

# CLONING VECTOR

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# WHAT IS CLONING VECTOR ?

- A cloning vector is a DNA molecule in which foreign DNA can be inserted or integrated and which is further capable of replicating within host cell to produce multiple clones of recombinant DNA.



# CHARACTERISTICS

- It should be able to replicate autonomously.
- Origin of replication
- Selectable markers
- Restriction sites



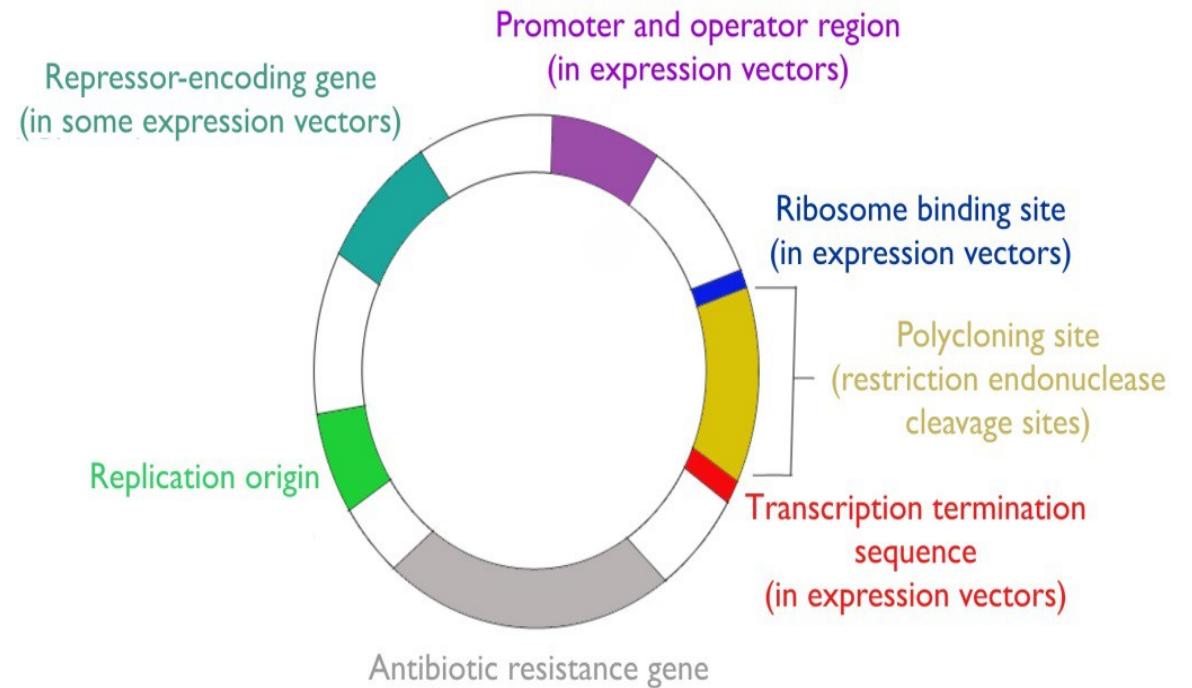
# TYPES

- Plasmid Vector
- Bacteriophage Vector
- Cosmid Vector
- Phagemid Vector
- Phasmid Vector
- Artificial Chromosome Vector



# 1. PLASMID VECTOR

- Plasmid is a DNA molecule , other than the artificial chromosome .
- Plasmid is a circular and double stranded DNA molecule and size ranges from 1 kb to over 250 kb.
- The plasmids can be single copy plasmids that are maintained as 1 plasmid DNA per cell or multicopy plasmids which are maintained 10-20 genome per cell.



# TYPES OF PLASMID VECTOR

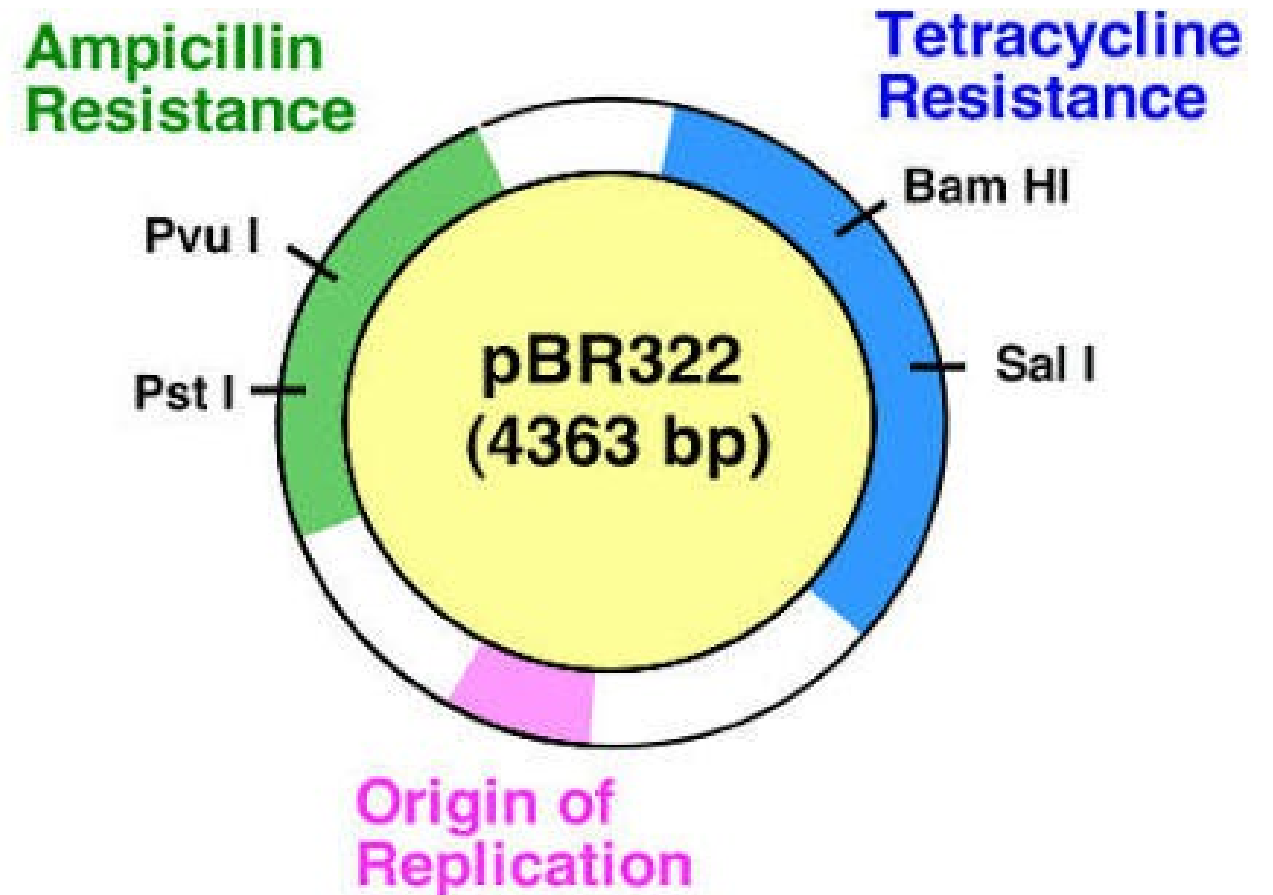
1. pBR322
2. pBR327
3. pUC
4. pSP64,pSP65
5. Agrobacterium plasmid vector



