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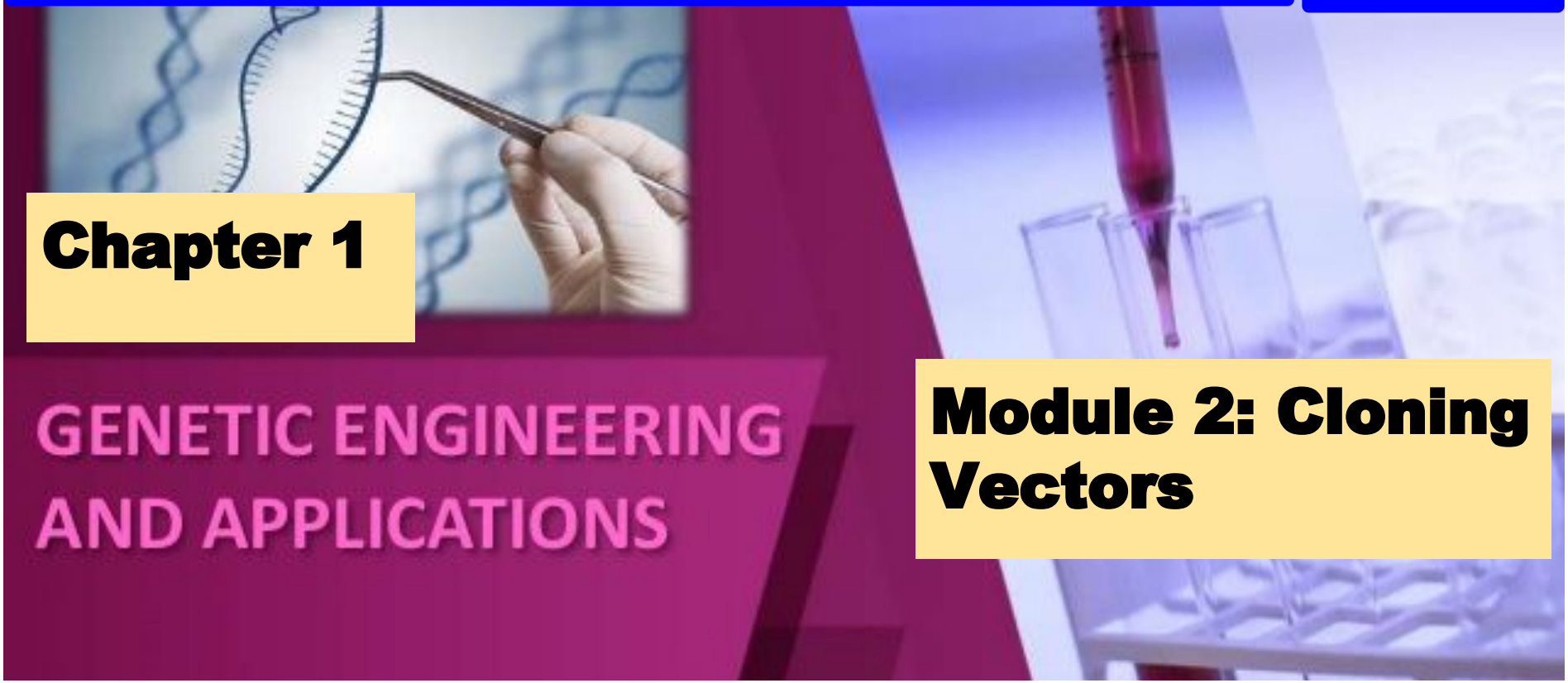

