Cloning Vectors

Vectors-the carriers of foreign DNA for cloning

1. Genetic engineering - Neelam Pathak
2. Gene Cloning and DNA Analysis (6th Edn) - T.A. Brown

CLONING VECTORS

Vectors are DNA molecules that act as vehicles for carrying a foreign DNA fragment when inserted into a host cell.

A cloning vector is a DNA molecule in which foreign DNA can be inserted or integrated and which is further capable of replicating within host cell to produce multiple clones of recombinant DNA.

A vector can be used for cloning to get DNA copies of the fragment or to obtain expression of the cloned gene (to get RNA or Protein)

Characteristics/ Necessary Properties of a Vector

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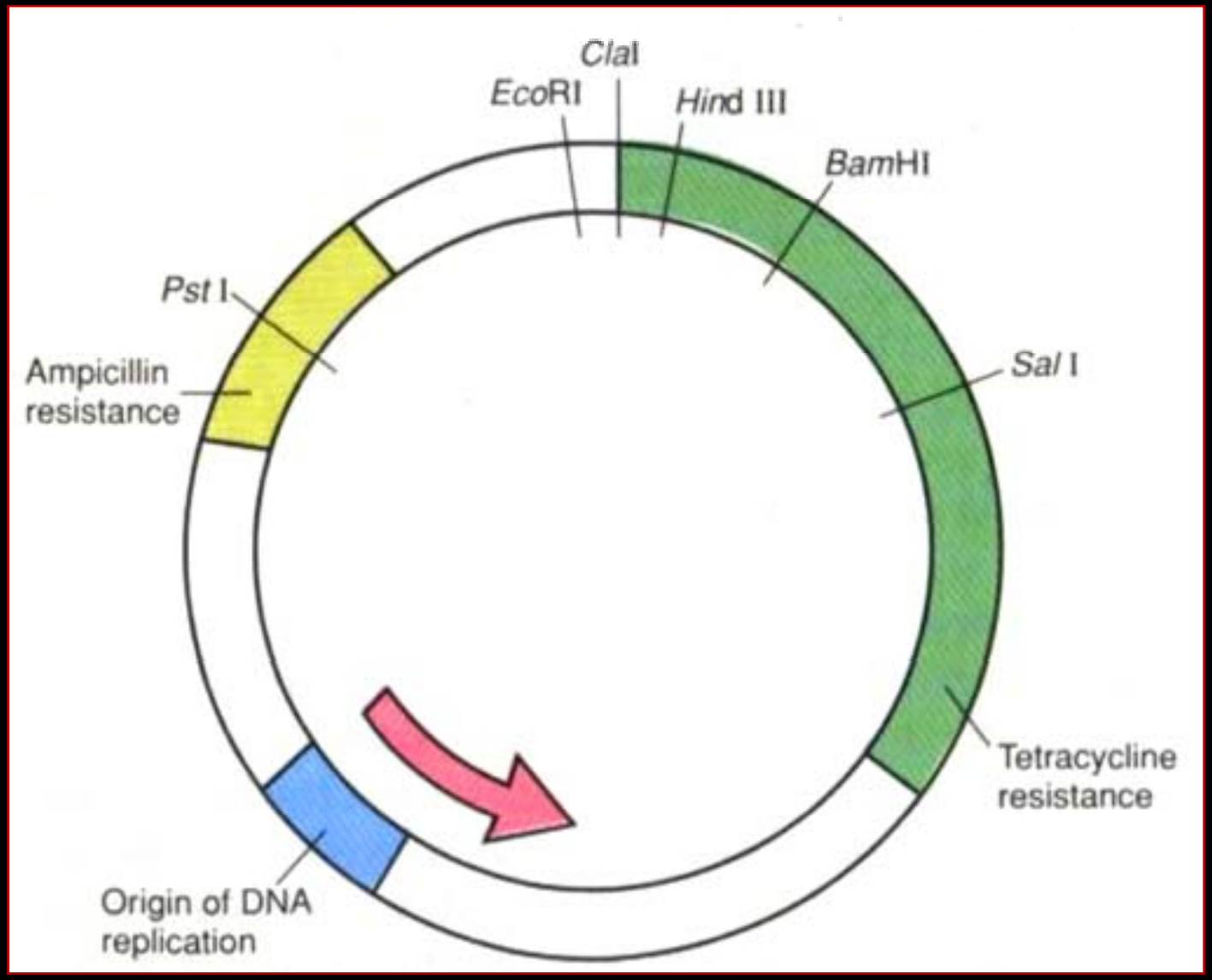
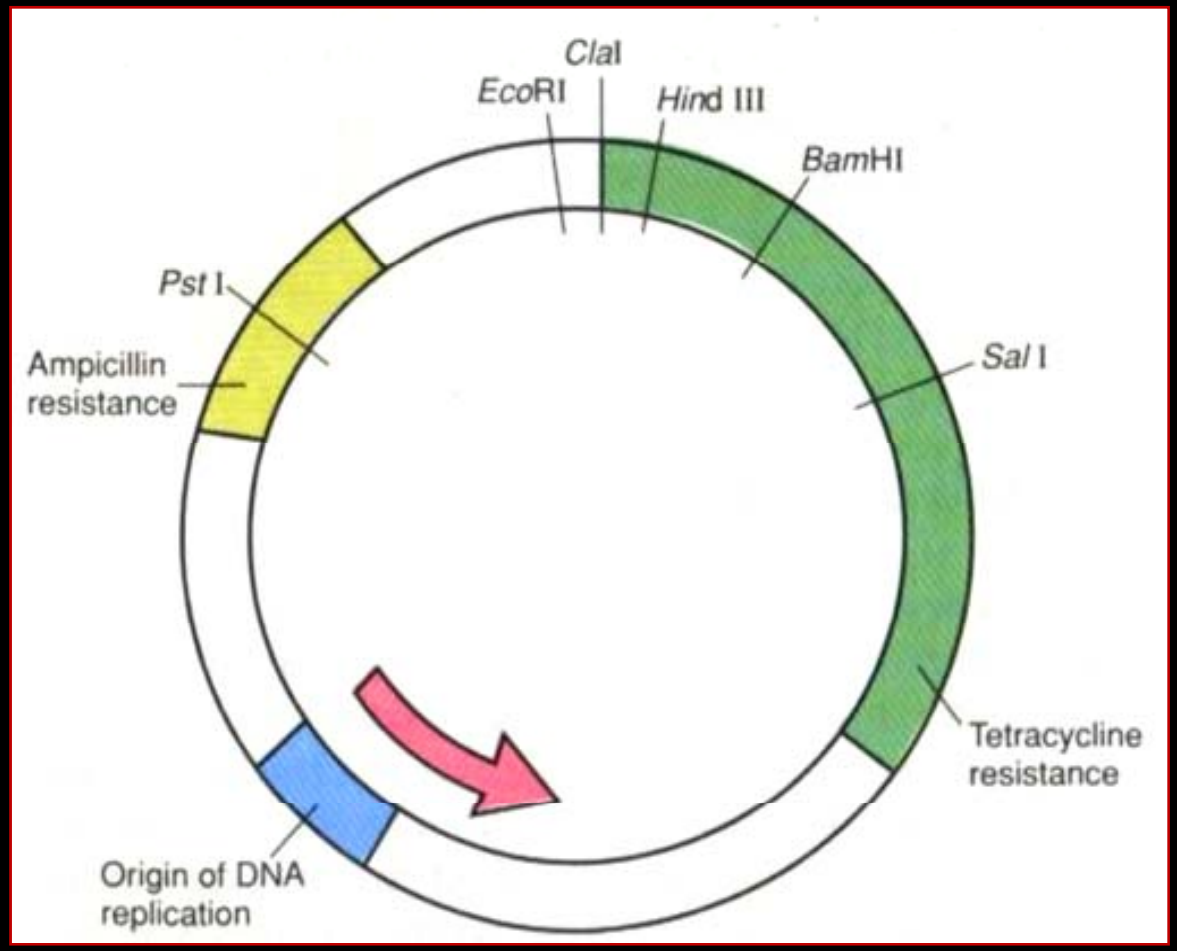
1. Capability of autonomous replication: vector should contain an origin of replication (ori) to enable independent replication using the host cell's machinery.

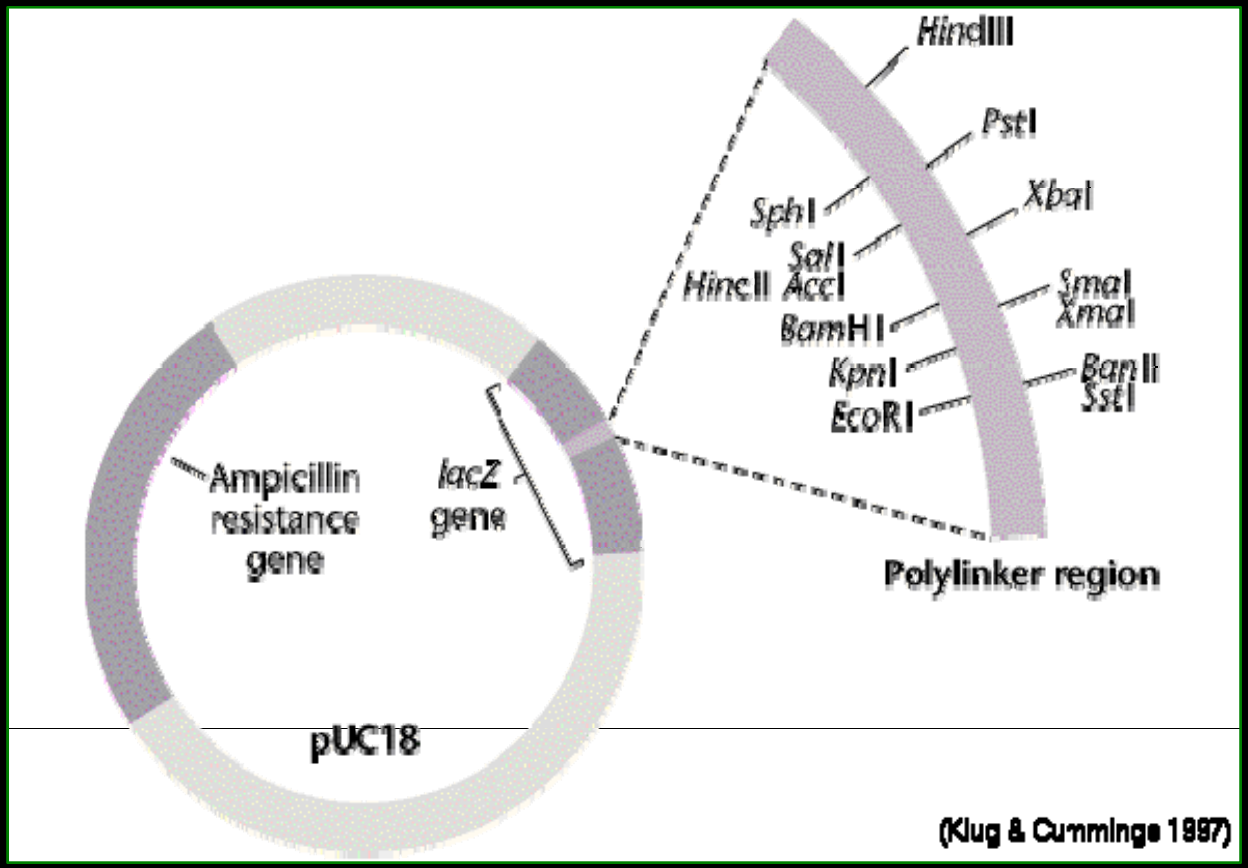
2. Small size: a cloning vehicle needs to be reasonably small in size and manageable. Large molecules tend to degrade during purification and are difficult to manipulate.


ClaI
EcoRI
Hind III
BamHI
Pst I
Ampicillin
resistance
Sal I
Tetracycline
resistance
Origin of DNA
replication

3. Presence of selectable marker genes: a cloning vector should have selectable marker gene. This gene permits the selection of host cells which bear recombinant DNA from those which do not bear rDNA. Eg. *Amp^R Tet^R, Neo^R, LacZ* genes.



4. Presence of unique restriction sites: It should have restriction sites, to allow cleavage of specific sequence by specific Restriction endonuclease. Unique restriction sites should be present on the vector DNA molecule either individually or as cluster (MCS- multiple cloning sites or Polylinker).

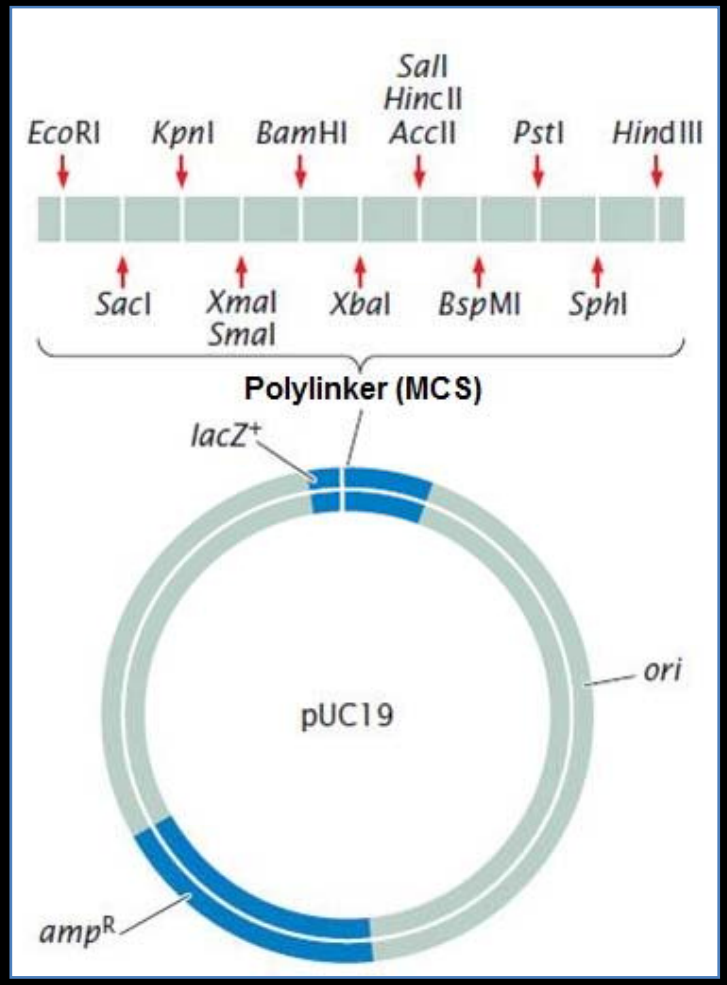


Polylinker or Multiple Cloning Site (MCS)

Since there are many different restriction enzymes, it would be convenient to have a wide range of restriction recognition sites in the vector; solved by inserting a polylinker into the vector.

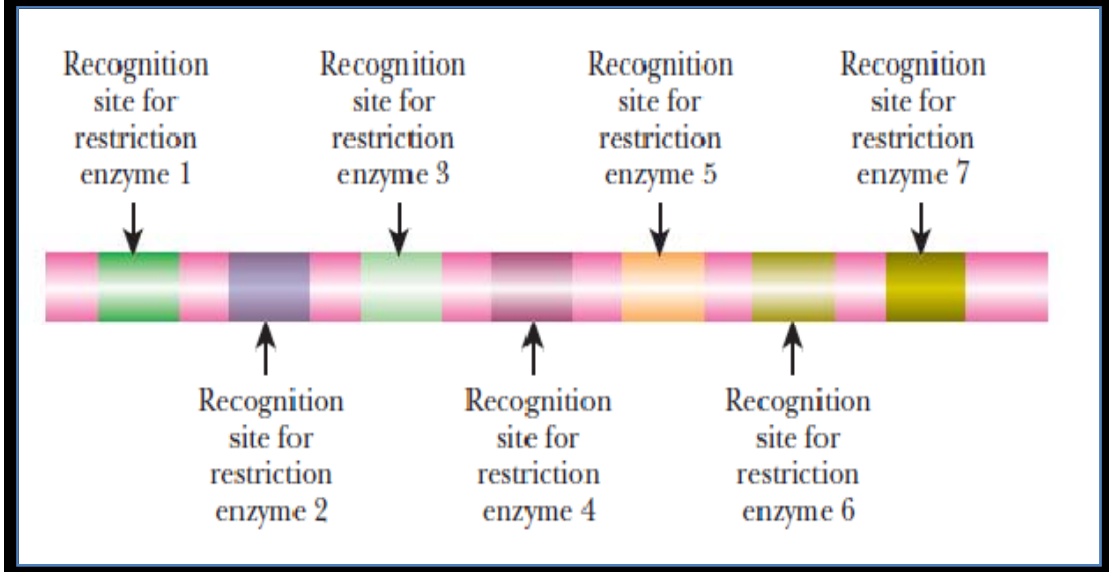
MCS is a stretch of artificially synthesized DNA, about 50 base pairs long, which contains cut sites for several (ususlly seven or eight) restriction enzymes.

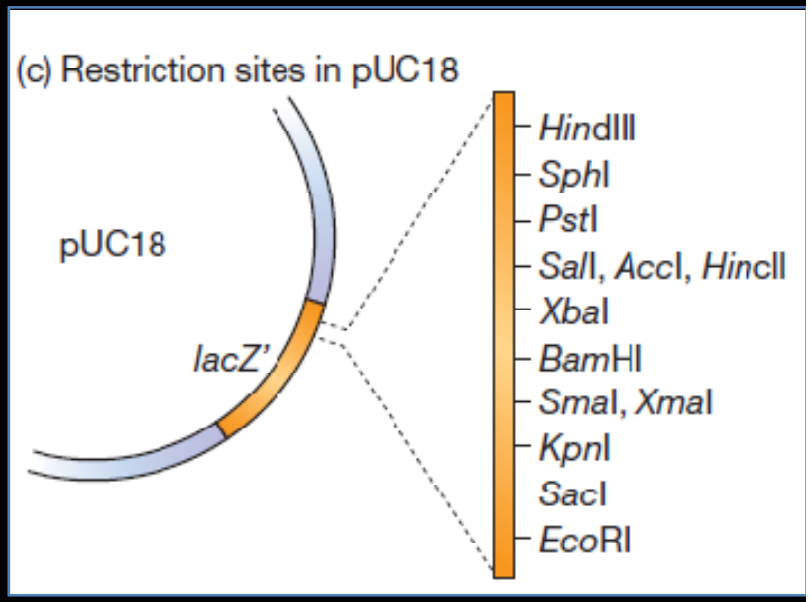
This allows a wide choice of restriction enzymes



Many plasmid vectors contain an artificial region of DNA that has many different restriction enzyme sites.

Such polylinkers site is designed so that all the restriction enzyme sites in the polylinker are unique, and the corresponding enzymes only cut the plasmid once.





5. **Ease of purification:** it should be easy to purify and handle.

6. **Minimum amount of nonessential DNA**

Types of Cloning Vectors

- ✓ Different types of cloning vectors are used for different types of cloning experiments (amplification/ expression)
- ✓ The vector is chosen according to the size and type of DNA to be cloned (smaller /larger fragments)
- ✓ Also depends on the host cell type used (prokaryotic/ eukaryotic)

PLASMID as CLONING VECTOR

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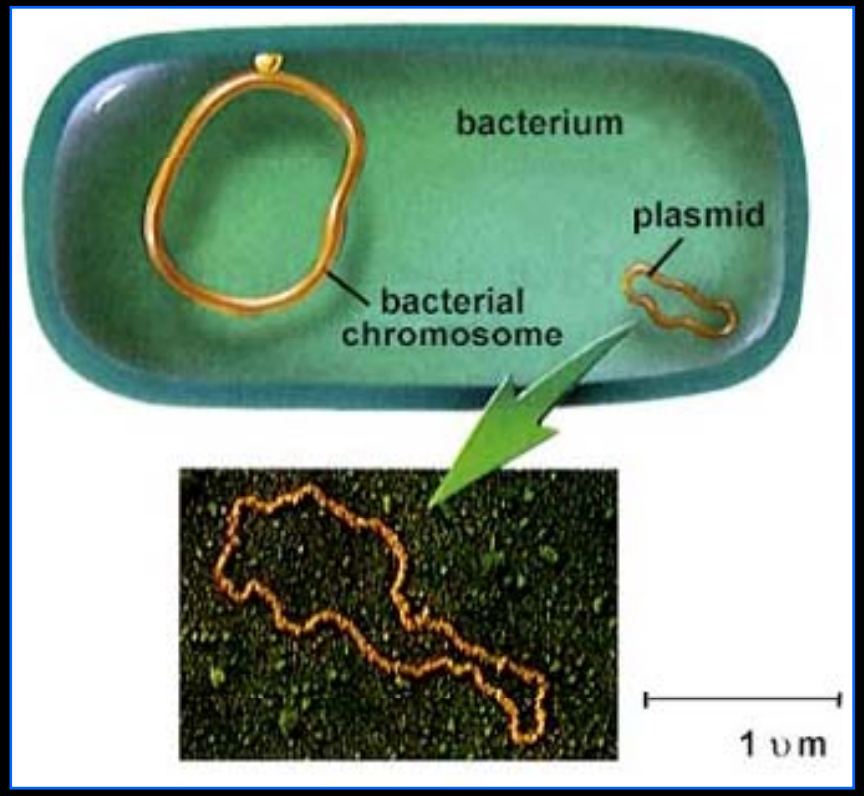
3. Genomes(3rd Edn) - T.A. Brown
4. Principles of gene manipulation and genomics -Primrose

Plasmids are self-replicating, double-stranded, circular DNA molecules that are maintained in bacteria as independent extra chromosomal entities.

Virtually all bacterial genera have natural plasmids.



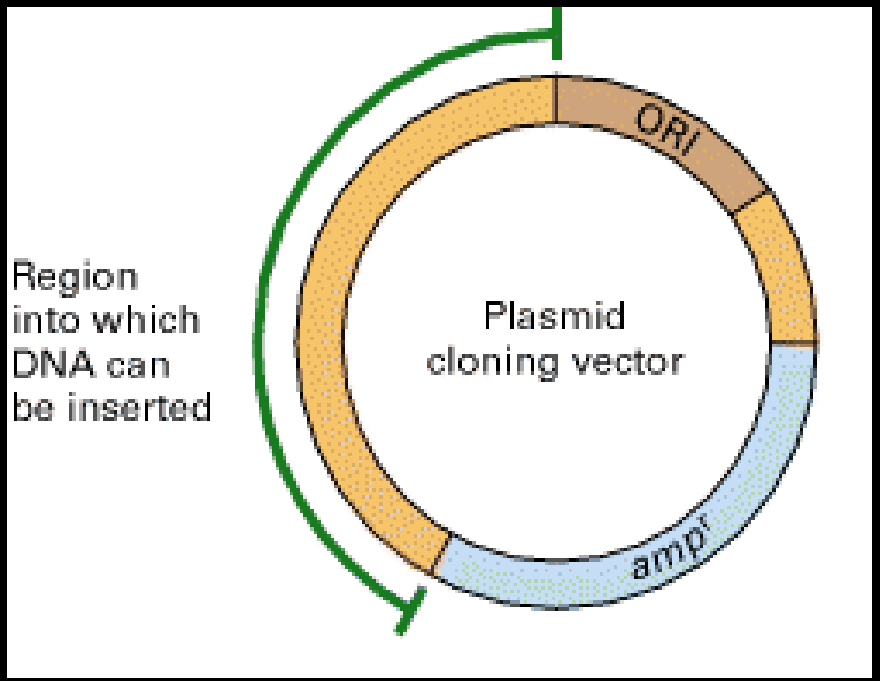
- ✓ Although they are not typically essential for bacterial survival, plasmids often carry genes that are advantageous under particular conditions.
- ✓ Plasmids can range in size from less than 1 kb to more than 500 kb.
- ✓ Each plasmid has an origin of replication to replicate inside the host cell
- ✓ plasmids are represented by 10 to 100 copies per host.
- ✓ Plasmid can carry DNA fragments as large as 10 kb.