# 1.pBR322

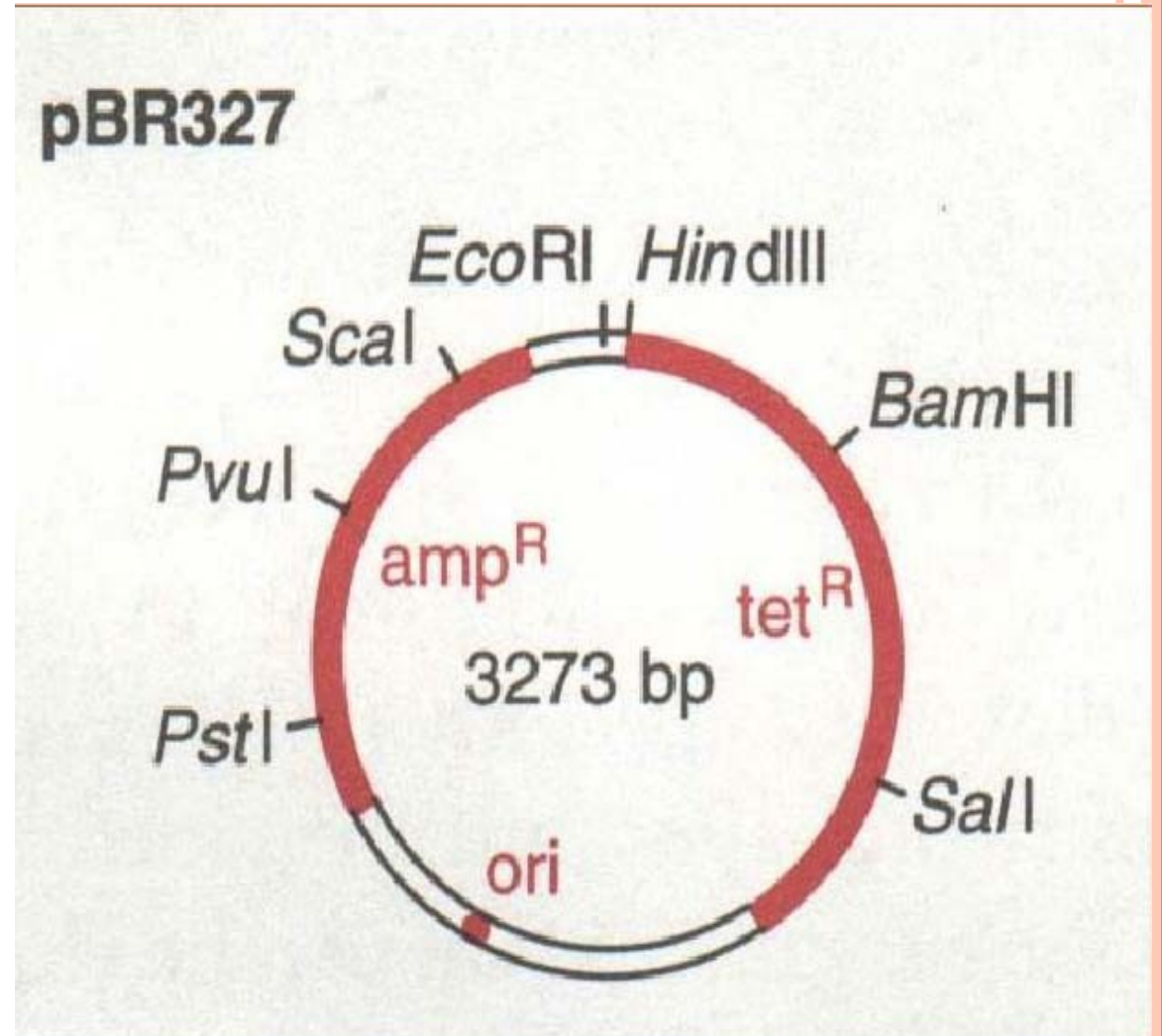
Contains:-

- ❑ Selectable markers –
  - Ampicillin resistance gene
  - Tetracycline resistance gene
  - Col E1 replication origin
  - Eco R1 site



## 2. pBR327

- It is derived from pBR322 by deletion of nucleotides between 1427 to 2516.
- These nucleotides are deleted to reduce the size of vector and eliminate sequences that were known to interfere with the expression of cloned DNA in eukaryotic cell.
- It contains genes for resistance against two antibiotics .

