Advance Genetic Engineering

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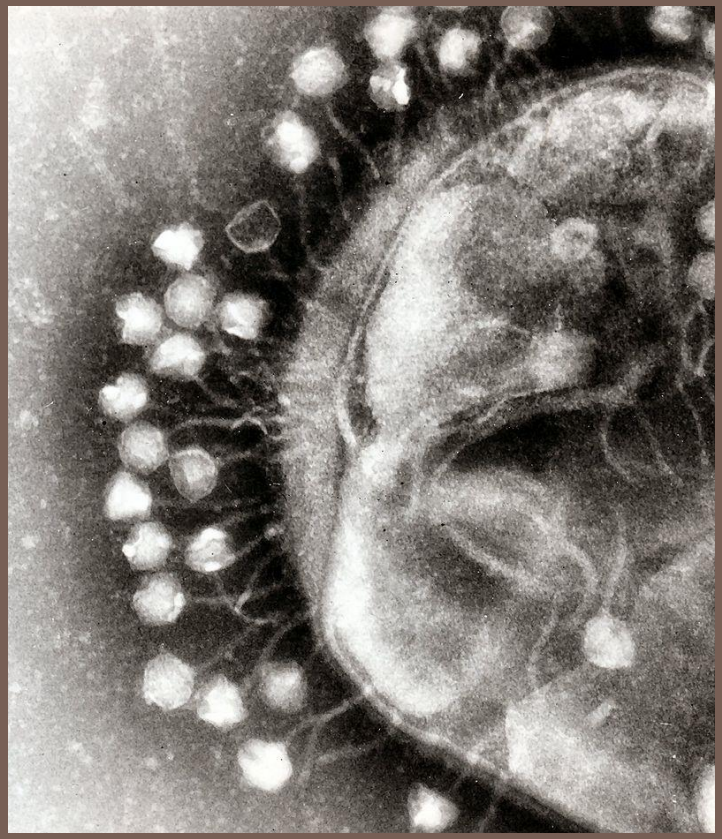
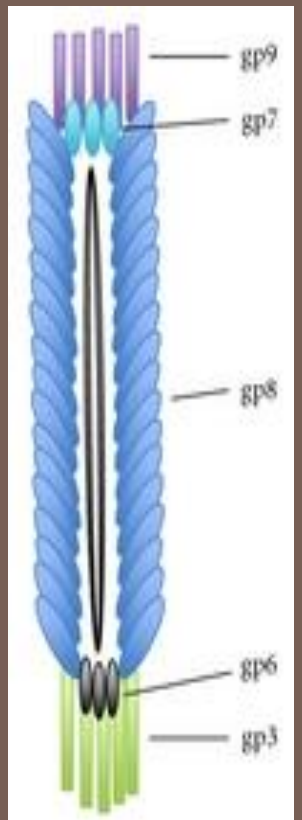
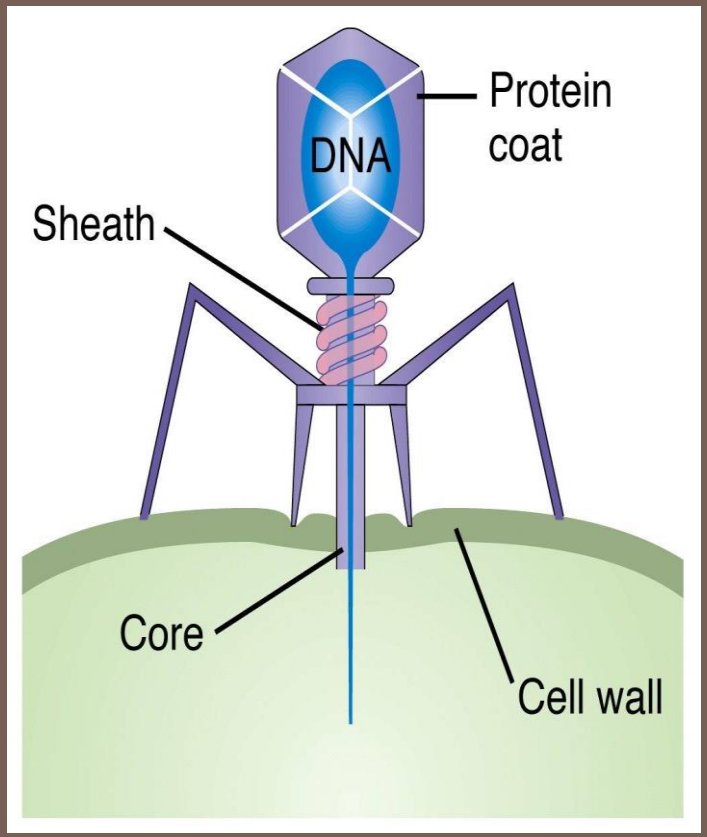
Bacteriophages







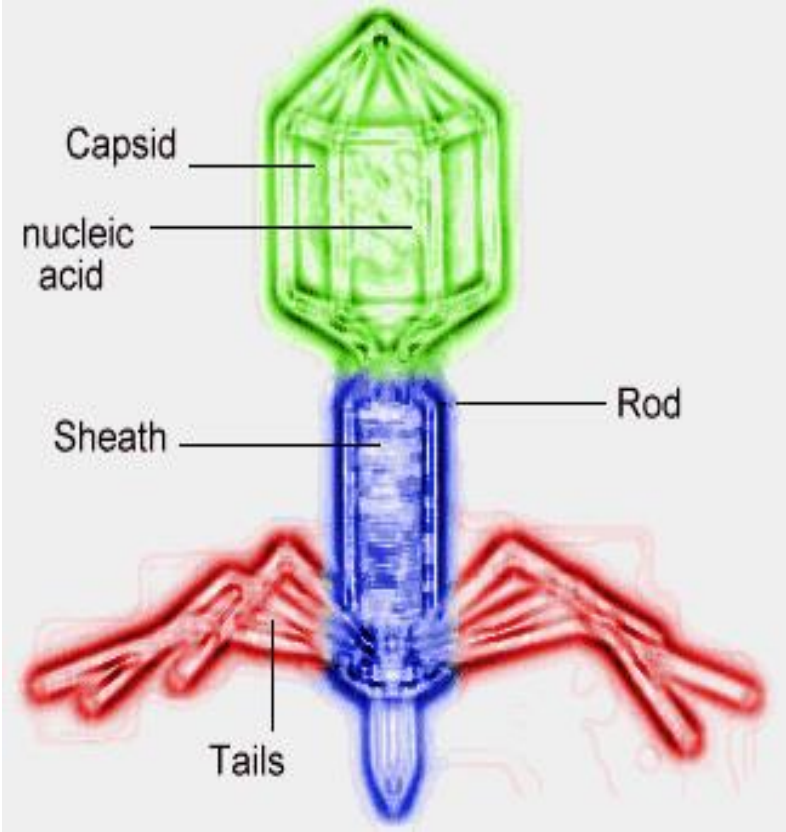
Phages are:

1. Bacterial viruses
2. Obligatory intraparasites..plasmids?
3. Bacteriophages are composed of proteins that encapsulate a DNAor RNA genome.
4. Their genomes ranging from 6 to 50 kb and may encode as few as four genes, and as many as hundreds of genes.
5. Replicate within bacteria by injecting their self leaving the capsule protein out side.

6. Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals and sea water.
7. Phages are classified by the International Committee on Taxonomy of Viruses(ICTV) according to morphology and nucleic acid into nineteen families. Of these, only two families have RNA genomes and only five families are enveloped. Of the viral families with DNA genomes, only two have single-stranded genomes. Eight of the viral families with DNA genomes have circular genomes, while nine have linear genomes.

Basics

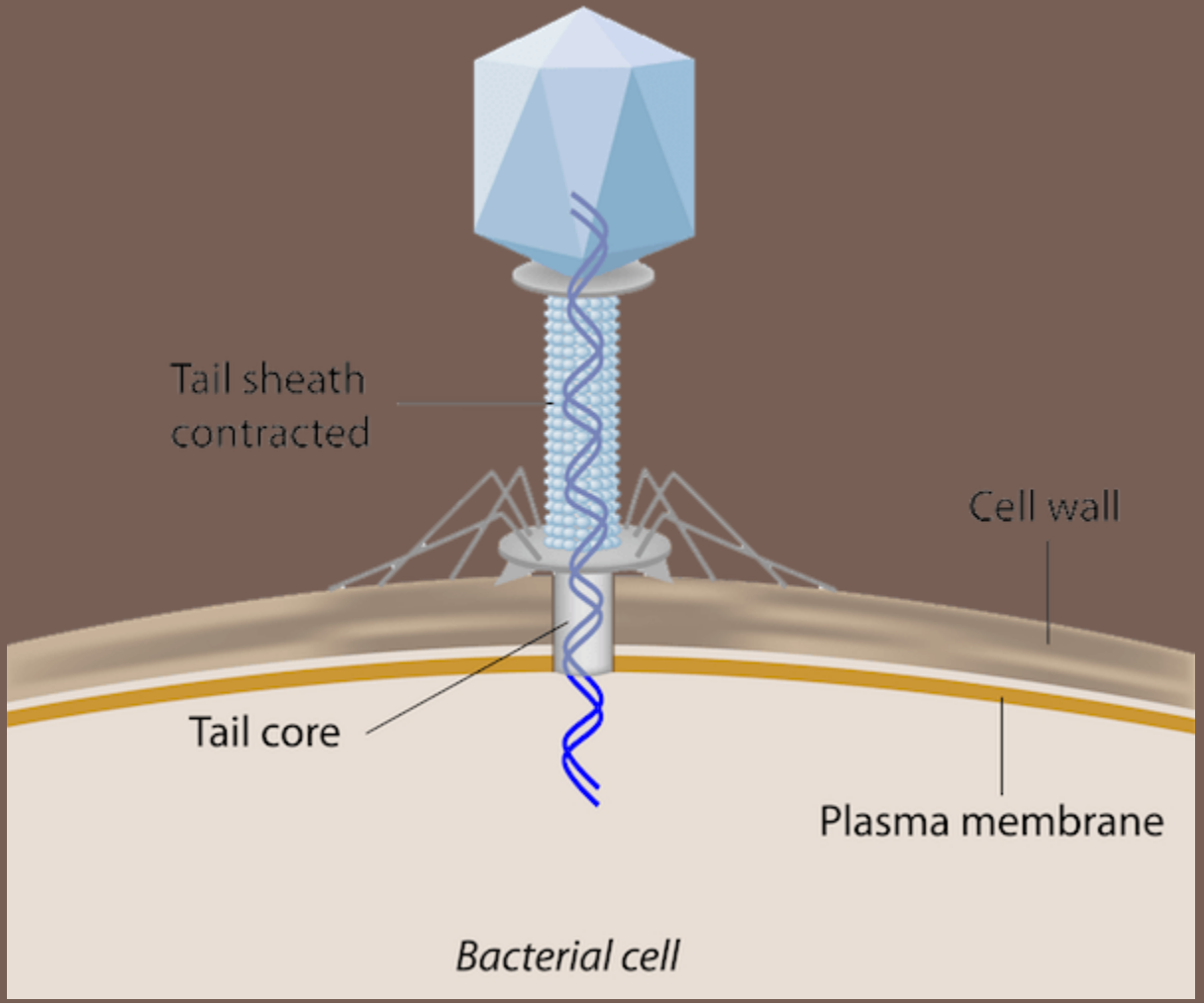
- Used for cloning foreign genes among other applications
- Proteins and peptides are fused to the **Capsid**(surface) of the phage
- The combination of the phage and peptide is known as a **Fusion Protein**

