



Phagemids and M13

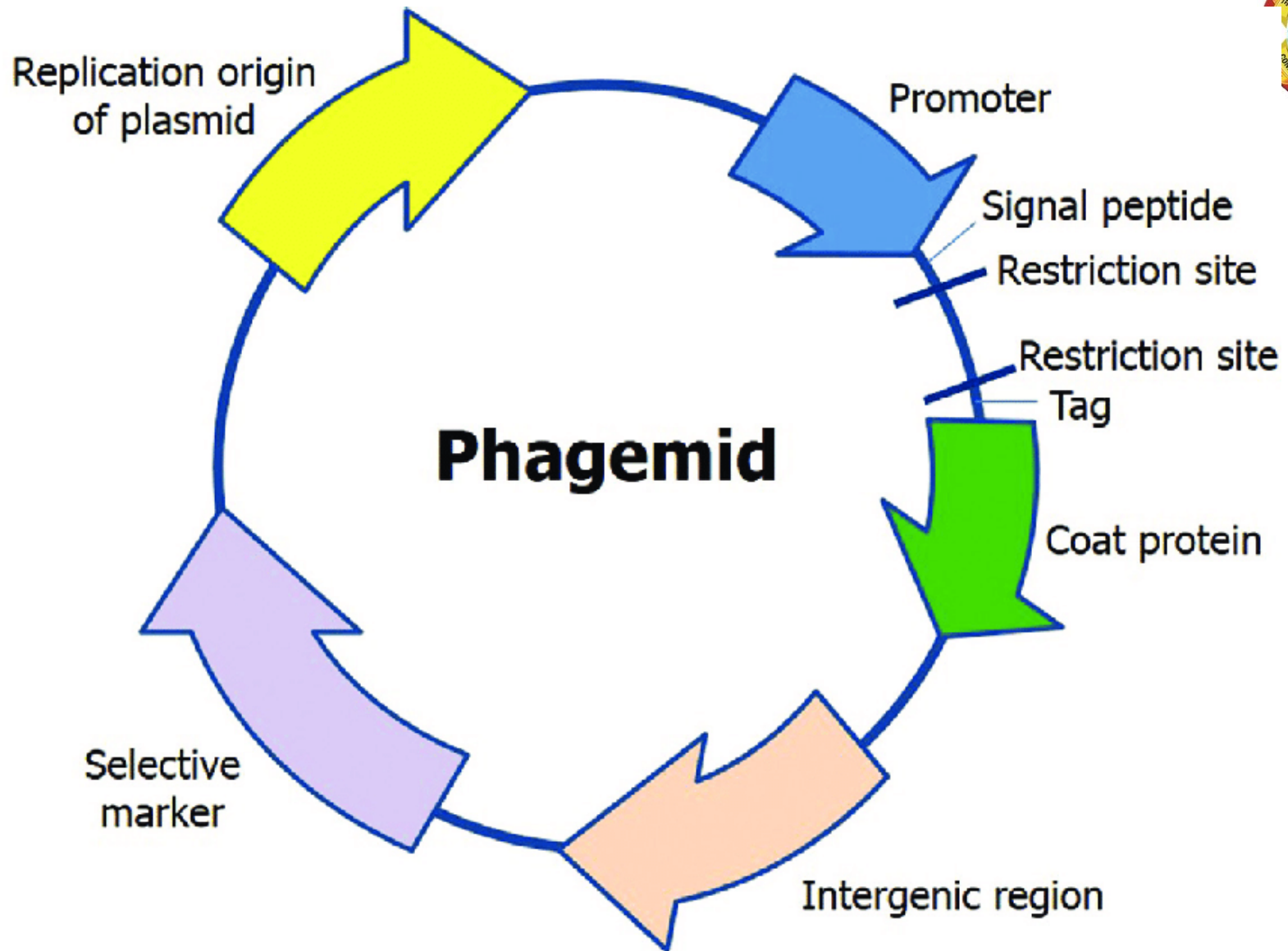


What is phagemid?

- ❑ A phagemid or phasmid is a hybrid plasmid that contains an f_1 origin of replication from an f_1 phage.
- ❑ It can be used as a type of cloning vector in combination with filamentous phage M13.
- ❑ A phagemid can be replicated as a plasmid, and also be packaged as single stranded DNA in viral particles.
- ❑ Phagemids contain an origin of replication (ori) for double stranded replication, as well as an f_1 ori to enable single stranded replication and packaging into phage particles.