Chapter 1

Module 2: Cloning Vectors

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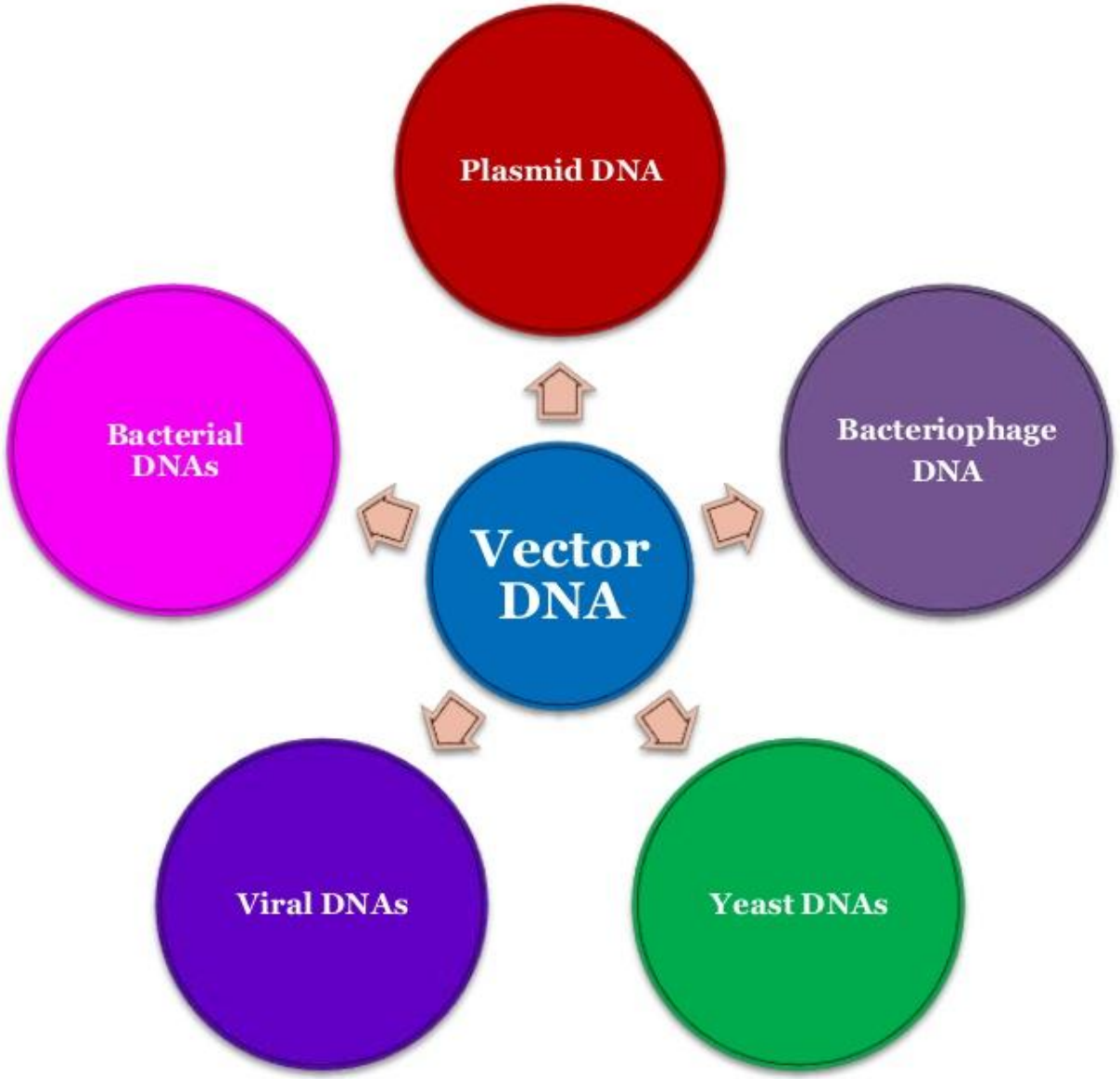
Assistant Prof. in Zoology

Vector DNA or Vehicle DNA

The DNA which acts as a carrier is a vehicle DNA



Types of vectors

- **Types of Vectors**
  - Bacterial plasmid vectors
  - Bacteriophage vectors
  - Cosmid vectors
  - Expression vectors
  - Bacterial Artificial Chromosomes (BAC)
  - Yeast Artificial Chromosomes (YAC)
  - Ti and Ri vectors

Characteristics of an prokaryotic vector

- Capable of autonomous replication independent of the main bacterial chromosome
- Easy to isolate, *i.e.* small.
- Non -toxic to host cells.
- Have space for foreign inserts.
- Have unique restriction sites for common restriction enzymes.
- Have convenient markers for selection of transformants, *e.g.* antibiotic resistance genes
- Be relaxed, *i.e.* multiple copies in a host cell.

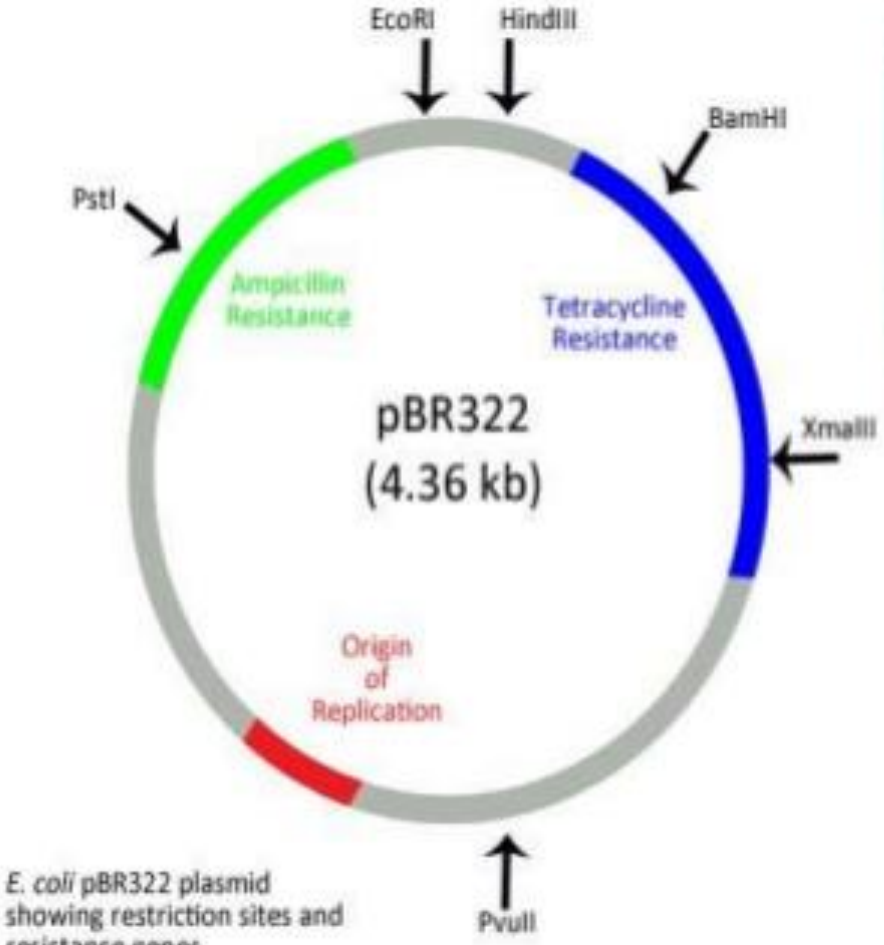
Practical Features of DNA Cloning Vectors

