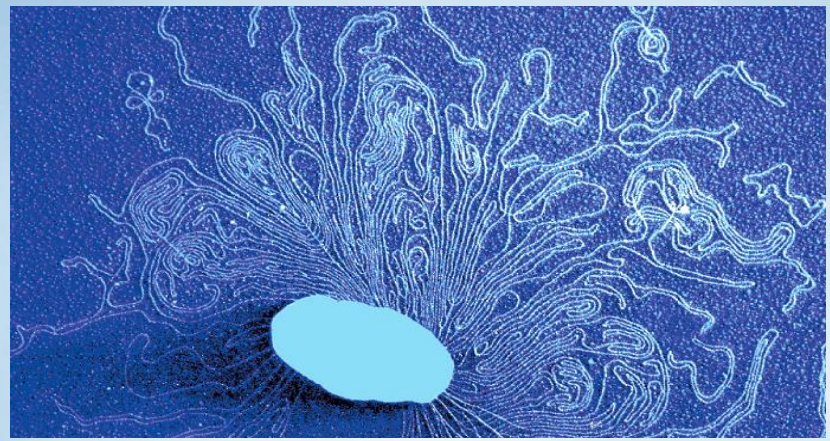
Cloning Vectors



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CLONING VECTORS

•Cloning vectors are DNA molecules that are used to "transport" cloned sequences between biological hosts and the test tube.

- Most vectors are genetically engineered.
- A vector is used to amplify a single molecule of DNA into many copes.

Cloning vectors share four common properties:

1. Ability to replicate.

2. Contain a genetic marker for selection.

3. Unique restriction sites to facilitate cloning of insert DNA.

4. Minimum amount of nonessential DNA to optimize cloning.

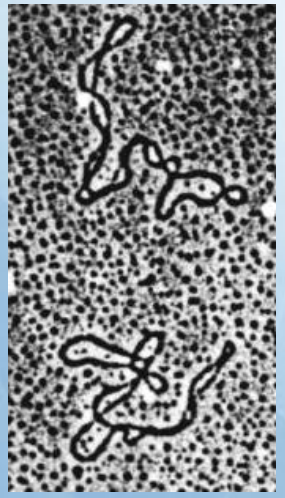
Types of vectors

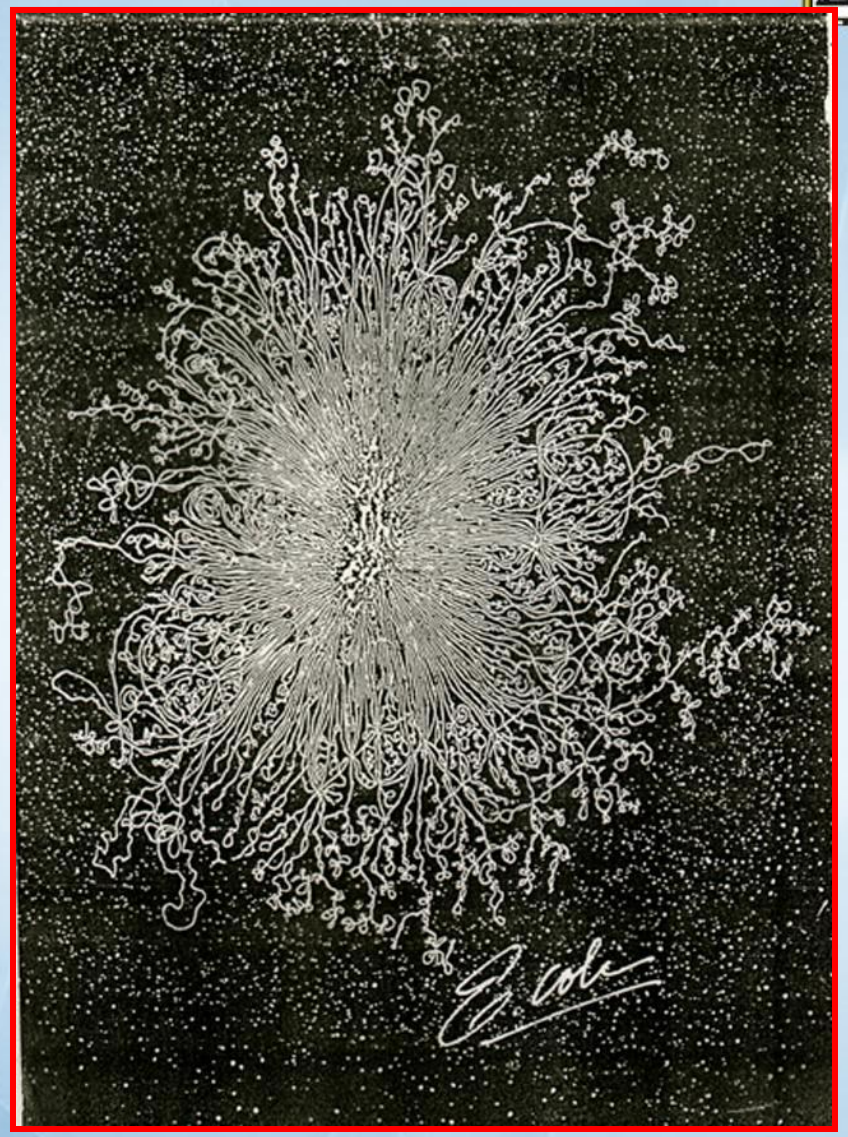
- Different types of cloning vectors are used for different types of cloning experiments.
- The vector is chosen according to the size and type of DNA to be cloned

	Maximum insert size (kilobases or kb [1000bp])
• Bacterial plasmid	6-12
• bacteriophage	25
• Cosmids	35
• yeast artificial chromosome	200-1000

Bacterial plasmids

- Most bacterial DNA is on a single large chromosome, but some DNA is in a small circle called a **plasmid**.



