COURSE BSc (BIOTECHNOLOGY) III YEAR

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PAPER TITLE: RECOMBINANT DNA TECHNOLOGY

Unit II CLONING VECTOR

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CLONING VECTOR

A cloning vector is a genome that can accept the target DNA and increase the number of copies through its own autonomous replication.

A cloning vector is a small piece of DNA that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes.

- The cloning vector may be DNA taken from a virus, the cell of a higher organism, or it may be the plasmid of a bacterium.
- **An ideal vector therefore contains features that allow for the convenient insertion or removal of a DNA fragment to or from the vector:**

1. Smaller in size less than 10 kb and Easy to purify
2. Must contain origin of replication
3. Ability to confer readily selectable phenotypic traits on host cells
4. Single sites for a large number of restriction endonucleases, preferably in genes with a readily scorable phenotype

Properties and construction of a vector DNA molecule

1. Capability of autonomous replication

- Bacterial and viral genomes contain only one origin of replication while eukaryotes contain multiple origins

2. Small size

- In small molecules the chances of occurrence of unique sites for restriction enzymes increases
- Efficiency of gene transfer is high with small vector molecules

3. Presence of selectable marker gene(s)

- For easy detection of recombinants
- E.g.: antibiotic resistant genes, *lac* z or resistant to toxins, etc

4. Presence of unique restriction enzyme sites or multiple cloning sites for inserting the target DNA

- Position of these restriction sites should be such that the insertion of a segment of DNA in any of these restriction sites bring about a phenotypic change in the characteristic of a vector molecule e.g.: loss of gene expression or loss of resistance to an antibiotic

5.Ease of purification

6. No effect on the replicative ability of vector due to insertion of target DNA

7. Ease of reintroduction into host cell with high efficiency

8. Biological containment

- Vectors should be biologically contained with no possibility of gene escape
- This can be achieved by non-conjugative and non-mobilized plasmid vectors

9.Presence of promoters and ribosome binding sites

10. Presence of two different origins of replication or broad host range origin of replication

E.g.: shuttle vectors that contain two different origins of replication

Types of Cloning Vectors

- They allow the exogenous DNA to be inserted, stored, and manipulated mainly at DNA level.
- Types

1. Plasmid vectors
2. Bacteriophage vectors
3. Cosmids
4. Phagemids
5. Fosmids
6. BACs & YACs

PLASMIDS

- Plasmids are circular molecules of DNA that lead an independent existence in the bacterial cell .
- Plasmids almost always carry one or more genes, and often these genes are responsible for a useful characteristic displayed by the host bacterium.
- Most plasmids possess at least one DNA sequence that can act as an **origin of replication**, so they are able to multiply within the cell independently of the main bacterial chromosome.
- A few types of plasmid are also able to replicate by inserting themselves into the bacterial Chromosome known as integrative plasmids or **episomes**.

PLASMID CLASSIFICATION

Classification of naturally occurring plasmids is based on the main characteristic coded by the plasmid genes. The five major types of plasmid according to this classification are as follows:

- **Fertility** or **F plasmids** carry only *tra* genes and have no characteristic beyond the ability to promote conjugal transfer of plasmids. example is the F plasmid of *E. coli*.
- **Resistance** or **R plasmids** carry genes conferring on the host bacterium resistance to one or more antibacterial agents, such as chloramphenicol, ampicillin, and mercury.
- **Col plasmids** code for colicins, proteins that kill other bacteria. e.g. ColE1 of *E. coli*.
- **Degradative plasmids** allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid, an example being TOL of *Pseudomonas putida*.
- **Virulence plasmids** confer pathogenicity on the host bacterium; these include the **Ti plasmids** of *Agrobacterium tumefaciens*, which induce crown gall disease on dicotyledonous plants.
- **Cryptic plasmids** are those that confer no identifiable phenotype on the host cell. Cryptic plasmids presumably carry genes whose characteristics are still unknown.

PLASMIDS TRANSFERABILITY

1. Conjugative Plasmid conjugative plasmids are small (20–200kb), self-replicating circular pieces of double-stranded DNA which encode their transfer by replication into another bacterial strain or species.
2. Non cojugative Plasmid are incapable of initiating conjugation, hence they can be transferred only with the assistance of **conjugative plasmids**.
3. Mobilizable Plasmid Mobilizable plasmids have an origin of transfer site (oriT), region essential for replication and a mobilization gene (mob)

E coli Natural Plasmids

Plasmid	Size (kb)	Host range	Antibiotic resistance	Additional marker genes showing insertional inactivation
RSF1010	8.6	Broad host range	Streptomycin and sulfonamides.	None
ColE1	6.6	Narrow host range	None	Immunity to colicin E1
R100	94.2	*E.Coli*K12,*Shigell a flexneri* 2b	Streptomycin, chloramphenycol, tetracycline	

ARTIFICIAL PLASMID VECTORS

Artificial plasmids vectors are classified into two broad types based on their use:

Cloning Vector :

A cloning vector is defined as a vector used for replication of a cloned DNA fragment in a host cell. These vectors are frequently engineered to contain "ori" – origin of replication sites particular to the host organism. Examples of commonly used cloning vectors are: pUC18, pUC19, pBluescript vectors etc.

Expression Vector:

A vector used for expression of a cloned DNA fragment in a host cell is called as an expression vector. These vectors are frequently engineered to contain regulatory sequences that act as promoter and/or enhancer regions and lead to efficient transcription of the insert gene. Commonly used expression vector series are: pET vectors, pBAD vetors etc.

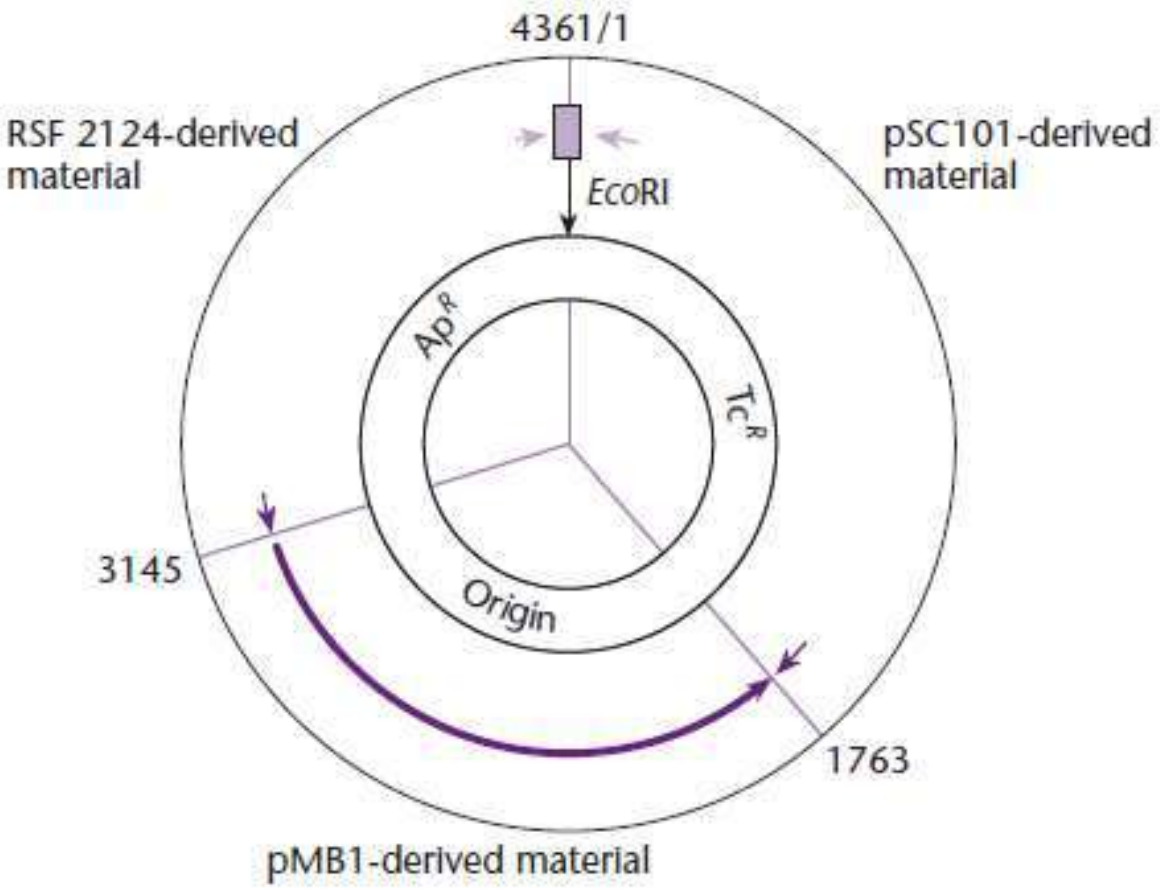
Shuttle vector:

Shuttle vectors can be propagated in two or more different host species (both in prokaryotes and eukaryotes). Hence, inserted DNA can be manipulated and replicated in two different cellular systems.