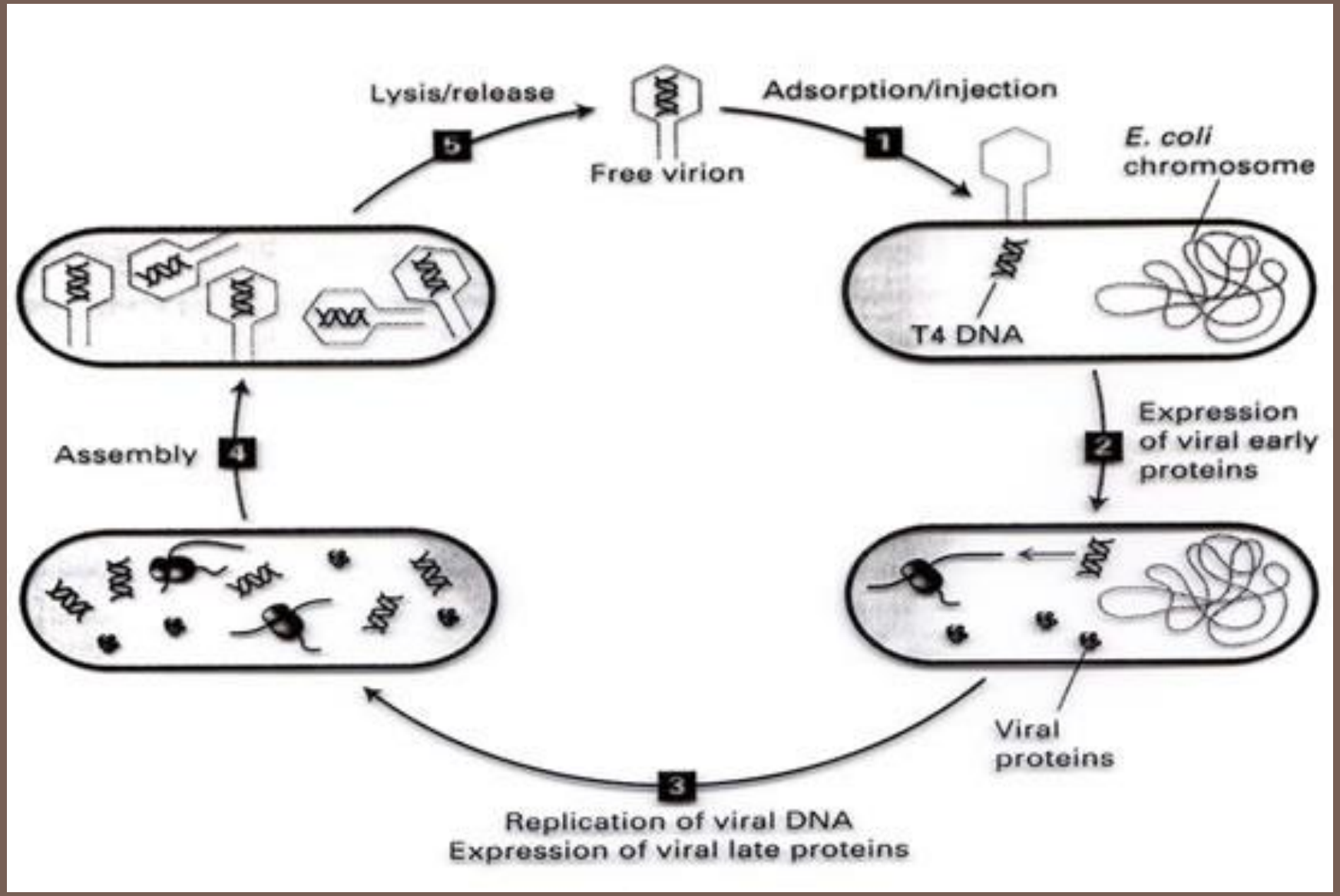


Phages in cloning:

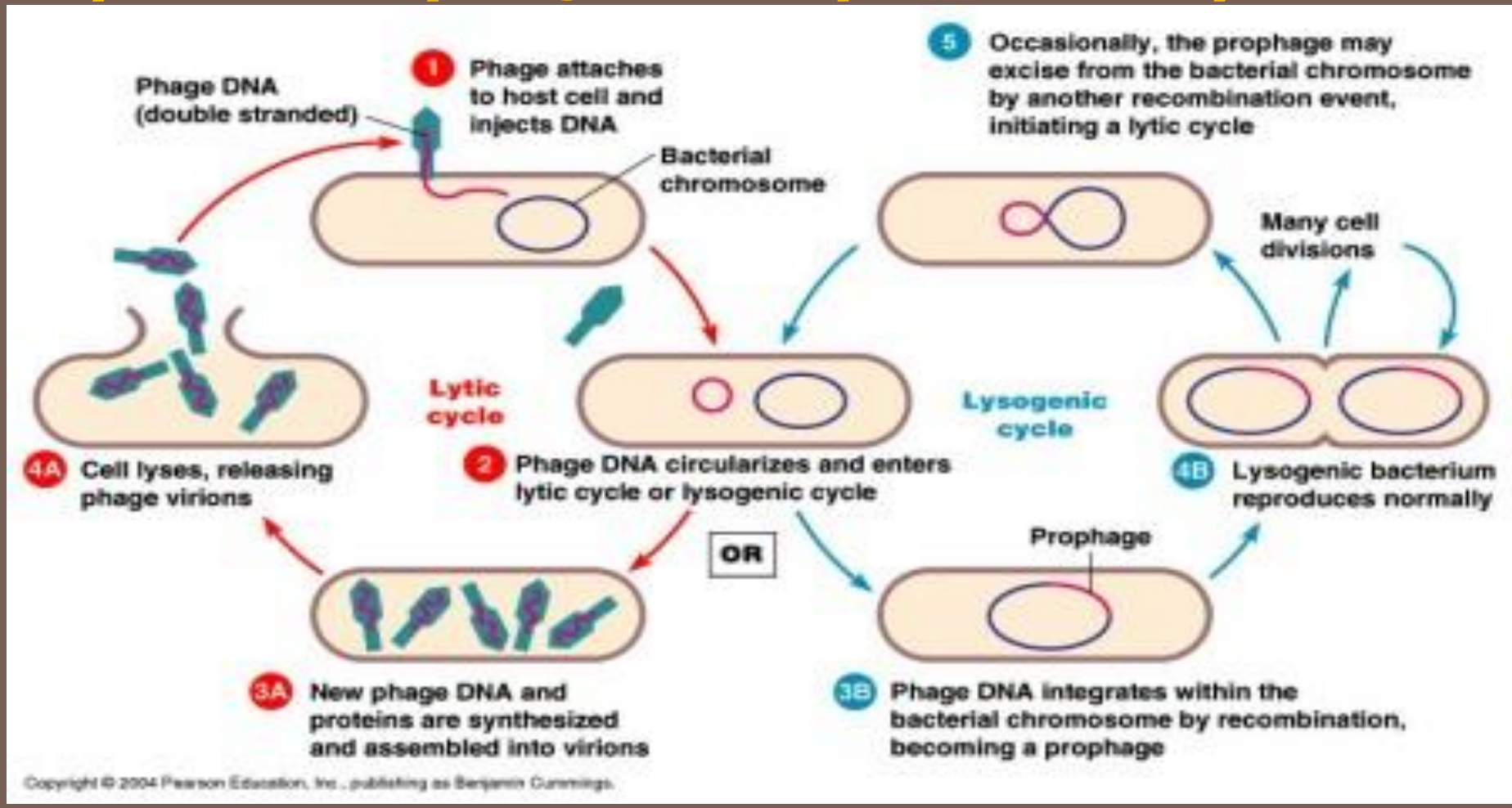
1. Phages are able to pick between 15-52kb DNA fragment which is 3 to 4 times the ability of plasmids.
2. Phages able to produce huge progeny comparing to that in plasmid replication.
3. Easy to extract.

