Gene transfer and homologous recombination in bacteria

Thomas Candela

Generalities

- **Gene flow promotes the genetic evolution and increases the microbial diversity.**

- **DNA acquisition may have a phenotypic impact if genes are involved in:**
  - Metabolism
  - Antigenic composition
  - Virulence
  - Antibiotic resistance

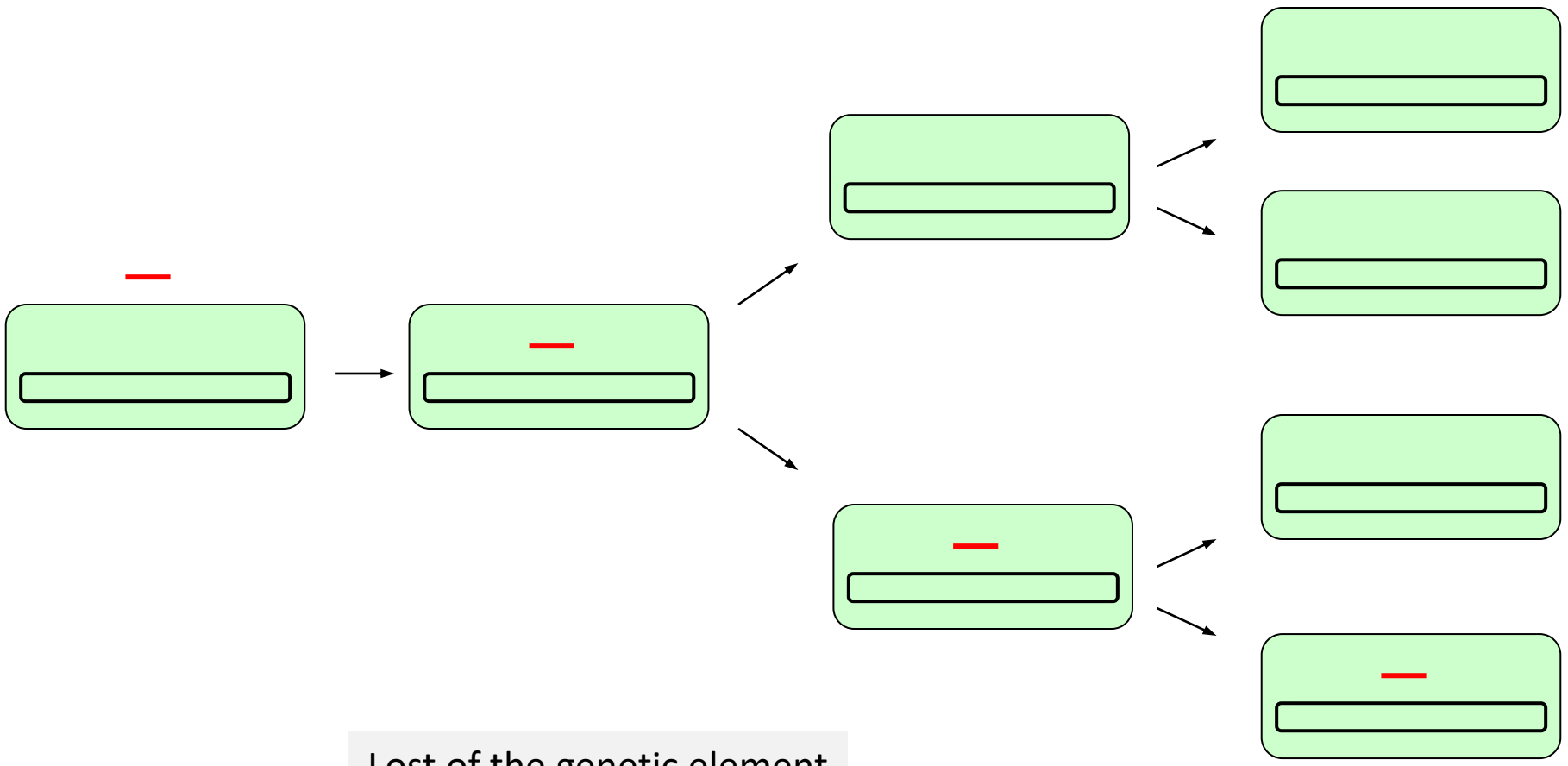
Generalities

- **A foreign DNA entry in bacteria must be followed by a specific genetic event to be acquired:**
  - Substitution (homologous recombination)
  - Addition :
    - ➢ auto replicative DNA (ex. : plasmids)
    - ➢ Integrative DNA

If DNA is not auto replicative or integrated, it will be diluted and lost among the bacterial population.

Lost of the genetic element

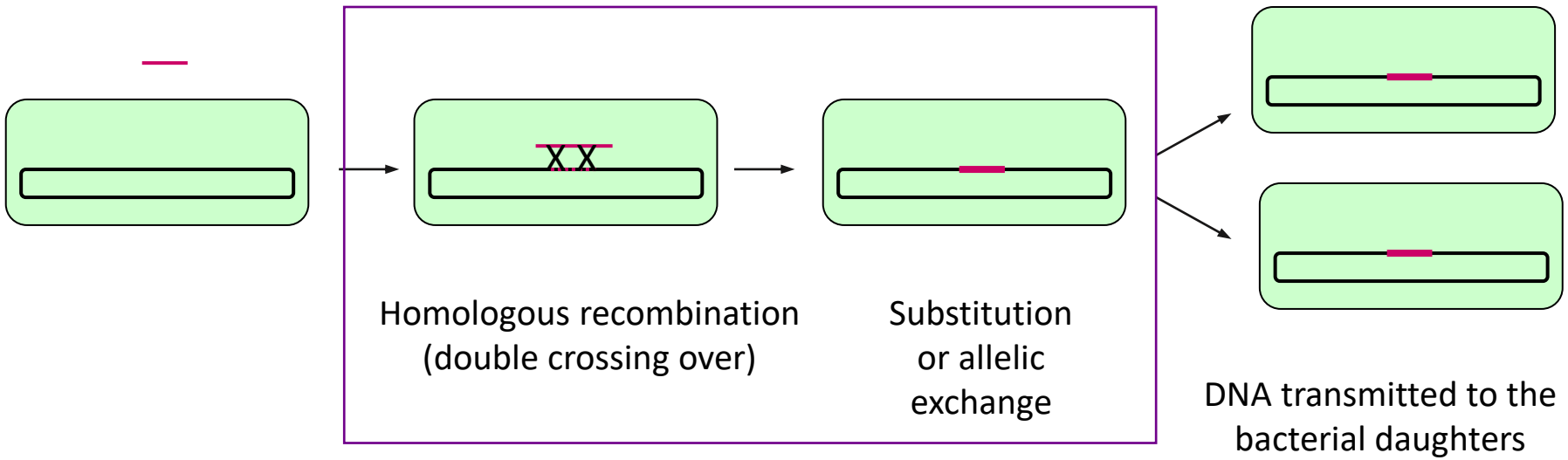
Acquisition followed by absence of integration or replication



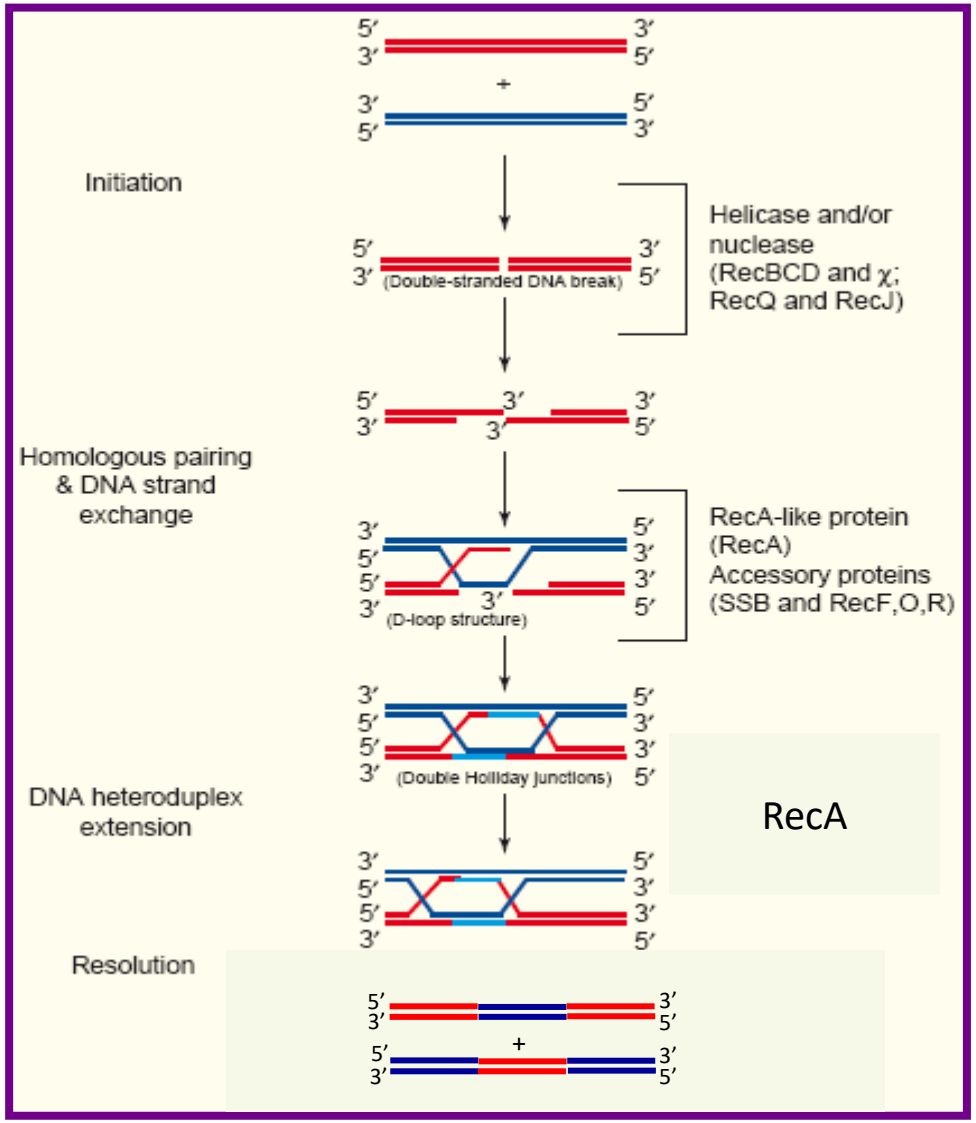
Lost of the genetic element

Acquisition by homologous recombination

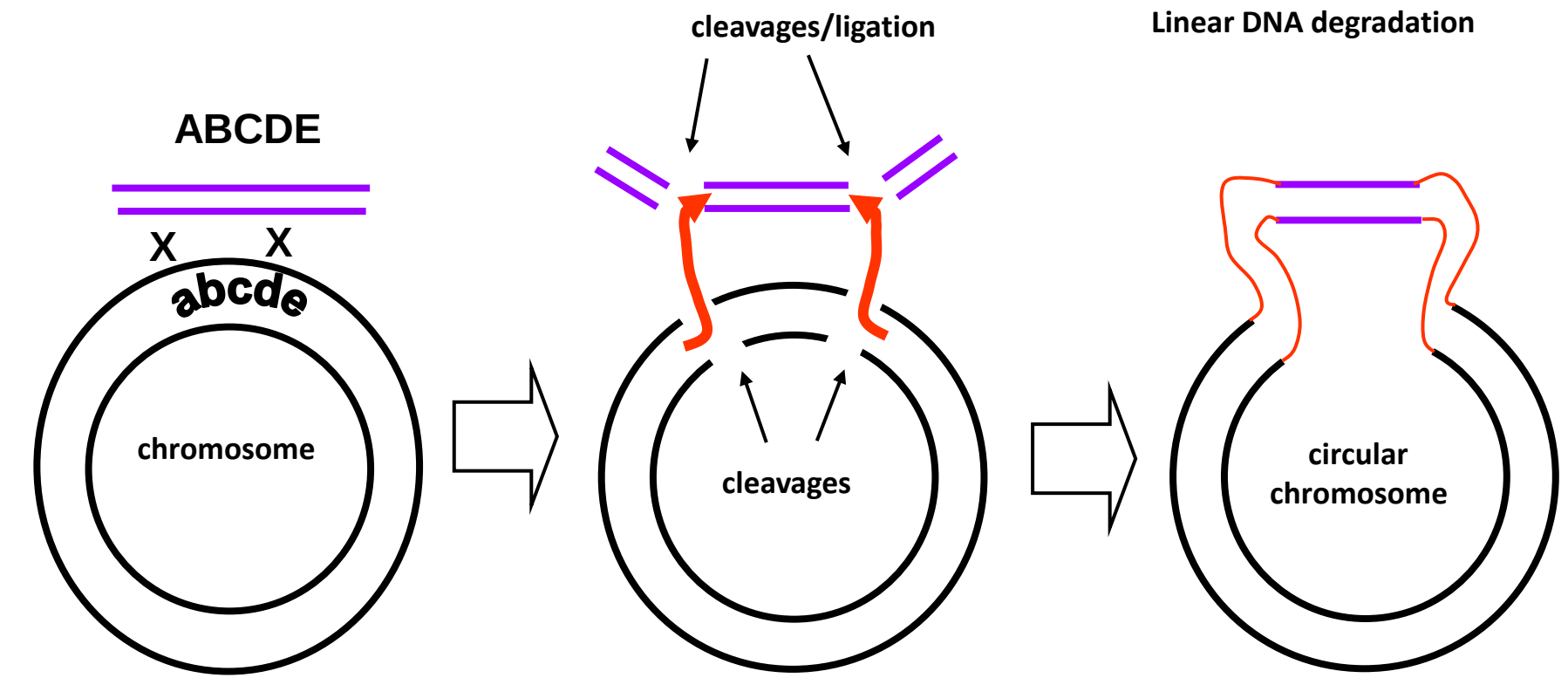
Acquisition of genetic DNA by substitution (homologous recombination)