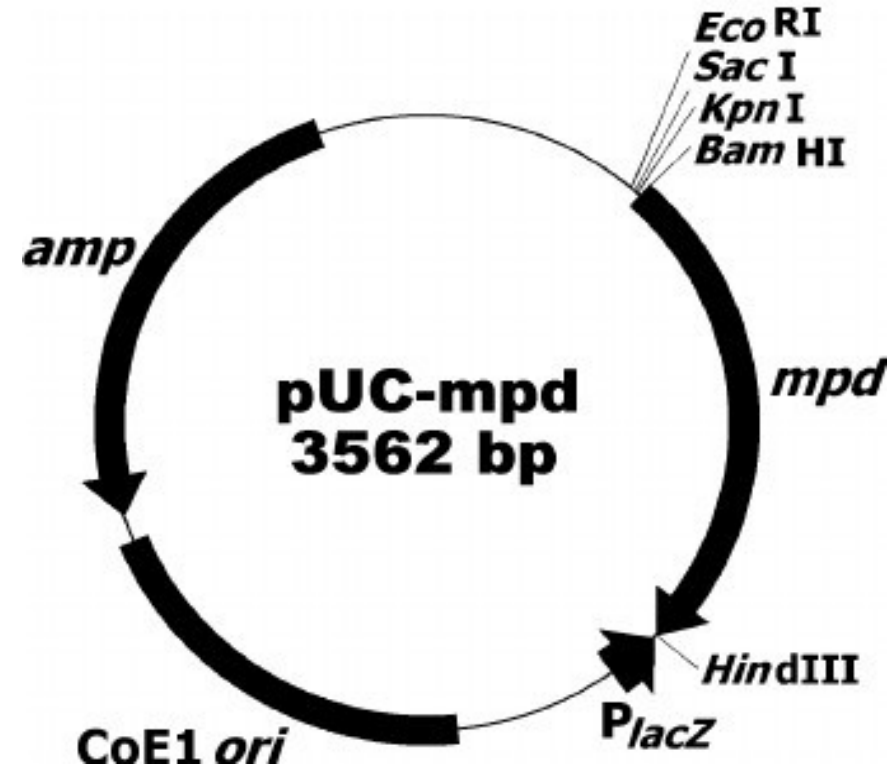




### 3. pUC Vector

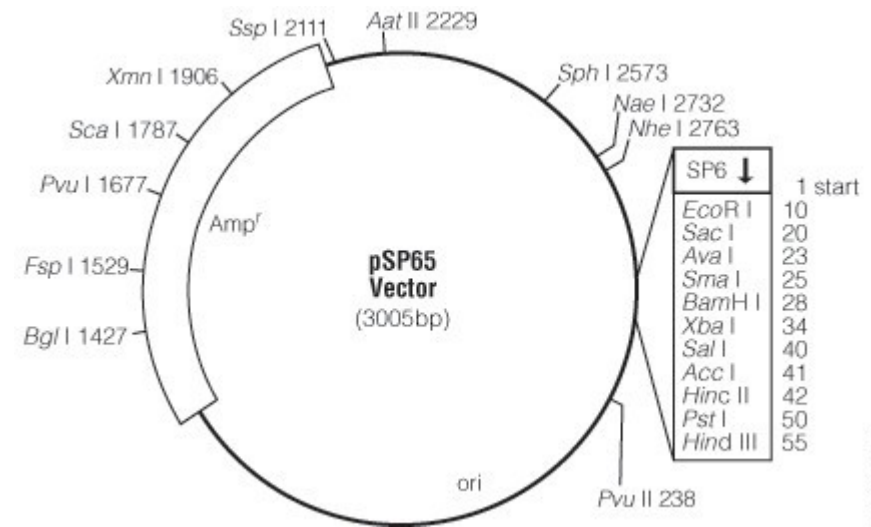
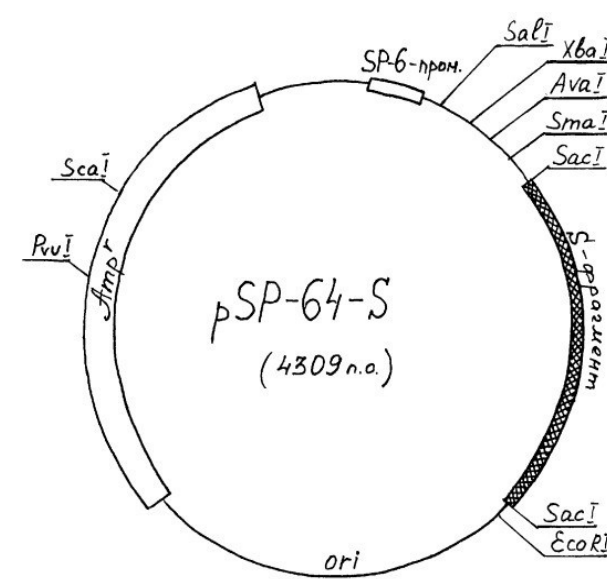
Contains :-

- ❑ Ampicillin resistance gene
- ❑ Multiple cloning site
- ❑ Col E1 (Origin)