Phages replication cycles



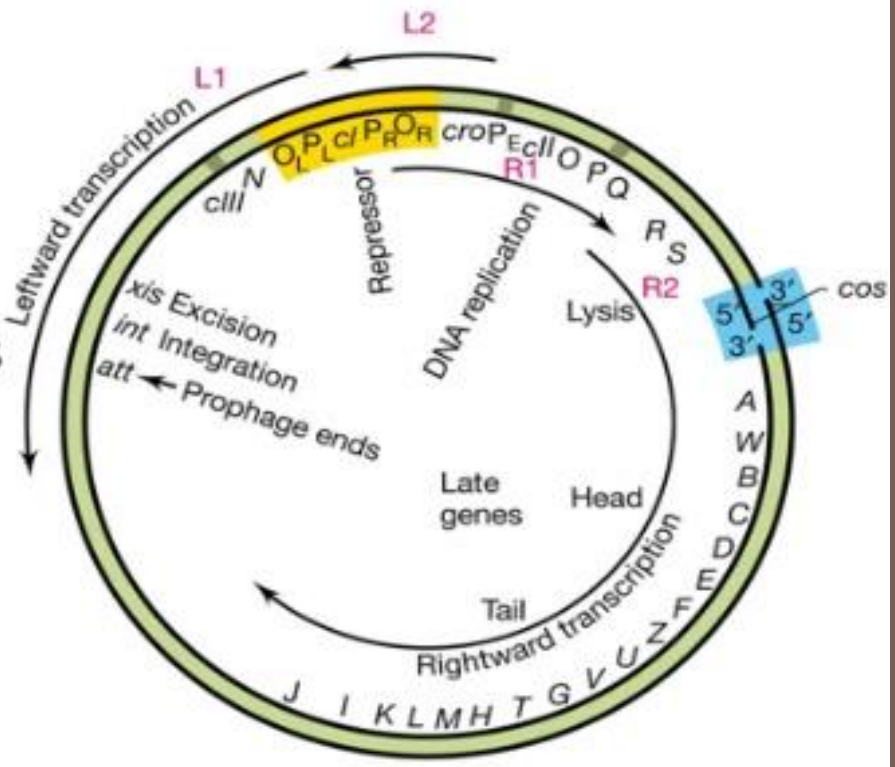

Lysis/release
5
Free virion
Adsorption/injection
1
E. coli
chromosome
T4 DNA
Expression
of viral early
proteins
2
Assembly
4
Viral
proteins
3
Replication of viral DNA
Expression of viral late proteins

lytic and lysogenic replication cycles



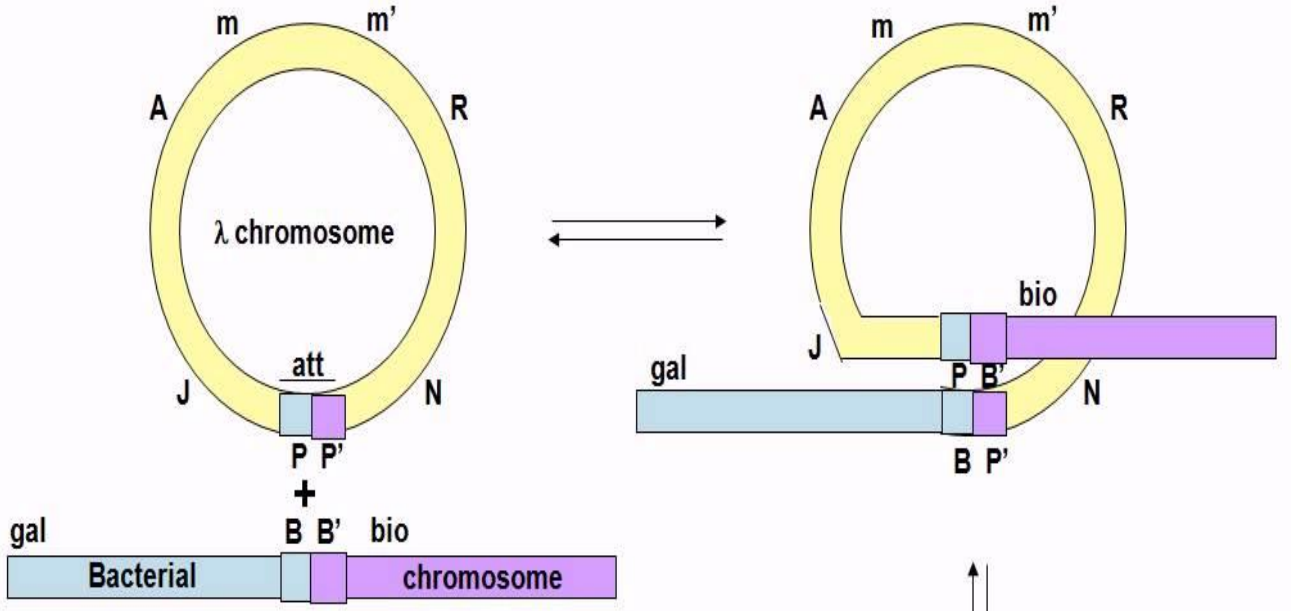
Lambda phage (λ) genome

- Two genes serve as the molecular switch.
- **Lambda repressor protein (CI):** activates the lysogenic pathway.
- **Cro protein:** activates the lytic pathway.