The simplest cloning vectors, and the ones most widely used in gene cloning, are those based on small bacterial plasmids.

Plasmid has the ability to clone reasonably large (about 100 bp-10 kbp) pieces of foreign DNA.

Initially naturally occurring plasmids were used as vectors.

Naturally occurring plasmids:
pSC101, pSF2124
Col E1, etc



However, naturally occurring (unmodified or nonengineered) plasmids often lack several important features that are required for a high-quality cloning vector.

Therefore naturally occurring plasmid cloning vectors have to be genetically modified.

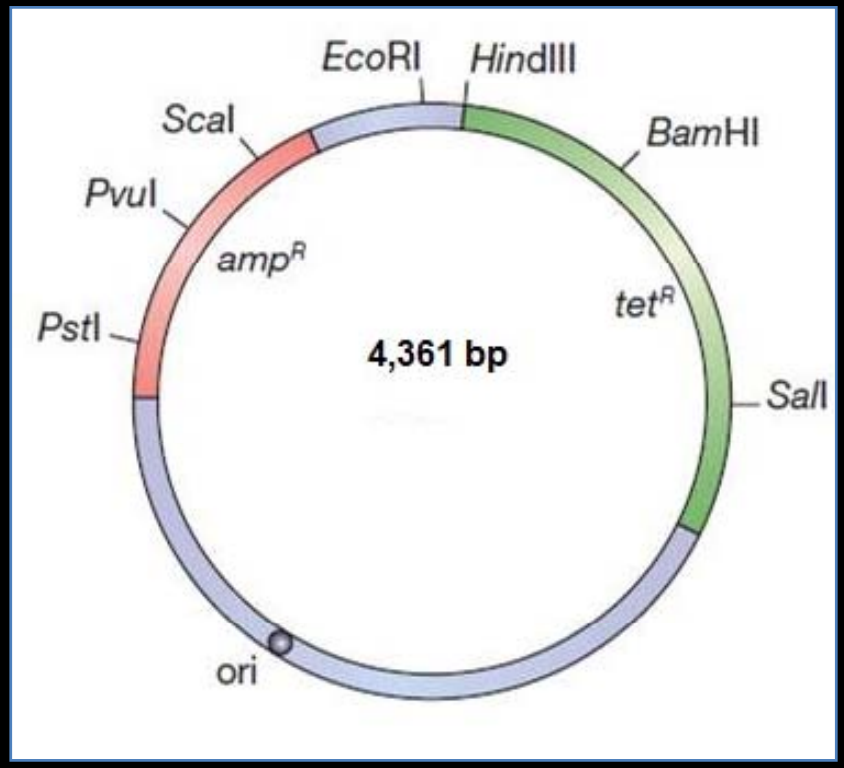
Modifications include;

- ✓ The size of plasmids are reduced
- ✓ Nonessential and toxin producing genes are deleted.
- ✓ Selectable marker genes are inserted (eg. antibiotic resistance genes)
- ✓ Addition of RE sites (MCS or Polylinker)

Eg: pBR322, pBR327, pUC8, pUC18, pUC19, etc.

pBR322

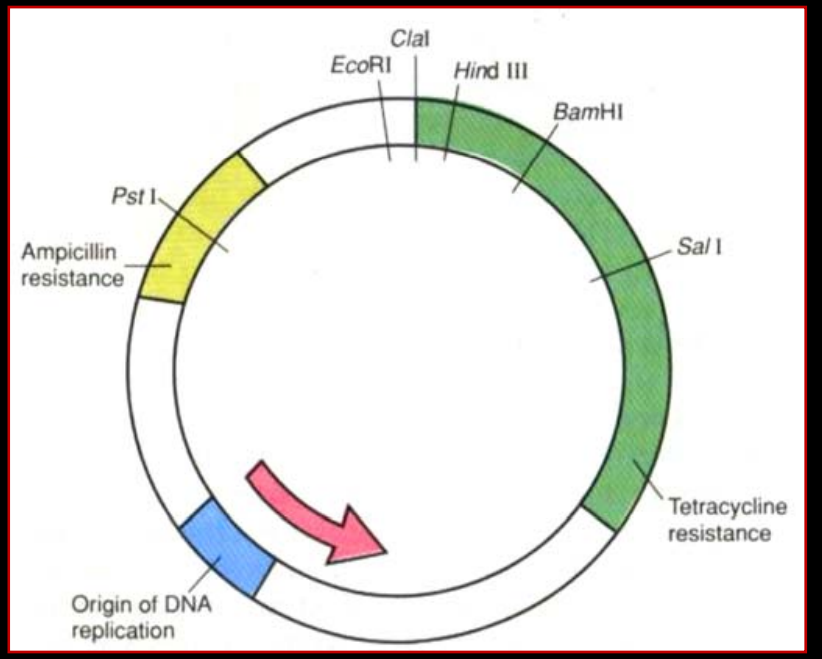
- ✓ vector was designed by Bolivar and Rodrigus in 1977
- ✓ pBR322 is 4,361 bp in size
- ✓ The name "pBR322" indicates
- ✓ lowercase "p"- for plasmid
- ✓ "BR" Bolivar and Rodriguez, who developed the plasmid
- ✓ 322 is a serial number

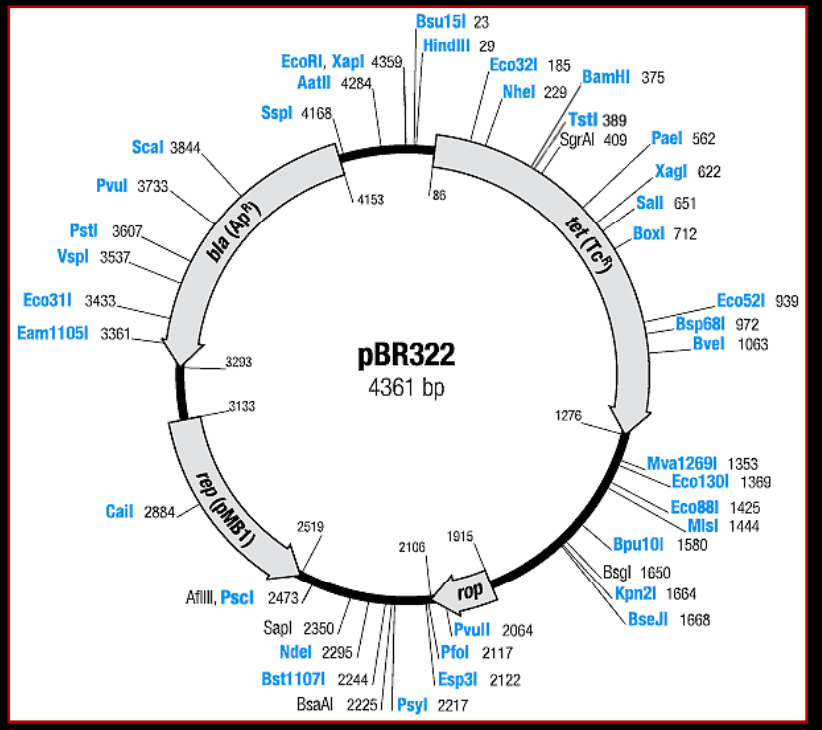


Useful properties:

1. **Small size** = <10 kb

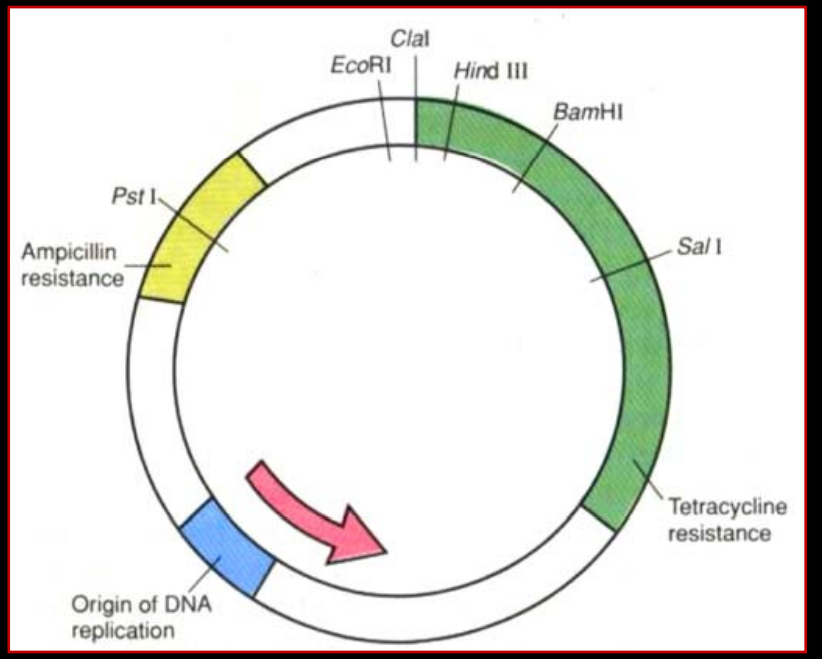
Easy to purify and handle

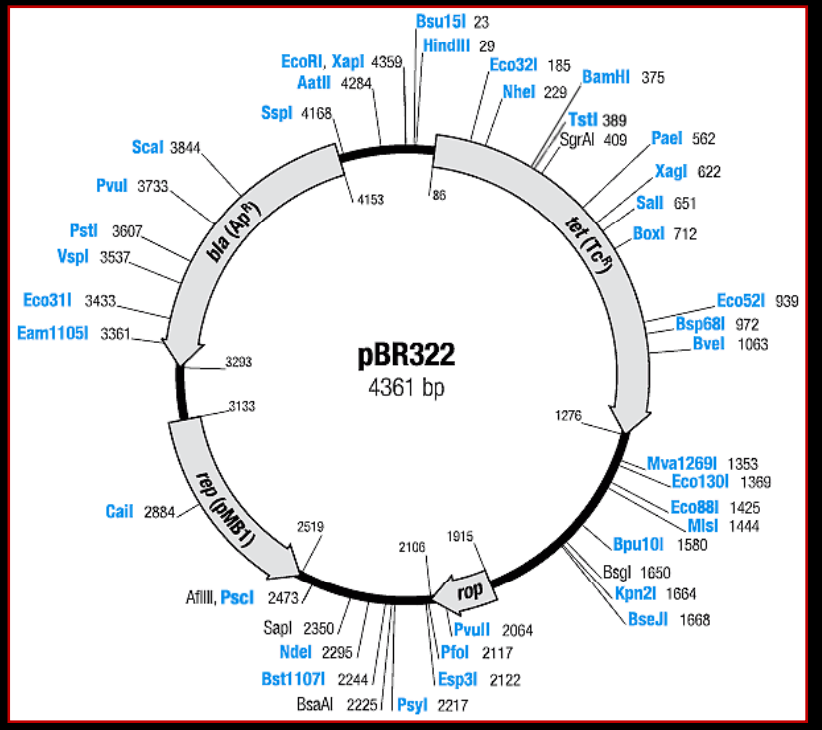




Useful properties:

2. Presence of two antibiotic resistance genes (Amp^R and Tet^R) – enables the selection of transformed cells from non-transformed cells and reccombinats from non-recombinants (by insertional inactivation)

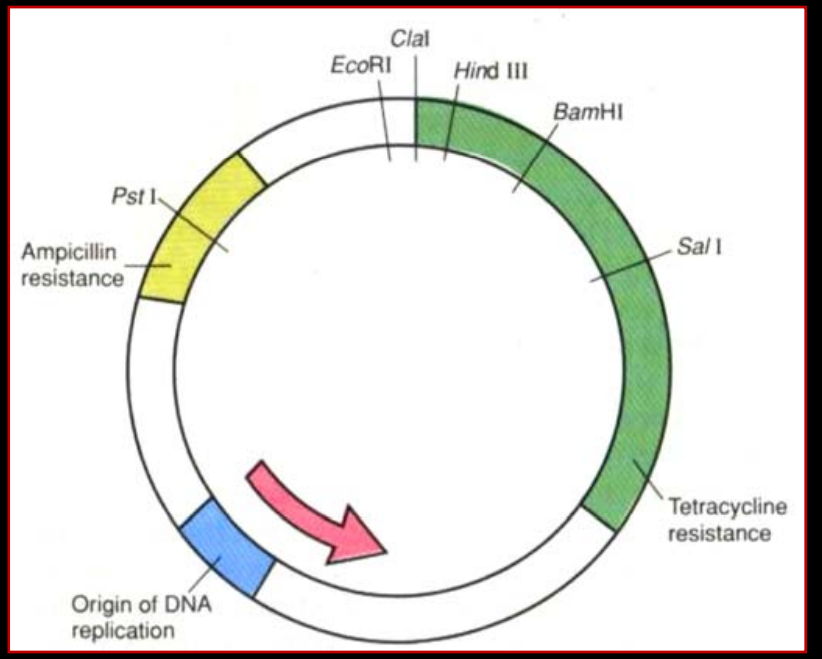


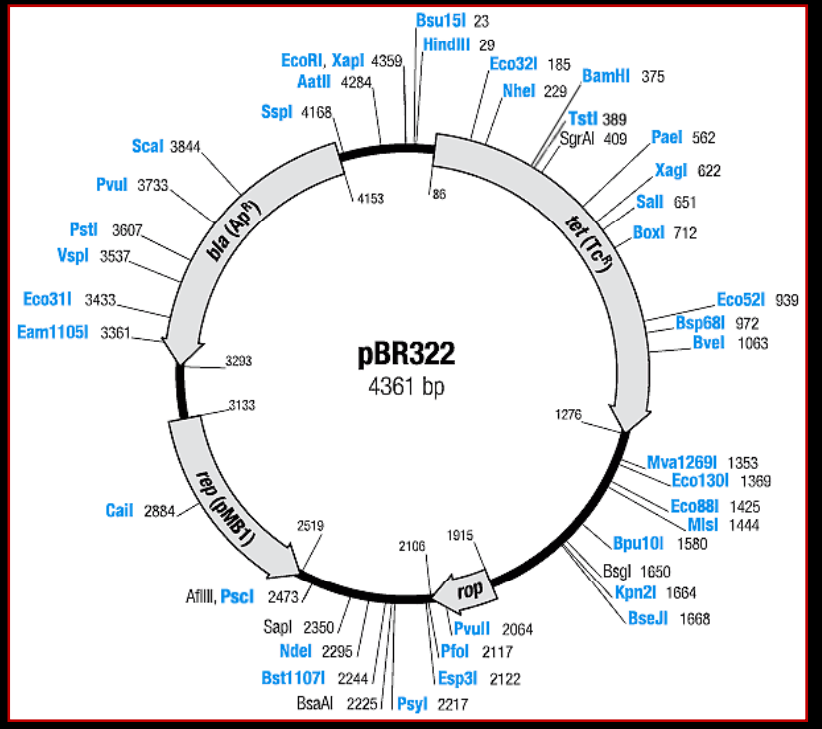


Useful properties:

3. A reasonably high copy number

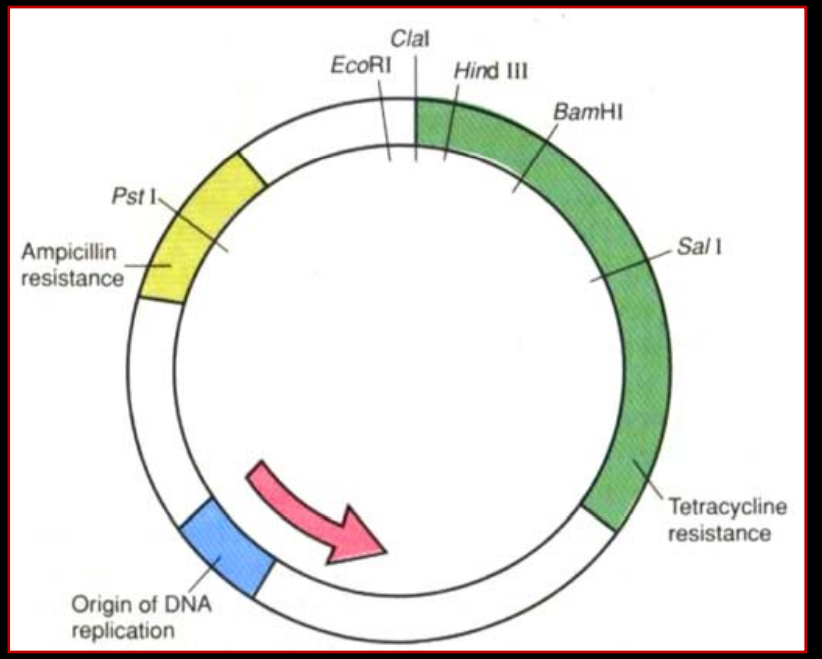
Generally there are about 15 molecules present in a transformed *E. coli* cell; this number can be increased upto 1000-3000 by plasmid amplification in the presence of chloramphenicol

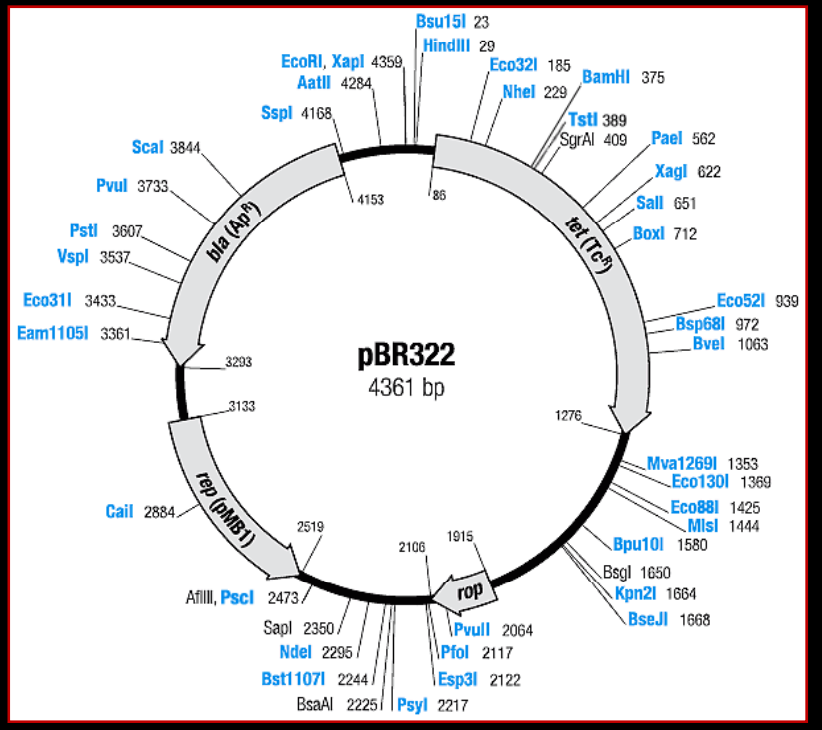




Useful properties:

4. Can take up ~6 kb of additional DNA

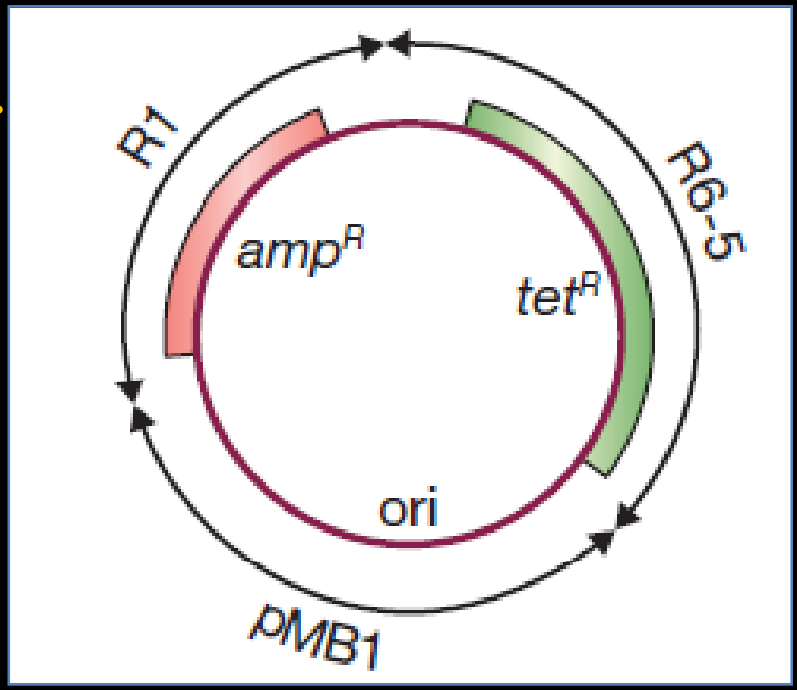


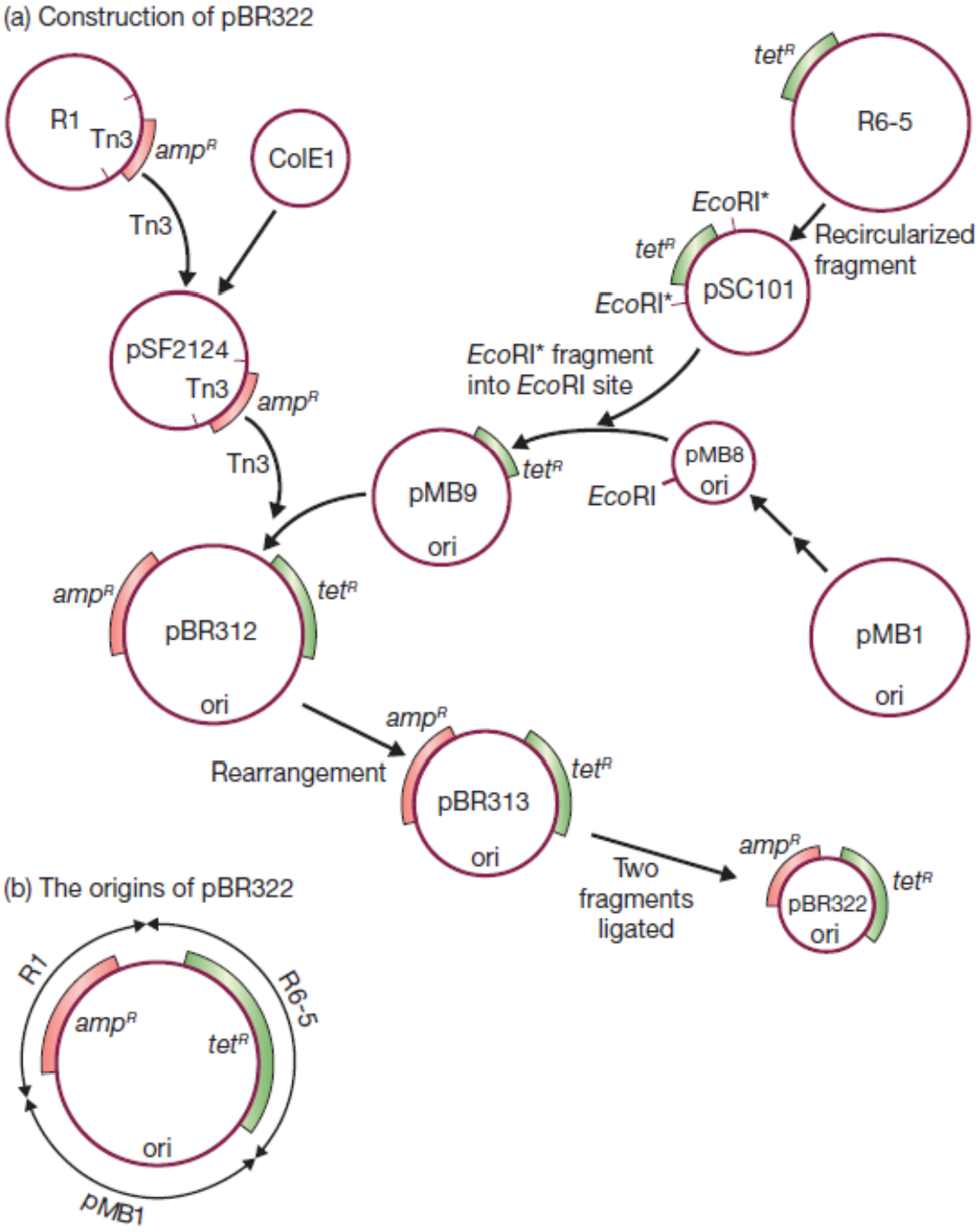


Pedigree of pBR322

pBR322 is derived from three different naturally occurring plasmids:

- ✓ *amp*^{R} gene from plasmid R1
- ✓ *tet*^{R} gene from plasmid R6-5
- ✓ origin of replication from pMB1




(a) Construction of pBR322
R1
Tn3
ampR
ColE1
Tn3
pSF2124
Tn3
ampR
Tn3
tetR
R6-5
EcoRI*
tetR
pSC101
EcoRI*
Recircularized fragment
EcoRI* fragment into EcoRI site
pMB9
ori
tetR
pMB8
ori
EcoRI
pMB1
ori
ampR
pBR312
ori
tetR
Rearrangement
ampR
pBR313
ori
tetR
Two fragments ligated
ampR
pBR322
ori
tetR
(b) The origins of pBR322
R1
R6-5
ampR
tetR
ori
pMB1

pBR327

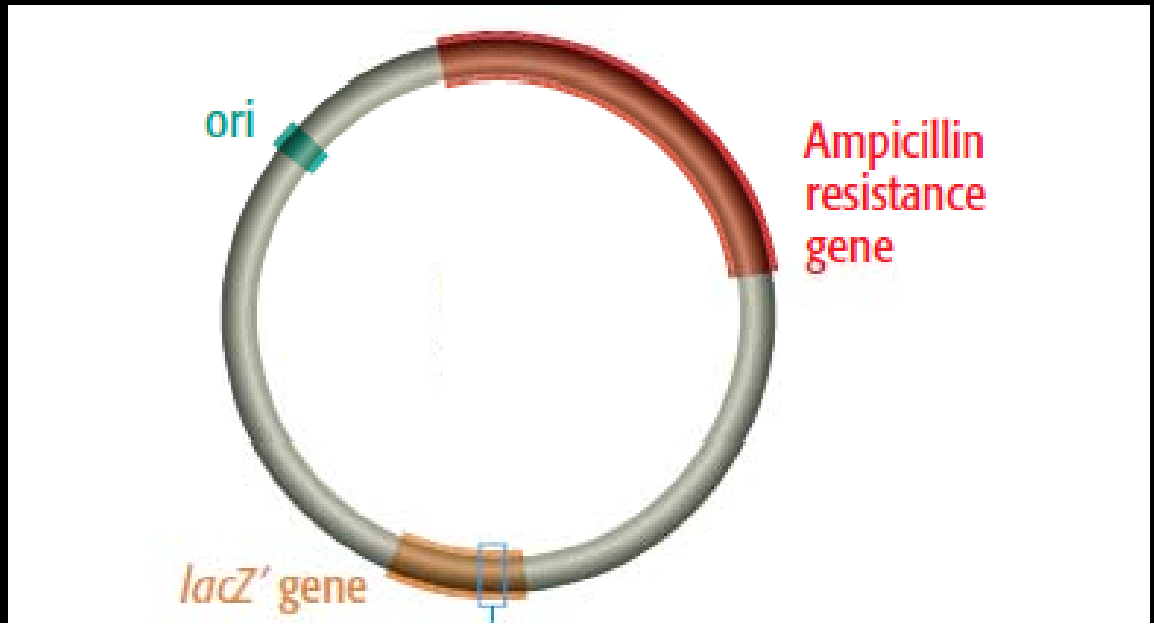
developed from pBR322 by several manipulations
pBR327 was produced by removing a 1089 bp segment from pBR322.

This deletion left the *amp*^{R} and *tet*^{R} genes intact
pBR327 has a higher copy number than pBR322, 30–45 copies per cell

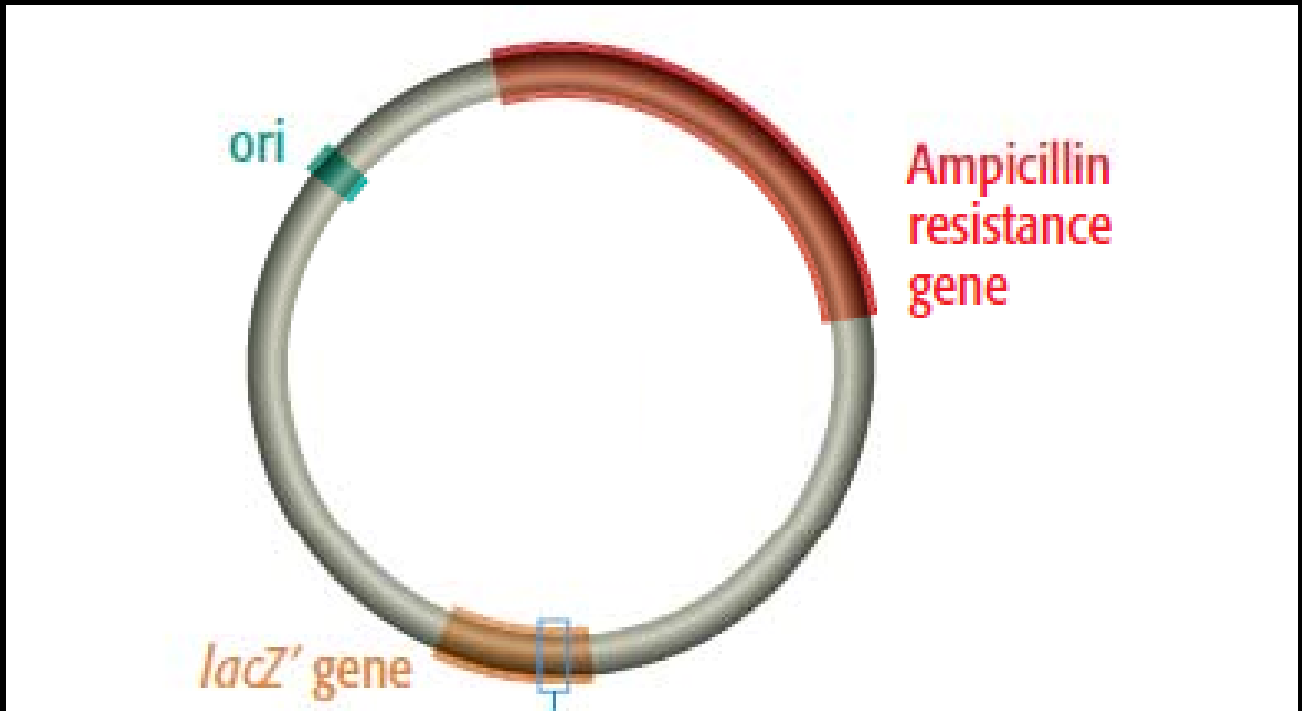
The higher copy makes this vector more suitable if the aim of the experiment is to study the function of the cloned gene.

pUC vectors

- ✓ The pUC is derived from pBR322
- ✓ Developed at the University of California in 1980s
- ✓ There are several vectors in pUC series – includes pUC8, pUC9, pUC18, pUC19, etc
- ✓ high copy cloning vector for replication in E. coli

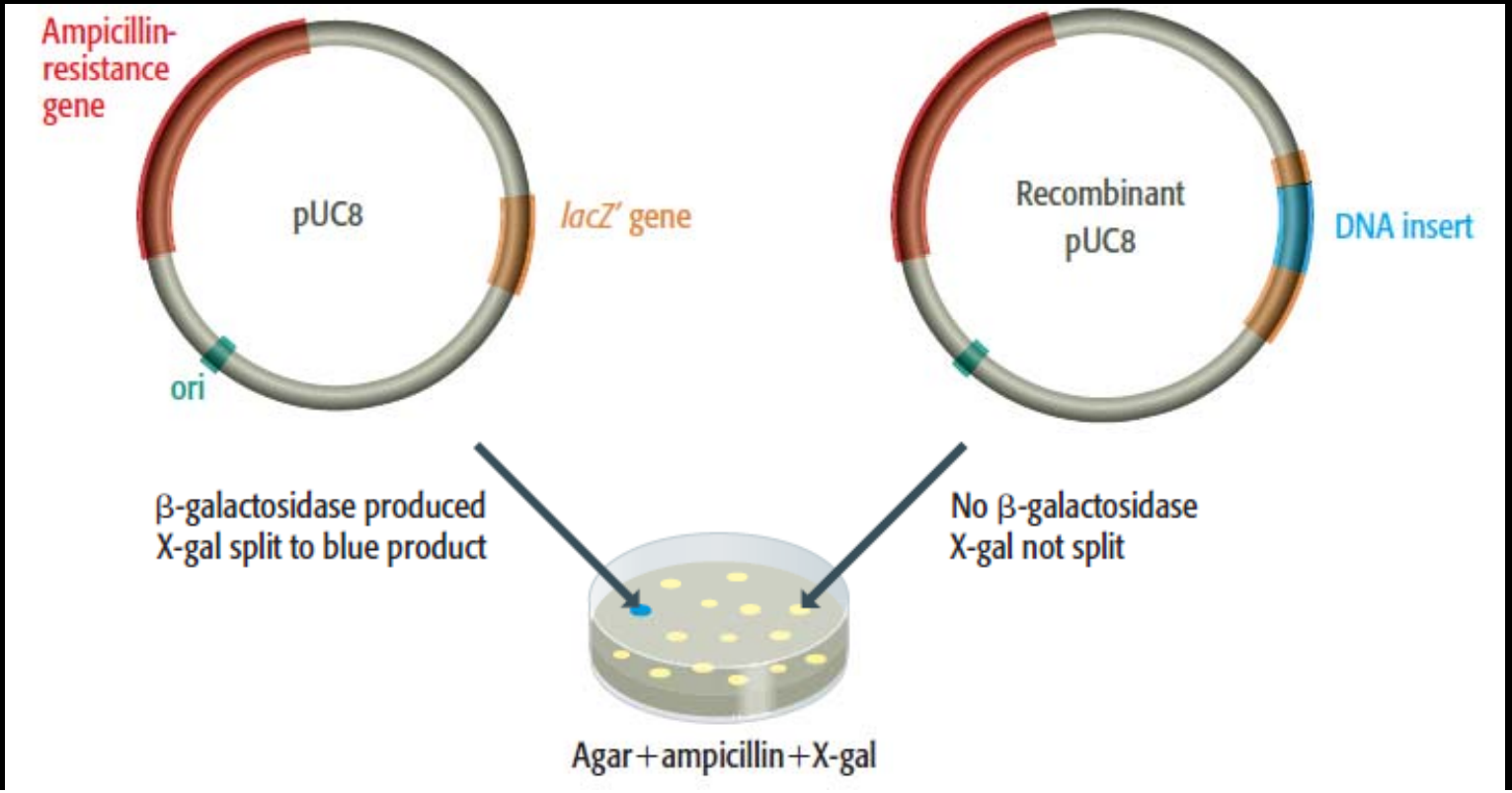


- ✓ constructed using the ampicillin resistance gene from the pMB1 and origin of replication from pBR322
- ✓ pUC is a high copy cloning vector for replication *in E. coli*
- ✓ pUC vectors generally contains an origin of replication, *lacZ'* and a *amp*^{R} gene
- ✓ The MCS is located within the *lacZ'* gene- enebles the selection of recombinants



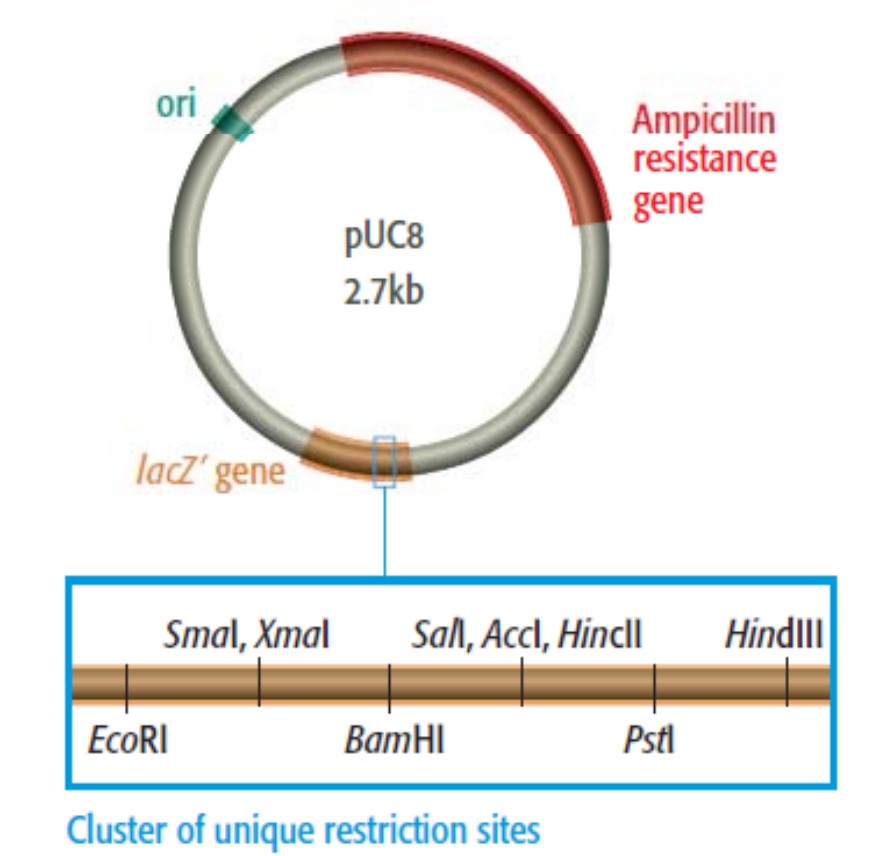
pUC suitable for "blue-white screening" technique

- ✓ The lacZ' gene contains a cluster of unique restriction sites, ligation of new DNA into any one of these sites results in insertional inactivation of the gene and hence loss of β-galactosidase activity. This is the key to distinguishing a recombinant plasmid one that contains an inserted piece of DNA from a nonrecombinant plasmid that has no new DNA.



pUC8

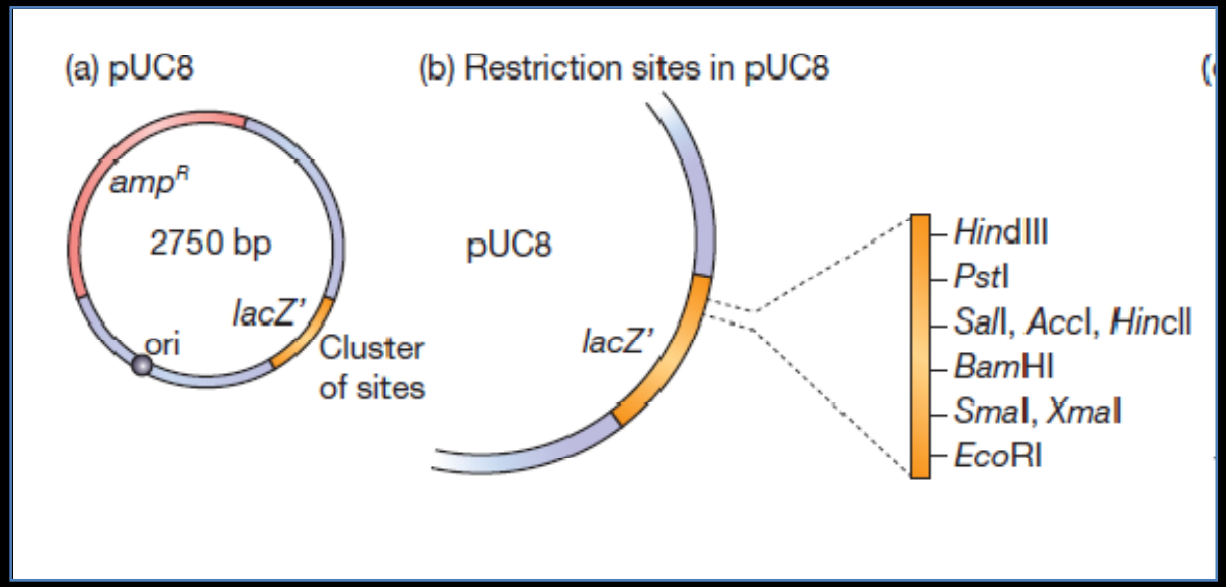
- ✓ pUC8 is descended from pBR322
- ✓ pUC8 is 2695 bps in size
- ✓ Contains an origin of replication, *lacZ* 'and the *amp*^{R}
- ✓ There is no restriction sites within the *amp*^{R} gene; all the cloning sites are clustered into the *lacZ* ' gene



Advantages of pUC

1. plasmid having a copy number of 500–700 even before amplification.
2. identification of recombinant cells can be achieved by a single step process, by plating onto agar medium containing amphicillin and X-galactose (BW screening).
3. clustering of the restriction sites, which allows a DNA fragment with different sticky ends to be cloned

4. Can carry upto 10 kbs foreign DNA



Disadvantages using plasmids

- Cannot accept large fragments
- Sizes range from 0 - 10 kb
- Standard methods of transformation are inefficient

Viruses as vectors

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2. Gene Cloning and DNA Analysis (6th Edn) - T.A. Brown
3. Molecular biology - David P Clark
4. Genomes(3rd Edn) - T.A. Brown
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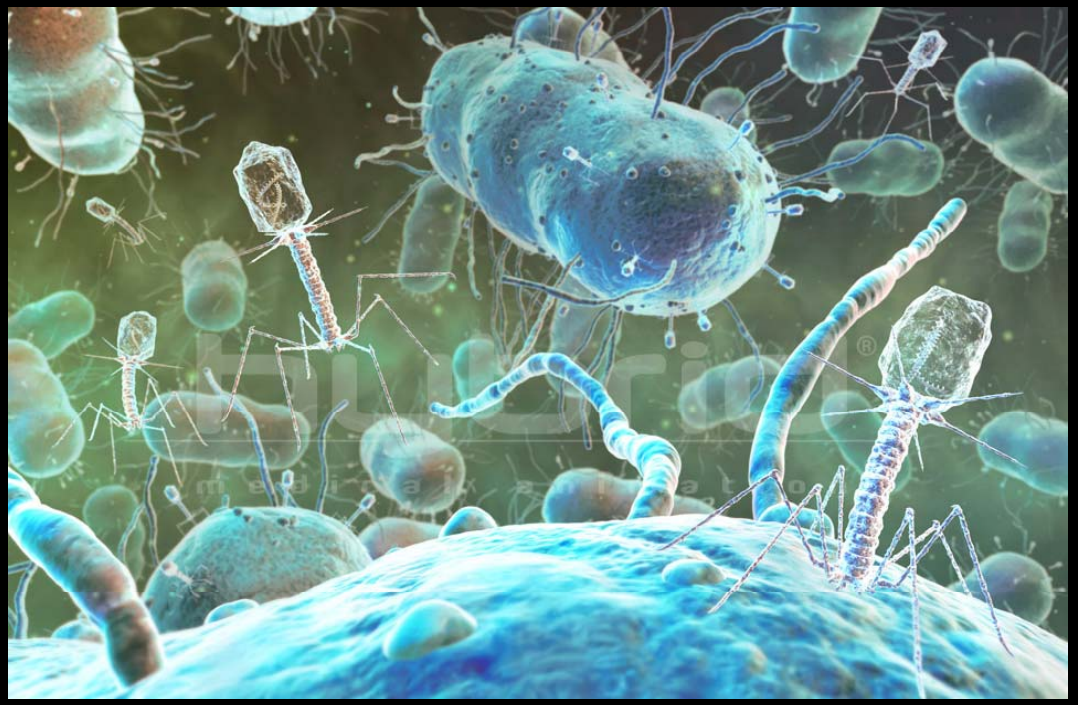
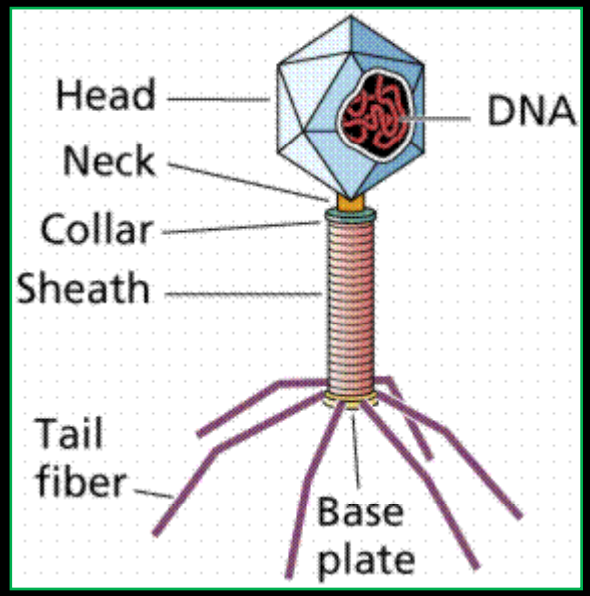


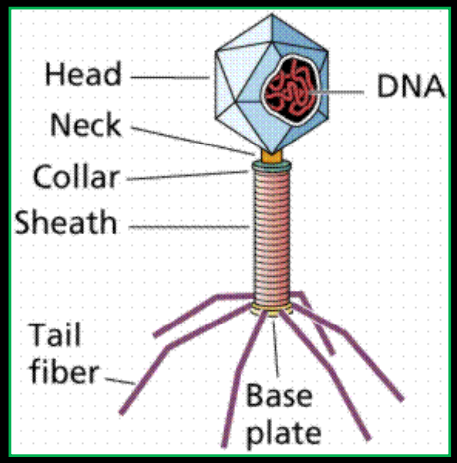
Advantages of phage vectors over plasmid vectors:

1. Highly efficient for the cloning of larger DNA fragments
2. Screening of the recombinant DNA molecule is easier

The most widely used *E. coli* phage vectors are λ phage and M13 phage



Bacteriophage Lambda Vectors



Bacteriophage lambda, which infects *E. coli*, has been widely used as a cloning vector.

