Genetic Adaptation II

Microbial Physiology

Module 3

Topics

- Topic 4: Transposable Elements
- Topic 5: Exchange of Genetic Material Between Organisms
- Topic 5a: Protection Against Foreign DNA

Aims and Objectives

- By the end of this module you should…
  - be able to distinguish between IS and Tn elements
  - understand the importance of transposable elements to the evolution and adaptation of microorganisms
  - have a detailed understanding of the three main mechanisms of DNA transfer and be able to distinguish between them
  - know how bacteria protect themselves against the introduction of foreign DNA

Last Time…

- Look at…
  - How mutations arise
  - Macrolesions
    - Insertions, deletions, inversions, duplications
  - Microlesions
    - Frame-shift mutations
    - Base substitutions
      - Transitions
      - Transversions
    - Point mutations
      - Silent
      - Missense
      - Nonsense

Last Time…

- Mutations can allow organisms to acquire new functions over many generations, under suitable conditions (selective pressures)

This time…

- Look at how organisms can acquire genetic material from other sources
  - Transposable elements
  - Other bacteria
    - Transformation
    - Transduction
      - Generalised
      - Specialised
    - Conjugation

Recombination

- Central to all the mechanisms of DNA transfer via transposable elements, transduction, transformation and conjugation
- Recombination can be…
  - homologous or non-homologous
    - non-homologous is also called site-specific
  - reciprocal or non-reciprocal

Homologous Recombination

- Occurs between DNA sequences that are the same or very similar
- Results in a reciprocal exchange of genetic material between the two sources
- Best example: crossing over that occurs between pairs of chromosomes
- Bacterial example: Gene mapping using conjugation of Hfr with F^- cells

Site-specific Recombination

- Exchange of material in sequences that show little or no sequence homology
- Exchange is non-reciprocal
  - DNA transfer only occurs one way
- Can only happen at specific sites in the genome
- Example: Prophage formation in bacteriophage λ

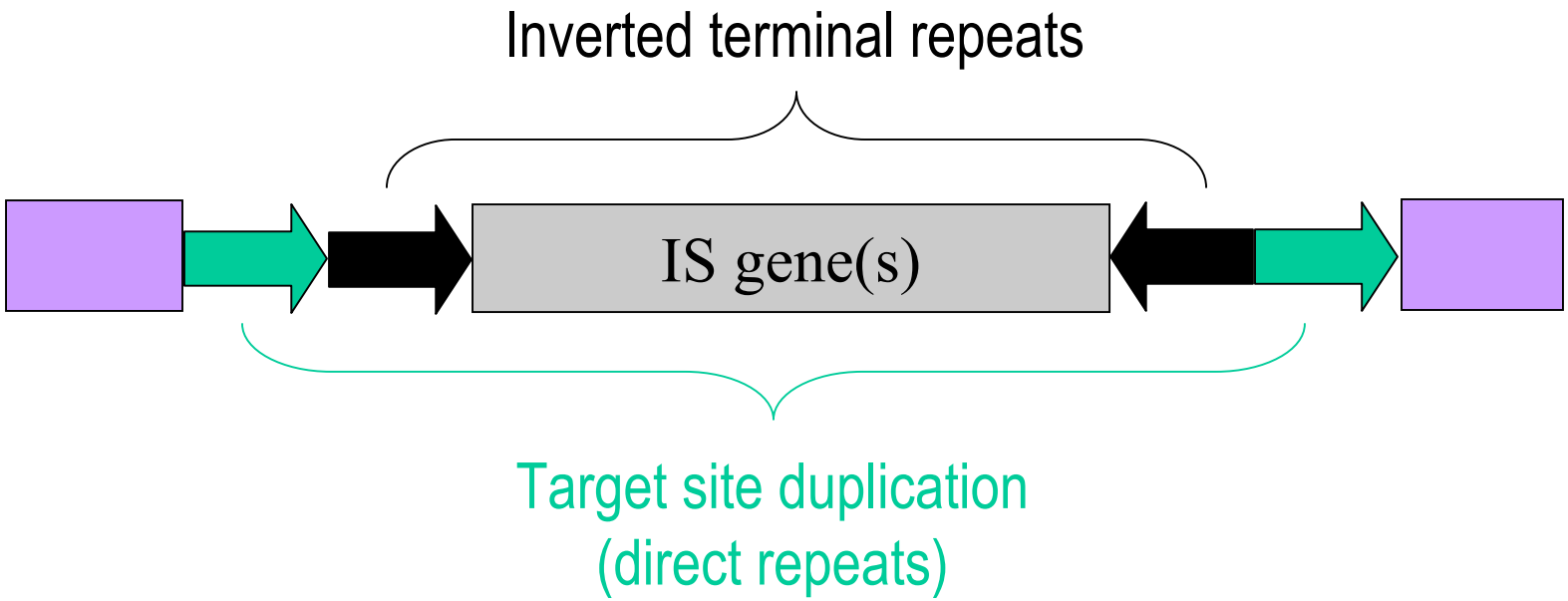
Topic 4: Transposable Elements

- Elements within the bacterial genome (chromosome and accessory genetic elements) that are capable of translocating to new locations
  - "Jumping genes"
  - Transfer generally occurs through non-homologous recombination
  - Some leave a copy in the original location
  - Some move without duplicating the sequence
- Two types of transposable genetic elements
  - Insertion sequences (IS)
  - Transposons (Tn)

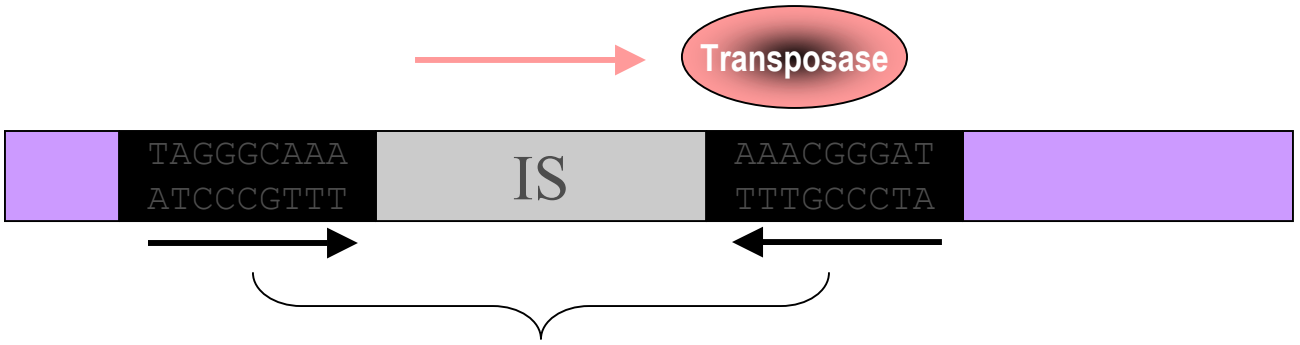
Insertion Sequences

- Simplest transposable elements
- Small
- Don’t code for structural proteins
- Code for transposase
  - Enzyme responsible for facilitating transposition
- Functions of the transposase
  - Helps create the insertion site
  - Joins the IS ends to the target site
  - Fills all the gaps created by insertion

