

A vibrant 3D graphic featuring the word 'welcome' in colorful, blocky letters. The letters are arranged in two rows: 'wel' on the top row and 'come' on the bottom row. The 'w' is pink, 'e' is green, 'l' is yellow, 'c' is blue, 'o' is yellow with a sunburst effect, 'm' is purple, and 'e' is green. The background is a light blue gradient with soft, out-of-focus blue roses and bokeh light effects.

welcome

# VECTORS: TYPES AND CHARACTERISTICS



E.g. **PLASMIDS**, **PHAGES**, **HYBRID VECTORS**,  
**ARTIFICIAL CHROMOSOMES**

# VECTORS

- ✓ Small DNA molecule capable of **self replication**.
- ✓ **Cloning vehicle** or **Cloning DNA**.
- ✓ Carrier of **DNA** fragment.
- ✓ E.g. Plasmid, Phage, Hybrid vector, Artificial Chromosomes.

# CHARACTERISTICS

- Self replication, multiple copies.
- Replication origin site.
- Cloning site.
- Selectable marker gene.
- Small size.
- Low molecular weight.
- Easily isolated & purified.
- Easily isolated into host cell.
- Control elements – promoter, operator, ribosome binding site.

# TYPES

Two types :-

## 1) Cloning Vectors

Propagation or cloning of DNA insert inside a suitable host cells.

Examples: **Plasmids, Phage or Virus**

- Obtaining millions of copies.

Uses :-

Genomic library.

Preparing probes.

Genetic Engineering Experiments.

- Selection of cloning vector depends on :-
  - (a) Objective of cloning experiment
  - (b) Ease of working.
  - (c) Knowledge existing about the vector.
  - (d) Suitability.
  - (e) Reliability.

## **2) Expression Vectors**

- **Express the DNA insert producing specific protein.**
- **They have prokaryotic promoter.**
- **Ribosome binding site.**
- **Origin of replication.**
- **Antibiotic resistance gene.**
- **Expression vectors with strong promoters.**
- **Inducible Expression Vectors.**
- **Eukaryotic expression vectors.**

## **VECTOR**

- **Plasmid**
- **Bacteriophages**
- **Cosmid**
- **Yeast Cloning Vectors**
- **Ti & Ri Plasmids**

## **TARGET HOST CELL**

**Bacteria, Streptomyces**

**Bacteria**

**Bacteria**

**Yeasts**

**Transformation of  
cloned gene in higher  
plants.**

# AGENTS USED AS VECTORS

- ❖ **PLASMIDS**
- ❖ **BACTERIOPHAGES**
- ❖ **COSMID**
- ❖ **ARTIFICIAL CHROMOSOME VECTORS**

**In 1973, Cohen described first successful construction of recombinant vector.**

**Plasmid PSC101 - Ecoli**



# PLASMID

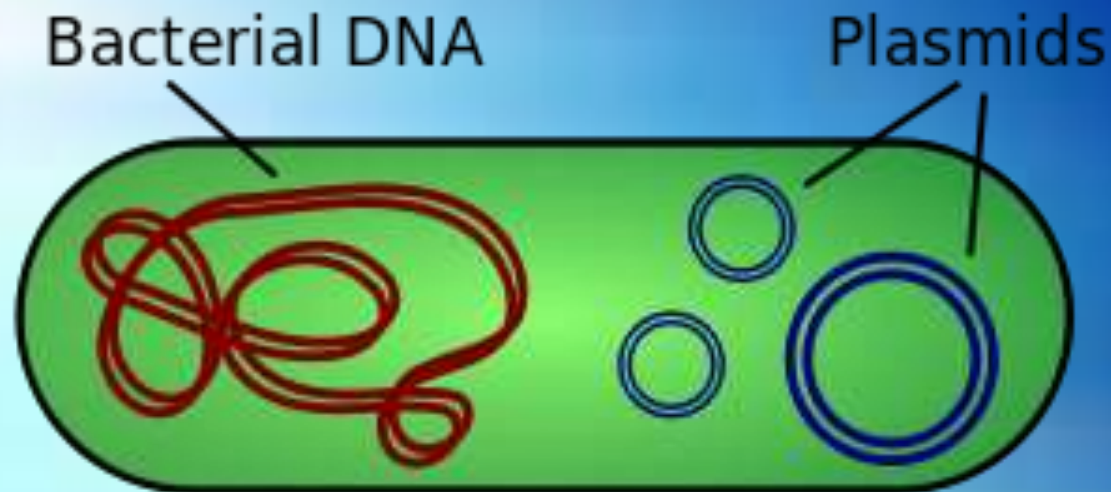
- ✓ Extra chromosomal DNA molecules.
- ✓ Self replicating.
- ✓ Double stranded.
- ✓ Short sequence of DNA.
- ✓ Circular DNA molecules.
- ✓ Found in prokaryotes.