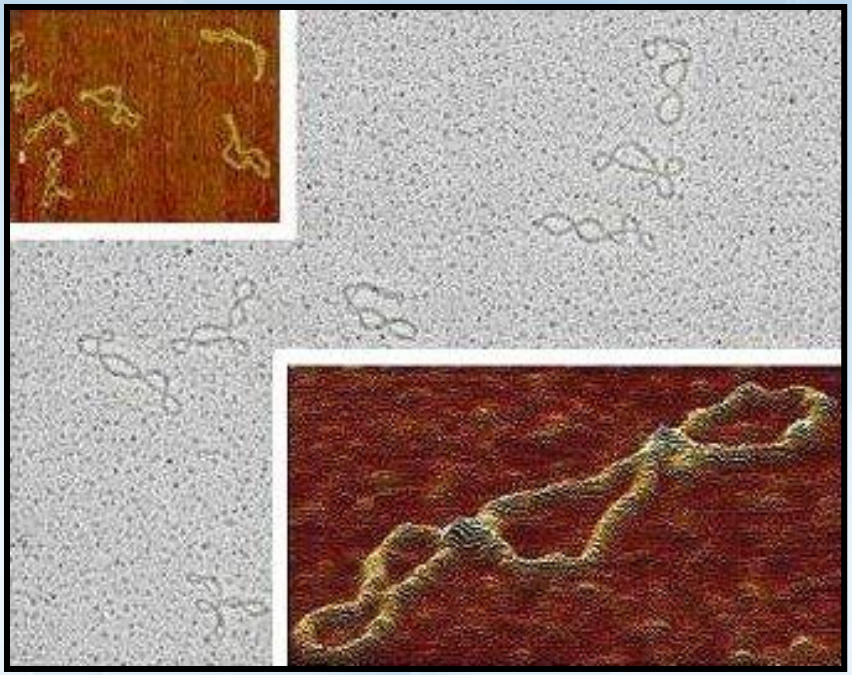


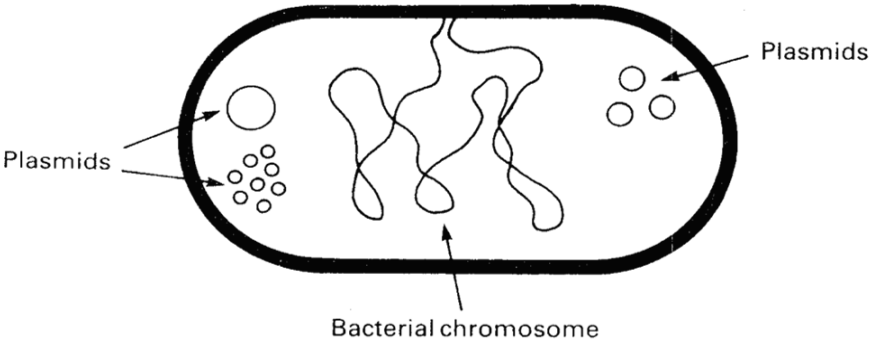
Bacterial Plasmids in Nature

Occur naturally in bacteria and usually carry genes that are useful but not essential to survival

There can be as many as several hundred copies of a single plasmid in each bacteria.

They can replicate independently of the host cell.





Plasmids: independent genetic elements found in bacterial cells.

Size and copy number

Table 2.1 Sizes of representative plasmids

Plasmid	Size		Organism
	Nucleotide length (kb)	Molecular wt (MDa)	
pBR345	0.7	0.46	*E. coli*
pBR322	4.362	2.9	*E. coli*
ColEl	6.36	4.2	*E. coli*
RP4	54	36	*Pseudomonas* + others
F	95	63	*E. coli*
TOL	117	78	*Pseudomonas putida*
pTiAch5	213	142	*Agrobacterium tumefaciens*

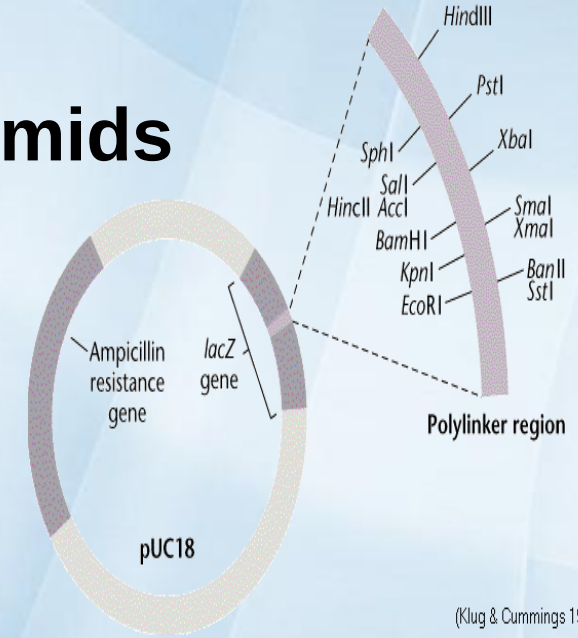
TABLE 4.2 Copy numbers of some plasmids

Plasmid	Approximate copy number
F	1
P1 prophage	1
RK2	4–7 (in *E. coli*)
pBR322	16
pUC18	~30–50
plJ101	40–300

PLASMID VECTORS

- Plasmid vectors are used to clone DNA ranging in size from several base pairs to several thousands of base pairs (100bp -10kb).

Features of many modern Plasmids



(Klug & Cummings 1997)

- Small size
- Origin of replication
- Multiple cloning site (MCS)
- Selectable marker genes
- Some are expression vectors and have sequences that allow RNA polymerase to transcribe genes
- DNA sequencing primers

SELECTIVE MARKER

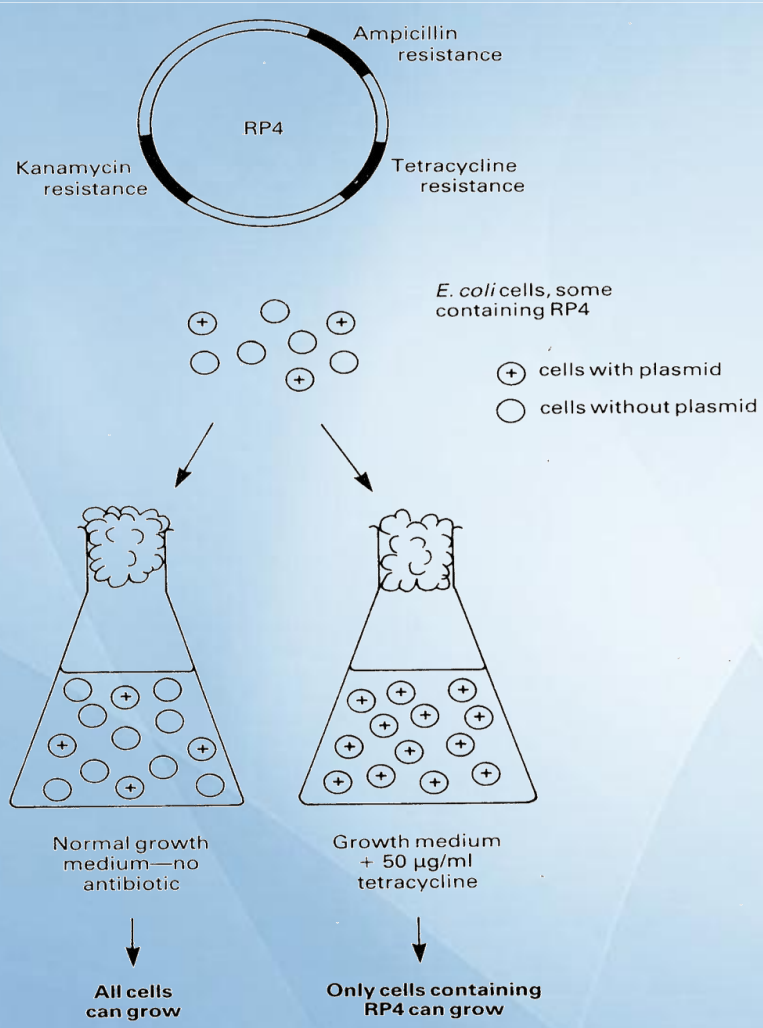
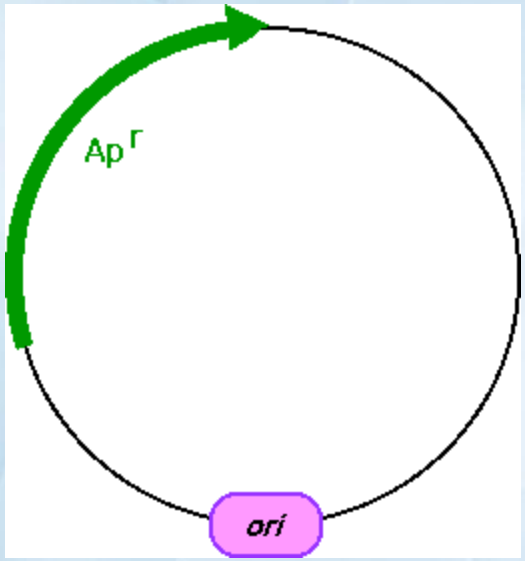
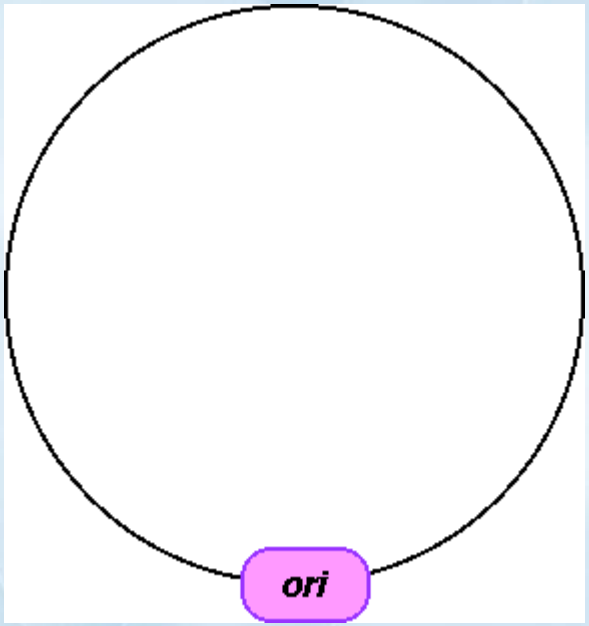