This system is called the **lambda repressor** switch

Insertion of Lambda Phage

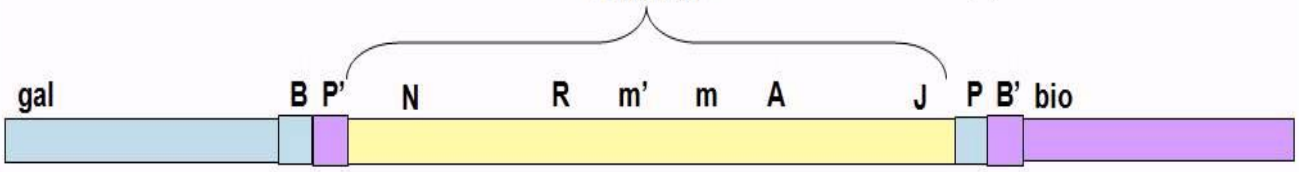


***attB* site in bacterial chromosome**

B--GCTTT(TTTATAC)TAA---B'

Integrase

Prophage



Lysogenic chromosome

***attP* site in bacteriophage lambda chromosome**

P--GCTTT(TTTATAC)TAA---P'

Lambda phage (λ) life cycle

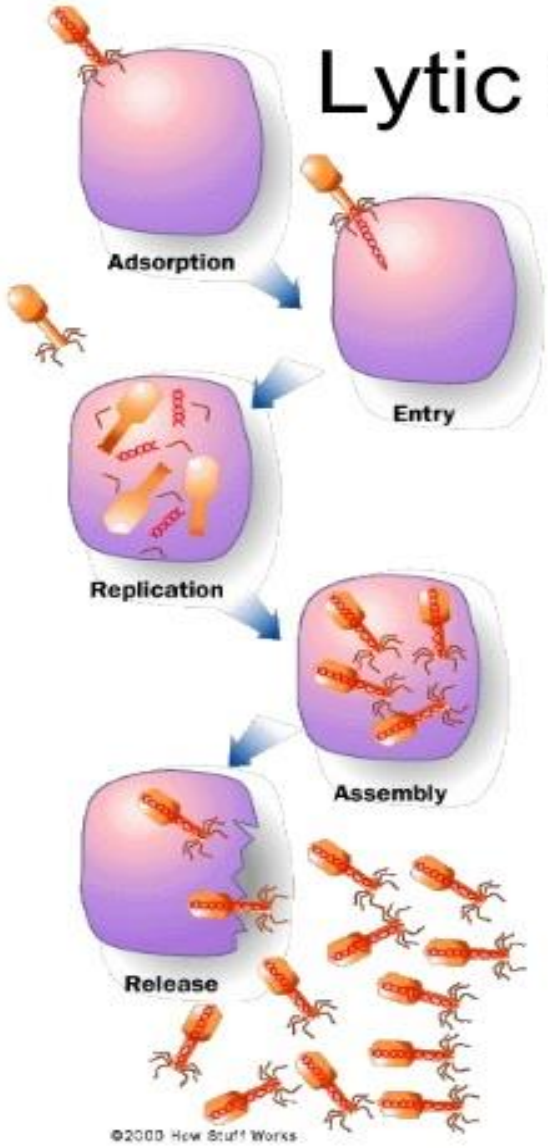
Lambda phage (λ) life cycle can take two forms

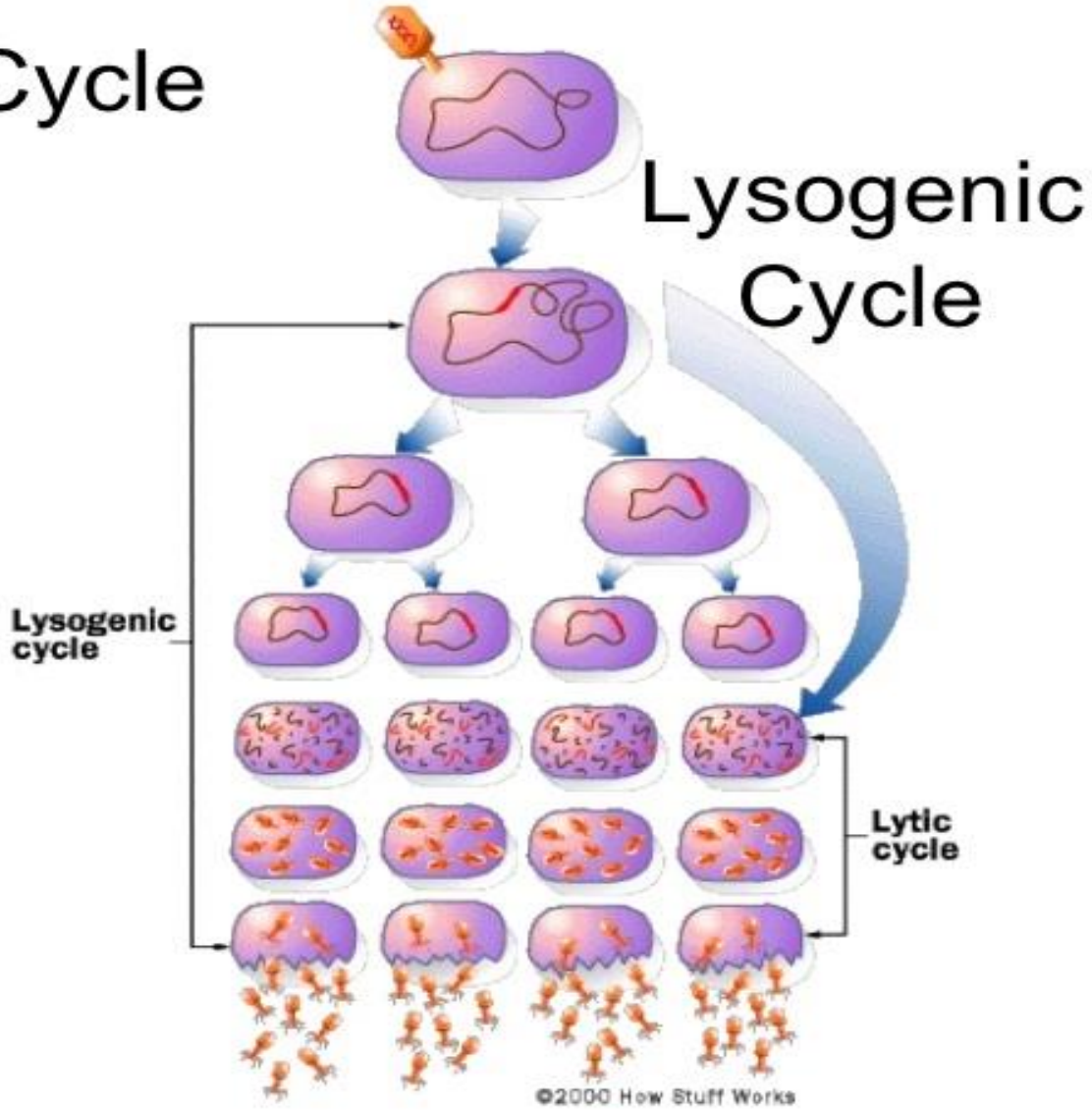
Lytic cycle

- Phage genome is replicated into many copies.
- Progeny assembles in phage particle and gets released.
- The host cell is destroyed (Lysed).

