

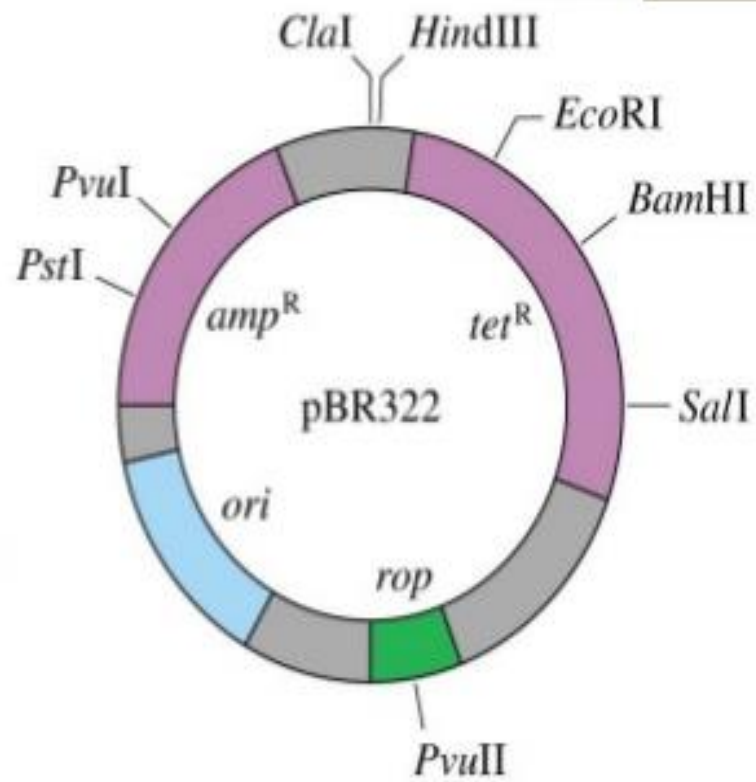


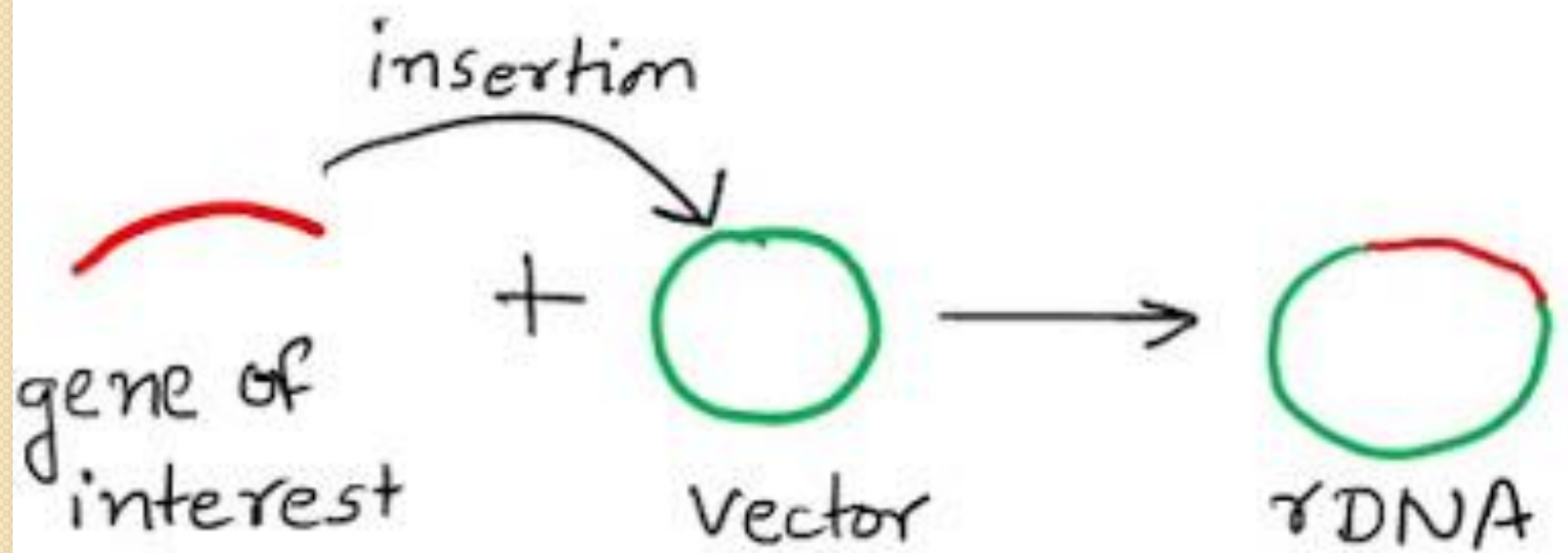
Vectors used in rDNA technology

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Vectors used in rDNA technology

- A vector is an area of DNA that can join another DNA part without losing the limit for self-replication
- Should be capable of replicating in host cell
- Should have convenient RE sites for inserting DNA of interest
- Should have a selectable marker to indicate which host cells received recombinant DNA molecule
- Should be small and easy to isolate





Types of vectors

1. **Cloning vector** : essentially stable & self duplicating DNA molecules to which foreign DNA fragments can be attached for introducing them to target cells. They may not express themselves through transcription & translation.
2. **Expression vectors** : allow the expression of the cloned gene to form the protein product. Commonly used for generating transgenic organisms in which cloned gene express to form the protein product.