Homologous recombination model



A pair number of crossing-overs produces a circular and viable recombinant



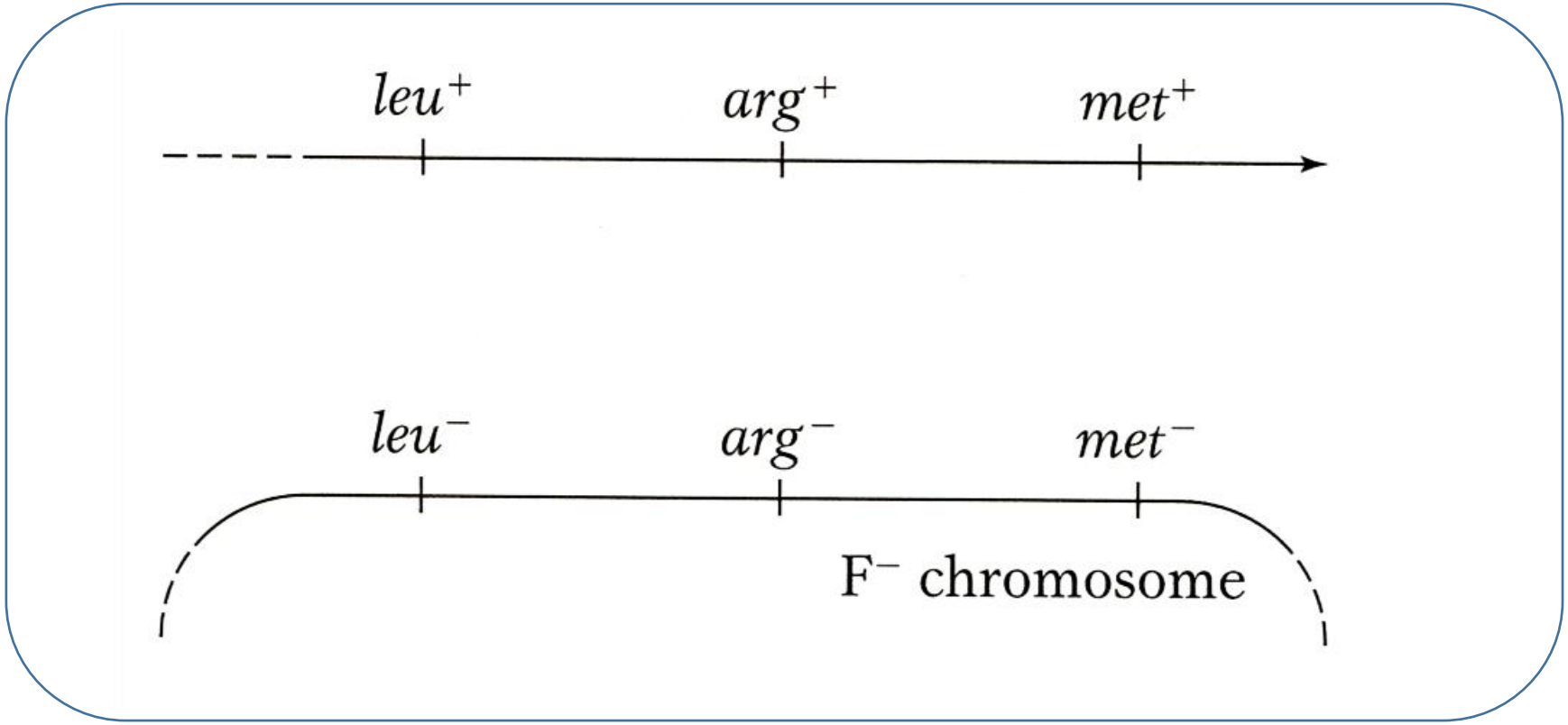
Homologous recombination

2 crossing-overs

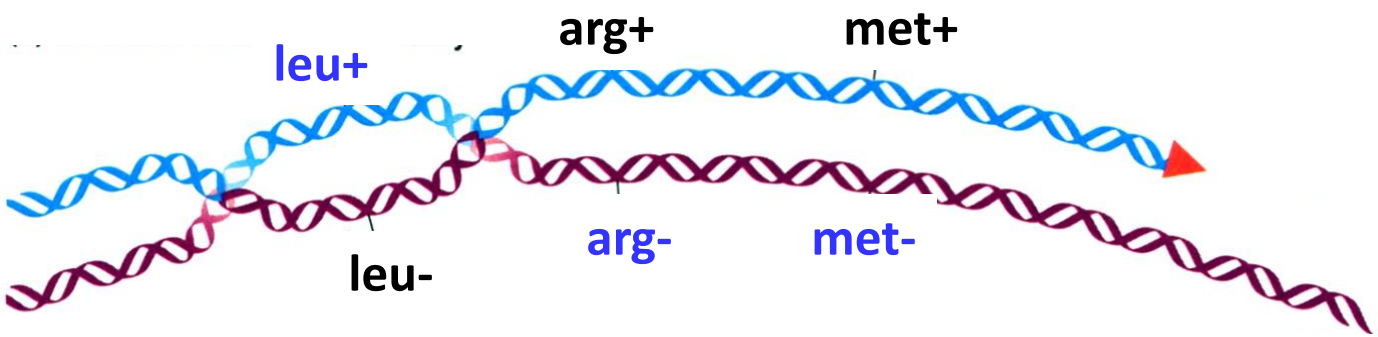
Viable recombinant

Genetic consequences of the transfer/ recombination

Parental genotype (donor) *leu+ arg+ met+*

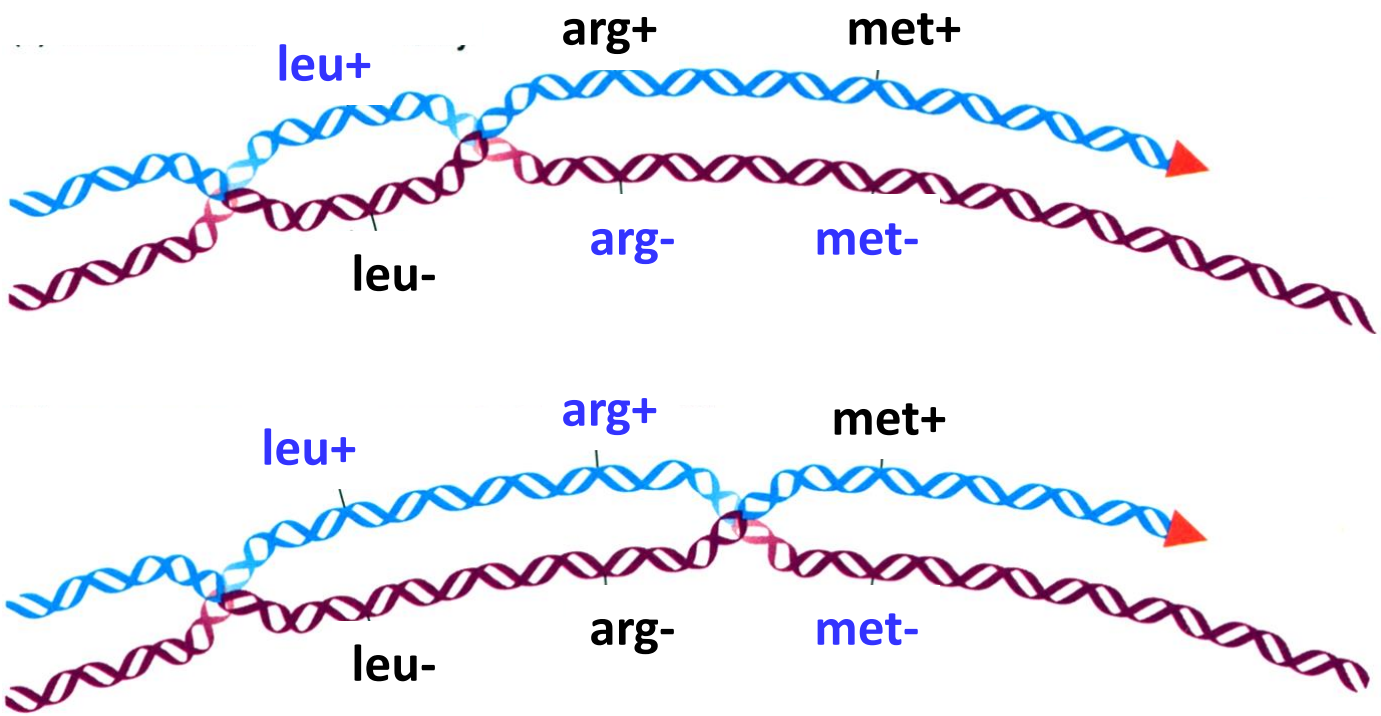


Parental genotype (recipient) *leu- arg- met-*



RECOMBINANTS

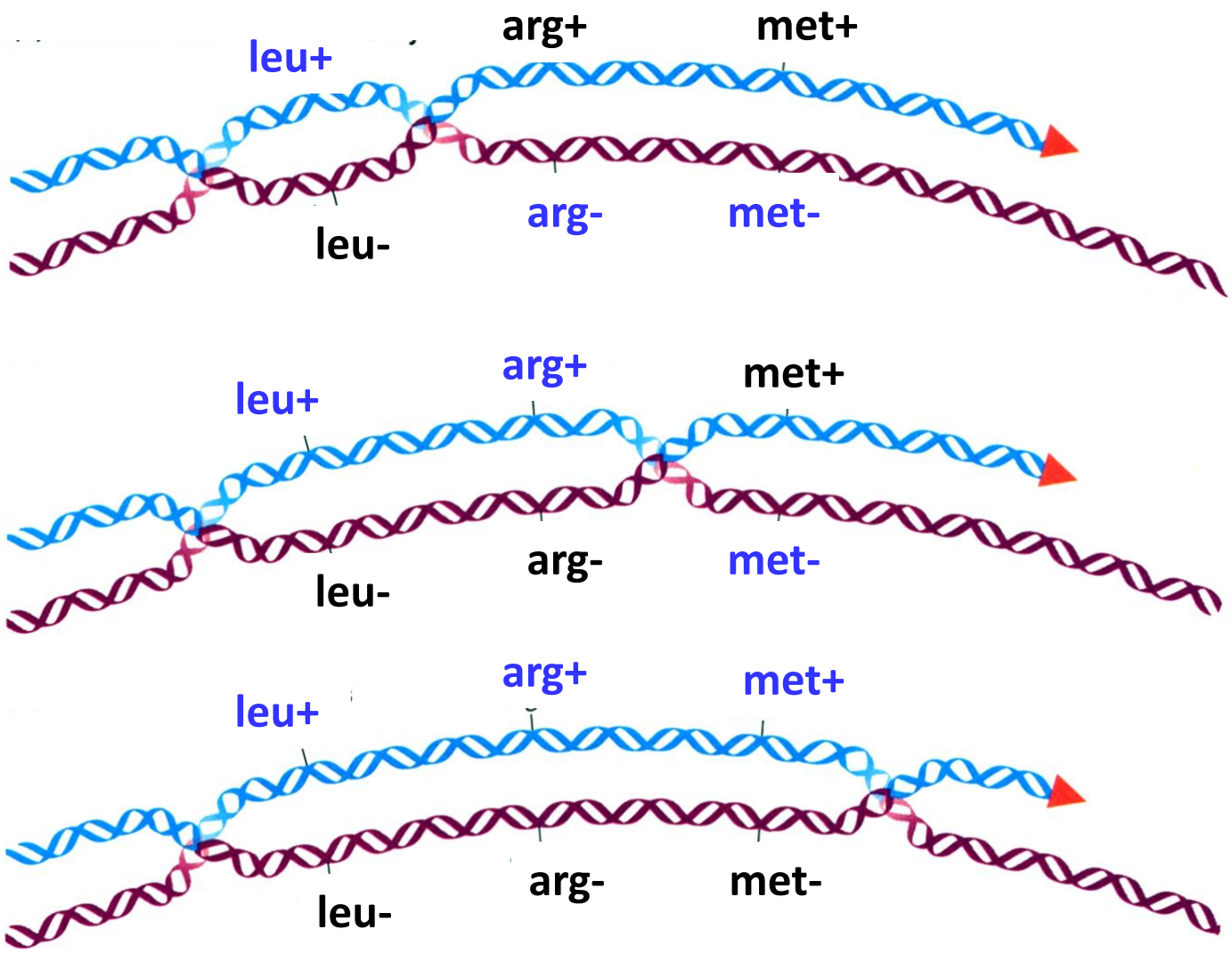
leu+ arg- met-



RECOMBINANTS

leu+ arg- met-

leu+ arg+ met-

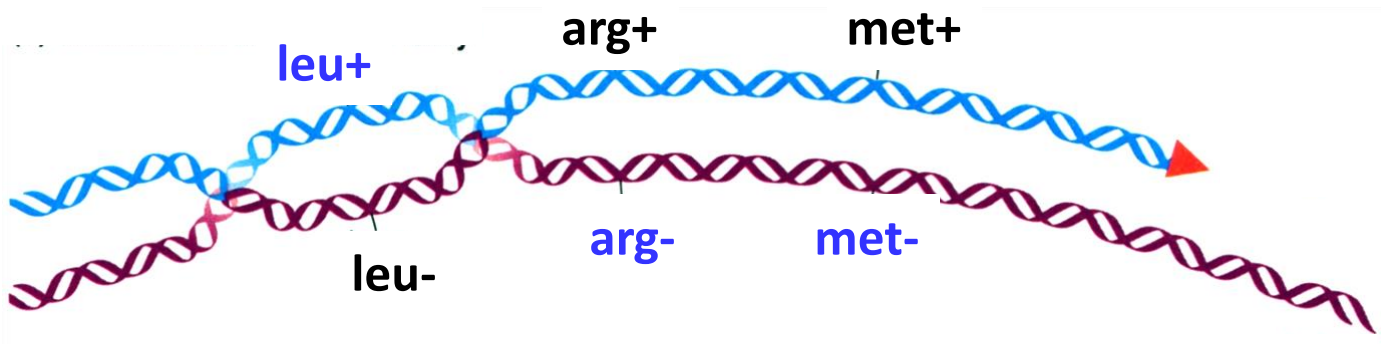


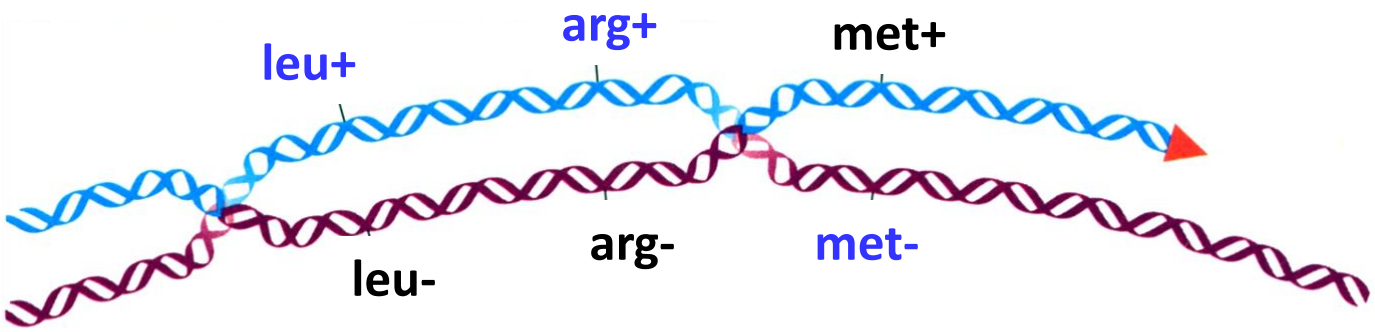
RECOMBINANTS

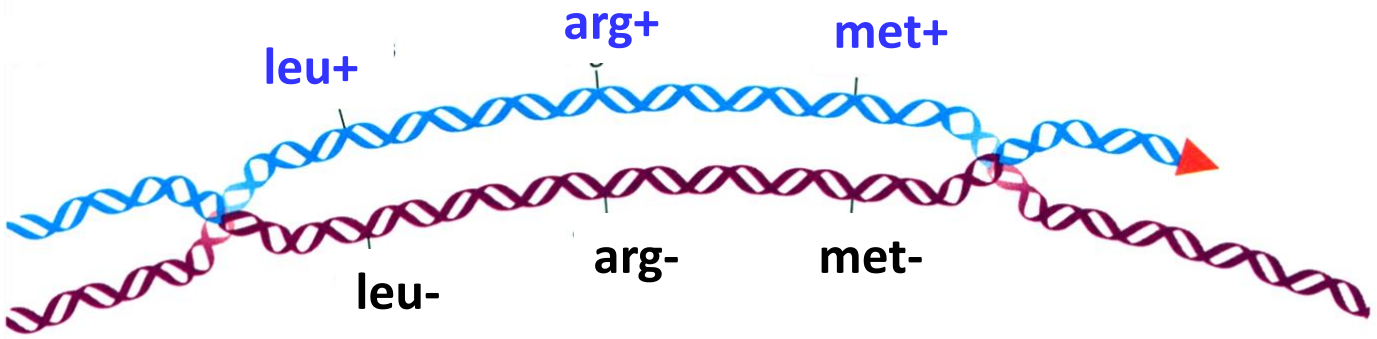
leu+ arg- met-

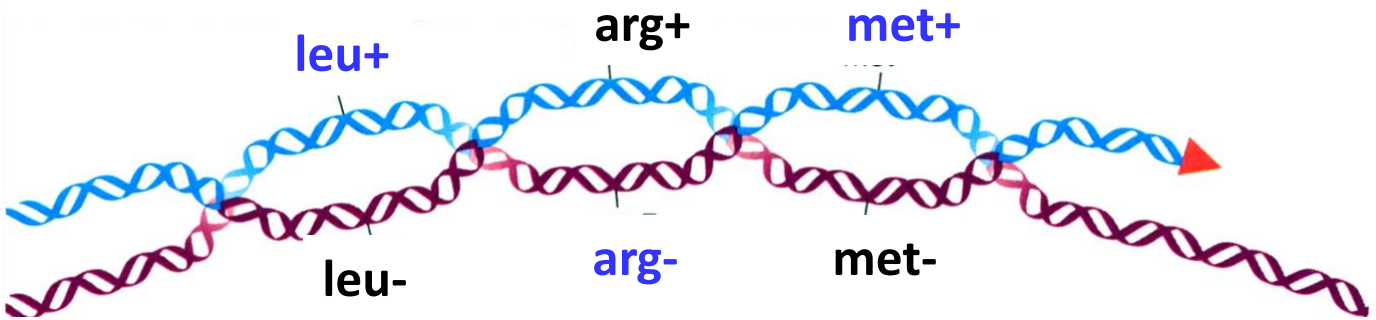
leu+ arg+ met-

leu+ arg+ met+









RECOMBINANTS

leu+ arg- met-

leu+ arg+ met-

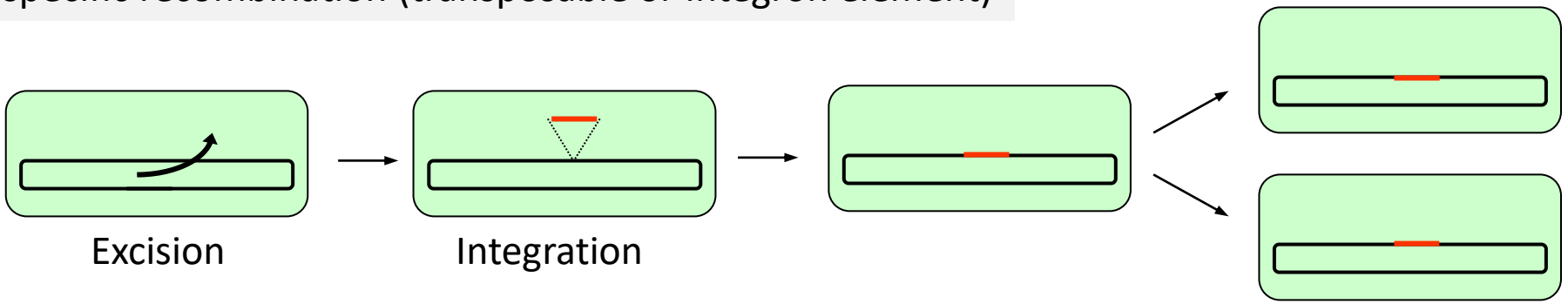
leu+ arg+ met+

leu+ arg- met+

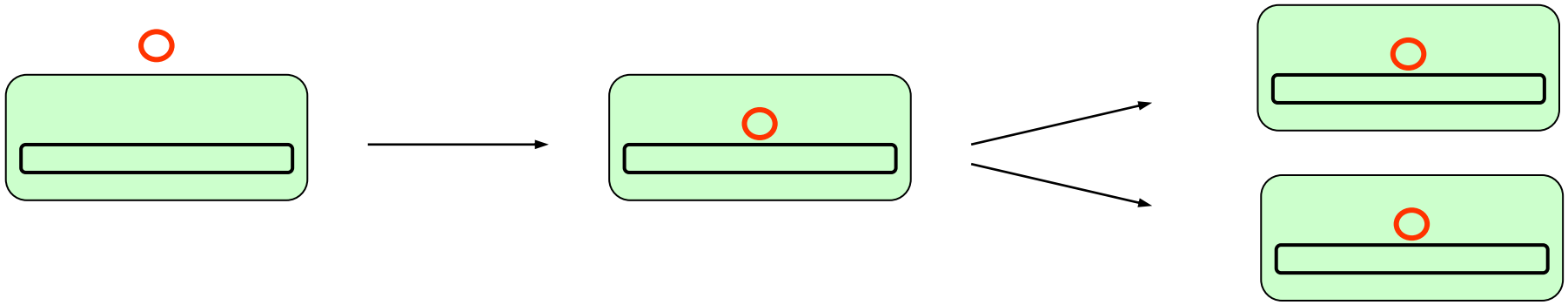
Acquisition through a genetic element

DNA addition in absence of homologous recombination

Site-specific recombination (transposable or Integron element)



