Cloning Vectors: pUC 19 and λ Phage

What is gene cloning?

- Gene cloning is the technique of producing of exact copies (clones) of a particular gene or DNA sequence.
- The DNA containing the target gene(s) is split into fragments using restriction enzymes.
- These fragments are then inserted into cloning vectors making the recombinant DNA molecules, which is then transferred into suitable host cells.
- Inside the host cell the inserted DNA fragment or gene undergoes replication making multiple copies of the target gene.

Cloning vector

Cloning vector is the central component of a gene cloning process.

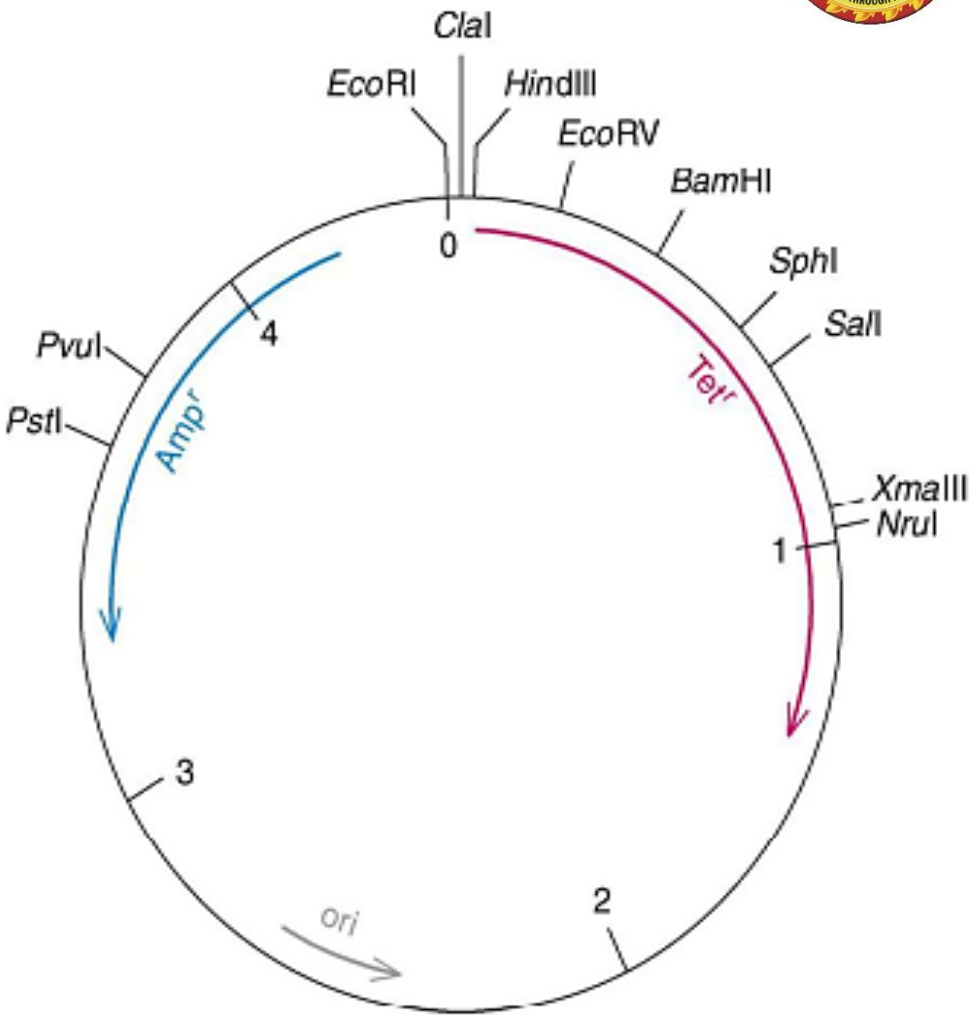
A small piece of DNA into which a foreign DNA fragment can be inserted.

The insertion of the fragment is carried out by treating the vector and the foreign DNA with a restriction enzyme that creates the same overhang, then ligating the fragments together.

Characteristics of a cloning vector

- Ori (Origin of replication) is a specific sequence of nucleotide from where replication starts.
- It should have selectable marker gene.
- It should have restriction sites: a synthetic multiple cloning site (MCS) can be inserted into the vector.
- Replicate inside the host cell to form multiple copies of the recombinant DNA molecule.
- Less than 10kb in size.

- **Origin of Replication: Allow the vector as well as the foreign DNA to amplify in the host cell**
- **Selectable Marker: Antibiotic resistance genes- Allow the host to grow on selective media; Can selectively amplify this specific vector in the host cell**
- **Multiple Cloning Sites: Allow insertion of foreign DNA**



pUC19 as a cloning vector

- ✓The pUC have been constructed by modifying the pBR322 vector structure.
- ✓pUC vector has a size of ~2.7 kb and smaller than pBR322.
- ✓They have higher copy numbers than pBR322.
- ✓In pUC19, ‘p’ indicates plasmid (type of vector), ‘UC’ stands for University of California (lab where it was developed), ’19’ represents the number to distinguish it from its predecessors like pUC9 and pUC18.