Lambda is a well-characterized virus with both lytic and lysogenic life cycles.

- Lambda DNA circularizes within the bacterial cell and the DNA inside the phage particle is linear
- The size of the lambda genome is 48.5 kb

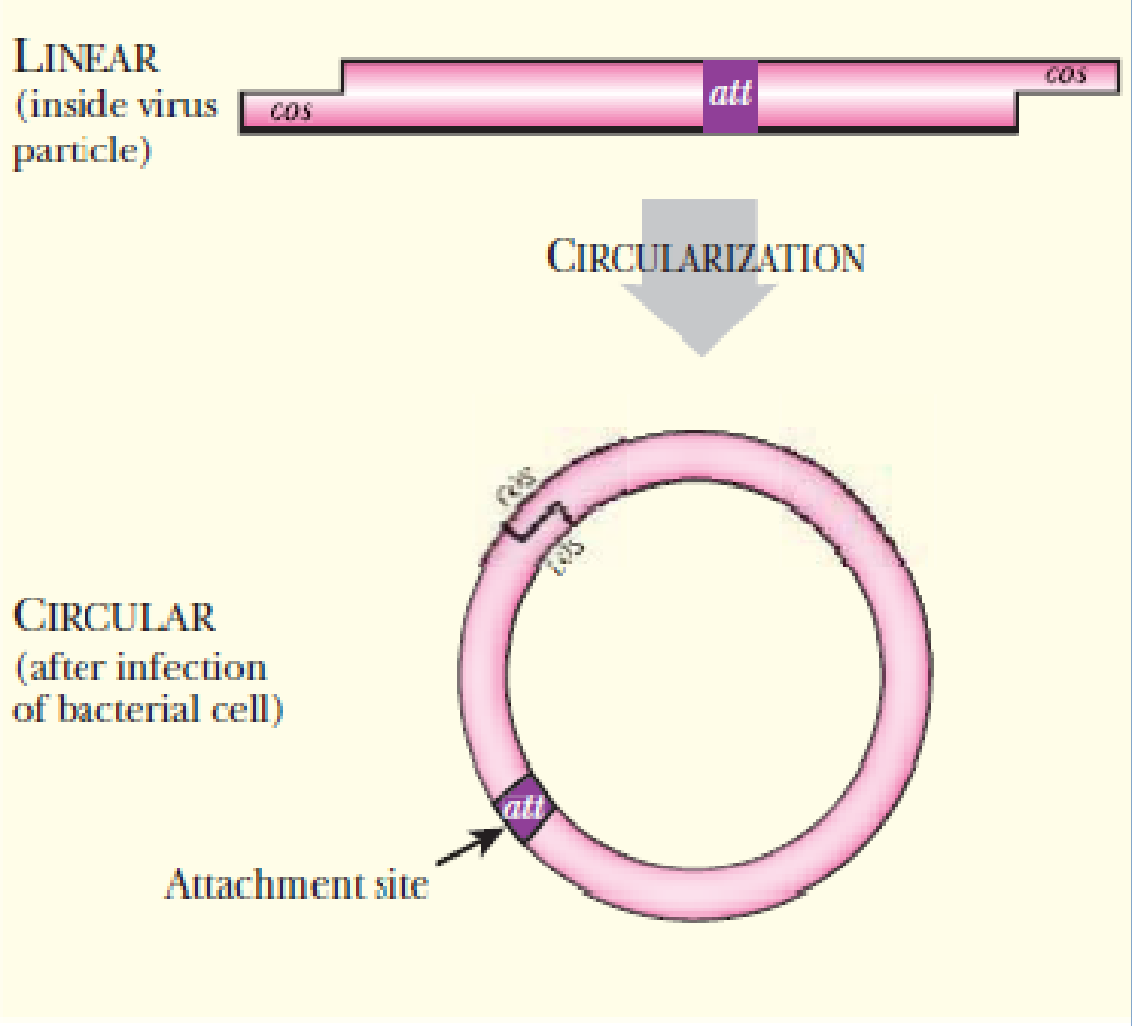
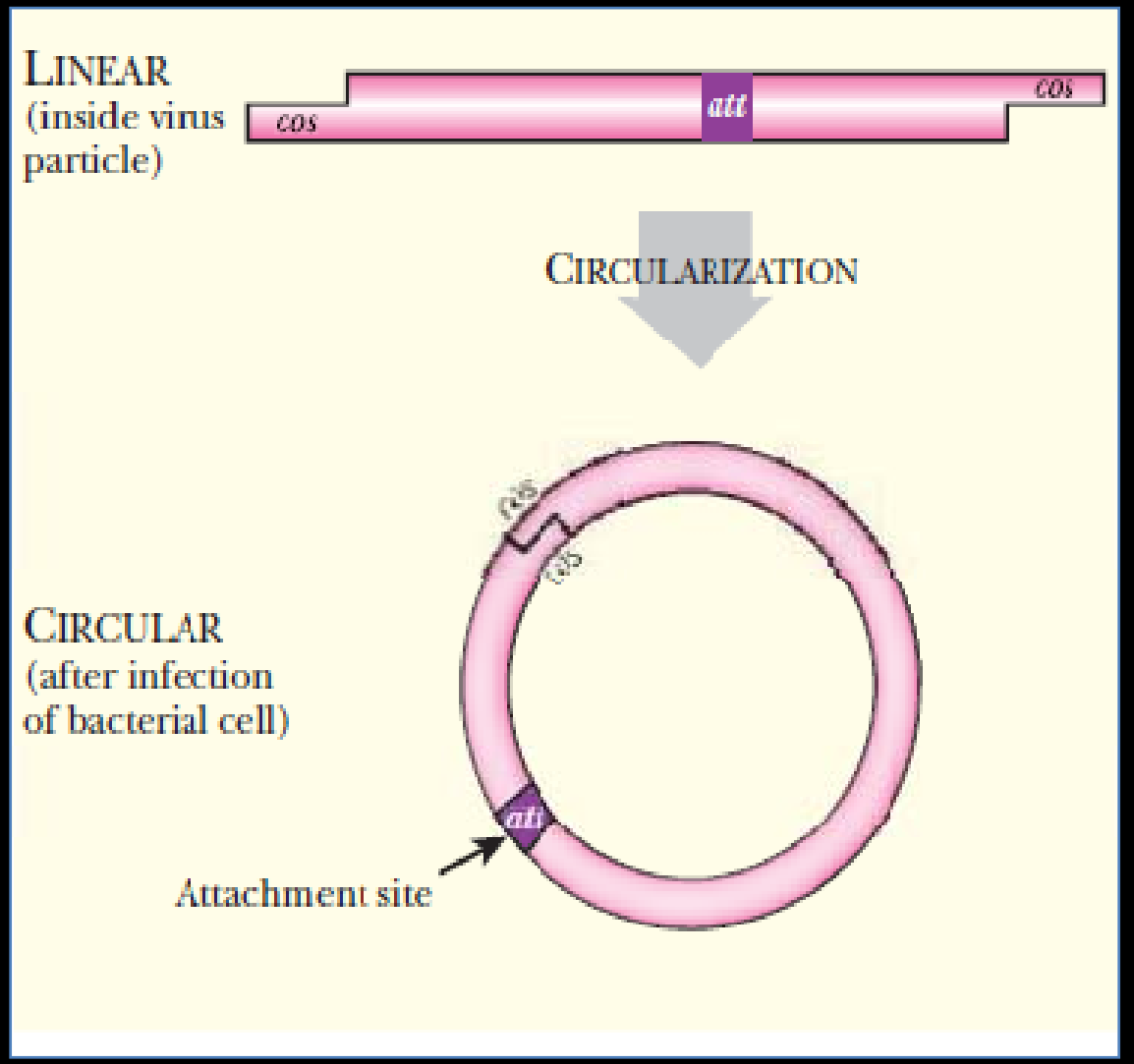


FIGURE 22.18 ***Lambda—Linear and Circular Genomes***

In the lambda phage particle, the genome is a linear DNA molecule with two *cos* sequences at each end. After the phage injects its DNA into the bacterial host, the DNA circularizes. The two cohesive ends base pair and are ligated together by bacterial enzymes so forming a circle.

At each end there are complementary 12 bp long overhangs known as *cos* sequences (cohesive ends).

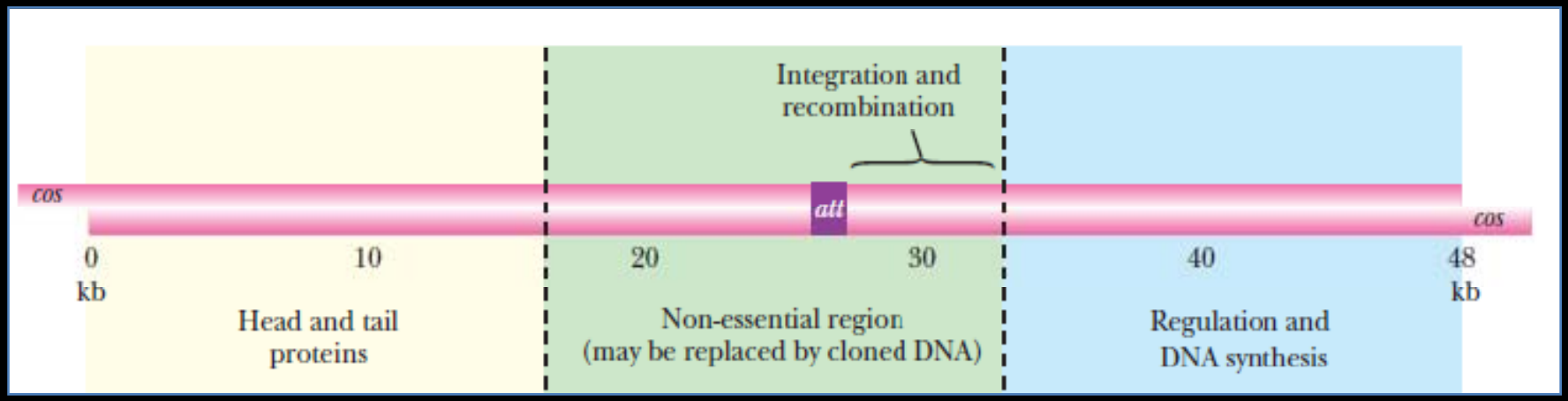
Once inside the *E. coli* host cell, these pair up and the cohesive ends are ligated together by host enzymes forming the circular version of the lambda genome.



Lambda phage particle can accommodate up to 52 kb of DNA

If a 15 kb DNA is removed from the genome, then up to 18 kb of new DNA can be cloned

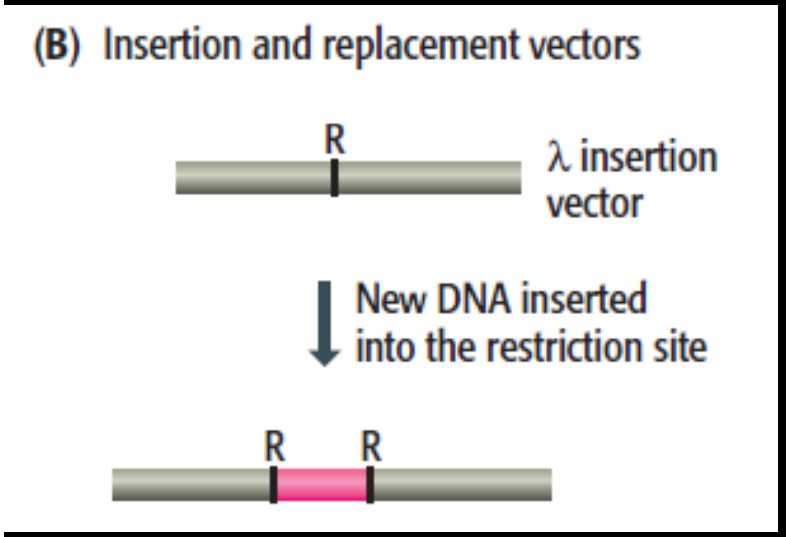
The middle region (~15 kb) of the lambda genome is non-essential and may be replaced with approximately 18 kb of foreign DNA.

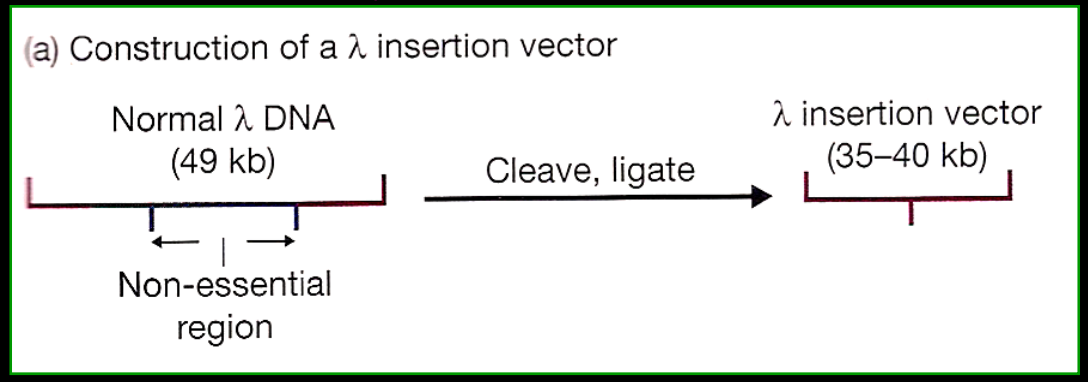


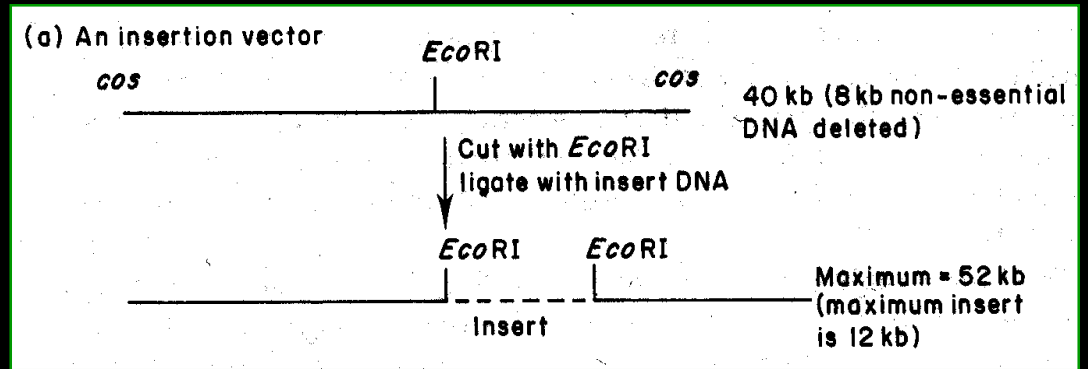
Two classes of λ phage vector: Insertion vectors and Replacement vectors

i) Insertion vector: In this type vector a large segment of the non-essential region is deleted, and the two arms ligated together, contains at least one unique RE site into which new DNA can be inserted e.g. **λgt10, λZAPII**

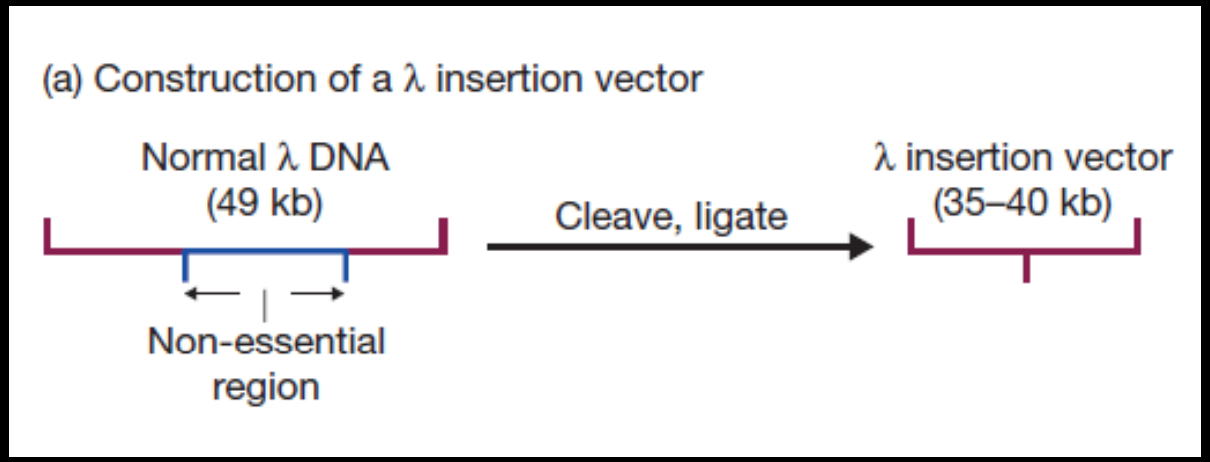
Insertion vectors offer limited scope for cloning large pieces of DNA







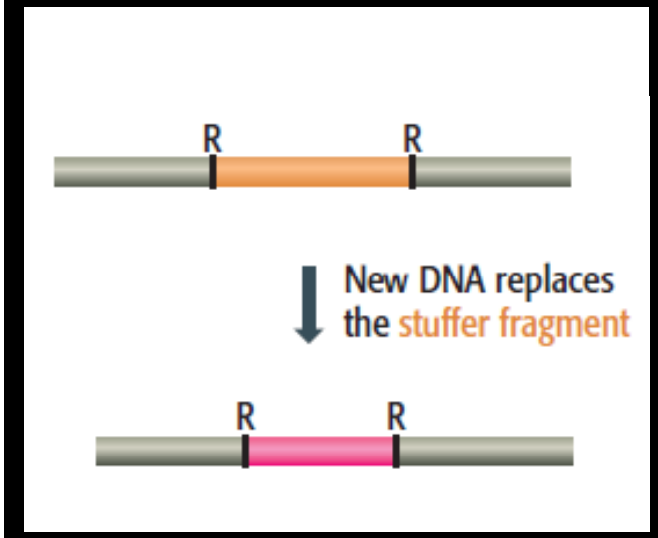
λ gt10 - can carry up to 8 kb of new DNA
λZAPII - can carry up to 10 kb of new DNA

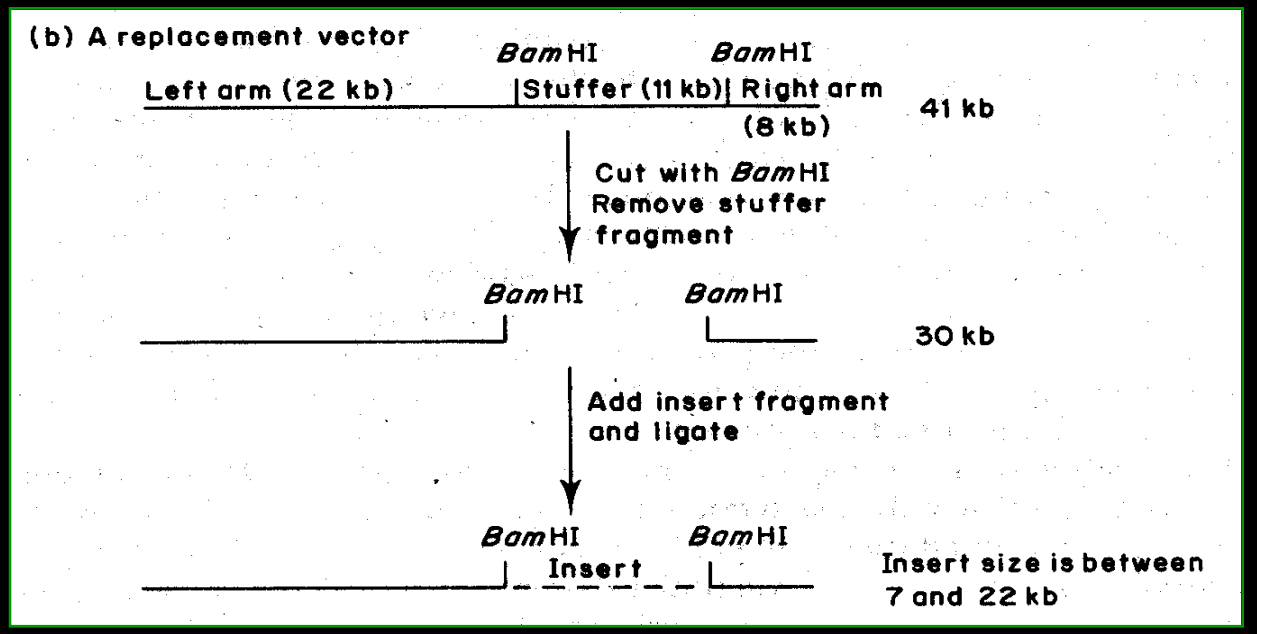


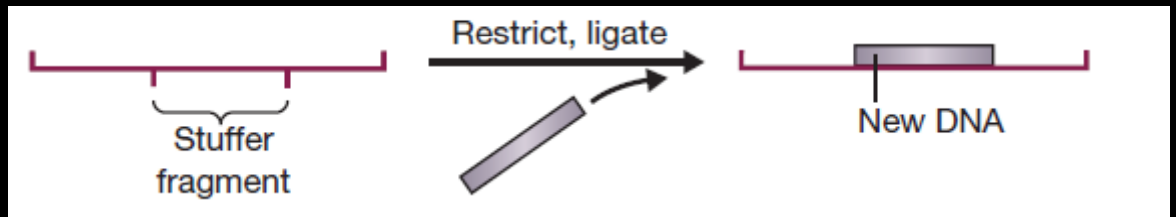
Replacement vectors: in which a central 'stuffer' fragment (non-essential DNA) is removed and replaced with the foreign DNA

Replacement vectors can generally carry larger pieces of DNA than insertion vectors.