Structural Features of IS



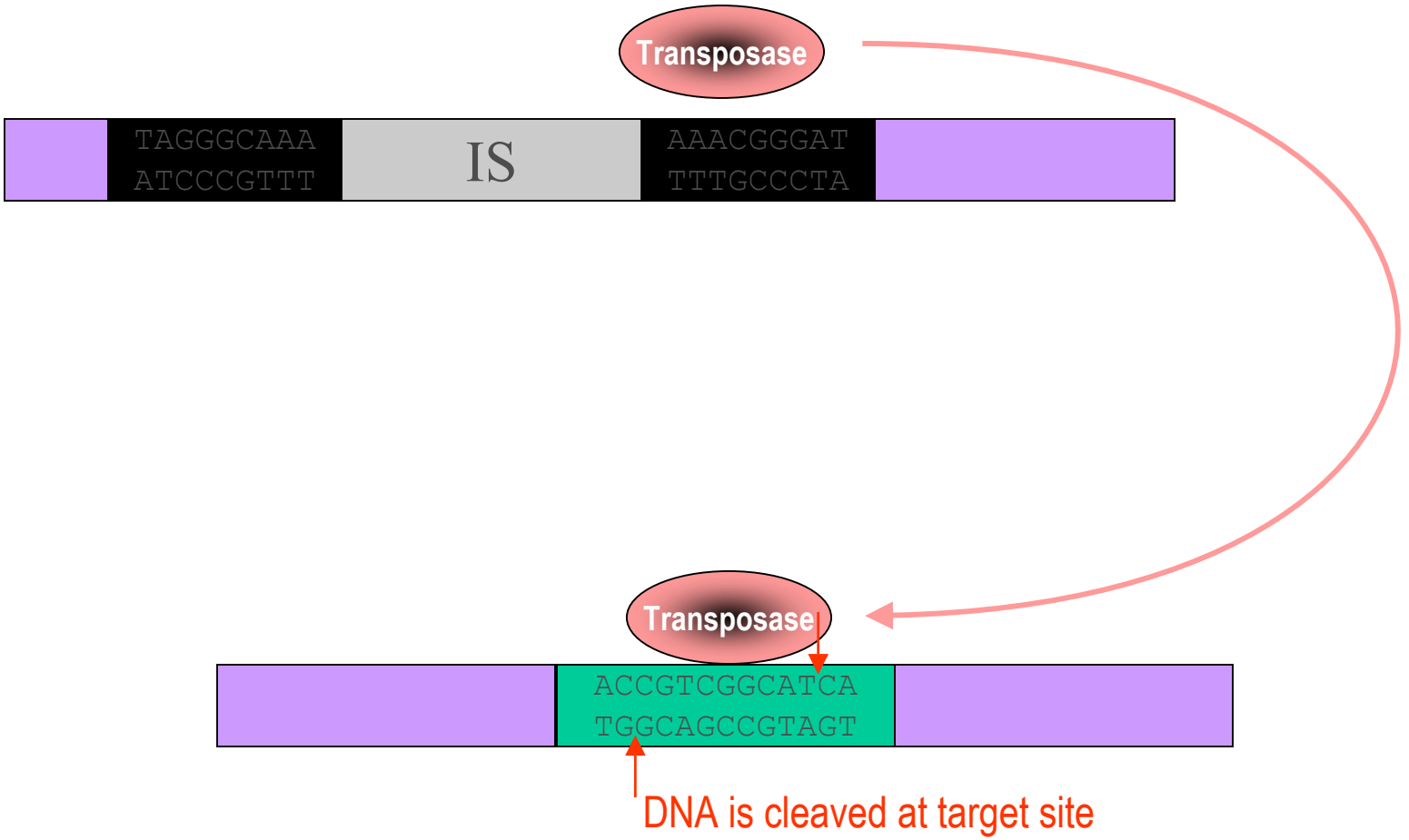
Mechanism of Transposition



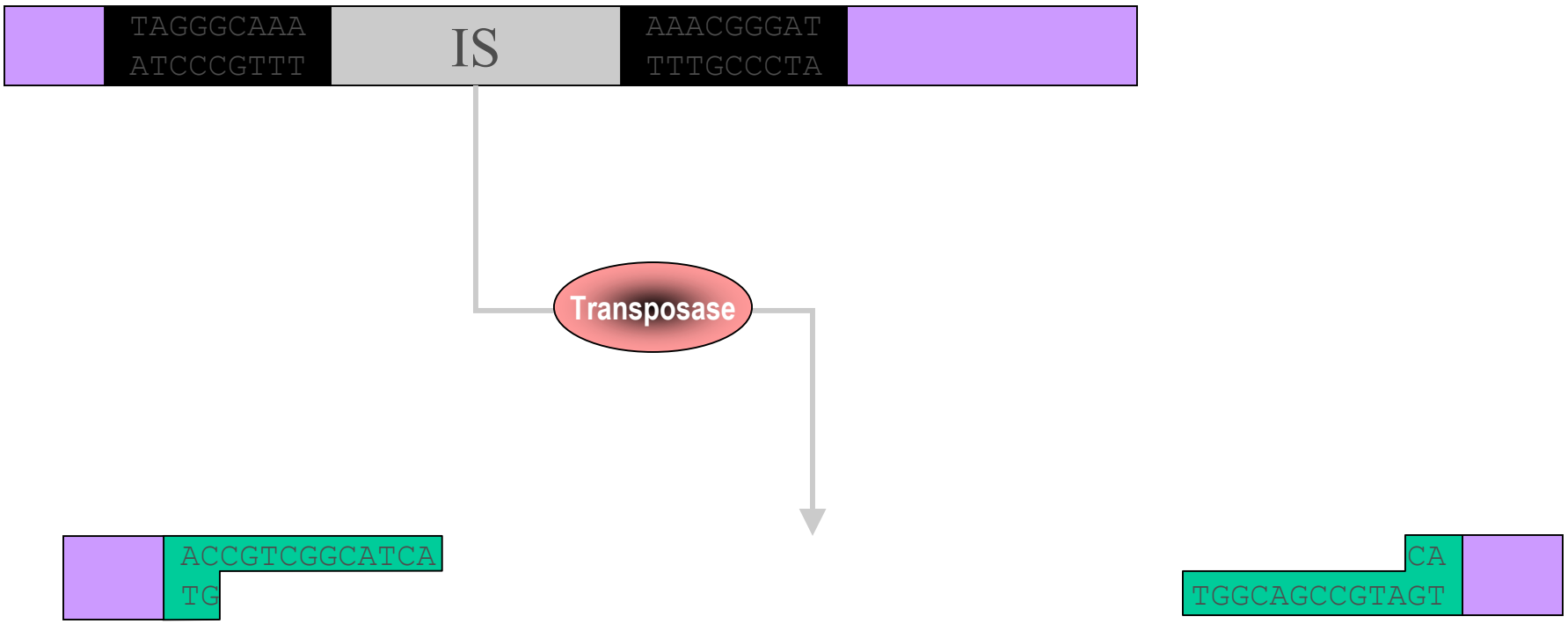
Inverted terminal repeats

Target site

Mechanism of Transposition

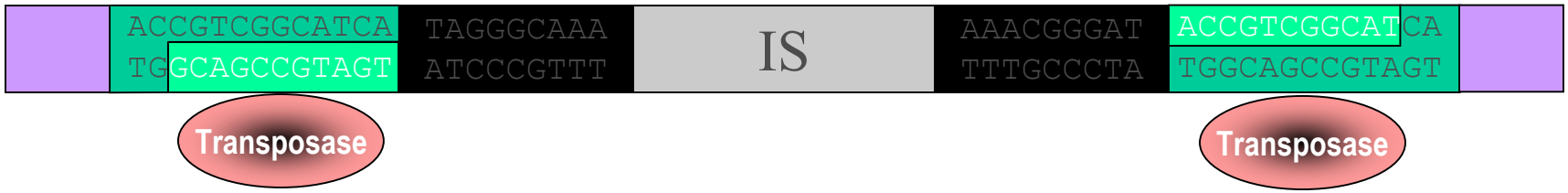


Mechanism of Transposition

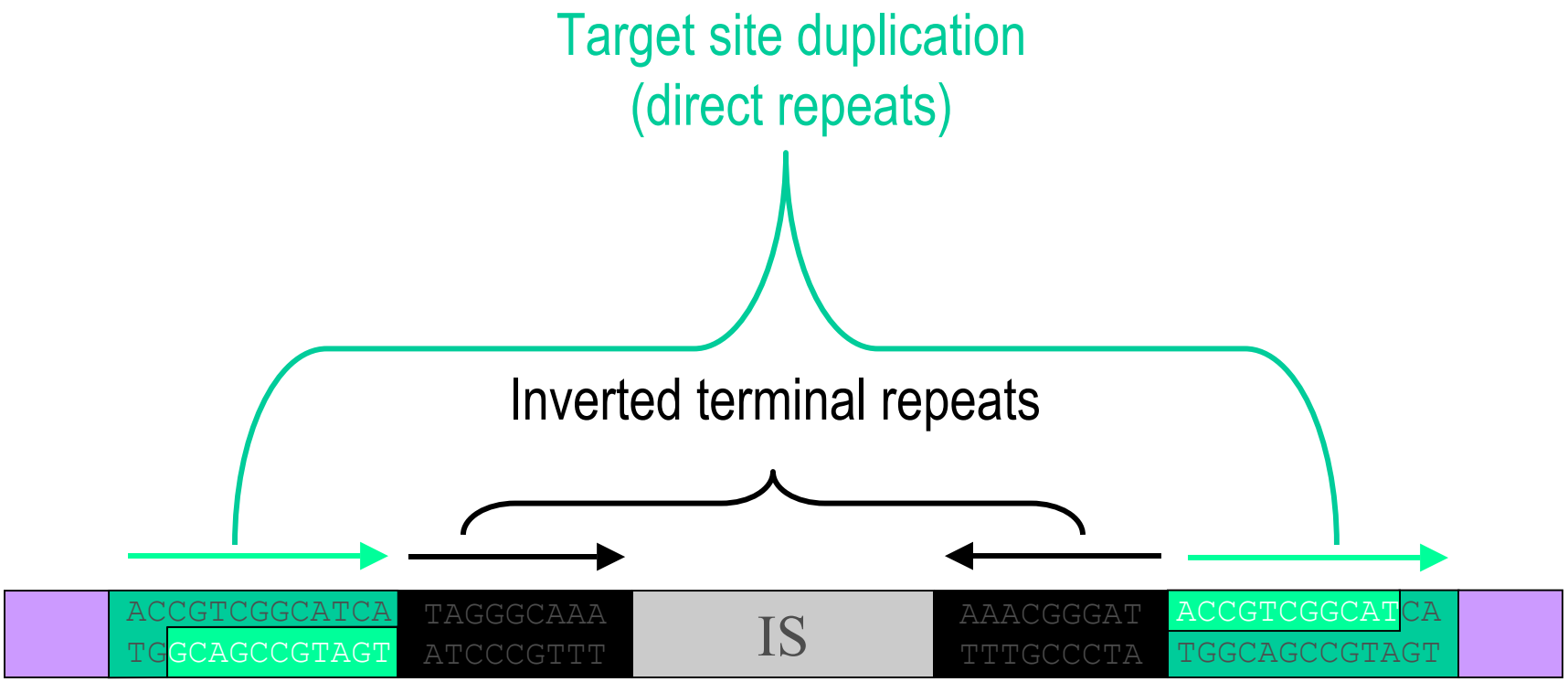


Mechanism of Transposition

Mechanism of Transposition



Mechanism of Transposition



IS General Features

- Inverted terminal repeats
  - Inverted repeats (generally)
  - 9 to 40 bp
- Target site duplications
  - Direct repeats
  - 2 to 13 bp

IS	Size (bp)	Target site duplication	Terminal Repeats
IS*1*	768	9	23, inverted
IS*2*	1 327	5	32, inverted
IS*3*	1 258	3-4	32, inverted

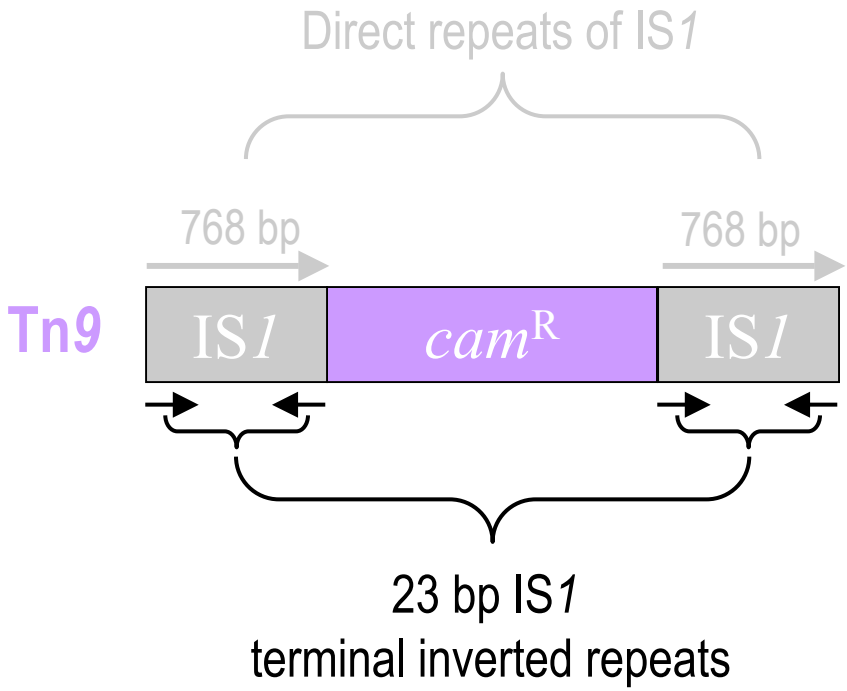
Functions of IS

- Chromosomes may contain multiple copies of IS elements
  - *E. coli* chromosome has 6-10 copies of IS*1*
- May also be present on plasmids
  - F plasmid has IS*2* and IS*3*
  - When also present on chromosome…
    - Homologous recombination between like Is'
    - Integration of F plasmid into chromosome
      - Hfr
        - Higher frequency recombinant
        - Promotes genetic exchange (including transposons)

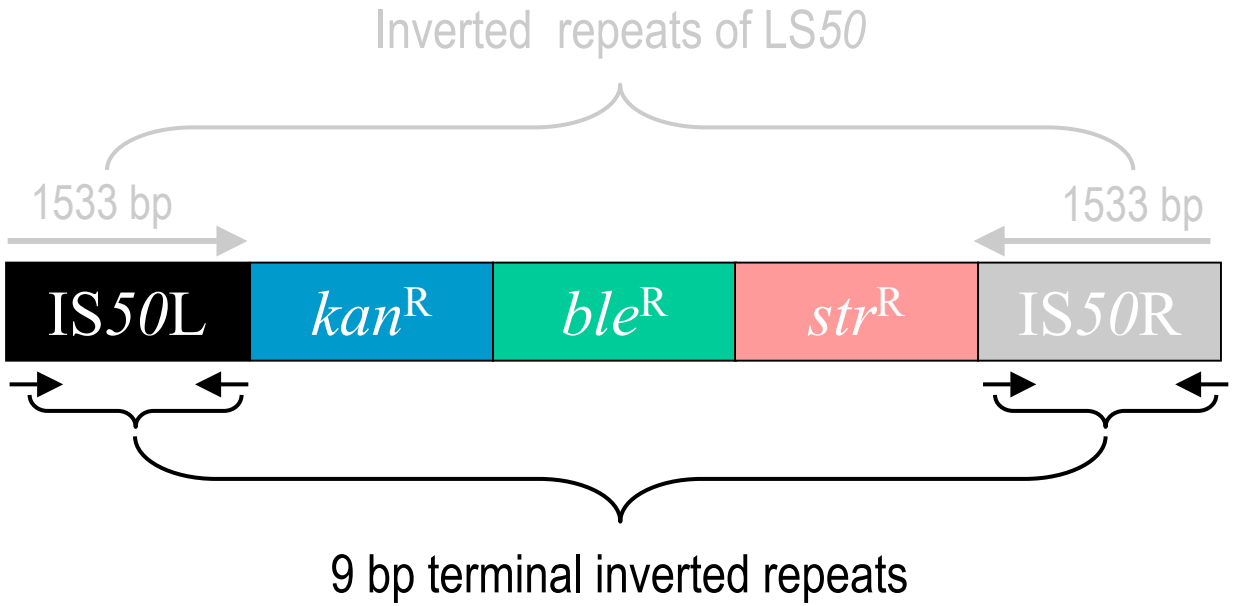
Transposons (Tn)

- Also called composite transposons
- More complex than IS
  - involve genes other than those required for transposition
  - larger
- Formed by the proximity of two ISs on a chromosome
  - sequence between the ISs is captured an transposed along with the IS elements

Tn*9*


Direct repeats of IS1
768 bp
768 bp
Tn9
IS1
camR
IS1
23 bp IS1
terminal inverted repeats