Figure 2.2 The use of antibiotic resistance as a selectable marker for a plasmid. RP4 (top) carries genes for resistance to ampicillin, tetracycline and kanamycin. Only those *E. coli* cells that contain RP4 (or a related plasmid) will be able to survive and grow in a medium that contains toxic amounts of one or more of these antibiotics.

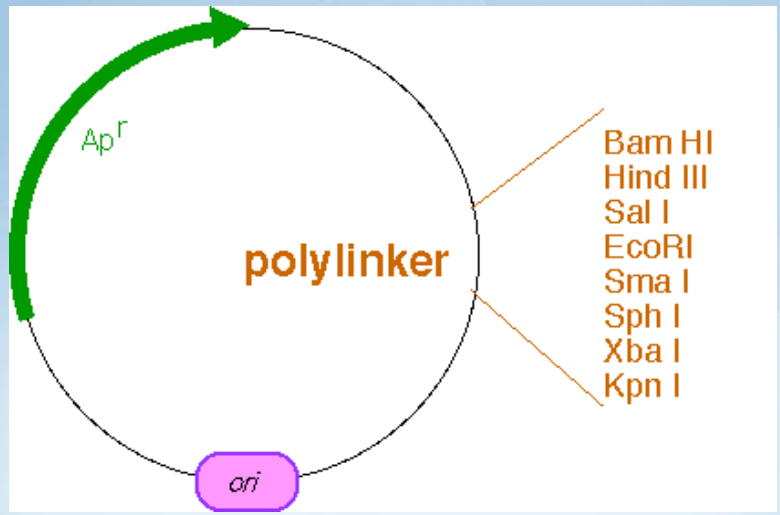


ORIGIN OF REPLICATION

- **Origin of replication** is a DNA segment recognized by the cellular DNA-replication enzymes.
- Without replication origin, DNA cannot be replicated in the cell.

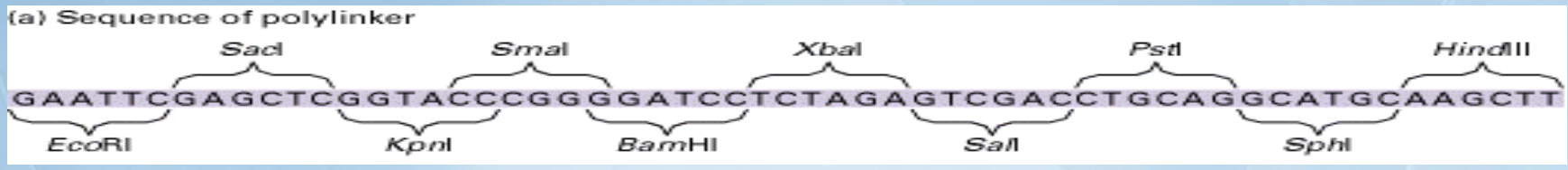


MULTIPLE CLONING SITE

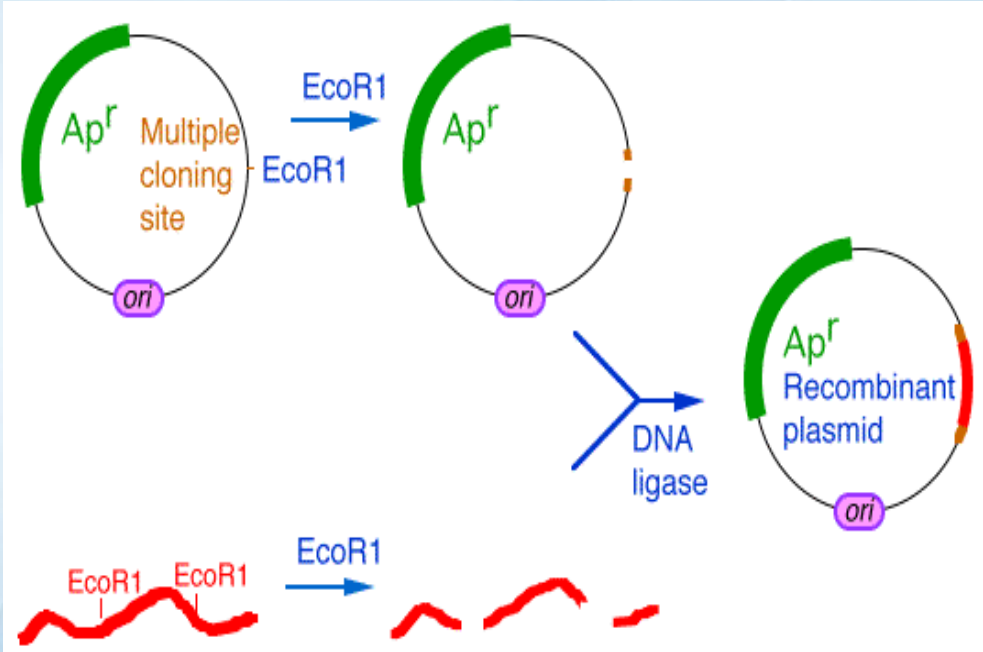


- Many cloning vectors contain a **multiple cloning site** or **polylinker**: a DNA segment with several unique sites for restriction endo- nucleases located next to each other
- Restriction sites of the polylinker are not present anywhere else in the plasmid.
- Cutting plasmids with one of the restriction enzymes that recognize a site in the polylinker does not disrupt any of the essential features of the vector