E.g. λEMBL4 - can carry up to 20 kb of inserted DNA



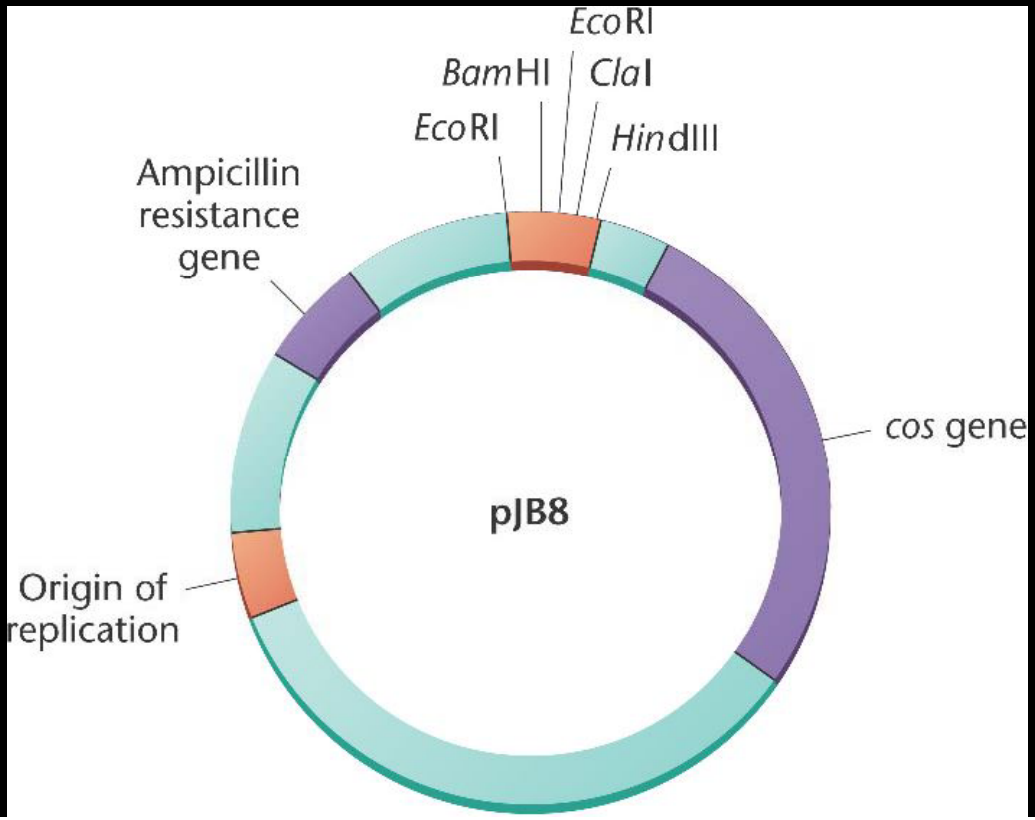




Cosmid

Cosmid is the hybrid of λ phages and bacterial plasmids and behave both as plasmids and as phages.

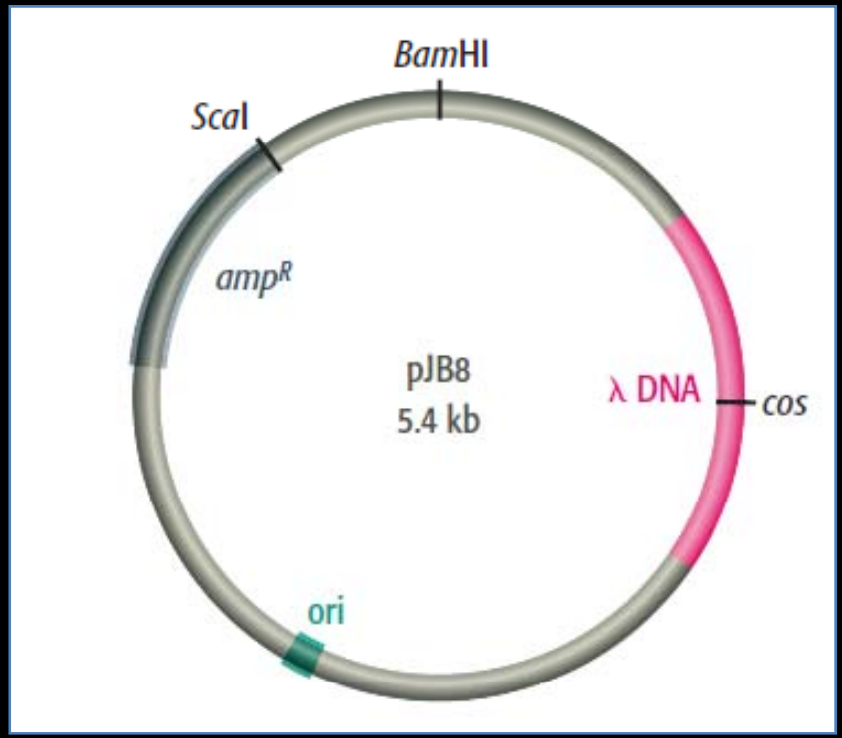
Cosmid is a small multicopy plasmid that carries lambda *cos* sites, ori, MCS, amp^R and can carry around 45 kb of DNA to be cloned.



Enzymes that package λ DNA into the phage protein coat need only the *cos* sites and *in vitro* packaging occurs not only with λ genomes, but also with any molecule that carries *cos* sites separated by 37-52 kb of DNA.

It can easily packed into λ phage heads like a phage with the *cos* sites.

They are able to replicate in the bacterial cell, like a plasmid and origin of replication allows to replicate in E.coli.

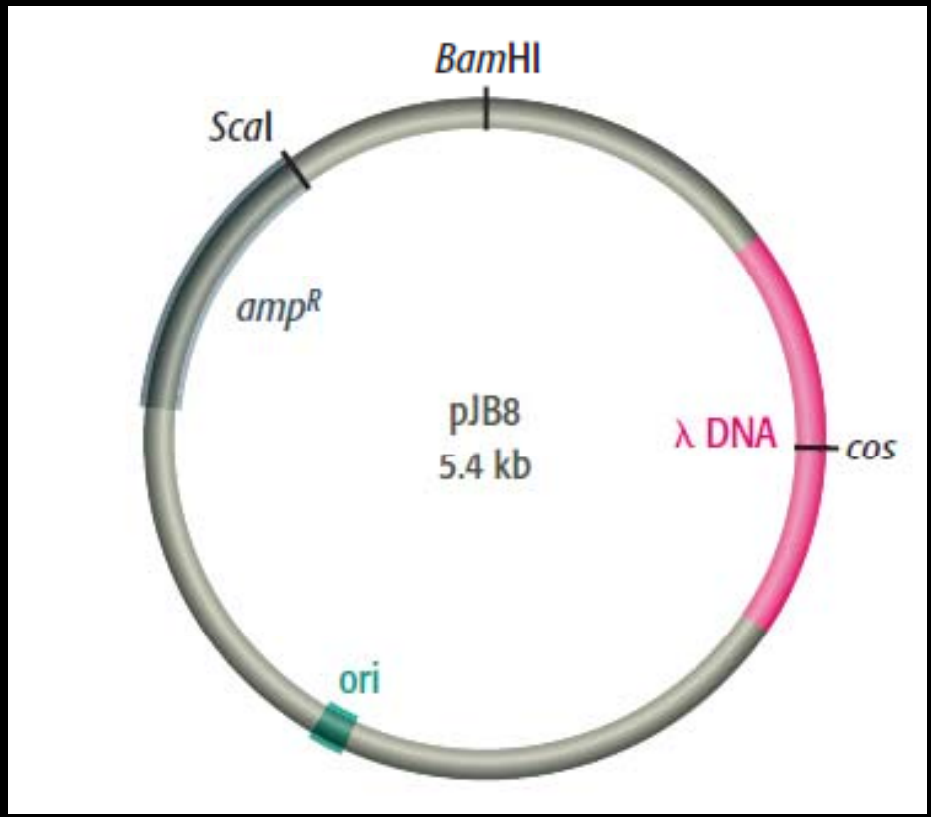


Usually cosmids size range from 5-7 kbp and it can carry ~45 kbp of foreign DNA to be cloned.

During the construction of cosmids, most of the λ structure is deleted, *cos* sites only remain.

This modified structure enables phage heads to be stuffed with donor DNA.

Eg. pJB8

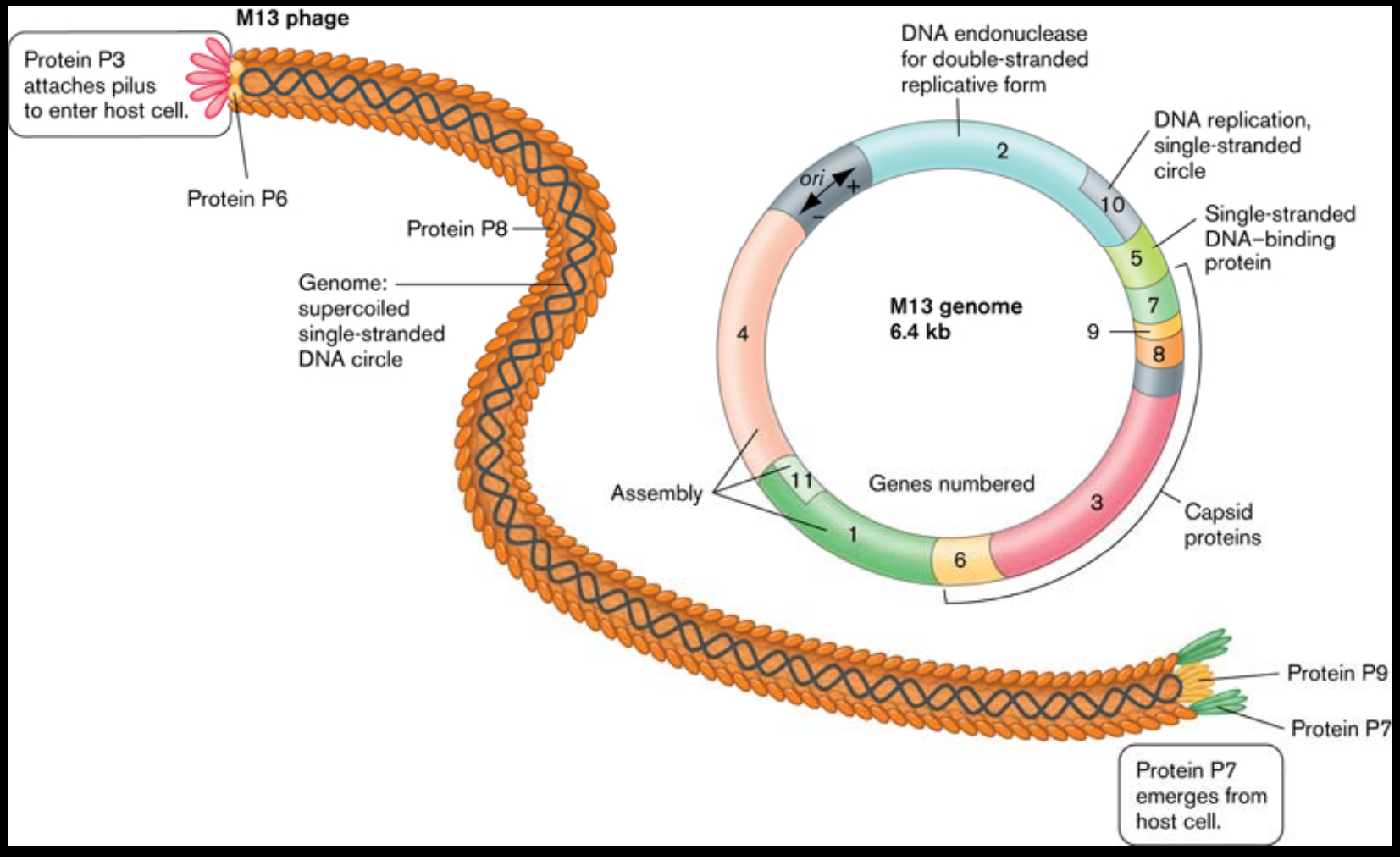


Advantages:

- High transformation efficiency
- Carry up to 45 kb DNA fragments
- Easy identification on the selective media
- Amplification and maintenance are easy

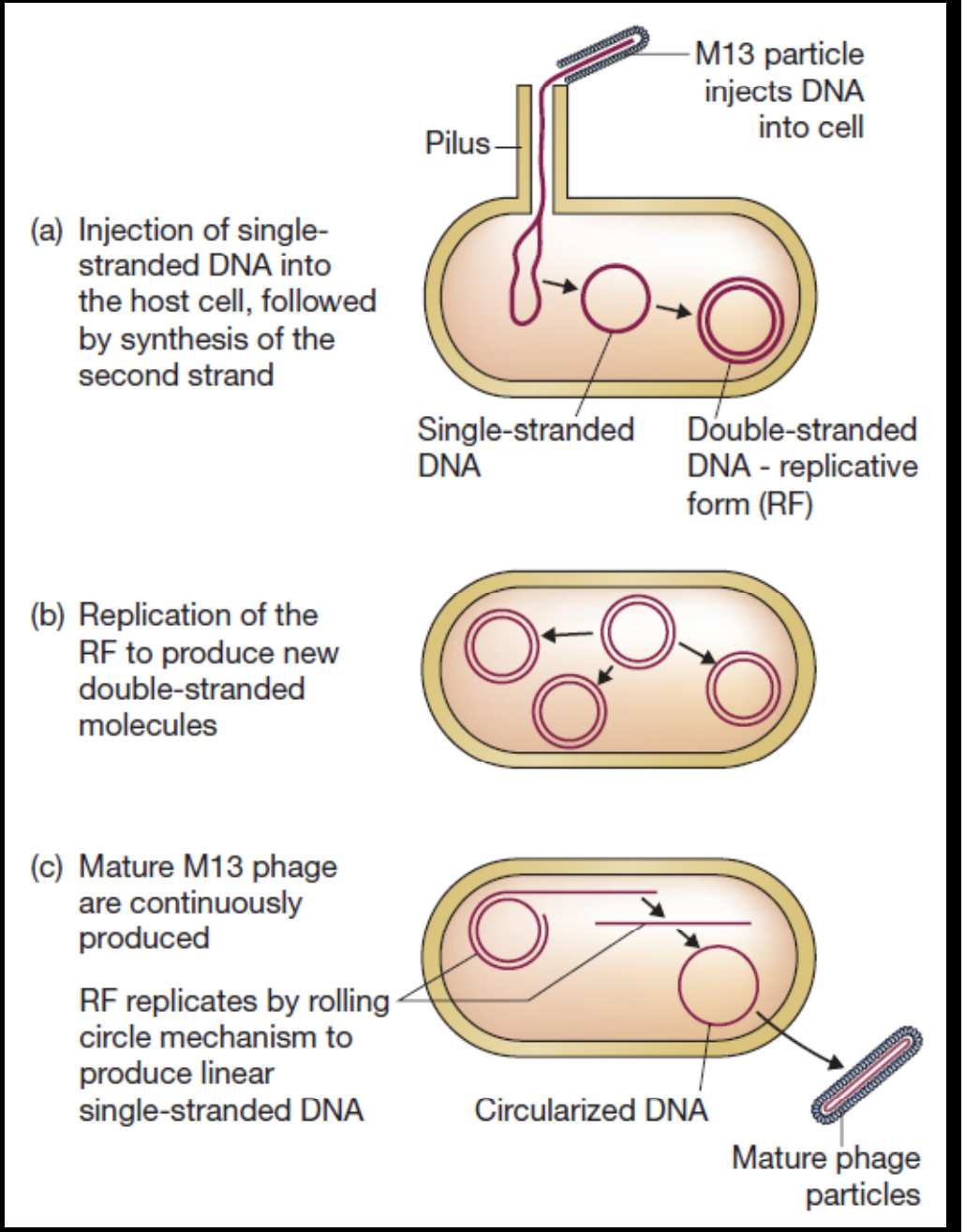
M13 phage vectors

M13 is a filamentous phage with 6407 bps of circular single-stranded DNA



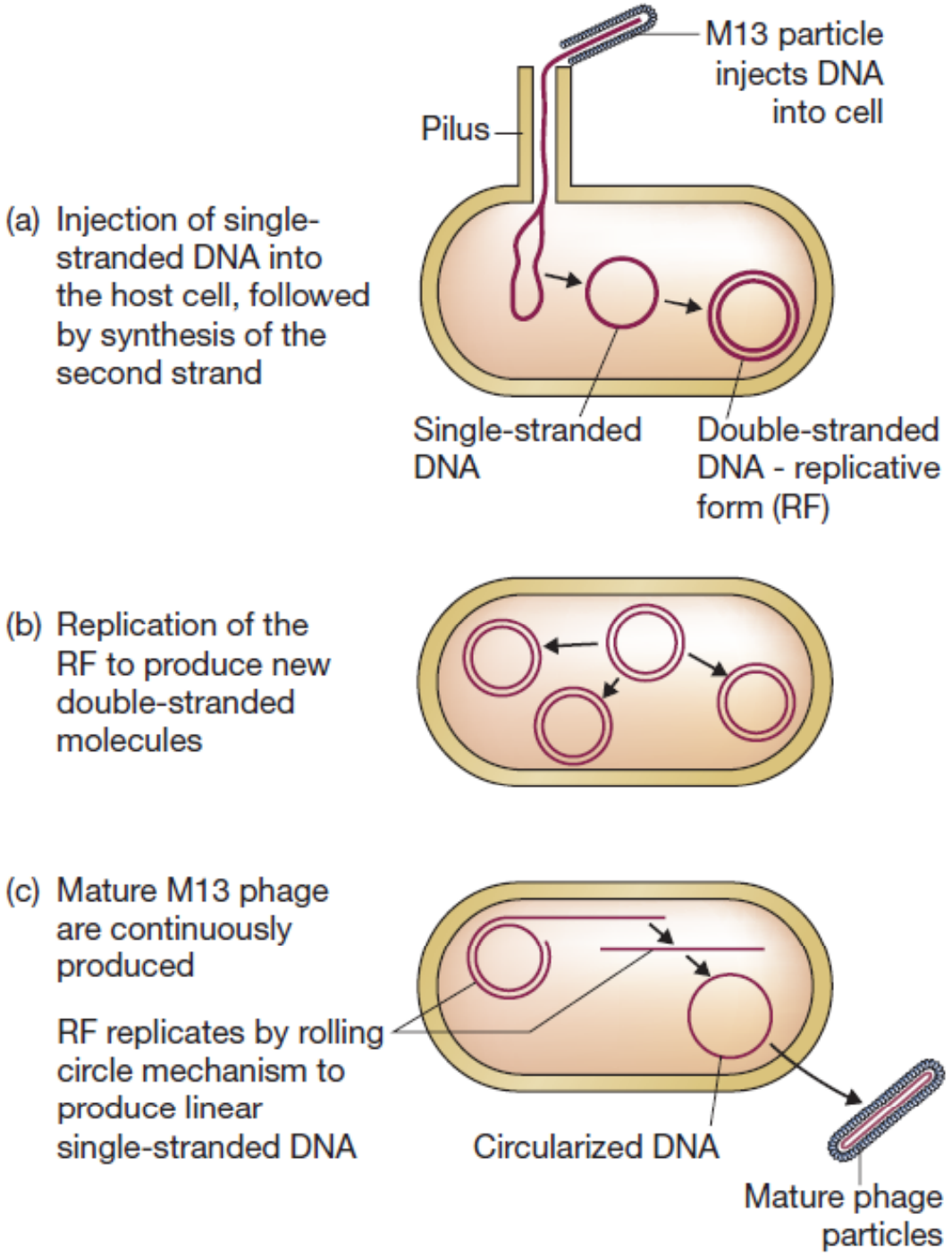
Life cycle of M13

Injection of an M13 DNA molecule into an E. coli cell occurs via the pilus.
Once inside the cell the single-stranded molecule acts as the template for synthesis of a complementary strand, resulting in normal double-stranded DNA (ds-RF).
This molecule is not inserted into the bacterial genome, but instead replicates until over 100 copies are present in the cell.



During the synthesis of new viral particles, the ds-RF replicates by rolling circle mechanism to produce linear ssDNA.

The linear ssDNA then circularize and packed in to the protein coat.



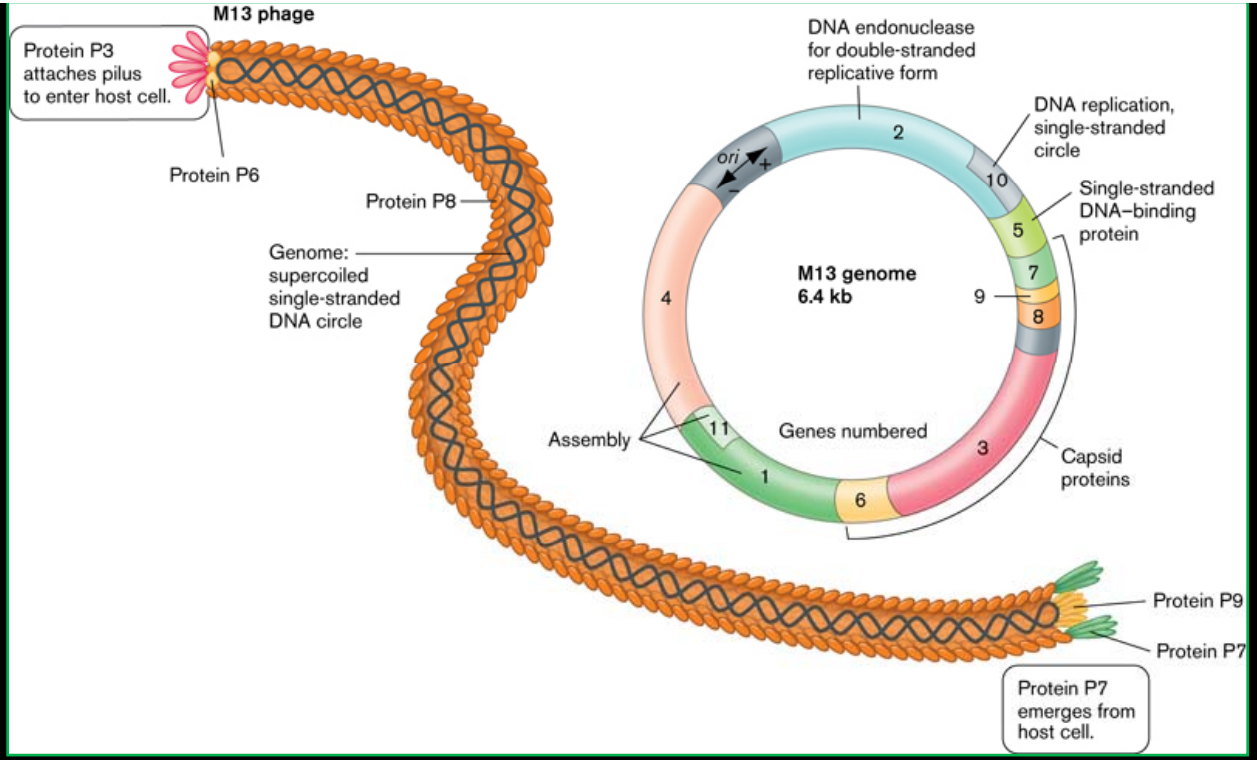
Several features of M13 it attractive as a cloning vector

1. The genome is less than 10 kb in size, well within the range desirable for a potential vector.

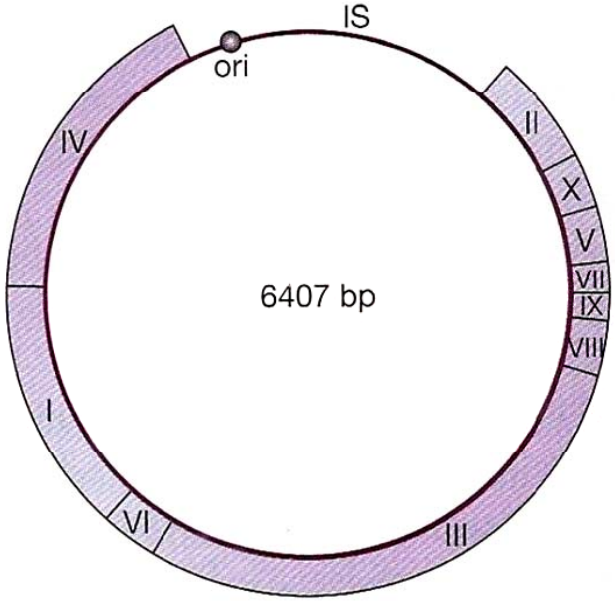
2. The ds replicative form (RF) of the M13 genome behaves very much like a plasmid, and can be treated as such for experimental purposes.