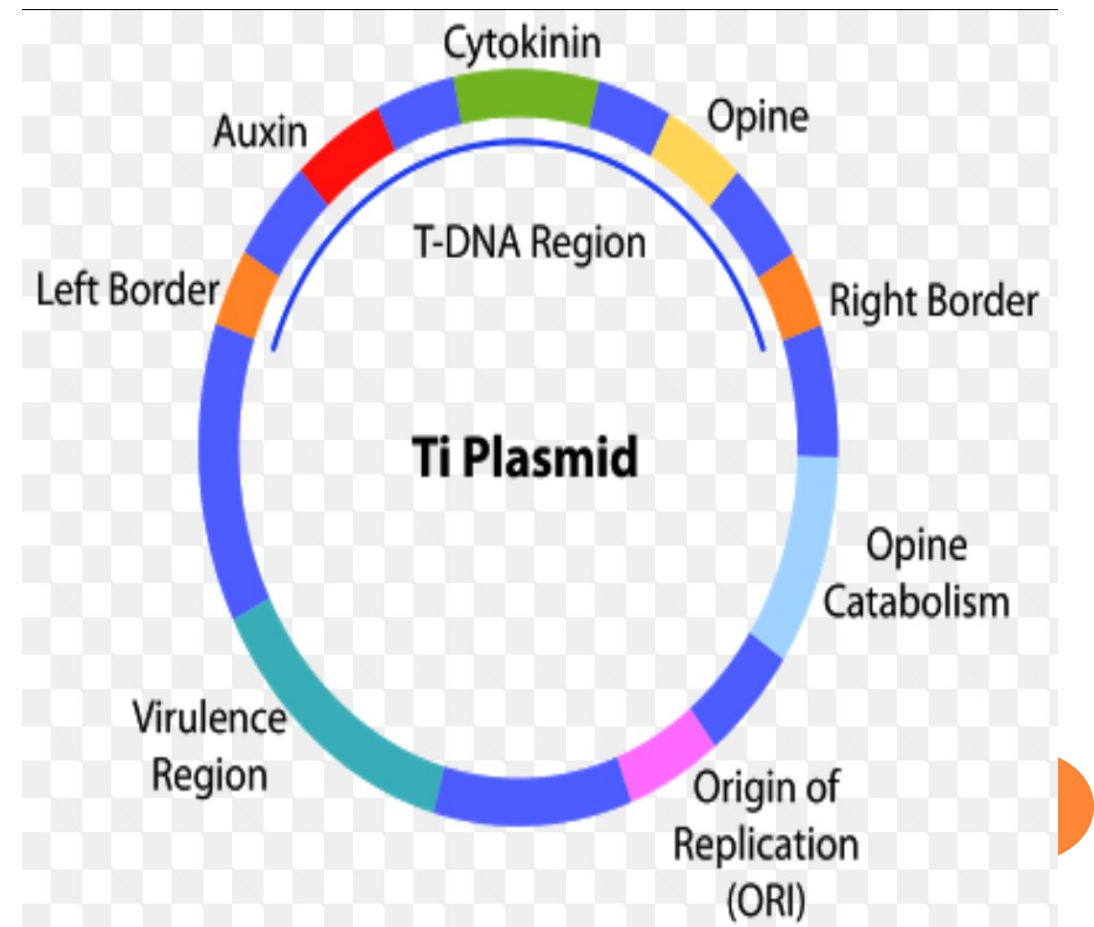
## 4. pSP64,pSP65

- These vectors are derivatives of pUC plasmid vector and carry promoters for phage RNA polymerase to allow transcription in vitro .
- pSP64 and pSP65 to contain the phage promoter SP6 and the poly cloning sites arranged in opposite orientation to allow transcription of desired strand from the cloned double stranded DNA.



## 5. Agrobacterium Plasmid Vector

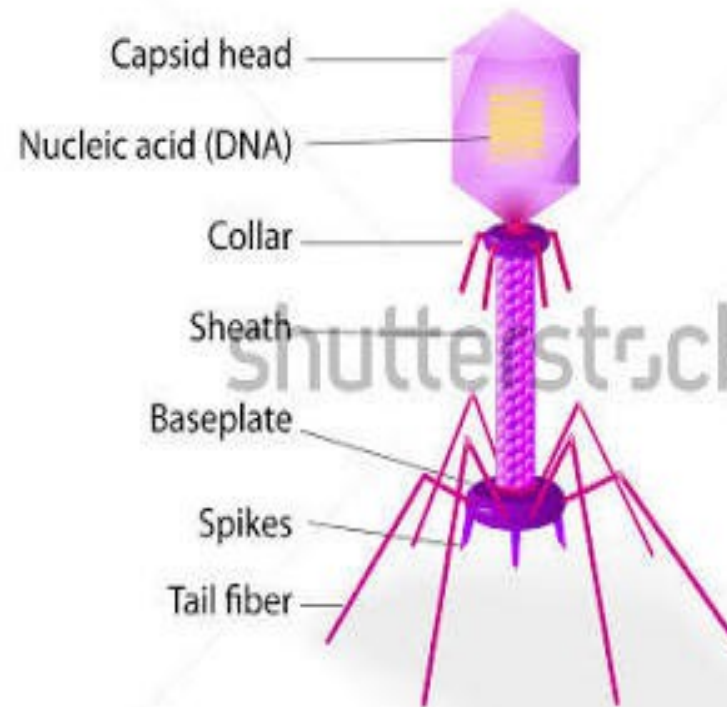
- In higher plants Ti plasmid of *Agrobacterium tumefaciens* or Ri plasmid of *Agrobacterium rhizogenes* are best known vectors.
- T-DNA from Ti or Ri plasmid of *Agrobacterium* is considered to be a very potential vector for cloning experiment.