MULTIPLE CLONING SITE

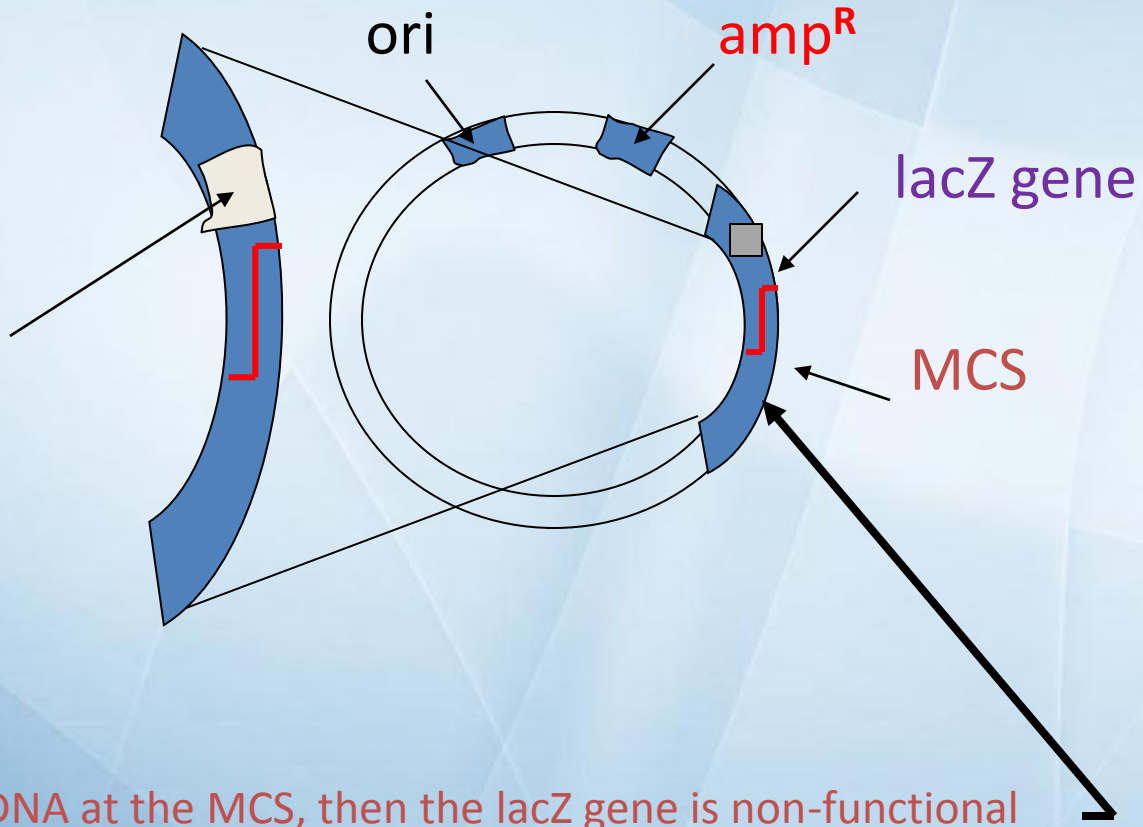


- **Gene to be cloned can be introduced into the cloning vector at one of the restriction sites present in the polylinker**



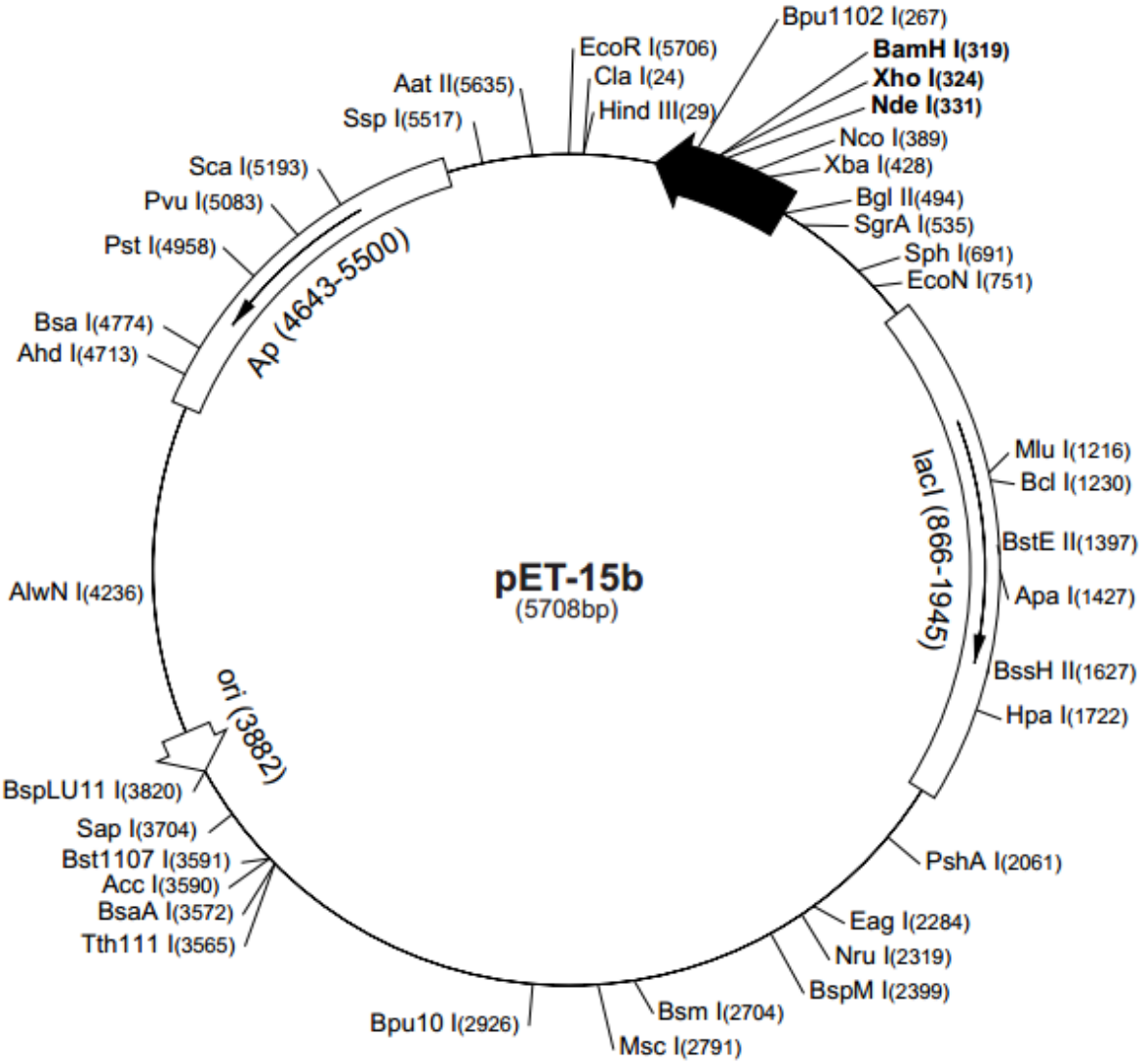
Practical Features of DNA Cloning Vectors (Plasmids)