- Size
- Origin of replication (ori)
- Multiple cloning site (MCS)
- Selectable marker genes
- RNA polymerase promoter sequences
- DNA sequencing primers

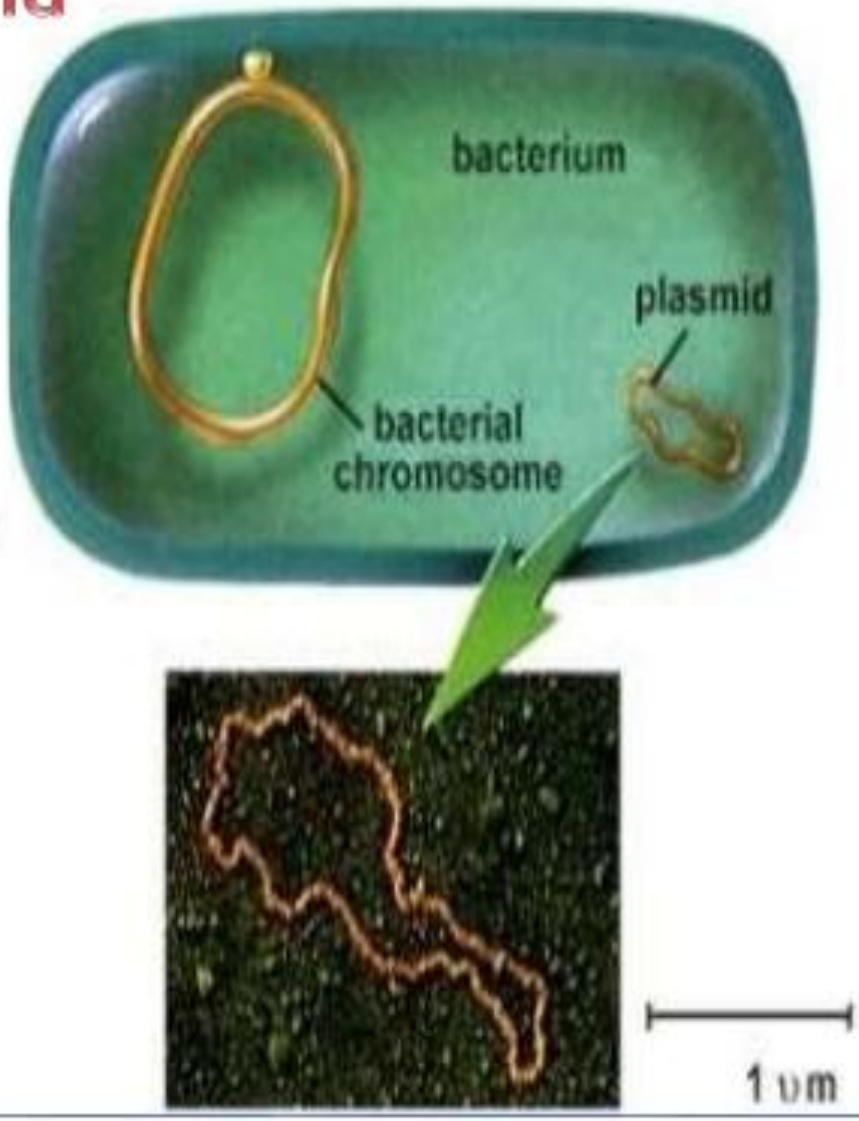
Plasmids

- **Plasmid DNA – small circular DNA found in bacteria**
- **They replicate autonomously.**
- **Easily purified**
- **Confer antibiotic resistance to host bacteria –allow easy identification.**
- **First type of cloning vector developed.**