3. The genes cloned with an M13-based vector can be obtained in the form of ssDNA. ssDNA is useful for several techniques such as DNA sequencing and *in vitro* mutagenesis, etc.

M13 phage as vectors



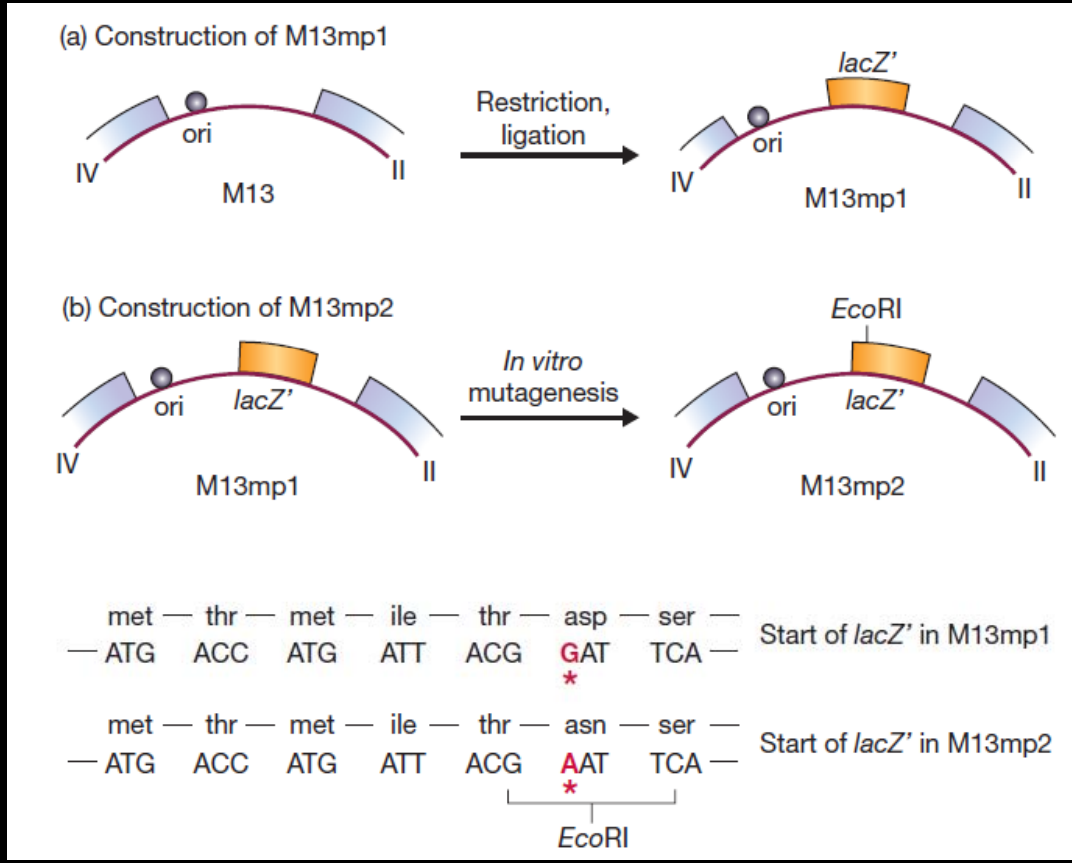
- The 6407 bp genome in M13 has 10 closely packed genes, each essential for the replication
- There is a 507 nt intergenic sequence into which only limited amount of foreign DNA could be inserted; thus only a limited scope for modifying M13 genome



Construction of M13 cloning vector:

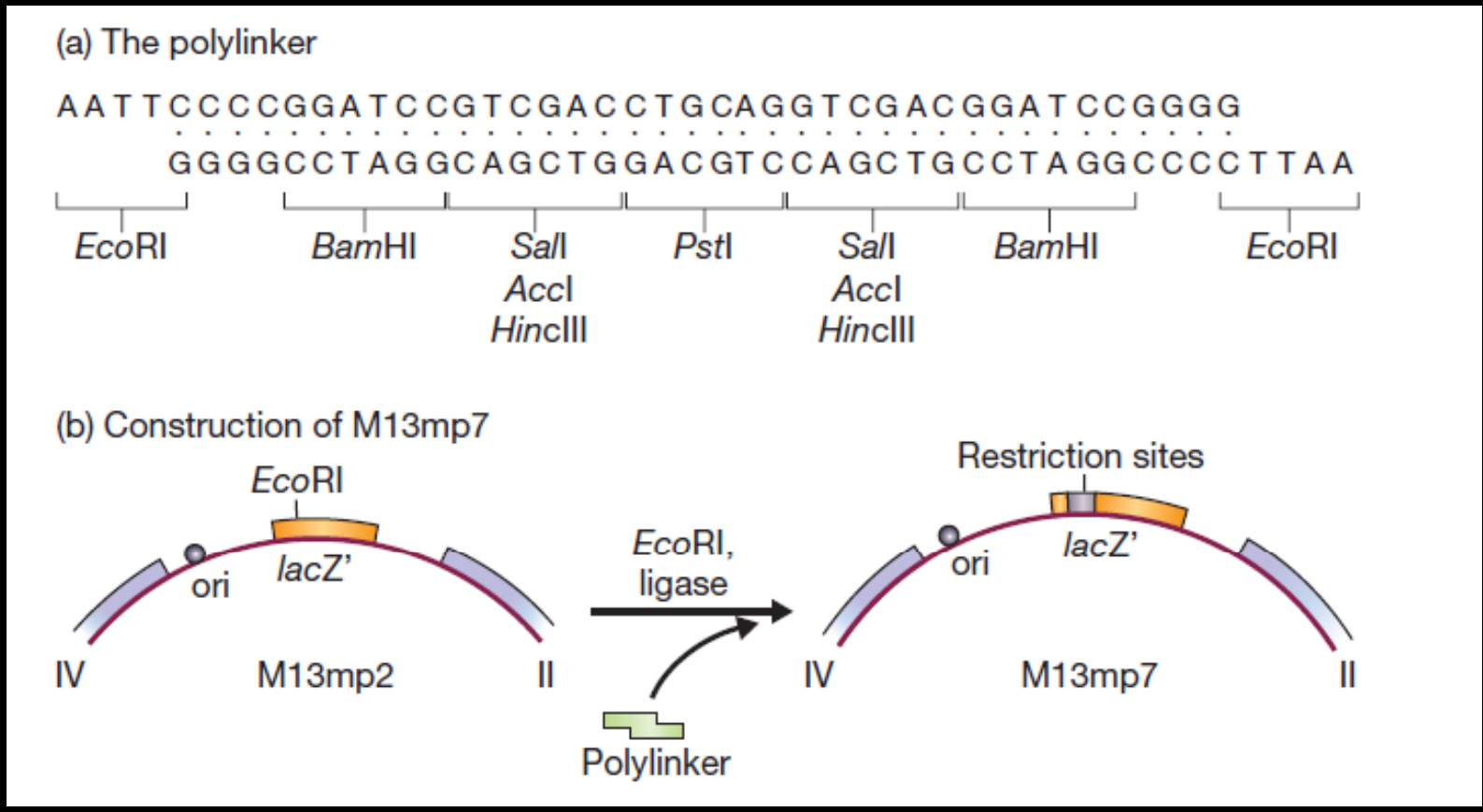
M13mp1: to the M13 phage genome, *lacZ'* gene is introduced into the intergenic sequence

M13mp2: The GGATTC sequence near the start of *lacZ'* gene in M13mp1 is converted to EcoRI site (GAATTC) by in vitro mutagenesis - result in M13mp2



Construction of M13 cloning vector:

M13mp7: Additional RE sites are introduced into the *lacZ'* gene of M13mp2– achieved by inserting a polylinker into the *Eco*RI site



M13 vectors

Advantage:

- Useful for producing single stranded versions of cloned genes

Disadvantage:

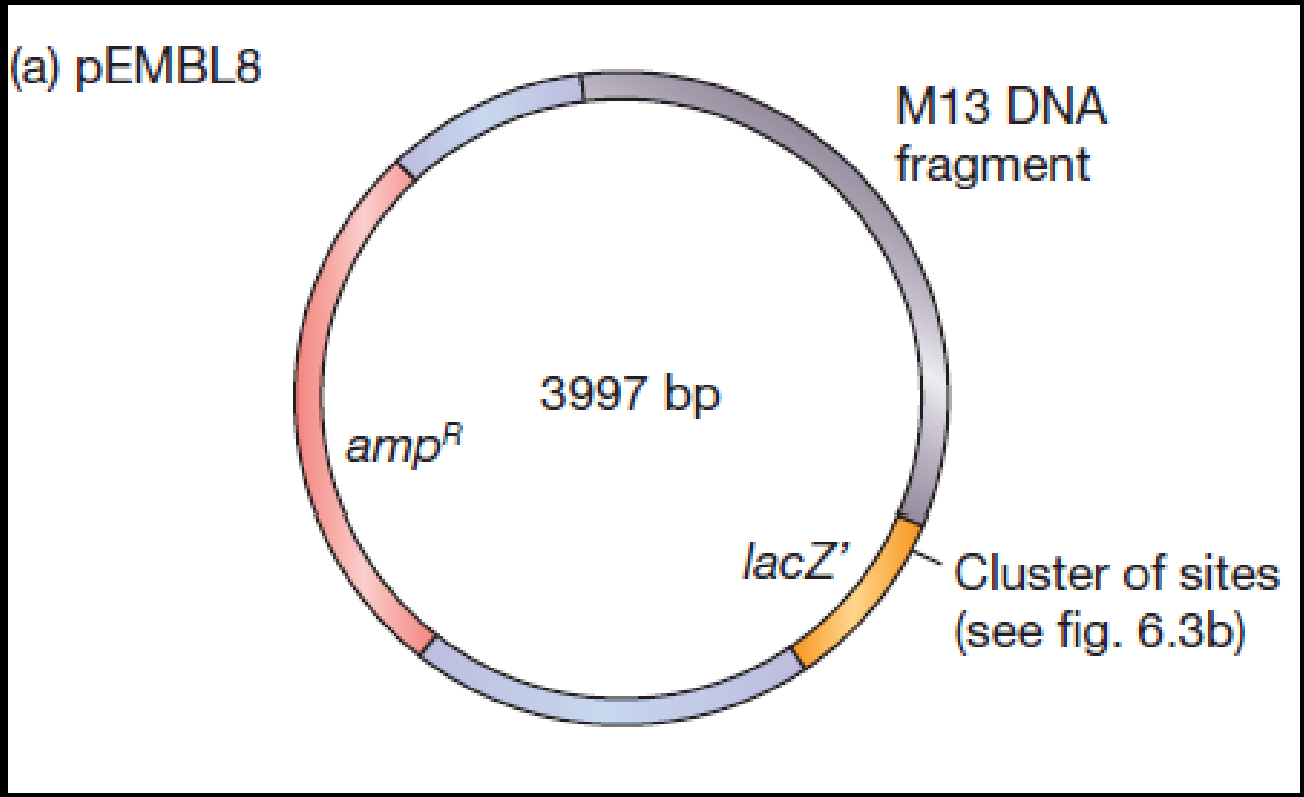
- Only 1.5 kb DNA can be efficiently cloned

Applications:

- ssDNA is useful for several techniques such as DNA sequencing and in vitro mutagenesis.

PHAGEMIDS

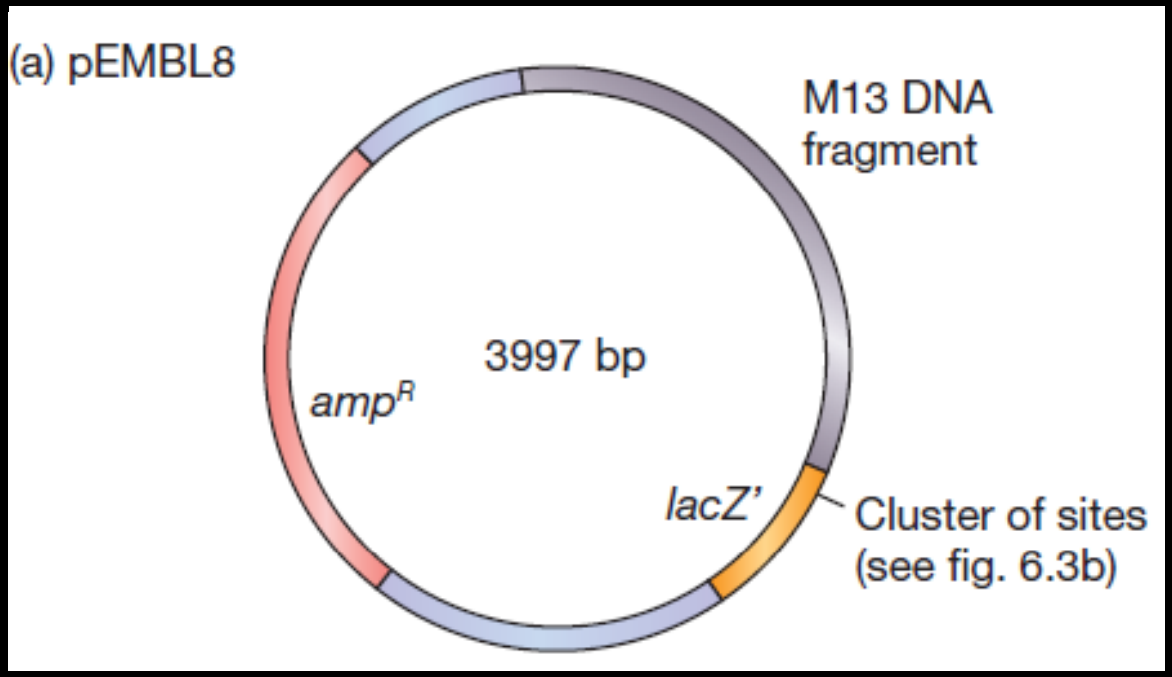
- ✓ Phagemid is a hybrid vector system, combining a part of the M13 genome with plasmid DNA.
- ✓ Phagemids can clone DNA fragments up to 3 kb
- ✓ Eg., pEMBL8

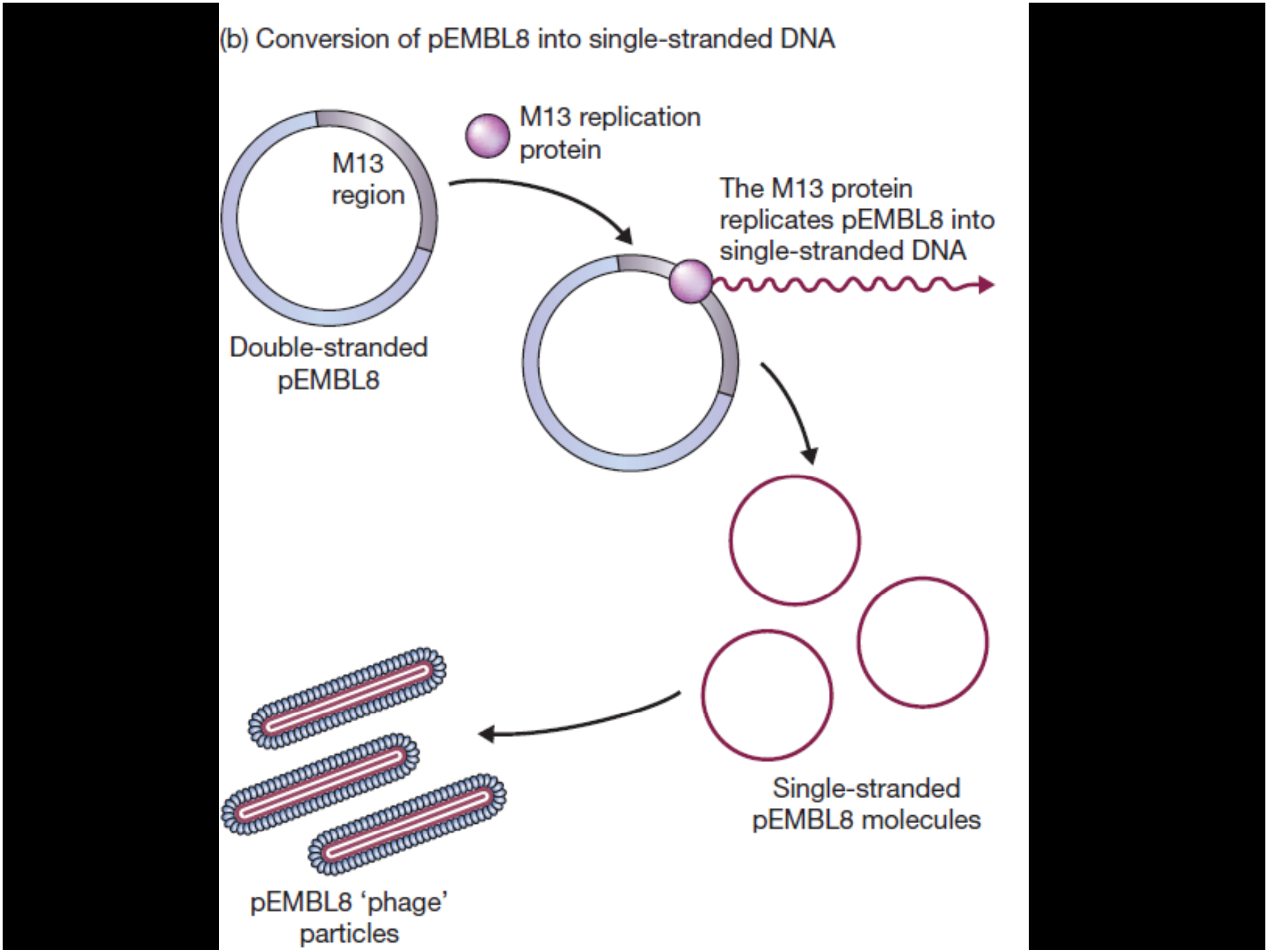


Eg., pEMBL8

pEMBL8 was made by combining regions of M13 and pUC8 vector systems

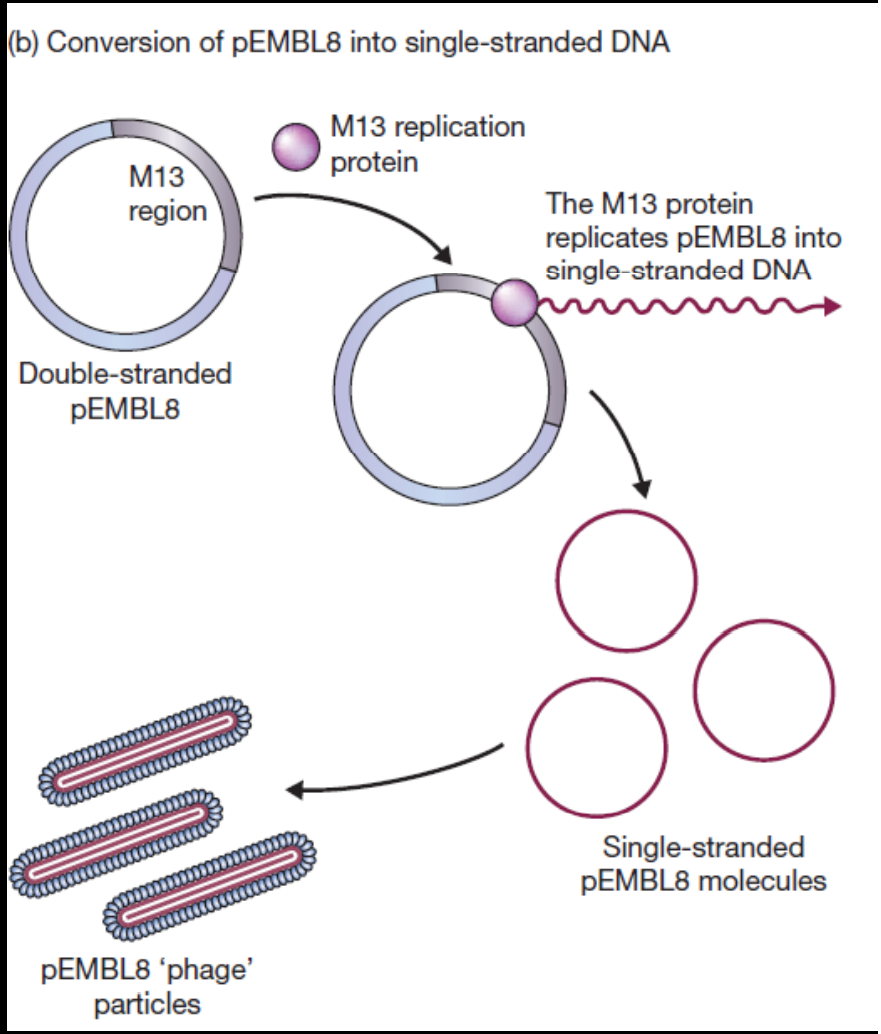
1. *ampR* gene from pUC8
2. *Lac Z'* gene with MCS from pUC8
3. 1300 bp fragment from M13 (single-stranded DNA synthesizing region)




(b) Conversion of pEMBL8 into single-stranded DNA
M13 region
M13 replication protein
The M13 protein replicates pEMBL8 into single-stranded DNA
Double-stranded pEMBL8
Single-stranded pEMBL8 molecules
pEMBL8 'phage' particles

The host *E. coli* cells are transformed with pEMBL8 and should be subsequently infected with a helper M13 phage, to provide the necessary replicative enzymes

ssDNA fragments up to 10 kb in length can be cloned within this systm

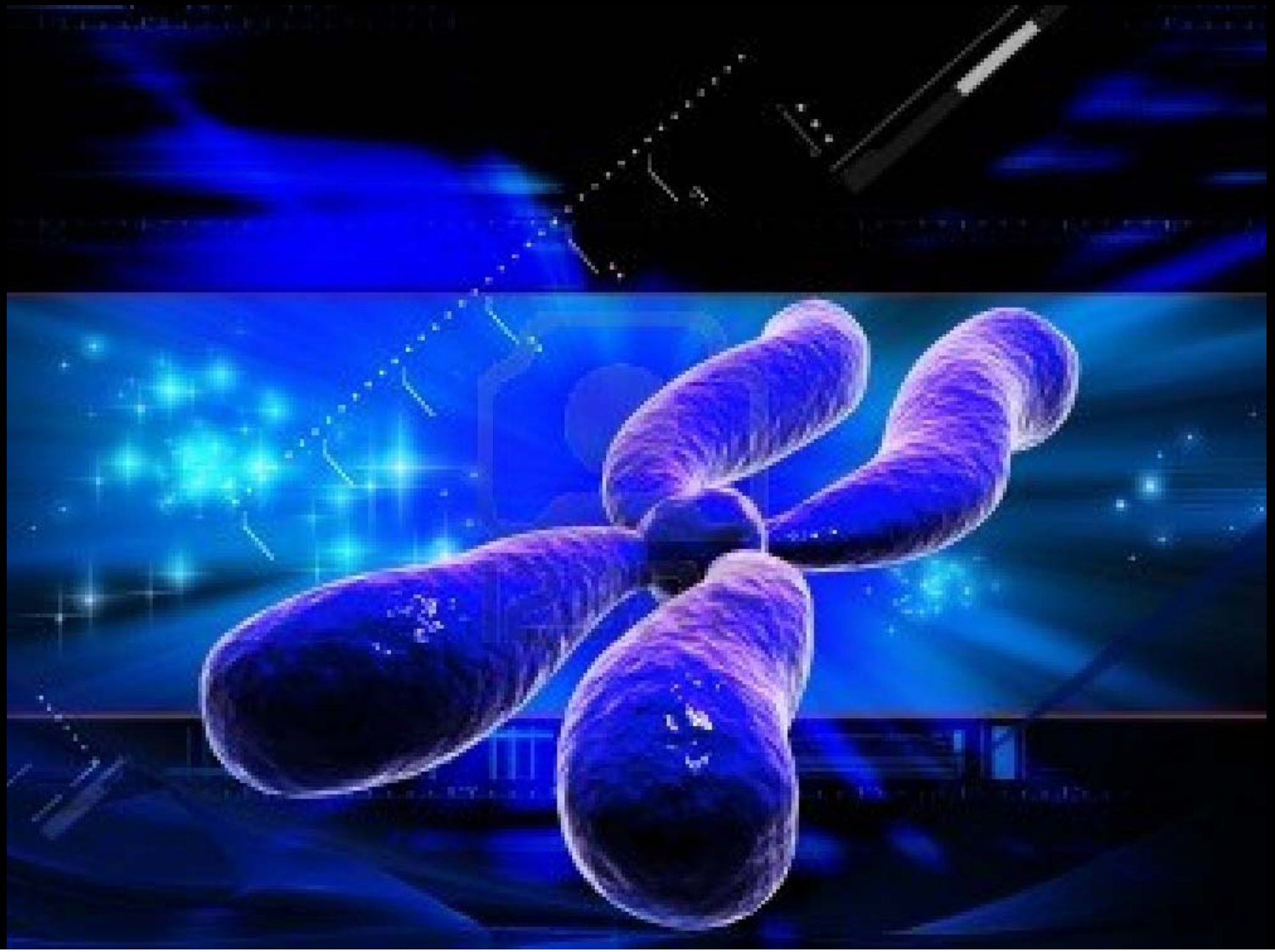


HIGH-CAPACITY VECTOR SYSTEMS

1. Genetic engineering - Neelam Pathak
2. Gene Cloning and DNA Analysis (6th Edn) - T.A. Brown
3. Molecular biology - David P Clark
4. Genomes(3rd Edn) - T.A. Brown
5. Principles of gene manipulation and genomics –Primrose
6. Genetics - Robert J Brooker

- ✓ A vector system that carries very large inserts (>100 kbp)
- ✓ helpful for the analysis and cloning of complex eukaryotic genomes.
- ✓ these types of vectors are used for creating libraries, genome sequencing, etc.