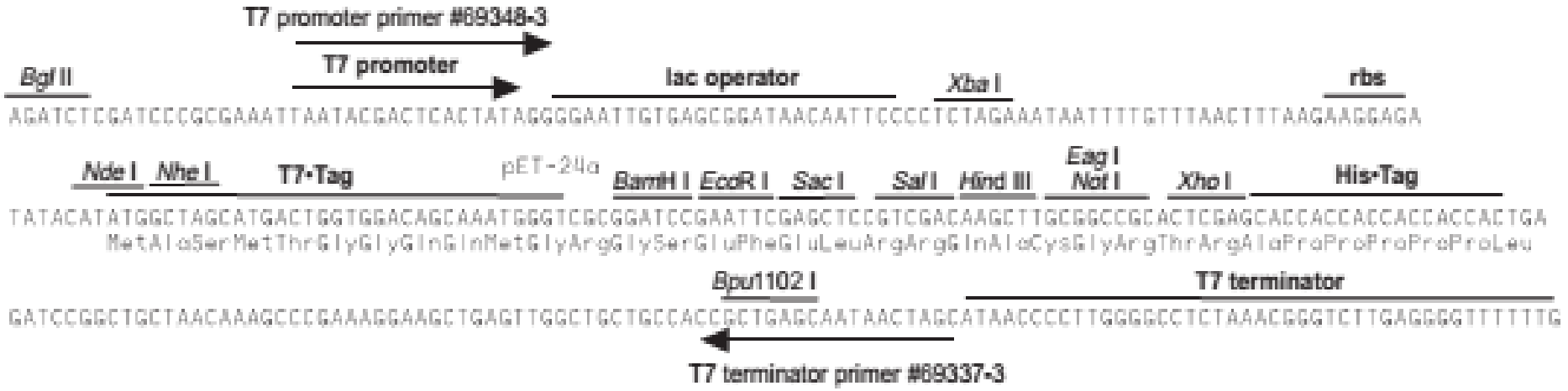
- origin of replication (ori)
- multiple cloning sites (MCS) or restriction sites
- selectable markers
- RNA polymerase promoter sequences



If plasmid picks up a foreign piece of DNA at the MCS, then the lacZ gene is non-functional

pET-15b sequence landmarks	
T7 promoter	463-479
T7 transcription start	452
His•Tag coding sequence	362-380
Multiple cloning sites	
(*Nde* I - *Bam*H I)	319-335
T7 terminator	213-259
lacI coding sequence	(866-1945)
pBR322 origin	3882
bla coding sequence	4643-5500



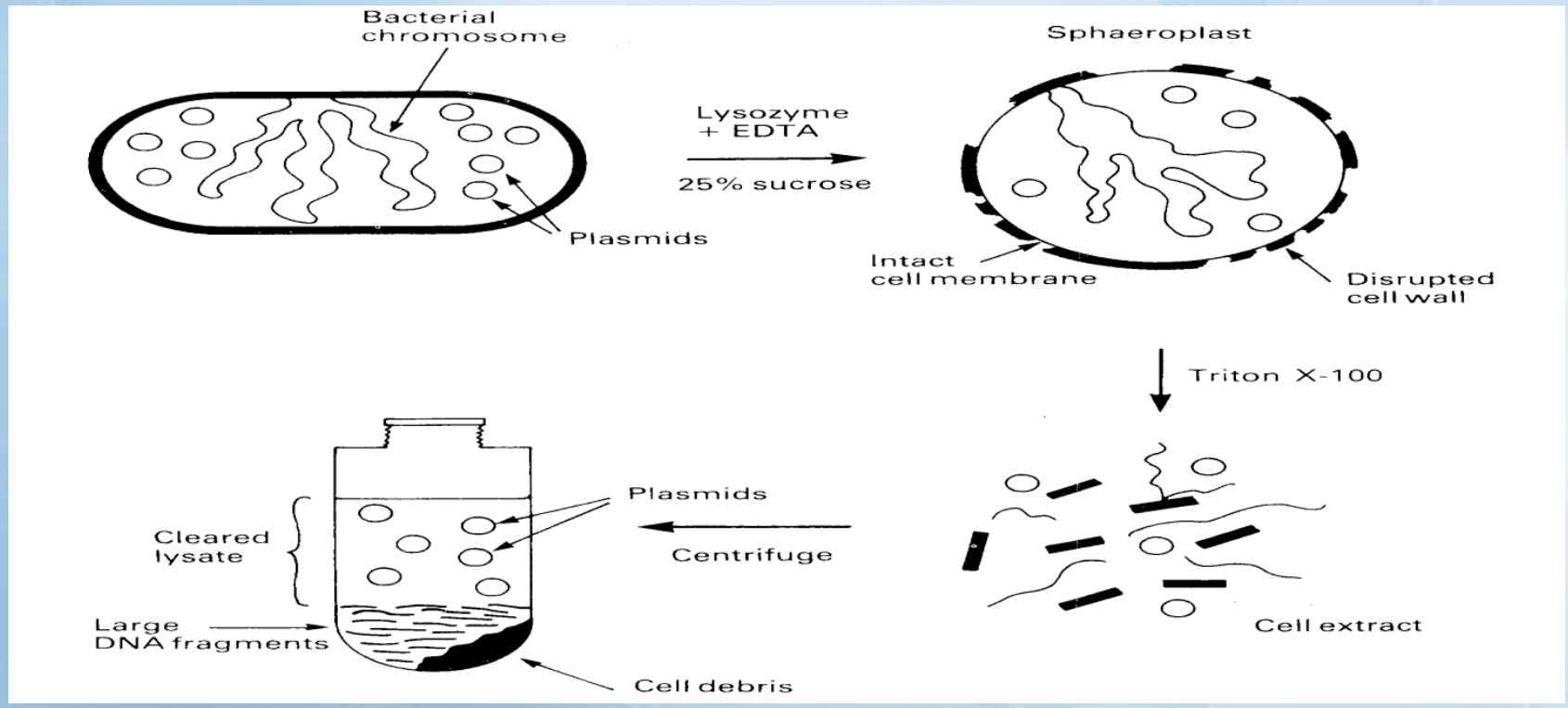

T7 promoter primer #69348-3
T7 promoter
Bgl II
lac operator
Xba I
rbs
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
Nde I
Nhe I
T7•Tag
pET-24a
BamH I
EcoR I
Sac I
Sal I
Hind III
Eag I
Not I
Xho I
His•Tag
TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA
MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeu
Bpu1102 I
T7 terminator
GATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
T7 terminator primer #69337-3

Plasmid Isolation from Bacteria

- تنميه المزرعة البكتيرية المحتوية على البلازميد على بيئة سائلة
- جمع الخلايا منها بالطرد المرطزى
- تحضير المستخلص الخلوى من هذة الخلايا (Cell extract)
- ويتم التخلص من البروتينات وازالة ال RNA.
- بالاضافة الى ذلك يجب فصل DNA البلازميد عن الكميات الكبيرة من DNA الكرموسومات البكتيرية الموجودة ايضا فى الخلايا.

Plasmid DNA isolation

- **- فصل DNA البلازميدات بناء على الحجم:**



2- فصل DNA البلازميدات بناء على الشكل:

Alkaline denaturation

استخدام الطرد المركزي في وجود الايثيديوم بروميد والسيزيوم كلوريد .