Plasmid

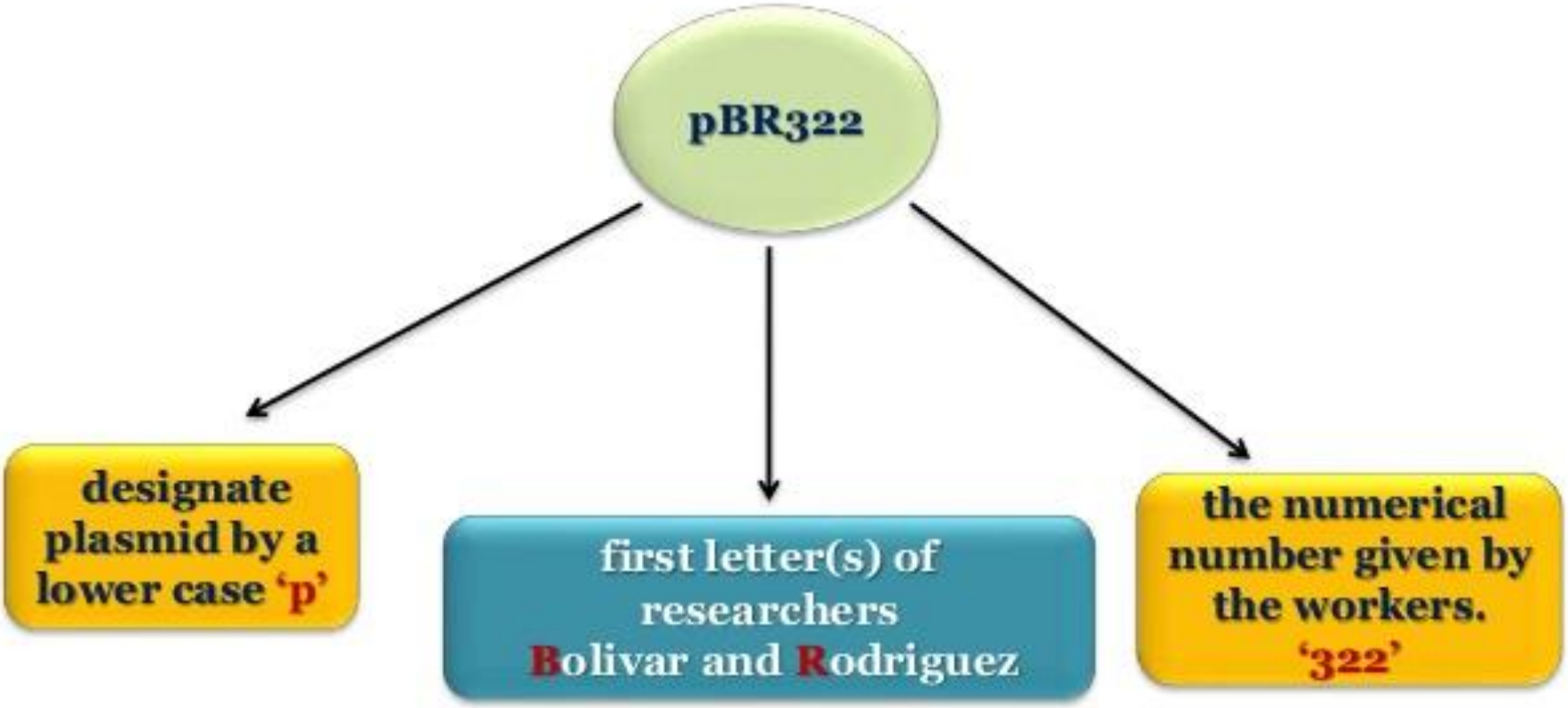


E. coli pBR322 plasmid showing restriction sites and resistance genes.



pBR322 ol E.coli is **the most popular and widely used plasmid vector**, and is appropriately regarded as the **parent or grand parent of several other vectors. (others pBR325,pBR328 and pBR329)**

Nomenclature of plasmids



Some plasmids are given **names of the places** where they are discovered e.g.

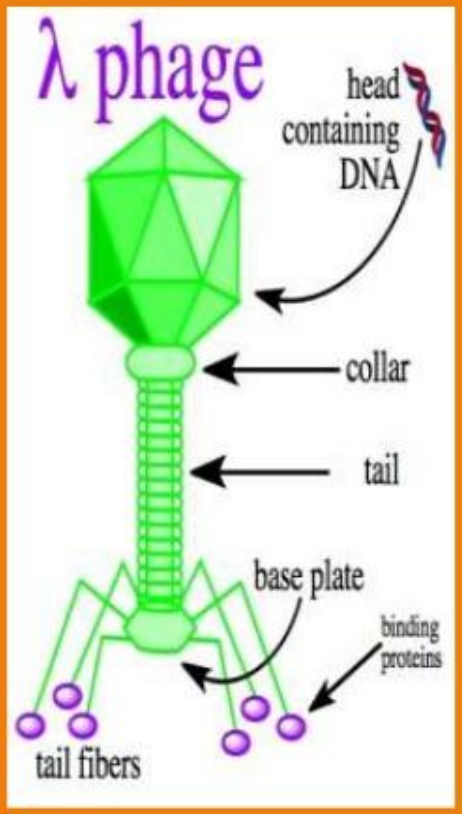
(**pUC** is plasmid from **U**niversity of **C**alifornia.)

BACTERIOPHAGE

- Bacteriophages or simply phages are the viruses that replicate within the bacteria
- Phages can take up larger DNA segments than plasmids.