# BACTERIOPHAGE VECTOR

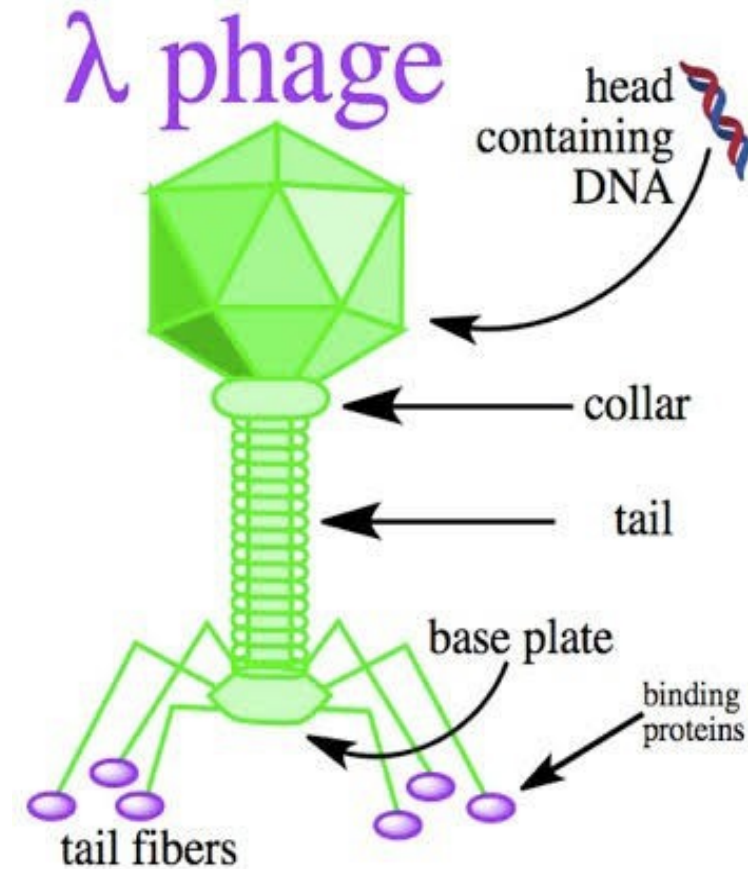
- Bacteriophages are viruses that attack bacteria .
- Several bacteriophages like Lambda ( $\lambda$ ) and M13 are used as cloning vectors .

## Structure of bacteriophage



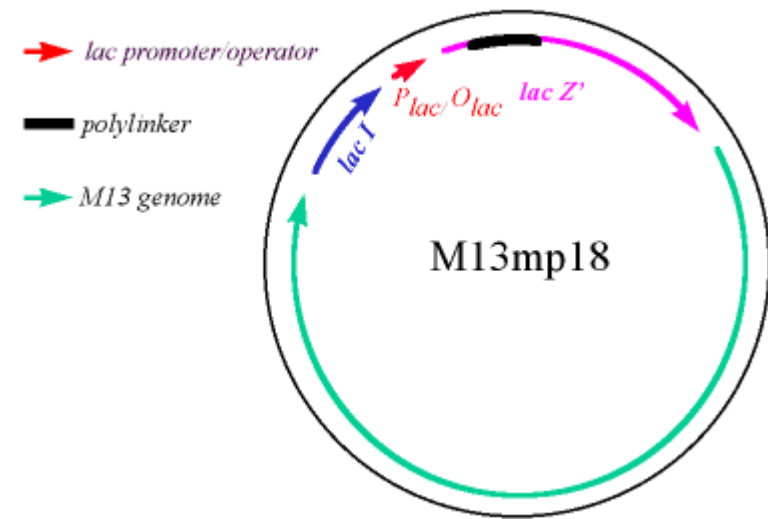
# Lambda ( $\lambda$ ) phage Vector:-

- High transformation efficiency , about 1000 times more efficient than plasmid vector.
- Origin of replication
- Genes for head and tail proteins
- Single stranded protruding cohesive ends
- Size is 48 - 502 bp



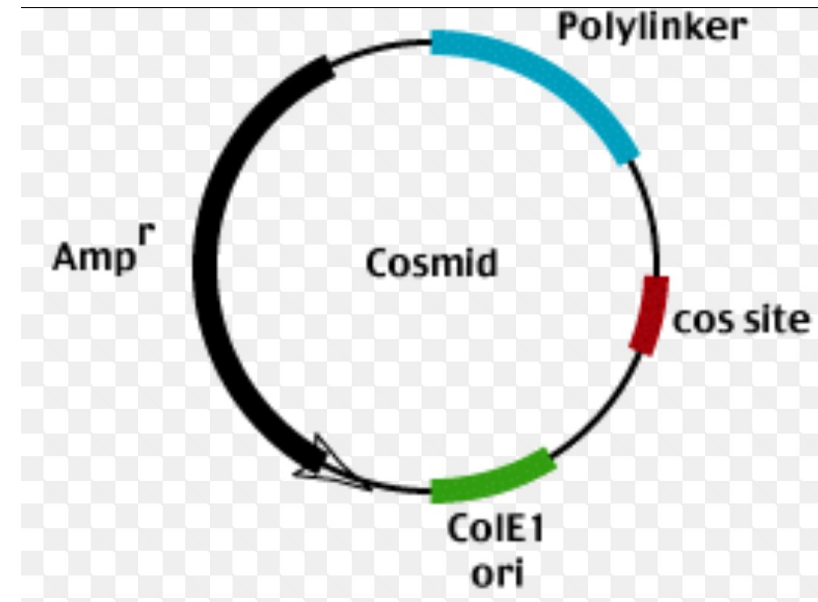
## ➤ Phage M13 Vector

- These vectors are used for obtaining single stranded copies of cloned DNA which are specially suited for DNA sequencing .
- They are derived from 6.4kb genome of E.coli , filamentous bacteriophage M13.
- This phage has a single stranded , linear DNA genome in phage particle which becomes converted into a double stranded , circular replicative intermediate with in the host cell