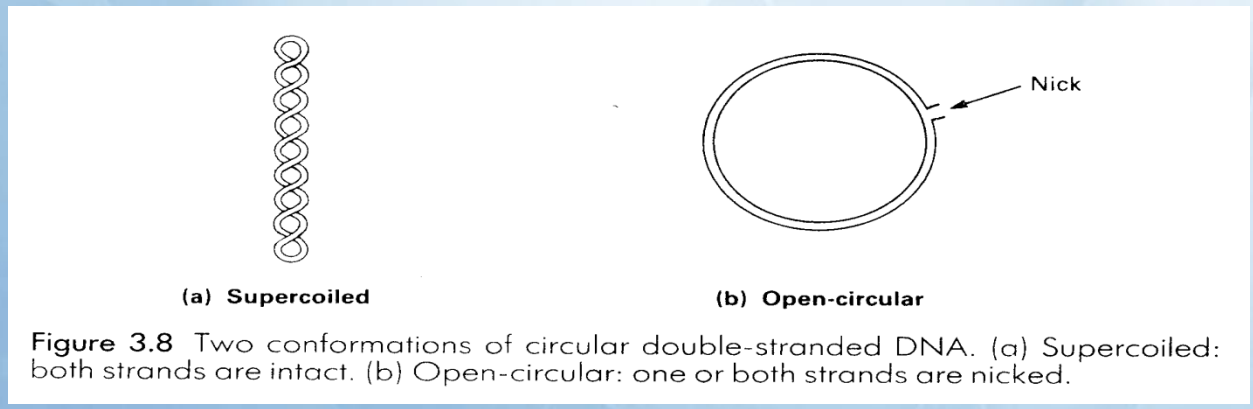
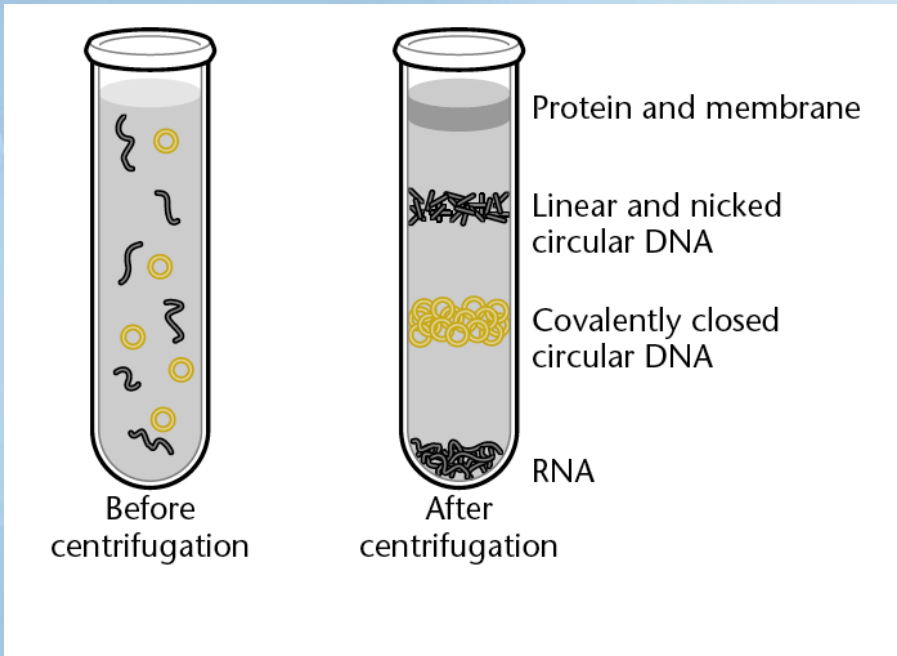
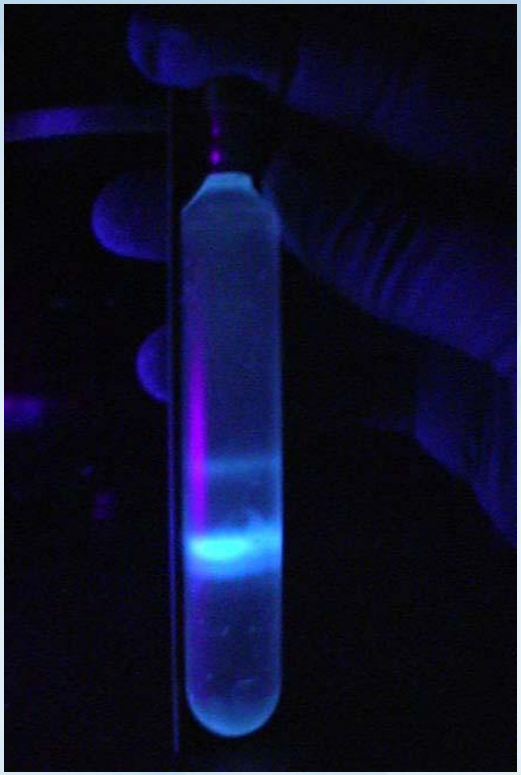


Figure 3.8 Two conformations of circular double-stranded DNA. (a) Supercoiled: both strands are intact. (b) Open-circular: one or both strands are nicked.

Conformations of Plasmid DNAs

"Old School method of purifying plasmid"





After 10 hrs centrifugation at 100,000 rpm (450,000 x*g*), two distinct bands, corresponding to **linear nuclear DNA** above and **circular DNA** below, are visible under ultraviolet light.

CsCl gradient with ethidium bromide and UV light.