## ➔ CHARACTERISTICS

- a. Minimum amount of DNA.
- b. Two suitable markers for identification .
- c. Single restriction site.
- d. More restriction enzyme.
- e. Size range 1kg – 200kg.
- f. Relaxed replication control.
- g. Restriction endonuclease enzyme.

# THREE TYPES OF PLASMID

1. **Fertility plasmids:-** can perform conjugation.
2. **Resistance plasmids:-** contain genes that build a resistance against antibiotics or poisons.
3. **Col plasmids:-** contain genes that code for proteins that can kill bacteria.

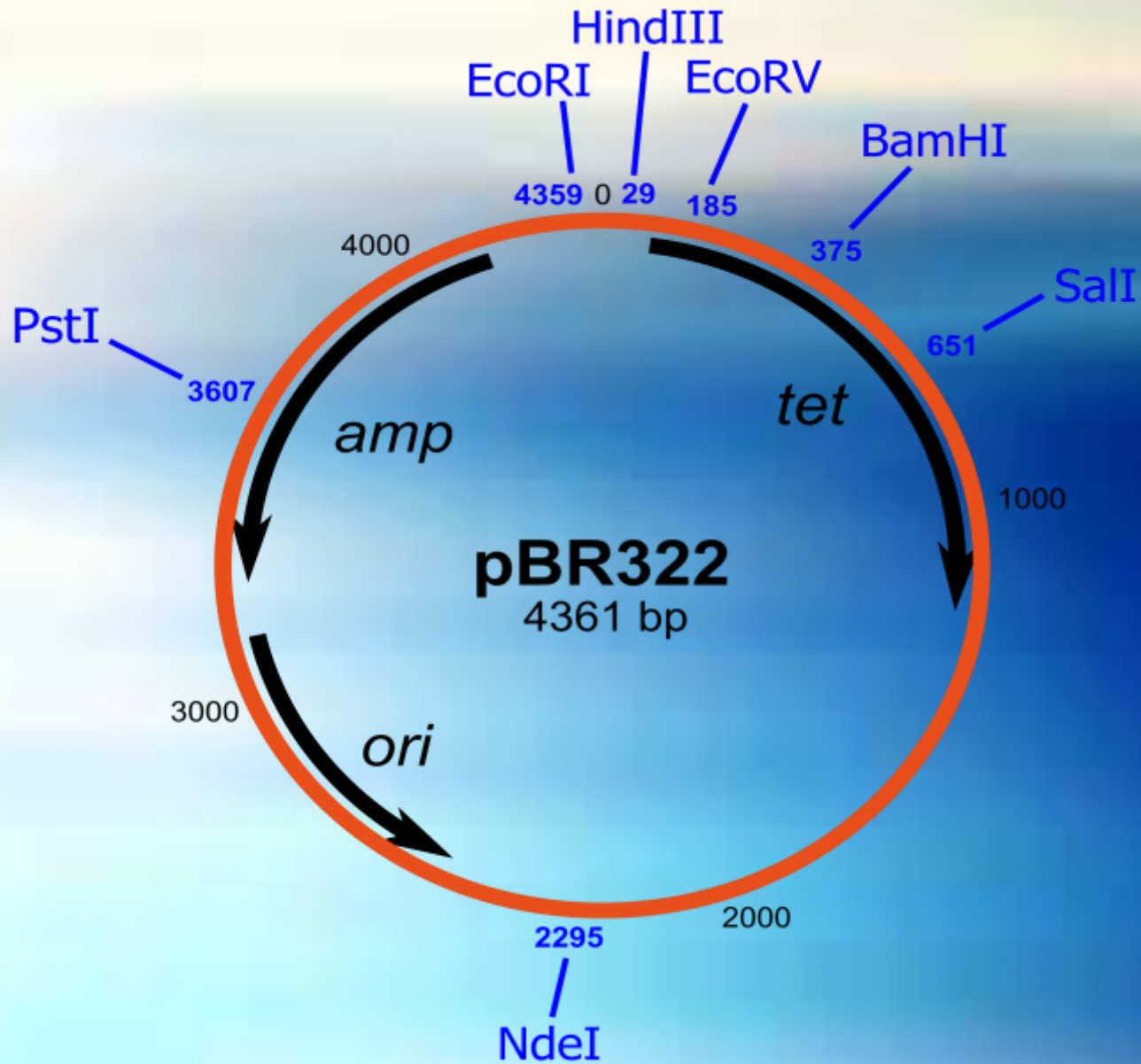


# EXAMPLES OF PLASMID VECTORS

- pBR322
- pBR327
- pBR325
- pBR328
- pUC8
- pUC9
- pUC12
- pUC13
- pGEM3Z

# PBR322

- **Cloning Vector.**
- **15 copies.**
- **Reconstructed plasmid.**
- **Derived from Ecoli plasmid- ColE1.**
  - PBR322** – 4362 base pairs
    - P** – denotes Plasmid
    - B** – Scientific Boliver