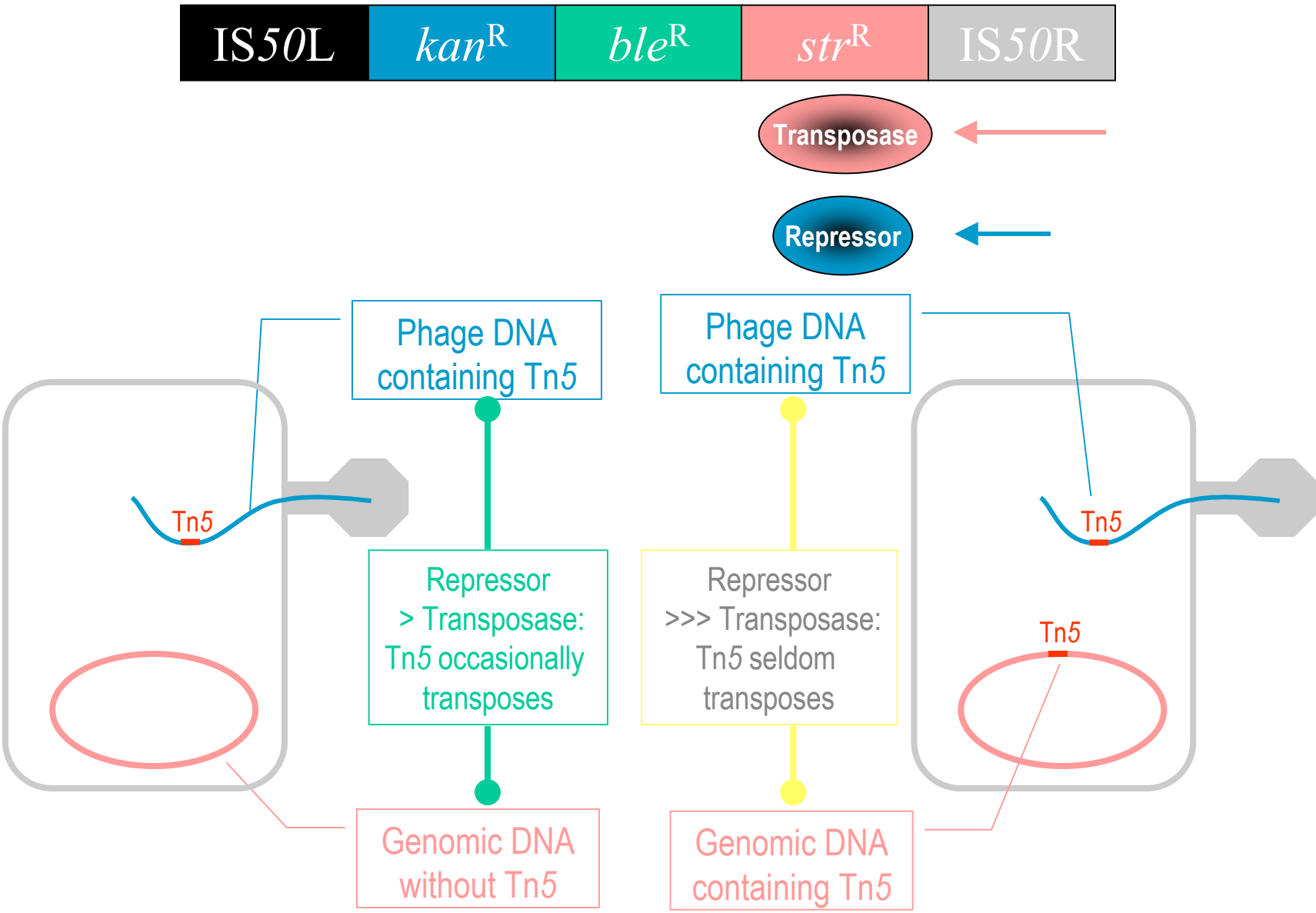
Tn5


Inverted repeats of LS50
1533 bp
1533 bp
IS50L
kanR
bleR
strR
IS50R
9 bp terminal inverted repeats

Regulation of Tn*5* transposition

- Different IS elements at each end
  - IS*50*L and IS*50*R
- IS*50*R produces 2 proteins
  - Transposase
  - Repressor
    - translated from start site within the transposase gene
    - more abundant than the transposase

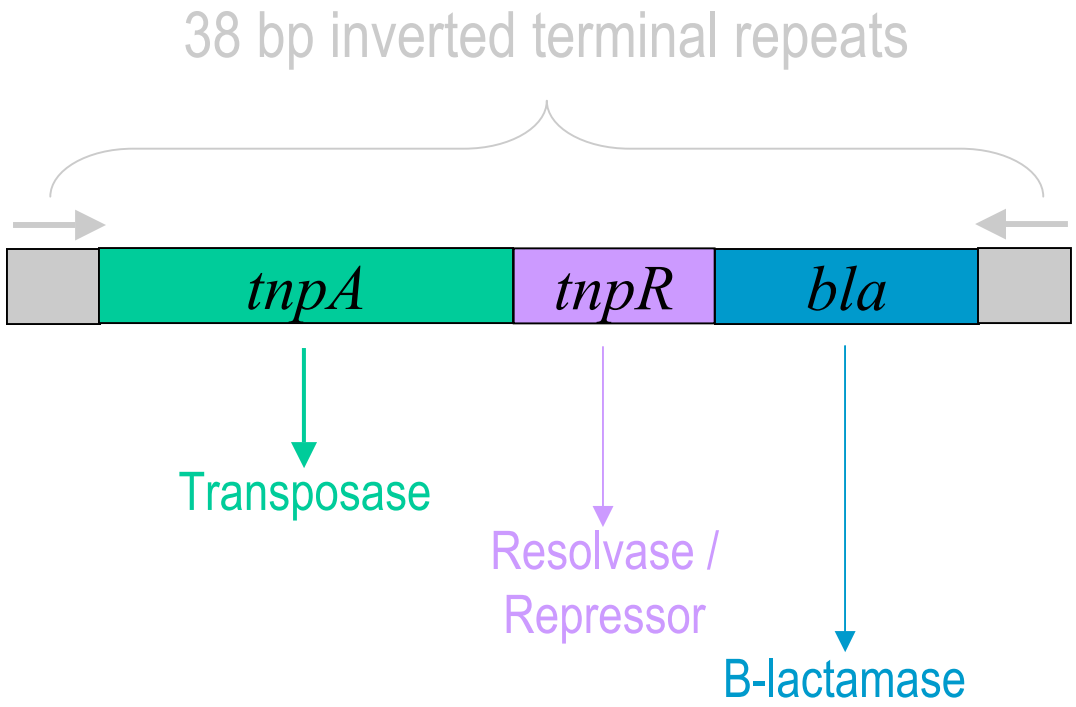
Regulation of Tn*5* transposition



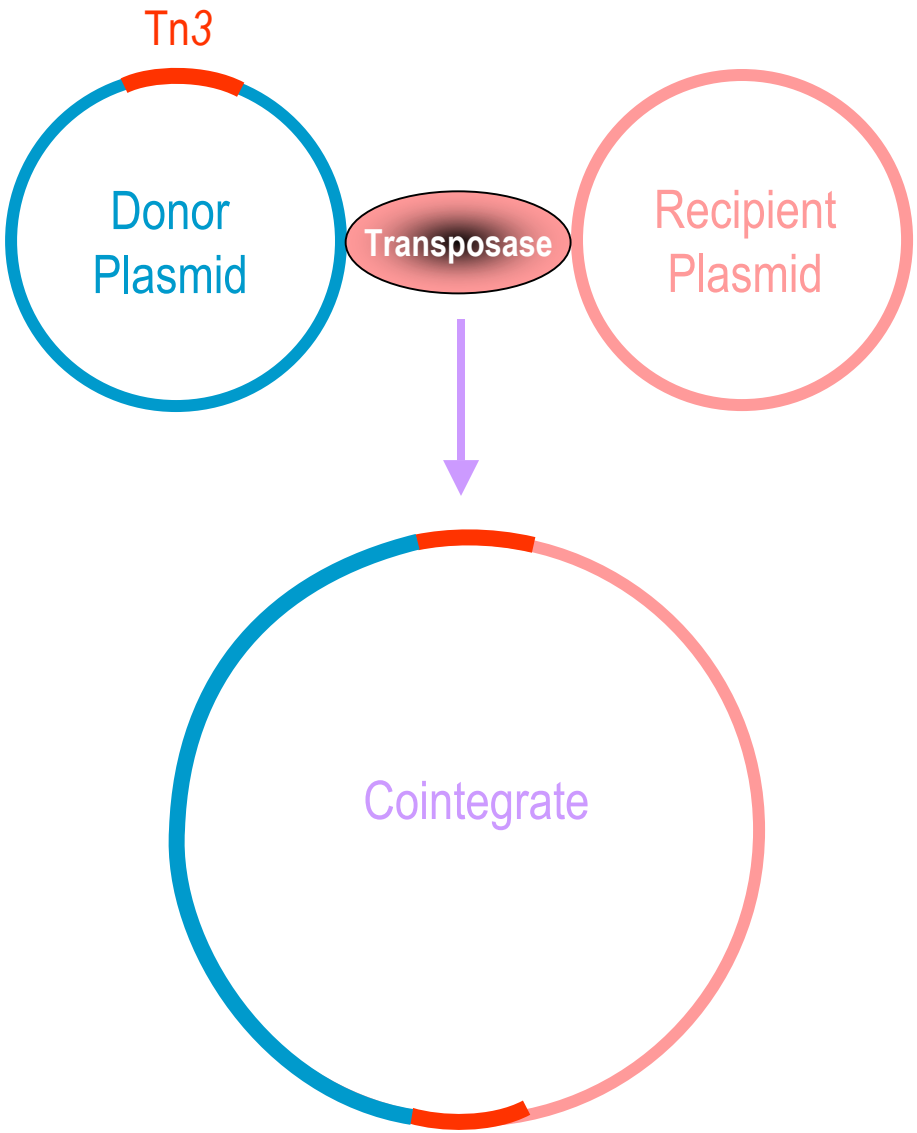
Tn*3* Elements

- Larger than IS elements
- Have inverted terminal repeats
- Produce target site duplication
- Carry genes other than other required for transposition
  - Not flanked by other IS elements
- Transposition is a two-step process
  - cointegration
  - resolution

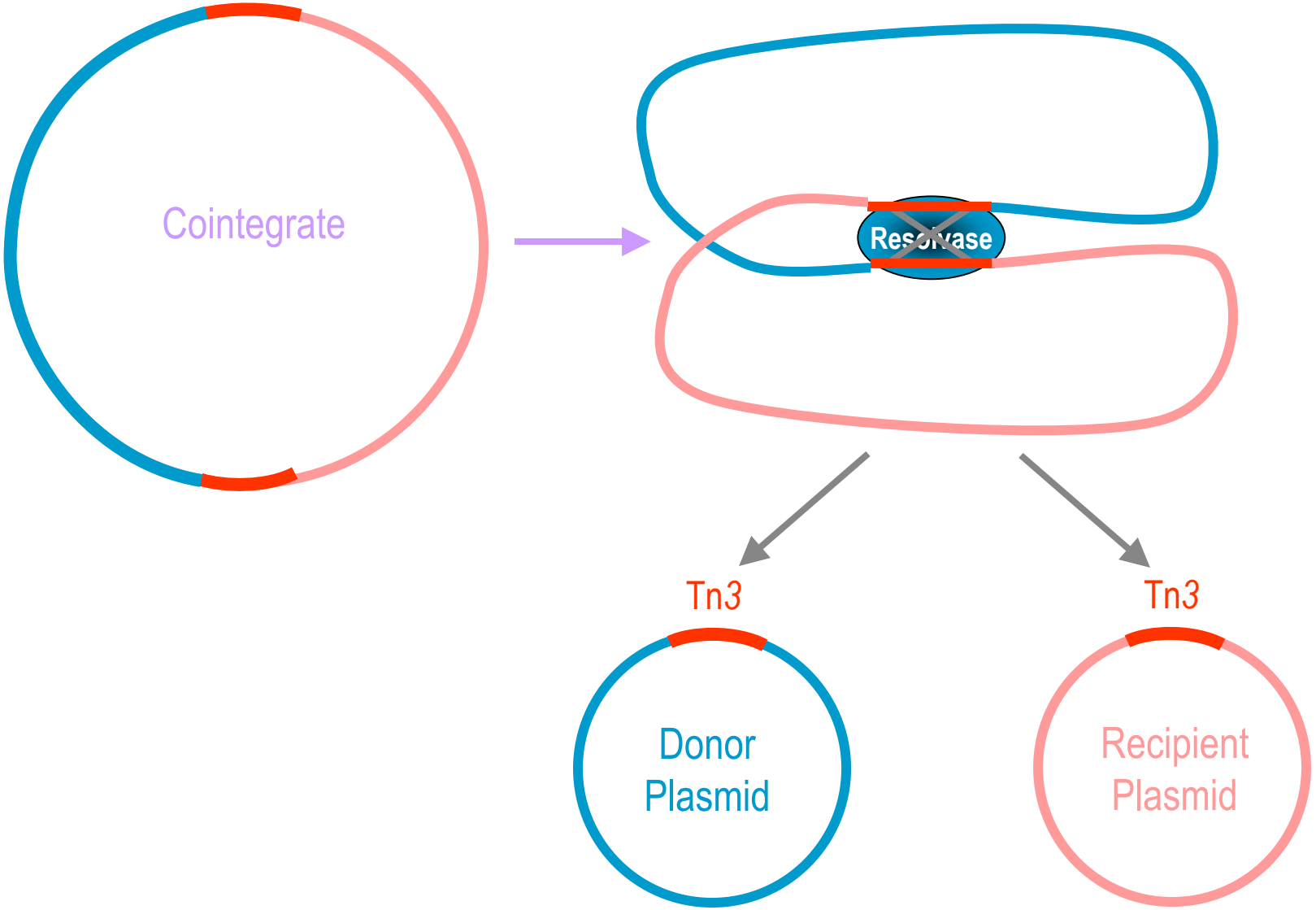
Tn*3* Structure



Tn*3* Transposition: Cointegration



Tn*3* Transposition: Resolution



Conservative and Replicative Transposition

- Conservative transposition
  - transposon is excised from one location and inserted into another
  - no duplication of transposon DNA
  - eg. Tn*5*
- Replicative transposition
  - transposon DNA is duplicated during the transposition process
  - source transposon remains in its original location
  - eg. Tn*A* transposon such as Tn*3*