- ❑ Similarly to a plasmid, a phagemid can be used to clone DNA fragments and be introduced into a bacterial host by a range of techniques, such as transformation and electroporation.
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- ❑ Phagemids contain an origin of replication (ori) for double stranded replication, as well as an f1 ori to enable single stranded replication and packaging into phage particles.



Typical structure map of a Phagemid.

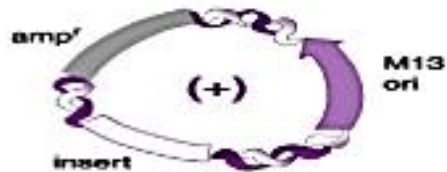


- ❑ However, infection of a bacterial host containing a phagemid with a 'helper' phage, for example VCSM13 or M13K07, provides the necessary viral components to enable single stranded DNA replication and packaging of the phagemid DNA into phage particles.
- ❑ The 'helper' phage infects the bacterial host by first attaching to the host cell's pilus and then, after attachment, transporting the phage genome into the cytoplasm of the host cell.
- ❑ Inside the cell, the phage genome triggers production of single stranded phagemid DNA in the cytoplasm. This phagemid DNA is then packaged into phage particles.

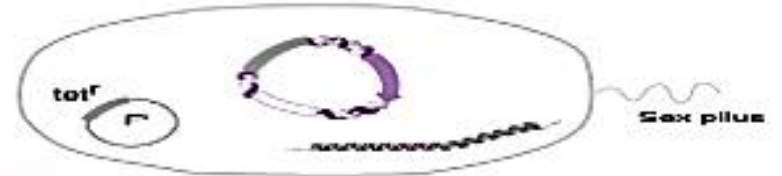
phagemid



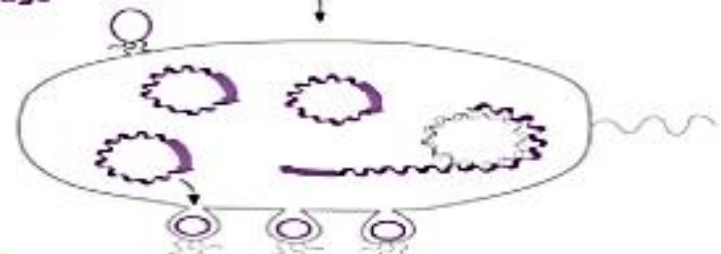
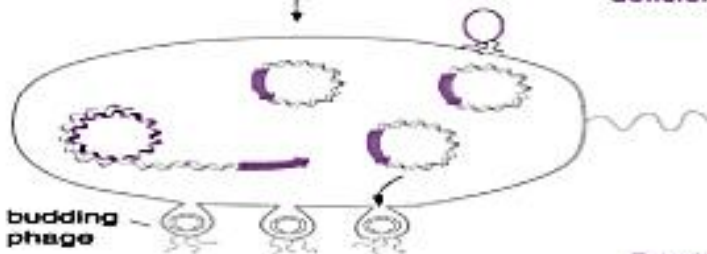
Helper phage genome



Transform F^{tet}
E. coli strain and
select for $\text{amp}^r + \text{tet}^r$



Infect with replication-
deficient M13 helper phage



budding
phage

Precipitate phage with
PEG and purify single
strand phage DNA

Pure (+) ss DNA

Pure (-) ss DNA



- ❑ The phage particles containing hybrids ssDNA are released from the bacterial host cell into the extracellular environment.
- ❑ Filamentous phages retard bacterial growth but, contrasting with the lambda phage and the T7 phage, are not generally lytic.
- ❑ Helper phages are usually engineered to package less efficiently (via a defective phage origin of replication) \ than the phagemid so that the resultant phage particles contain predominantly phagemid DNA.
- ❑ F1 Filamentous phage infection requires the presence of a pilus so only bacterial hosts containing the F-plasmid or its derivatives can be used to generate phage particles.