    - R** - Rodriguez
    - 322** – number given to distinguish.
  - **Ampilicin resistance gene derived from RSF2124.**
  - **Tetracycline resistance gene from PSC101.**
  - **Origin of replication from PMB1.**
  - **Two selectable markers**
    - amp<sup>r</sup> tet<sup>r</sup>**



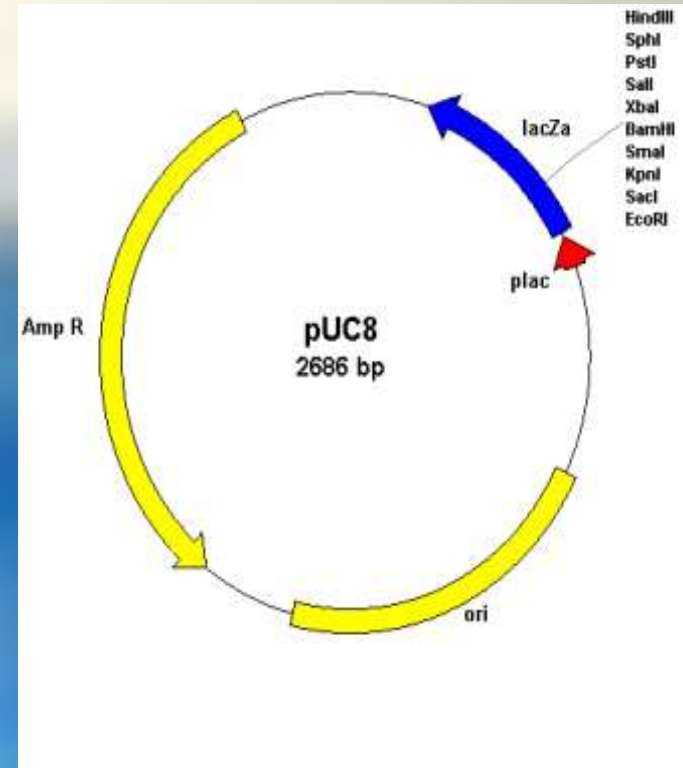
Structure of E.Coli plasmid cloning vector pBR322

# PBR327

- ❖ Derived from PBR322.
- ❖ 30 – 40 copies.
- ❖ Expression plasmid.
- ❖ Ampilicin resistance gene.
- ❖ Tetracycline resistance gene.
- ❖ Deletion of nucleotide between 1427 & 2516.

# pUC8

- ✓ Popular Ecoli cloning vector.
- ✓ Derivative of pBR322.
- ✓ Two parts derived:-
  - Ampicillin resistance gene.
  - ColEI – origin of replication.
- ✓ 2700 base pairs.
- ✓ lac Z gene derived from Ecoli.
- ✓ Polylinker sequence having unique restriction sites
- lies in lac region.