Lysogenic cycle

- Phage genome **IS NOT** replicated.
- Phage genome is integrated in the host genome.
- No progeny is produced.
- The host cell is not destroyed.
- Replication of phage genome achieved when the bacterial cell replicates.


Lytic Cycle
Adsorption
Entry
Replication
Assembly
Release
©2000 How Stuff Works


Lysogenic Cycle
Lysogenic cycle
Lytic cycle
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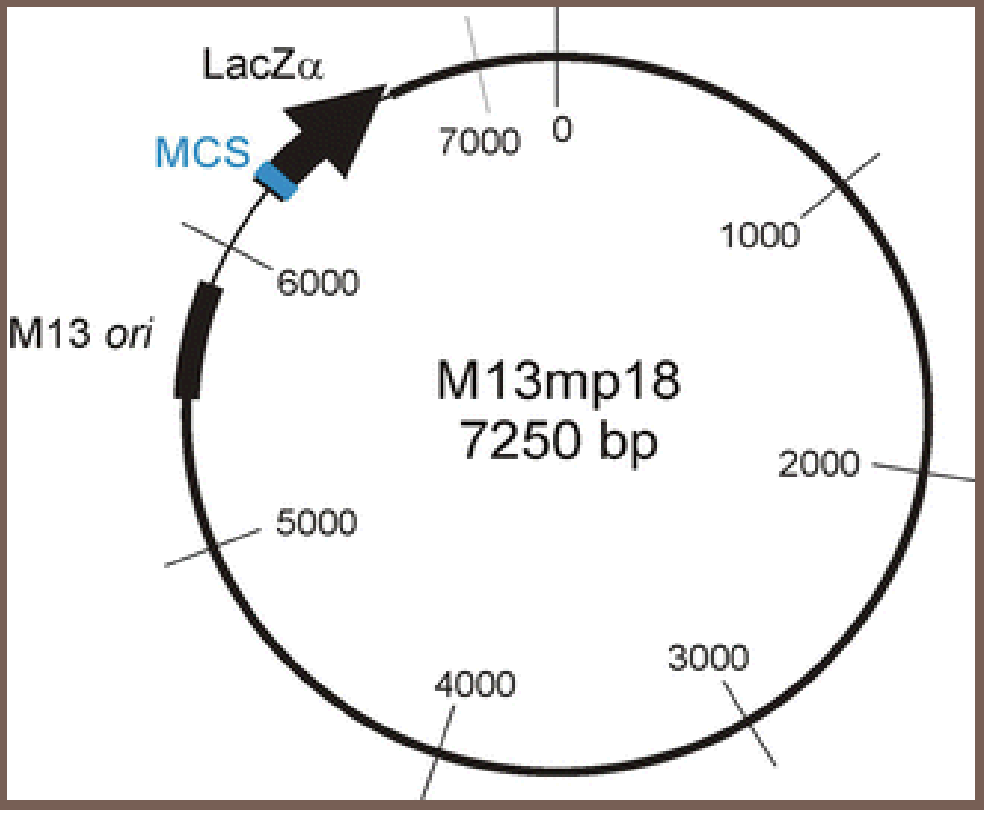
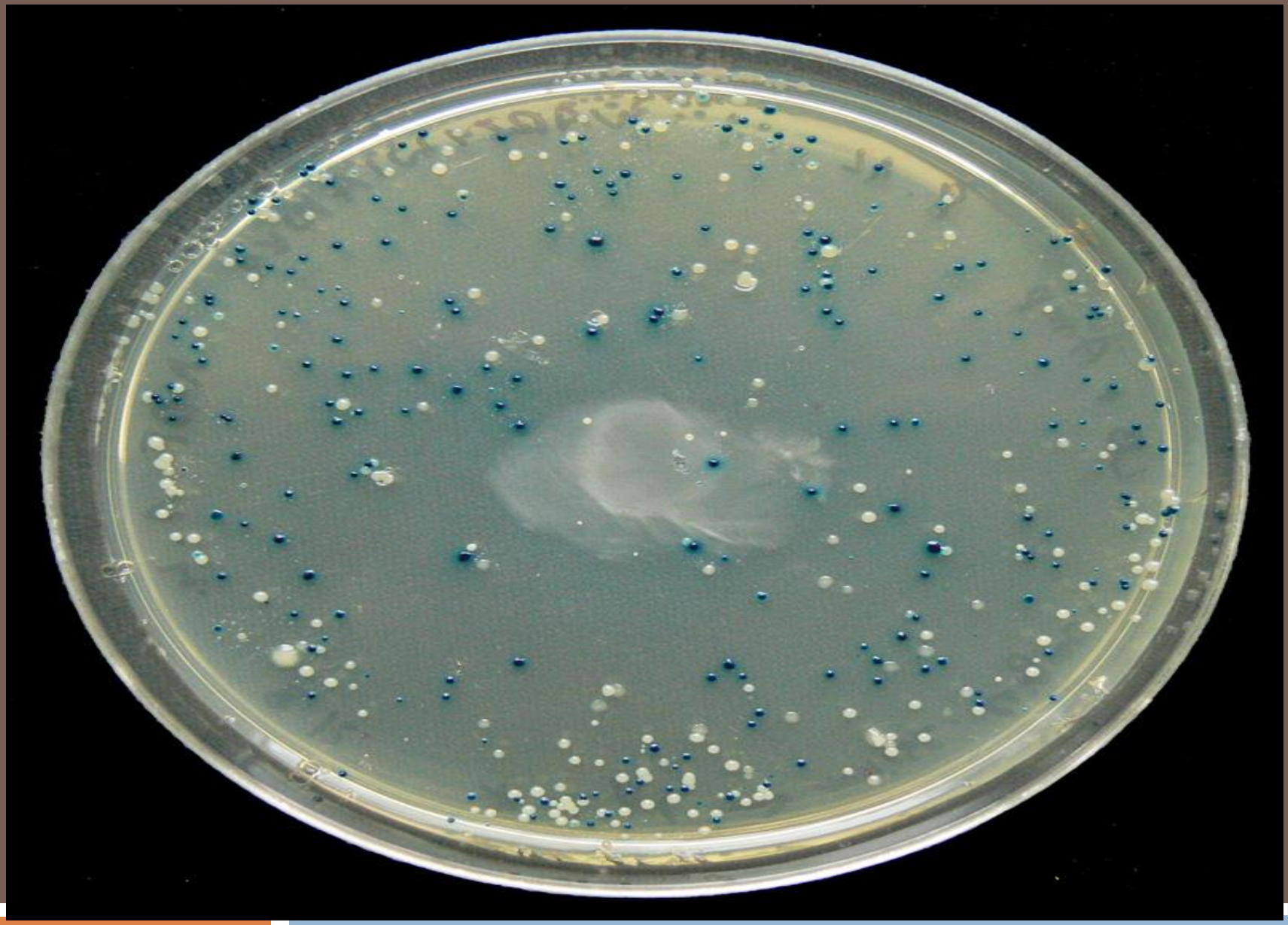
Phages cloning strategies