Most commonly used phages are bacteriophage λ(phage λ) and bacteriophage M13 (phage M13)

Phage Vectors



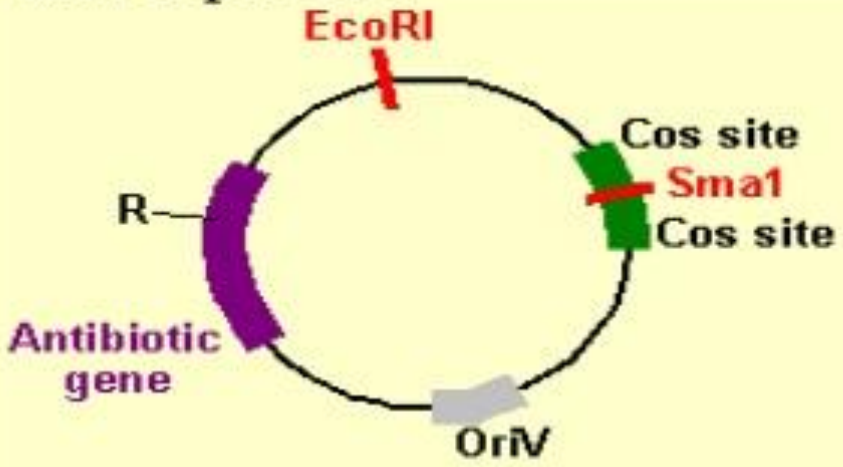
- **Two types of phage vectors have been extensively developed-λ and M13.**
- **phage vectors have engineered phage genomes previously genetically modified to include restriction sites.**
- **after insertion of foreign DNA, the recombinant phage genome is packaged into the capsid and used to infect host cells**

COSMIDS

- Cosmids are the vectors having characteristics of both **plasmid and bacteriophage** .

Fragment of phageλ DNA including COS site + plasmid = cosmid

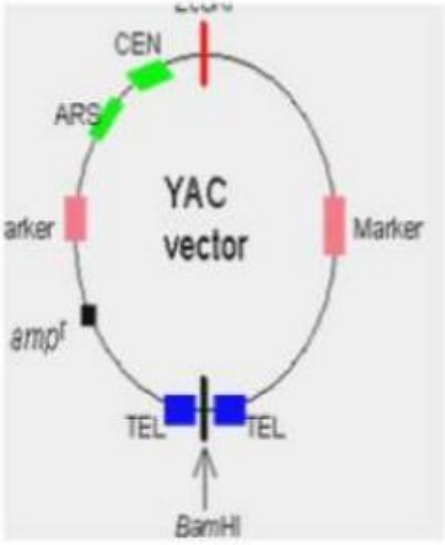
- Cosmids can carry larger fragments of foreign DNA than plasmids.
- A foreign DNA of 40 kb can be inserted in to cosmids.
- Once inside the host cell, cosmids behave just like plasmids and replicate.



KEY

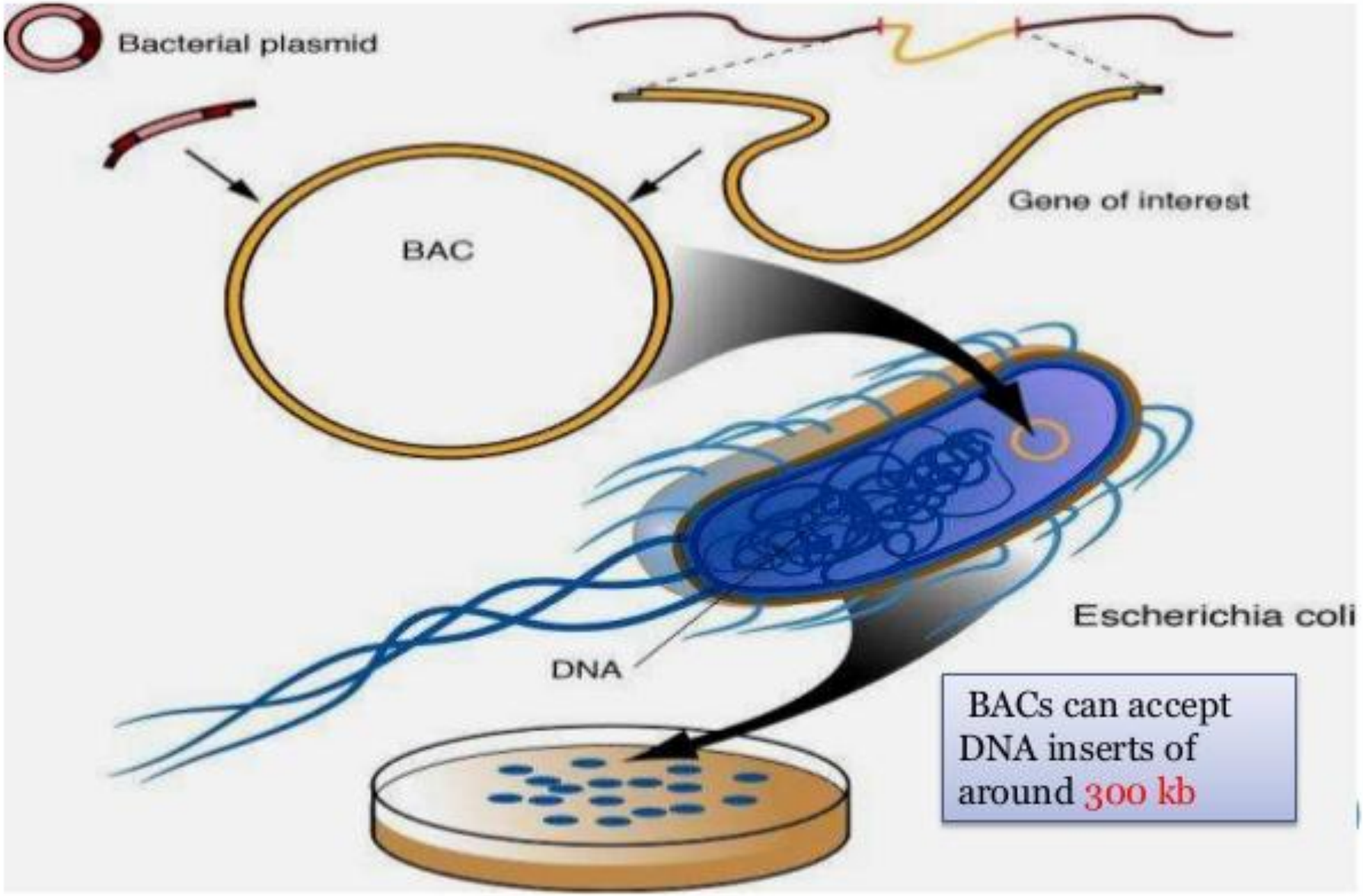
OriV - origin of replication.
Cos sites - provide blunt ends.
R - recombinant site
EcoRI, SmaI } - Restriction endonuclease recognition sequence.