Autonomous replication



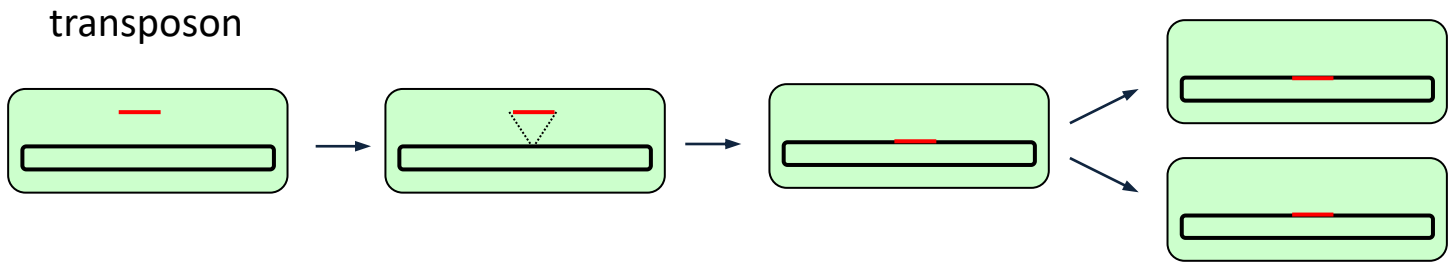
Transposable elements

Transposon

- Transposons are DNA sequences able to change their location in the genome.
- They are not replicative
- The genes in the transposon encode the necessary enzymes to allow transposition and may also encode other functions like antibiotic resistance, virulence factors

Transposon

- Transposition is a mechanism of fast evolution. It consists in DNA addition defined size in the genome (bacterial chromosome or plasmid). It works in the absence of sequence homology (illegitimate recombination).



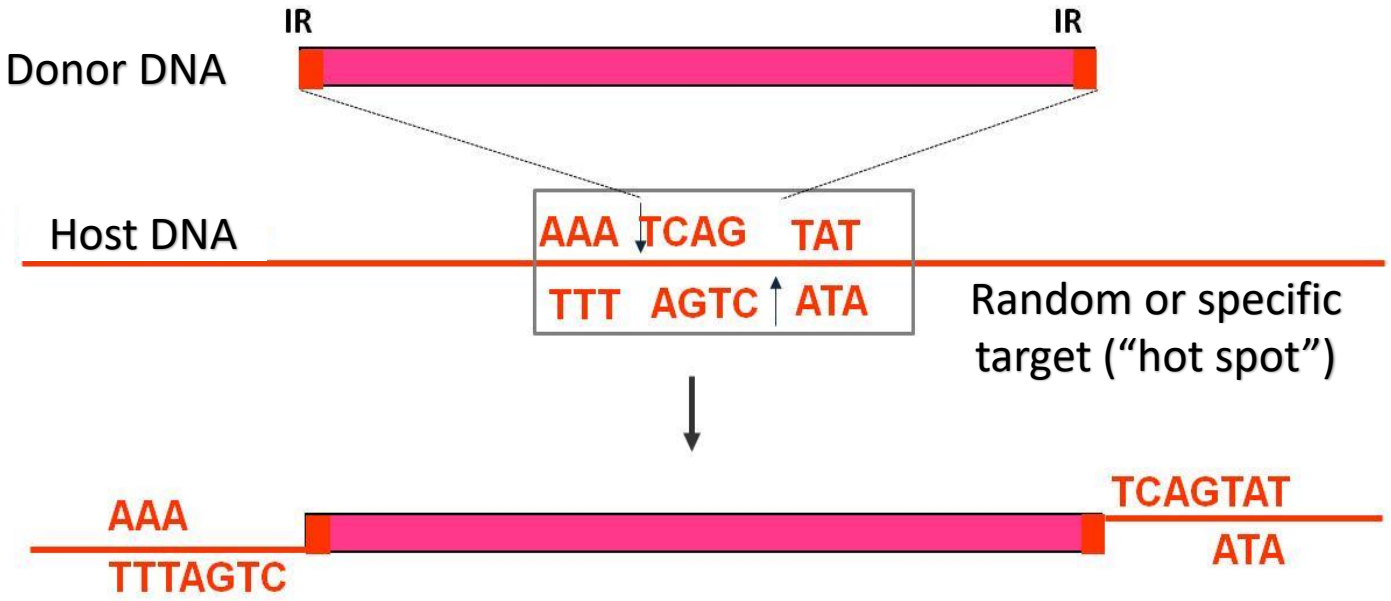
- Transposable elements have a transposase responsible for the illegitimate recombination.

Transposon properties

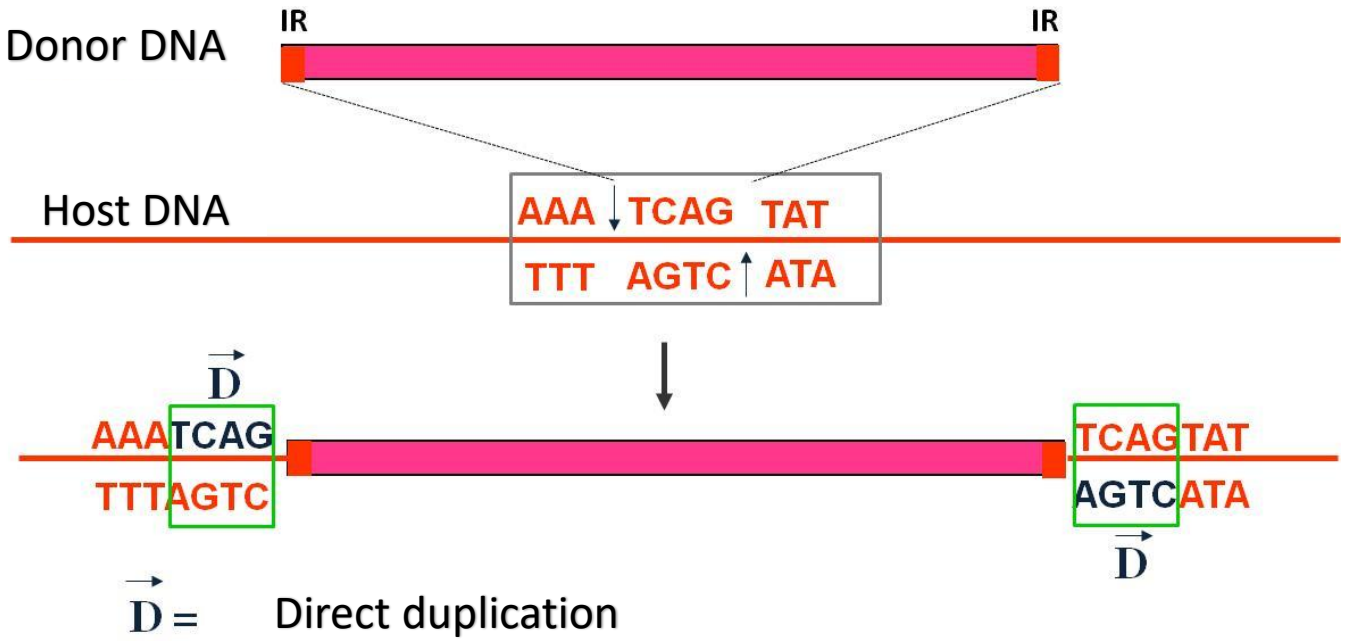
- Able to move from a site to another of the genome
- Able to move from the chromosome to a plasmid or vice versa in the bacterium
- May bring some new genes (resistance, virulence)
- May modify the gene expression:
  - Positively :
    - Repressor inactivation
    - Insertion in a negative regulatory sequence
    - Bring a promoter
  - Negatively :
    - Insertion in a gene
    - Insertion in a positive regulatory sequence
- May provoke genomic modifications.

Transposition mechanism

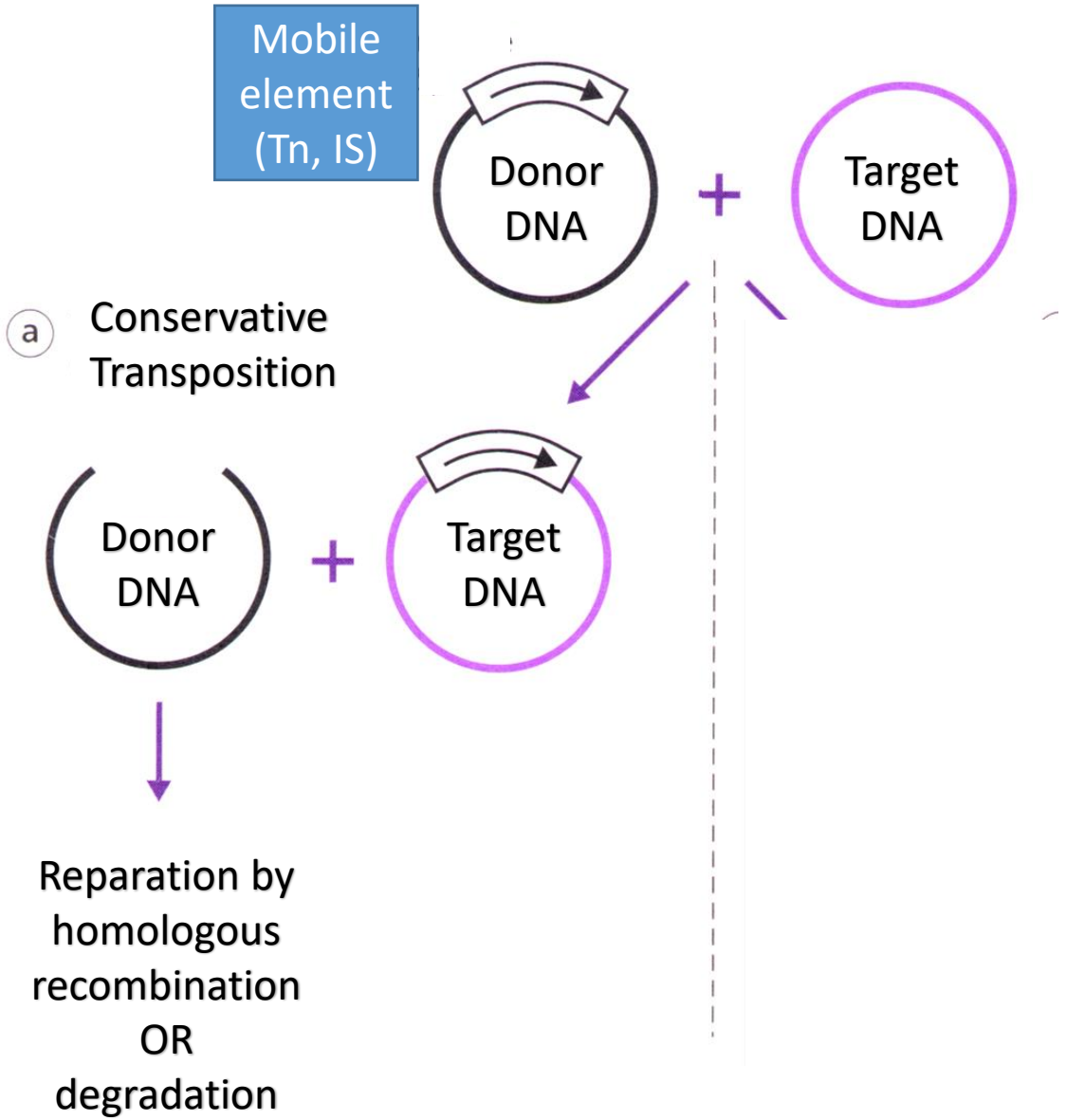
- Transposition implies the presence of inverted and repeated sequences named IR



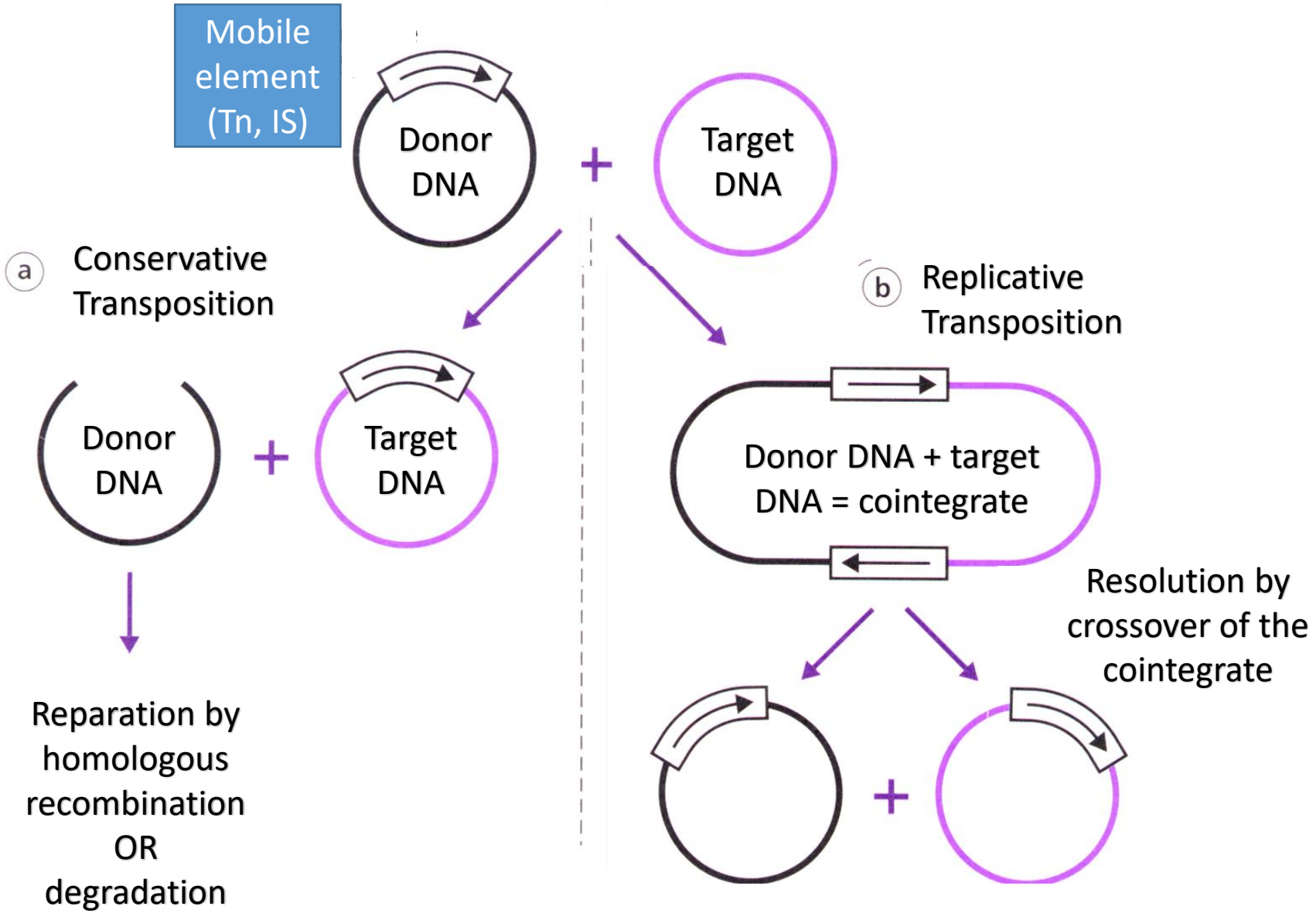
Transposition mechanism



Conservative transposition



Conservative and replicative transpositions



Different types of transposable elements

- Insertion sequence (IS)
  - The simplest transposable elements
  - Size : 1 to 2 kb

IR= repeated inversed sequences (10 to 40bp)

Transposase: excises the transposon and cleaves the targeted DNA

- Composite transposons

Transposon Tn*10*

Both IS may be separated by several genes. This genes are moved by the two IS.