1. Insertional inactivation assay



M13+
Lambda charonA16
Lambda CI



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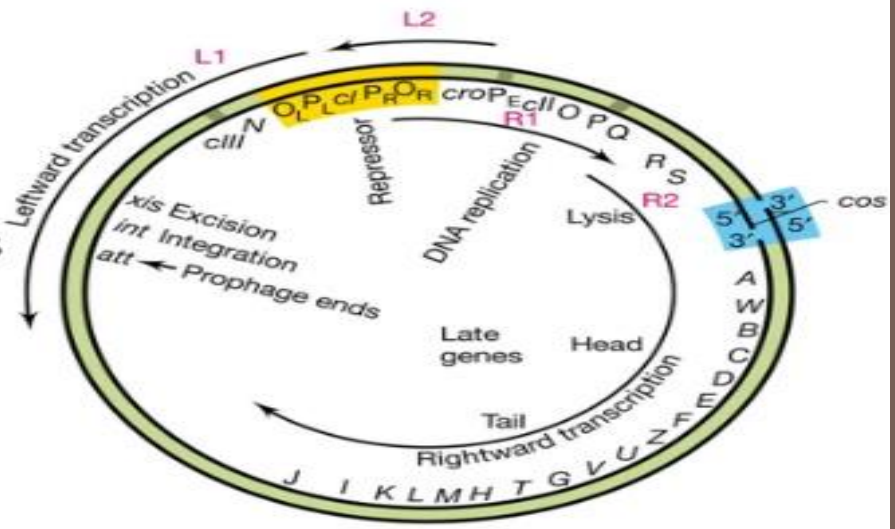


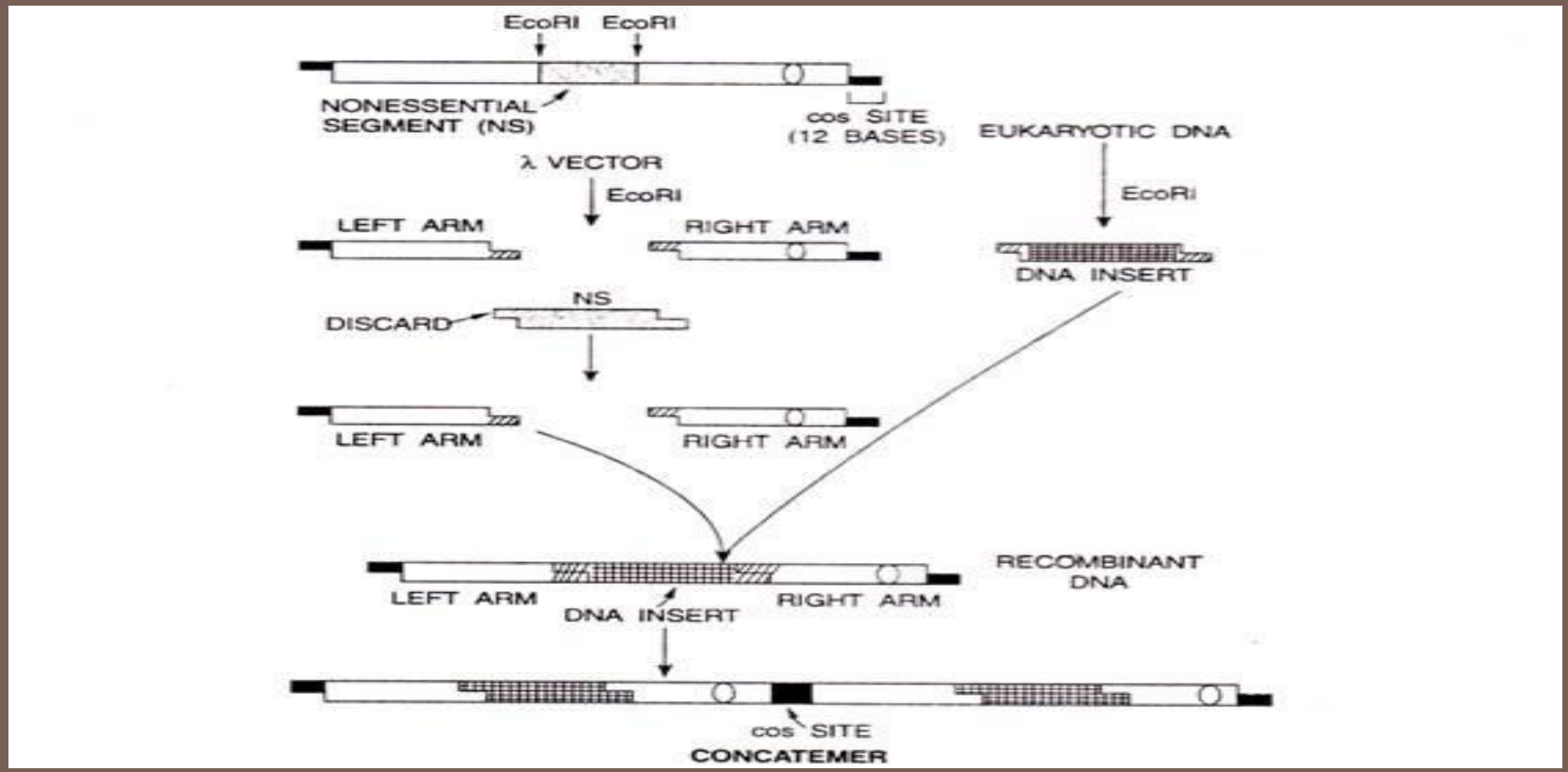
This system is called the **lambda repressor switch**