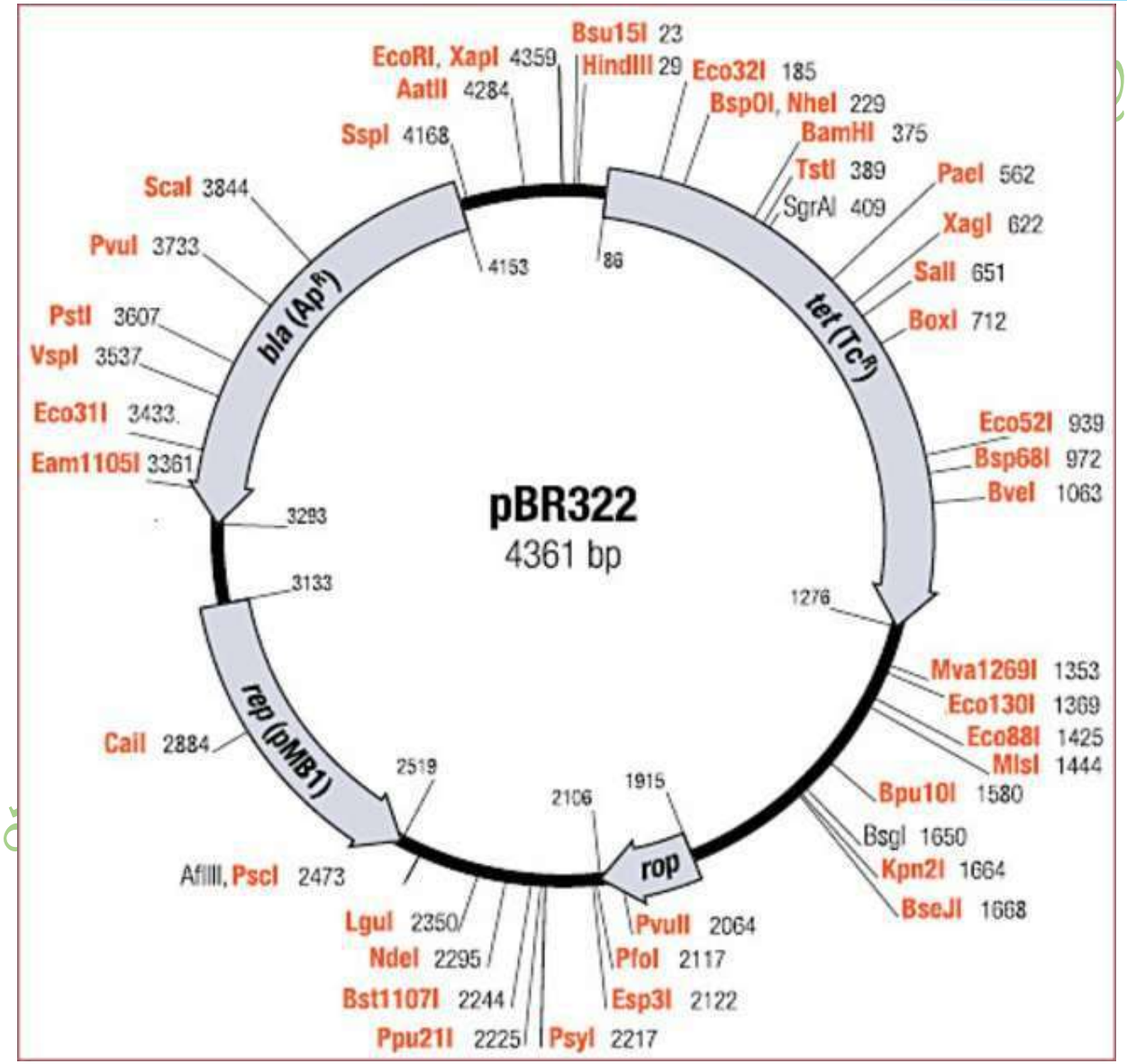
PLASMID pBR322

- **pBR322** is a plasmid and was one of the first widely used *E. coli* cloning vectors.
- It is Created in 1977 in the laboratory of Herbert Boyer at the University of California San Francisco, it was named after Francisco Bolivar Zapata, the postdoctoral researcher who constructed it.
- The p stands for "plasmid," and BR for "Bolivar" and "Rodriguez."
- pBR322 is 4361 base pairs in length and has two antibiotic resistance genes – the gene *bla* encoding the ampicillin resistance (Amp^R) protein, and the gene *tetA* encoding the tetracycline resistance (Tet^R) protein.
- It contains the origin of replication of pMB1, and the *rop* gene, which encodes a restrictor of plasmid copy number.
- The plasmid has unique restriction sites for more than forty restriction enzymes.
- Eleven of these forty sites lie within the Tet^R gene. There are two sites for restriction enzymes HindIII and ClaI within the promoter of the Tet^R gene.
- There are six key restriction sites inside the Amp^R gene.

THE ORIGINS OF PLASMID PBR322



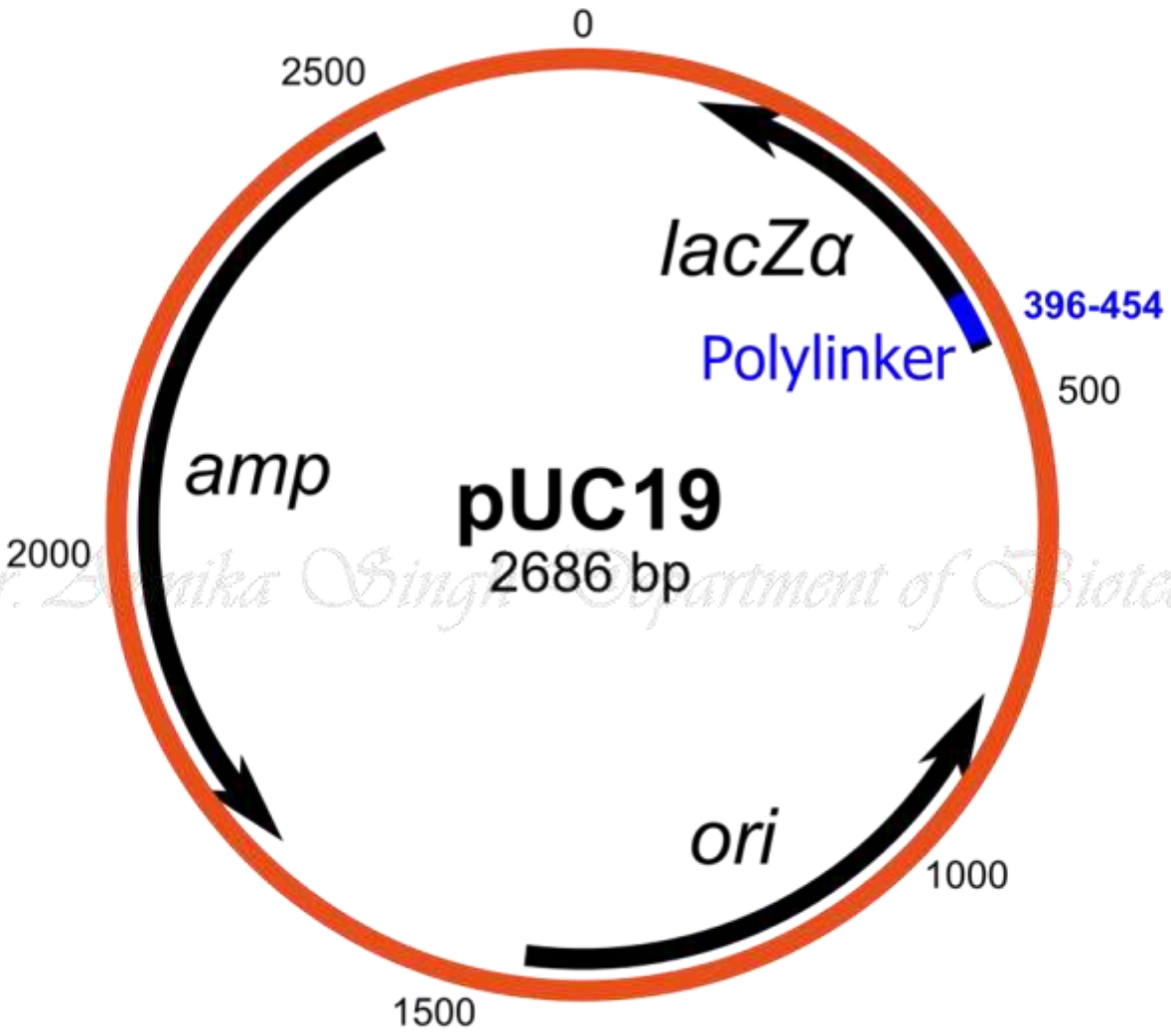
PLASMID pBR322



pUC19

- **pUC19** is one of a series of plasmid cloning vectors created by Joachim Messing and co-workers.
- The designation "pUC" is derived from the classical "p" prefix (denoting "plasmid") and the abbreviation for the University of California, where early work on the plasmid series had been conducted.
- It is a circular double stranded DNA and has 2686 base pairs.
- The multiple cloning site (MCS) region is split into codons 6-7 of the lacZ gene, providing for many restriction endonucleases restriction sites.
- In addition to β-galactosidase, pUC19 also encodes for an ampicillin resistance gene (amp^R).
- The *ori* site, or origin of replication, is derived from the plasmid pMB1.
- pUC19 is small but has a high copy number.
- The high copy number is a result of the lack of the *rop* gene and a single point mutation in the ori of pMB1.