Artificial Chromosomes



Artificial Chromosomes

For large eukaryotic genomes, researchers often begin with vectors that can accept chromosomal DNA inserts of very large size.

most plasmid and viral vectors can accommodate inserts only a few thousand to perhaps tens of thousands of nucleotides in length.

If a plasmid or viral vector has a DNA insert that is too large, it will have difficulty with DNA replication and is likely to suffer deletions in the insert.

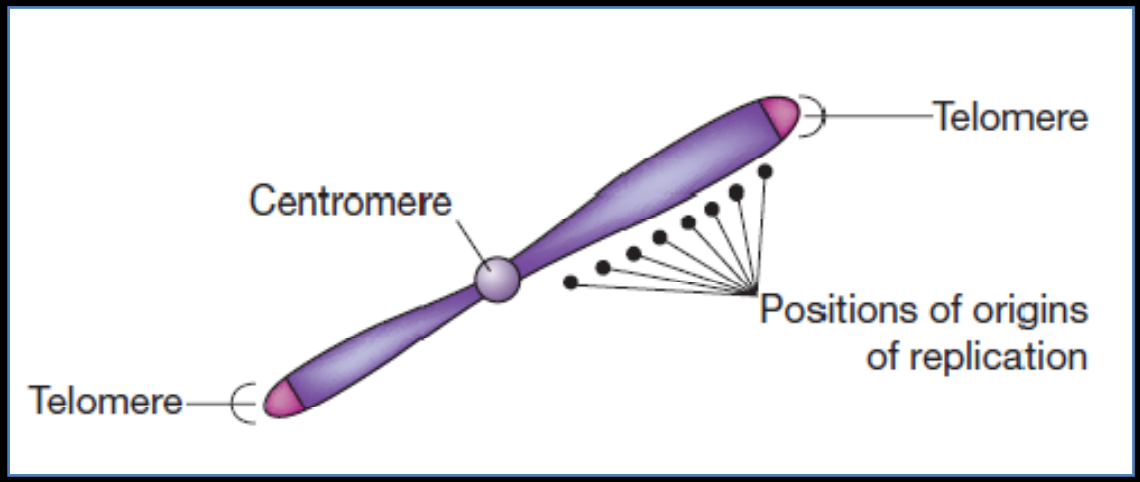
Analysis of the genomes of higher organisms requires the cloning of much larger fragments than for bacteria.

Therefore larger DNA fragments require special vectors.

"artificial chromosomes" have been developed to carry huge lengths of eukaryotic DNA.

key components of artificial chromosomes:

- ✓ **Centromere:** required for the chromosome to be distributed correctly to daughter cells during cell division.
- ✓ **Telomeres:** structures at the ends of a chromosome, which are needed in order for the ends to be replicated correctly and which also prevent the chromosome from being nibbled away by exonucleases.
- ✓ **Origins of replication:** which are the positions along the chromosome at which DNA replication initiates.

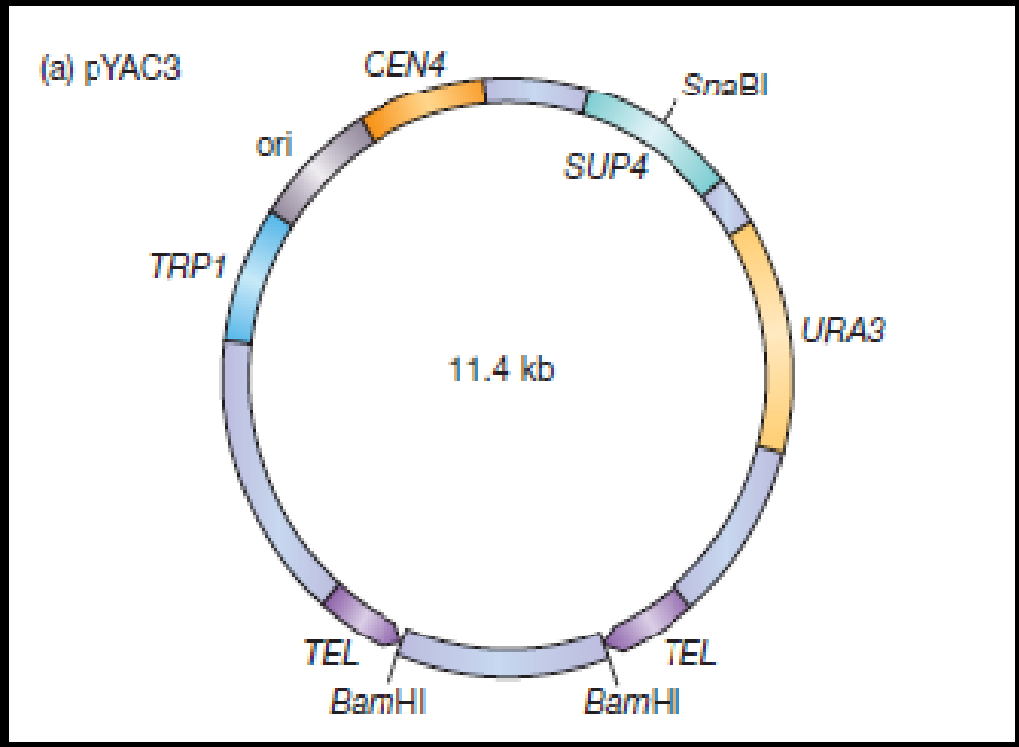


These individual components are isolated, joined together *in vitro*, creating the artificial chromosome.

Types of artificial chromosomes: YAC, BAC, PAC and HAC.

Yeast Artificial Chromosome (YAC)

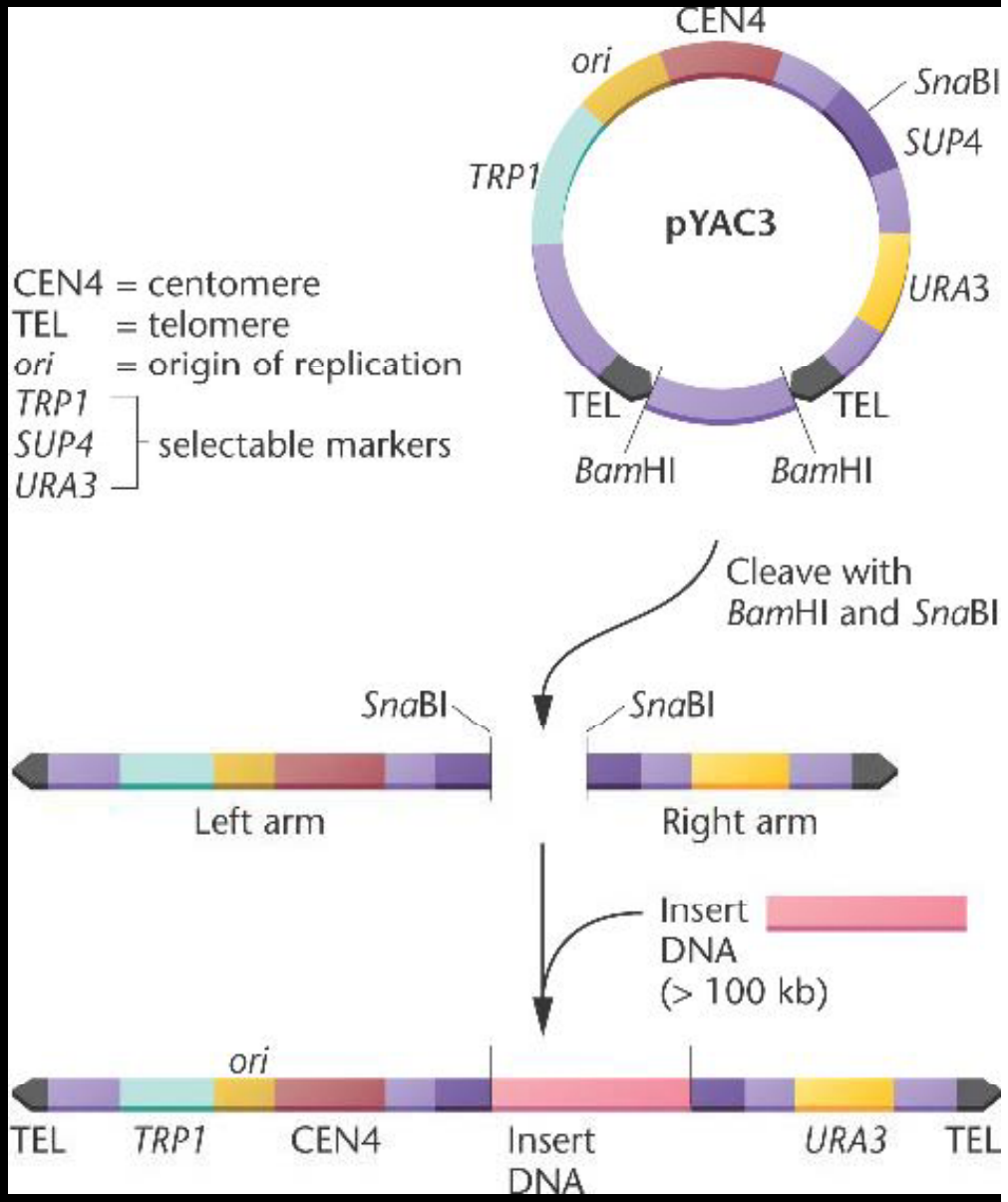
- ✓ first type of artificial chromosome to be developed
- ✓ Developed by David Burke, Georges Carle, and Maynard Olson in 1987.
- ✓ Huge segments of DNA, up to 2,000 kb or perhaps 2 million bp can be carried on YACs.
- ✓ Eg: pYAC3



YAC vectors – Example: pYAC3

pYAC3 is essentially a **pBR322 plasmid** into which a number of yeast genes have been inserted.

three genes, **URA3, SUP4** and **TRP1** function as the selectable **markers**

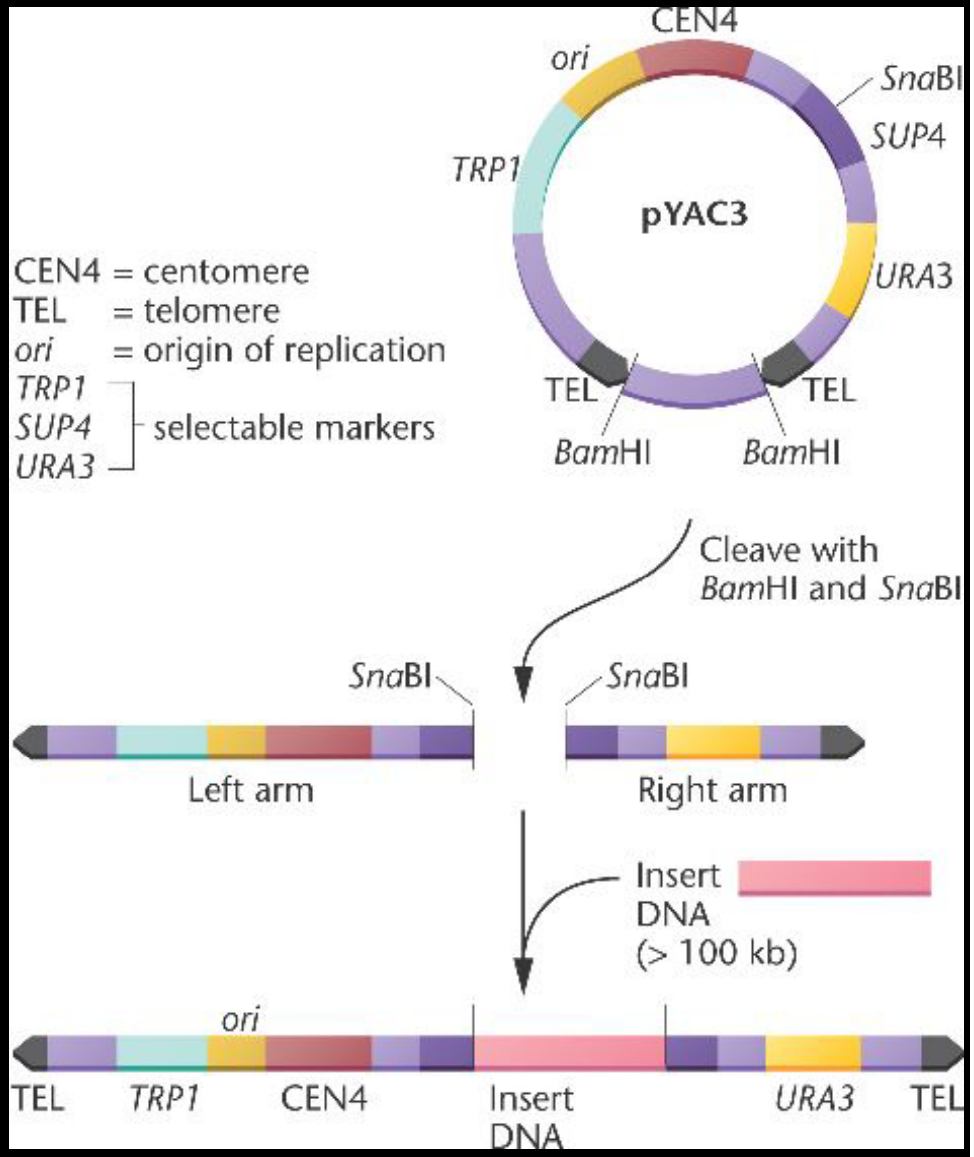


CEN4 is the centromere sequence of the chromosome

the telomeres, is provided by the two sequences called TEL.

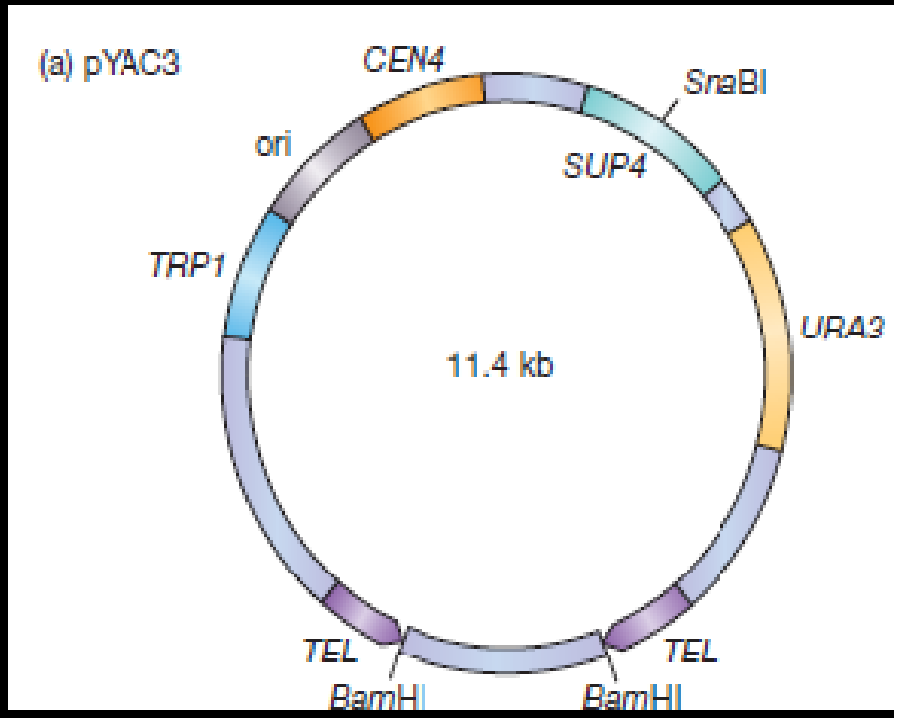
ORI is the bacterial origin of replication and ARS is the yeast origin of replication

Selectable marker is SUP4 into which new DNA is inserted for cloning

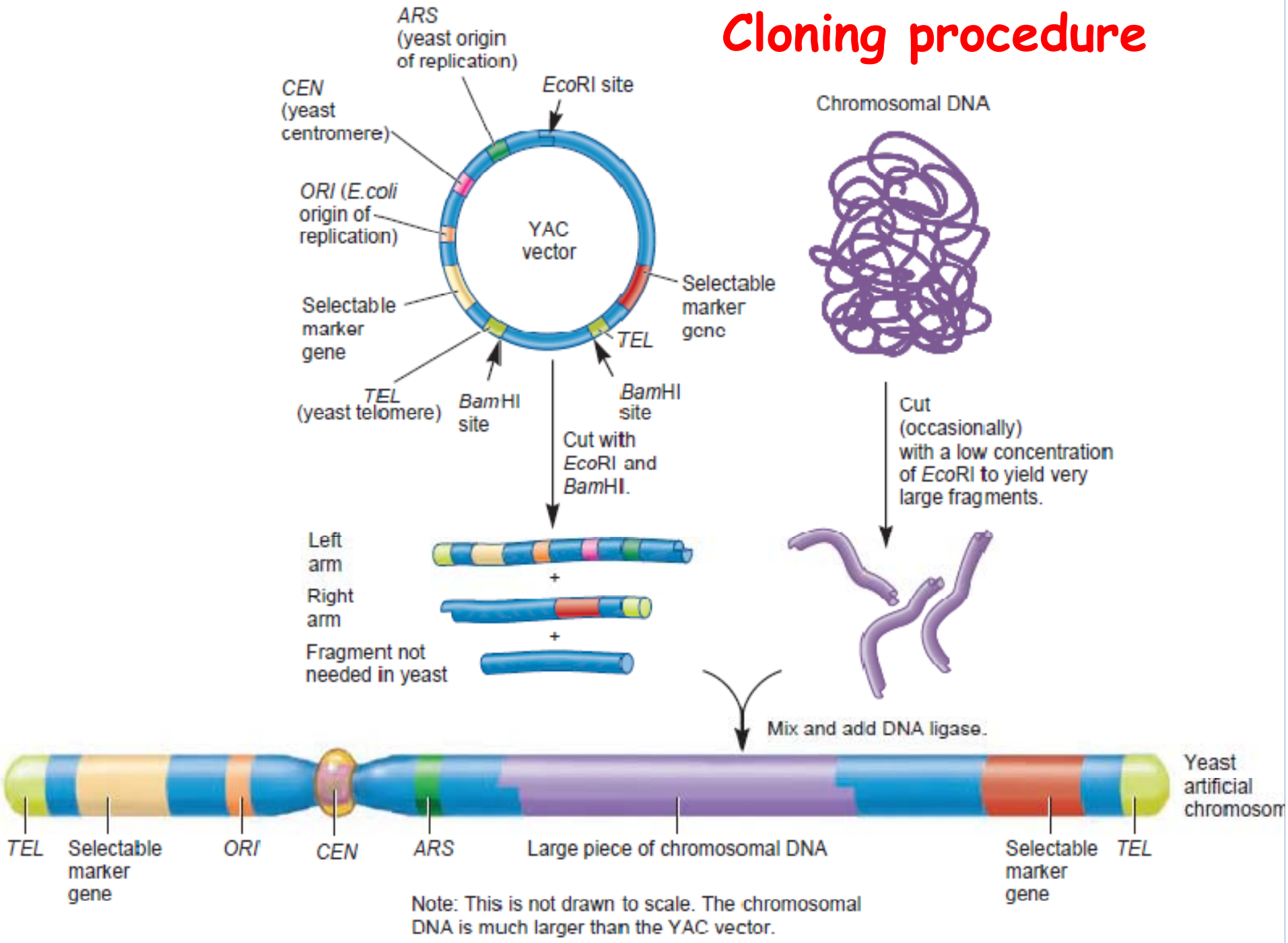


structure of a YAC vector (pYAC3)

- ✓ YAC vector contains two telomeres (*TEL*),
- ✓ centromere (*CEN*),
- ✓ bacterial origin of replication (*ORI*),
- ✓ yeast origin of replication (*ARS*),
- ✓ selectable markers (*TRP1'*, *URA3* & *SUP4*) and
- ✓ unique cloning sites that are each recognized by a single restriction enzyme.