Construction of pUC19

➢**Origin of Replication (*Ori*)**:

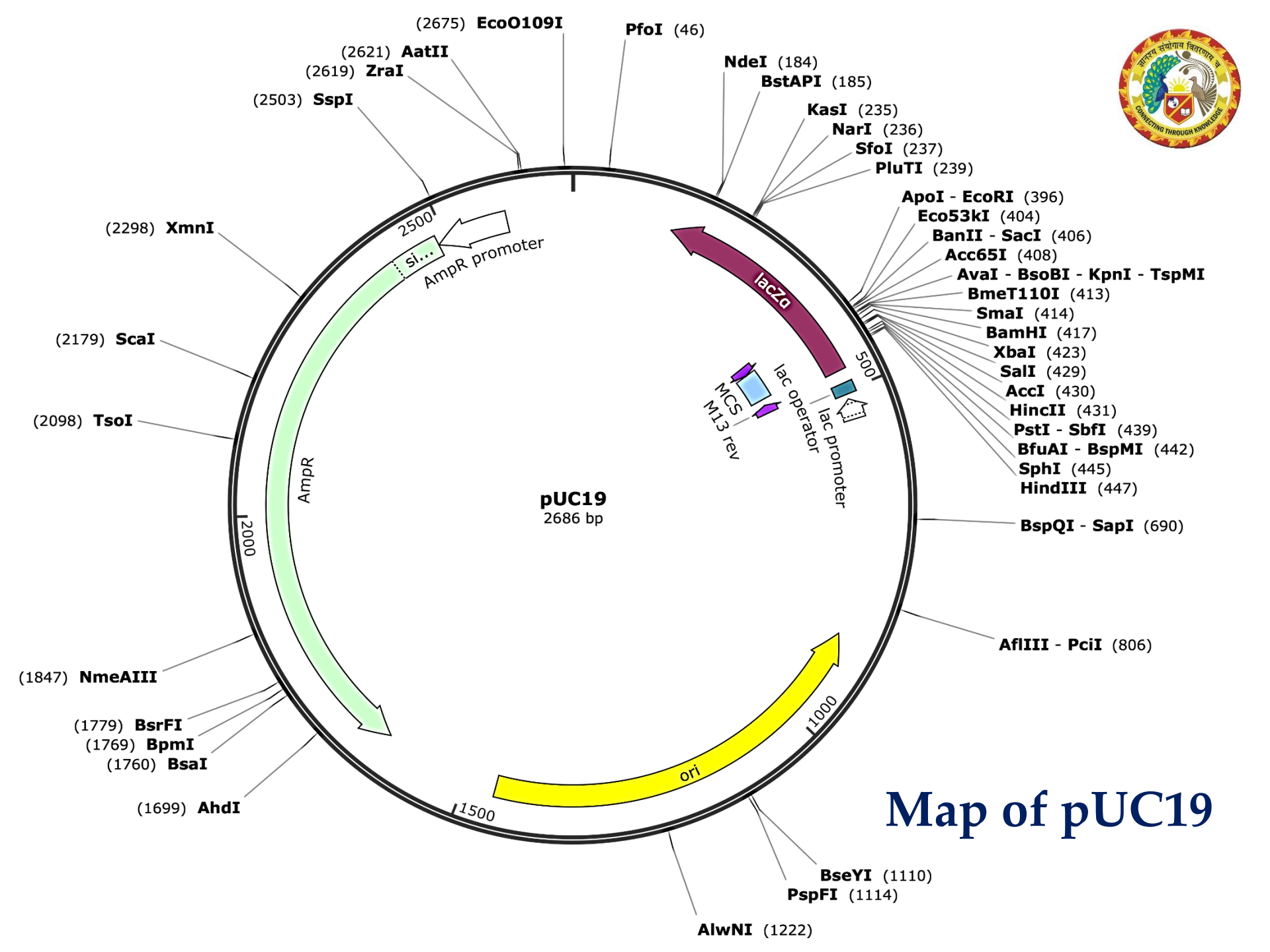
It is derived from the *Ori* of pBR322 with a chance mutation to increase the copy number of the plasmid.

➢**Selectable Marker**:

It has an Ampicillin resistant (Amp^R) gene as a selectable marker for the screening of trnasfromants.