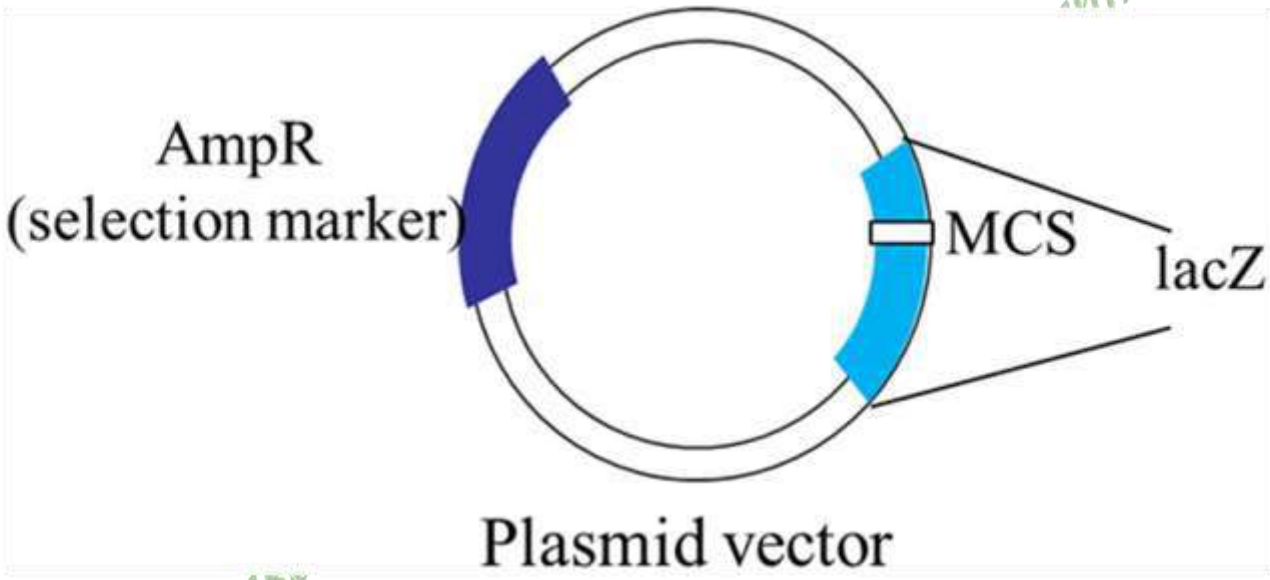
pUC19



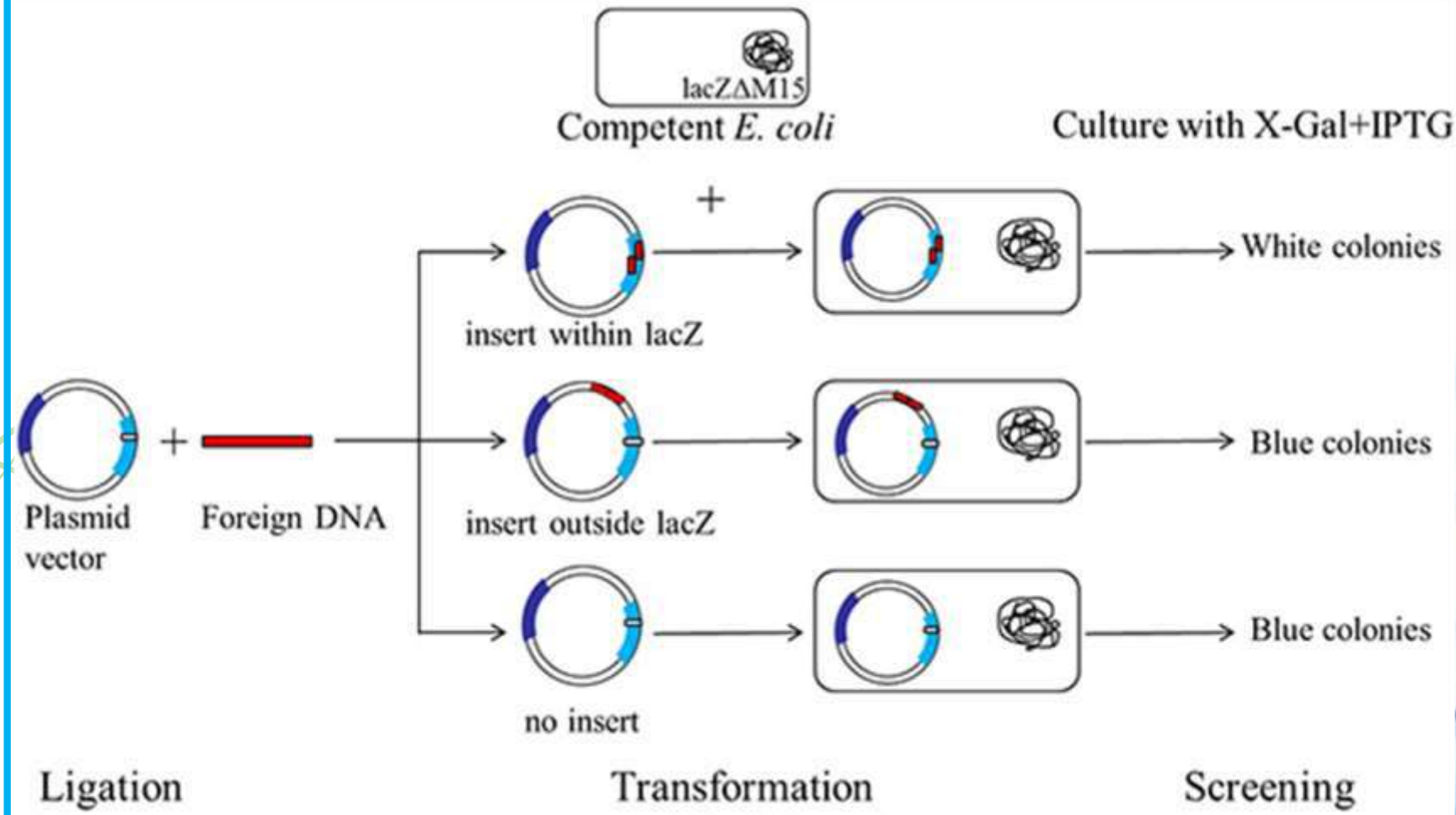
BLUE-WHITE SCREENING

- A multiple cloning site (MCS) is present within the lacZ sequence in the plasmid vector. This sequence can be nicked by restriction enzymes to insert the foreign DNA.
- **When a plasmid vector containing foreign DNA is taken up by the host *E. coli*, the α-complementation does not occur, therefore, a functional β-galactosidase enzyme is not produced.** Insertion of foreign DNA into the MCS located within the *lac Z* gene causes **insertional inactivation.**
- If the foreign DNA is not inserted into the vector or if it is inserted at a location other than MCS, the lacZ gene in the plasmid vector complements the lacZ deletion mutation in the host *E. coli* producing a functional enzyme.
- Both the fragments can together hydrolyse X-gal (5-bromo-4-chloro-3-indolyl- beta-D-galactopyranoside) and form blue colonies when grown on media where it is supplemented.
- Thus bacteria carrying recombinant plasmids in the MCS cannot hydrolyse X-gal, giving rise to white colonies, which can be distinguished on culture media from non-recombinant cells, which are blue

BLUE-WHITE SCREENING



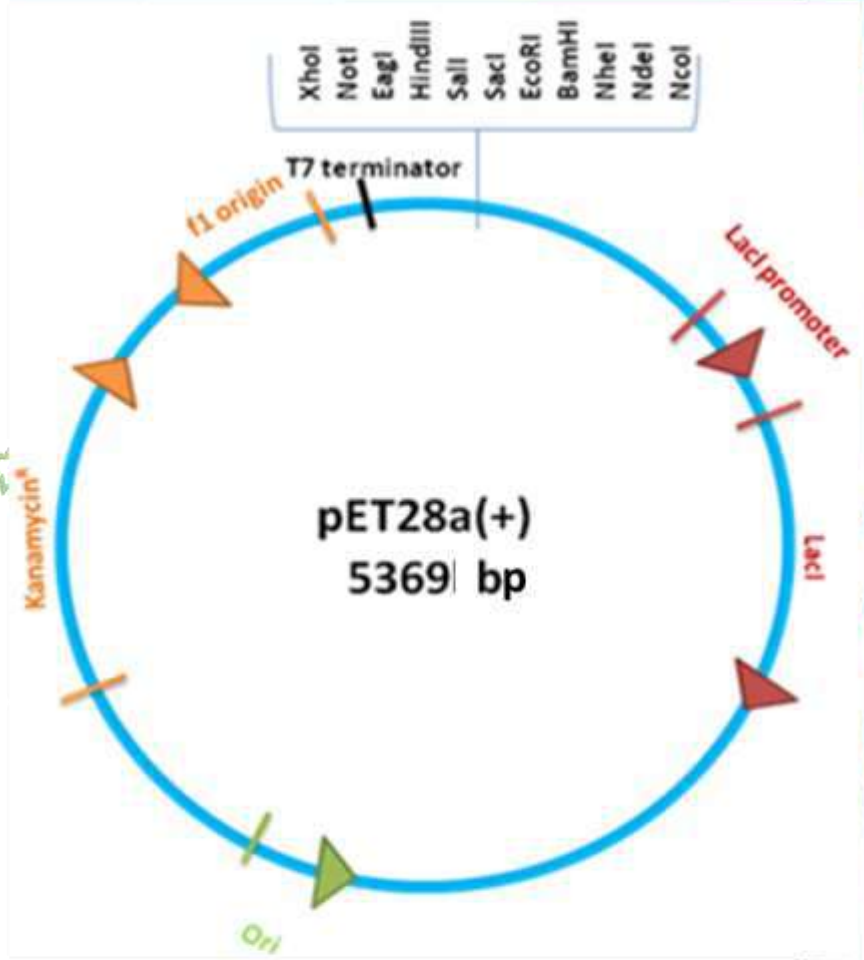
BLUE-WHITE SCREENING



pET VECTORS

pET *E. coli* T7 Expression Vectors

- The pET System is the most powerful system for the cloning and expression of recombinant proteins in *E. coli*.
- The original pET vector system was constructed by Studier and colleagues. That plasmid is developed at Novagen with enhanced characteristics.
- Target genes are cloned under strong T7 bacteriophage promoter.
- The expression of the target protein is inducible by providing T7 RNA polymerase in the host cell as an inducing signal.
- Ampicillin and kannamycin resistance genes are available in pET vectors as selection marker.
- pET28 and pET32 are the most commonly used pET vectors.



PHAGE VECTORS

- To insert DNA fragments of more than 10 kb, normally plasmids are not the suitable vehicles, a new class of vectors based on bacteriophages can be use. various bacteriophages available such as λ, T4, T5, and T7 phages; the λ phage gained favourable attention due to its unique life cycle.
- **λ phage**
- Bacteriophage λ contains ~49kb (48.5 kbp) of DNA and has a very efficient mechanism for delivering its genome into a bacterium.
- Genes of the central region are concerned with recombination (e.g. *red*) and the process of lysogenization
- Much of this central region, including these genes, is not essential for phage growth and can be deleted or replaced without seriously impairing the infectious growth cycle. Its dispensability is crucially important, for the construction of vector derivatives of the phage.
- Two key features contribute to its utility as a vector to clone larger DNA fragments:
- **1.** One-third λ genome is nonessential and could be replaced with foreign DNA. Approximately 24.6kb of λ genome can be deleted, hence maximum insert size could be upto 26 kb.
- **2.** Packing of DNA in phage could only take place if the size is between 40 and 52 kb long, a constraint that can be used to ensure packaging.

λ BACTERIOPHAGE

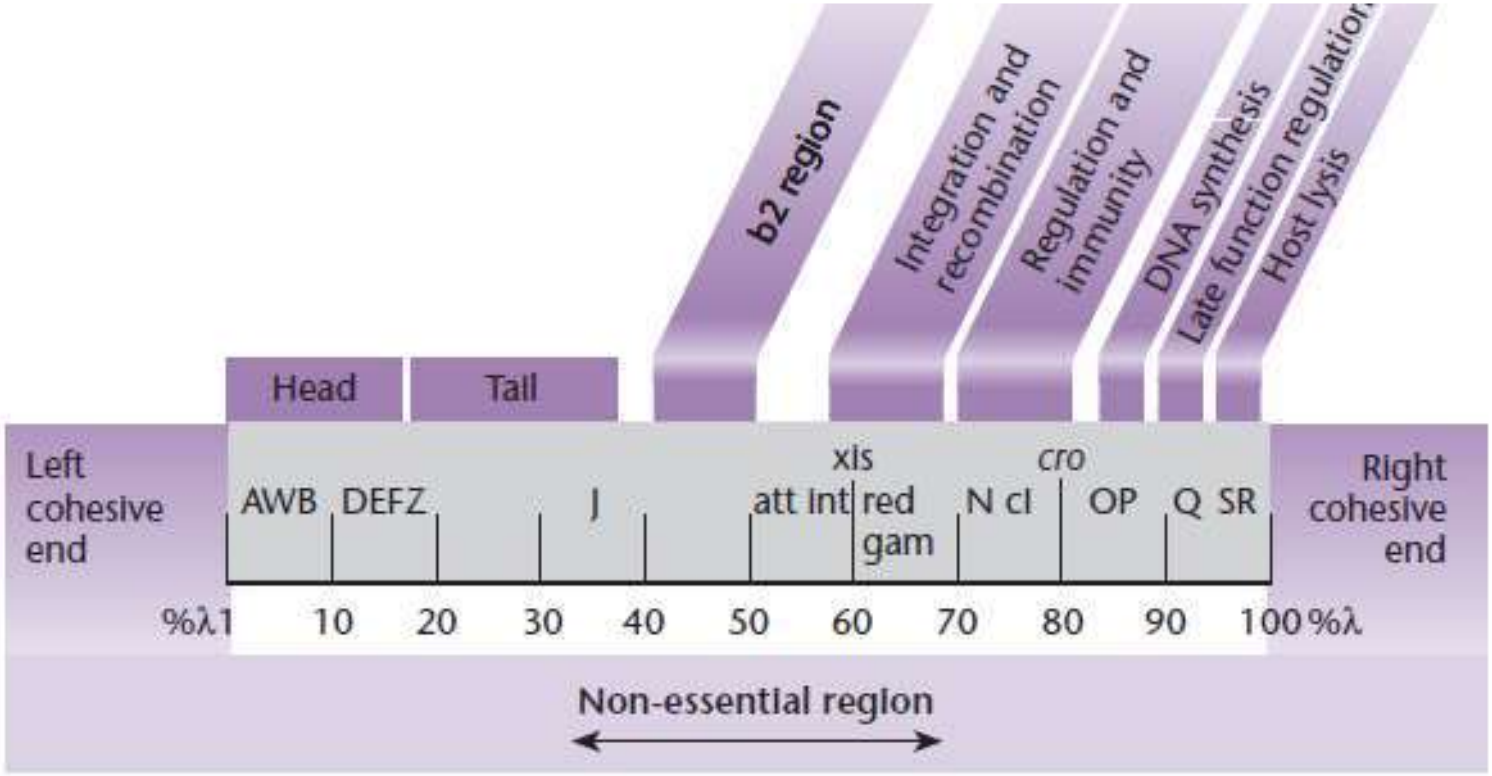
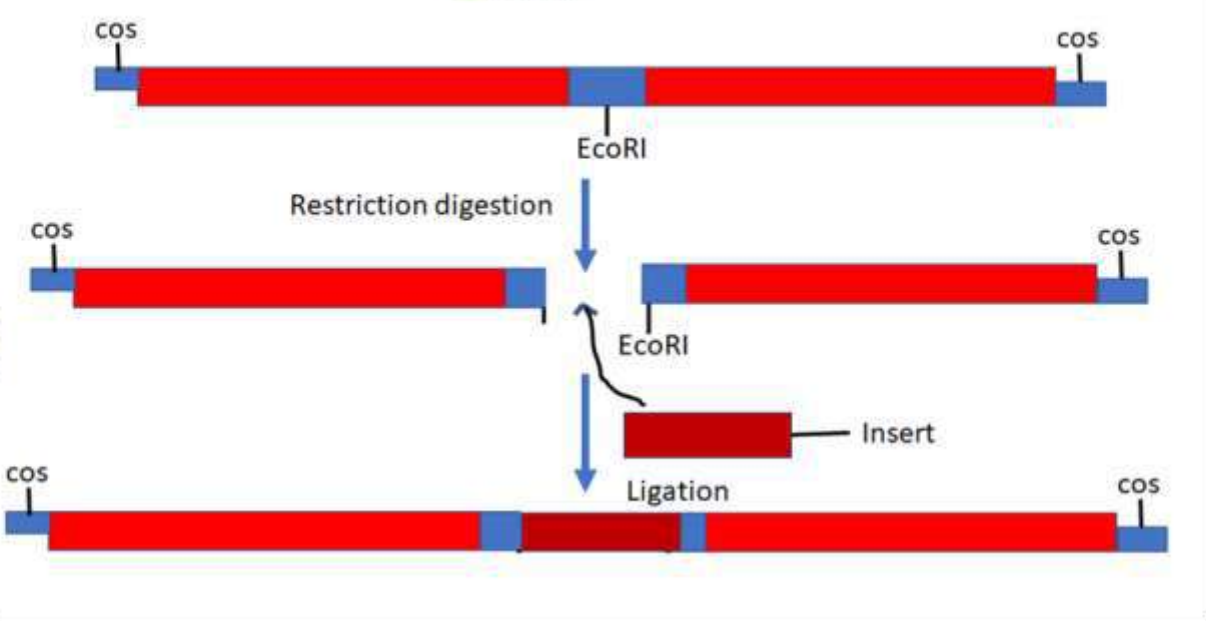