Different types of transposable elements

- Unitary transposons

Transposon Tn*3* (≈ 5 kb)

TnpA = Transposase
TnpR = Resolvase
BlaT = ß-lactamase

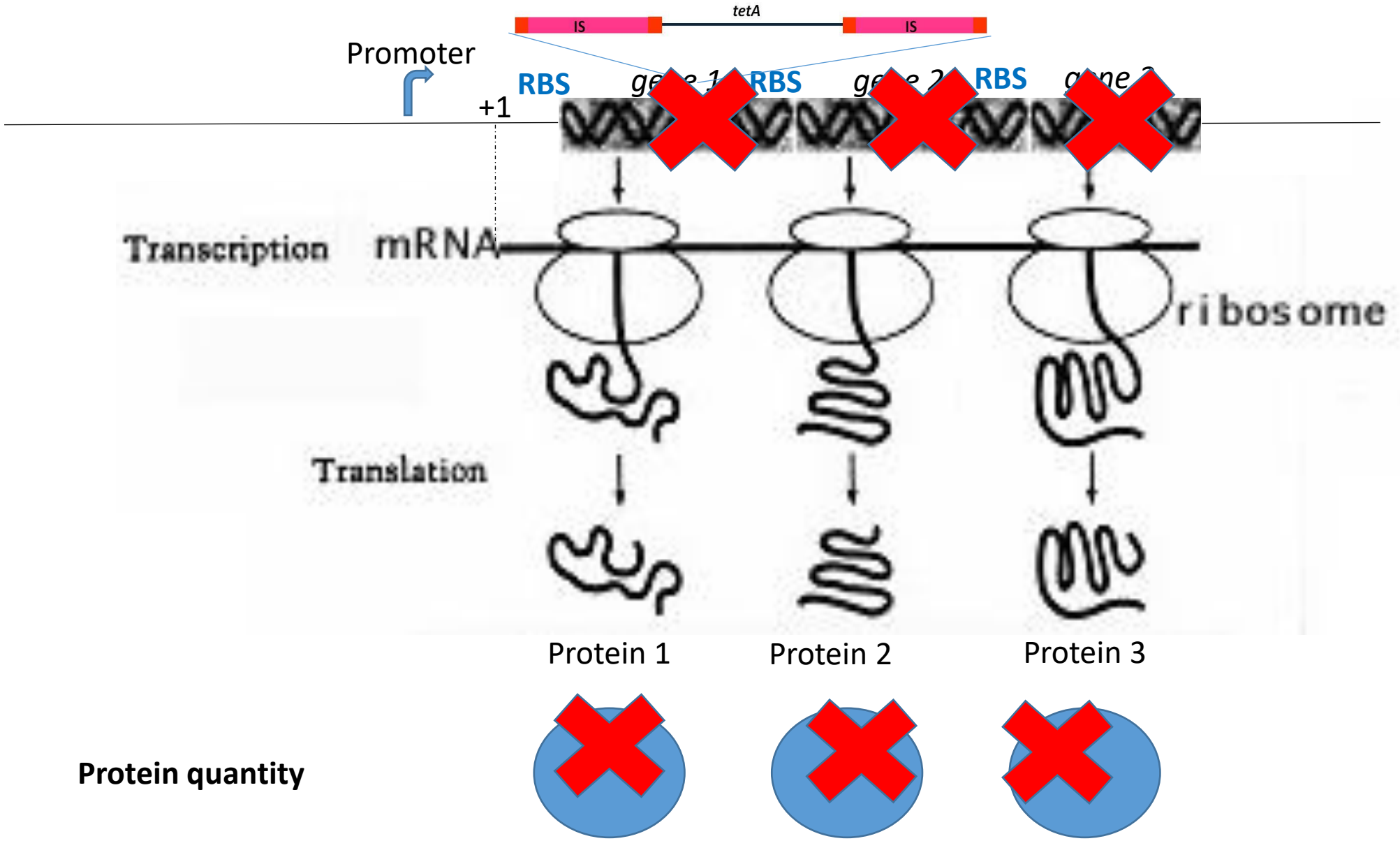
- Conjugative transposons
  - Big transposons (> 16 kB)
  - Able to transfer from bacteria to bacteria by conjugation between several species

Importance of transposable elements in therapeutic

- **Transposons carry several resistance genes:**
  - Enzymes *bla* that inactivate the ß-lactamines
  - Enzymes that modify aminosides
  - RNA methylases
  - Glycopeptide resistance
    - Resistance of type VanA : Tn*1546* = derived from Tn*3*
    - Resistance of type VanB : Tn*1549* = conjugative transposon

- **Polar mutations**, consequences on gene transcription
  - Example : addition of one promoter (some IS)

Polar mutation



Protein quantity

Integrons

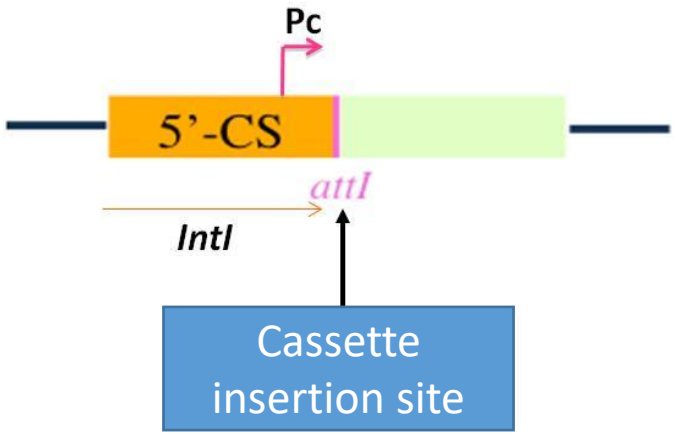
Definition

- Integrons constitute a gene capture and expression system under cassettes form. Integrons are not mobiles by them selves, but they are frequently found on transposons or plasmids.

- Cassettes are small mobile elements that are non-replicatives, but able to be integrated or excised by a specific recombination mechanism made by an integrase.

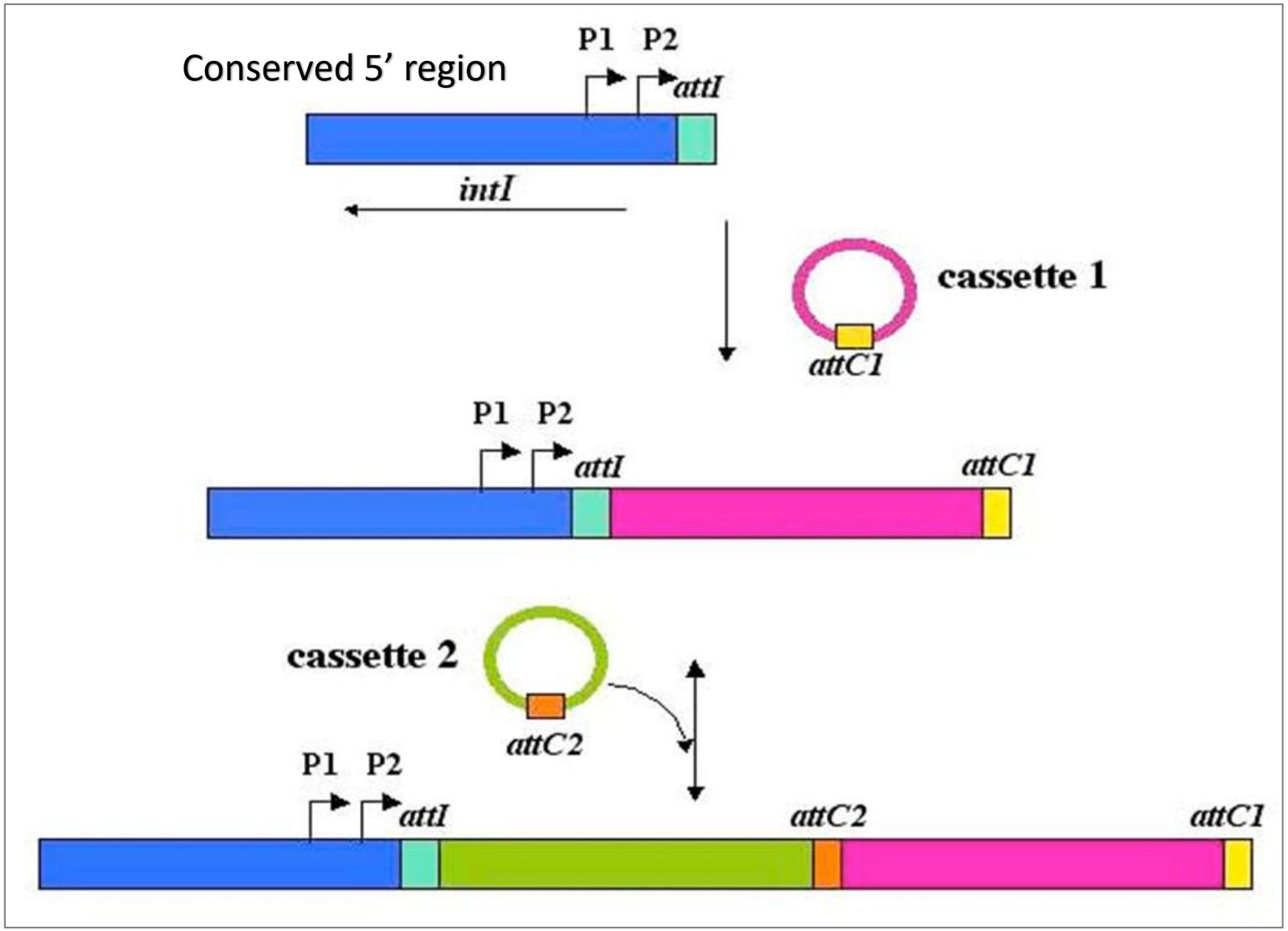
Integron structure

- A functional platform is constituted by :
  - *intI* (5'region) encode an integrase
  - *attI, a* recombination specific site
  - Pc, a strong promotor



- Different classes of integron exist. They are defined according to the gene nature of the integrase.
- Cassettes do not encode a functional gene of the integron.

Insertion des cassettes



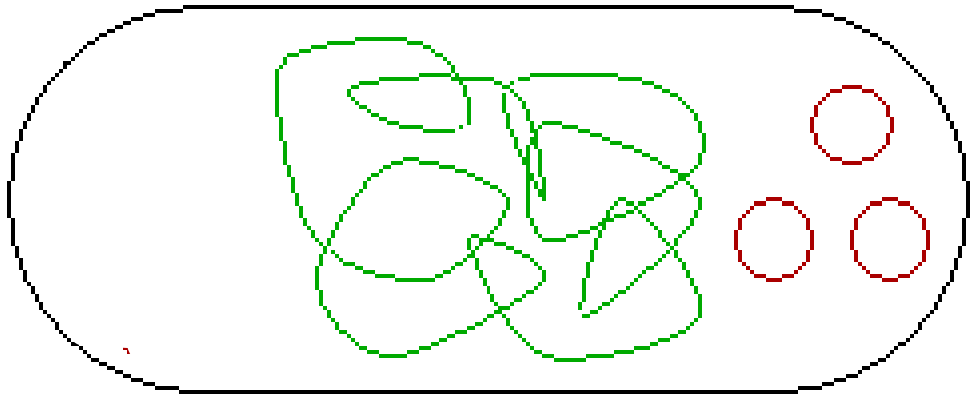
Plasmids

Plasmids – definition

The name of **plasmid was introduced** by Joshua Lederberg in 1952

A **plasmid is a DNA molecule**:

- Distinct from the chromosomal DNA
- Non-essential to the cell survive
- Able to replicate in an autonomous manner

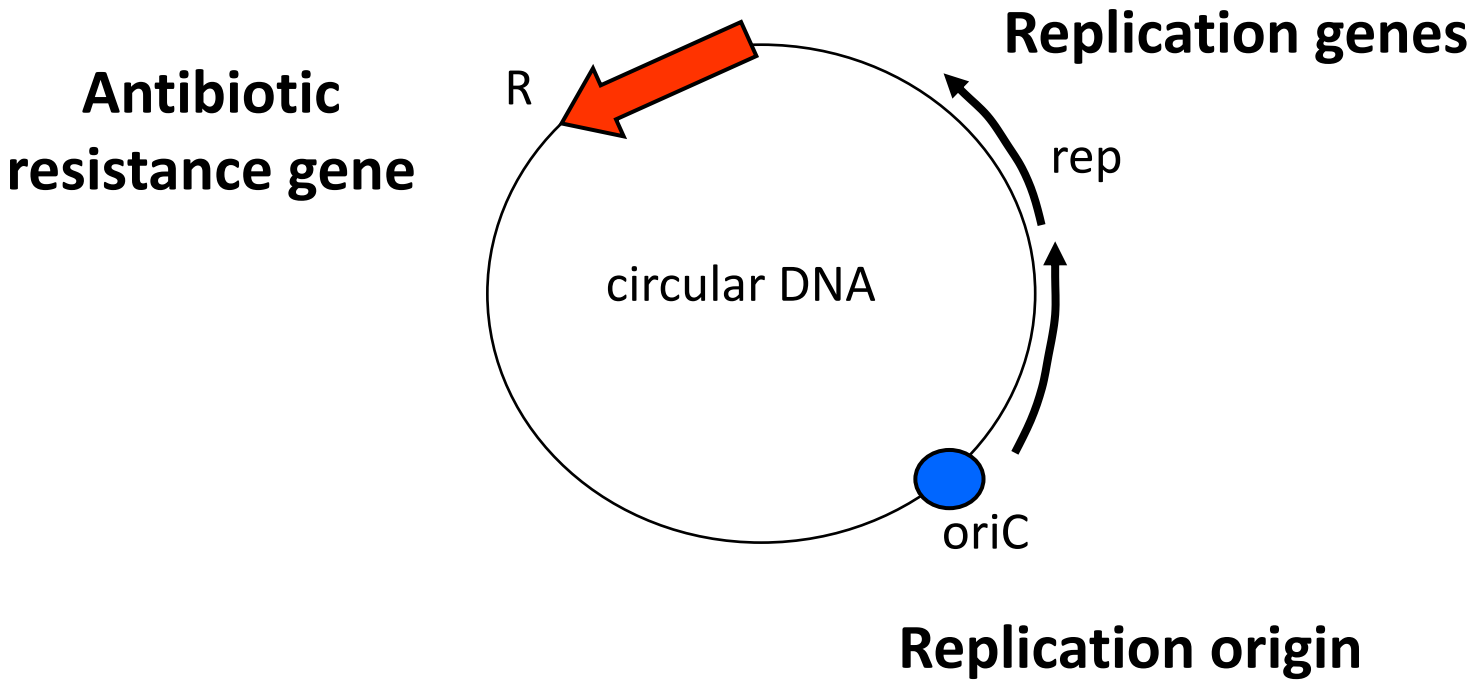


Plasmids – structural aspects

- **Size**– from 2 to 100kb and more

- **Forme**: circular (for the majority) or linear (rare)