# PHAGE

- ✓ Cloning large DNA fragments.
- ✓ Linear Phage molecule.
- ✓ Efficient than plasmid.
- ✓ Used in storage of recombinant DNA.
- ✓ Commonly used Ecoli phages :-

$\lambda$  phage

M13 Phage



# BACTERIOPHAGE VECTORS

- ❑ Cloning Vectors.
- ❑ It infects bacteria.
- ❑ Commonly used Ecoli phages :-

$\lambda$  phage

M13 Phage

## Lambda phage vector

- ❑ Genome size is 48,502 bp.
- ❑ High transformation efficiency.
- ❑ 1000 times more efficient than the plasmid vector.
- ❑ Origin of replication.
- ❑ Genome linear in head.
- ❑ Single- stranded protruding cohesive ends of 12 bases.
- ❑ Cos site – site of cleavage of phage DNA.

## Phage M13 Vectors

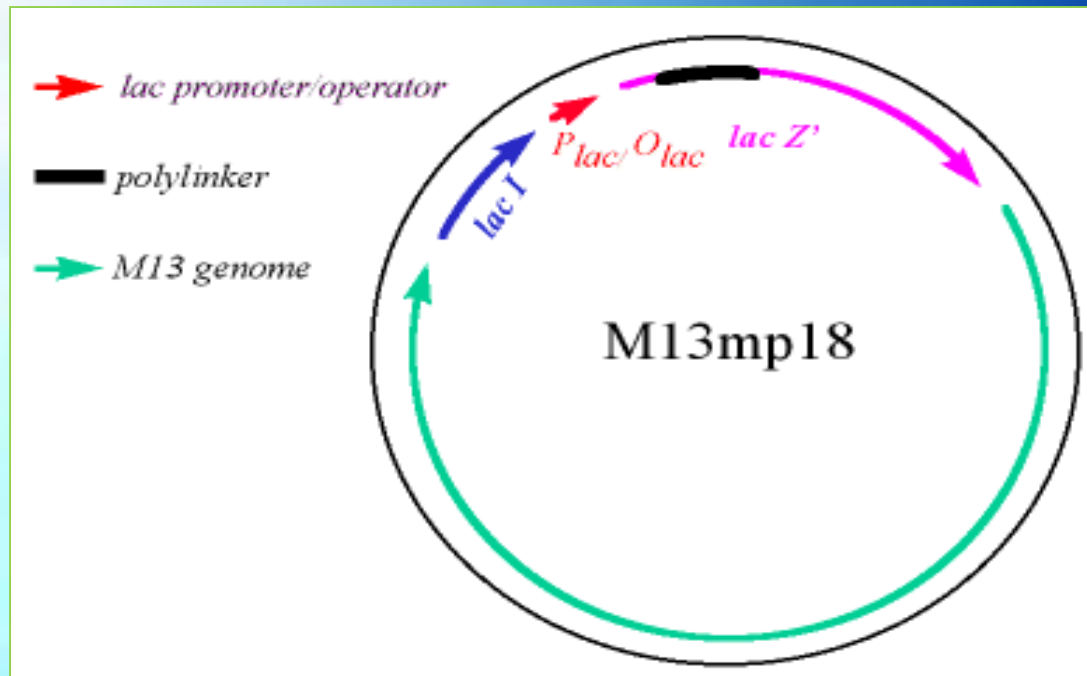
Filamentous bacteriophage of Ecoli.

Used for obtaining single stranded copies.

DNA sequencing.

Single stranded.

Inside host cell become double stranded.



# HYBRID VECTOR

- Component from both plasmid & phage chromosomes.
- ✓ Helper phage provided.
- ✓ Developed in 1978 by Barbara Hohn & John Collins.
- ✓ 30 – 40 Kb
- ✓ Origin of replication, cloning site, marker gene, DNA cos site.
- ✓ Smaller than plasmid.
- ✓ Use – construction of genomic libraries of eukaryotes.
- ✓ e.g. **Cosmid**