Fig. Map of the λ chromosome, showing the physical position of some
genes on the full-length DNA of wild-type bacteriophage λ.
Clusters of functionally related genes are indicated.

λ BACTERIOPHAGE

Insertion vectors:

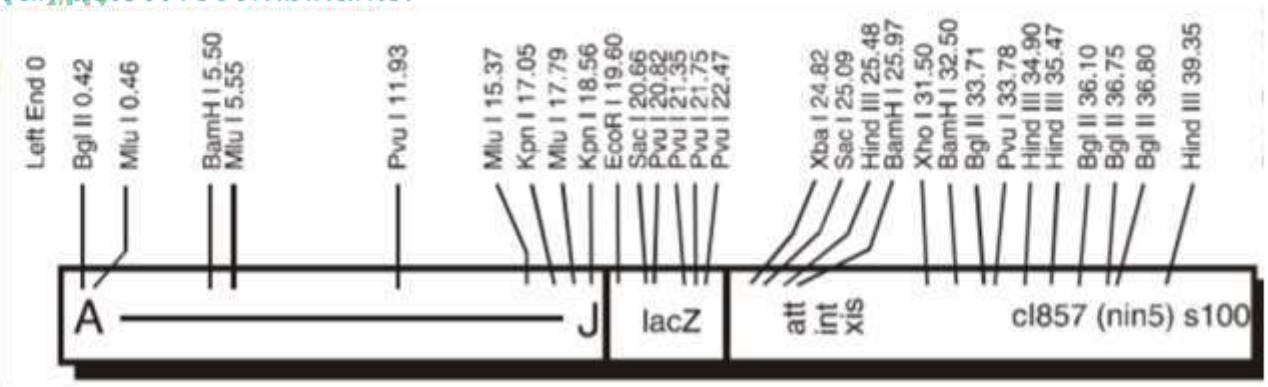
- Foreign DNA sequence is inserted into the λ genome without any significant change of the wild type genome.
- Can be used to clone smaller DNA molecule, Smaller insert size (upto ~10kb).
- They may contain a multiple cloning site inserted in *lacZ* system for screening of recombinant bacterial colonies. Eg: λ ZAP, λ gt etc.



Lambda gt11 VECTOR

- Lambda gt11 is widely used for cloning small inserts up to 7.2kb.
- The vector is a linear double stranded DNA that has a unique EcoRI site located near the carboxyl terminus of the *lacZ* gene in the phage repressor gene *cI*.
- Inserts cloned into the EcoRI site disrupt the *lacZ* gene, allowing for blue/white recombinant selection. Inserts can then be sequenced[2] and in-frame cloned segments can be expressed as β-galactosidase fusion proteins under control of the *lacZ* promoter.
- In order to clone cDNA, a linker with an EcoR I site is ligated to the phage. Libraries may be screened with nucleic acid probes.

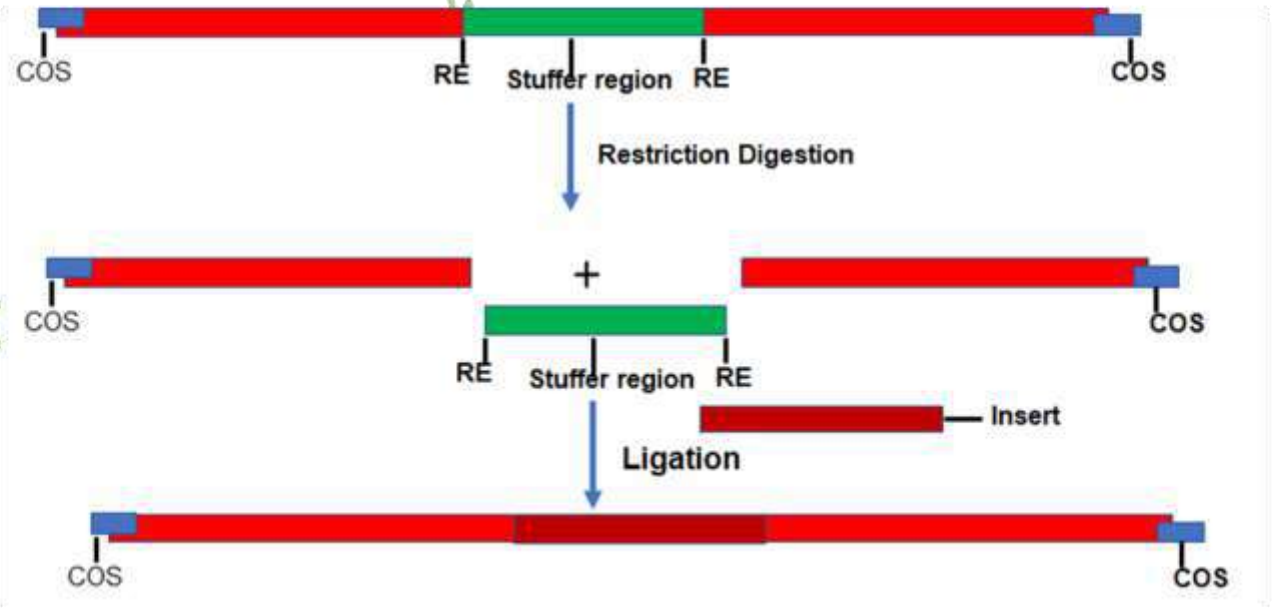
- cDNA library and genomic library construction.
- Cloning up to 7.2 kb.
- Repressor inactivation produces clear plaques for recombinant selection.
- Nucleic acid probes can detect recombinants.



λ BACTERIOPHAGE

Replacement vectors:

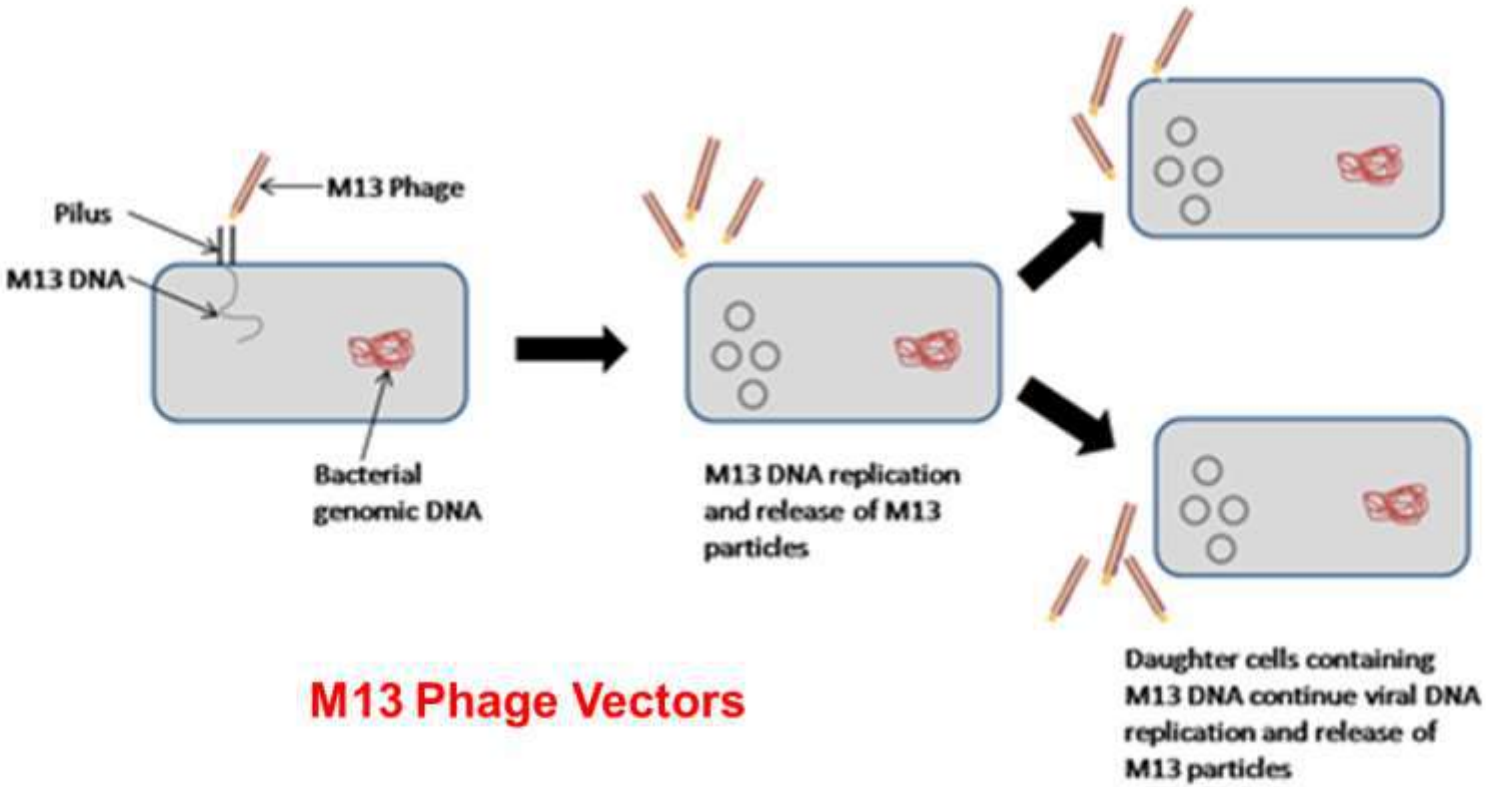
- Full length λ molecule having two identical restriction sites flanked by "stuffer fragment".
- Stuffer fragment is replaced by foreign DNA during restriction cloning.
- The vector without the foreign insert cannot be packaged due to the size limitation (smaller than the required).
- Insert size ranges between 10-23 kb.
- Eg. λ EMBL 3, λ EMBL 4, λ DASH etc.