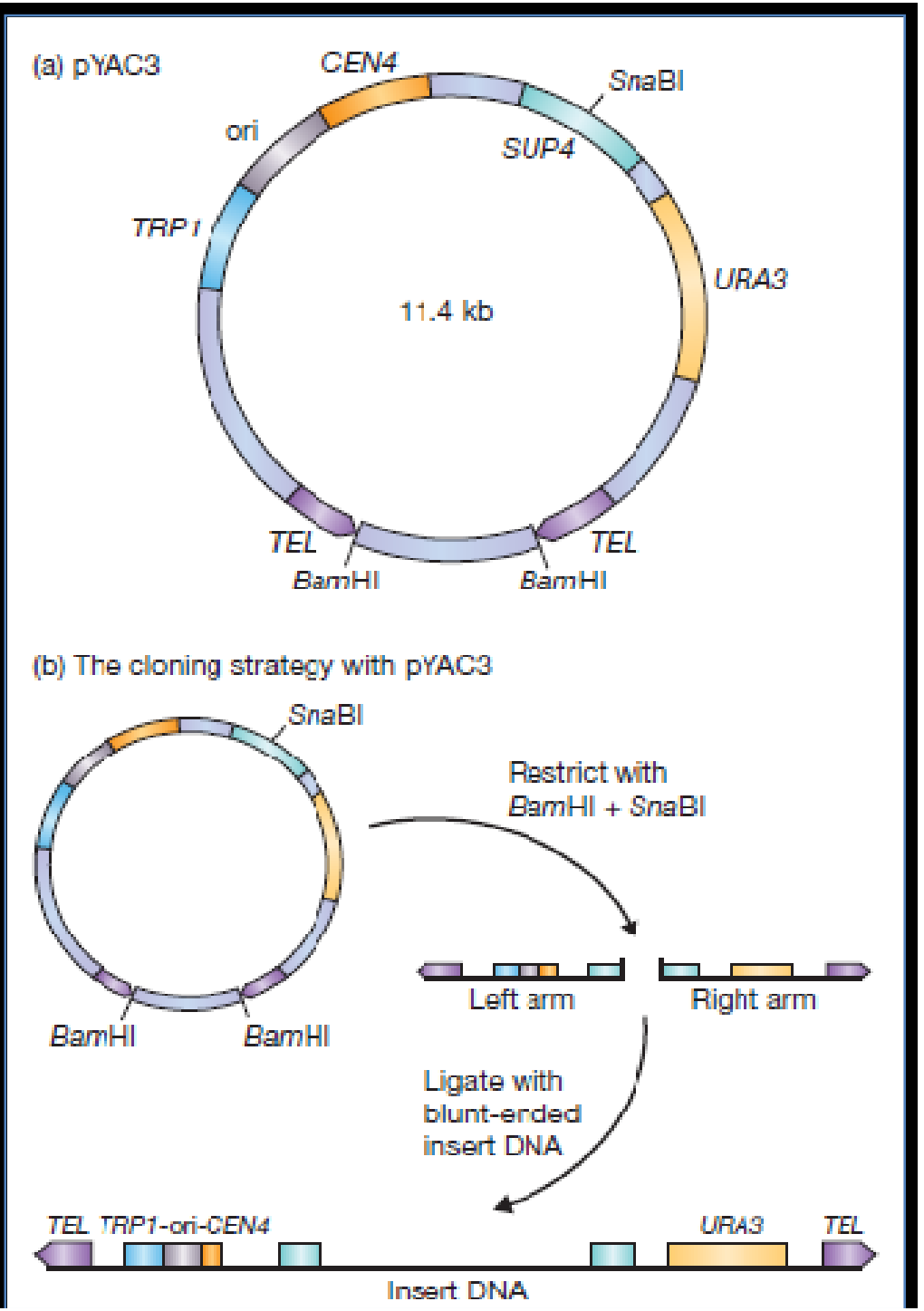
Cloning procedure



The YAC has two forms, a circular form for growing in bacteria, and a linear form for growing in yeast.

The circular form can be manipulated and grown like any other plasmid in bacteria since it has a bacterial origin of replication and an antibiotic resistance gene.

In order to use this in yeast, the circular form is isolated and linearized such that the yeast telomere sequences are on each end.



Applications of YAC vectors

- Cloning vehicles that propagate in eukaryotic host cells as eukaryotic chromosomes

- Can be routinely used to clone 600 kb fragments, and special types can handle DNA up to 1400 kb length

- Allows to study the functions and expression of genes that are very long

- Under some circumstances, YACs can be propagated in mammalian cells, enabling functional analysis to be done in the organism in which the gene normally resides

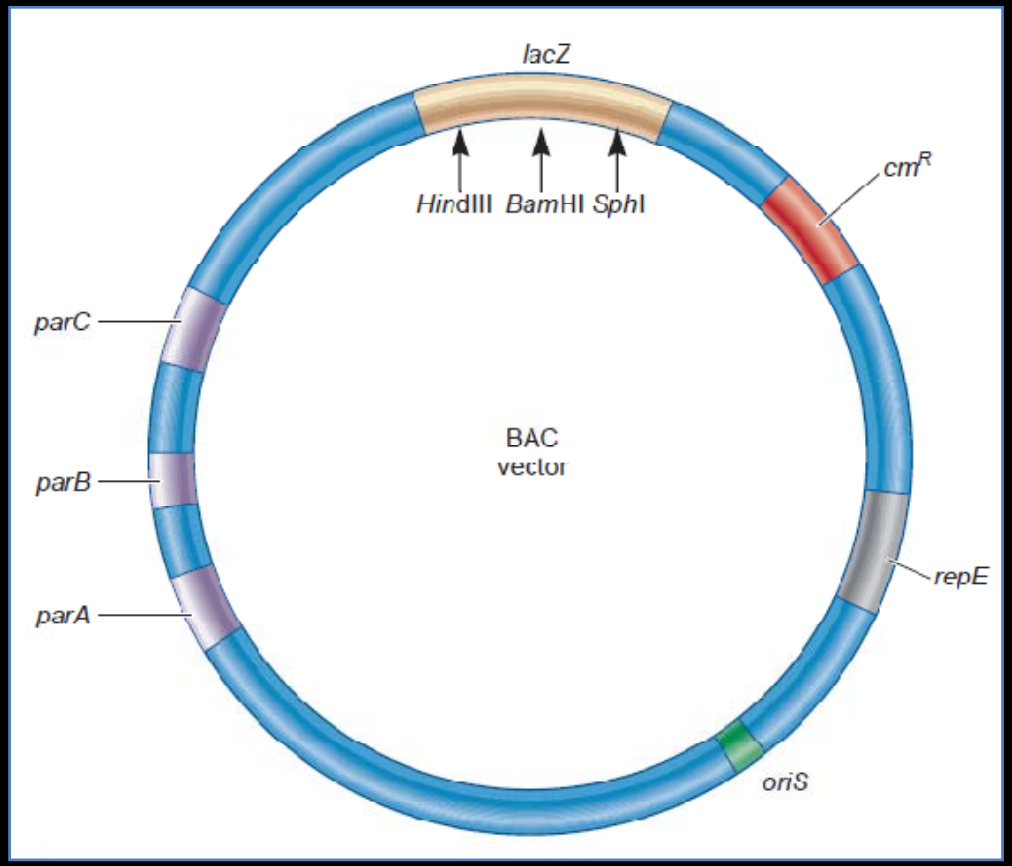
- YACs are very important in the creation of gene libraries

Disadvantages:

- **Insert instability** – cloned DNA sometimes become rearranged by intramolecular recombination

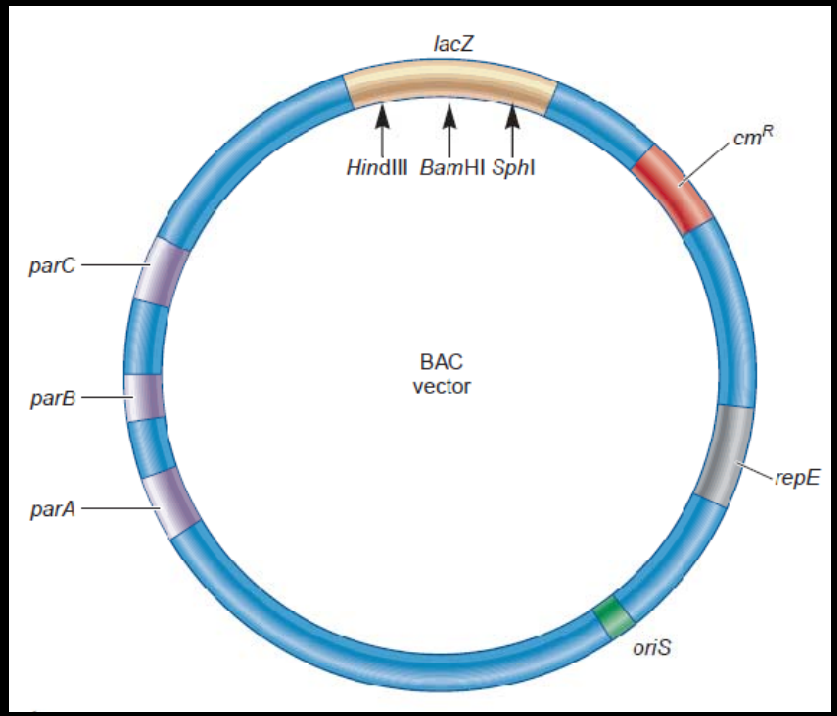
Bacterial Artificial Chromosomes (BAC)

- BACs were developed from F plasmid of *E.coli.*
- BACs can contain inserts up to 300 kbp or longer.



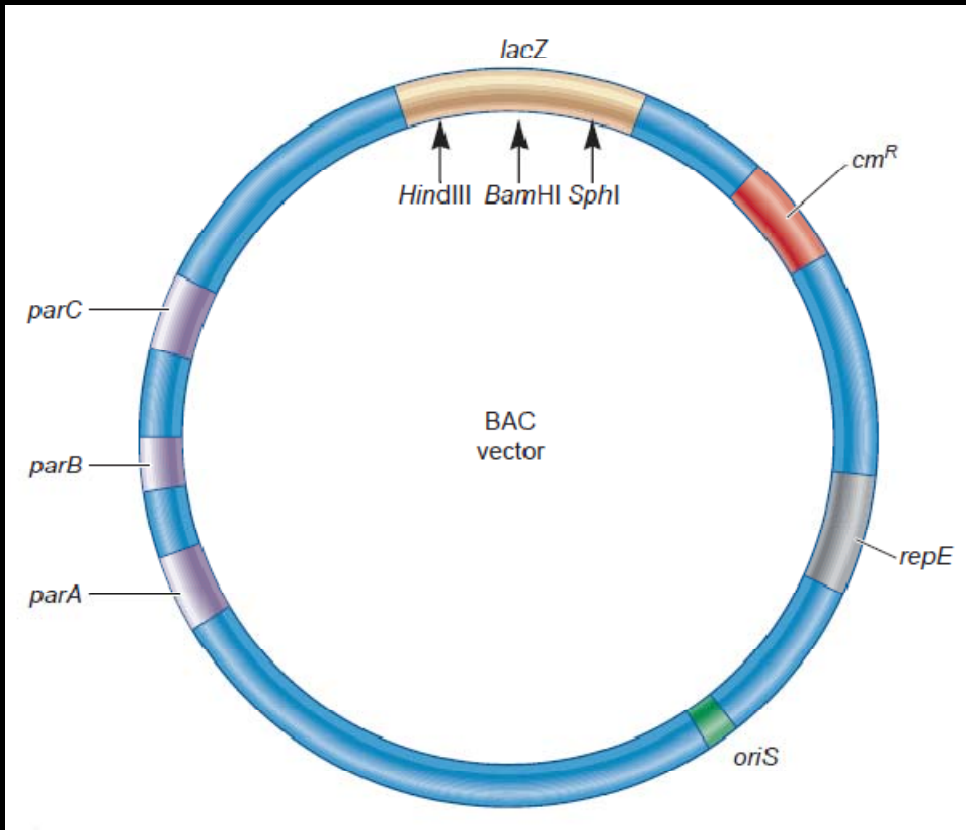
Structure

- ✓ origin of replication is *oriS*
- ✓ the *repE* gene encodes a protein essential for replication at *oriS*.
- ✓ *parA*, *parB*, and *parC* genes encode proteins required for the proper segregation of the vector into daughter cells.
- ✓ chloramphenicol resistance gene (cmR) *and* lac Z' genes are the selectable markers
- ✓ Lac Z' contains MCS into which the foreign DNA to be cloned is inserted



contains unique restriction enzyme sites, such as *Hin*dIII, *Bam*HI, and *Sph*I; are located within the *lacZ* gene.
BACs have low copy number origin of replication and allow replication of clones at one copy per cell.

The rDNA molecules are introduced into host cell by electroporation.



P1-derived Artificial Chromosome (PAC)

- ✓ Another cloning vector used for larger eukaryotic DNA segments is the PAC.
- ✓ PAC combine the features of bacteriophage P1 vectors and BACs
- ✓ PACs have insert capacity in range of 70- 300 kb (usually ~150 kb).

- ✓ PACs have a low copy number origin of replication allow replication of clones at one copy per cell.
- ✓ The rDNA molecules are introduced into host cell by electroporation.
- ✓ Vector with foreign DNA to be cloned are ligated and packaged into phage and allowed to infect *E.coli.*

Human Artificial Chromosome (HAC)

- ✓ HAC is a microchromosome, with 6-10 Mbp in size and able to carry large DNA segments.
- ✓ First appeared in 1997

- ✓ A human artificial chromosome (HAC) is a microchromosome that can act as a new chromosome in human cells.
- ✓ That is, instead of 46 chromosomes, the cell could have 47 with the 47th being very small, roughly 6-10 megabases in size, and able to carry new genes.
- ✓ They are useful in expression studies as gene transfer vectors and are a tool for elucidating human chromosome function.
- ✓ HACs considered as a promising system for gene delivery and expression with a potential to overcome many problems caused by the use of viral-based gene transfer systems.

There are two approaches for the construction of HAC:

1. bottom-up approach
2. top-down approach

bottom-up approach

Also known as assembly approach:

The "bottom-up construction" strategy involves the *de novo* construction of HACs by introducing necessary DNA elements for the maintenance of chromosome function into cells.

involved generating HACs by introducing defined chromosomal sequences including teleomere, centromere, origins of replication, etc.

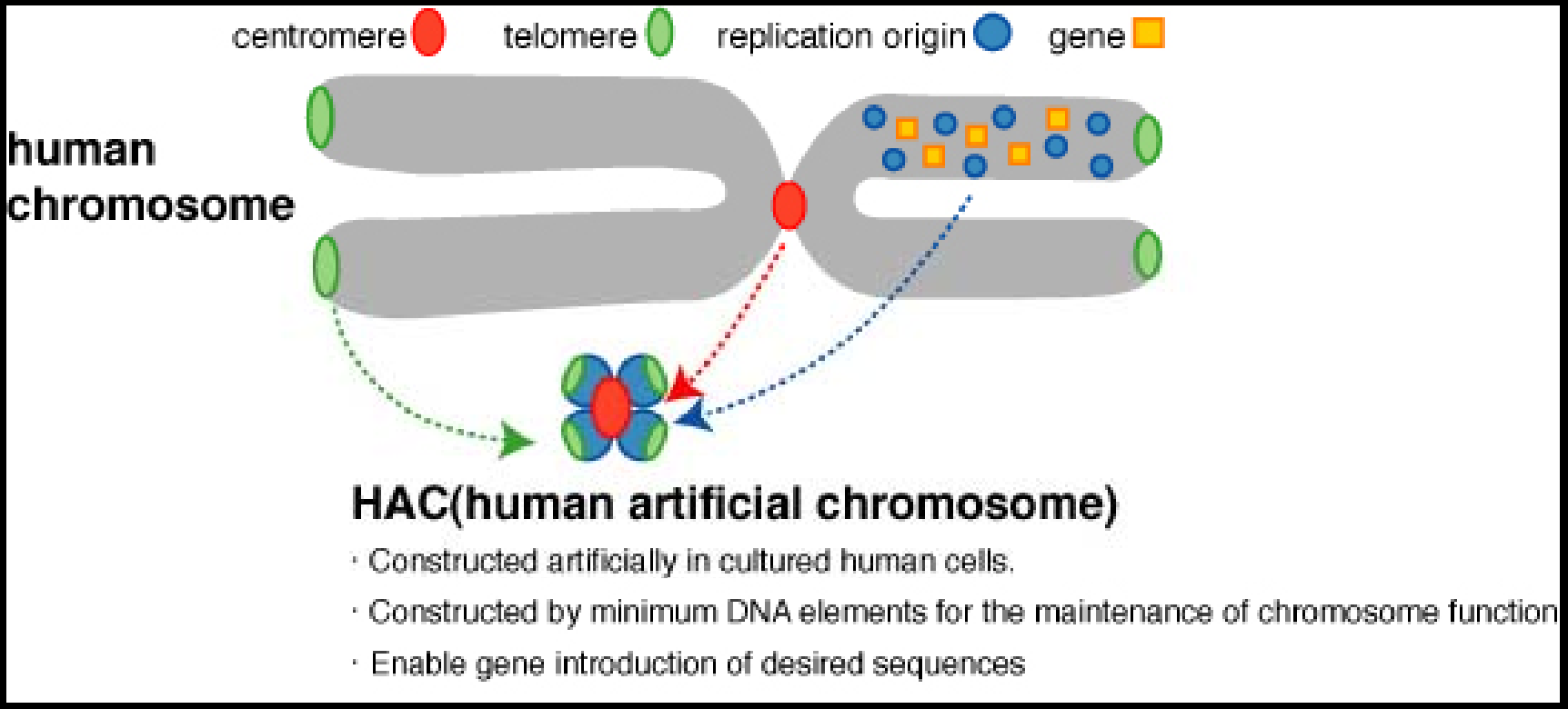
top-down' approach

Also known as TACF (telomere associated chromosome fragmentation)

"top-down construction" refers to the truncation of natural chromosomes into smaller sizes

involved modifying natural chromosomes into smaller defined microchromosomes in cultured cells.

- ✓ Within the cell, HAC maintains only one copy per cell.
- ✓ In 2011, human artificial chromosome called **21HAC** was reported - is based on a stripped copy of human chromosome 21, producing a chromosome 5Mb in length



SHUTTLE VECTORS & EXPRESSION VECTORS

1. Genetic engineering - Neelam Pathak
2. Molecular biology - David P Clark

SHUTTLE VECTORS

It is possible to insert two origins of replication into plasmid, one for E.coli and other for the chosen host.

Such vectors thus able to replicate in E.coli using one origin of replication and in chosen host using alternative replication origin.

Such a vector is called shuttle vector because it can be transferred back and forth between two different species. E.g. bacteria and yeast, or mammalian cells and bacteria.

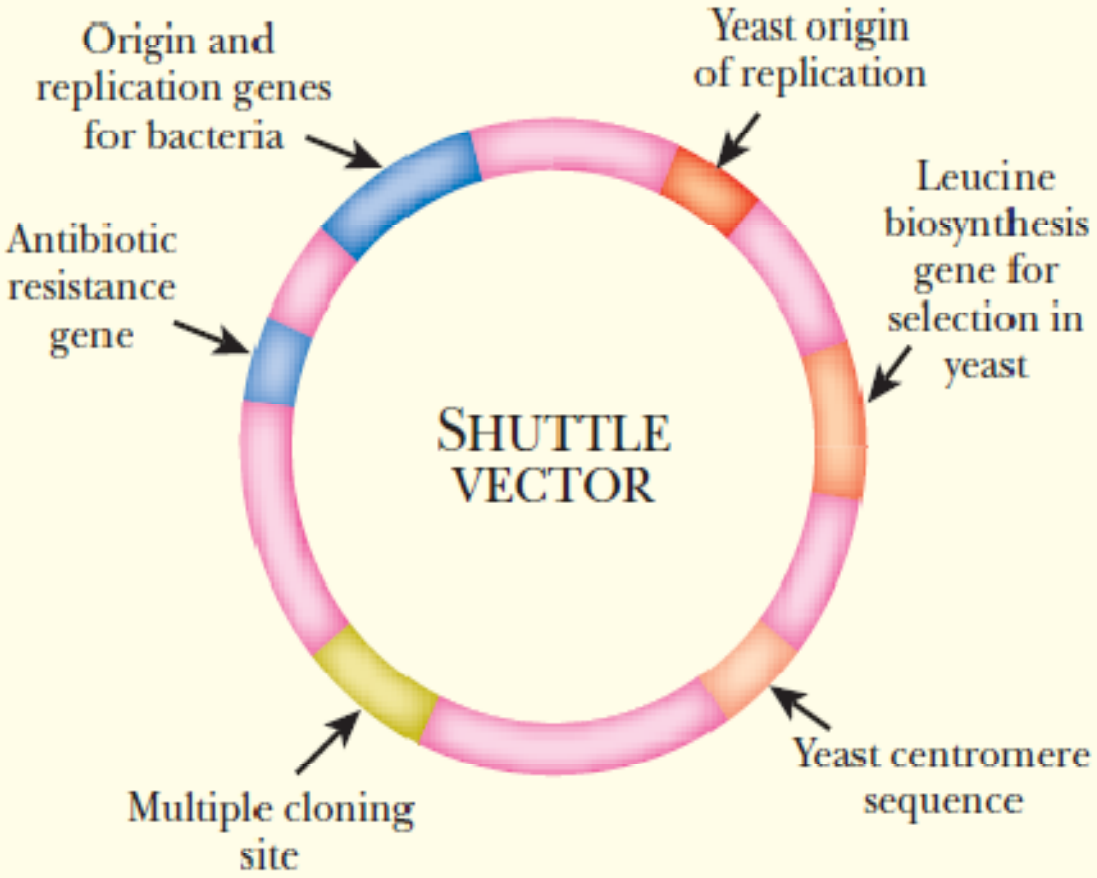
Shuttle vectors can replicate in two different organisms, and have the appropriate origins of replication.

Hence one can clone a gene in bacteria, may be modify it or mutate it in bacteria, and test its function by introducing it into yeast or animal cells.

The first such vector was derives from fusion between the E.coli vector pBR322 and the *Saccharomyces* plasmid pC194 and pUB110.

FIGURE 22.17 ***Shuttle Vector for Yeast***

In order for a shuttle vector to grow in both yeast and *E. coli*, it must have several essential elements: two origins of replication, one for *E. coli* and one for yeast; a yeast centromere sequence so that it is partitioned into the daughter cells during yeast replication ; selectable markers for both yeast and *E. coli*; and a multiple cloning site for inserting the gene of interest.



EXPRESSION VECTORS

Once a gene has been cloned into a vector it may or may not be expressed.

If both structural gene and promoter were cloned on the same segment of DNA the gene may well be expressed.

On the other hand, if only the structural gene was cloned then expression will depend on whether a promoter is provided by the plasmid.

Expression vectors are specifically designed to place the cloned gene under the control of a strong promoter.

Basic Features of Expression Vectors:

1. Efficient and Regulatable Promoter for Transcription

Eg:1) *lac* UV promoter and its inducer (IPTG)
Eg:2) lambda left promoter (PL) and its repressor (cI protein)

2. Translational Signal sequences (Ribosome binding site, start codon, and stop codon)

3. High copy number: more the copy number of vector, more the amount of cloned gene and hence more the product formation.

FIGURE 22.31 ***Expression Vectors Can Have Tightly Regulated Promoters***

An expression vector contains sequences upstream of the cloned gene that control transcription and translation of the cloned gene. The expression vector shown uses the *lac* promoter, which is very strong, but inducible. To stimulate transcription, an artificial inducer molecule called IPTG is added. IPTG binds to the LacI repressor protein which then detaches from the DNA. This allows RNA polymerase to bind. Before IPTG is added to the culture, the LacI repressor prevents the cloned gene from being expressed.