# Genetics Engineering (Zoo-455)

## Cloning Vectors

Lecture-4

# Cloning vectors:

- Most vectors are derived from plasmids or viruses
- DNA molecule which is able to replicate several time in its self when a foreign DNA is integrated and produces a plenty copy of the recombinant DNA

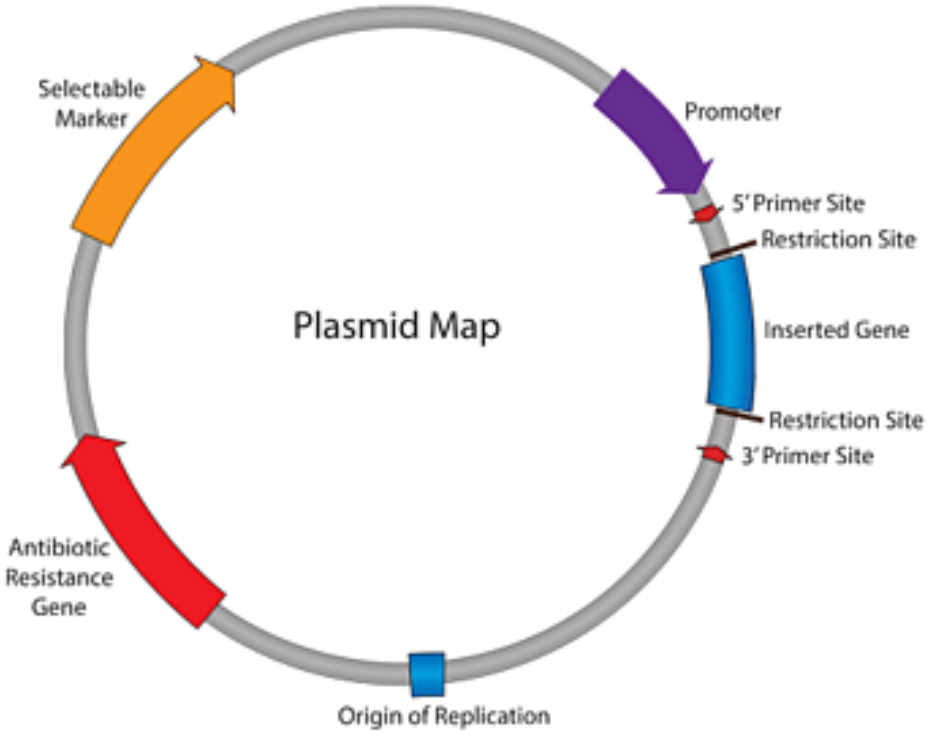
# Types of cloning vectors:

1) Plasmids.
2) Bacteriophages.
3) Bacterial artificial chromosomes.
4) Yeast artificial chromosomes.
5) Mammalian artificial chromosomes.

# Common structure of vectors:

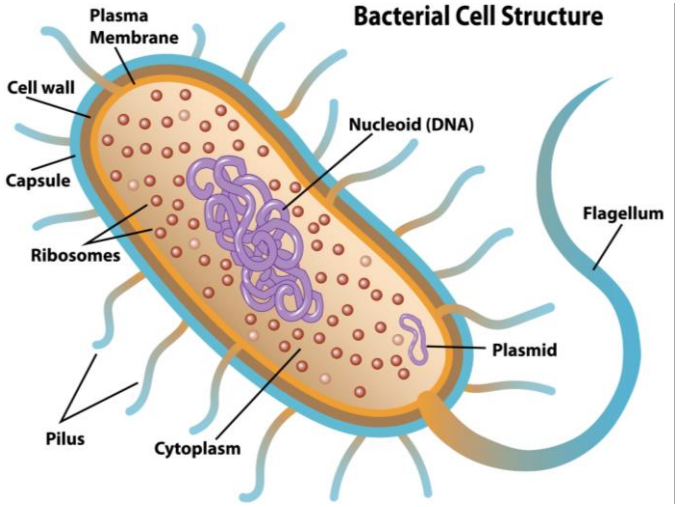
Every plasmid has following essential elements:

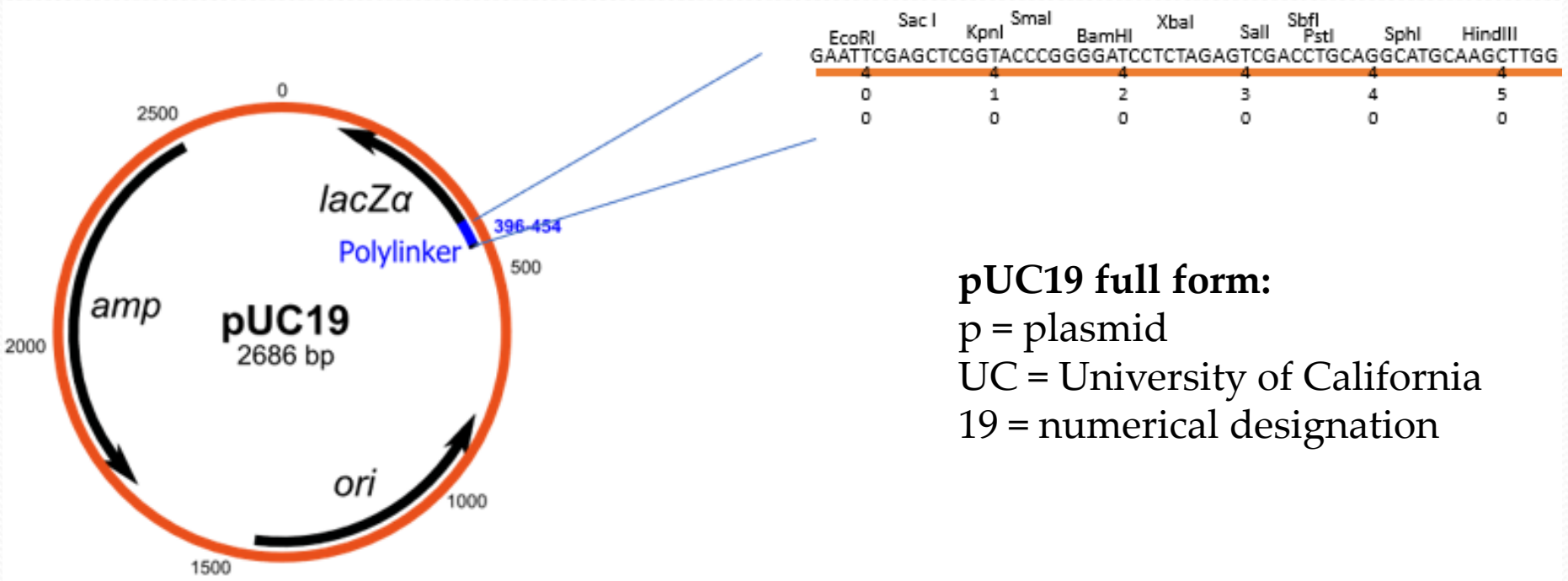
1) **Origin of replication (OR) :** This refers to a specific location in the strand where the replication process begins. In plasmids, this region is A=T rich region as it is easier to separate the strands during replication.
2) **Selectable marker site :**This region consists of antibiotic resistance genes which are useful in the identification and selection of bacteria that contain plasmids.
3) **Promoter region :** This drives the transcription of the foreign insert.
4) **Primer binding site:** This is the short sequence of single-strand DNA which is useful in DNA amplification and DNA sequencing.
5) **Multiple cloning sites (Polylinker):** Short segment containing a several restriction enzyme sites enabling easy insertion of foreign DNA.



