TRANSFORMATION AND TRANSDUCTION

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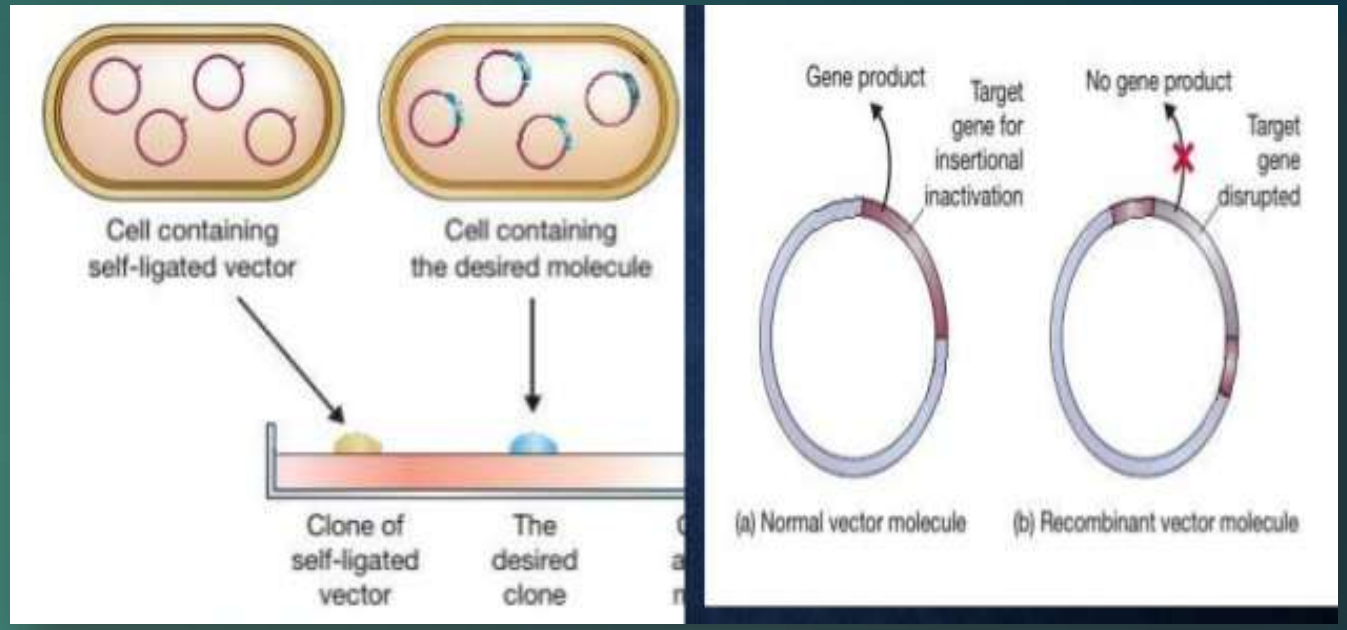
TRANSFORMATION:

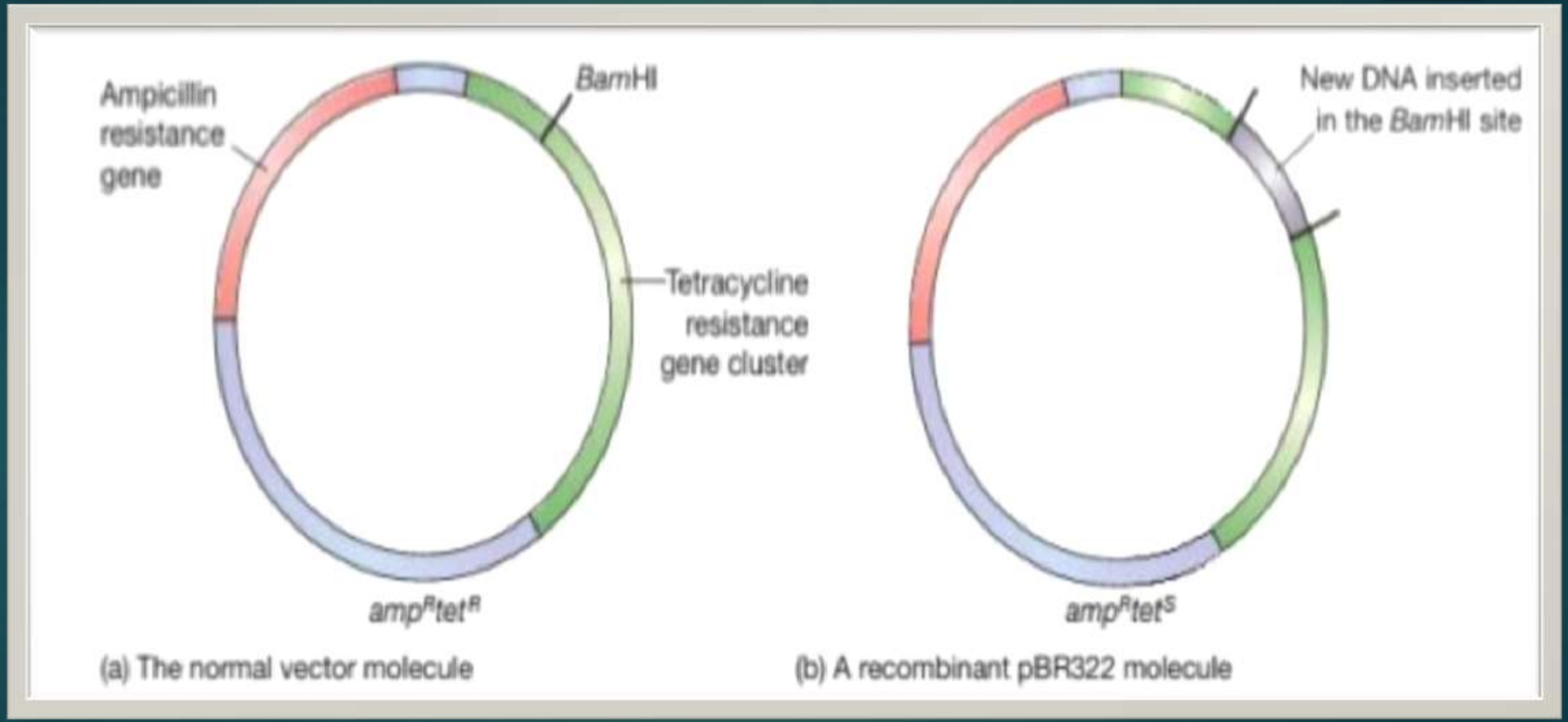
- The uptake of DNA by bacterial cells
- Most species of bacteria are able to take up DNA molecules from the medium in which they grow
- Some time it will be degraded .but ocassionally it is able to survive and replicate in the host cell (when it is plasmid)