Artificial Chromosomes

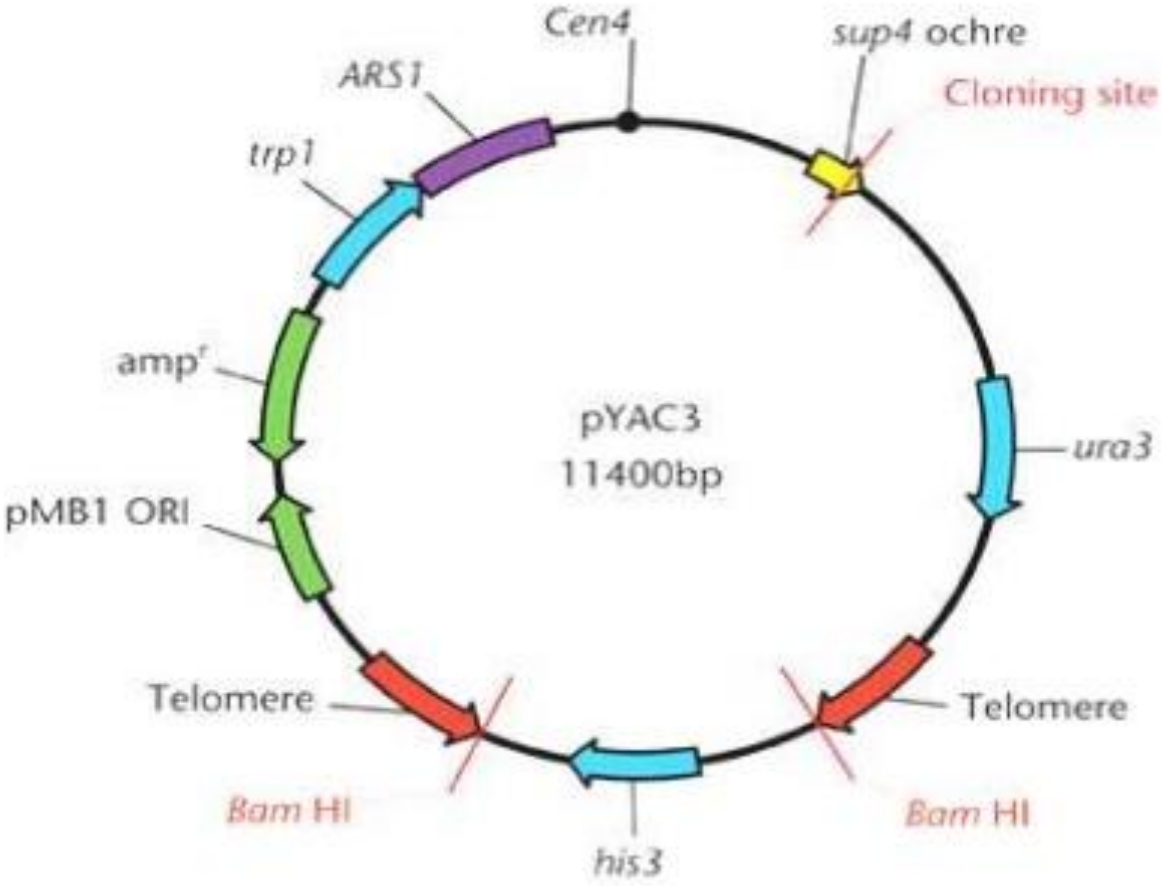


- large fragments of DNA can be cloned.
- Mapping of genes is easier.
- One copy of YAC is present per cell.
- yeast artificial chromosomes (YACs)
- bacterial artificial chromosomes (BACs)
- played important role in the human genome project