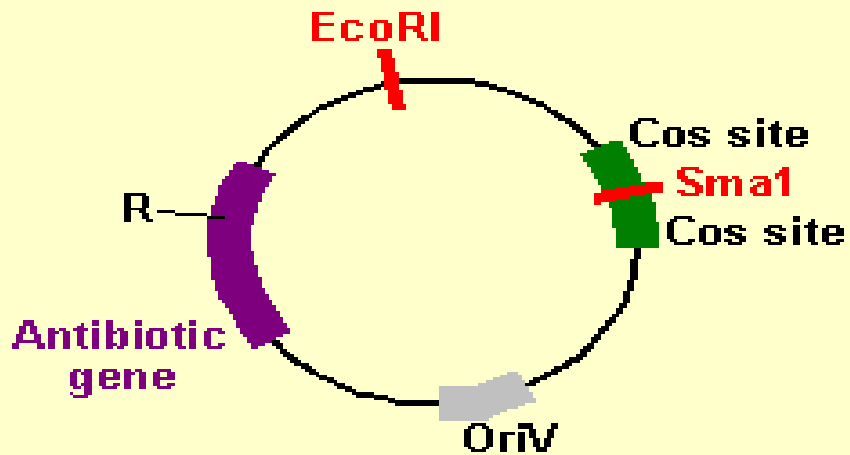
# COSMIDS

- Combine parts of the lambda chromosome with parts of plasmids.
- Contain the *cos* sites of  $\lambda$  and plasmid origin of replication.
- Behave both as plasmids and as phages.
- **Cosmids** can carry up to 50 kb of inserted DNA.

## Structure of Cosmid

- Origin of replication (*ori*).
- Restriction sites for cleavage and insertion of foreign DNA.
- Selectable marker from plasmid.
- A **cos** site - a sequence yield cohesive end (12 bases).
- **Ampicillin resistance** gene (*amp*).

# Basic Features of a Cosmid



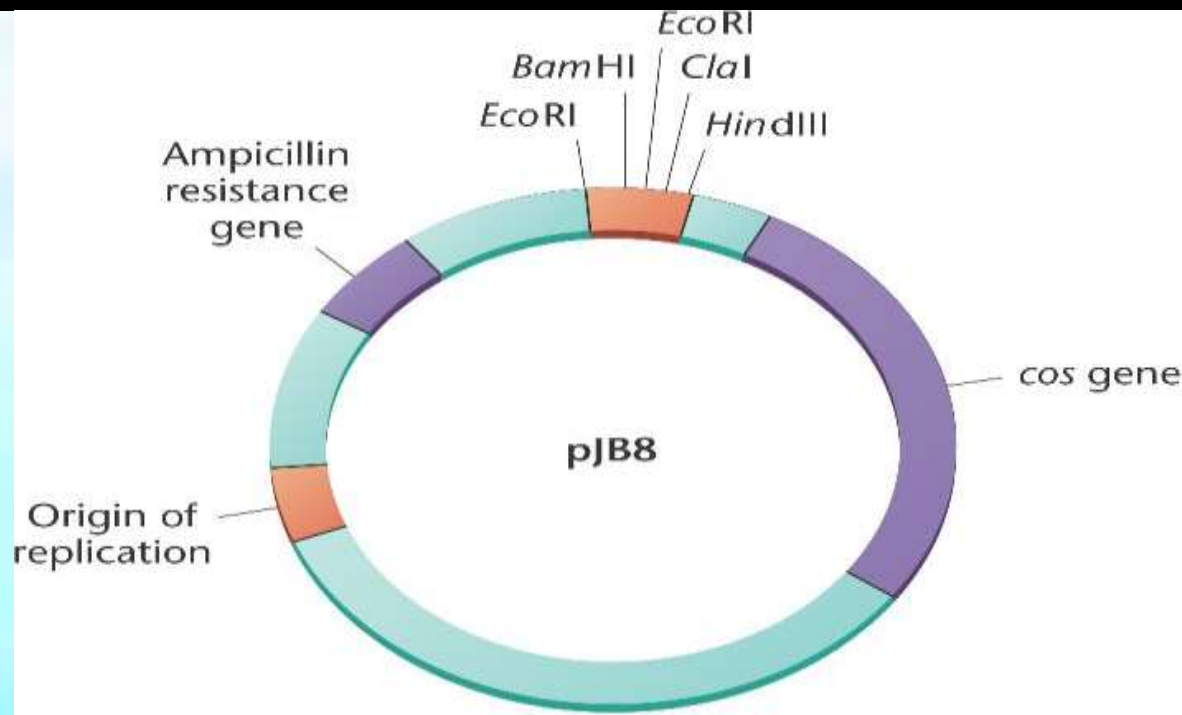
## KEY

OriV - origin of replication.

Cos sites - provide blunt ends.

R - recombinant site

EcoRI } - Restriction endonuclease  
SmaI } - recognition sequence.