➢**Scorable Marker**:

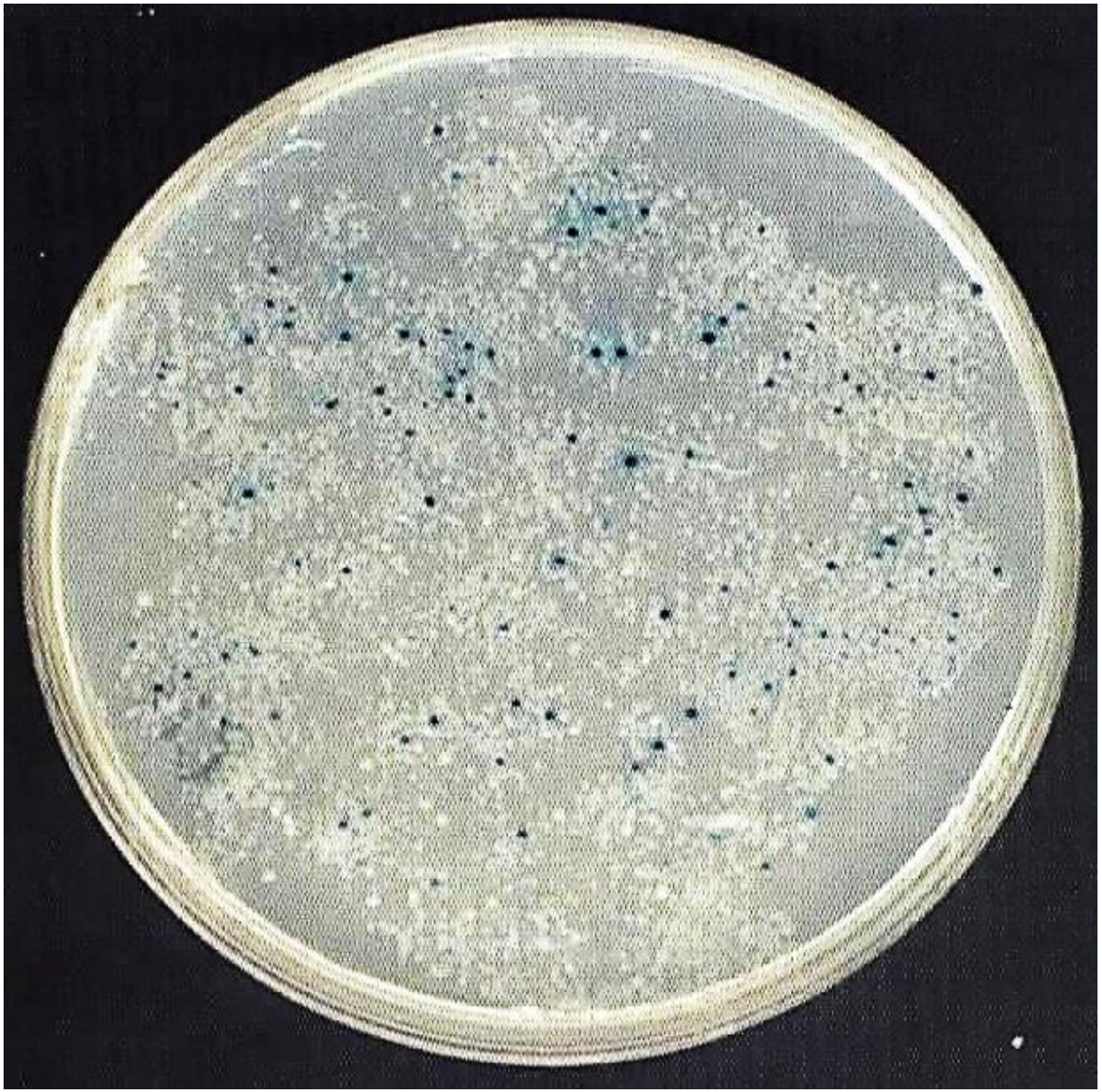
The *LacZ* gene having multiple cloning sites (MCS) within its sequence act as the scorable marker to screen out positive or negative transformants on the basis of blue-white screening.


(2675) EcoO109I
PfoI (46)
(2621) AatII
(2619) ZraI
NdeI (184)
BstAPI (185)
(2503) SspI
KasI (235)
NarI (236)
SfoI (237)
PluTI (239)
ApoI - EcoRI (396)
Eco53kI (404)
BanII - SacI (406)
Acc65I (408)
AvaI - BsoBI - KpnI - TspMI
BmeT110I (413)
SmaI (414)
BamHI (417)
XbaI (423)
SalI (429)
AccI (430)
HincII (431)
PstI - SbfI (439)
BfuAI - BspMI (442)
SphI (445)
HindIII (447)
(2298) XmnI
2500
si...
AmpR promoter
lacZα
500
(2179) ScaI
MCS
M13 rev
lac operator
lac promoter
(2098) TsoI
AmpR
pUC19
2686 bp
2000
BspQI - SapI (690)
AflIII - PciI (806)
(1847) NmeAIII
1000
(1779) BsrFI
(1769) BpmI
(1760) BsaI
ori
(1699) AhdI
1500
BseYI (1110)
PspFI (1114)
AlwNI (1222)