BACTERIAL ARTIFICIAL CHROMOSOME

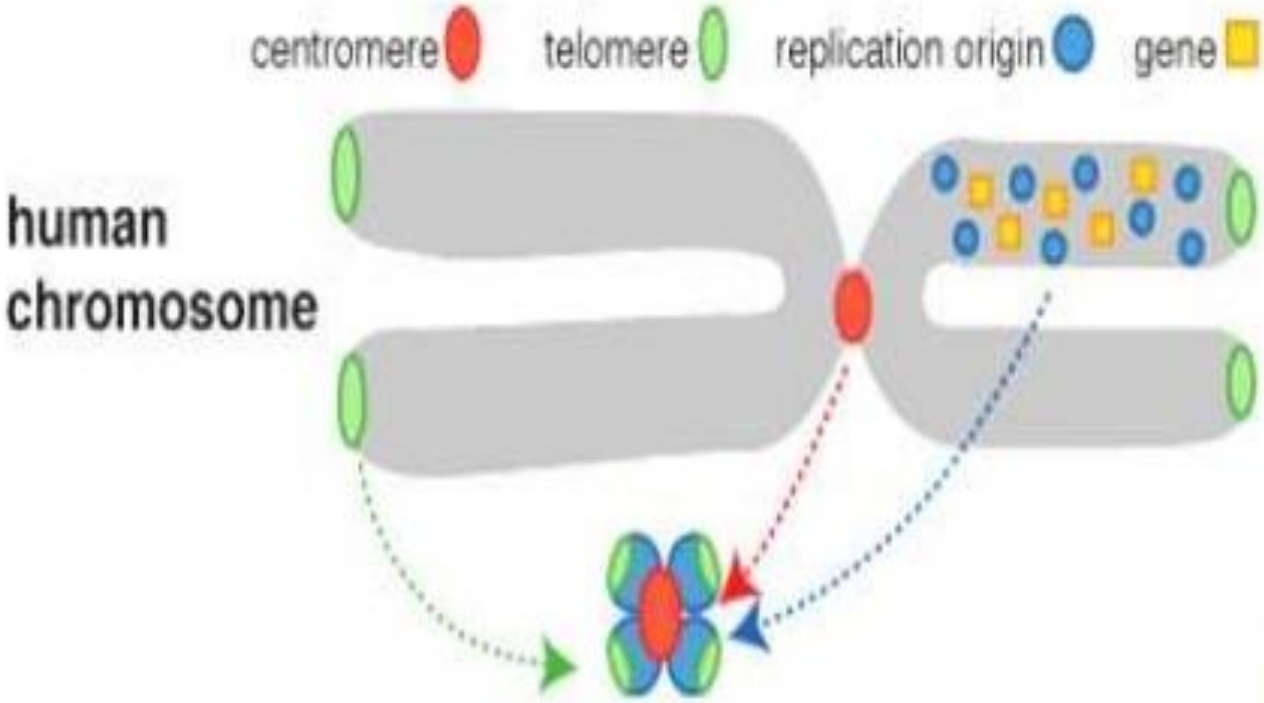


YEAST ARTIFICIAL CHROMOSOME



❖ Yeast artificial chromosome (YAC) is a synthetic DNA that can accept large fragments of foreign DNA (particularly human DNA).

HUMAN ARTIFICIAL CHROMOSOME (HAC)



H. Willard 1997

- **synthetically produced vector DNA**, possessing the characteristics of human chromosome
- Advantage with HAC is that it can carry **human genes that are too long.**
- HAC can carry genes to be introduced into the cells in **gene therapy.**

Choice of vector

✓ The size of the foreign DNA is very important in the choice of vectors.

✓ The efficiency of this process is often crucial for determining the success of cloning.

- ❑ Phage λ ⟶ E.Coli (5-25kb)
- ❑ Cosmid λ ⟶ E.Coli (35-45kb)
- ❑ Plasmid artificial chromosome (PAC) ⟶ E.Coli (100-300kb)
- ❑ Bacterial artificial chromosome (BAC) ⟶ E.Coli (100 -300kb)
- ❑ Yeast chromosome ⟶ **S. cerevisiae (200-2000kb)**

Host cell types

Host cell types

Prokaryotic hosts	Eukaryotic hosts
Bacteria E . Coli Bacillus sp. Pseudomonas sp. Streptomyces sp.	Yeast Algae fungi
	Yeast - Saccharomyces Fungi- Aspergillus, Neurospora Algae - Chlamydomonas