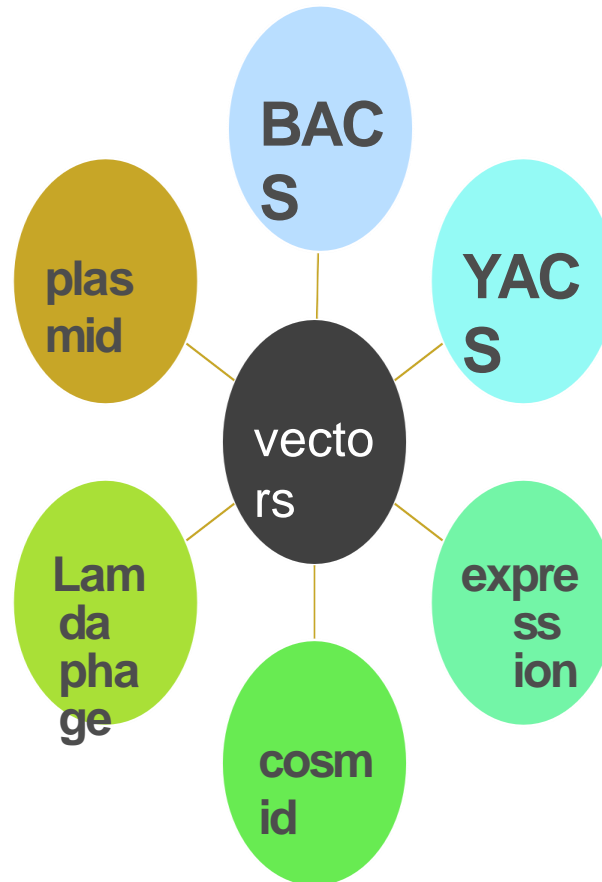
Features of cloning vectors

- Small size, convenient for easy isolation, handling & transmembrane transport.
- Ability for autonomous replication
- Presence of replication ori for autonomous replication.
- Unique target size or restriction site.
- Presence of **cutting site** & **recognition site** outside the replicon for restrictases, cleavage at the cutting site produces a gap, called cloning site, for the splicing of the donor DNA fragment.
- Presence of **marker gene**.
- Absence of transfer genes and mobilizing genes.

Common cloning vectors

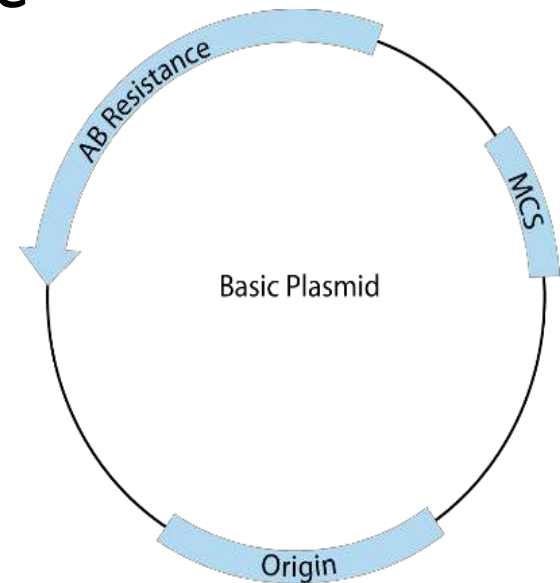
- **Bacterial systems** : Plasmids [PBR322, Ti plasmid] & phages [host cell is a bacteria – phage lamda, phage m13]
- **Animal systems** : Animal viruses.
- **Plant systems** : Plant viruses.
- **Plasmid** – Phage hybrids [cosmids & phasmids]
- **Artificial chromosome vectors** – YAC & BAC
- **Transposable elements.**

Vectors used in rDNA technology



Plasmid vector

- Plasmids are small, extra-chromosomal double stranded circular DNA molecules that are separate from the rest of the chromosome.
- They replicate independently of the bacterial chromosome.
- Useful for cloning DNA inserts less than 20 kb (kilobase pairs).
- Inserts larger than 20 kb are lost easily in the bacterial cell.



Properties of plasmids

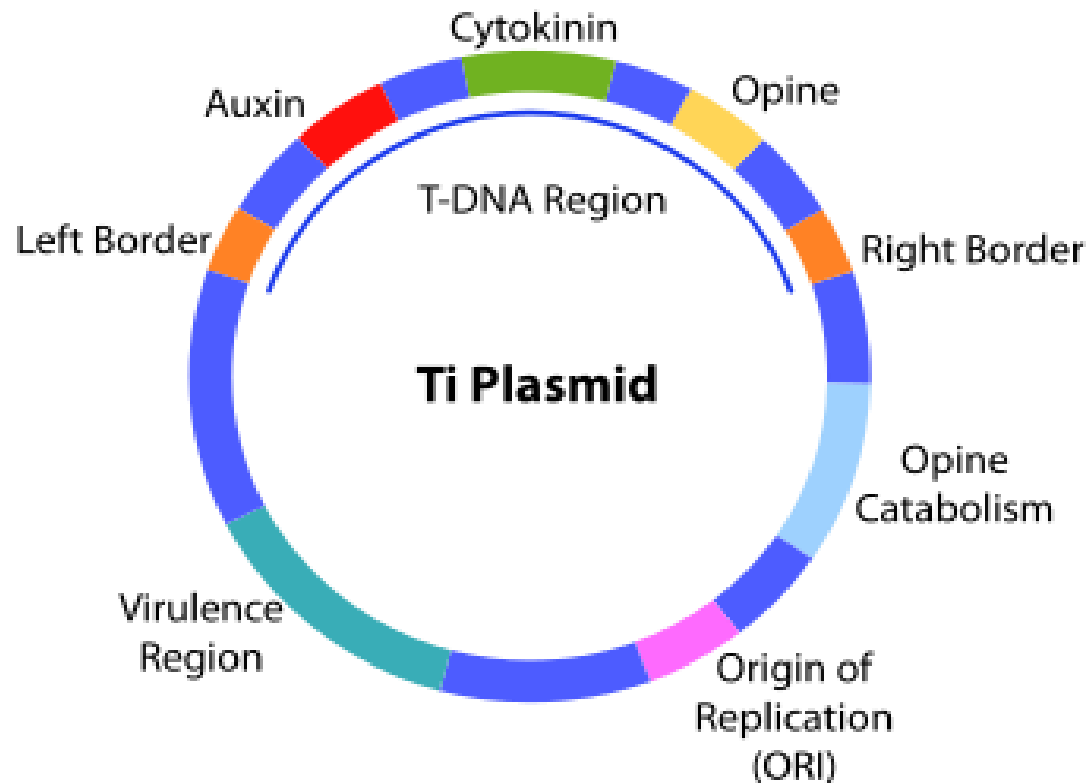
- Contain a **replication ori, selectable marker gene** [antibiotic resistant gene], **unique restriction site** or **cloning site**.
- **Replication of plasmids** : replicate autonomously due to the presence of origin of replication.
- **Size of plasmid** : 1kb to 200kb. Small sized plasmids are advantageous in genetic engineering.
- **Copy number** : no of molecules of plasmid found in a single bacterial cell is termed as copy number. Specific for given plasmid.