M13 PHAGE VECTORS

- The filamentous phages only infect strains of enteric bacteria harboring F pili.
- M13 phage is filamentous phage that infects *E. coli* via F-pilus .
- The genome is a single stranded circular DNA of size ~6.4kb surrounded by a proteinaceous coat.
- Replication of phage DNA does not result in host-cell lysis.
- Up to 1000 phage particles may be released into the medium per cell per generation
- The DNA strand present in phage is called plus (+) strand. After entering to *E. coli* host, it converts into double stranded DNA molecule called **replicative form (RF)** by utilizing bacterial machinery.
- M13 phage as cloning vector can be obtained in both single stranded as well as double stranded form.
- Replicative form double stranded vector are modified and replicated inside *E. coli* host similar to a plasmid vector.

M13 PHAGE VECTORS

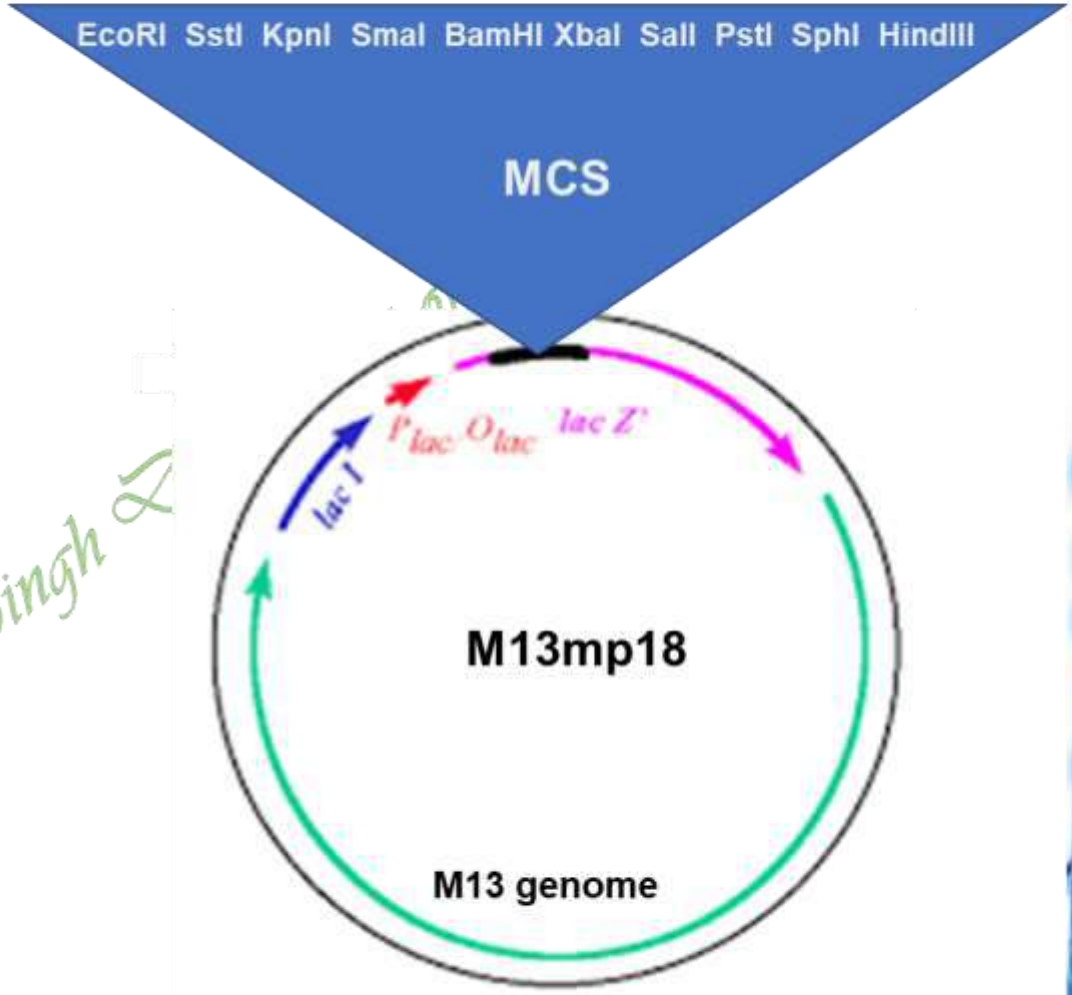


M13 Phage Vectors

M13 PHAGE VECTORS

Features and Benefits

- DNA cloning vectors
- Single stranded DNA serves as a sequencing template.



CHIMERIC VECTORS

- **Chimeric Vectors** are improved vectors with high insert capacity and transformation efficiency in comparison to plasmid and phage alone and many of them combine elements from both plasmids and phages.
- **Chimeric Vectors possess the properties of plasmid and phage both.**

Examples:

- **Cosmids**
- **Phagemids**
- **Fosmids**
- **PACs**
- **BACs**

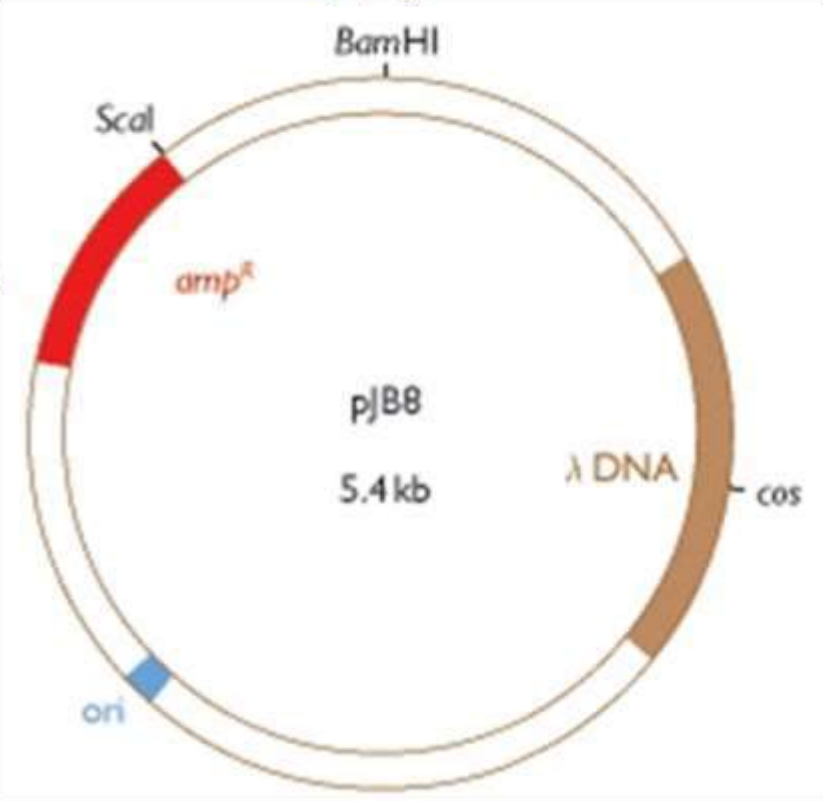
Cosmid

- Cosmids are vectors that are hybrids of λ phages and plasmids, and their DNA can replicate in the cell like that of a plasmid or be packaged like that of a phage.
- A cosmid, first described by **Collins** and **Hohn** in 1978, is a type of hybrid plasmid with a bacterial "ori" sequence and a " *cos"* sequences derived from the lambda phage.
- *Cos* site is the sequence required by a DNA molecule in order to be recognized as a 'λ genome' by the proteins that package DNA into λ phage particles.
- Cosmid DNA containing particles are as transmittable as real λ phages, but once inside the cell, the cosmid cannot control synthesis of new phage particles and instead replicates as a plasmid.
- **New DNA insert of size upto 44 kb can be inserted.**

Cosmid

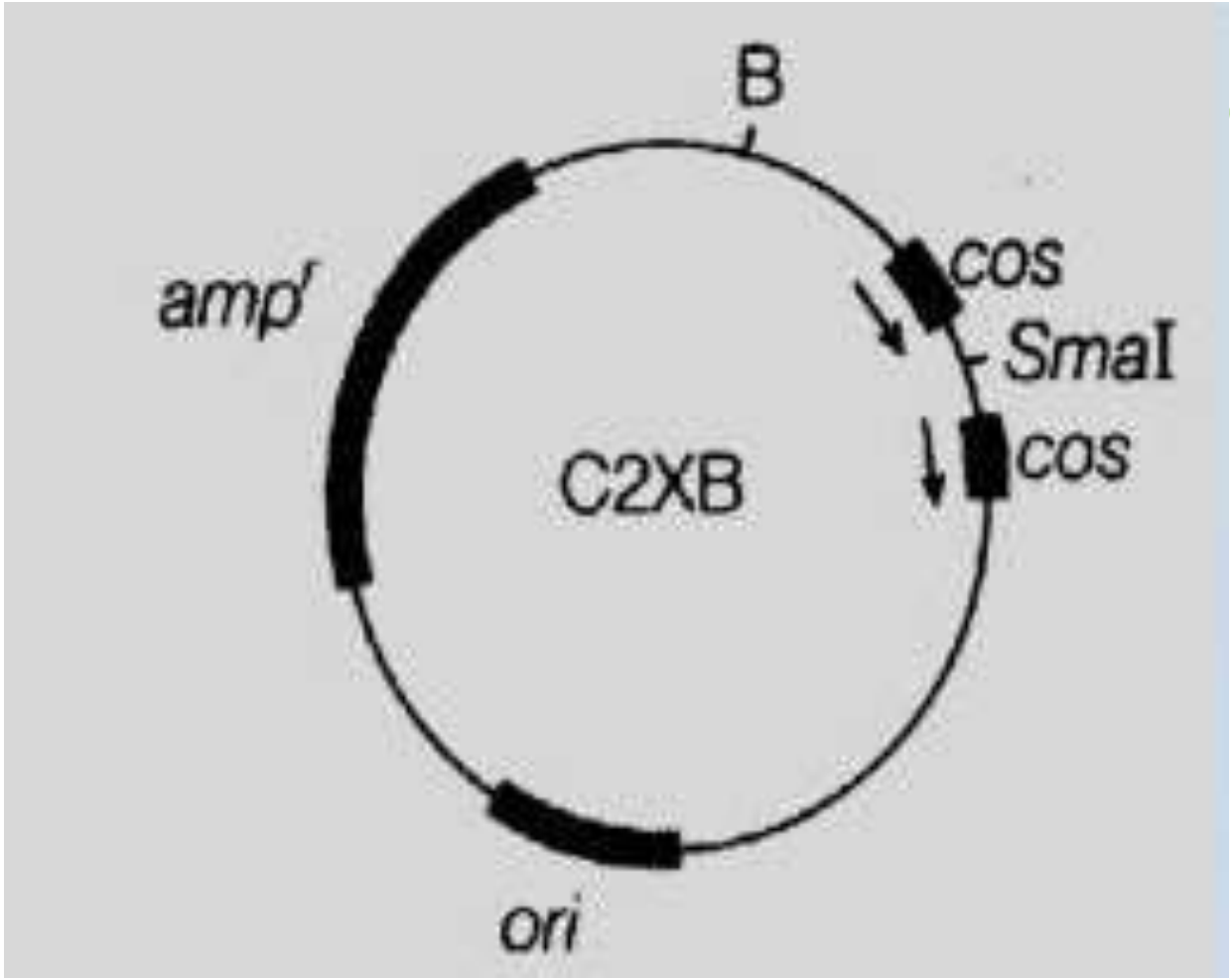
pJB8 is 5.4 kb in size and carries the ampicillin-resistance gene (amp^R), a segment of λ DNA containing the *cos* site, and an *Escherichia coli* origin of replication (ori)

