside the tet^r gene region allow it to grow on tet containing media after transformation in *E.coli* and also non recombinant pSC101 will grow on the same condition leads to detection of recombinant colony difficult.
- These were the draw back of using pSC101 as a cloning vehicle.
- That leads to the construction of plasmid in the laboratory.

pBR322

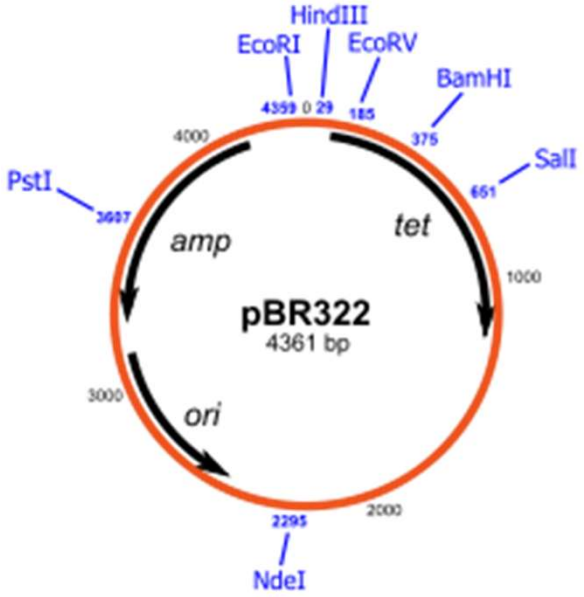
- Artificial plasmid vector
- Best known cloning vehicle
- p= plasmid, B= F. Bolivar and R= R. Rodriguez
- 322 distinguishes this plasmid from the other plasmids developed in the same laboratory, e.g., pBR325, pBR327 and pBR328



pBR322 continued…

- The creation of this artificial vector was done by using different parts of certain naturally plasmids.
- Plasmids pBR322 contains two selectable markers; ampicillin and tetracycline genes of RSF2124 and pSC101, respectively
- *Ori* sequence derived from pMB1 is a ColE1 like plasmid
- Total length is 4363 bp

- There are over 40 enzymes with unique cleavage sites on the pBR322 plasmid
- Tetracycline resistant gene contains 11 Restriction sites
- Promoters contain *cla*I and *Hin*dIII restriction enzymes restriction sites
- Ampicillin contain 6 restriction sites for 6 different restriction enzymes
- Thus, cloning in pBR322 with aid of any one of those 19 enzymes will result in insertional inactivation of either ampicillin or the tetracycline markers
- However, cloning in other unique sites doesn't permit the easy selection of recombinant, because neither of the antibiotic determinant is inactivated.

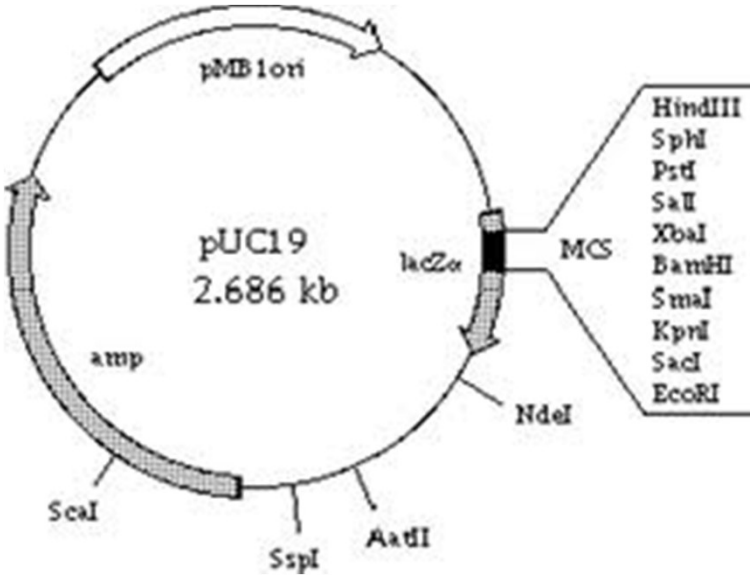


Useful features of pBR322

- Small size 4.4 kb enables easy purification and manipulation
- Two selectable markers (amp and tet) permits easy selection of recombinant DNA
- High copy no. of 15 copies per cell, which be amplified up to 1000 to 3000 when protein synthesis is blocked e.g., by applying chloramphenicol
- Theses feature made it popular cloning vector of late seventies

pUC vector

- Cloning vector
- pUC name derived from firstly prepared in university of California.
- Size ~2700bp
- Possesses ampicillin resistance gene
- *Ori* sequence derived from pBR322
- lacz gene derived from *E.coli*
- With in the *lacz* gene MCS is presents



Blue white screening

- When DNA fragments are cloned in this (MCS) region of pUC. The lac gene is inactivated called **insertional inactivation**.
- X-gal and IPTG
- Recombinant pUC will produce white colony
- Non-recombinant pUC will produce blue colony

Acknowledgement and Suggested Readings:

1. Gene Cloning and DNA Analysis: An Introduction; Sixth Edition ; T. A. Brown; Wiley – Blackwell Publications
2. Principles of Gene Manipulation; Sixth Edition; Sandy B Primrose, Richard M Twyman and Robert W. Old; Wiley – Blackwell Publications
3. Biotechnology: Applying the Genetic Revolution; David P. Clark and Nanette J. Pazdernik; Academic Press (Elsevier)

Thanks