# 1- Properties of Plasmids:

- Present in bacterial cells.
- The first vectors used in gene cloning
- It is a small extra circular DNA, double stranded, self-replicating.
- Can be inserted into bacterial cells by a process called transformation.
- Up to 15 kb DNA fragment can be inserted into the plasmid.
- Carry a marker gene for antibiotic resistance.
- Cell that contains the plasmid will grow in presence of the selectable corresponding antibiotic supplied in the media.
- pBR322, pUC19, are examples of some plasmid vectors



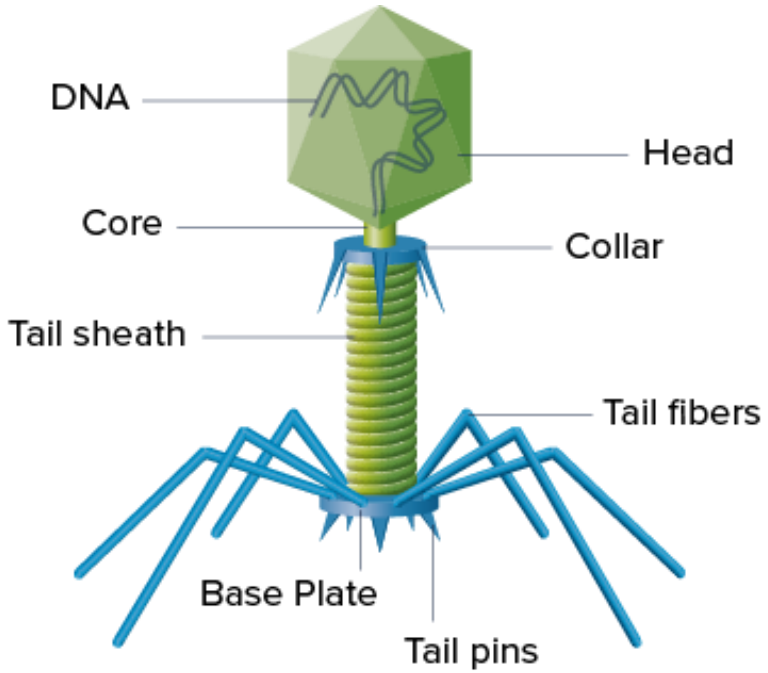



**pUC19 full form:**
p = plasmid
UC = University of California
19 = numerical designation

- ❑ pUC19 is a common cloning vector.
- ❑ A small double-stranded circle
- ❑ Contains 2686 bp in length.
- ❑ Contains ampicillin resistance and *lac*Z gene.
- ❑ Contains 54 base-pair *polylinker* (*multiple cloning site*) within *lac*Z gene.
- ❑ Contains origin of replication (ori).

# 2- Properties of Bacteriophages:

- It is the viruses that infect bacteria.
- They are not able to infect eukaryotic cell.
- They are more efficient than plasmids for cloning large DNA inserts.
- Using bacteriophage as a vector, a DNA fragment of size up to 50 kb can be transformed.
- Phage λ (Lambda) and M13 phage are commonly used bacteriophages in gene cloning
- Lambda has 49 kb (double-stranded DNA).
- If Lambda crosses cell membrane of *E. coli,* then it will degrade by restriction enzyme before adding methyl group.
- If methylase add first methyl group to the lambda, the lambda DNA then will not recognize by restriction enzyme; therefore, it will continue to grow.



## 3- Bacterial artificial chromosomes (BACs):

- BAC vectors are DNA constructs that are used for cloning DNA in bacteria.
- The length of inserted gene 75-300 kb can be inserted into the BAC vectors.
- Bacterial machinery does not have post-translational mechanism.