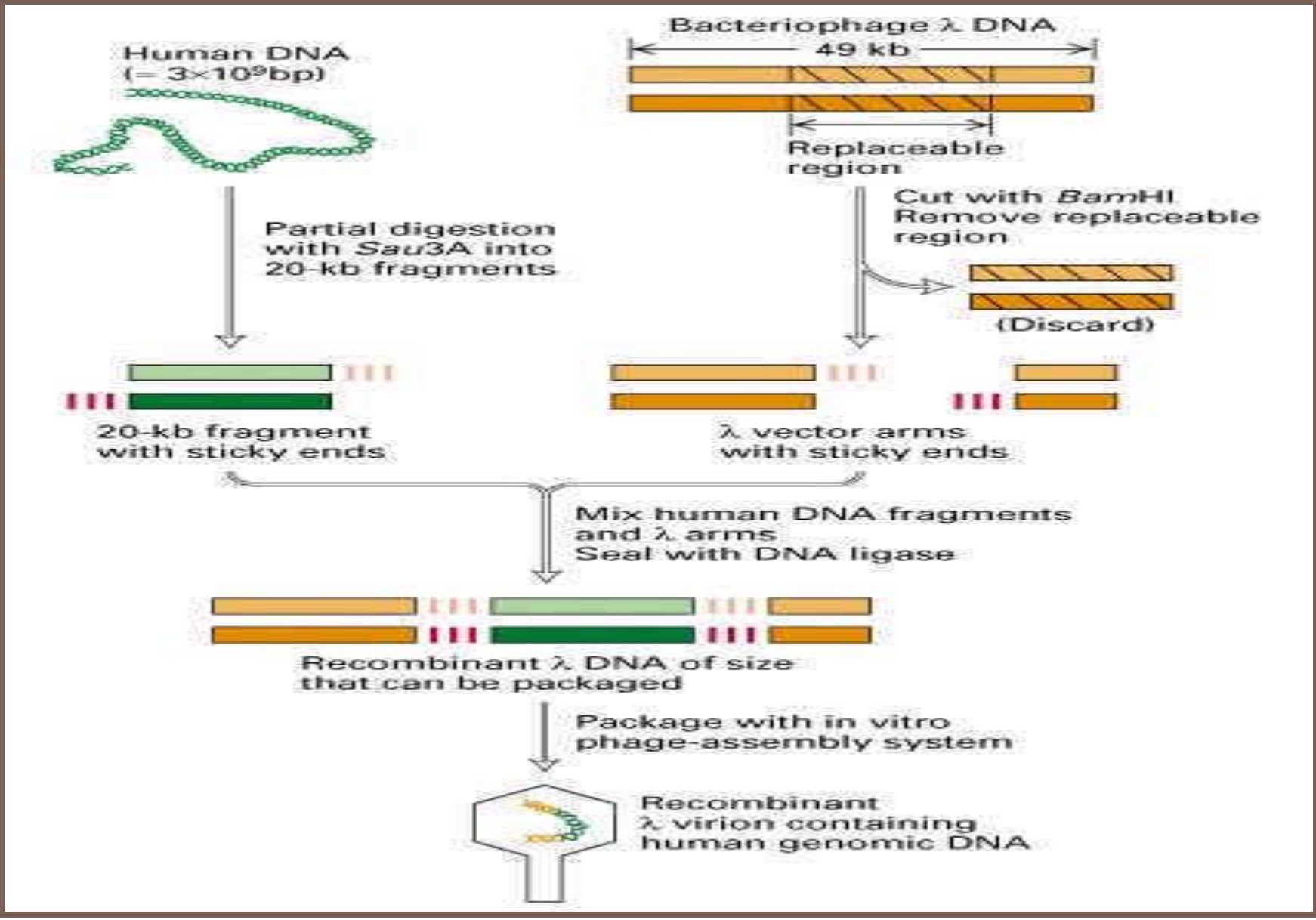
Non hybrids look turbid……. Hybrids look clear

Insertional without inactivation assay

1. Modified lambda phage + DNA fragment(Hybrid).....E.coli p2(SP+)……SP-
Non hybrid phage ……....E.coli p2(SP+)……SP+

2.




Human DNA
(= 3×10⁹bp)
Partial digestion
with Sau3A into
20-kb fragments
20-kb fragment
with sticky ends
Bacteriophage λ DNA
49 kb
Replaceable
region
Cut with BamHI
Remove replaceable
region
(Discard)
λ vector arms
with sticky ends
Mix human DNA fragments
and λ arms
Seal with DNA ligase
Recombinant λ DNA of size
that can be packaged
Package with in vitro
phage-assembly system
Recombinant
λ virion containing
human genomic DNA

Phage derivatives

1. **Lambda phage**
2. **EMBL 4 & 5**
3. **Mu**
4. **M13**


تجري بعون الله تعالى مناقشة أطروحة الدكتوراه
للطالبة رفيف علاء سعيد
عن أطروحتها الموسومة
(العلاقة بين نظام التصحيح الجيني في بكتريا القالون مع نظام التصحيح الجيني في
المرضى المصابين بسرطان القالون)
The correlation between mismatch repair system in E. coli andmismatch repair
system in patients with colorectal cancer
وذلك يوم الخميس المصادف ٢٠١٦/٢/٢٥ الساعة التاسعة والنصف صباحاً في كلية الطب
الأستاذ الدكتور
محمد عبد كاظم حسن
الأستاذ الدكتور
كريم شعلان الأعرجي
الأستاذ الدكتور
عبدالحسين مويت الفيصل
الأستاذ المساعد الدكتور
زينب حمود مجيمس