Types of plasmids

1. **Conjugative or transmissible plasmids** : Plasmids carry a set of transfer genes [tra genes] that enable plasmid to transfer from one bacterium to another.
2. **Non-conjugative plasmids** : plasmids do not carry a set of tra genes. They replicate autonomously but cannot transfer to another bacterium.
3. **Relaxed plasmids** : plasmids have multiple copy numbers.
4. **Stringent plasmids** : plasmids have a limited number of copies per cell.

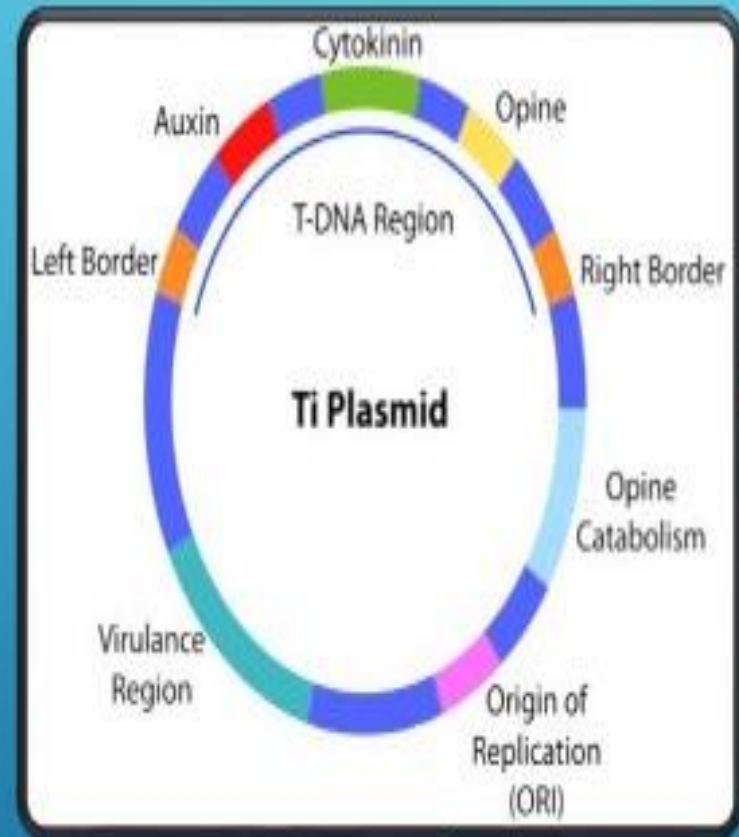
Ti plasmid


- Large conjugative mega plasmid.
- Induction of tumour like crown gall in plants.
- Used as vectors for the transfer of foreign DNA in to host cells.



FEATURES OF TI PLASMID

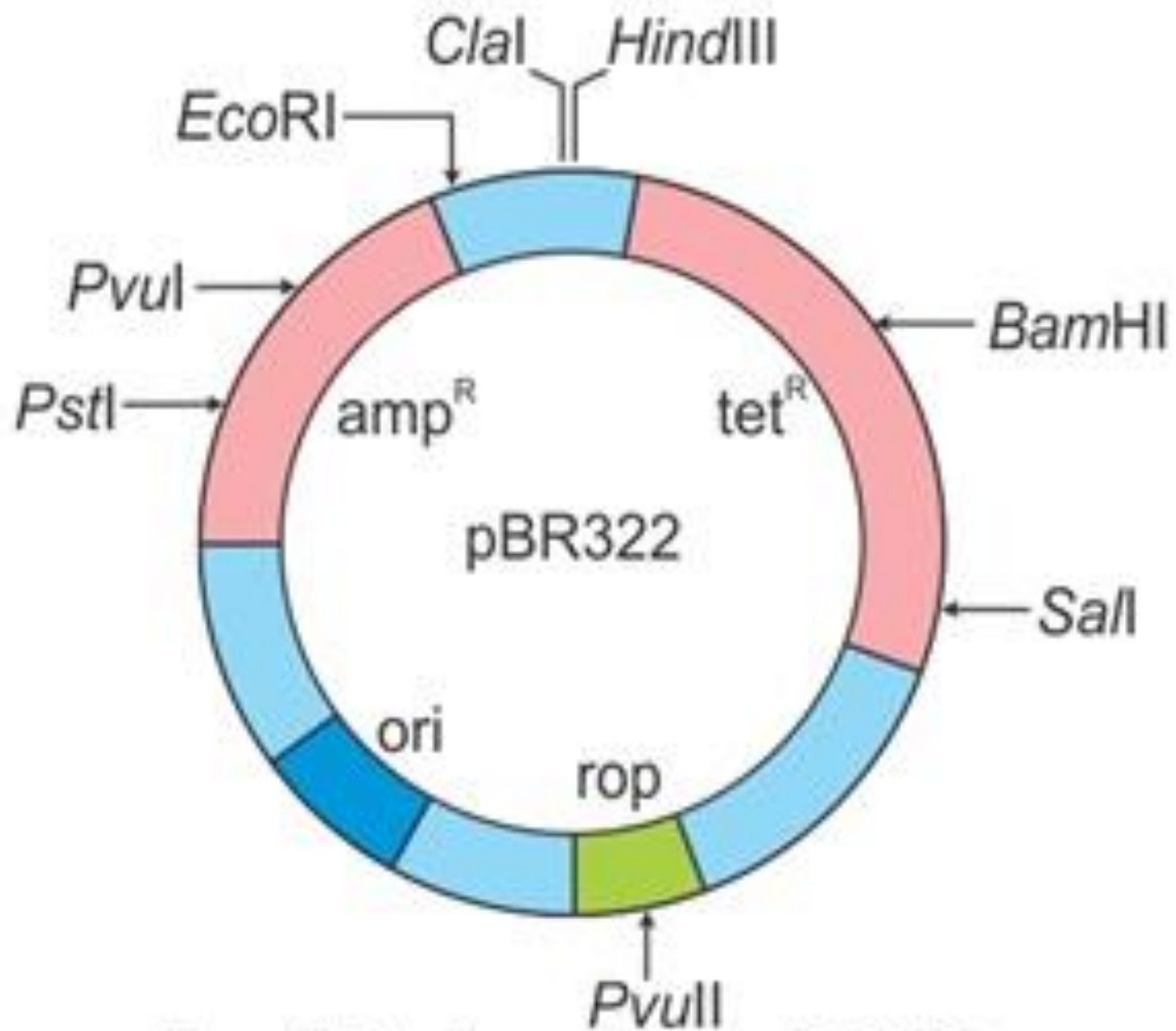
- ❖ It is responsible for crown gall disease
- ❖ It has 200 kb
- ❖ It has 8 genes (*vir*, *shi*, *roi*, *nos*, *noc*, *ocs*, *occ* and *ori V*)
- ❖ A part of Ti-plasmid, T DNA, integrates into the plant chromosomal DNA
- ❖ The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the right border and terminated at the left border and requires the *vir* genes of the Ti plasmid.