- However, cosmids can carry DNA inserts about three times as large as those carried by λ itself (as large as about 45 kb).



Schematic representation of pJB8

C2XB Cosmid Cloning Scheme

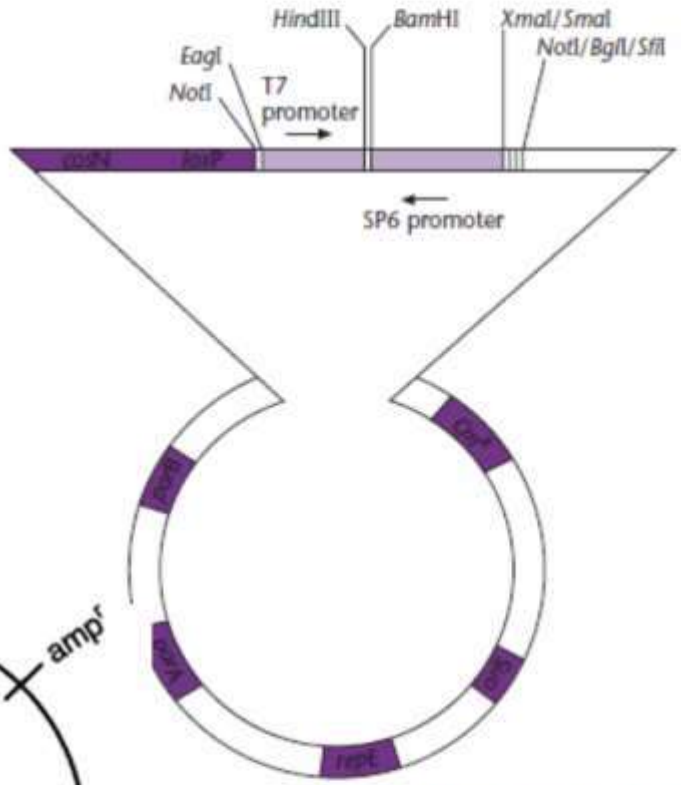


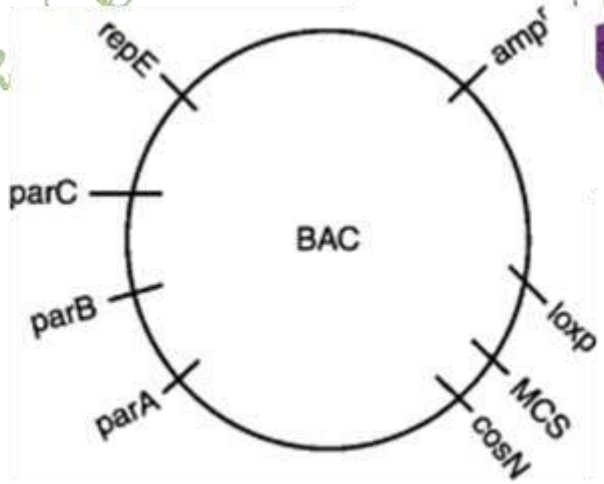
BAC system

- Shizuya *et al.* (1992) have developed a bacterial cloning system for mapping and analysis of complex genomes.
- This BAC system (bacterial artificial chromosome) is based on the single-copy sex factor F of *E. coli*.
- This vector includes the λ *cos* N and P1 *lox*P sites, two cloning sites (*Hin*dIII and *Bam*HI), and several G+C restriction enzyme sites (e.g. *Sfi*I, *Not*I, etc.) for potential excision of the inserts.
- The cloning site is also flanked by T7 and SP6 promoters for generating RNA probes.
- This BAC can be transformed into *E. coli* very efficiently, thus avoiding the packaging extracts that are required with the P1 system.
- BACs are used to construct genome libraries with an average insert size of 125 kb
- They are capable of maintaining genomic fragments of greater than 300 kb for over 100 generations with a high degree of stability

Structure Of A BAC Vector

- a BAC vector derived from a mini- F plasmid.
- The *ori*S and *rep*E genes mediate the unidirectional
- replication of the F factor, while *par*A and *par*B maintain the copy number at a level of one or two per genome. Cm*R* is a chloramphenicol-resistance marker.
- *Cos*N and *lox*P are the cleavage sites for λ terminase and P1 *cre* protein, respectively.
- *Hind*III and *Bam*HI are unique cleavage sites for inserting foreign DNA.





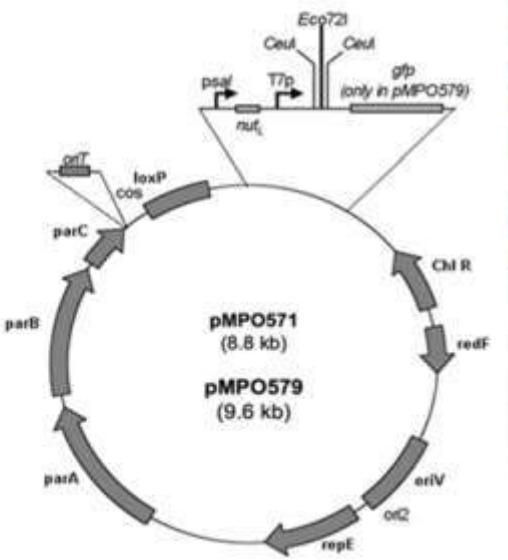
(Adapted from Shizuya *et al*. 1992.)

Table Maximum DNA insert possible with different cloning vectors

Vector	Host	Insert size
λ phage	*E. coli*	5–25 kb
cosmids	*E. coli*	35–45 kb
P1 phage	*E. coli*	70–100 kb
PACs	*E. coli*	100–300 kb
BACs	*E. coli*	300 kb
YACs	*Saccharomyces cerevisiae*	200–2000 kb

Fosmid

- Fosmids are similar to cosmids, however they are primarily based on bacterial F-plasmid, first developed by **Simon and co-workers**, in the year 1992,
- They carry the F plasmid origin of replication and a λ *cos* site, can carry up to 40 kb of insert DNA.
- The cloning vector is limited, as a host (usually *E. coli*) can only contain one fosmid molecule. Low copy number offers higher stability as compared to high copy number cosmids.
- Fosmids have high structural stability and have been found to maintain human DNA effectively even after 100 generations of growth. It is ideal to use a fosmid vectors for constructing genomic and meta-genomic libraries.

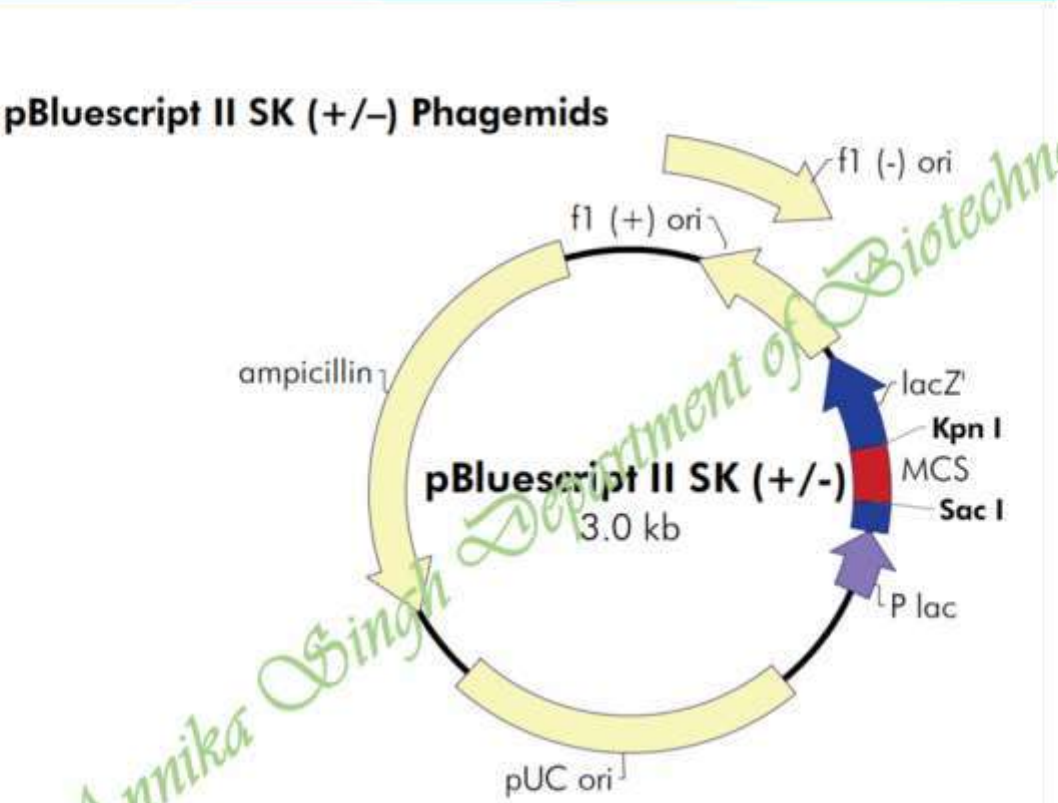


Phagemid vectors

Phagemid

- Phagemids are cloning vectors developed as a hybrid of the filamentous phage M13 and plasmids to produce a vector that can get packed as a phage particle but also can propagate as a plasmid.
- The components present in a phagemid vector are:
  - Origin of replication (ori) of a plasmid.
  - Intergenic region (IG region) which contains the packaging signal for the phage particle and also has replication origin of M13 phage.
  - A gene encoding phage coat protein.
  - A selection marker.
  - Restriction enzyme recognition sites.