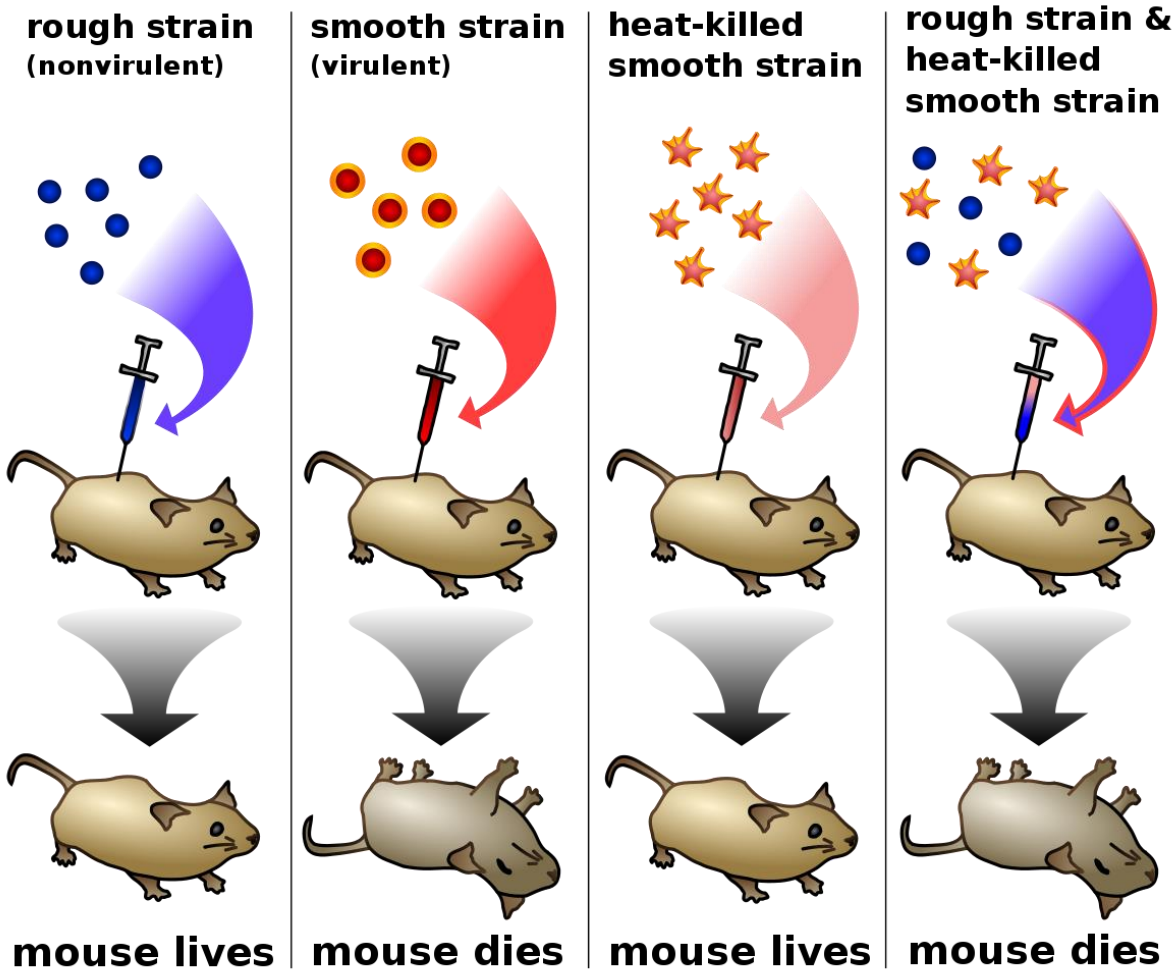
- **Copy number:** 1 to more than 100

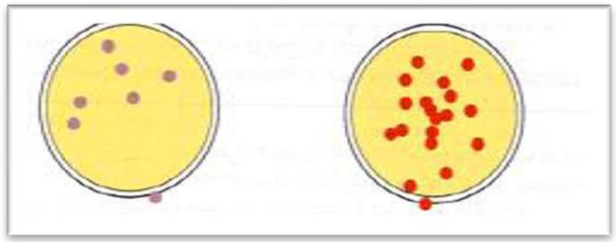


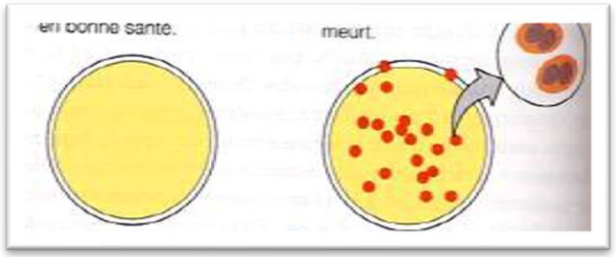
Gene transfer mechanisms from bacteria to bacteria

Transformation

Natural transformation **discovered by F. Griffith in 1928**





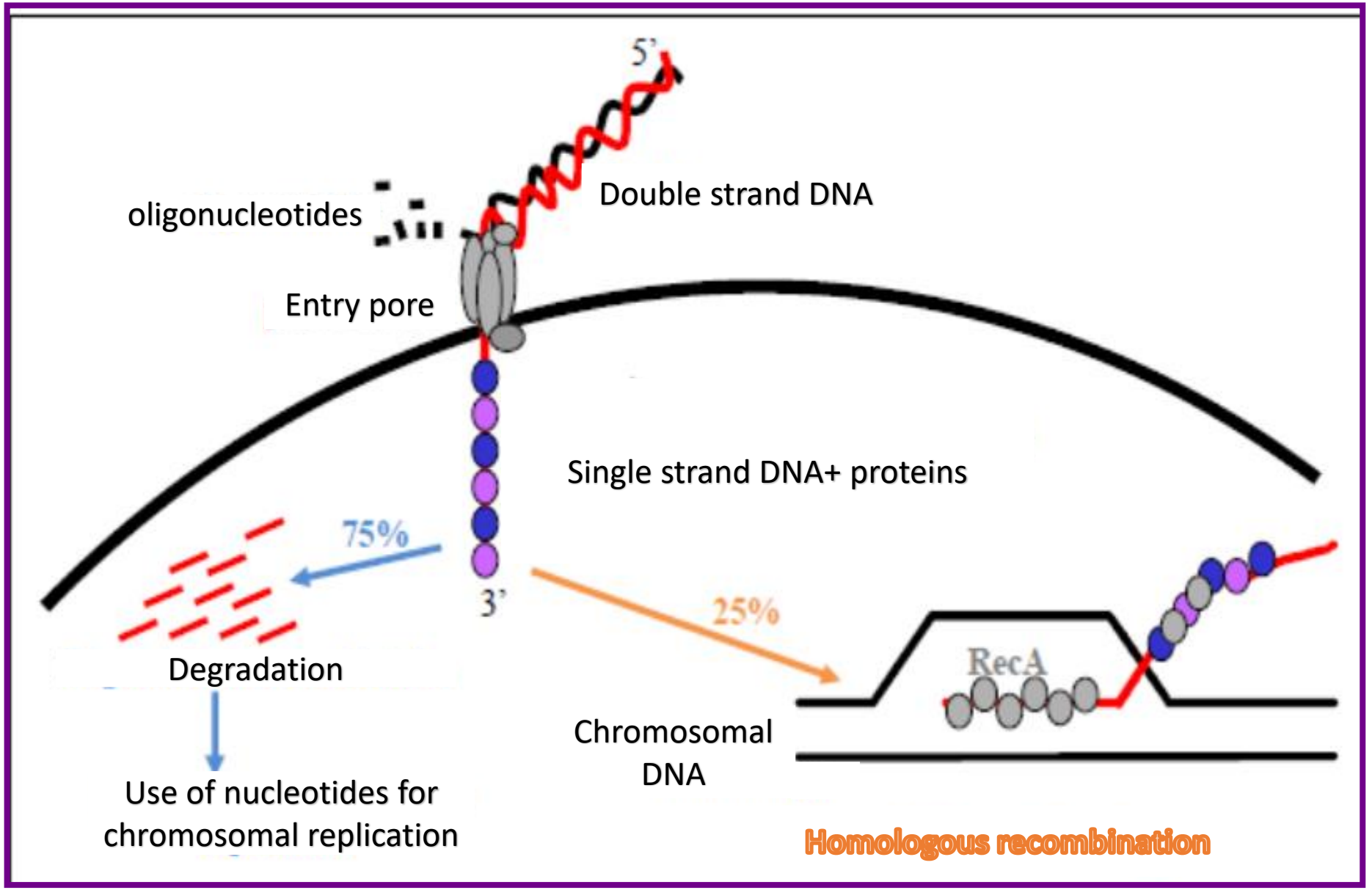


Natural transformation

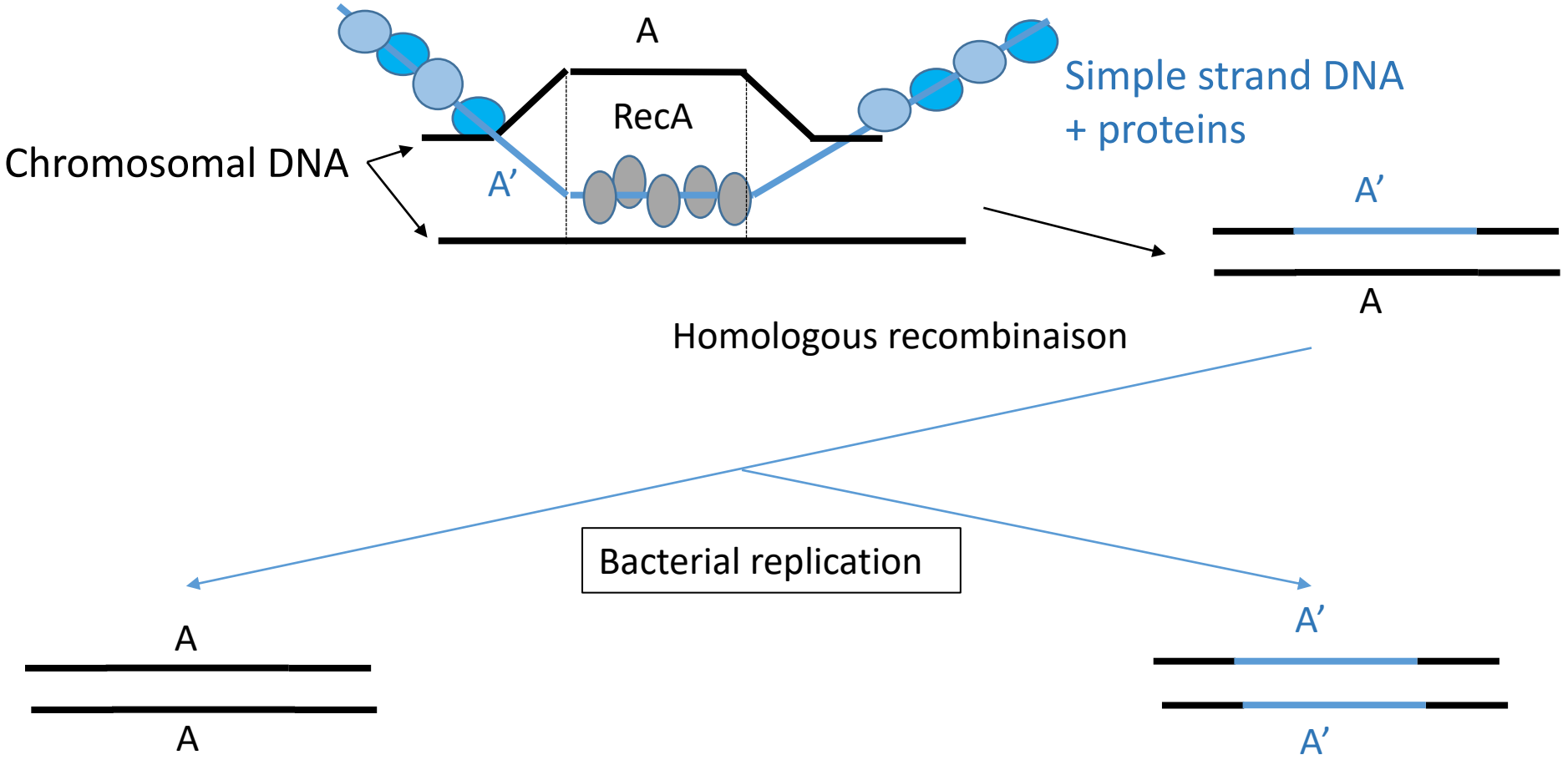
- **Introduction in a competent bacteria of a free DNA.**

- **Species able to perform natural transformation:**
  - *Streptococcus pneumoniae*
  - *Bacillus subtilis*
  - *Haemophilus influenzae*
  - *Helicobacter pylori*
  - *Neisseria meningitidis*
  - *Neisseria gonorrhoeae,*
  - *Vibrio cholerae,…*

Natural transformation mechanism

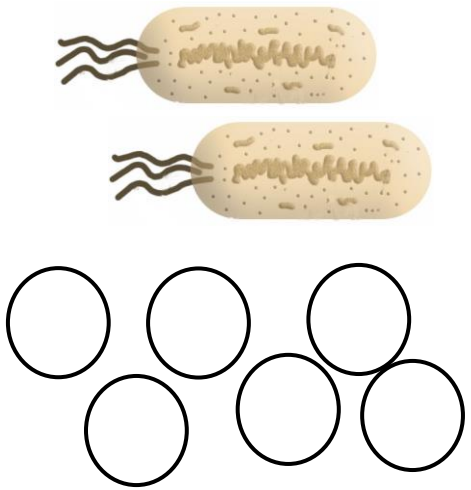


Natural transformation mechanism



E. coli : absence of natural transformation

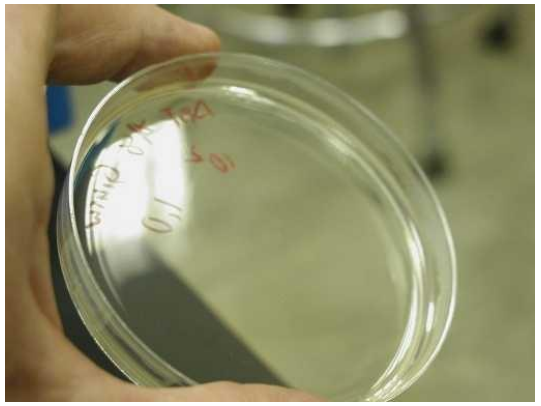
E. coli



Plasmid carrying the gene resistance A

speading

SELECTION
medium + antibiotic A



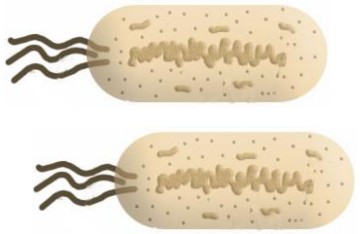
NO colonies : gene resistance A is not acquired

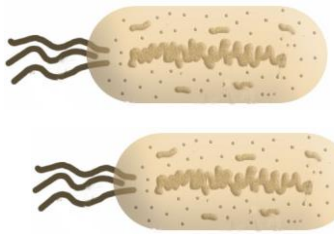
In laboratoiry, *E. coli* is made COMPETENTS and is therefore transformable