M13 Phage

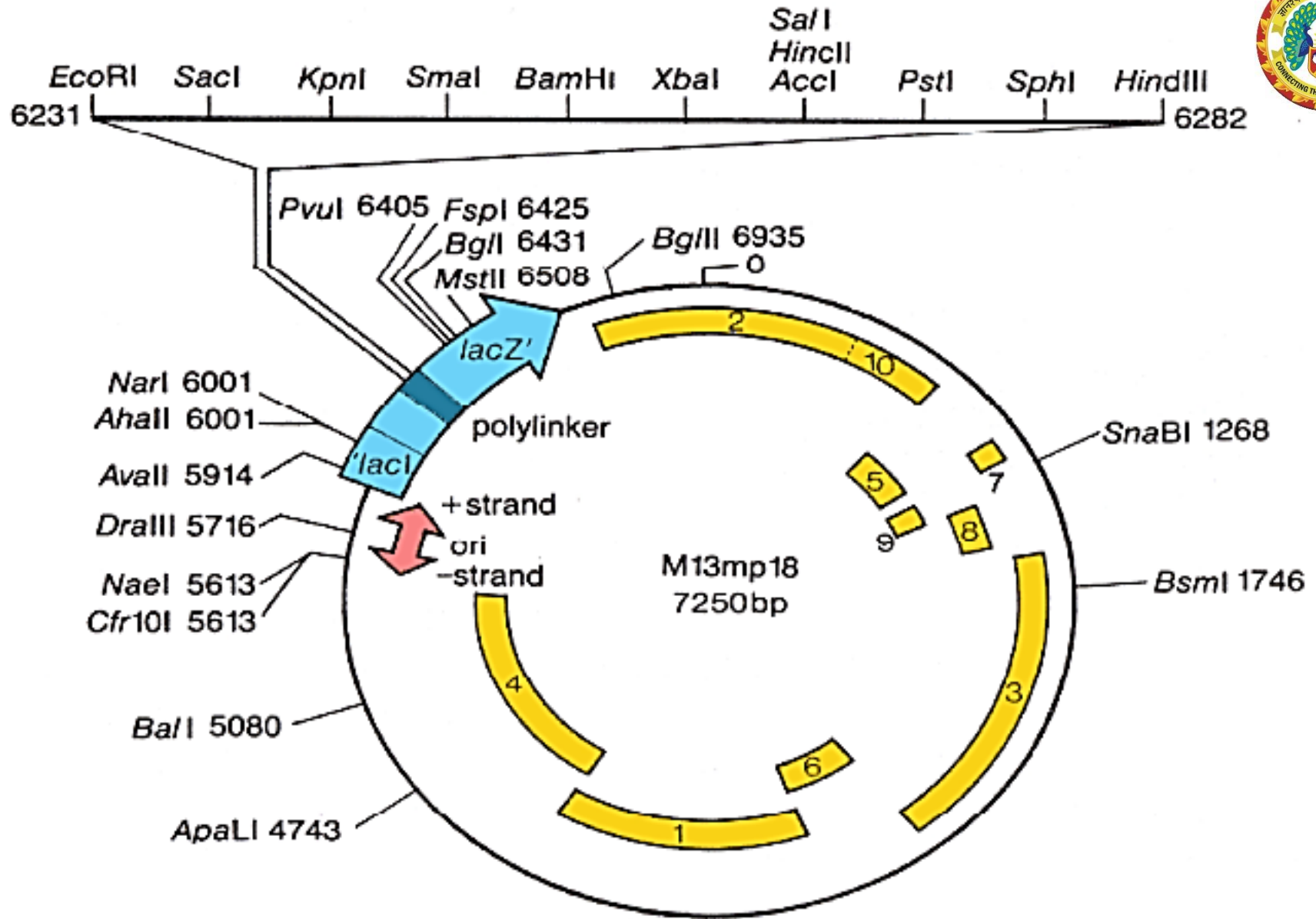


- ❑ It is 6.4 kb in length, circular and consists of a single stranded DNA molecule and is used as a cloning vector because its less than 10kb size.
- ❑ The double stranded replicative form of the genome acts like a plasmid.
- ❑ Genes cloned can be obtained in the form of single stranded DNA which is helpful in gene sequencing and in vitro mutagenesis.
- ❑ It is easily prepared from the culture of infected E. coli cells and infects only the F⁺ bacteria.

M13 as vector



- ❑ M13mp1- The lac Z gene was introduced in the intergenic sequence (IS) and it does not have any unique restriction site in the gene.
- ❑ M13mp2- This vector was constructed by including the EcoR1 site in the lac Z gene. This was done by a single nucleotide change in GGATTC near the start of the lac Z gene to GAATTC by in vitro mutagenesis. The β -galactosidase enzyme remains functional in the vector.
- ❑ M13mp7- It was formed by inserting a polylinker into the EcoR1 site of lac Z gene of M13mp2. A polylinker is a short nucleotide sequence that consists of number of restriction sites (EcoR1, BamH1, Sal1 and Pst1) and has a EcoR1 sticky ends. This polylinker does not disrupt the function of Lac Z gene.



Typical structure map of a M13 vector.