Important Note re Transposition

- Transposition IS (essentially) a recombination event
  - Generally does not involve homologous sequences
    - i.e. is non-homologous
  - Does not use the general recombination system
  - Happens at specific DNA sequences
    - Site-specific recombination
    - transposase used instead of RecA

Properties of IS and Tn elements

Name	Size (bp)	Direct Repeats	Terminal Repeats	Genes Carried
IS*1*	768	9 bp	23 bp, inverted	None
IS*2*	1327	5 bp	32 bp, inverted	None
IS*3*	1400	3-4 bp	32 bp, inverted	None
Tn3	4957	5 bp	38 bp, inverted	*amp*^{R}
Tn*5*	5700	9 bp	1400 bp (IS*50*), inverted	*kan*^{R}, *ble*^{R}, *str*^{R}
Tn*9*	2500	9 bp	768 (IS*1*), direct	*cam*^{R}
Tn*10*	9300	9 bp	1400 bp (IS*10*), inverted	*tet*^{R}

Significance of Transposable Elements

- Medical significance
  - transmission of antibiotic resistance genes
  - passage of genes from chromosome to plasmid
    - from plasmid to other cells
    - cells need not be the same strain
  - process occurs in pathogenic strains of
    - *Staphylococcus*, *Enterococcus*, *Neisseria*, *Shigella* and *Salmonella*
  - bacteria can acquire resistance to multiple antibiotics
    - MDR strains
      - multiple drug resistant

Phase Variation

- Phenomenon observed in *Salmonella*
- Associated with flagella formation
- Flagella are usually composed of a single protein
  - flagellin
- Phase variation results in flagella proteins being one of two different types
- Which protein is produced depends on the orientation of an invertible DNA segment

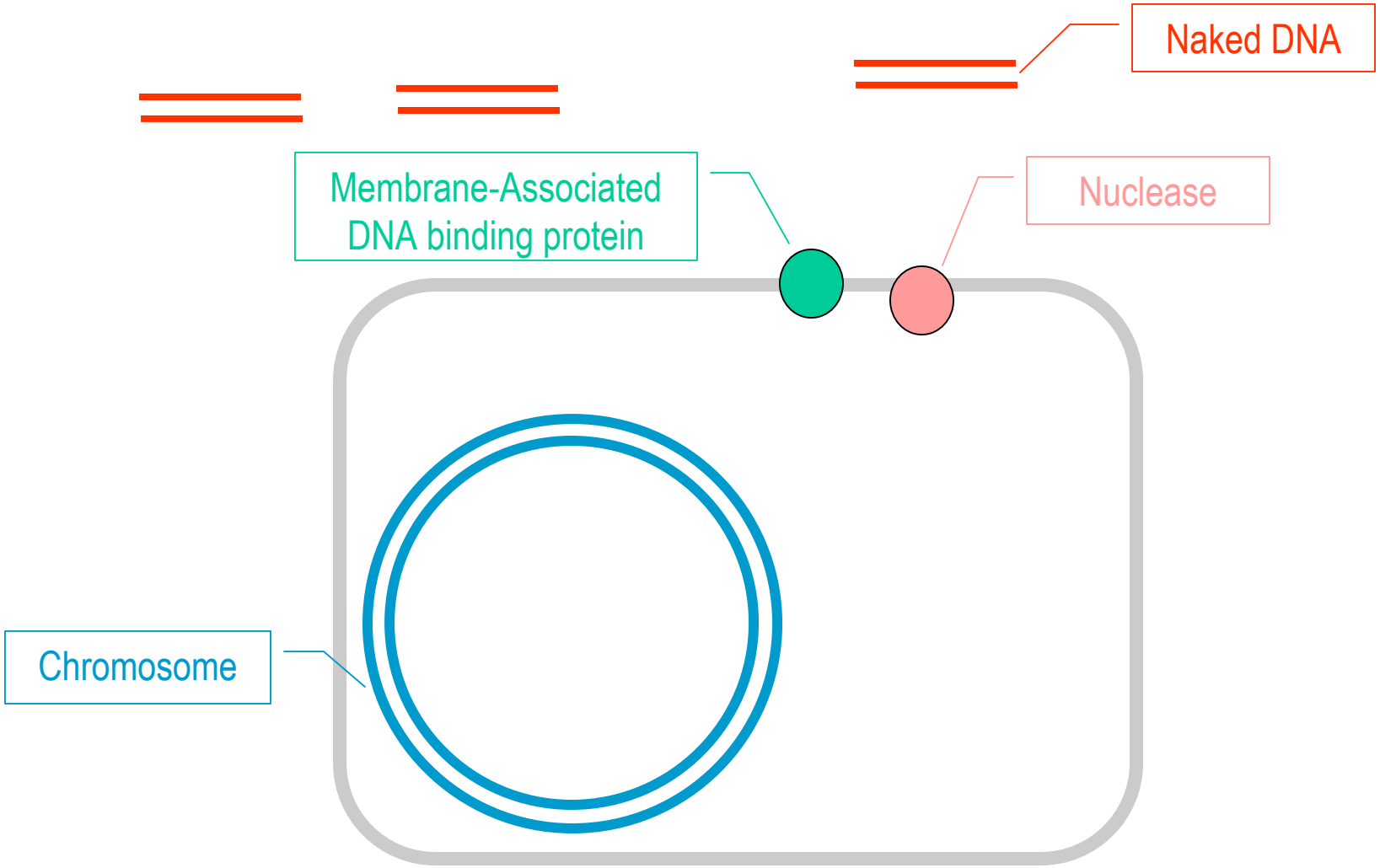
Topic 5: Exchange of Genetic Material between Organisms

- Mutations are essential for the generation of microbial diversity
- The ability to test combinations of mutations under selective pressure accelerates the evolutionary process
- Process accelerated by the ability of bacteria to acquire DNA from other sources
- Three mechanisms
  - Transformation
  - Transduction
  - Conjugation

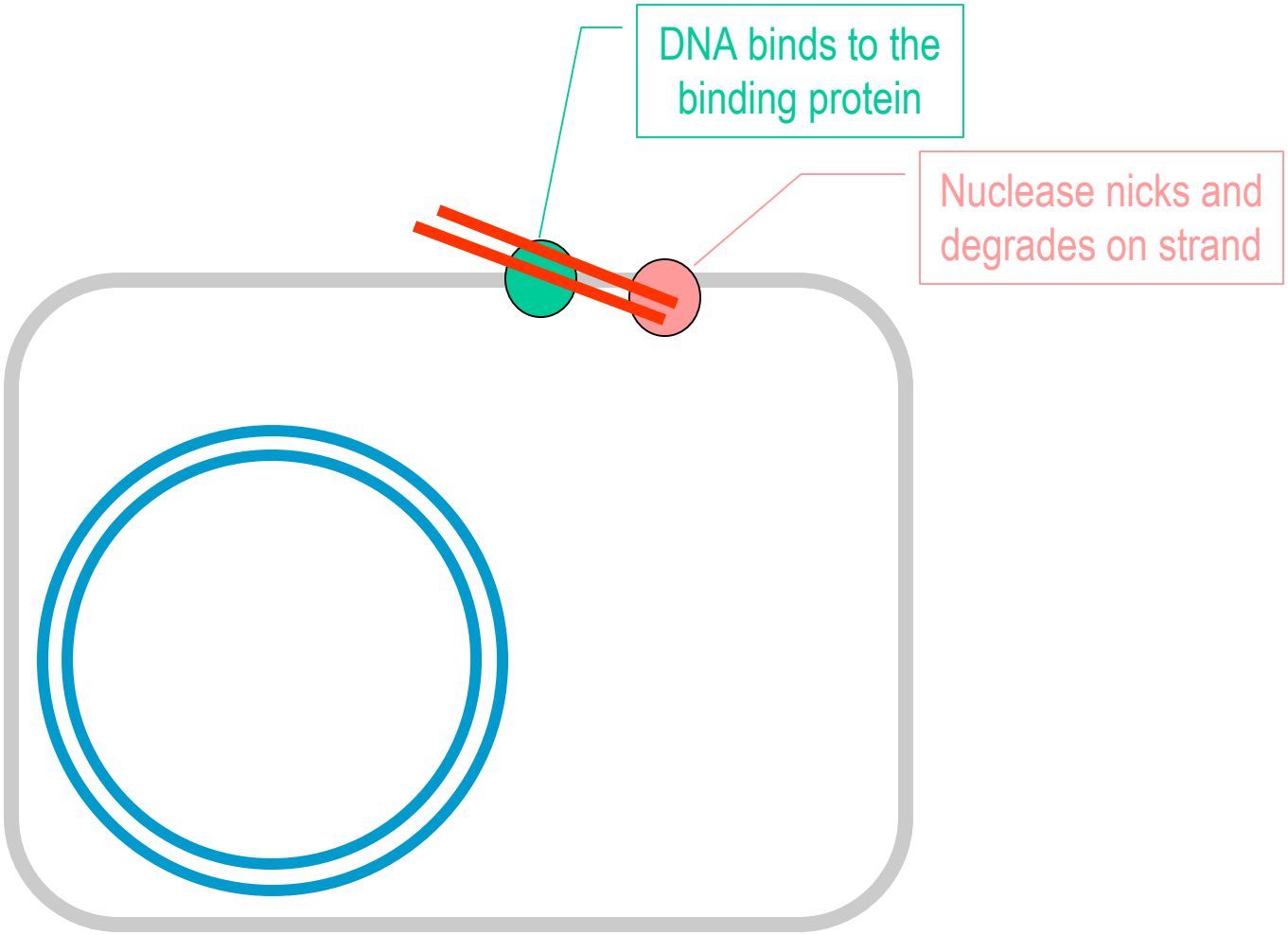
Transformation

- The uptake of naked DNA from the environment into a recipient cell
  - free DNA is usually the result of lysis of the donor cell
- Only competent cells can be transformed
  - secrete competency factors which induce 8-10 proteins required for transformation
- Competency depends on
  - growth stage
  - best if dividing at maximal rate
  - competency can be increased by cations and temperature