Map of pUC19

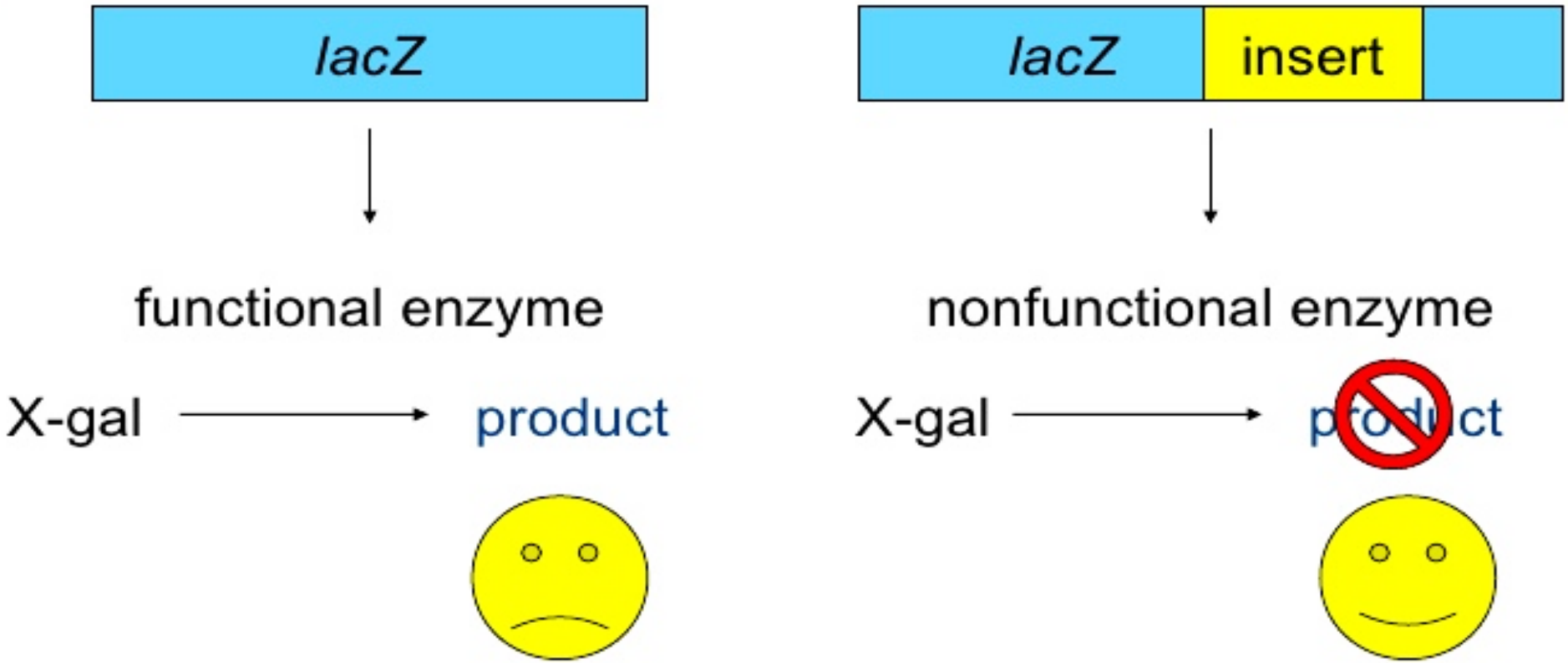
Blue-White Screening

- ✓Transformed bacterial colonies (host) with recombinant plasmids appears white on the selective media with ampicillin, while colonies with non-recombinant plasmids are blue.
- ✓Use the concept of lac operon as the vector contains a portion of it coding the β-galactosidase.
- ✓X-gal is supplemented with the medium, which is a substrate of β-galactosidase and turns blue in the presence of functional β-galactosidase.
- ✓Insertion of foreign DNA into the MCS disrupts the lac operon, β-galactosidase becomes non-functional and the colonies fail to turn blue, but appear white.



Blue and while colonies on the ampicillin and X-gal LB media

Blue/White Color Screening



Molecular mechanism of blue/white screening in pUC19.