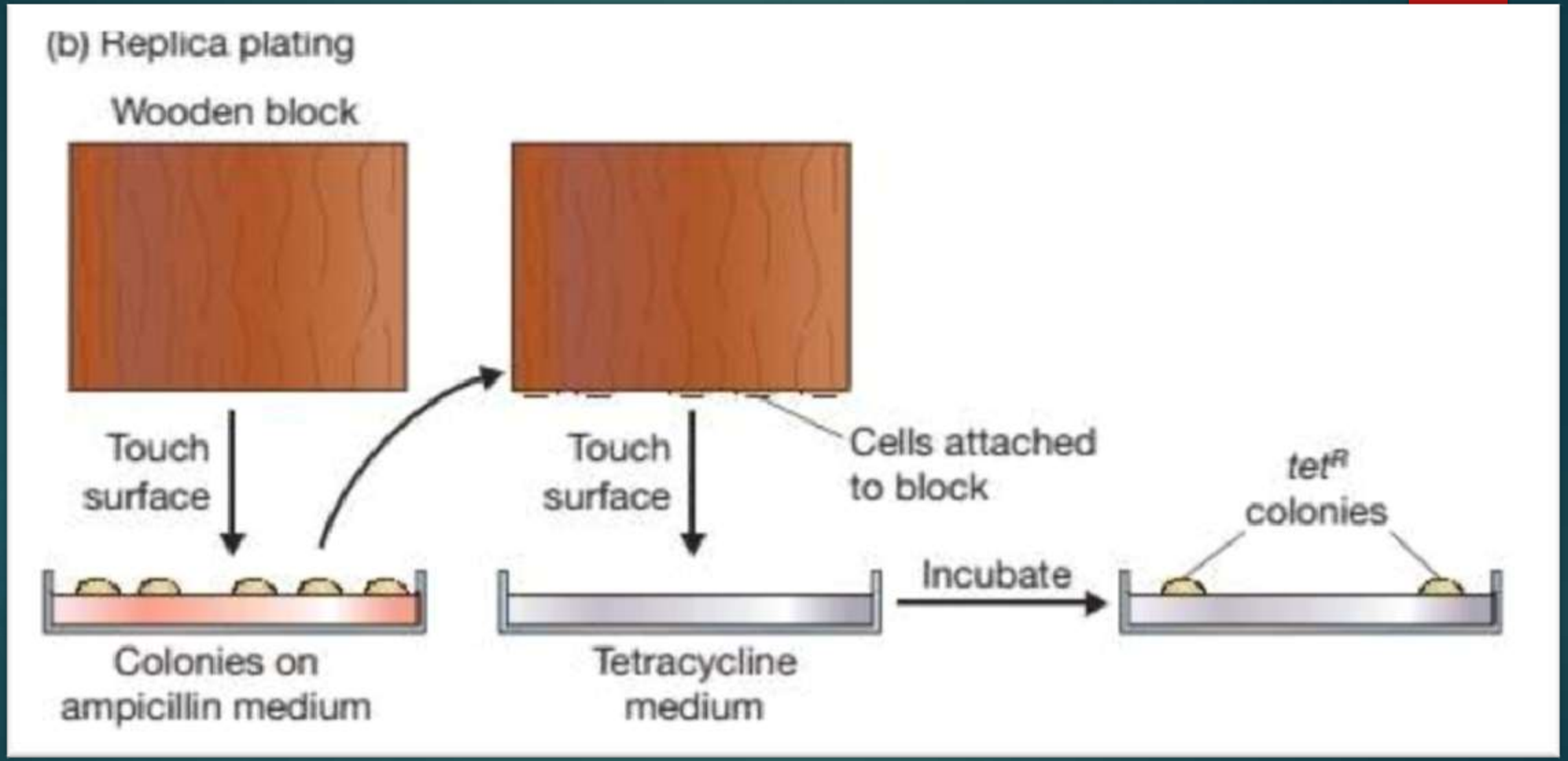
IDENTIFICATION OF RECOMBINANTS:

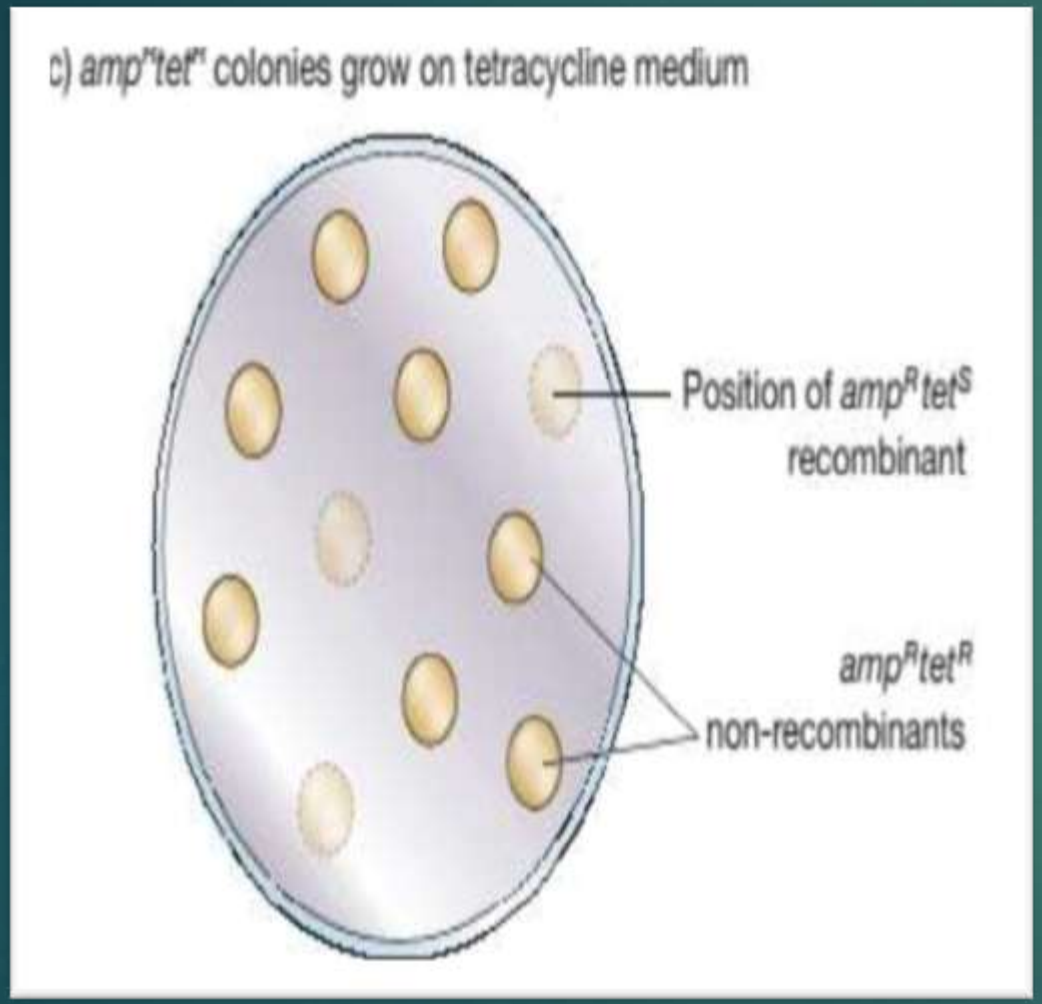
- Plating on a selective medium enables transformants to be distinguished from non transformants.
- The next problem is to determine which of the transformed colonies comprise cells that contain recombinant DNA molecules, and which contain self-ligated vector molecules.
- With most cloning vectors, the insertion of a DNA fragment into the plasmid destroys the integrity of one of the genes present on the molecule.
- Recombinants can therefore be identified because the characteristic coded by the inactivated gene is no longer displayed by the host cells

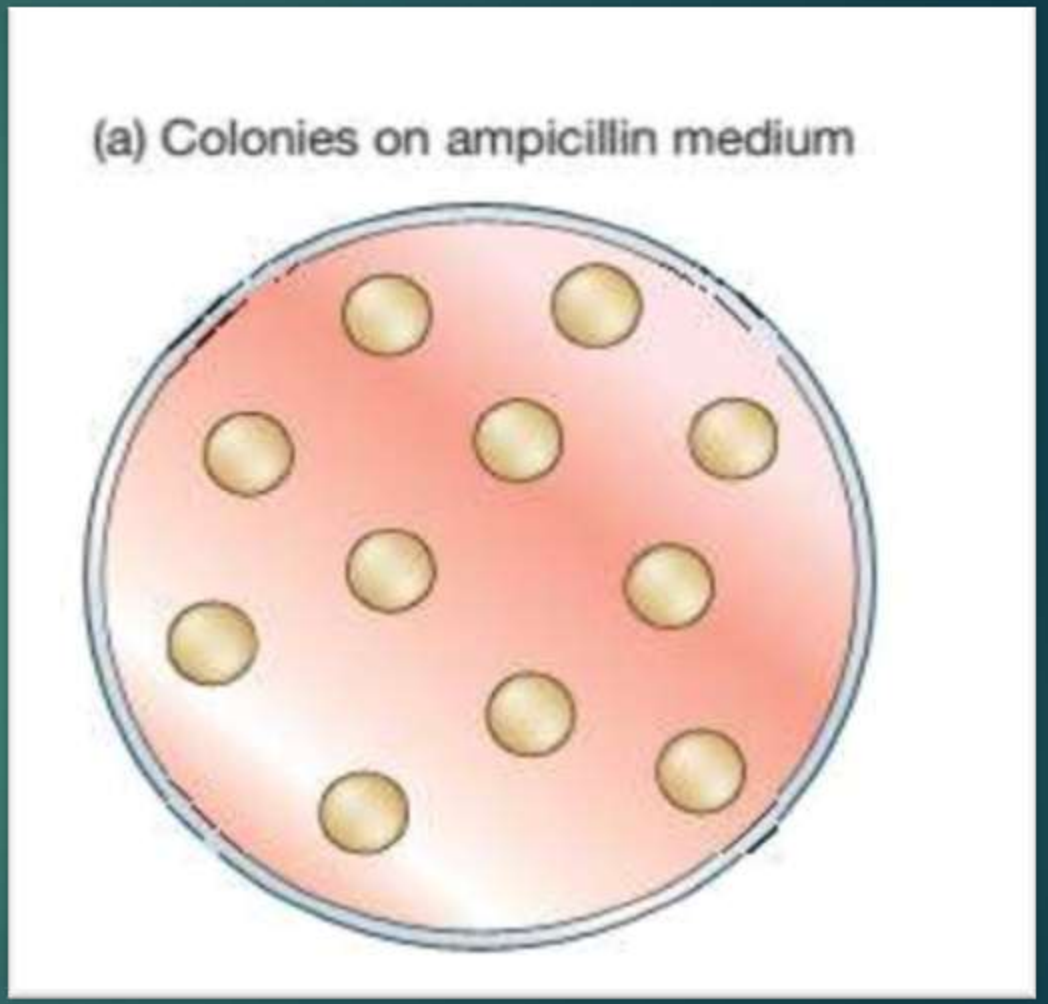
- To identify the recombinants the colonies are replica platedonto agar medium that contains tetracycline.
- After incubation, some of the original colonies regrow, but others do not Those that do grow consist of cells that carry the normal pBR322 with no inserted DNA and therefore a functional tetracycline resistance gene cluster (ampR tetR)
- Reference back to the original ampicillin agar plate reveals the positions of these colonies, enabling samples to be recovered for further study.