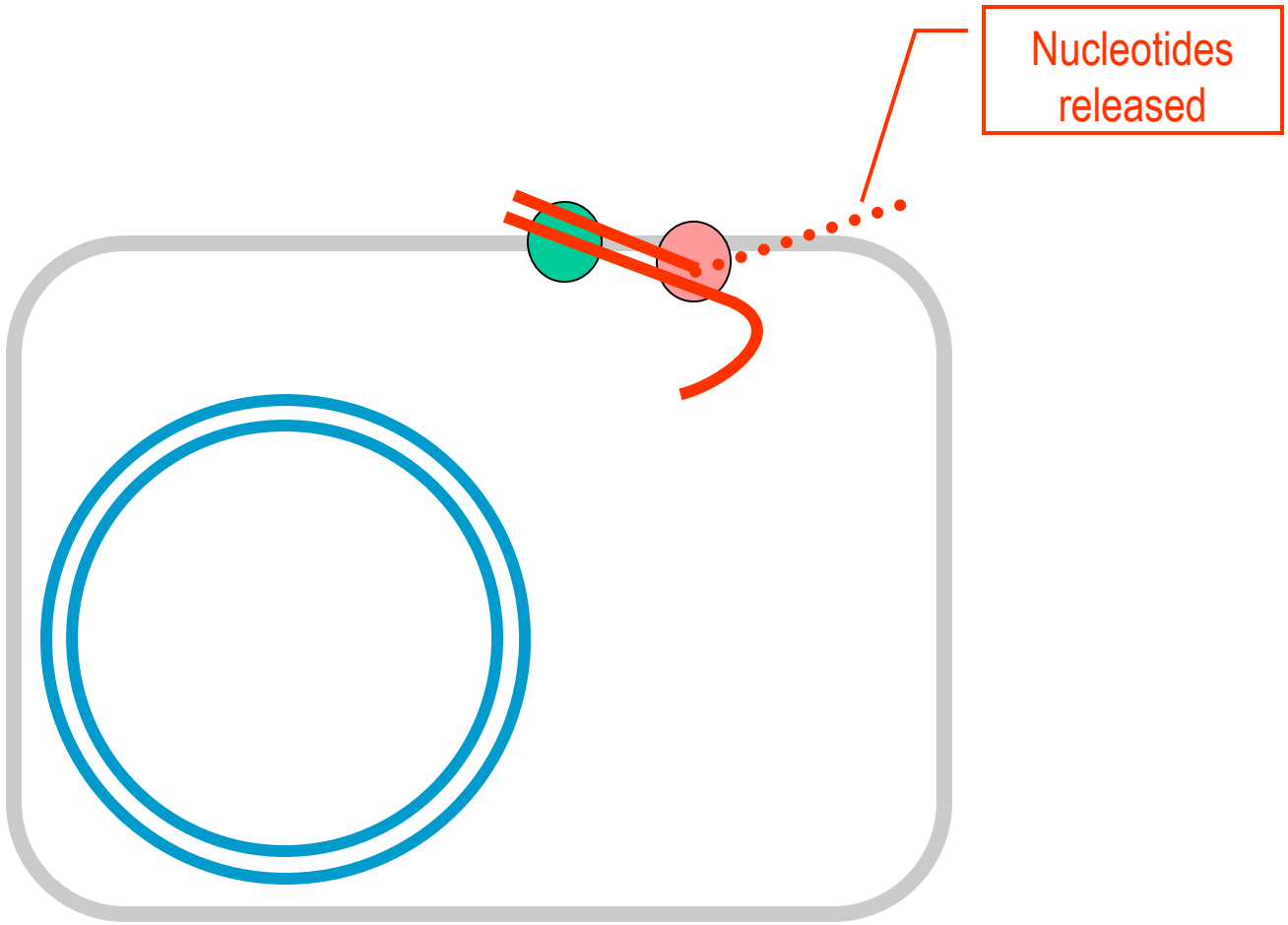
Mechanism of Transformation in *Streptococcus*



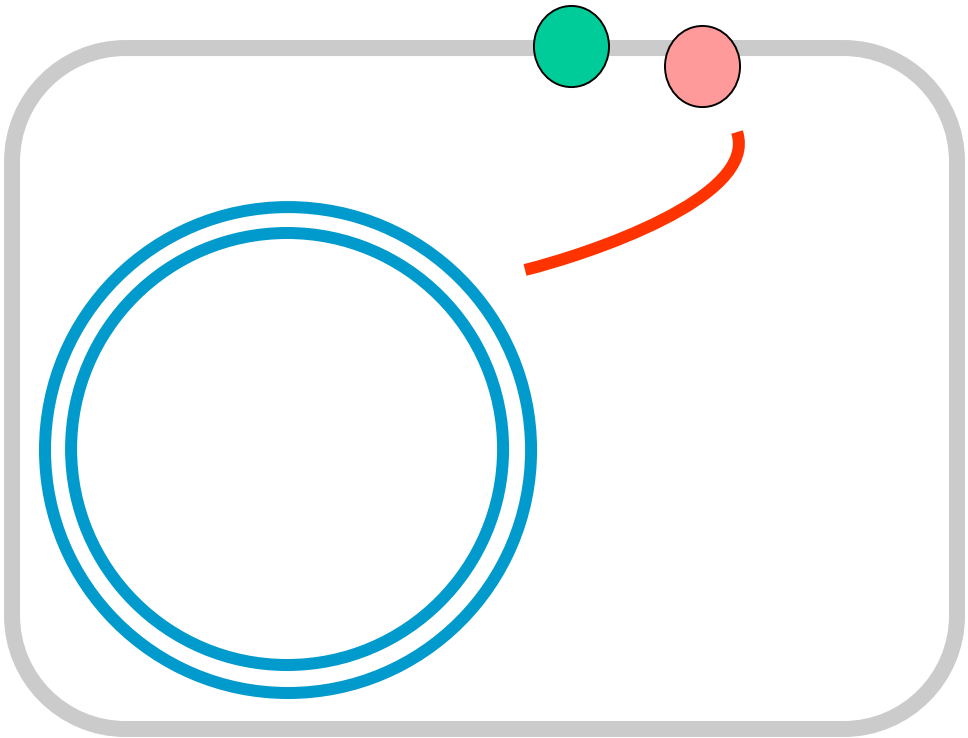
Mechanism of Transformation in *Streptococcus*



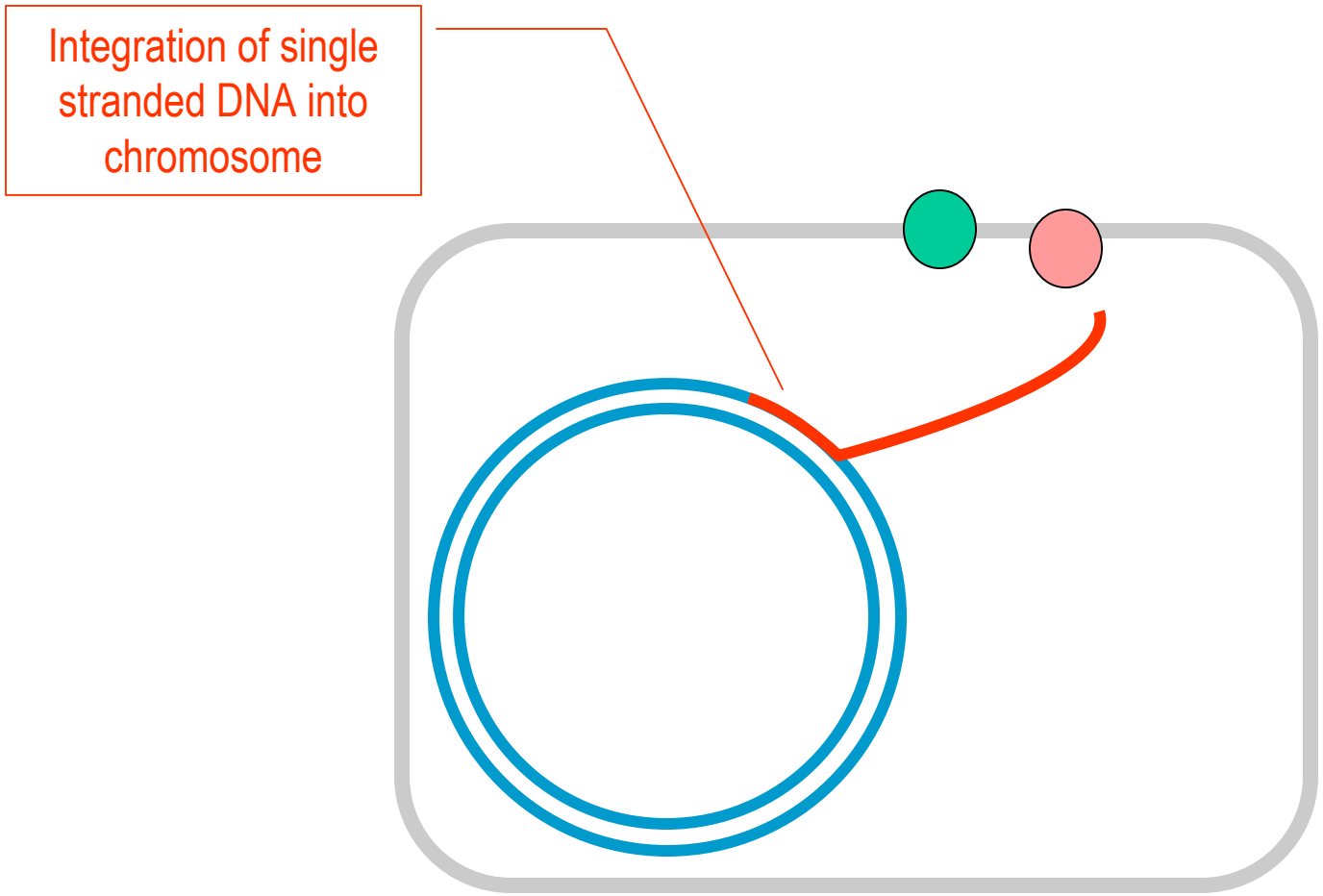
Mechanism of Transformation in *Streptococcus*



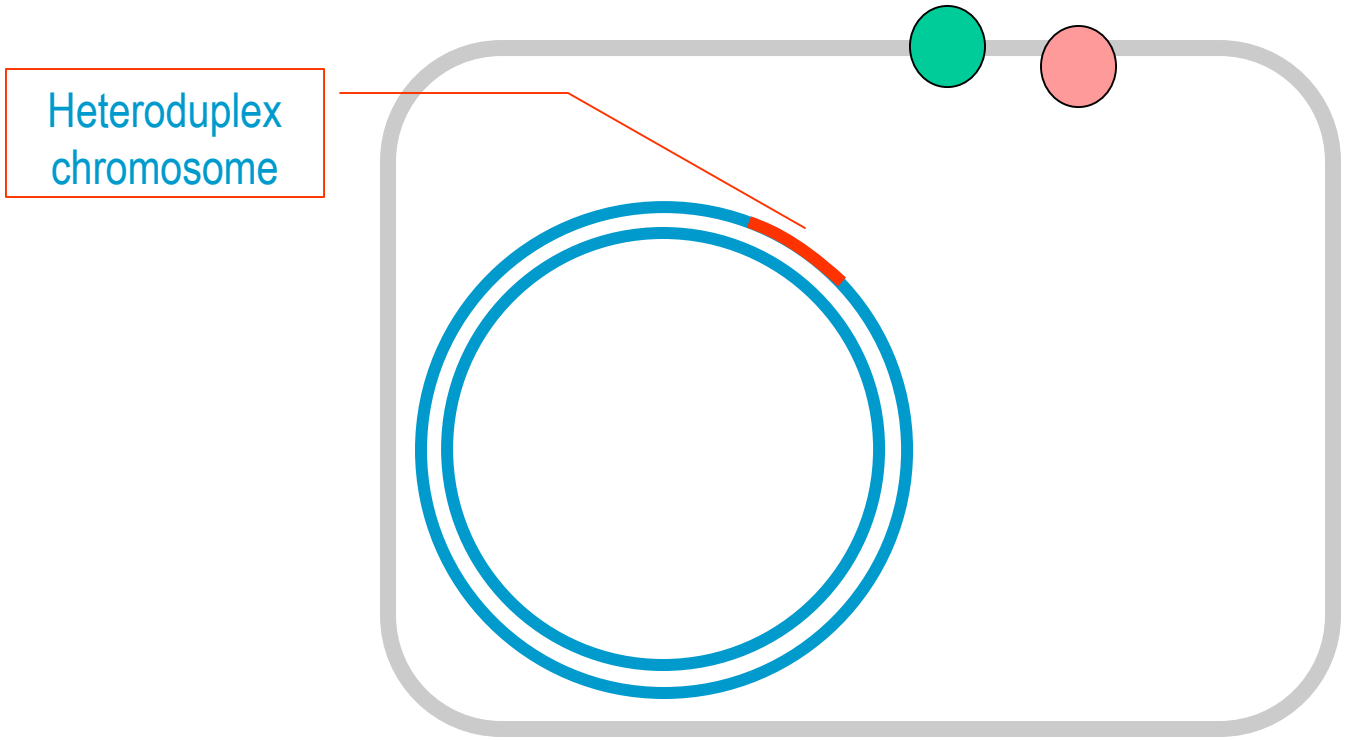
Mechanism of Transformation in *Streptococcus*