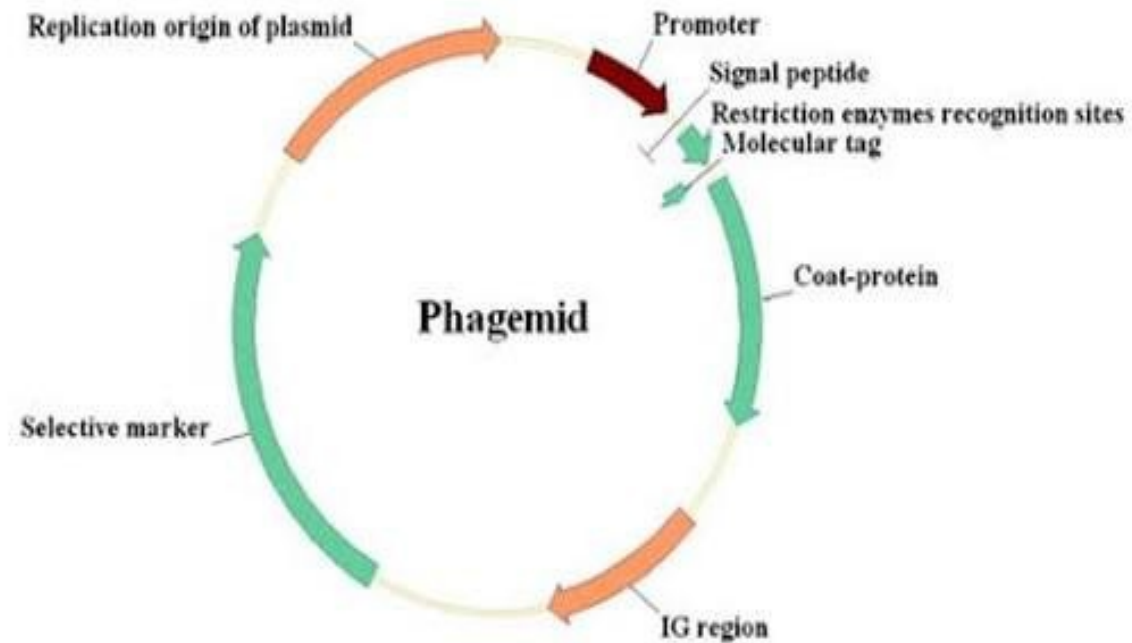
### 3. COSMID VECTOR

- Combine part of the Lambda chromosome with parts of plasmids.
- An origin of replication (Ori).
- A cos site (a sequence yield cohesive end )
- An ampicillin resistance gene (amp)
- Restriction site for cloning .
- Cosmid can carry up to 50 kb of inserted DNA.



## 4. Phagemid Vectors

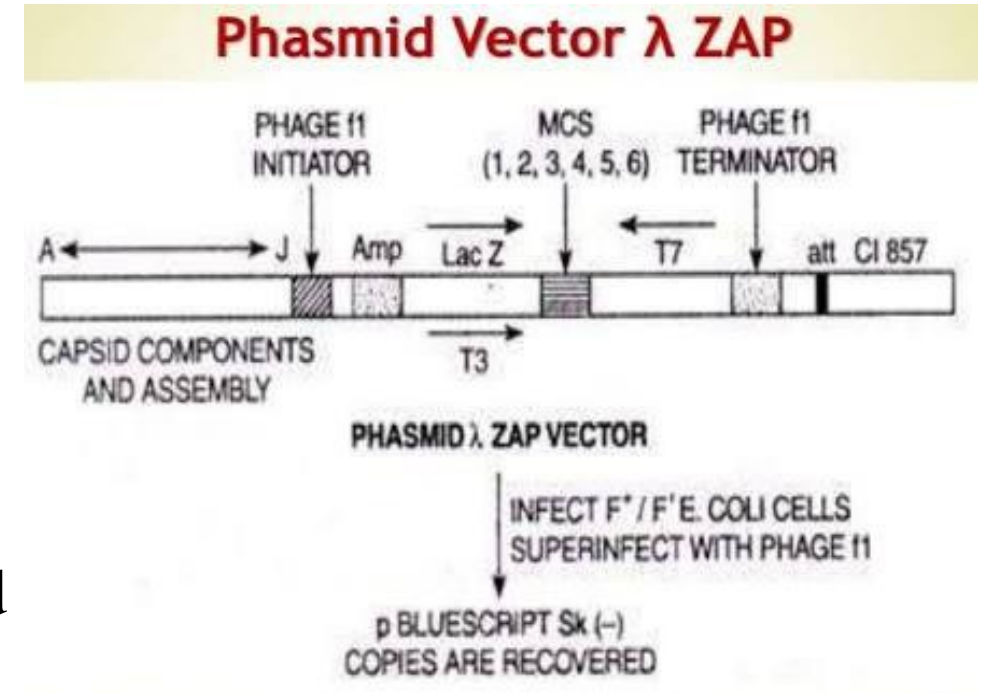
- A plasmid vector that contains origin of replication from a phage, in addition to that of plasmid is called Phagemid.
- P Blue script SK is a phagemid vector of 2958 base pairs derived from pUC19 . It contain ;
  1. Phage N13 origin of replication
  2. A portion of lac Z gene driven by lac promotor.
  3. A multi[ple cloning site (MCS) within lac Z gene .