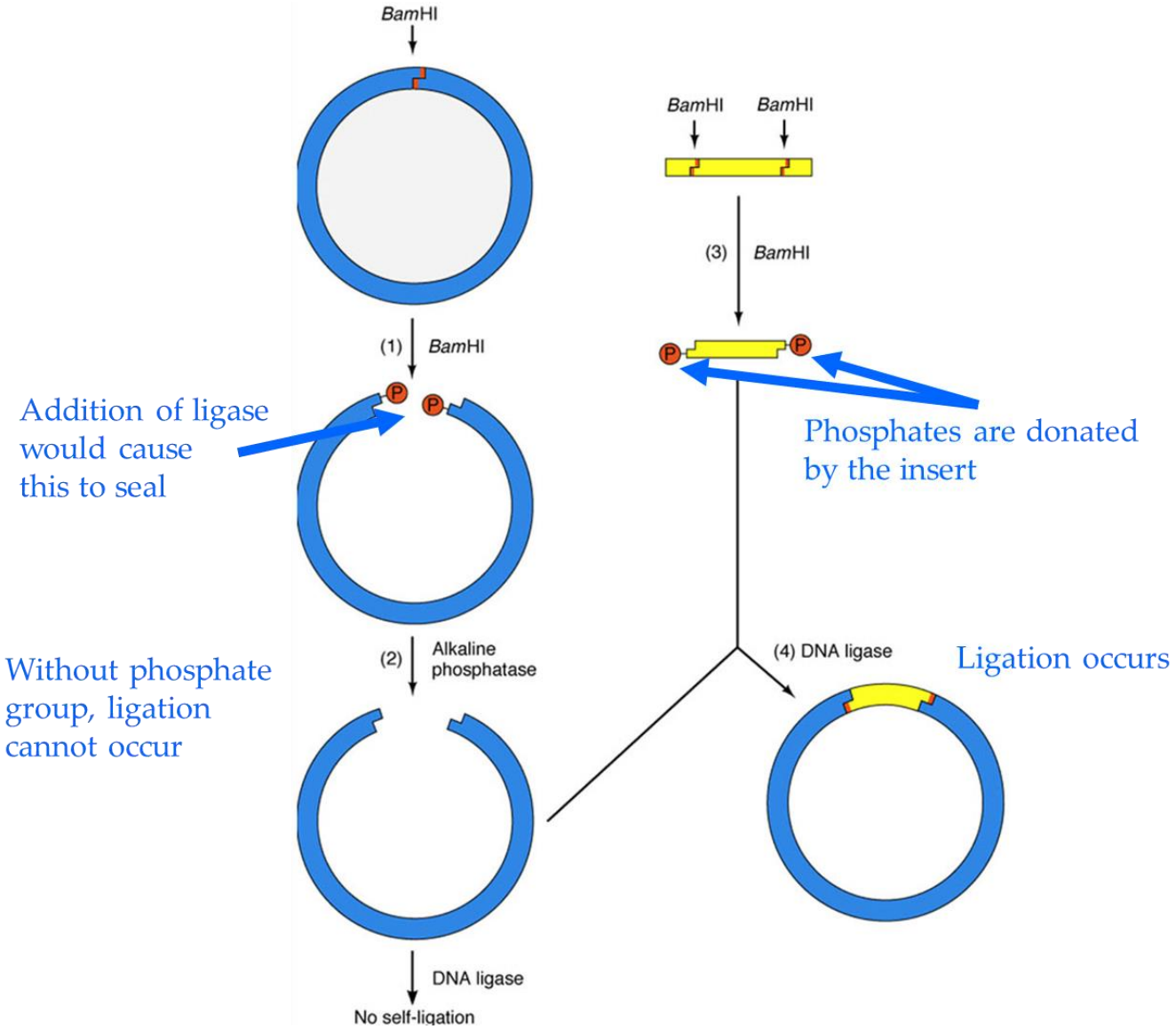
## 4- Yeast artificial chromosomes (YACs):

- YAC vectors are DNA constructs that are used for cloning DNA in yeasts.
- The length of inserted gene 100-3000 kb can be inserted in these vectors.
- Yeast machinery has post-translational mechanisms that are useful in the expression of eukaryotic proteins.

# 5- Mammalian artificial chromosomes (MACs):

- MACs are still under development.
- MACs are microchromosomes that can act as a new chromosome in a population of human cells
- MACs range in size from 6 to 10 Mb that carry new genes introduced by human researchers.
- MACs can be used as vectors in transfer of new genes, studying their.

# Digestion and ligation of insert DNA into plasmid:



# Questions?