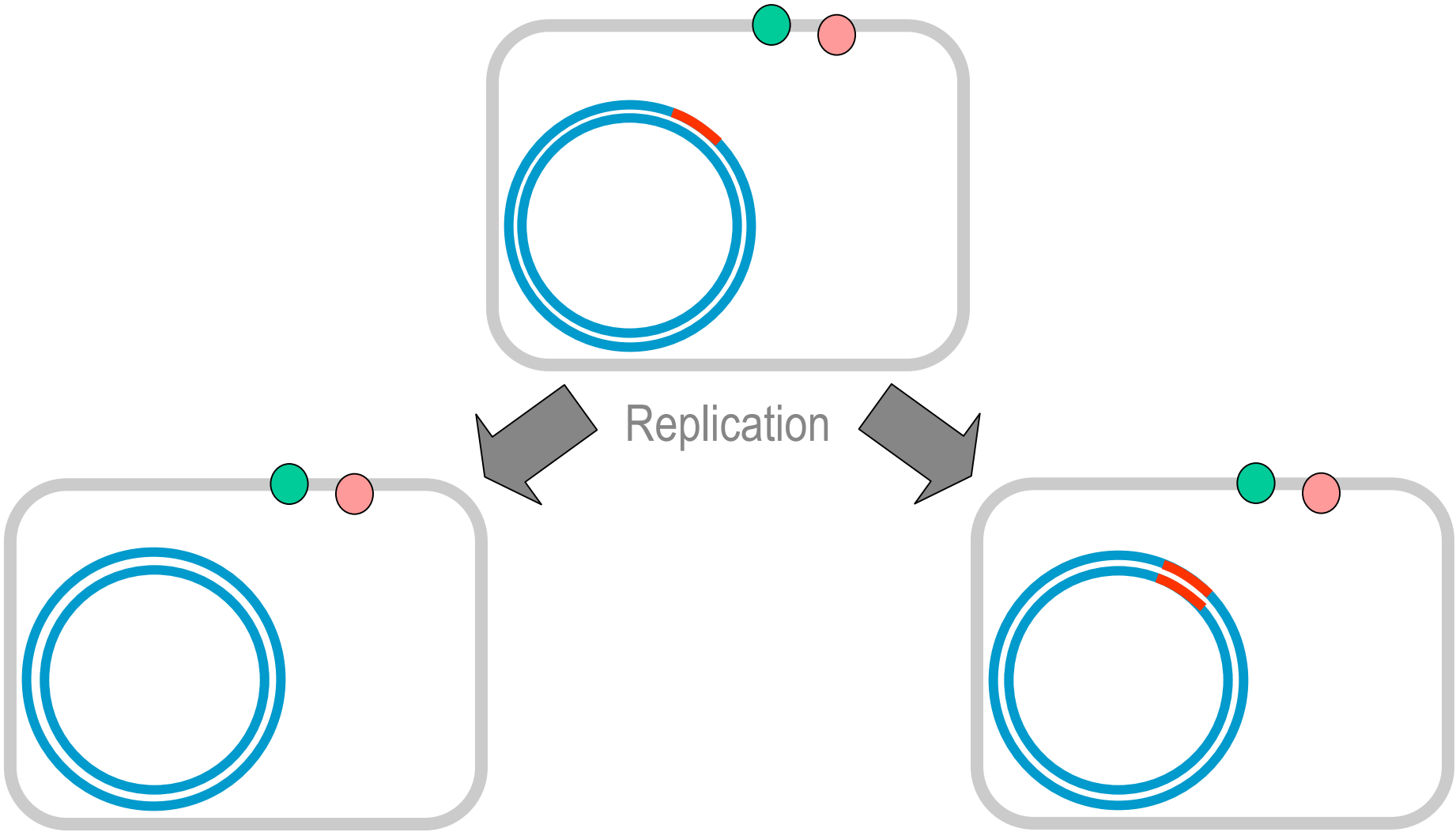
Mechanism of Transformation in *Streptococcus*



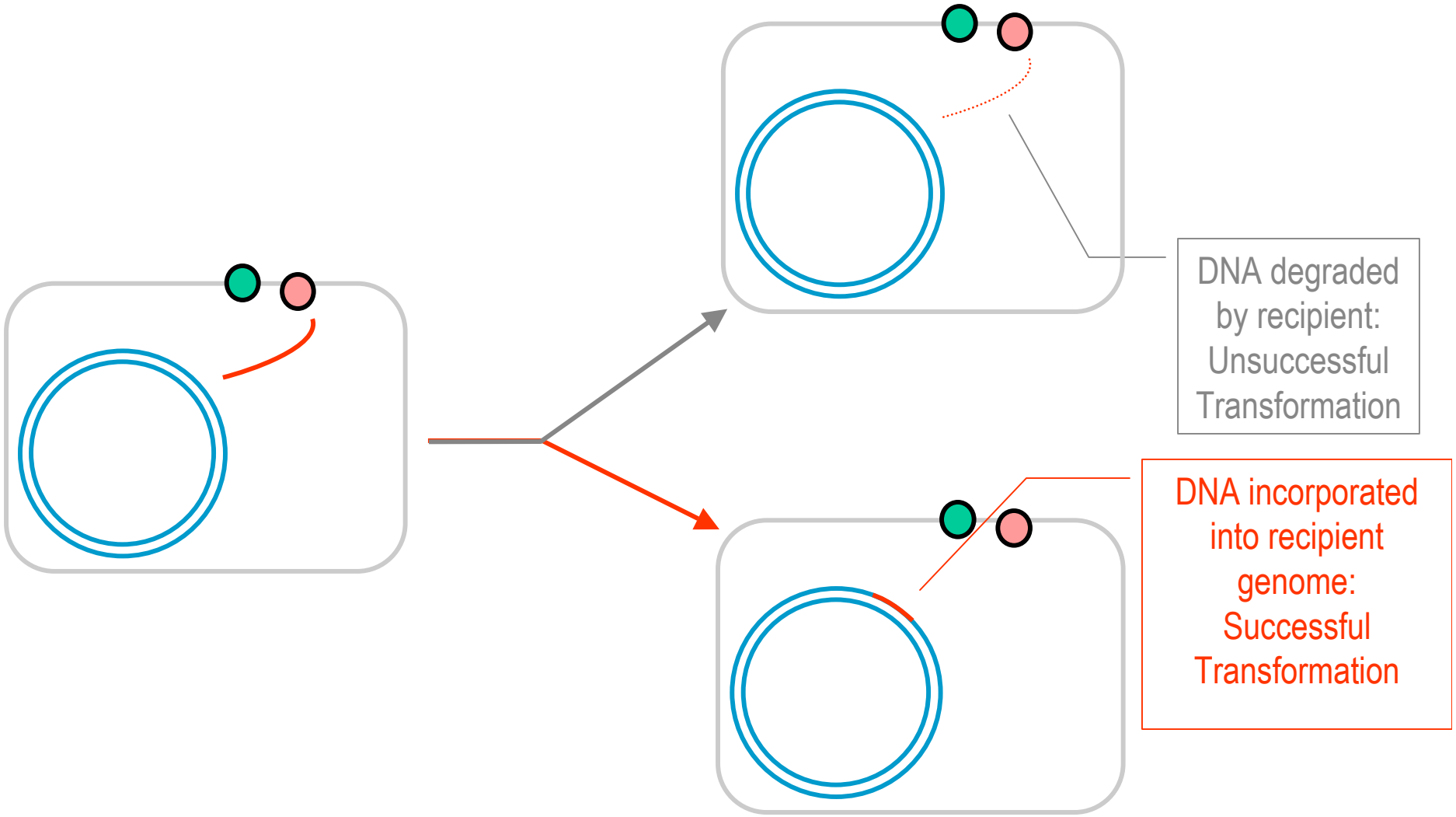
Mechanism of Transformation in *Streptococcus*



Mechanism of Transformation in *Streptococcus*



Successful Transformation



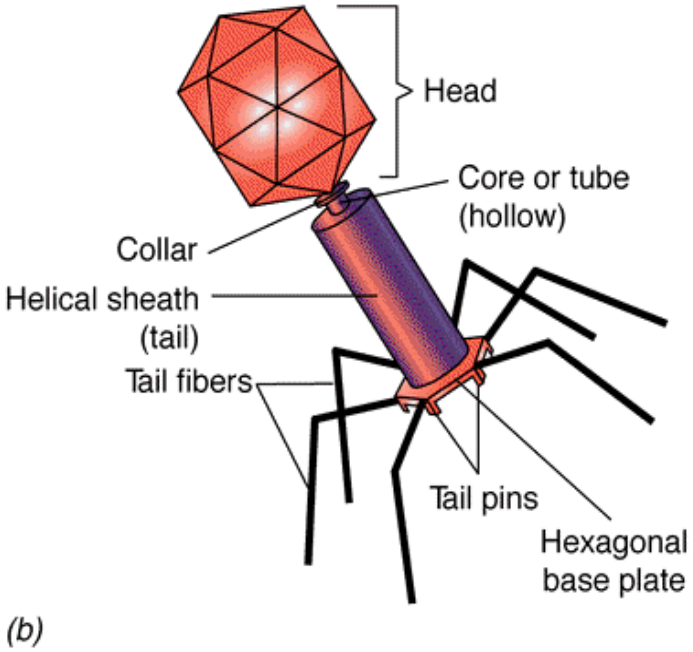
Transformation Conclusion

- Transformed plasmid DNA need not recombine to be maintained by the cell

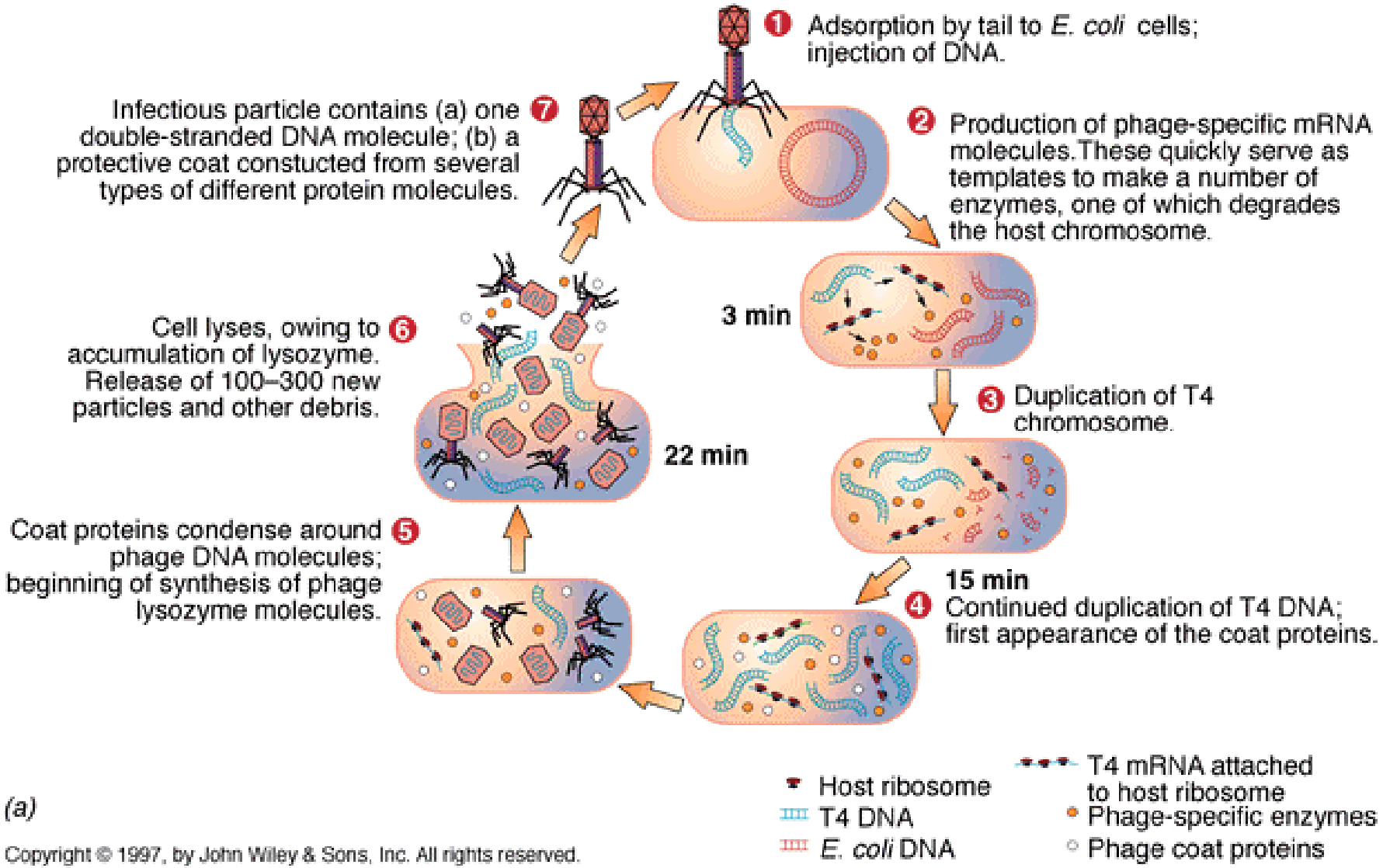
Natural Transformation	
Gram Positive	*Streptococcus pneumoniae*
	Bacillus subtilus
	Bacillus cereus
Gram Negative	*Neisseria gonorrheae*
	Haemophilus influenzae
	Pseudomonas stuzeri
Artificial Transformation	
	Escherichia coli
	Salmonella Typhimurium
	Pseudomonas aeruginosa