- 
- Different strains of agrobacterium contain different **Ti plasmids which code for the production of different opines.**
 - The genes on the Ti plasmids that are not transferred to plants during gall formation encode the proteins responsible for the uptake & catabolism of opines.
 - Ti plasmid has a **central role** in crown gall formation.
 - The portion of the Ti plasmid that are not transferred to plants during gall formation is **called transfer DNA [T-DNA]**
 - It is responsible for **tumorous genotype[gall]**.
 - T region of the plasmid is flanked by **a pair of 25bp** direct repeats, called **border repeats.**

PBR 322

- First artificial cloning vector developed from E.coli plasmid.
- P - plasmid
- B,R – researchers behind the discovery, bolivar & rodriguez
- 322 – numerical representation.
- gene for ampicilin resistance – derived from RSF21
- Ori - pMB1



***E. coli* cloning vector pBR322**

Virus vectors

- **Animals**

- Simian virus
- Bovine papilloma virus
- Vaccinia virus
- Retroviruses
- Adenoviruses

- **Plants**

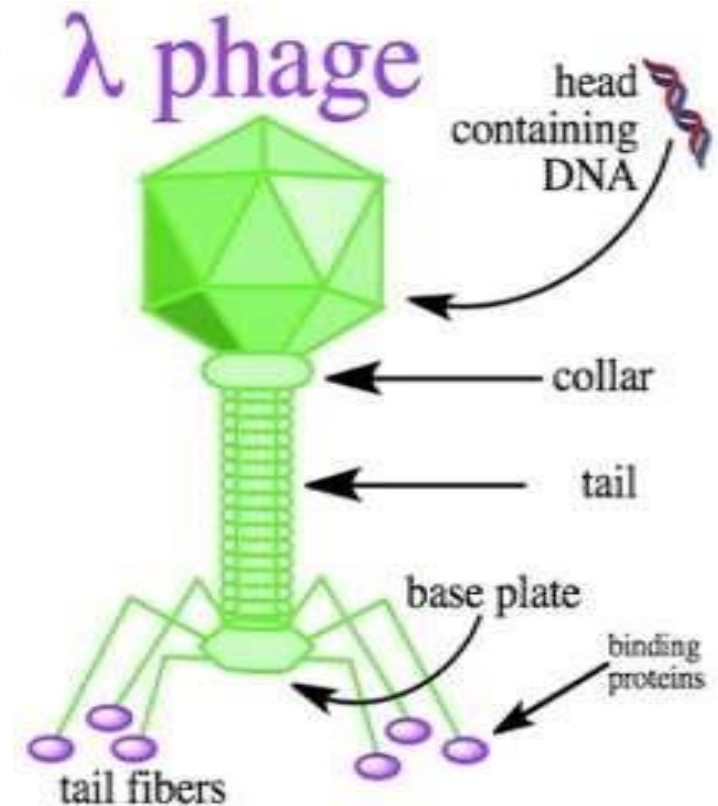
- Cauliflower mosaic virus
- Tobacco mosaic virus
- Gemini viruses

Phage vectors

- Lamda phages
- M13 phages

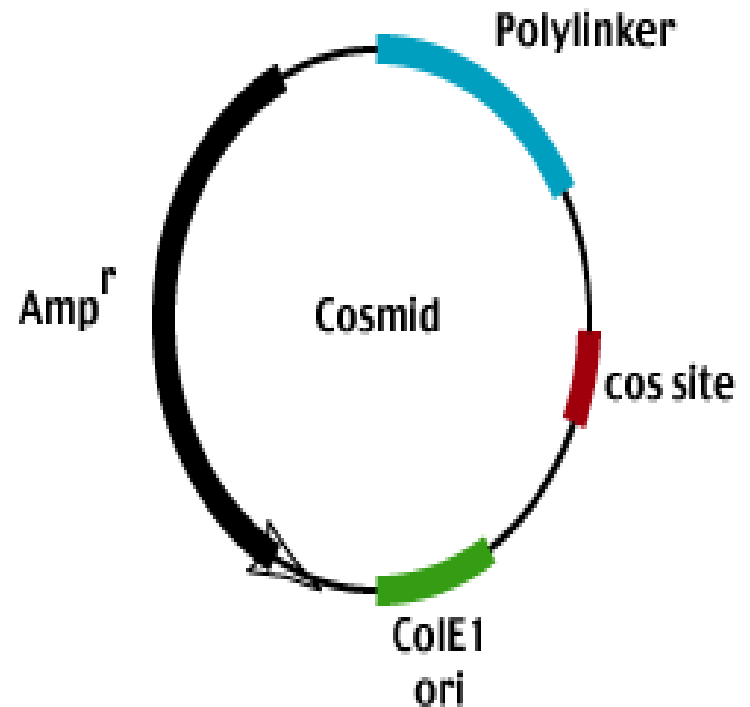
Lamda phage vector

- Lamda phage vectors are recombinant infections, containing the phage chromosome in addition to embedded "outside" DNA.
- All in all, phage vectors can convey bigger DNA groupings than plasmid vectors.