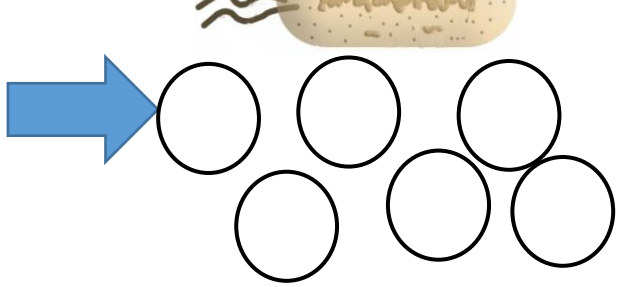
treatement

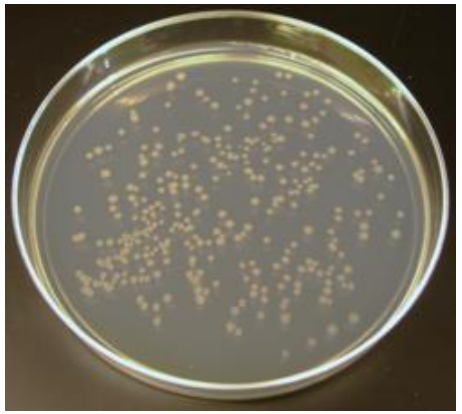
Competent *E. coli*

selection









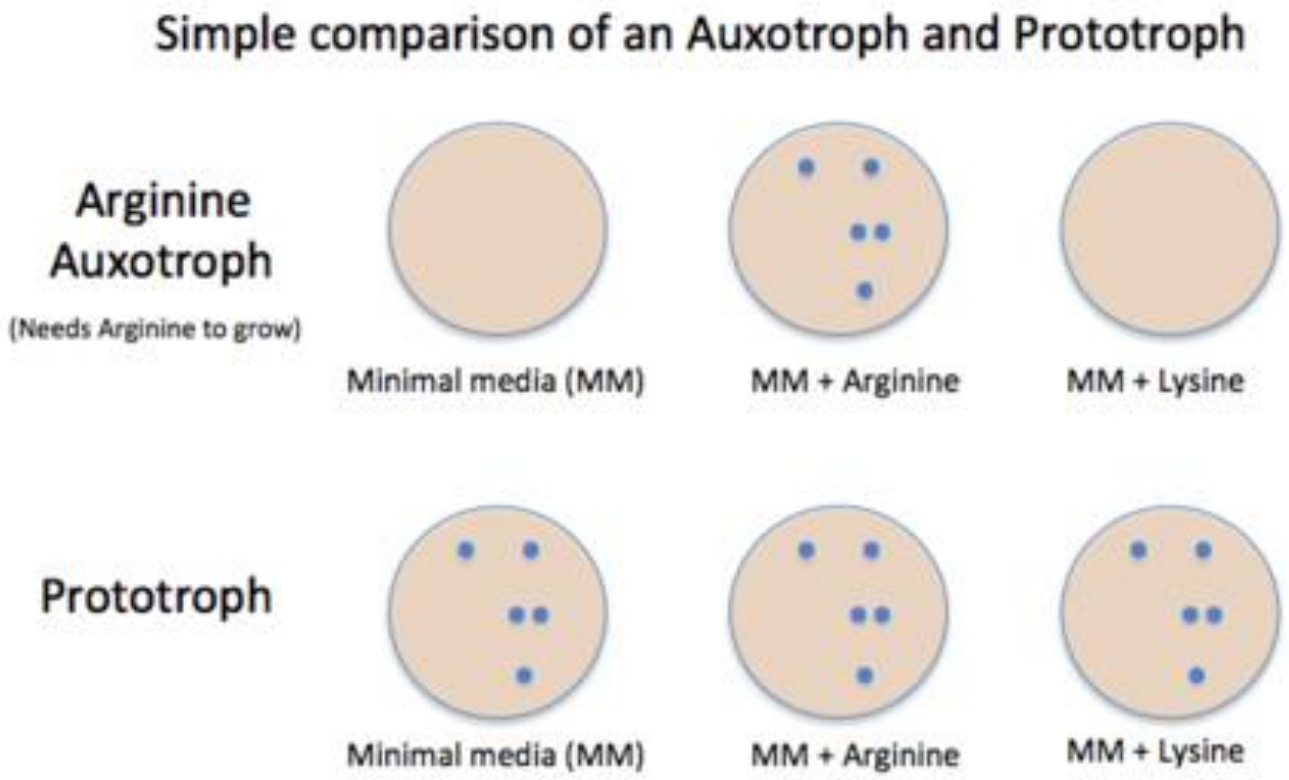
CaCl2, or
PEG, or
electroporation

Plasmid carrying the gene resistance A

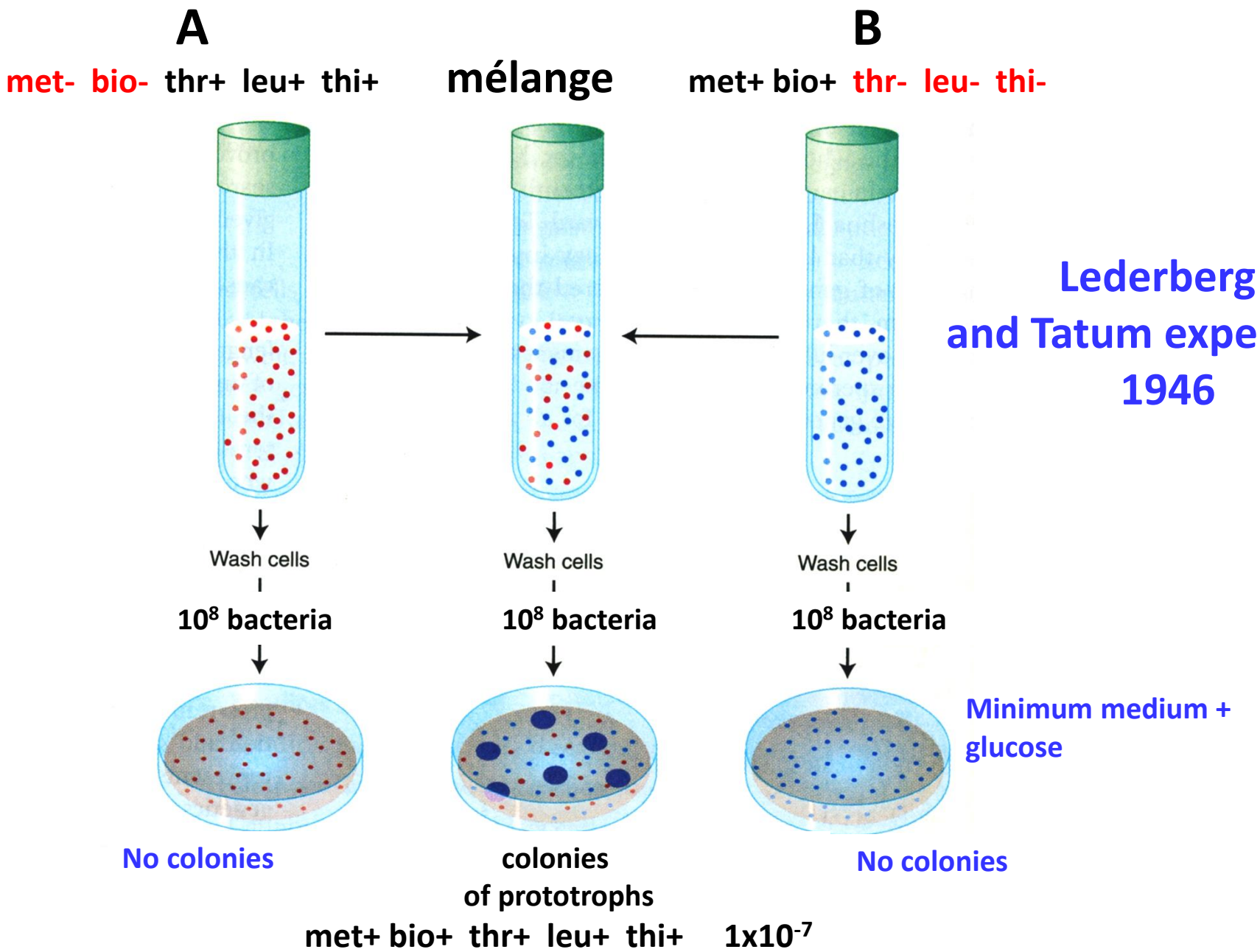
Medium + antibiotic A

Conjugation

Reminder

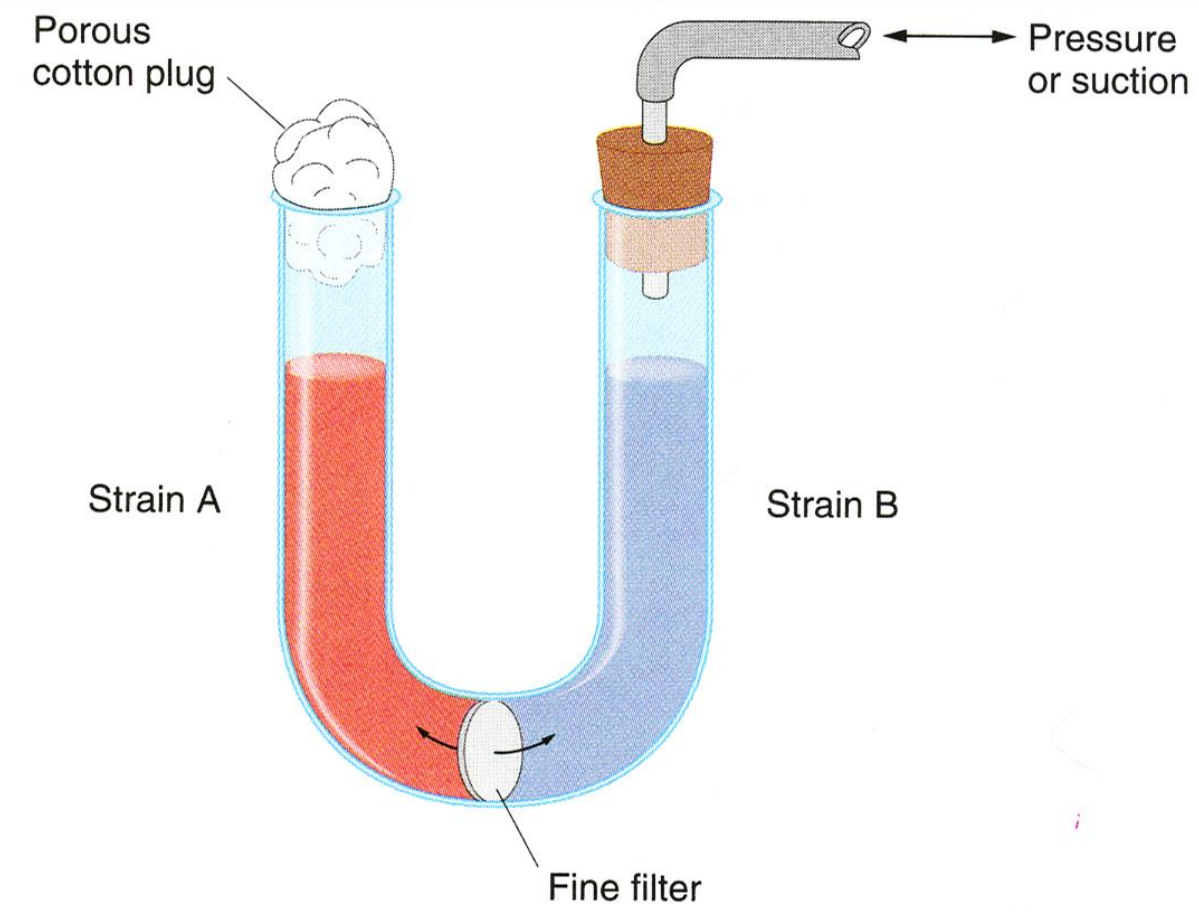

Simple comparison of an Auxotroph and Prototroph
Arginine Auxotroph
(Needs Arginine to grow)
Minimal media (MM)
MM + Arginine
MM + Lysine
Prototroph
Minimal media (MM)
MM + Arginine
MM + Lysine

Discovery of conjugation

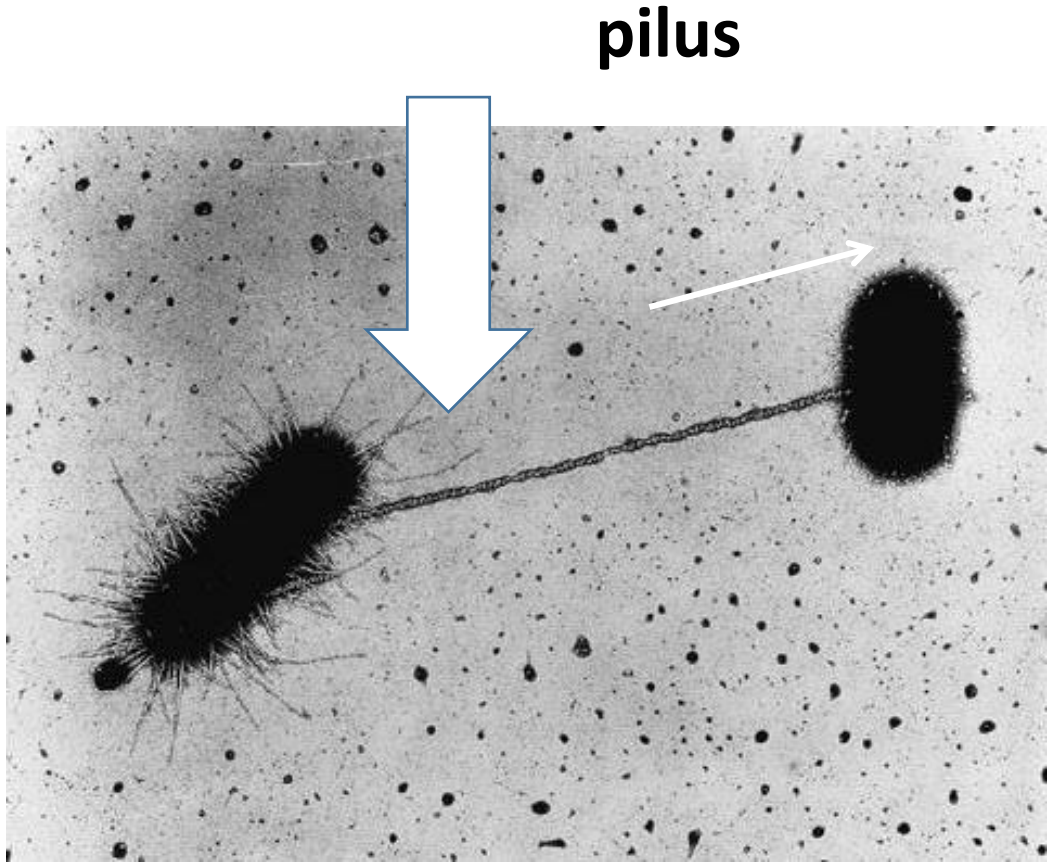


Lederberg and Tatum experiment 1946

A direct contact between bacteria is needed to promote the transfer by conjugation

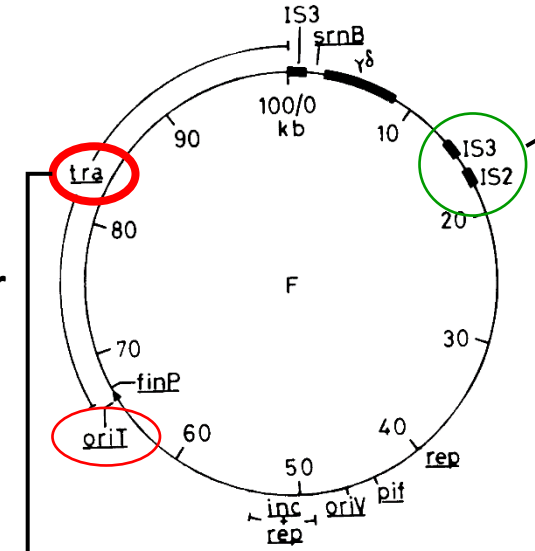


Direct contact between bacteria



Genes necessary for conjugation

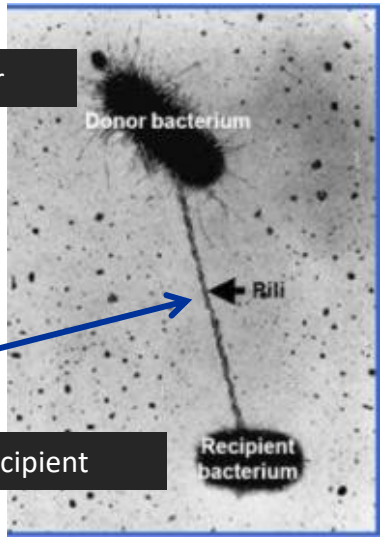
- **Example of F Factor, conjugative plasmid model**
  - **Found in donor bacteria**
  - **Size ≈ 100 kB**
  - ***tra* region**
    - **Pili synthesis that are necessary for the transfer by conjugation**
    - **Possible integration of the F factor in the bacterial chromosome : Hfr bacteria**



Integration factors in the chromosome

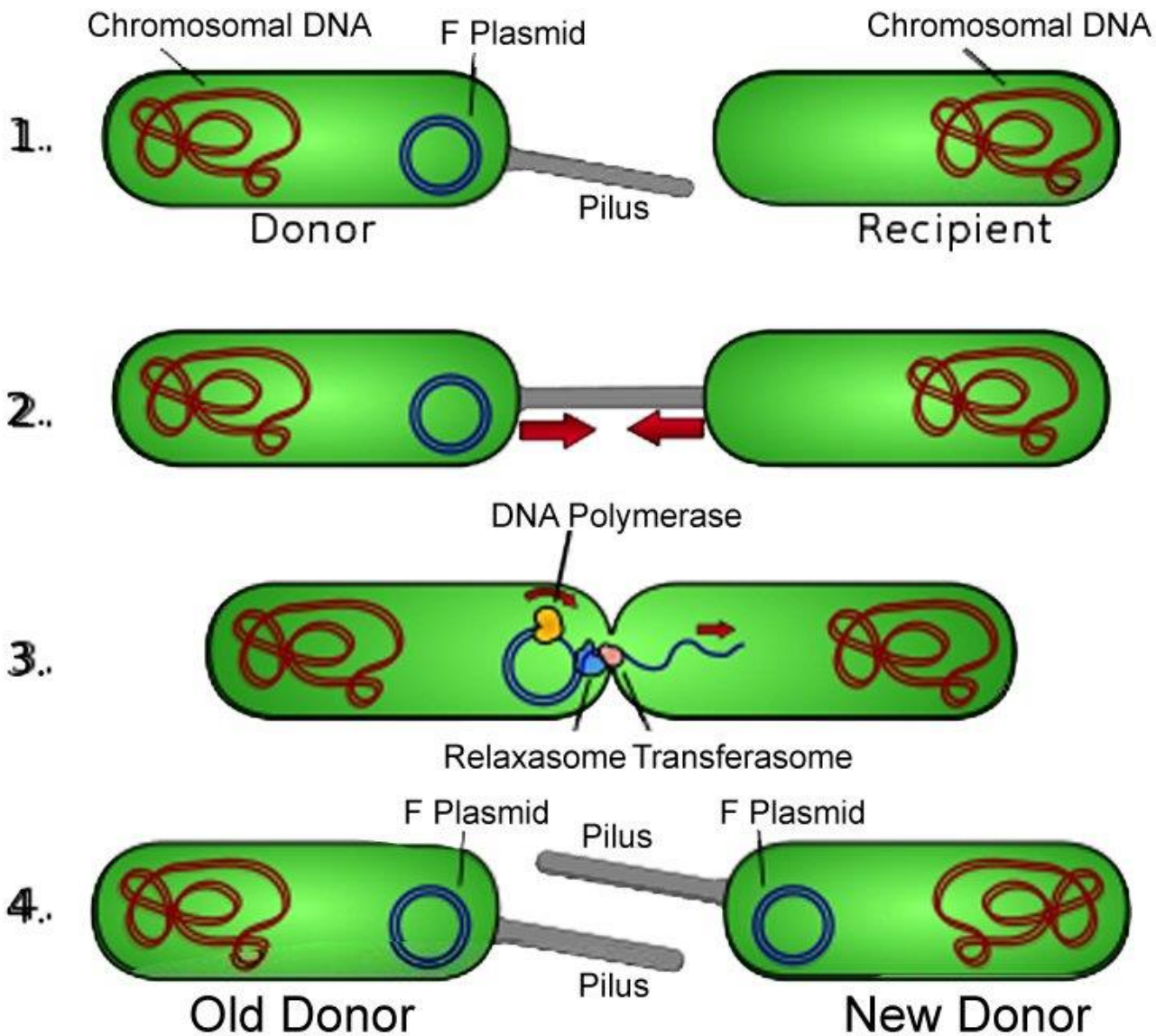
- Genes for pili synthesis
- Genes for DNA transfert
- Regulation genes,…