α-Complementation

LacZ→ Beta galactosidase (Homotetramer)

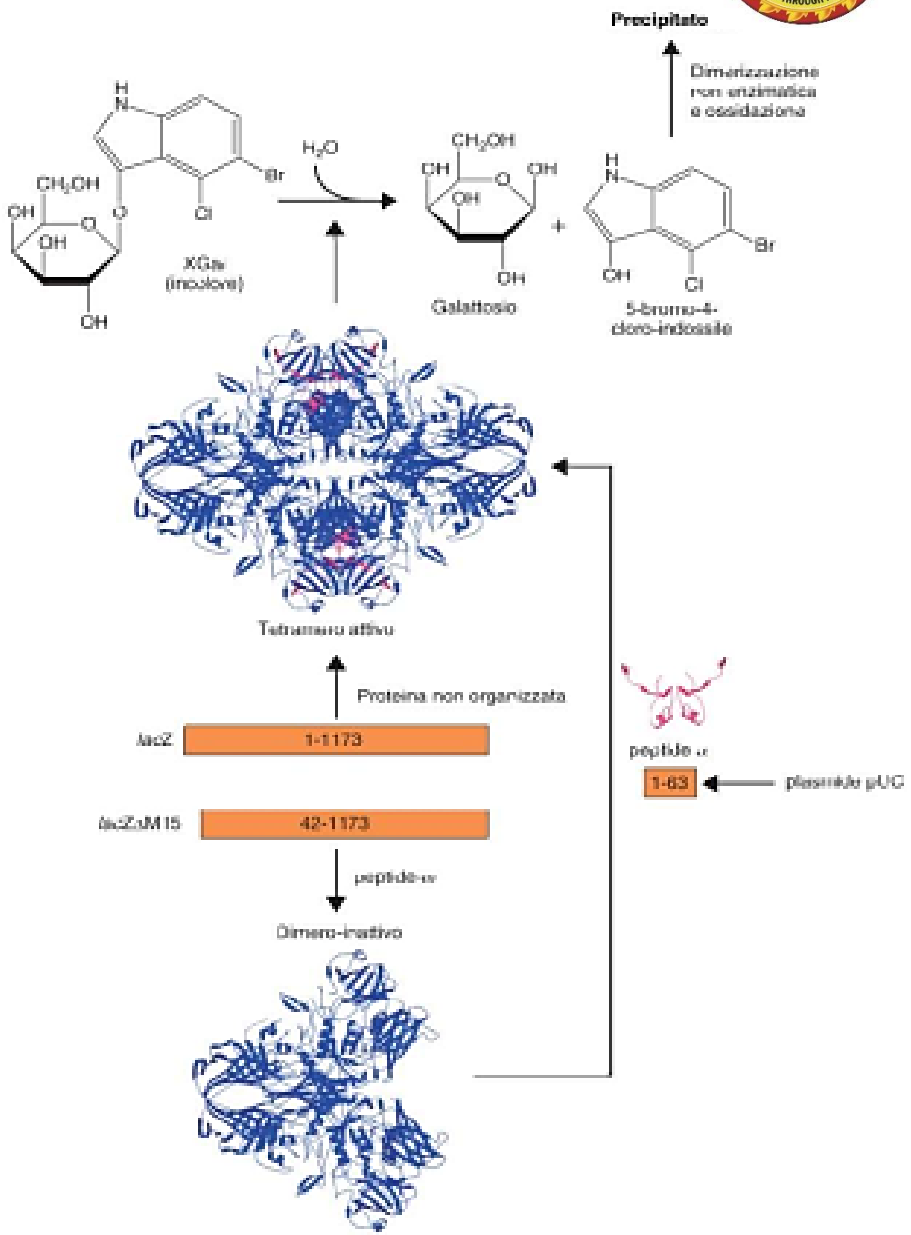
- 1021aa → 3,1 kbp
- Bacteria carry mutant allele (LacZΔM15) lacking N-terminal domain → inactive protein

- Alpha peptide carried by vector plasmid
- MCS inserted into LacZ alpha peptide →

✓ With insert = white colonies

✓ Without insert = blue colonies

- Exploits X-Gal (5-bromo-4-cloro-3-indonyl-Betagalactoside), a chromogenic substrate analog to galactose



Advantages of pUC19 vector

- ✓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 pUC19 vector

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

λ-Phage

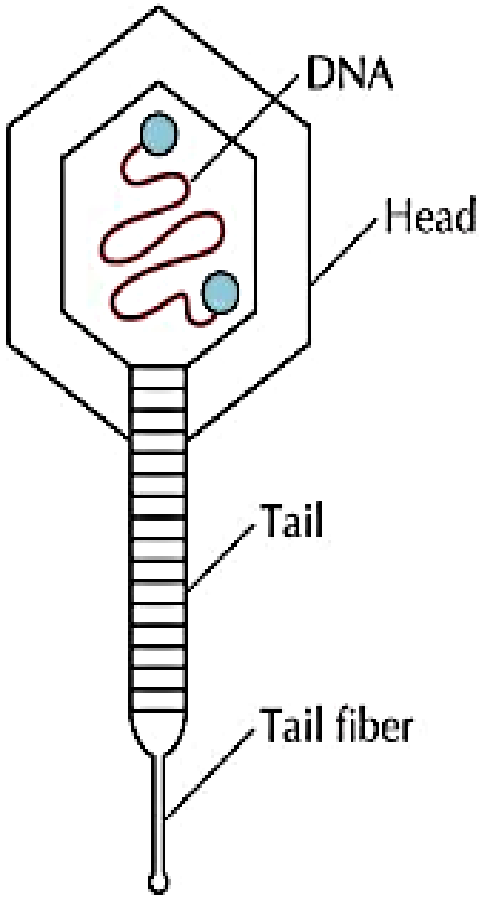
- It is 49kb in size and is used as a cloning vector because:

✓ The genes related in terms of function are clustered together in the genome and allows the genes to be switched on and off as a group rather than individually.