Phagemid vectors



- The pBluescript II phagemids (plasmids with a phage origin) are cloning vectors designed to simplify commonly used cloning and sequencing procedures, including the construction of nested deletions for DNA sequencing, generation of RNA transcripts in vitro and site-specific mutagenesis and gene mapping.

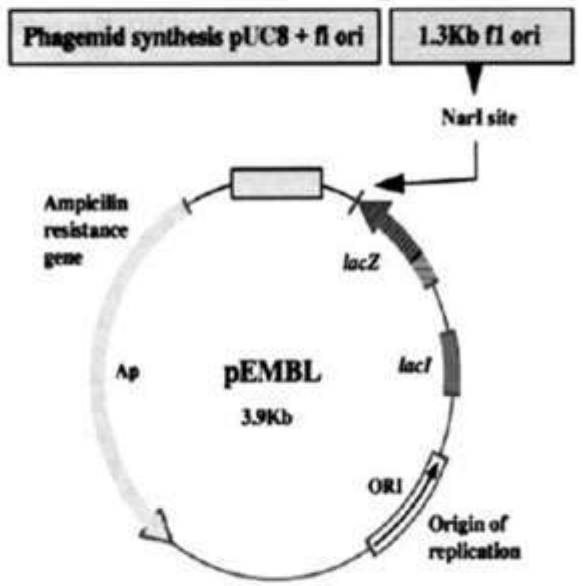
Phagemid vectors

pEMBL

One of the first hybrid phagmid vectors was pEMBL constructed in 1983. They are characterized by the presence of –

1) The *bla* gene as selectable marker for ampicillin resistance.
2) A short segment coding for the alpha-peptide of beta-galactosidase (lacZ) and containing a MCS.
3) The intragenic (IG) region of phage F1.

- These vectors have been used successfully for DNA sequencing with the dideoxy method.
- However, the pEMBL plasmids have the advantage of being smaller than M13 vectors, and the purification of DNA is simpler.
- In addition, long inserts have a higher stability in pEMBL plasmids than M13 vector.



RECOMBINANT DNA TECHNOLOGY

Yeast Plasmid Vectors

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Yeast Cloning Vectors

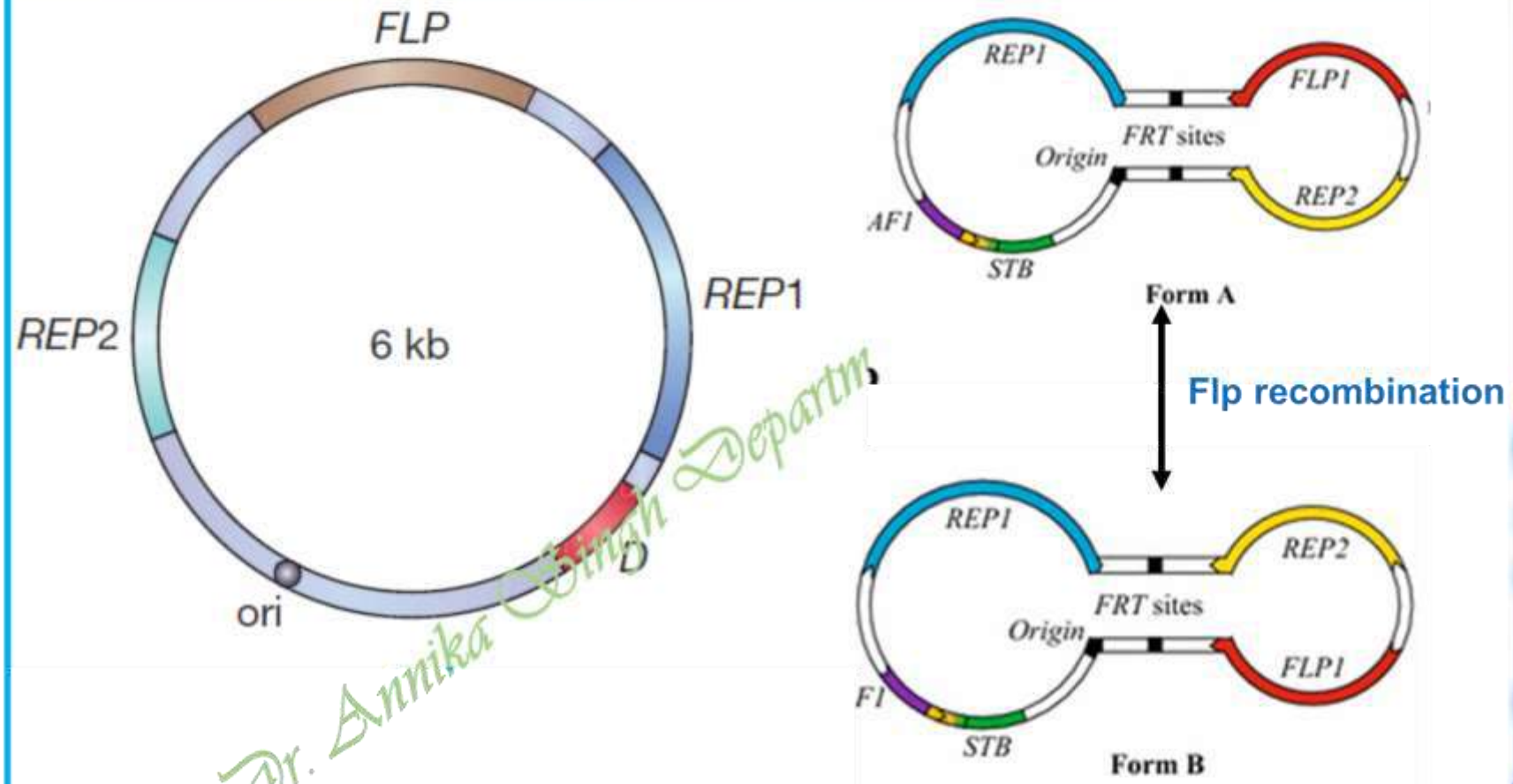
Why yeast vectors?

- Yeast are eukaryotes and thus contain complex internal cell structures similar to those of plants and animals.
- Unlike bacteria, yeast can post-translationally modify proteins .
- Yeast have rapid growth, ease of replica plating and mutant isolation, a well-defined genetic system, and a highly versatile DNA transformation system.
- Yeast have both a stable haploid and diploid state which is useful for genetic analysis, as well as an efficient mechanism of homologous recombination to facilitate simple gene replacement/mutation.
- Yeast expression plasmids should contain all the necessary components to allow shuttling between *E. coli* and yeast cells.
- the vectors must contain a yeast-specific origin of replication (ORI) and a means of selection in yeast cells, in addition to the bacterial ORI and antibiotic selection markers.

2 µm Plasmid

- The 2 µm plasmid is an excellent basis for a cloning vector.
- It is 6 kb in size, which is ideal for a vector, and exists in the yeast cell at a copy number of between 70 and 200.
- Replication makes use of a plasmid origin, several enzymes provided by the host cell, and the proteins coded by the *REP1* and *REP2* genes carried by the plasmid.
- a normal yeast gene is used as selectable marker gene , generally one that codes for an enzyme involved in amino acid biosynthesis.
- An example is the gene *LEU2*, which codes for β-isopropylmalate dehydrogenase, one of the enzymes involved in the conversion of pyruvic acid to leucine.
- The host must be an auxotrophic mutant that has a non-functional *LEU2* gene.
- Selection is possible because transformants contain a plasmid-borne copy of the *LEU2* gene, and so are able to grow in the absence of the amino acid (minimal medium)
- Only transformed cells are able to survive and form colonies.

2 µm Plasmid



FRT- Flp recognition targets; STB also known as REP3, partitioning locus

REP1 and *REP2* are involved in replication of the plasmid, and *FLP* codes for a protein that can convert the A form of the plasmid (shown here) to the B form, in which the gene order has been rearranged by intramolecular recombination.

Yeast Selectable Marker Genes

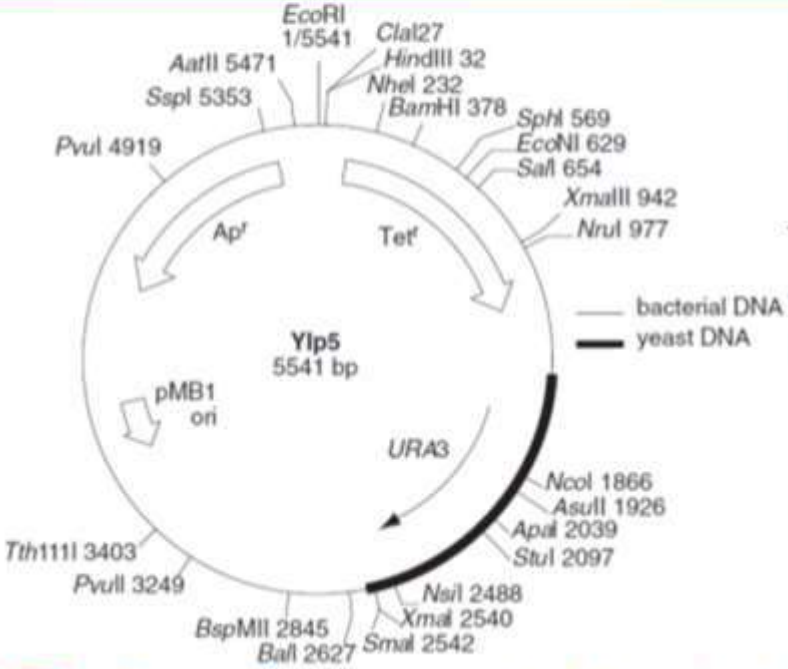
- **URA3** is a gene on chromosome V in *Saccharomyces cerevisiae*(yeast)
- **URA3** is often used as a "marker gene", that is, a gene to label chromosomes or plasmids.
- **URA3** encodes Orotidine 5'-phosphate decarboxylase **(ODCase)**, which is an enzyme that catalyzes one reaction in the synthesis of pyrimidine ribonucleotides
- ***LEU2,*** which codes for β-isopropylmalate dehydrogenase, one of the enzymes involved in the conversion of pyruvic acid to leucine.
- ***TRP1*** gene, which is involved in tryptophan biosynthesis codes for Phosphoribosylanthranilate isomerase; catalyzes the third step in tryptophan biosynthesis
- **SUP4** tRNATyr gene is stimulated by tyrosyl tRNA synthetase (TyrRs) Tyrosine tRNA (tRNA-Tyr), can mutate to suppress ochre nonsense mutations SUPpressor
- ***GAL2*** Galactose uptake by yeast cells is via a permease encoded by the *GAL2* gene.