Ampicillin resistance gene
BamHI
Tetracycline resistance gene cluster
$amp^R tet^R$
(a) The normal vector molecule
New DNA inserted in the BamHI site
$amp^R tet^S$
(b) A recombinant pBR322 molecule


(b) Replica plating
Wooden block
Touch surface
Touch surface
Cells attached to block
tet^R colonies
Incubate
Colonies on ampicillin medium
Tetracycline medium


c) amp^R tet^R colonies grow on tetracycline medium
Position of amp^R tet^S recombinant
amp^R tet^R non-recombinants

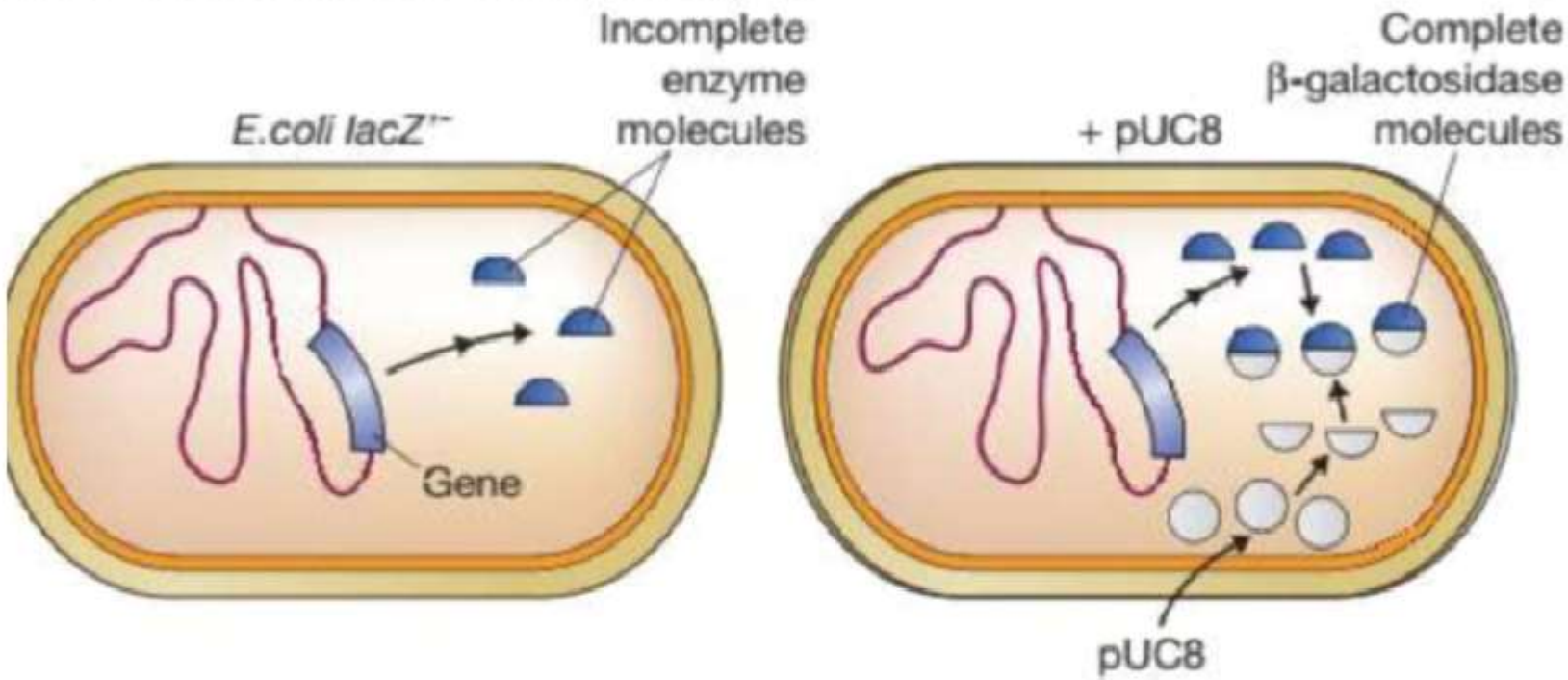

(a) Colonies on ampicillin medium

INSERTIONAL INACTIVATION DOES NOT ALWAYS INVOLVE ANTIBIOTIC RESISTANCE

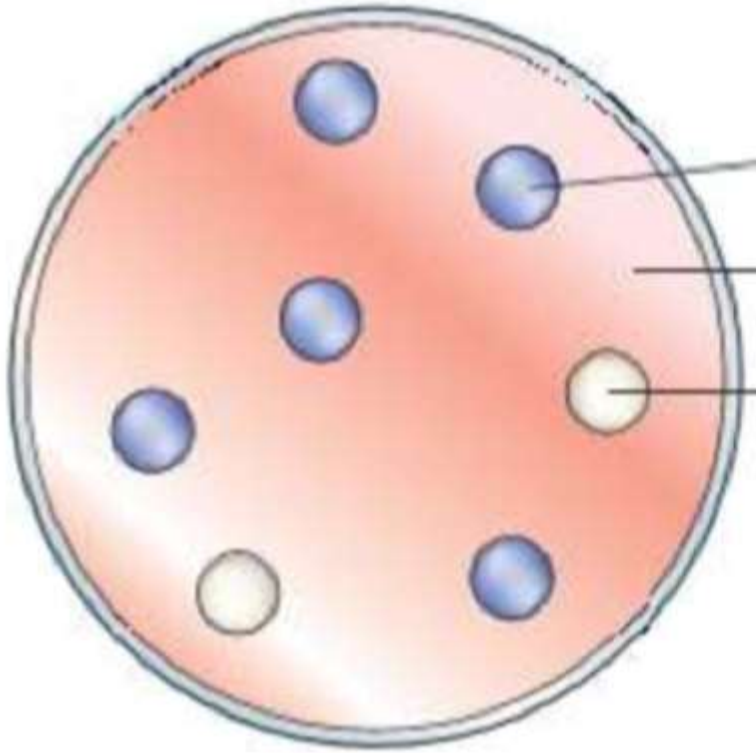
- The insertional inactivation of an antibiotic resistance gene provides an effective means of recombinant identification, but it is inconvenient due to the need to carry out two screenings: one with the antibiotic that selects for transformants; and a second screen, after replica plating, with the antibiotic that distinguishes recombinants.
- Most modern plasmid vectors therefore make use of a different system. For example
- pUC8 which carries the ampicillin resistance gene and a gene called lacZ′, which codes for part of the enzyme β-galactosidase.
- Cloning with pUC8 involves insertional inactivation of the lacZ 'gene, with recombinants identified because of their inability to synthesize β-galactosidase.
- A cloning experiment with pUC8 involves the selection of transformants on ampicillin agar, followed by screening for β-galactosidase activity to identify recombinants.