Transduction

- Transfer of DNA from a donor to a recipient via a bacteriophage (bacterial virus)
- Uses errors in the life cycle of the bacteriophage to transfer the DNA
- Two classes of transduction
  - generalised
  - specialised

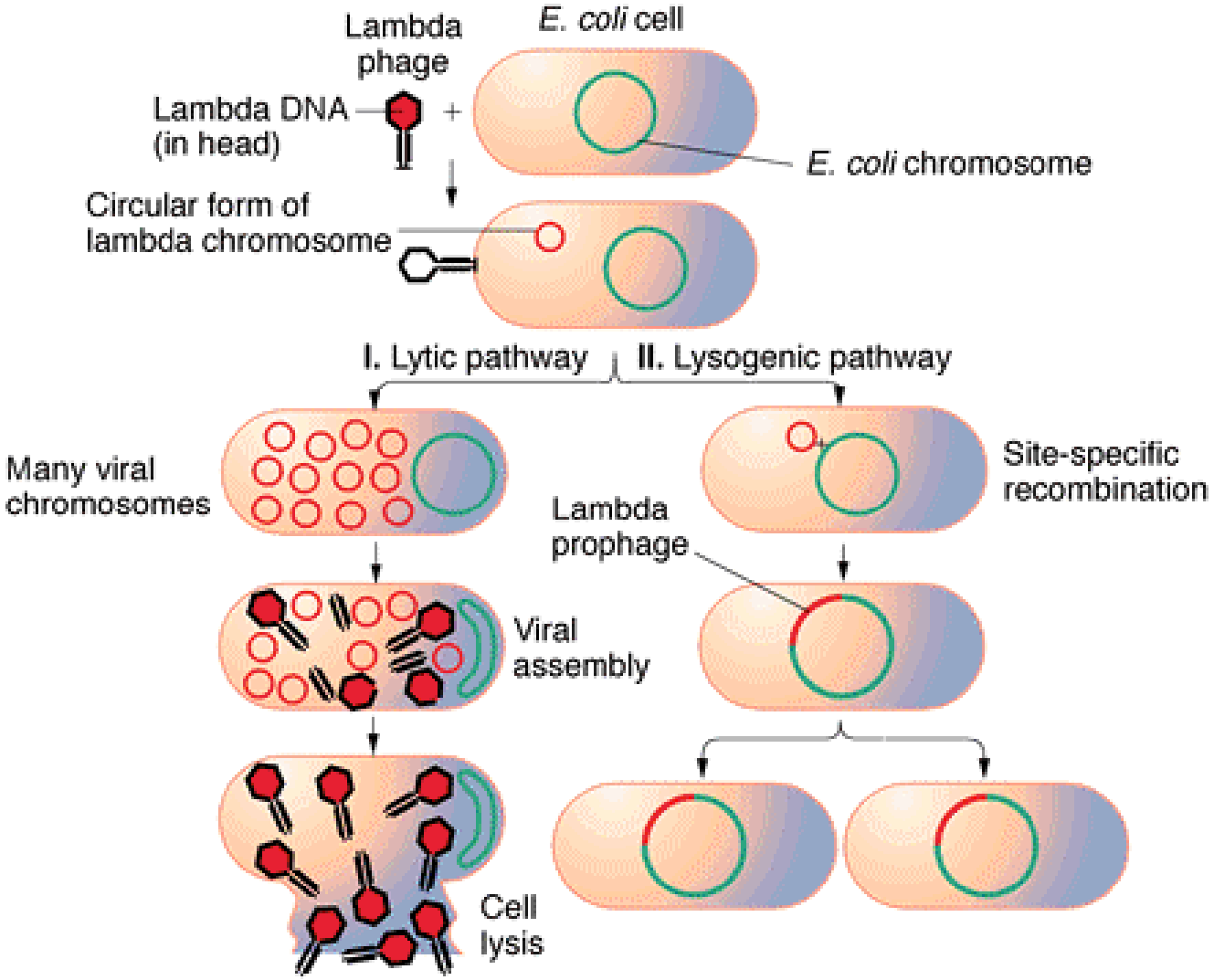


Bacteriophage Lifecycles: Lytic



(a)

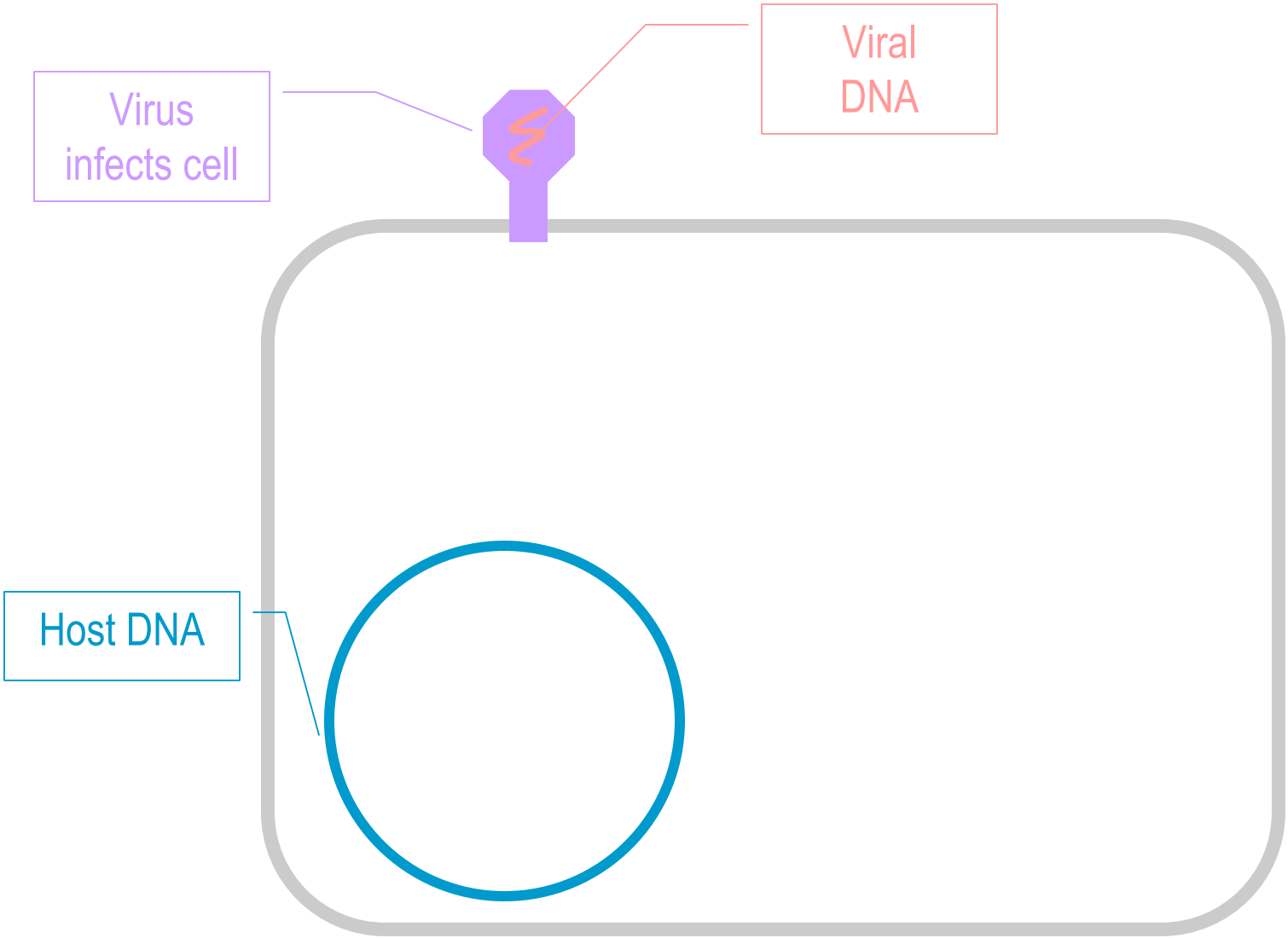
Bacteriophage Lifecycles: Lysogeny



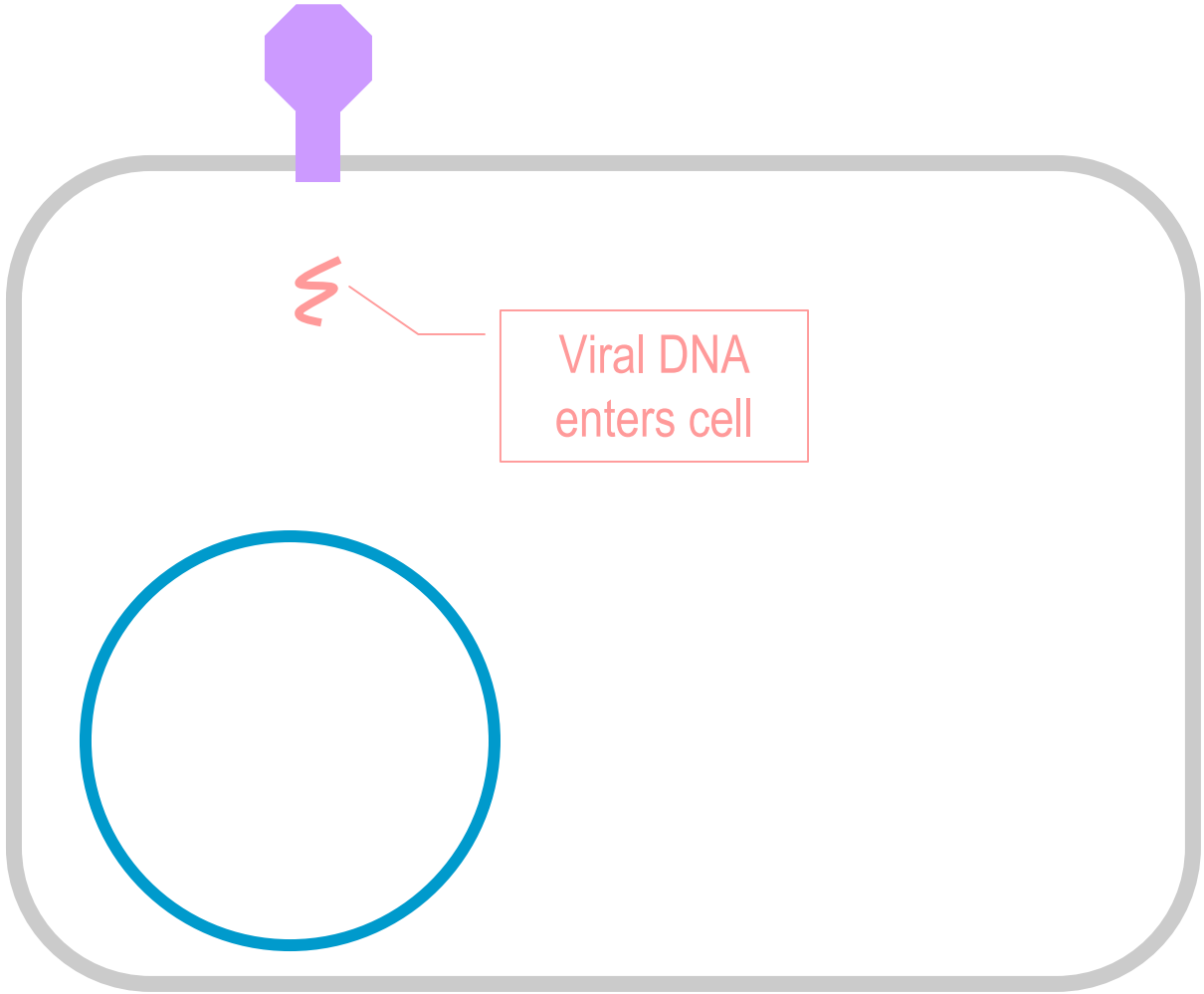
Generalised Transduction

- Replication cycle of virulent bacteriophage has 3 phases
  - invasion
  - replication
  - release
- During infection…
  - host DNA is fragmented
  - occasionally packaged instead of phage DNA
    - defective phage
    - can be any DNA fragment
- Defective phage can infect
  - can’t replicate or induce lysis