## 5. Phasmid Vector

- These vectors are  $\lambda$  insertion vectors consisting of shortened linear  $\lambda$  genome containing DNA replication and lytic function + the cohesive end of phage.
- A good example of phasmid  $\lambda$  ZAP. The main features of  $\lambda$  ZAP are as below:-
  - i. The DNA insert is placed with MCS located in the lac Z gene of P blue script SK(-).
  - ii. It is suitable for cloning of cDNA using EcoRI linker.
  - iii. Integration of DNA insert inactivates lac Z gene.



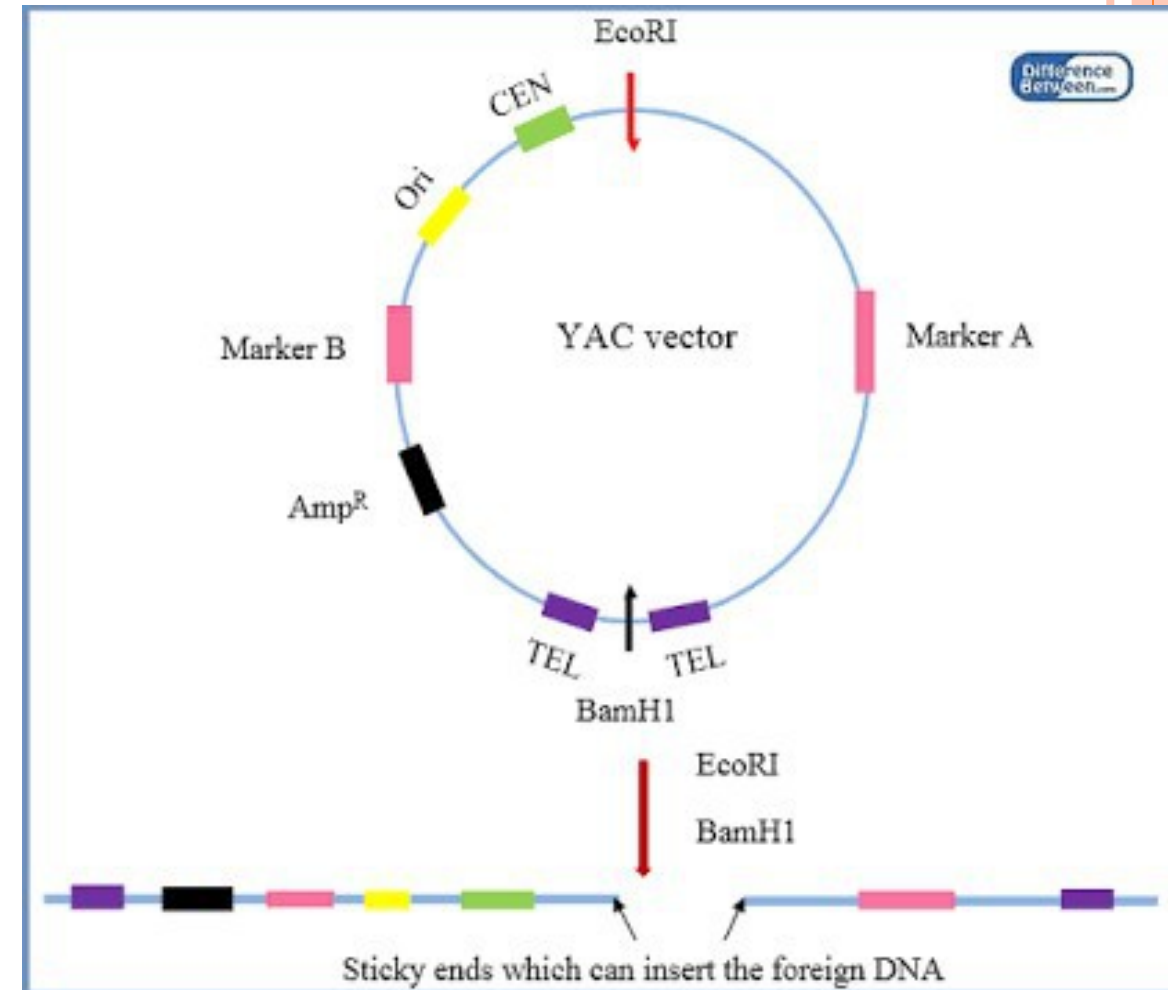
## 6. Artificial chromosome vector

- These vectors are circular or linear vector that are stably maintain usually 1-2 copies per cell.
- There are several types of such vectors ;
  1. Yeast Artificial Chromosome [YAC]
  2. Bacterial Artificial Chromosome [BAC]
  3. Mammalian Artificial Chromosome [MAC]
  4. Human Artificial Chromosome [HAC]