- Cells that harbour a normal pUC8 plasmid are amp R and able to synthesize β-galactosidase. Recombinants are also ampR but unable to makeβ-galactosidase.
- Screening for the presence or absence of β-galactosidase is in fact quite easy.
- Rather than assay for lactose being split to glucose and galactose, the test is for a slightly different reaction that is also catalysed by β-galactosidase.
- This involves a lactose analogue termed X-gal which is broken down by β-galactosidase to a product that is coloured deep blue.
- If X-gal is added to the agar, along with ampicillin, then non-recombinant colonies, the cells of which synthesize β-galactosidase, will be coloured blue, whereas recombinants with a disrupted lacZ 'gene and unable to makeβ-galactosidase, will be white.

a) The role of the modified *E. coli lacZ* gene



(b) Screening for pUC8 recombinants



Blue colony = non-recombinant

Agar + X-gal + IPTG

White colony = recombinant

Blue colonies = β-galactosidase synthesized

X-gal → blue product

White colonies = β-galactosidase not synthesized

X-gal → no blue product

NOT ALL SPECIES OF BACTERIA ARE EQUALLY EFFICIENT AT DNA UPTAKE:

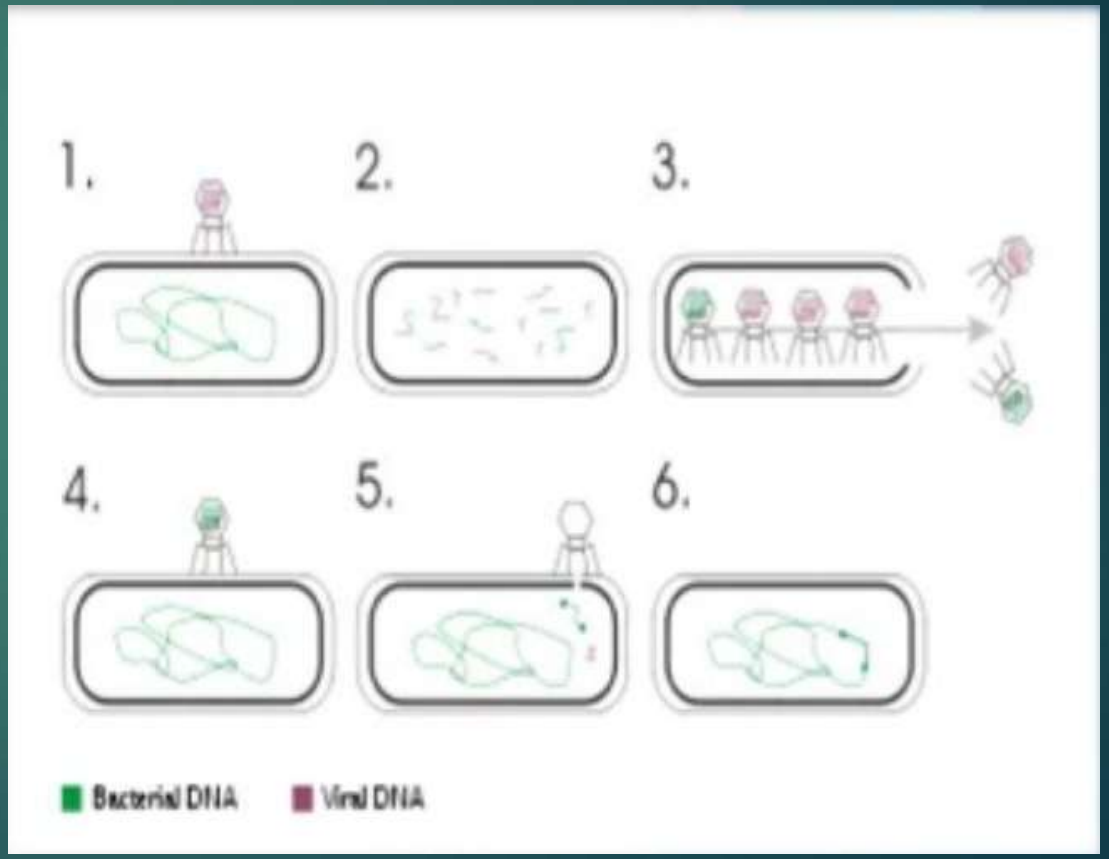
- In nature, transformation is probably not a major process by which bacteria obtain genetic information
- In labortary some spceies can be easily transformed (Bacillus and streptococcus)
- Most species of bacteria, including *E. coli,* take up only limited amounts of DNA under normal circumstances
- To transform these species efficiently, the bacteria have to undergo some form of physical and/or chemical treatment that enhances their ability to take up DNA

TRANSDUCTION:

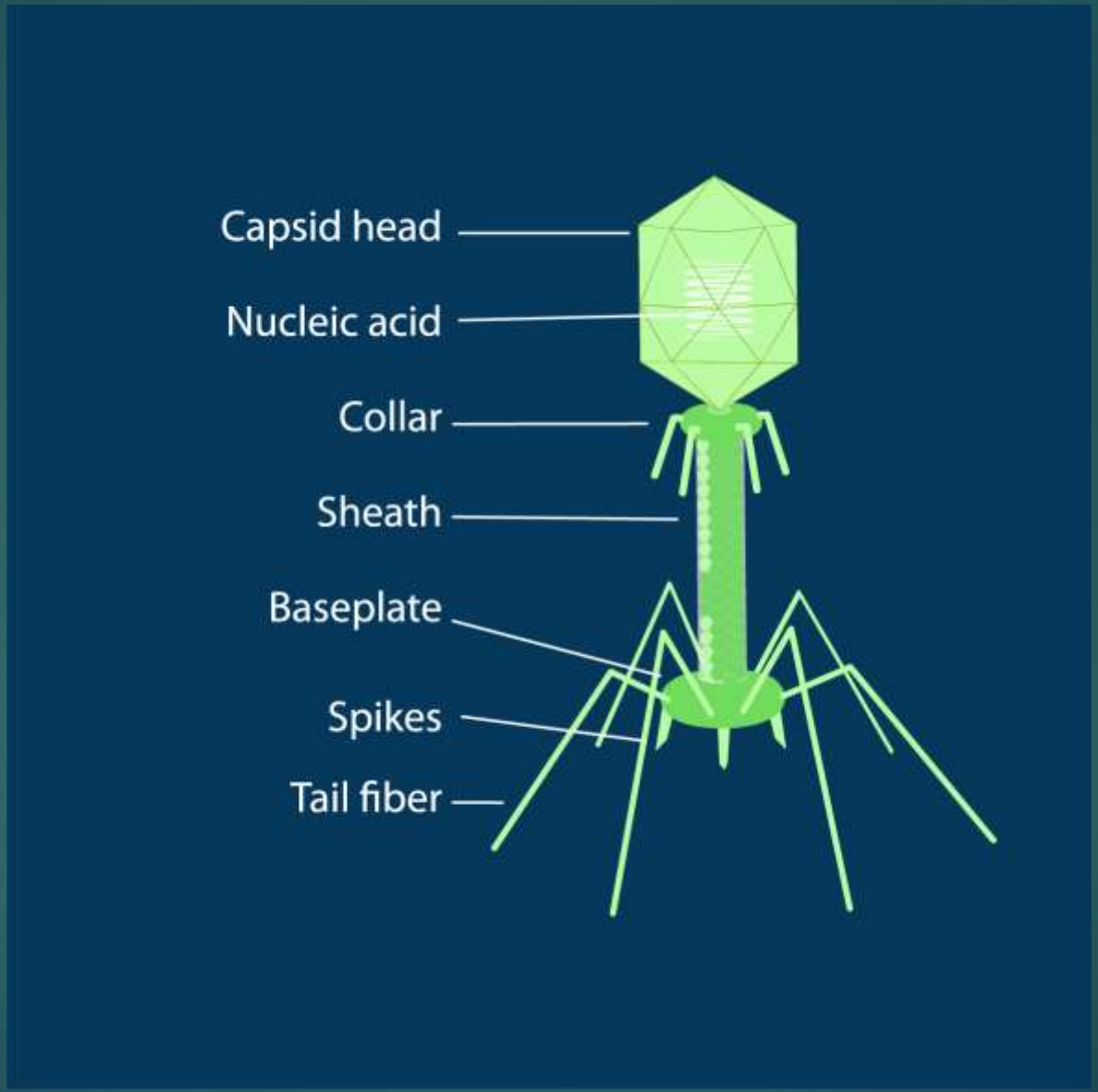
- It refers to the process whereby foreign DNA is introduced into another cell via a viral vector.

or

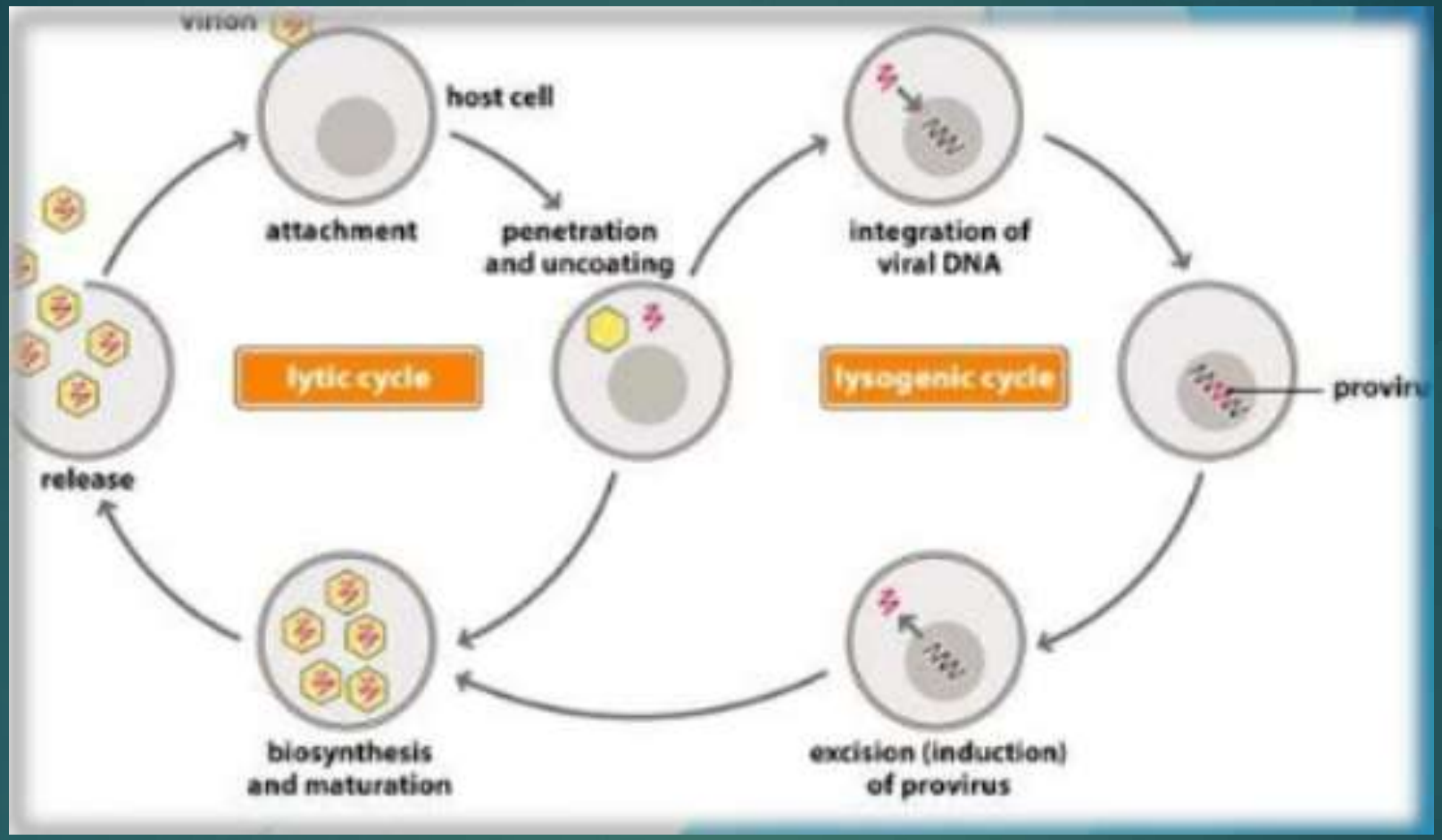
- It is the process by which DNA is transferred from one bacterium to another by a virus.