✓ The linear double stranded DNA molecule has a stretch of 12 nucleotides at its either ends which act as sticky ends or cohesive ends (cos sites)

✓ They can base pair to form a circular DNA molecule which is important for insertion into the bacterial genome.

✓ Another role of cos sites is in the formation of large number of λ DNA molecules by rolling circle mechanism of replication (Catenane).

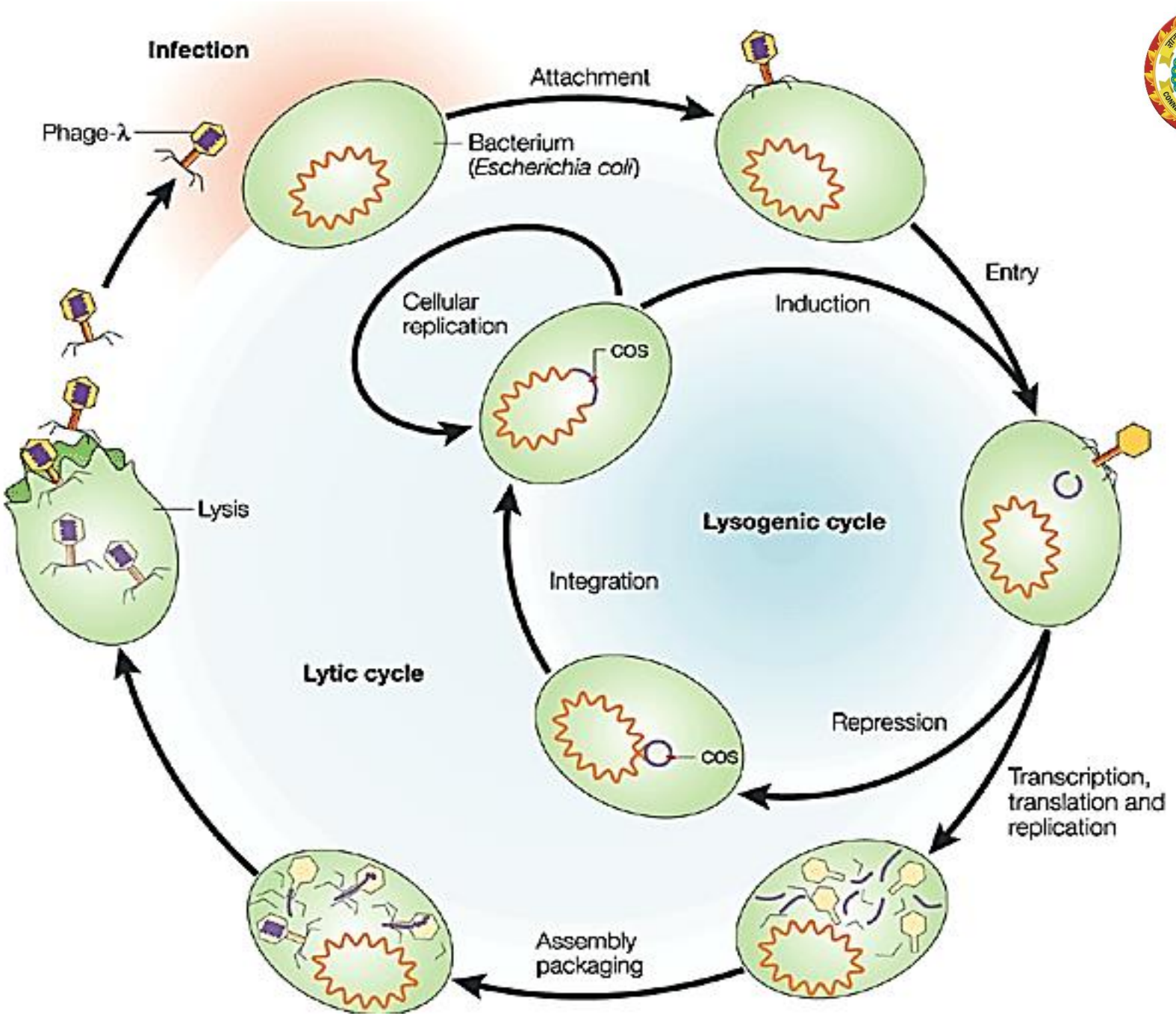


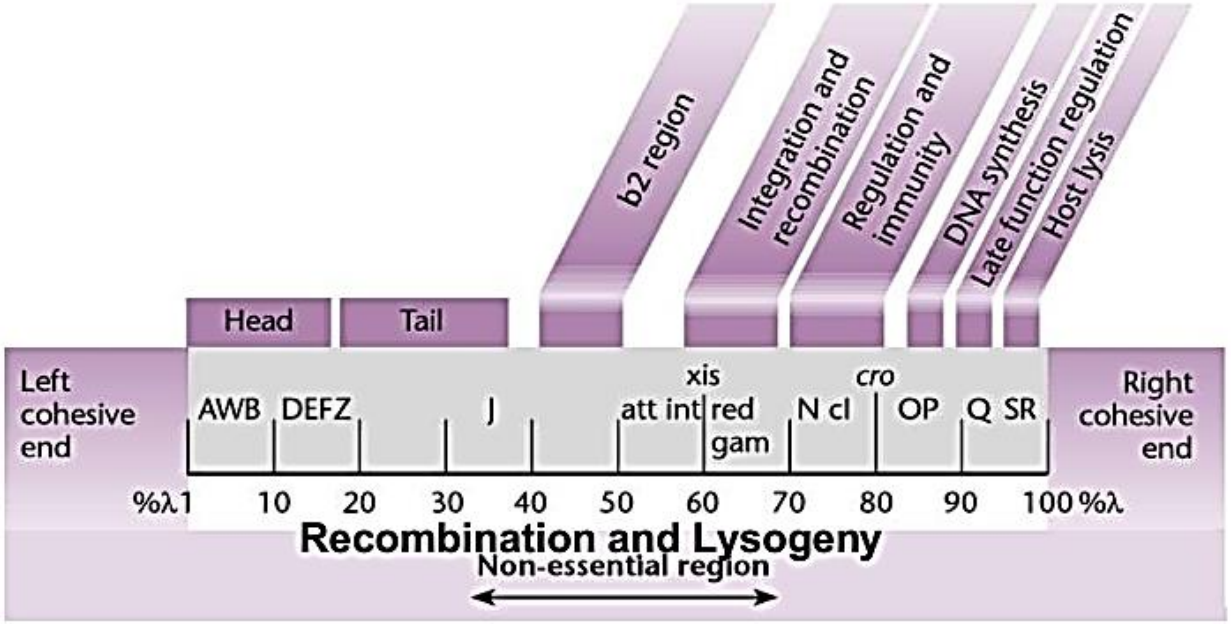
λ Phage Cloning Vector

- "Head and Tail" phage, very well-studied
- Large, linear genome of ~49.0 kb

- Two lifestyle modes
  - Lytic: replicative mode
  - Lysogenic: latent mode