Donor

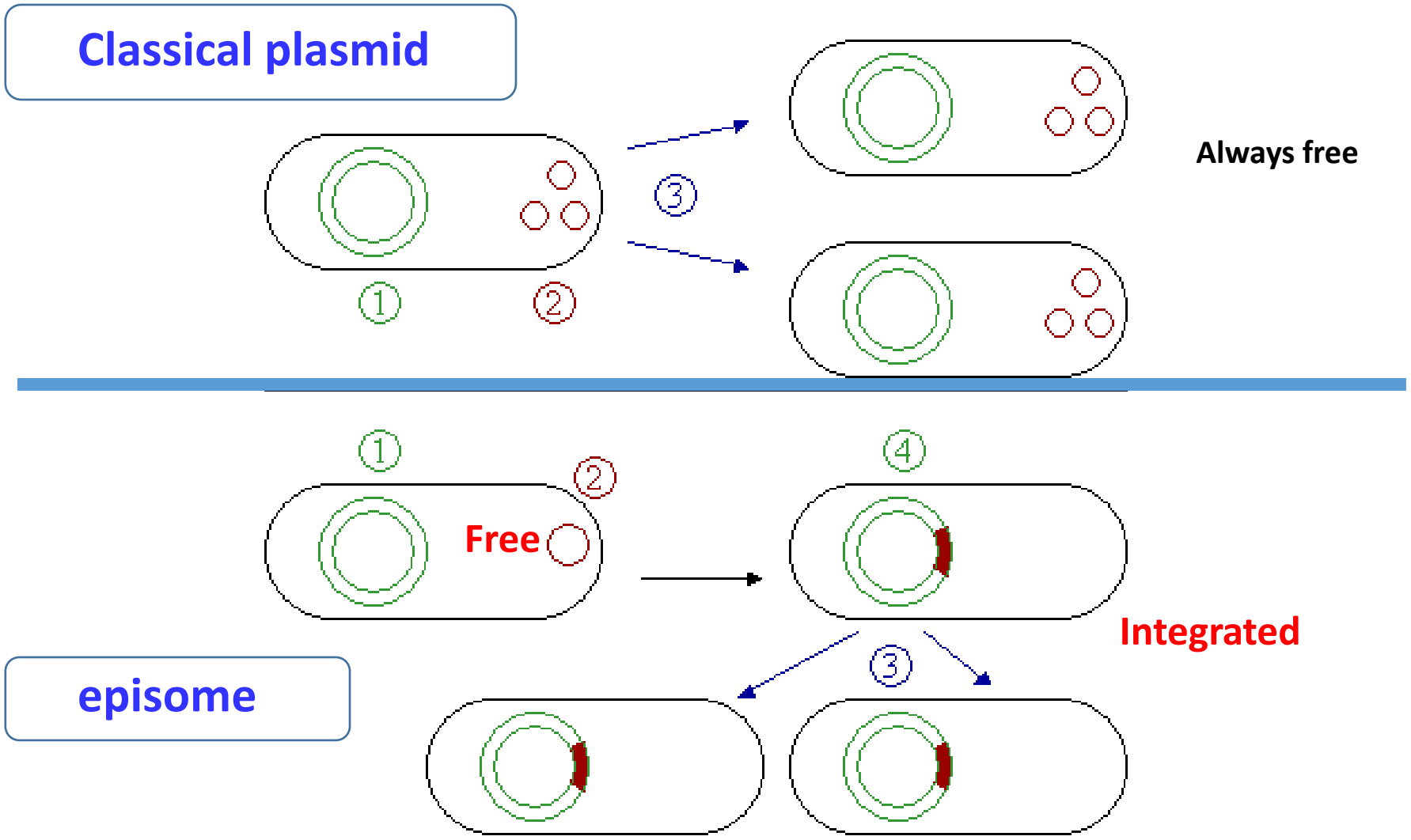


recipient

Conjugation mechanism



Episome

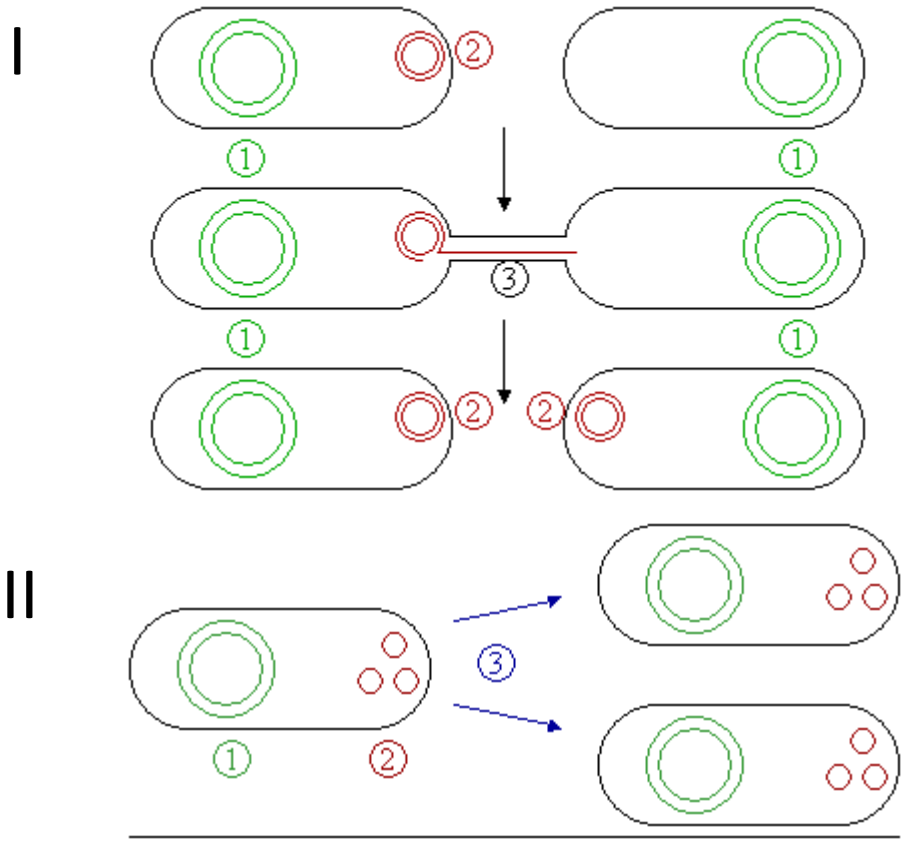


Example of F factor

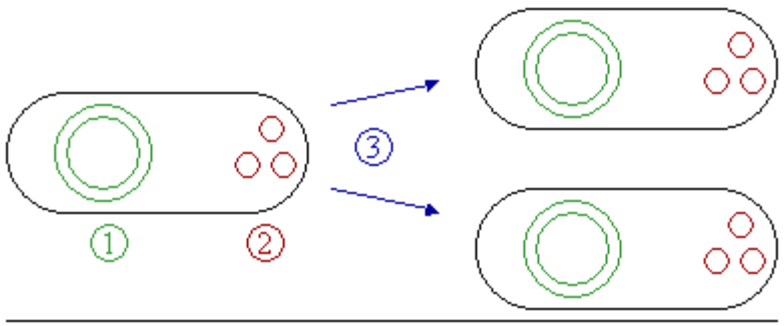
Two groups of plasmids

- **Conjugatives** plasmids
- **Non conjugatives** plasmids

Conjugative plasmid
Transfer by conjugation, then replication



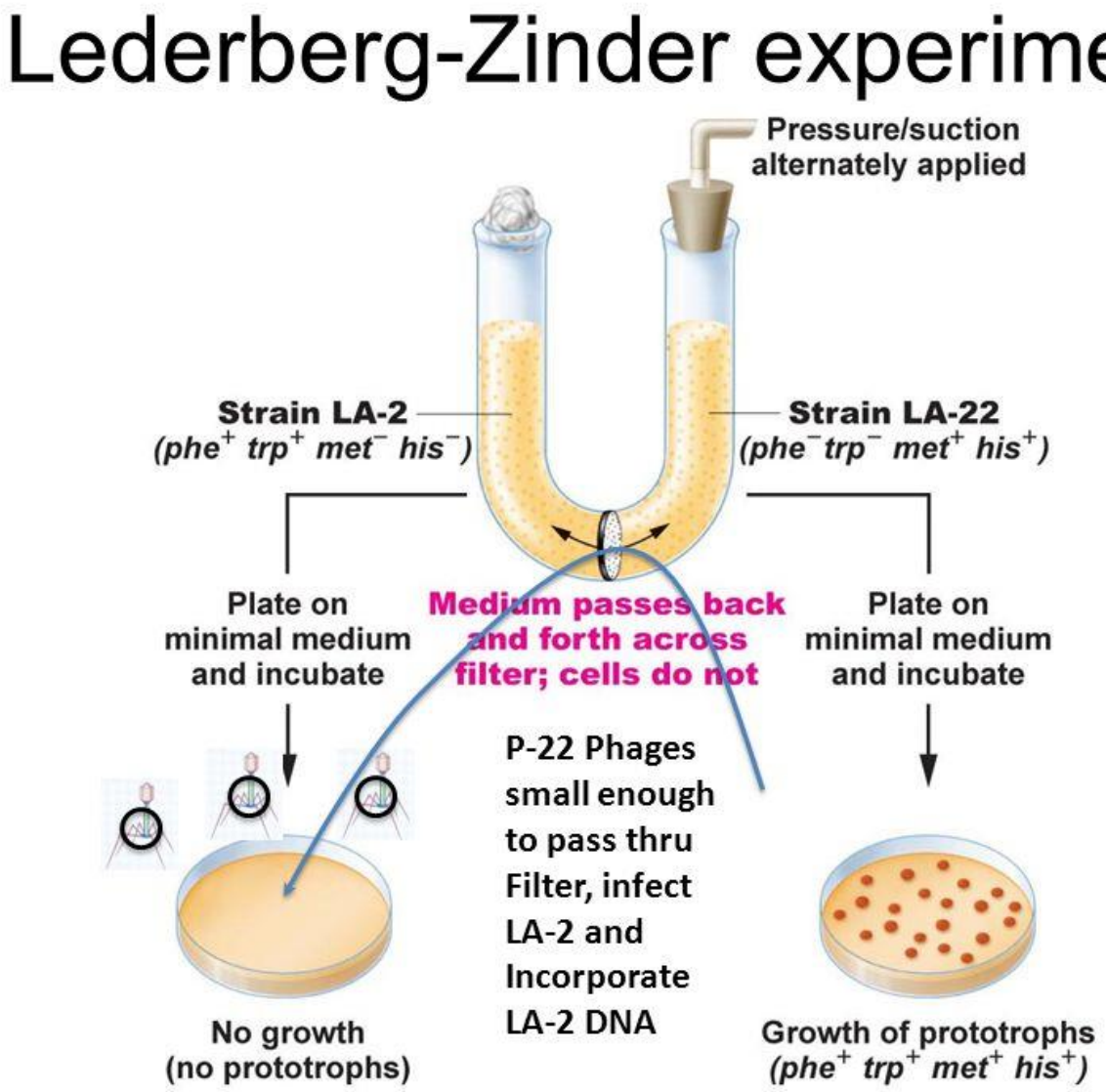
Non conjugative plasmid
No transfer, only replication

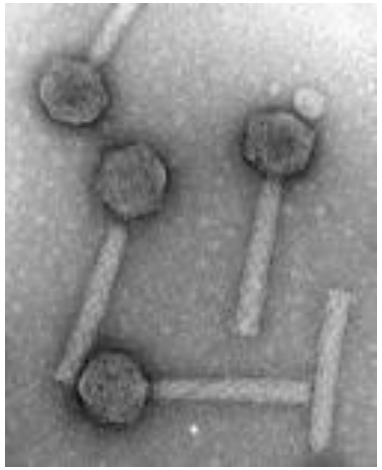


Transduction

Discovery

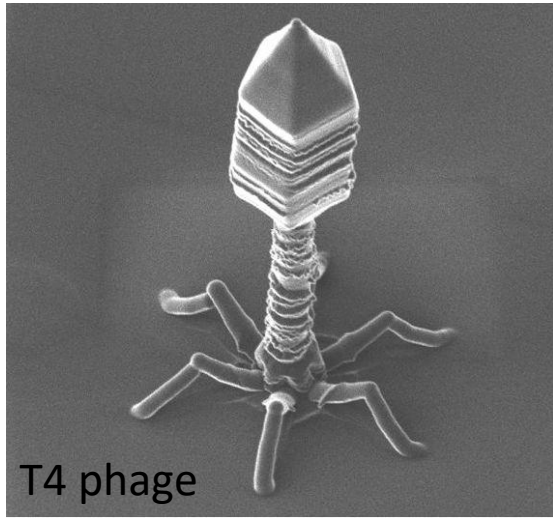
Introduction in a recipient bacterium of DNA tha is carried by a bacteriophage



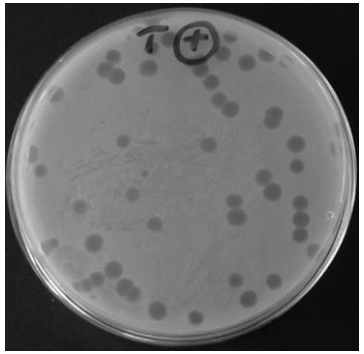


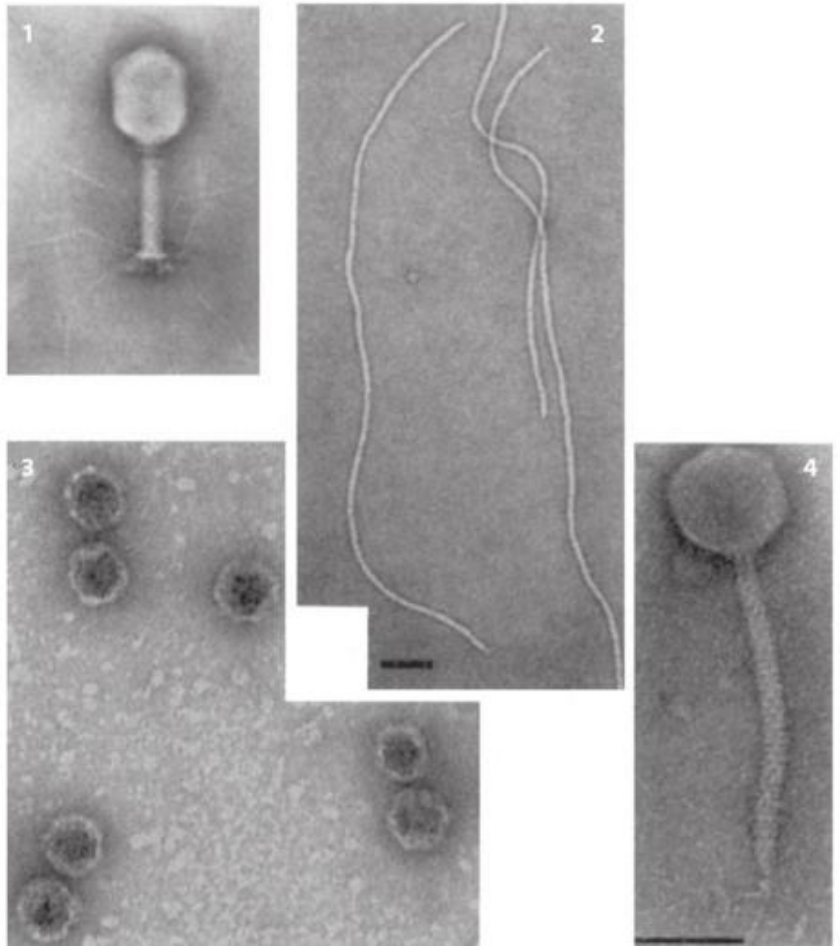
Phages

- Morphology
  - T2 and T4 phages, infecting *E. coli*
    - Head + nucleic acids
    - Tail + fixation system
  - Filamentous phages