# ARTIFICIAL CHROMOSOME

✓ Linear or Circular.

✓ 1 Or 2 copies per cell.

➤ Different types –

Bacterial Artificial Chromosome (BAC)

Yeast Artificial Chromosome (YAC)

P1 derived artificial chromosome (PAC)

Mammalian Artificial Chromosome (MAC)

Human Artificial Chromosome. (HAC)

■ YAC – Cloning in yeast

■ BAC & PAC – Bacteria

■ MAC & HAC – Mammalian & Human cells.

# BACTERIAL ARTIFICIAL CHROMOSOME

- **1<sup>st</sup> BAC Vector – PBAC108L.**
- **Cloning of large regions of eukaryotic genome.**
- **Origin of replication from bacterium Ecoli F -factor.**
- **BAC vectors are pBACe3.6, pBeloBAC11.**
- **Used in analysis of genomes.**
- **Host for BAC is mutant strain.**

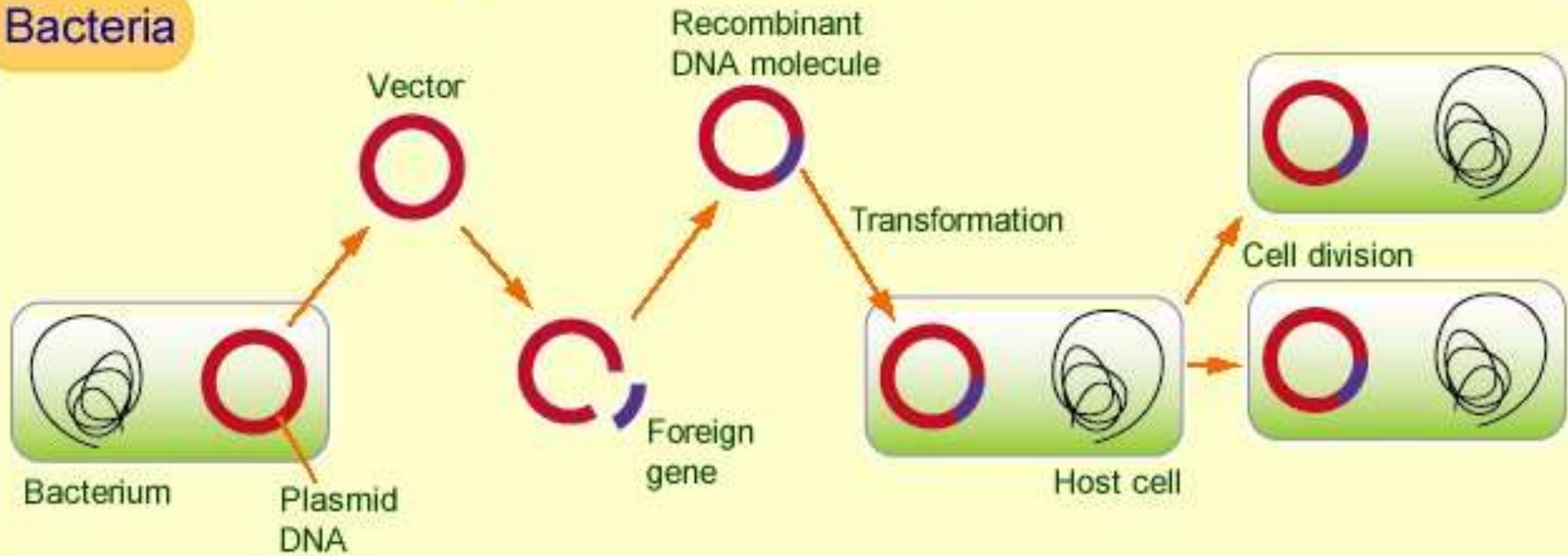
# YEAST ARTIFICIAL CHROMOSOME

- Linear Plasmid Vector.
- Clone large DNA segment ( 100 – 1400kb).
- Occurring two forms:-
  - Circular – grows in bacteria.
  - Linear – multiplies in yeast cells.
- **pYAC3** - first YAC developed.
- It contains :-
  - ARS sequence – replication
  - CEN4 sequence – centromeric function
  - TRP1 & URA3 – 2 selectable markers
- Use – mapping complex eukaryotic chromosome .



# Gene Cloning with Different Vectors

## Bacteria



## Virus