- It has a large size genome (49kb) and only 3kb new DNA can be inserted because if the size of the molecule is more than 52kb then it can not be packaged into the head of the phage.
- The phage has more than one recognition sequence for almost all the restriction endonucleases. So the use of any restriction enzyme will break the phage DNA into number of small fragments.
- Despite these disadvantages, λ phages are used to clone large DNA (5kb to 25kb) molecules.

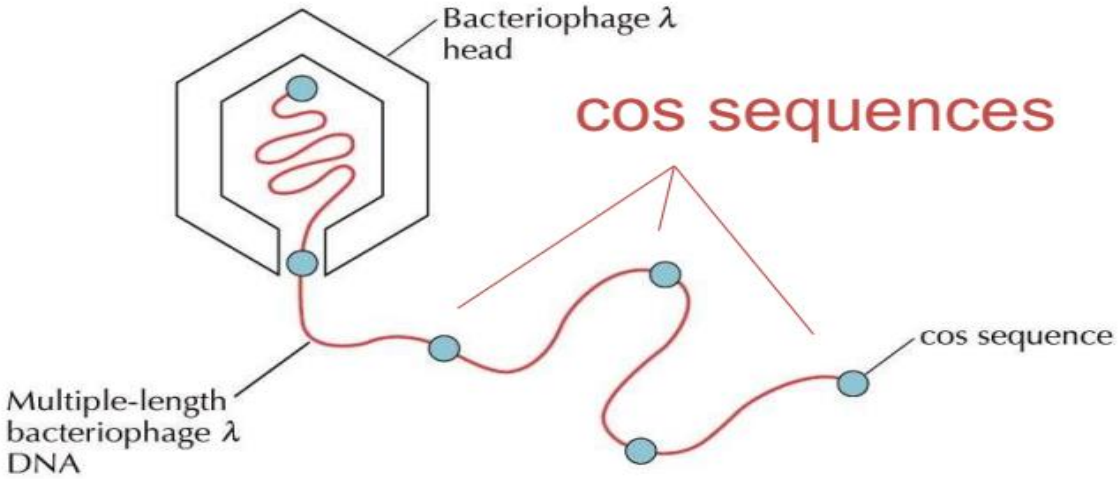

Infection
Attachment
Phage-λ
Bacterium
(Escherichia coli)
Entry
Induction
Cellular
replication
cos
Lysis
Lysogenic cycle
Integration
Lytic cycle
cos
Repression
Transcription,
translation and
replication
Assembly
packaging