Alkaline denaturation

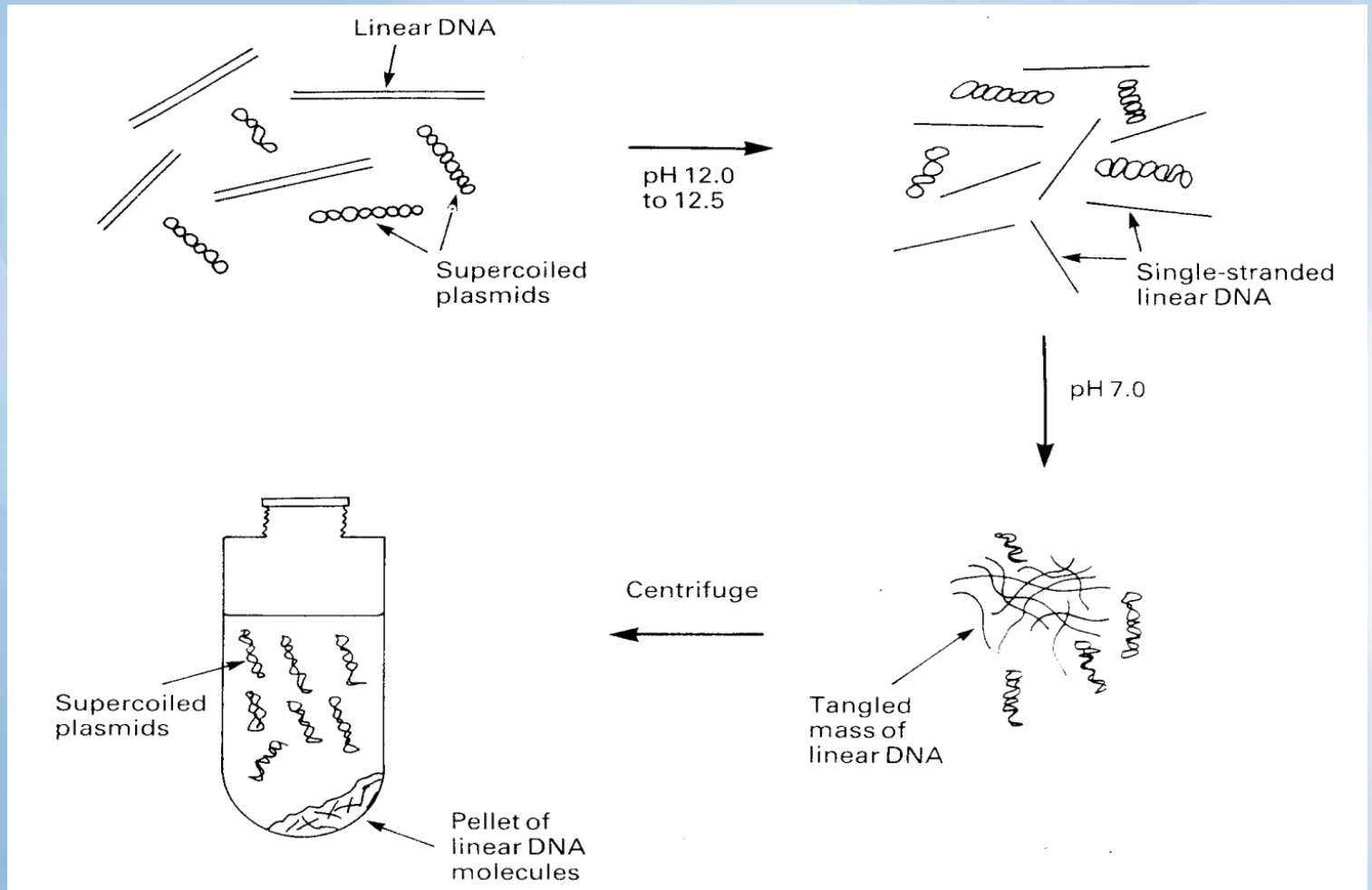
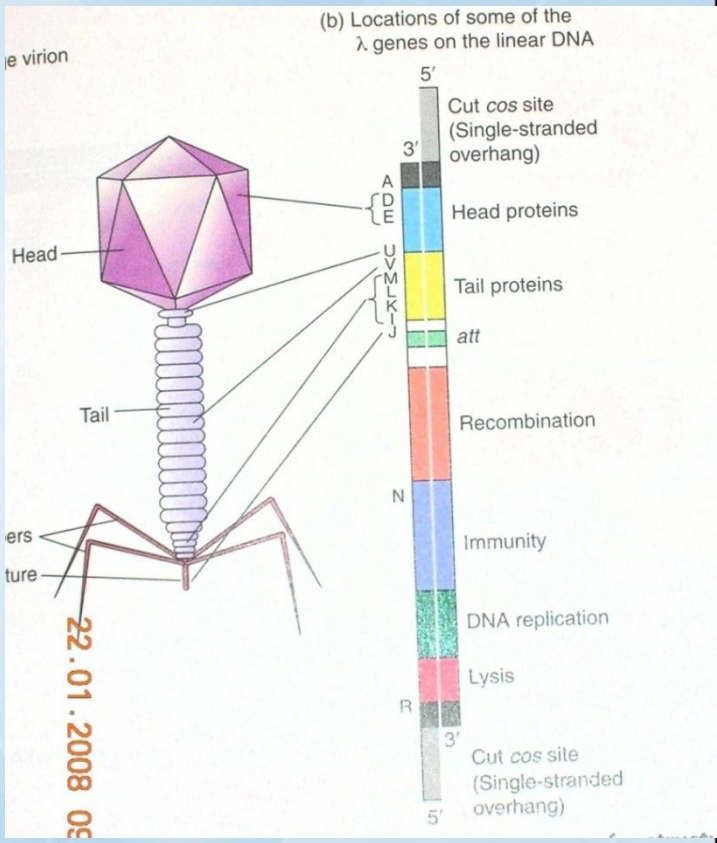


Figure 3.9 Plasmid purification by the alkaline denaturation method.