Cosmid vector

- Cosmids are hybrids of phages and plasmids that can carry DNA fragments up to 45 kb.
- They can replicate like plasmids but can be packaged like phage **lambda**

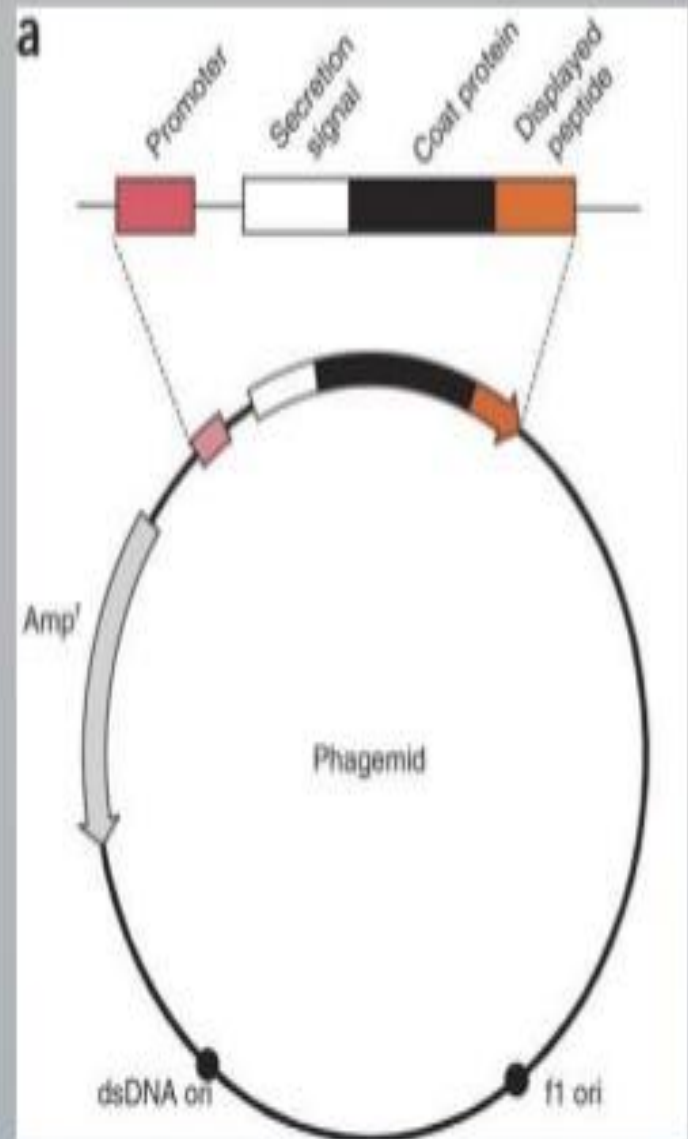


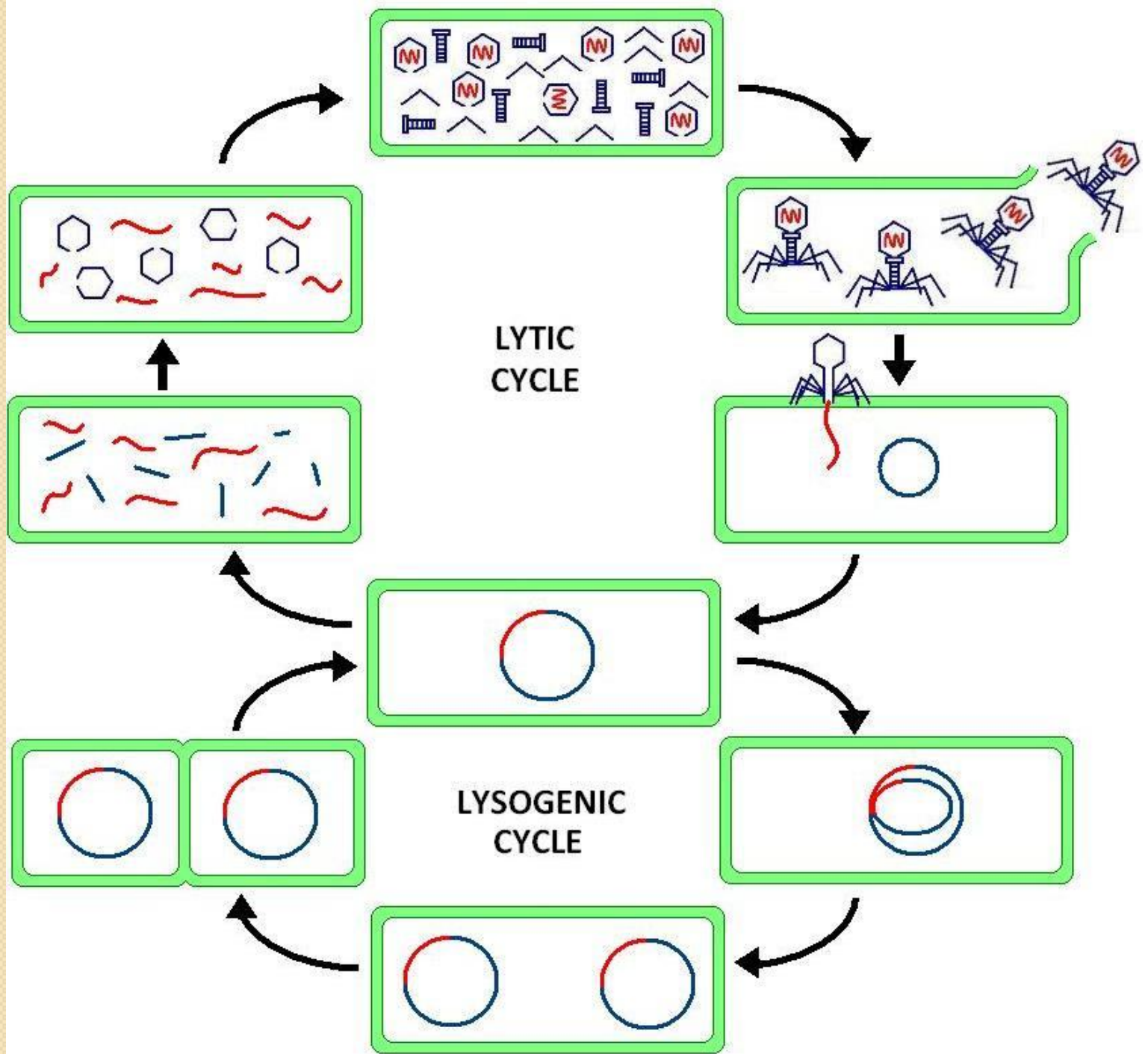
Phasmids

- Bifunctional plasmid-phage hybrid vectors.
- Have replicative powers of both plasmids & phages.
- Replicate non-lytically as a plasmid or lytically as a phage.
- They can behave as plasmids, as phages & also as prophages.

Phagemid

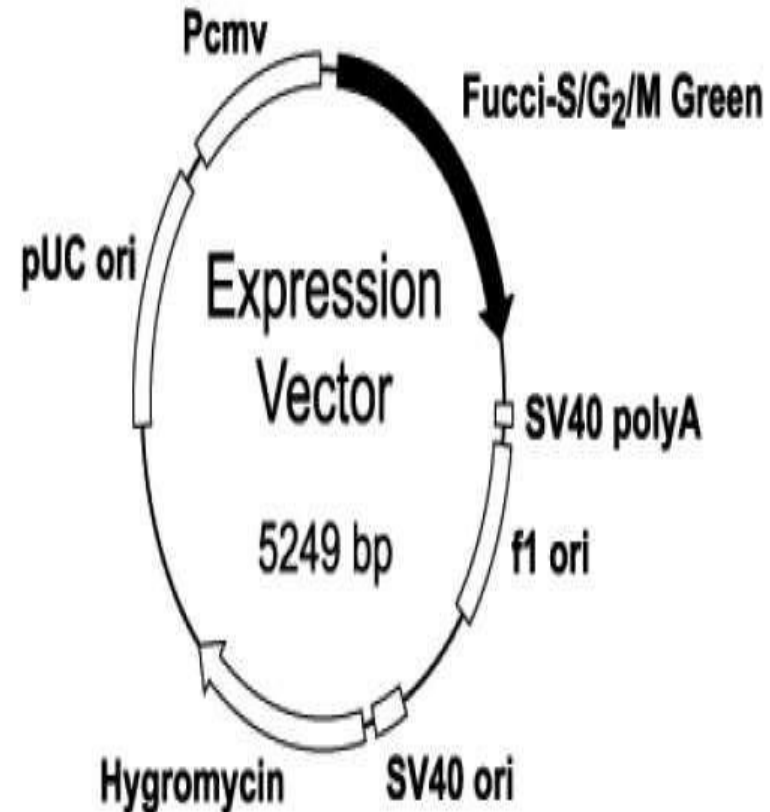
- A **phagemid** or **phasmid** is a plasmid that contains an f1 origin of replication from an f1 phage.
- It can be used as a type of cloning vector in combination with filamentous phage M13.