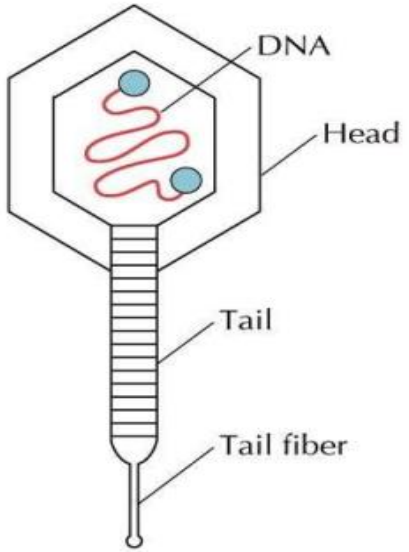


- **Cos site:** At the ends short (12bp) ss- complementary region "cohesive or sticky" ends--- circulation after infection
- **Left Arm:** Structural genes for head and tail
- **Central Region:** genes for lysogenic growth and recombination/insertion of genome into baterial genome
- **Right Arm:** genes involved in DNA replication and lytic growth
- **Only 30 kb is required for lytic growth.**
- **Thus, one could clone 19 kb of "foreign" DNA.**
- **Packaging efficiency 78%-105% of the lambda genome.**

Phage λ cloning vectors:

- Engineered version of bacteriophage λ (infects *E. coli*).
- Central region of the λ chromosome (linear) is cut with a restriction enzyme and digested DNA is inserted.
- DNA is packaged in phage heads to form virus particles.
- Phages with both ends of the λ chormosome and a 37-52 kb insert replicate by infecting *E. coli*.
- Phages replicate using *E. coli* and the lytic cycle.
- Produces large quantities of 37-52 kb cloned DNA.
- Like plasmid vectors, large number of restriction sites available; phage λ cloning vectors are useful for larger DNA fragments than pUC19 plasmid vectors.

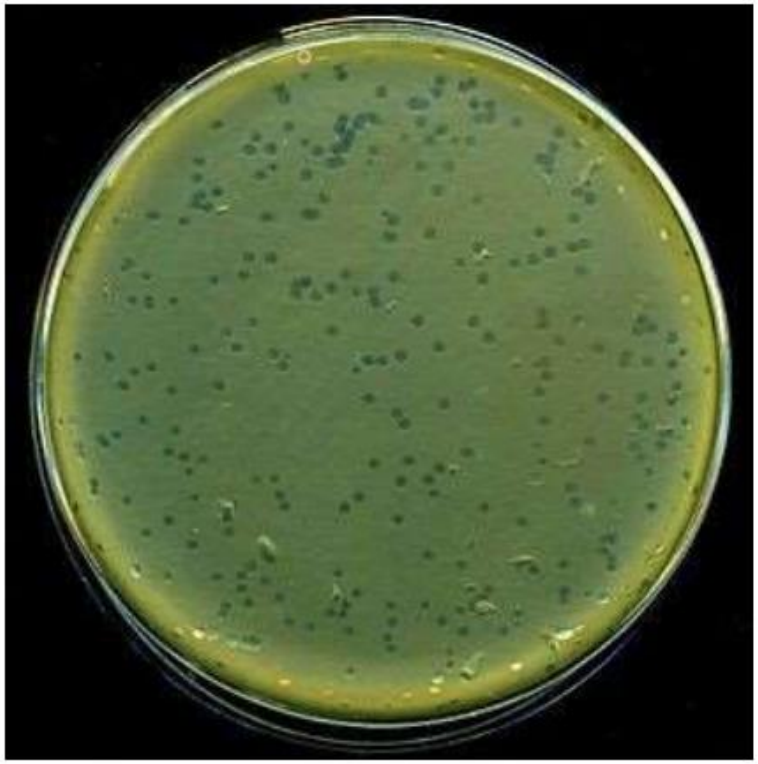




- ***The infection process is about thousand times more efficienct than transformation with plasmid vectors.***

- **10^6 tansformed colonies per microgram of plasmid vector**

- **10^9 plaques per microgram of recombinant Lambda vector**



Types of λ vector

✓Mostly of two types as per their usages:

Insertion Vectors

- In these vectors the non-essential region is deleted and the two arms ligated together. The vector possesses one unique restriction site.

Replacement Vectors

- This vector has two recognition sites for the restriction endonuclease used for cloning.
- These sites replace the segment of DNA (Stuffer fragment) from the vector genome by the DNA to be cloned.
- These vectors can carry large pieces of DNA than insertion vectors.