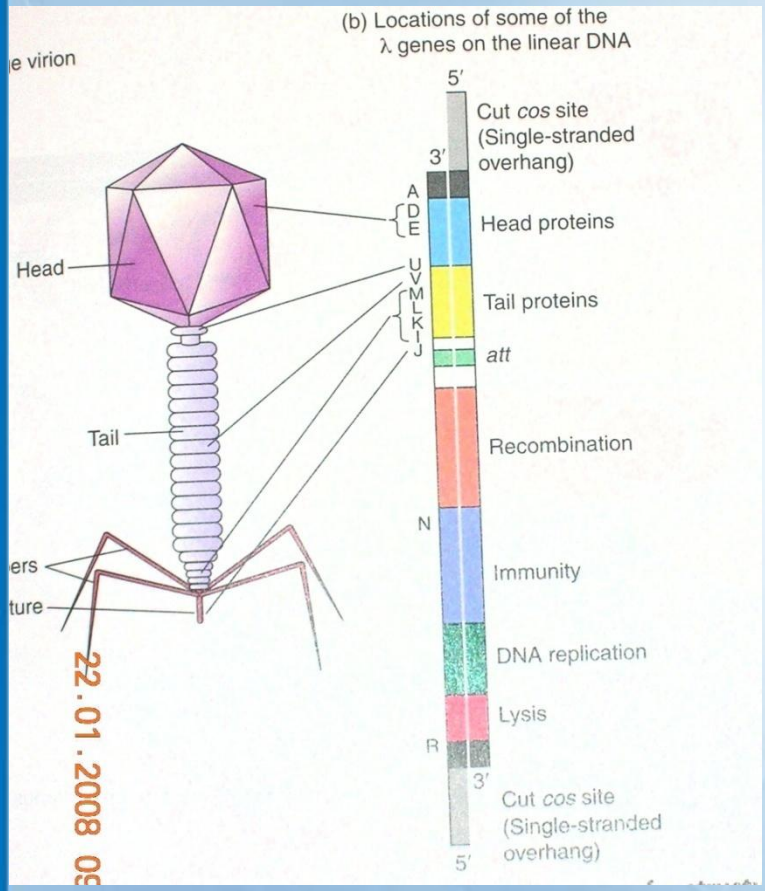
OTHER TYPES OF CLONING VECTORS

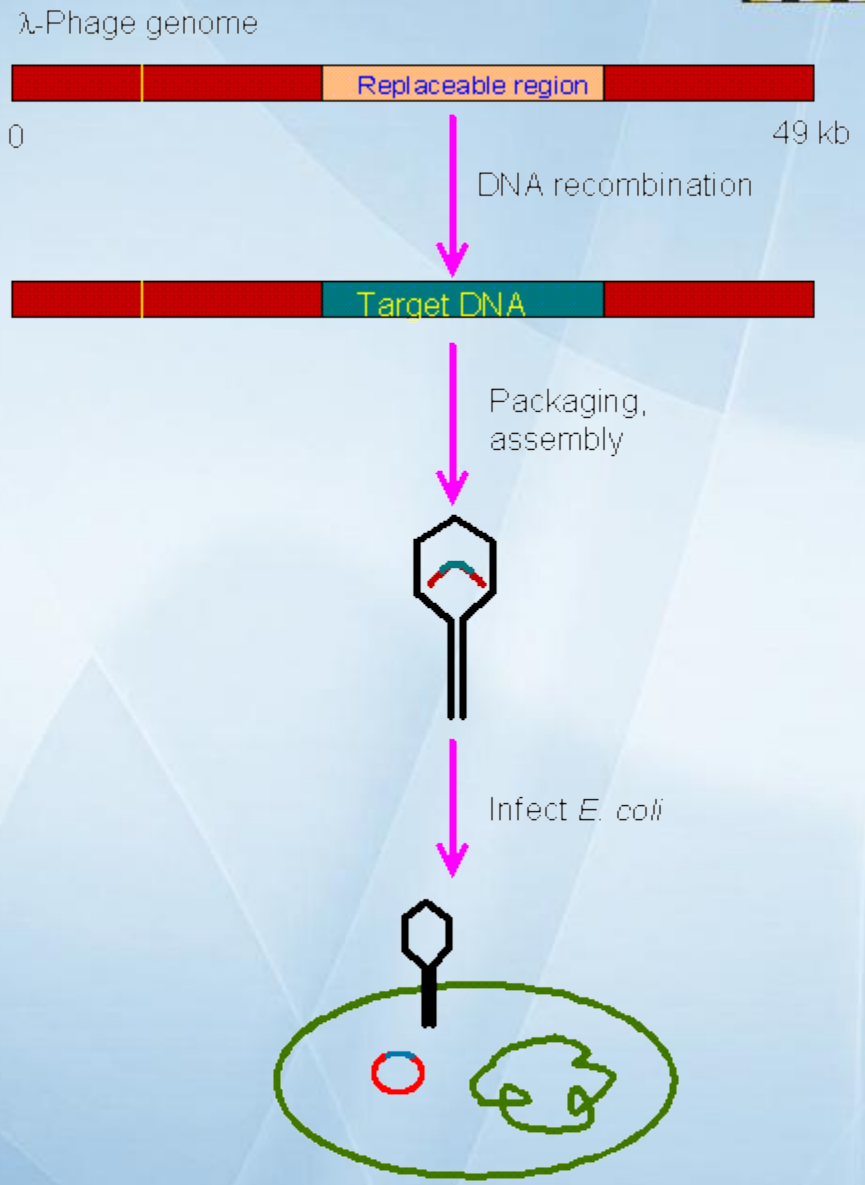
BACTERIOPHAGE LAMBDA

- **Phage lambda** is a **bacteriophage** or **phage**, i.e. bacterial virus, that uses *E. coli* as host.
- Its structure is that of a typical phage: **head, tail, tail fibres.**
- **Lambda viral genome:** 48.5 kb linear DNA with a 12 base ssDNA "sticky end" at both ends; these ends are complementary in sequence and can hybridize to each other (this is the **cos** site: **co**he**s**ive ends).
- **Infection:** lambda tail fibres adsorb to a cell surface receptor, the tail contracts, and the DNA is injected.
- The DNA circularizes at the **cos** site, and lambda begins its life cycle in the *E. coli* host.

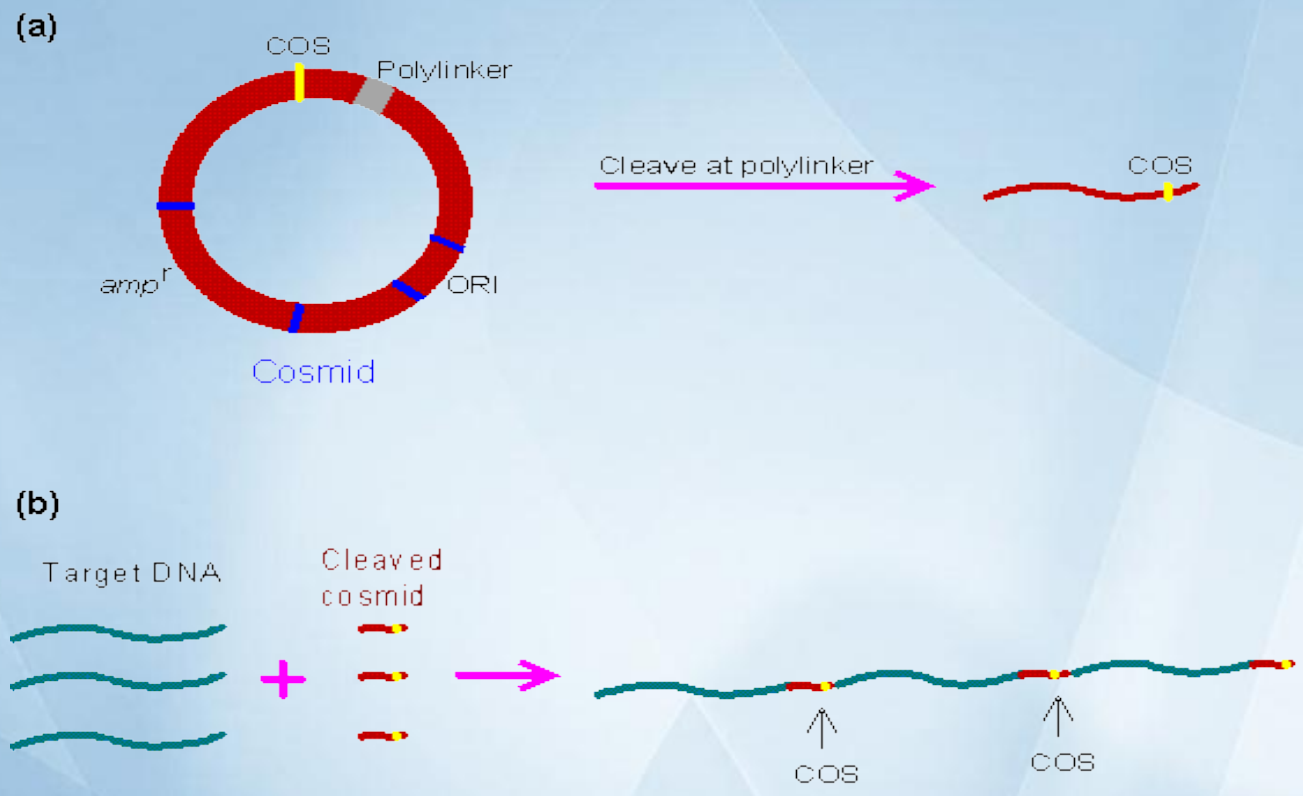


BACTERIOPHAGE LAMBDA





COSMID VECTOR



- The cosmid vector is a combination of the plasmid vector and the COS site which allows the target DNA to be inserted into the λ head. It has the following advantages:
  - High transformation efficiency.
  - The cosmid vector can carry up to 45 kb whereas plasmid and λ phage vectors are limited to 25 kb.

Yeast Artificial Chromosomes

The yeast artificial chromosome (YAC) vector is capable of carrying a large DNA fragment (up to 200 Kb), but its transformation efficiency is very low.