- The experiments implicated a bacteriophage as the vector or transducing agent.
- • What is Bacteriophage?
- • The virus that infect the bacteria are known as bacteriophage. Most bacteriophage, the virulent phages, undergo a rapid lyric growth cycle in their host cells. They inject there nucleic acid, usually DNA, into the bacterium, where it replicates rapidly and also directs synthesis of new phage proteins.


Capsid head
Nucleic acid
Collar
Sheath
Baseplate
Spikes
Tail fiber

- Transduction occurs by either the lytic or lysogenic cycles.
- In a lytic infection, the host cells fills with virions and bursts.
- The result is cell death.
- Lysogenic infections are also known as latent infections.
- The viral genome becomes incorporated into the host cell's DNA.
- It can remain this way for an extended period.
- The host cell lives.

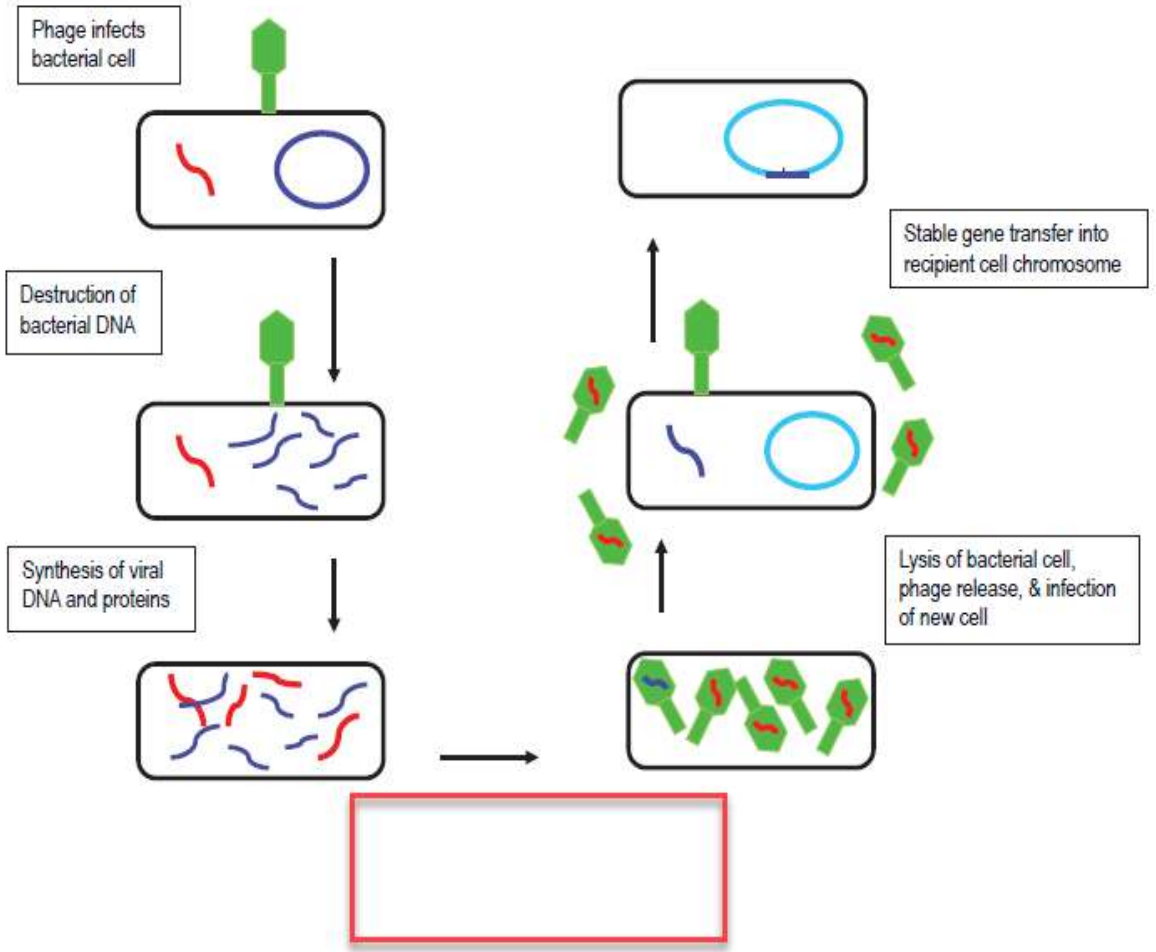

virion
host cell
attachment
penetration
and uncoating
integration of
viral DNA
provir
lytic cycle
lysogenic cycle
release
biosynthesis
and maturation
excision (induction)
of provirus

Types of transduction:

GENERALIZED TRANSDUCTION:

If all fragments of bacterial DNA (I.e from any region of the bacterial chromosome) have a chance to enter a transducing phage, the process is called generalized transduction.