Two types of host-vectors

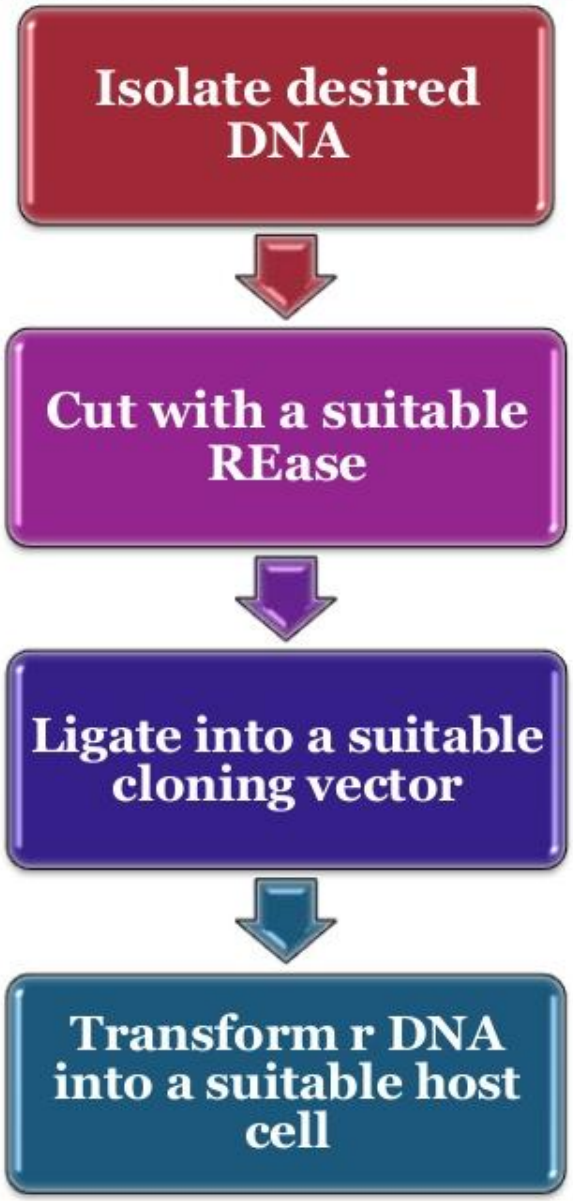
Cloning vector

- **Propagation of DNA inserts**

Expression vector

- **Production of proteins**

Making of r DNA



Molecular Cloning / DNA cloning

Molecular cloning refers to the process of making multiple DNA molecules.

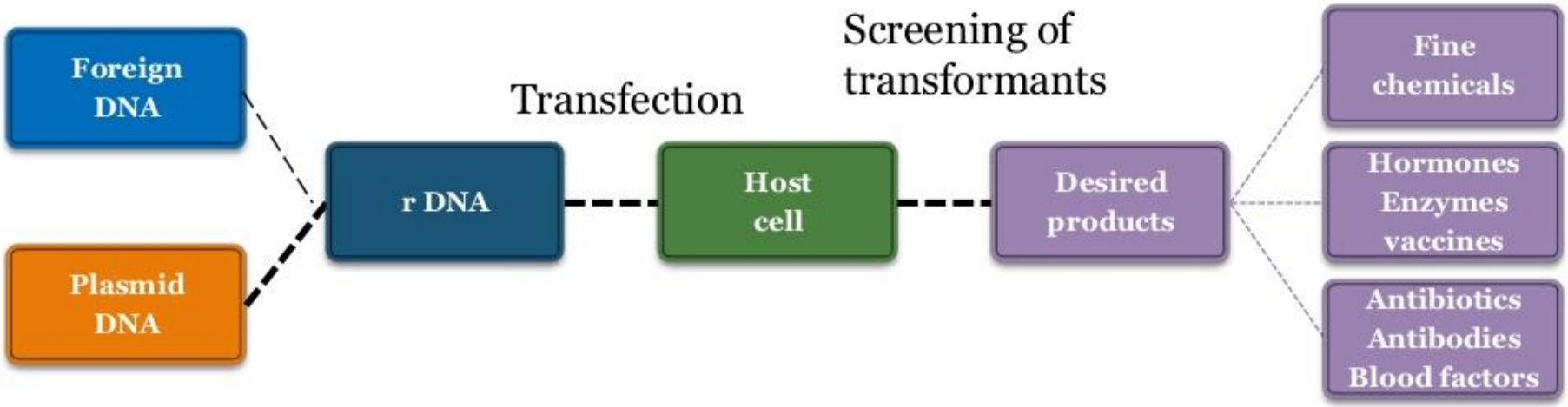
Step 1– fragmentation - breaking apart a strand of DNA

Step 2 – ligation-gluing together pieces of DNA in a desired sequence.

Step 3 –Transfection - inserting the newly formed DNA into cells.

Step 4-Screening / selection – selecting out the cells that were successfully tranfected with the new DNA

Recombinant DNA cloning procedure



DNA cloning protocol – 7 steps

1. Choice of host organisms and cloning vector
2. Preparation of vector DNA
3. Preparation of DNA to be cloned
4. Creation rDNA.
5. Introduction of rDNA into the host organism.
6. Selection of organisms containing rDNA.
7. Screening for clones with desired DNA inserts and biological properties.

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THE END

THANKS FOR LISTENING

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