- A **phagemid** can be replicated as a plasmid, and also be packaged as single stranded DNA in viral particles.





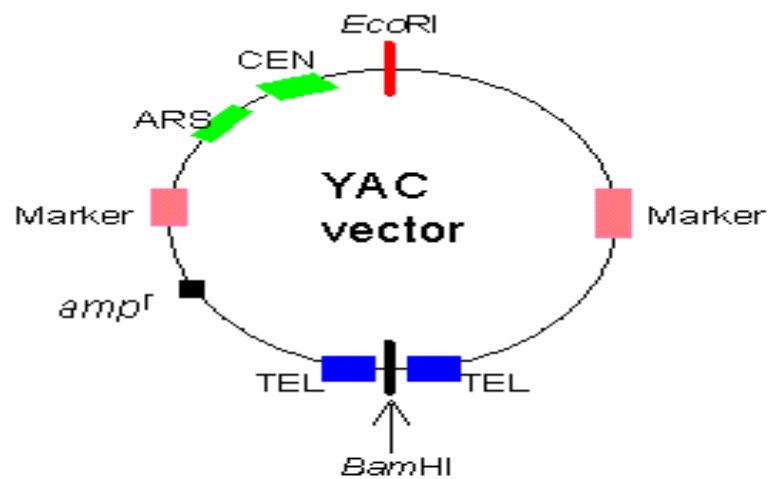
Expression vectors

- Expression vectors are vectors that **carry host signals that facilitate the transcription and translation of an inserted gene.**
- They are very useful for expressing eukaryotic genes in bacteria.

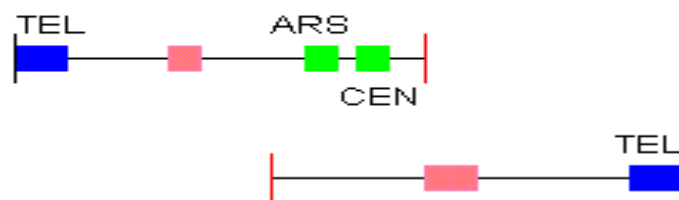


Yeast artificial chromosomes (YACS)

- Yeast artificial chromosomes (YACS) are yeast vectors that have been engineered to contain a centromere, telomere, origin of replication, and a selectable marker.
- They can carry up to 1,000 kb of DNA.
- they are useful for cloning eukaryotic genes that contain introns.