T4 phage

- Study of phages
  - Liquid medium
    - Bacteria → high turbidity
    - Bacteria + Phage → lysis, decrease of turbidity
  - Solid medium
    - Bacterial Lysis plaque
    - Phage titer

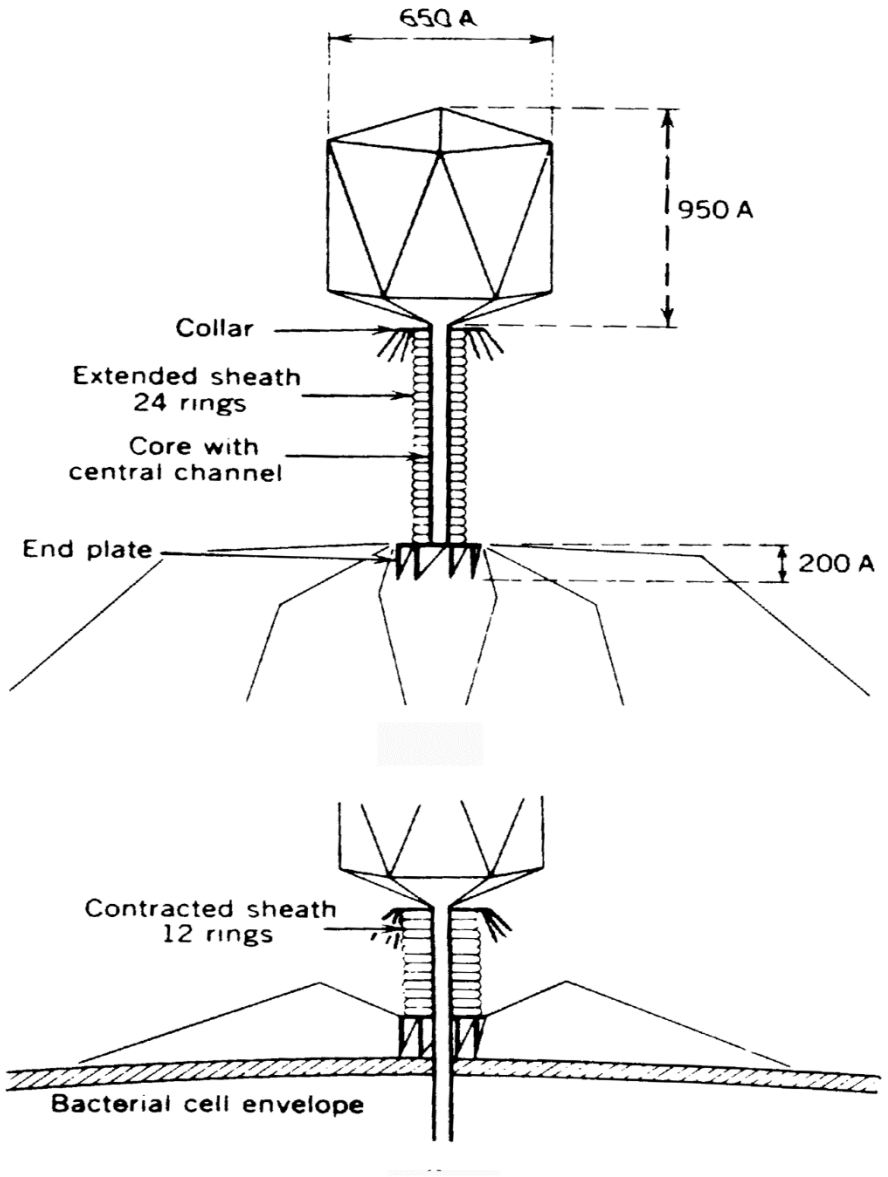




1. Caudal phage 2. Filamentous phage 3. Icosaedric phage 4. T1 phage from P. Singleton 2005, Dunod

Attachment and DNA penetration

- Attachment on a specific surface receptor (irreversible) => determine the phage's host range
  - Example : LamB porine of *E. coli* and λ phage
- Cell wall perforation
- Contracting of the sheat
- DNA Injection



Two types of mechanisms:

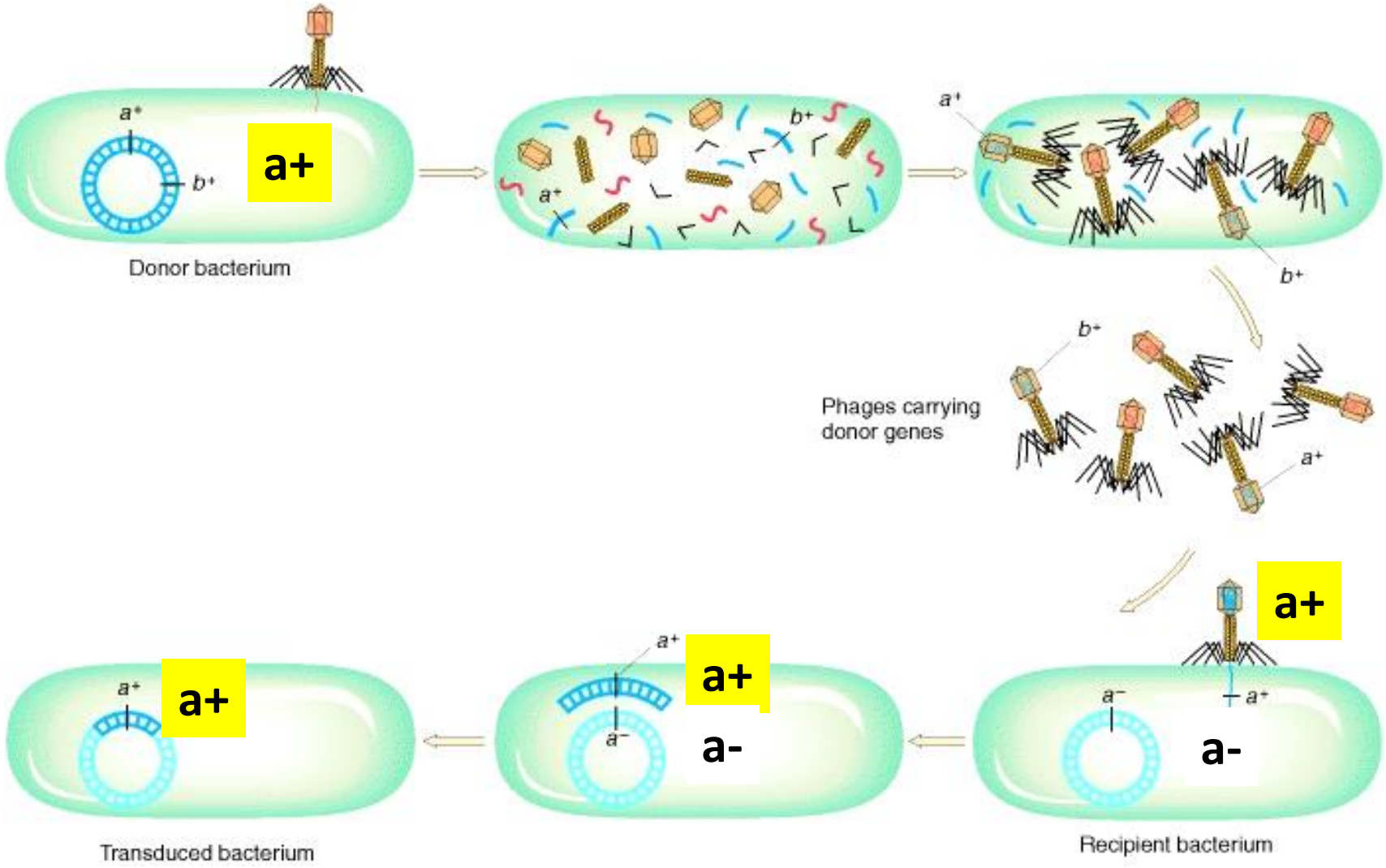
Generalized transduction or non specific

Ex: P1 bacteriophage of *Escherichia coli* able to transfer several bacterial genes

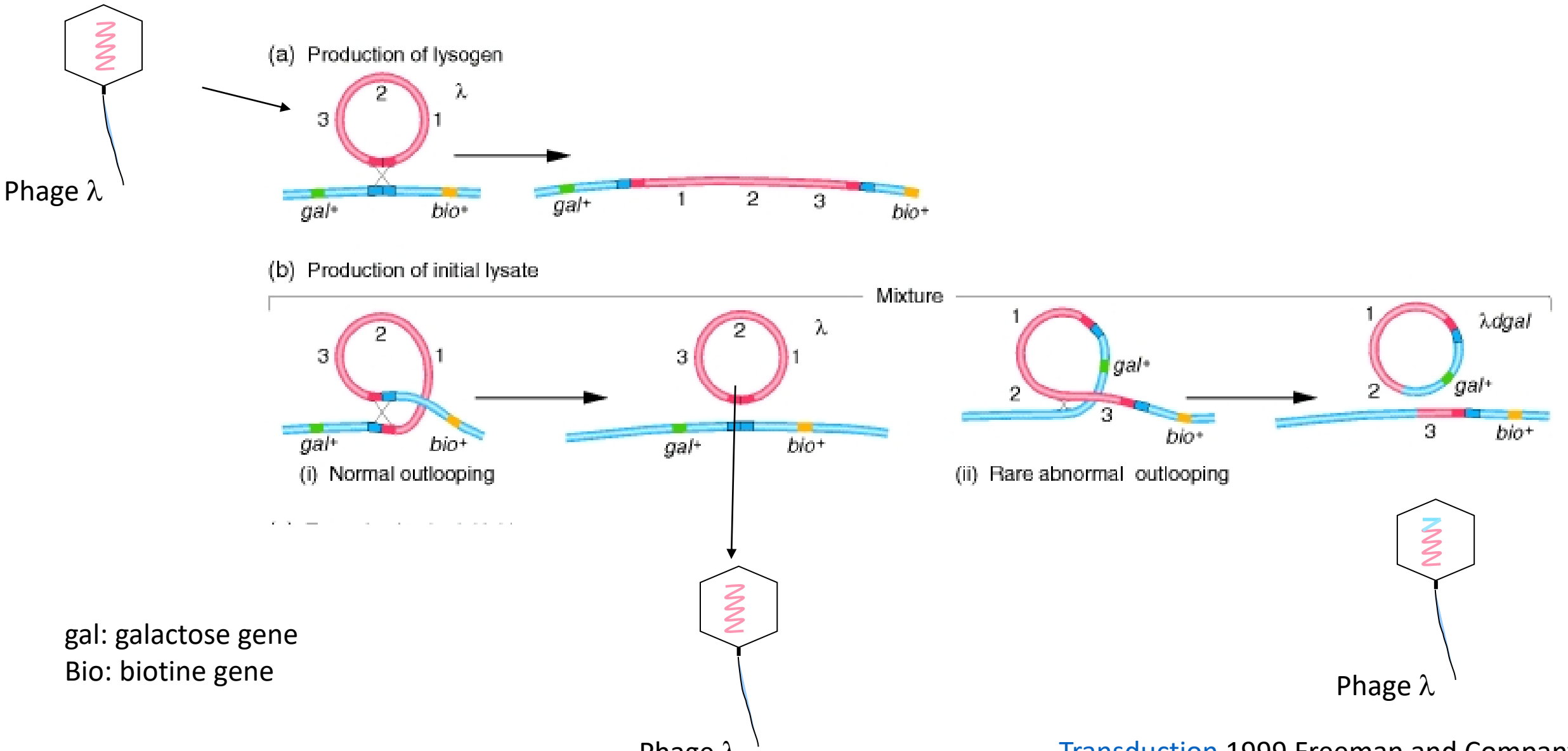
Specialized transduction

Ex: λ bacteriophage (temperate phages) of *Escherichia coli* that is able to transfer some metabolism properties *:* galactose metabolism or biotine synthesis (genes that are closed to the attachement site *attB*)

Generalized transduction: phage P1



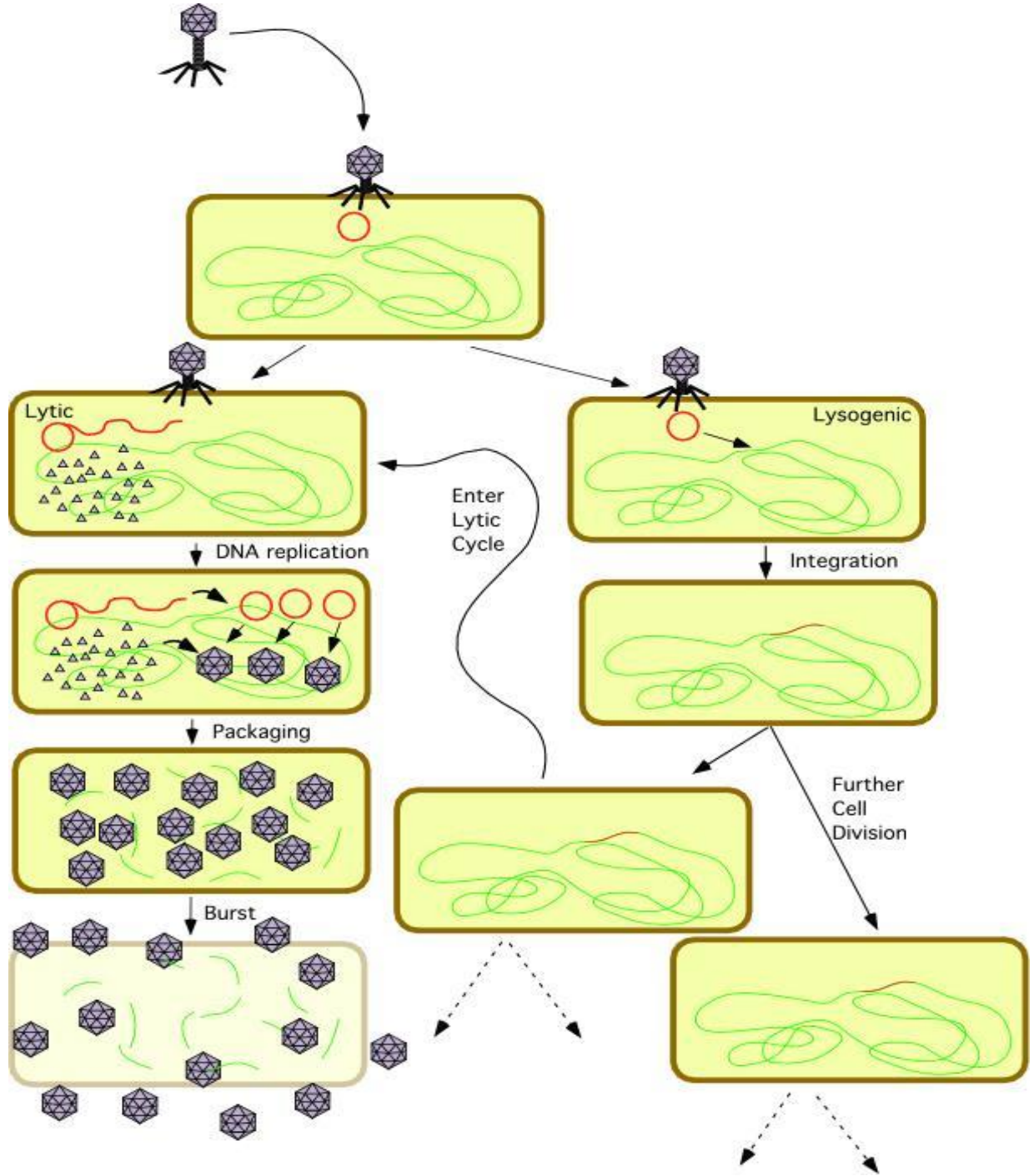
Specialized transduction



gal: galactose gene
Bio: biotine gene

Transduction 1999 Freeman and Company

Multiplication cycle of phages



Use of genetic transfer in molecularbiology and cloning in laboratories

- **Transformation :**
  - Plasmid transformation
  - cloning
- **Conjugation :**
  - Use of conjugative plasmids to transfer DNA from Bacteria (E. coli) to another species (example: *Bacillus, Staphylococcus, Clostridium*….)
- **Transduction :**
  - Transfer of DNA to a mutant to another strain
- **Transposition :**
  - Random mutagenesis