Yeast Plasmid Vectors

The four main types of yeast plasmids are defined below:

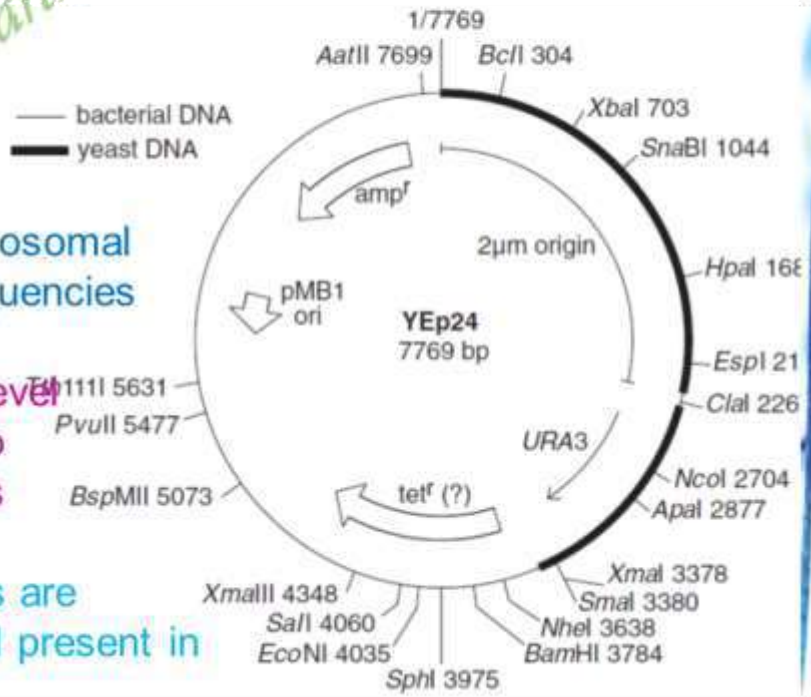
- ***Yeast Integrating plasmids (Ylp)***: These plasmids lack an ORI and must be integrated directly into the host chromosome via homologous recombination.
- ***Yeast Episomal plasmids (YEp)***: These are most similar to bacterial plasmids and are considered "high copy". A fragment from the 2 micron circle (a natural yeast plasmid) allow for 50+ copies to stably propogate per cell. The copy number of these vectors can also be controlled if specific regulatable elements are included
- ***Yeast Replicating plasmids (YRp)***: These vectors contain an Autonomously Replicating Sequence (ARS) derived from the yeast chromosome. As the name suggests, these vectors can replicate independently of the yeast chromosome; however, they tend to be unstable and may be lost during budding.
- ***Yeast Centromere plasmids (YCp)***: These are considered low copy vectors and incorporate part of an ARS along with part of a centromere sequence (CEN). These vectors replicate as though they are small independent chromosomes and are thus typically found as a single copy. Unlike the ARS vectors, CEN vectors are stable without integration.

Yeast Plasmid Vectors



Yeast Integrating plasmids (YIp): These plasmids lack an ORI and must be integrated directly into the host chromosome via homologous recombination.

YEp vectors (yeast episomal plasmids), contain sequences from a naturally occurring yeast plasmid called the 2µm circle.



YEp : These 2µm sequences allow extrachromosomal replication and confer high transformation frequencies (~10^4 to 10^5 transformants/µg DNA).

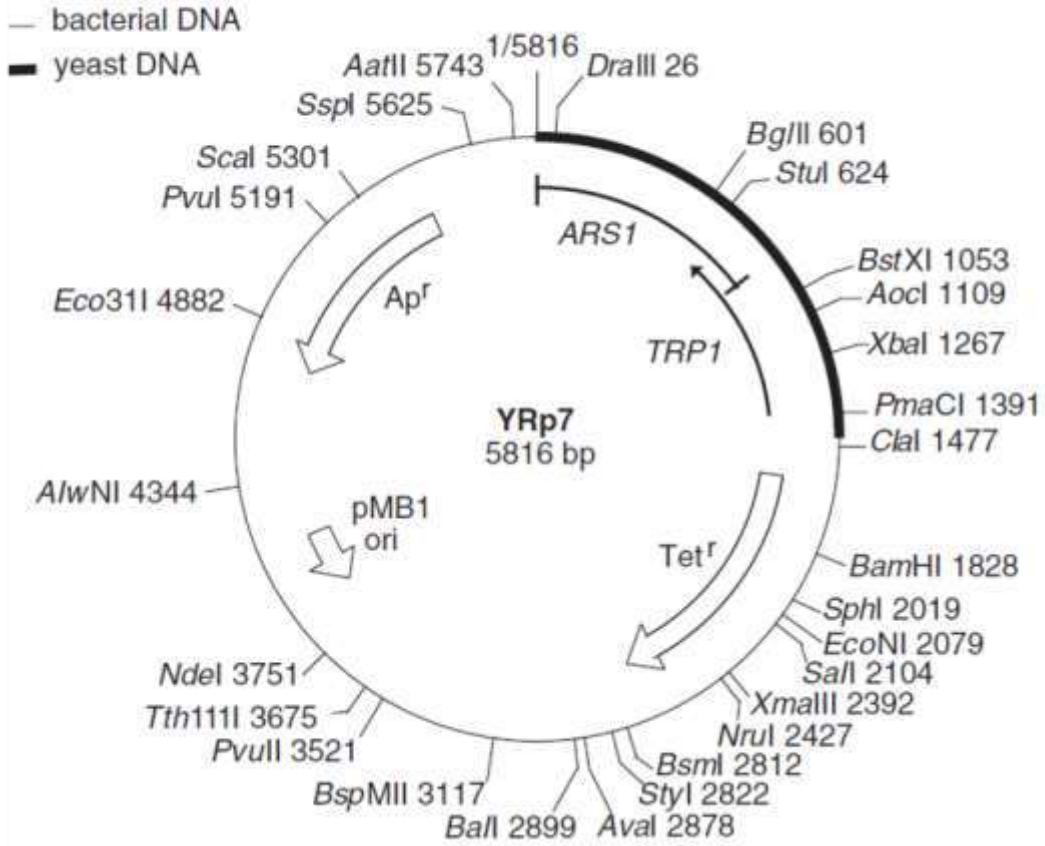
These plasmids are commonly used for high-level gene expression in yeast, due to their ability to be propagated relatively stably through mitosis and meiosis in high copy number.

If the 2µm-encoded *REP1* and *REP2* functions are present transformants are relatively stable and present in high

Yeast Plasmid Vectors

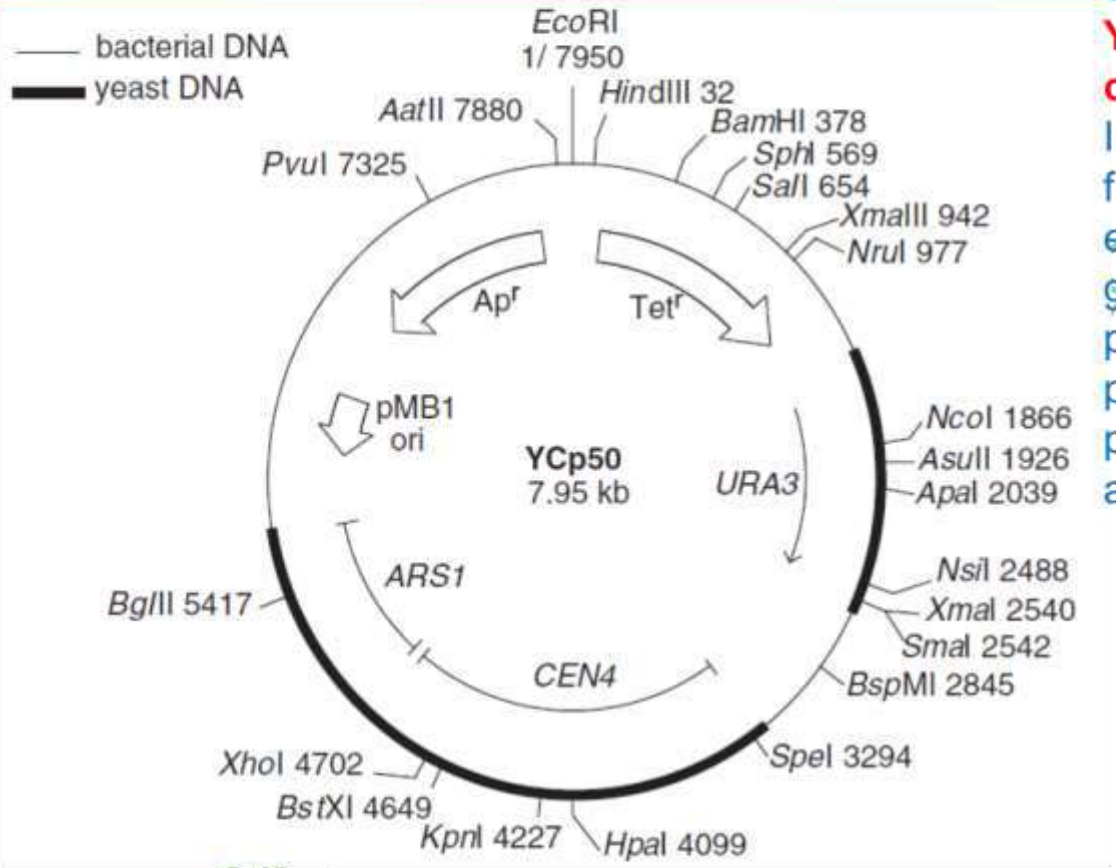
YRp plasmids

- These vectors contain an Autonomously Replicating Sequence (ARS) derived from the yeast chromosome
- have high frequencies of transformation (10^3 to 10^4 transformants/µg DNA), but transformants are very unstable both mitotically and meiotically.
- Despite the fact that ***ARS*-containing** plasmids replicate only once during the cell cycle,



- YRp plasmids can be present in high copy number (up to 100 copies per plasmid-bearing cell, although the average copy number per cell is 1 to 10).

Yeast Plasmid Vectors



YCp plasmids (yeast centromeric plasmids)

Incorporation of DNA segments from yeast centromeres (*CEN* elements) into YRp plasmids, to generate vectors called YCp plasmids (yeast centromeric plasmids), greatly increases plasmid stability during mitosis and meiosis.

- Such plasmids—present in 1 to 2 copies per cell—have a loss rate of approximately 1% per generation and show virtually no segregation bias.
- During meiosis, *CEN* plasmids behave like natural chromosomes, generally segregating in a $2^{+}:2^{-}$ ratio.

Yeast Artificial Chromosomes: YAC

- First described in 1983 by **Murray** and **Szostak**, a yeast artificial chromosome has sequences to exist inside *E. coli* as a circular plasmid and contains sequences to maintain as linear nuclear chromosome in yeast.
- As YAC vectors can accommodate **100-500 kb** of insert DNA. The number of clones in a genomic library can be greatly reduced.
- YAC vectors have following elements:
  - ***E. coli* origin of replication**
  - **Yeast origin of replication**
  - **Elements of eukaryotic yeast chromosome (centromere and telomere region)**
  - **Selection markers for both the host.**
- YAC vector is initially propagated as circular plasmid inside bacterial host utilizing bacterial *ori* sequence.

Circular plasmid is cut at specific site using restriction enzymes to generate a linear chromosome with two telomere sites at terminals.

The linear chromosome is again digested at specific site with two arms with different selection marker.

Yeast Plasmid Vectors