Digest with *Bam*HI and *Eco*RI



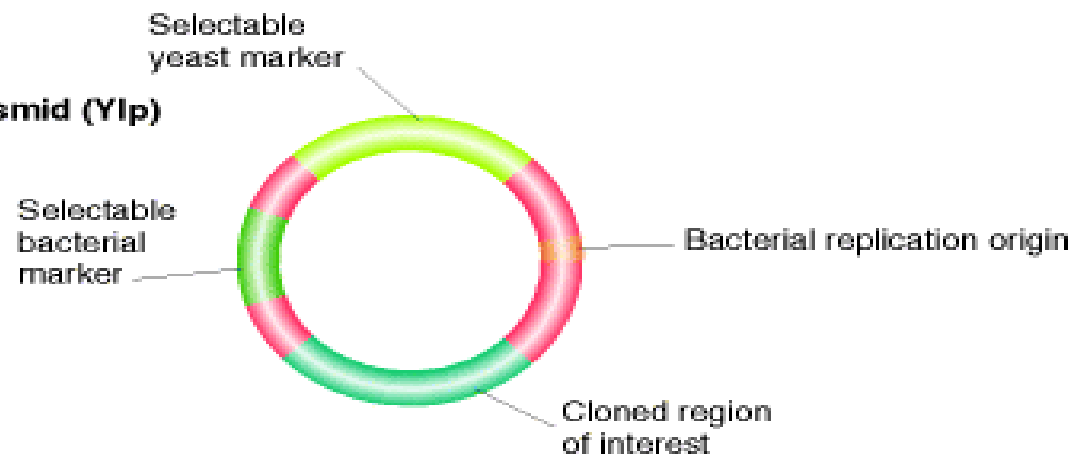
Digest with *Eco*RI



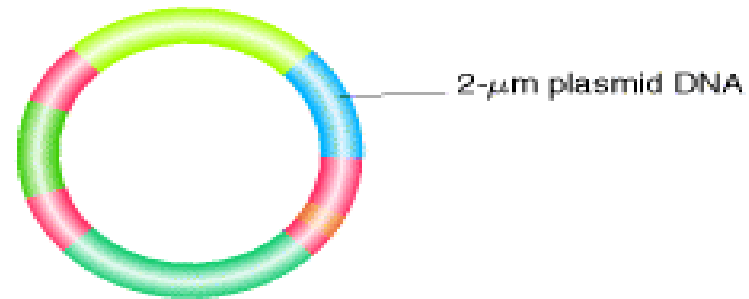
Ligate



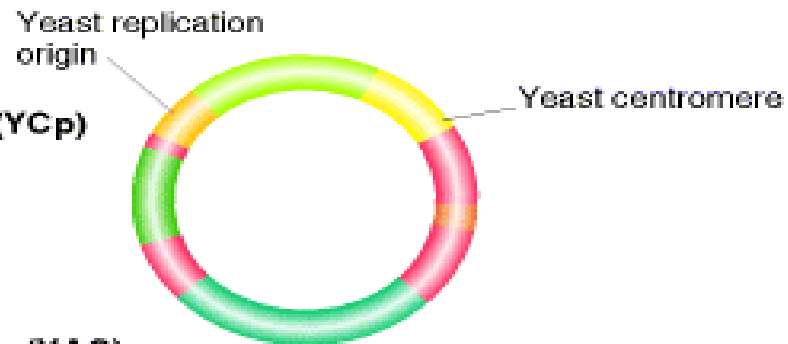
(a) Yeast integrative plasmid (YIp)



(b) Yeast episomal plasmid (YEp)



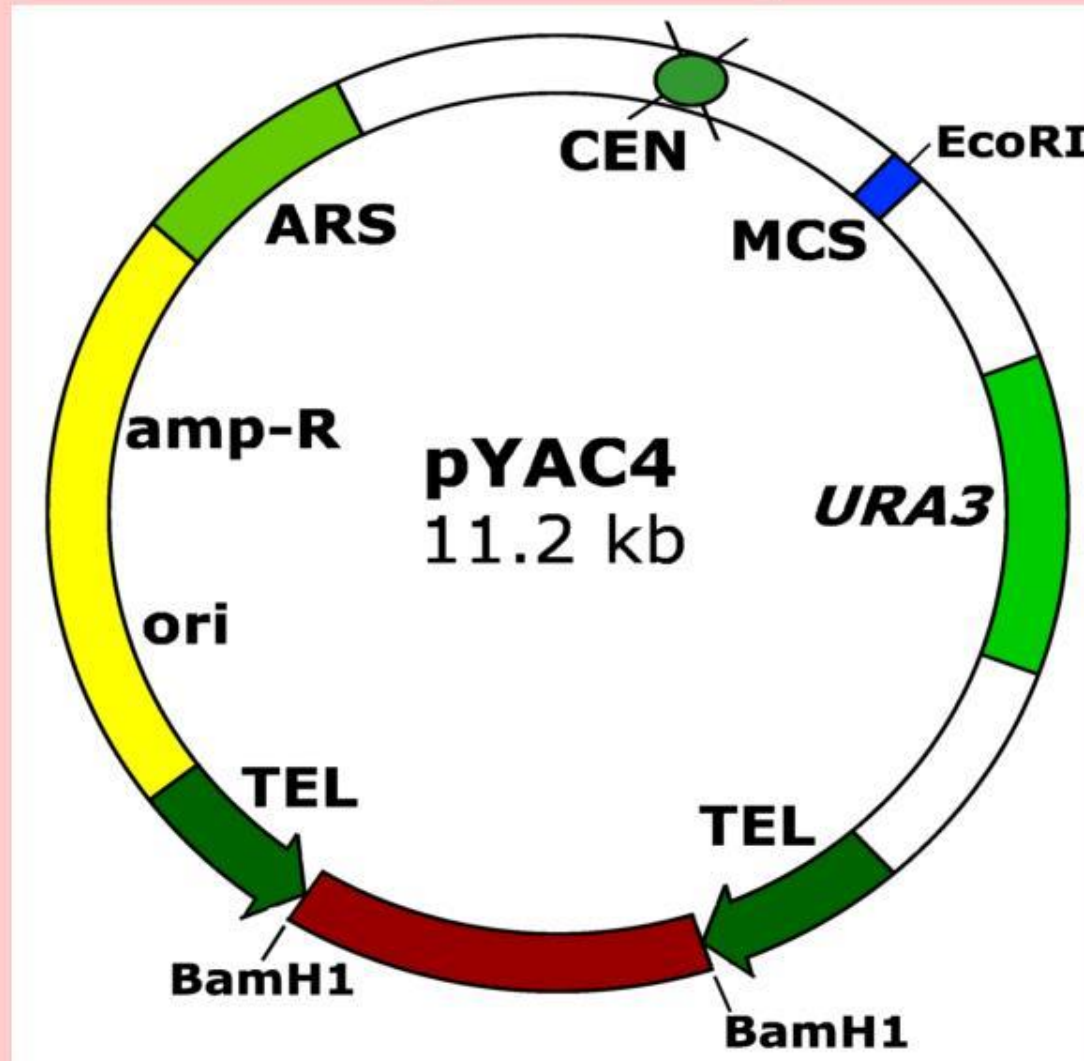
(c) Yeast centromere plasmid (YCp)