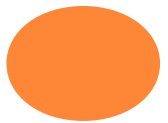
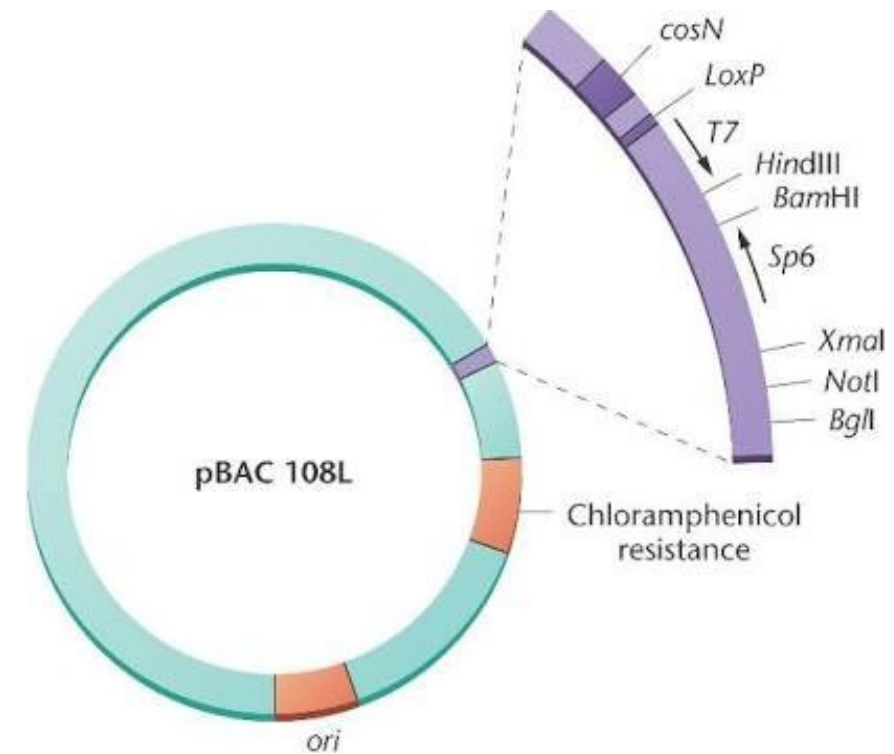
# Yeast Artificial Chromosome

- YAC are genetically engineered chromosome derived from the DNA of the Yeast , *Saccharomyces cerevisiae*.
- YAC vectors allow the cloning, within yeast cells , of fragments of foreign genomic DNA that can approach 500 kbp in size.



# Bacterial Artificial Chromosome

- A BAC is a DNA construct, based on a functional fertility plasmid, used for transforming and cloning in bacteria, usually E.Coli.
- They are capable of carrying approximate up to 300 kbp of inserted DNA sequence.



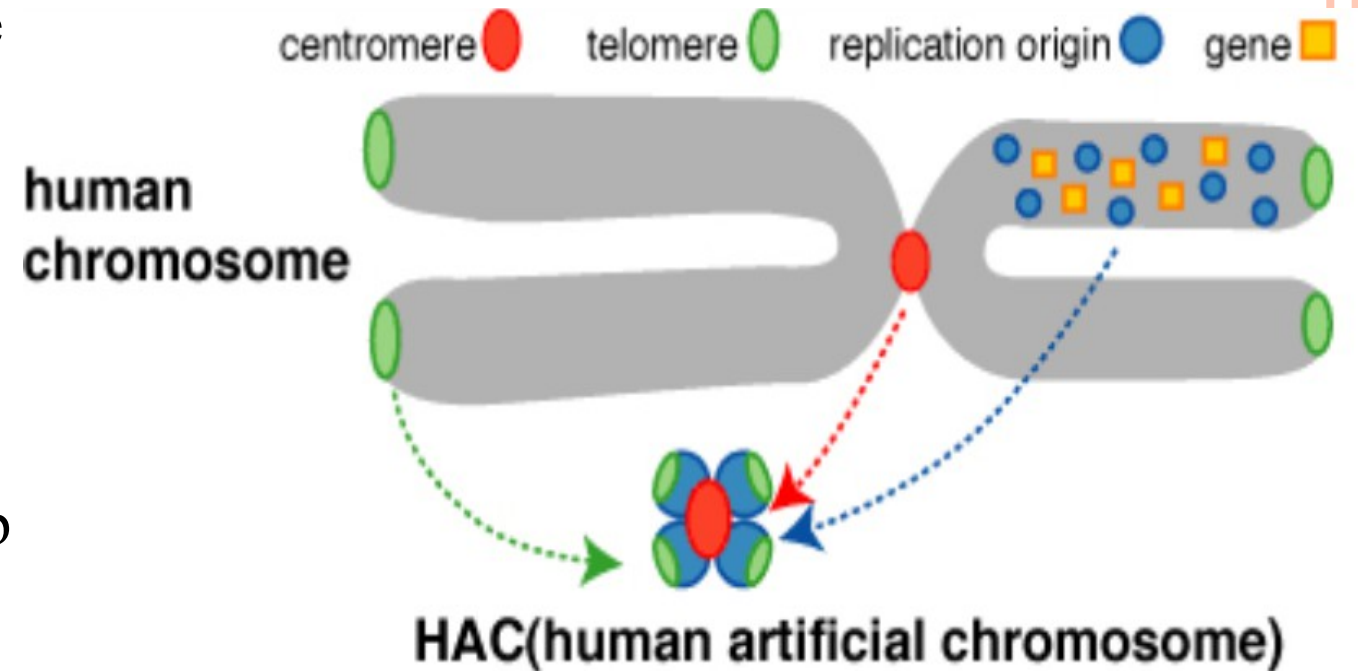
# Mammalian Artificial Chromosome

- MAC are produced by mammalian telomere and some centromeric sequences.



# Human Artificial Chromosome

- A HAC is a microchromosome that can act as a new chromosome in a population of human cells.
- That is, instead of 46 chromosomes, the cells could have 47 with the 47th being very small, roughly 6-10 megabases (Mb) in size instead of 50-250 Mb for natural chromosomes, and able to carry new genes introduced by human researchers.



# Applications

- A particular gene can be isolated and its nucleotide sequence is determined.
- Control sequence of DNA can be identified and analysed.
- Protein / Enzyme / RNA function can be investigated .
- Mutation can be identified example gene defects related to specific disease.
- Organisms can be engineered for specific purposes example Insulin production