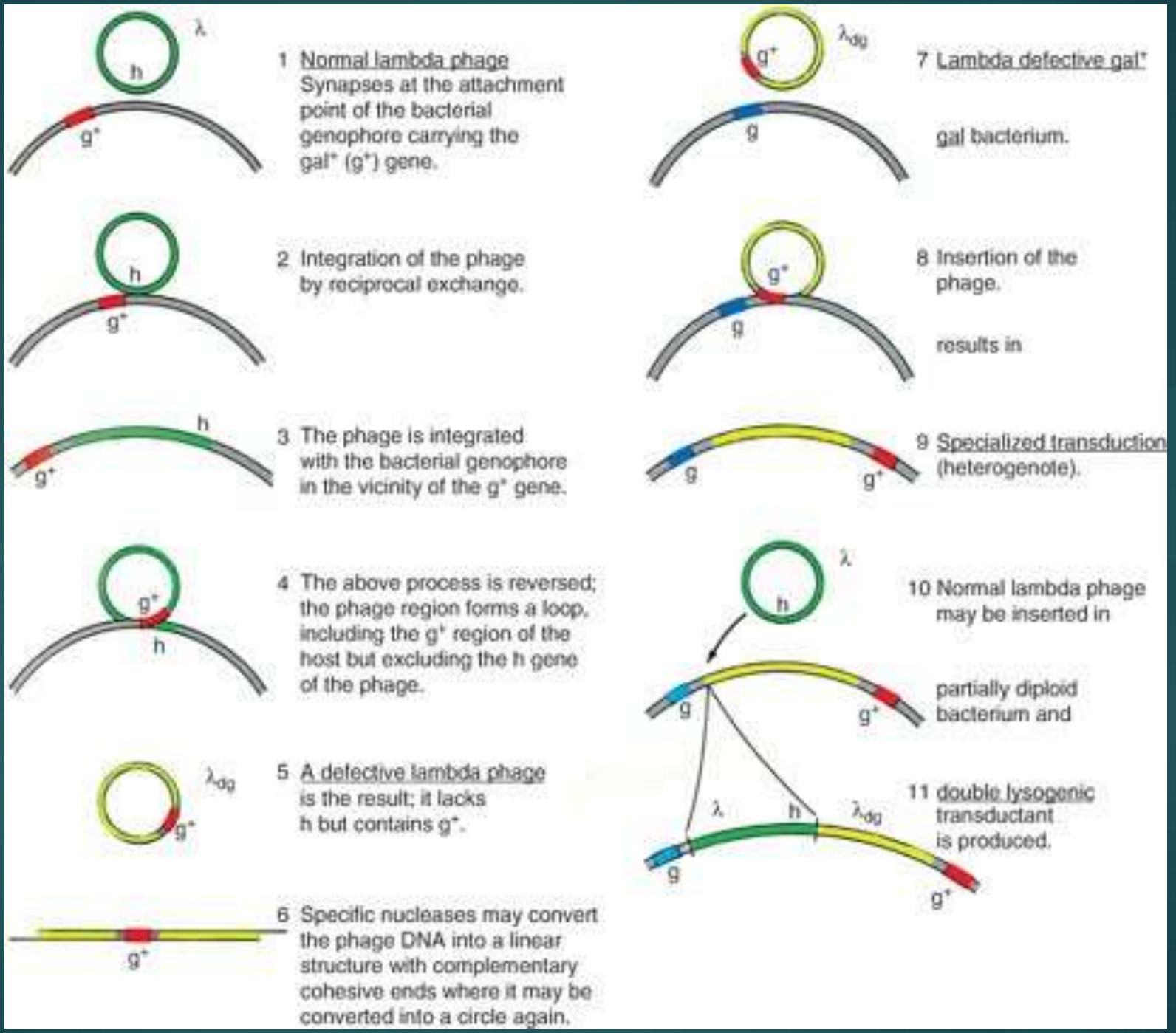
Generalized transduction



Courtesy of M. Mulks (MSU)

SPECIALIZED TRANSDUCTION:

Bacterial genes can also be transduced by bacteriophage in another process called specialized transduction in which certain temperate phage strains can transfer only a few restricted genes of the bacterial chromosome.


λ
h
g+
1 Normal lambda phage
Synapses at the attachment point of the bacterial genophore carrying the gal+ (g+) gene.
2 Integration of the phage by reciprocal exchange.
3 The phage is integrated with the bacterial genophore in the vicinity of the g+ gene.
4 The above process is reversed; the phage region forms a loop, including the g+ region of the host but excluding the h gene of the phage.
λdg
5 A defective lambda phage is the result; it lacks h but contains g+.
6 Specific nucleases may convert the phage DNA into a linear structure with complementary cohesive ends where it may be converted into a circle again.
7 Lambda defective gal+
gal bacterium.
8 Insertion of the phage.
results in
9 Specialized transduction (heterogenote).
10 Normal lambda phage may be inserted in
partially diploid bacterium and
11 double lysogenic transductant is produced.

GENERAL USES OF THE TERM AND APPLICATIONS:

- More generally, transduction is the process by which genetic material e.g: DNA or siRNA, is inserted into a cell by a virus.
- Common techniques in molecular biology are the use of viral vectors(including bacteriophages).
- Some medical applications are:-
- It provide resistance to anti-biotic drugs.
- Helps in the correction of genetic diseases by direct modification of genetic errors.
- It is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.


Thank You!