(d) Yeast artificial chromosome (YAC)

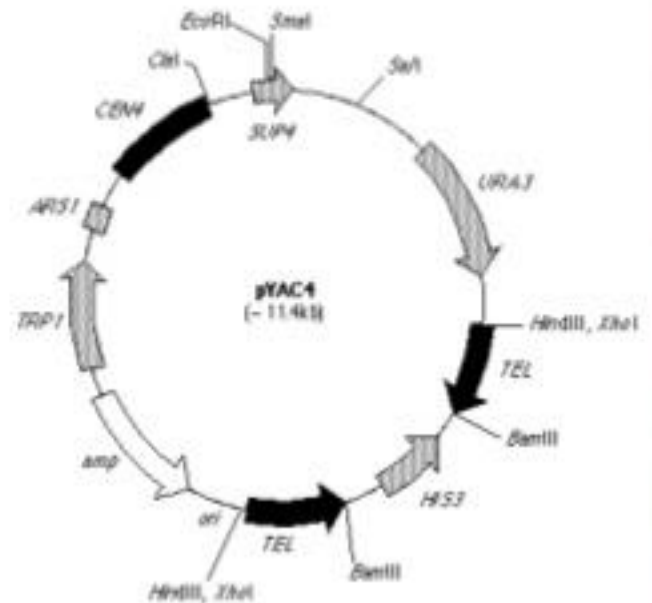


Yeast Artificial Chromosomes (YACs)



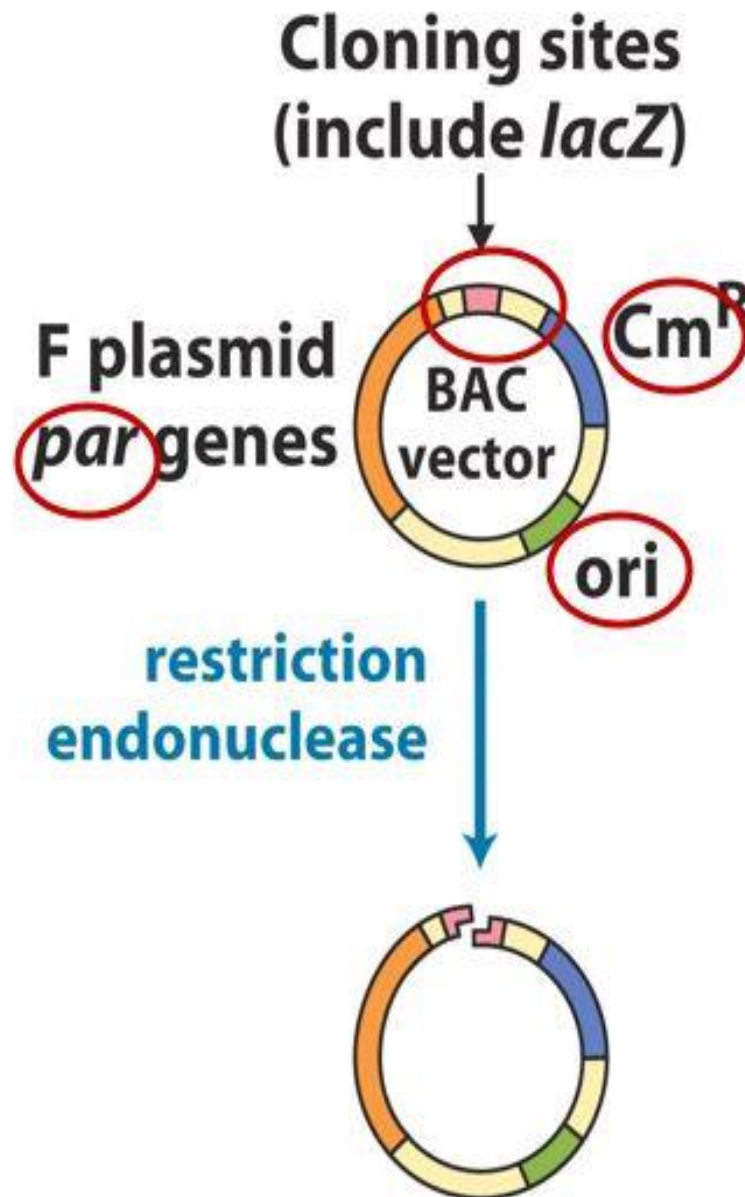
Components of YAC

- The vector contains several elements of typical yeast chromosomes including a yeast centromere (CEN4), yeast autonomously replicating sequence (ARS1), yeast telomere (TEL), genes for YAC selection in yeast, bacterial replicating origin and a bacterial selectable marker.



Bacterial artificial chromosomes (BACS)

- Bacterial artificial chromosomes (BACS) are bacterial plasmids derived from **the F plasmid**.
- They are capable of carrying up to 300 kb of DNA.



Artificial Chromosomes allow for cloning of large pieces of DNA

Bacterial Artificial Chromosome

1. *ori* allows for replication in bacteria,
2. *par* helps segregate BAC evenly between daughter cells,
3. *lac Z* allows for detection of insert,
4. Cm^R allows for selection of transformed cells.