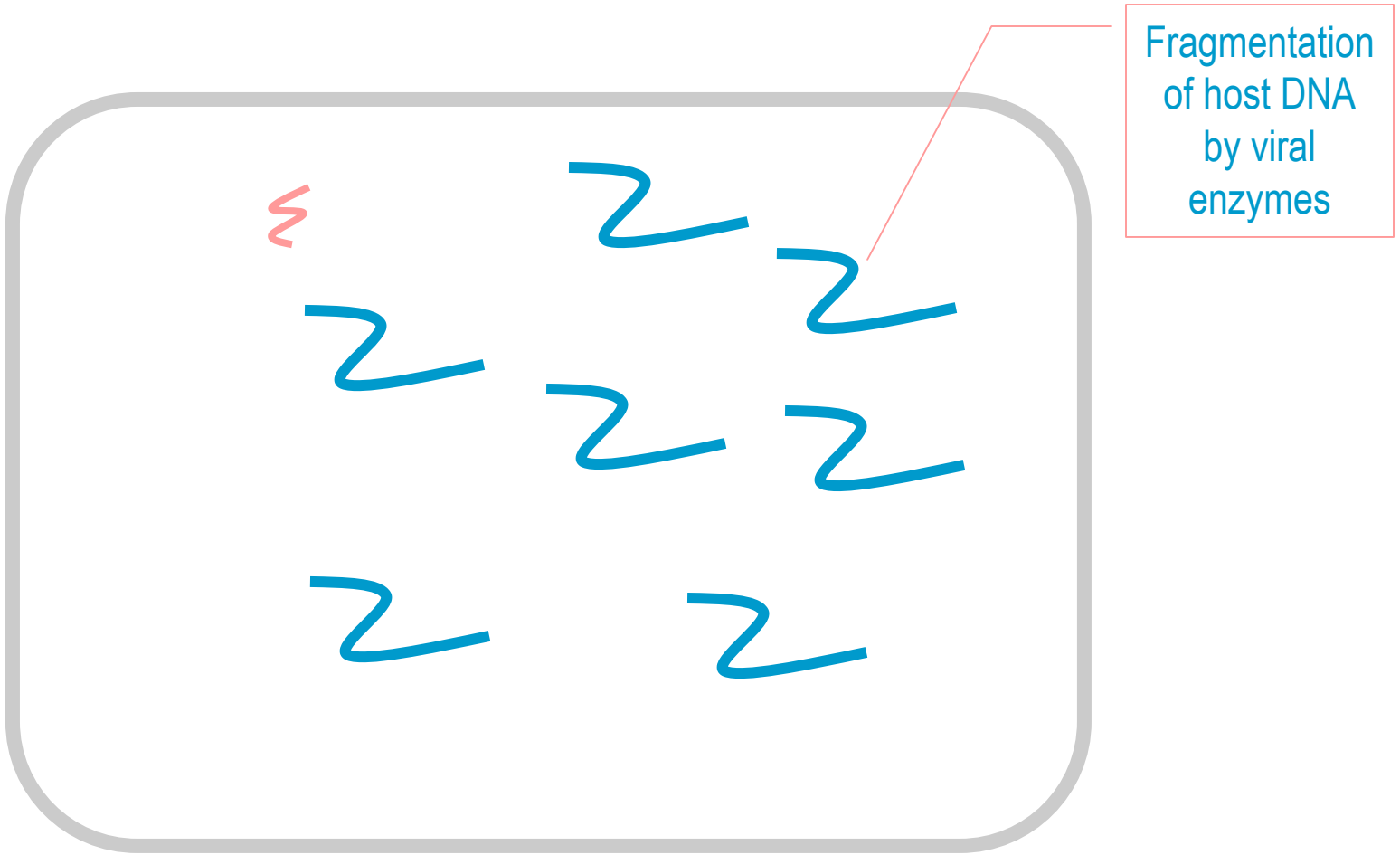
Generalised Transduction Mechanism



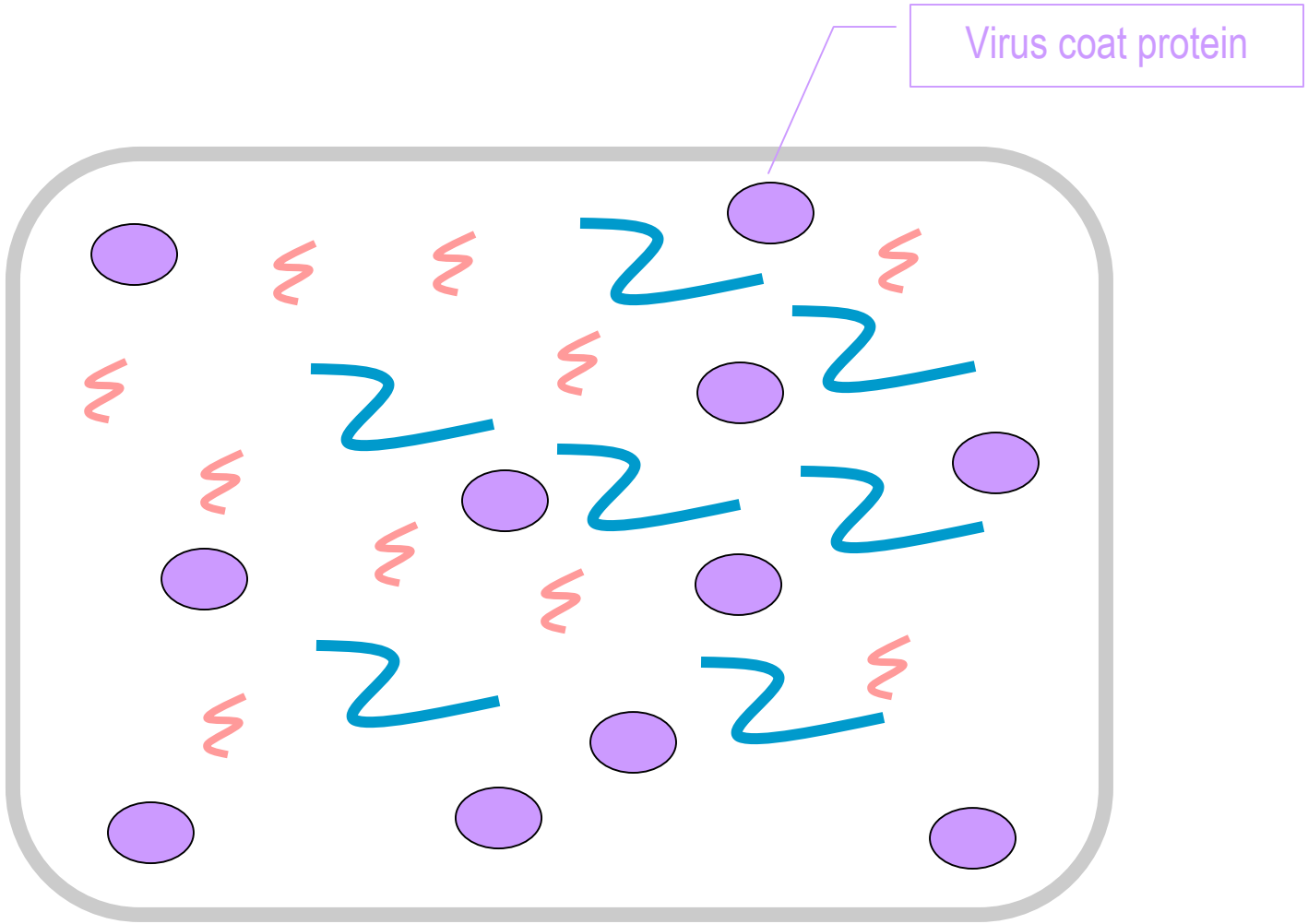
Infection



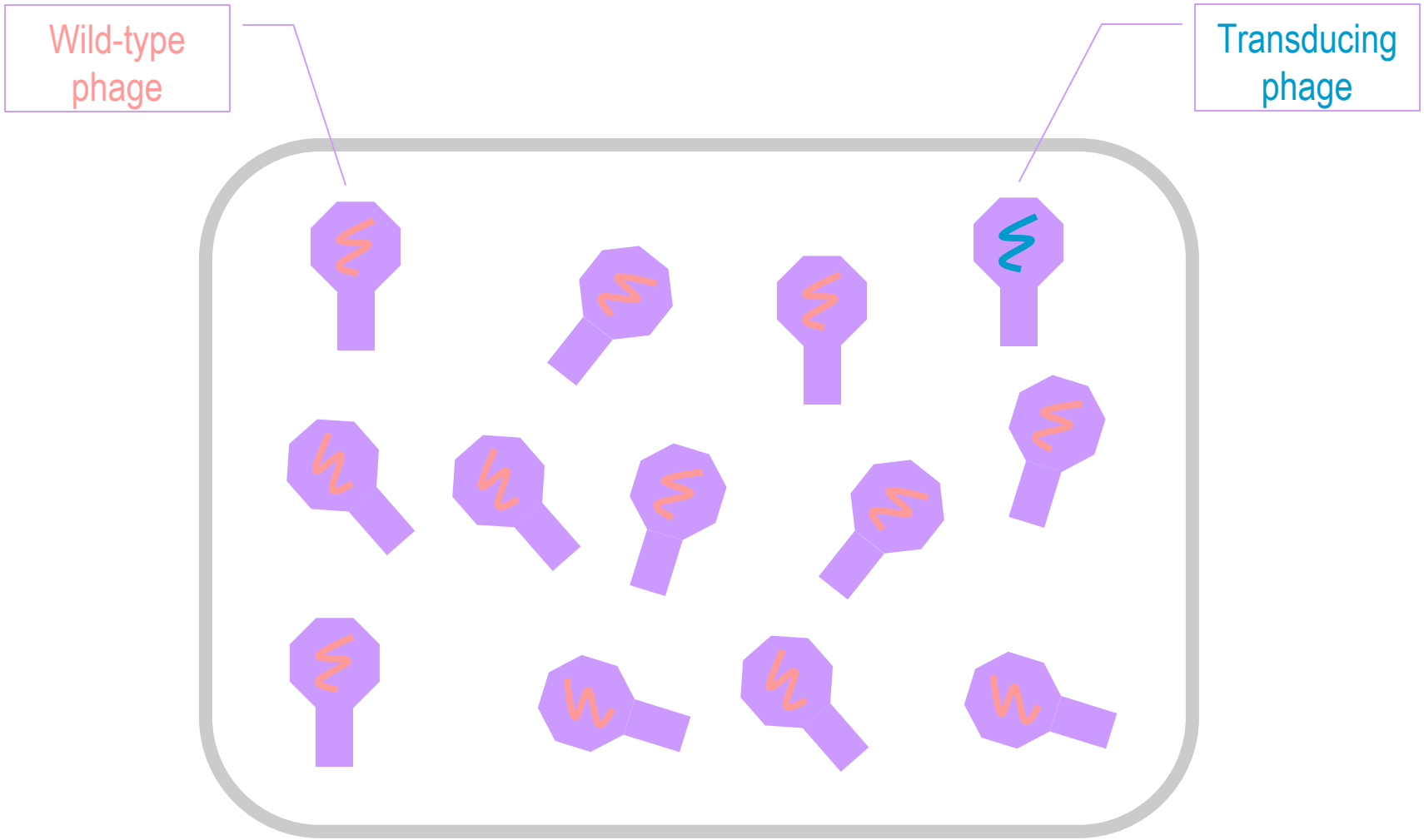
Degradation of Host DNA



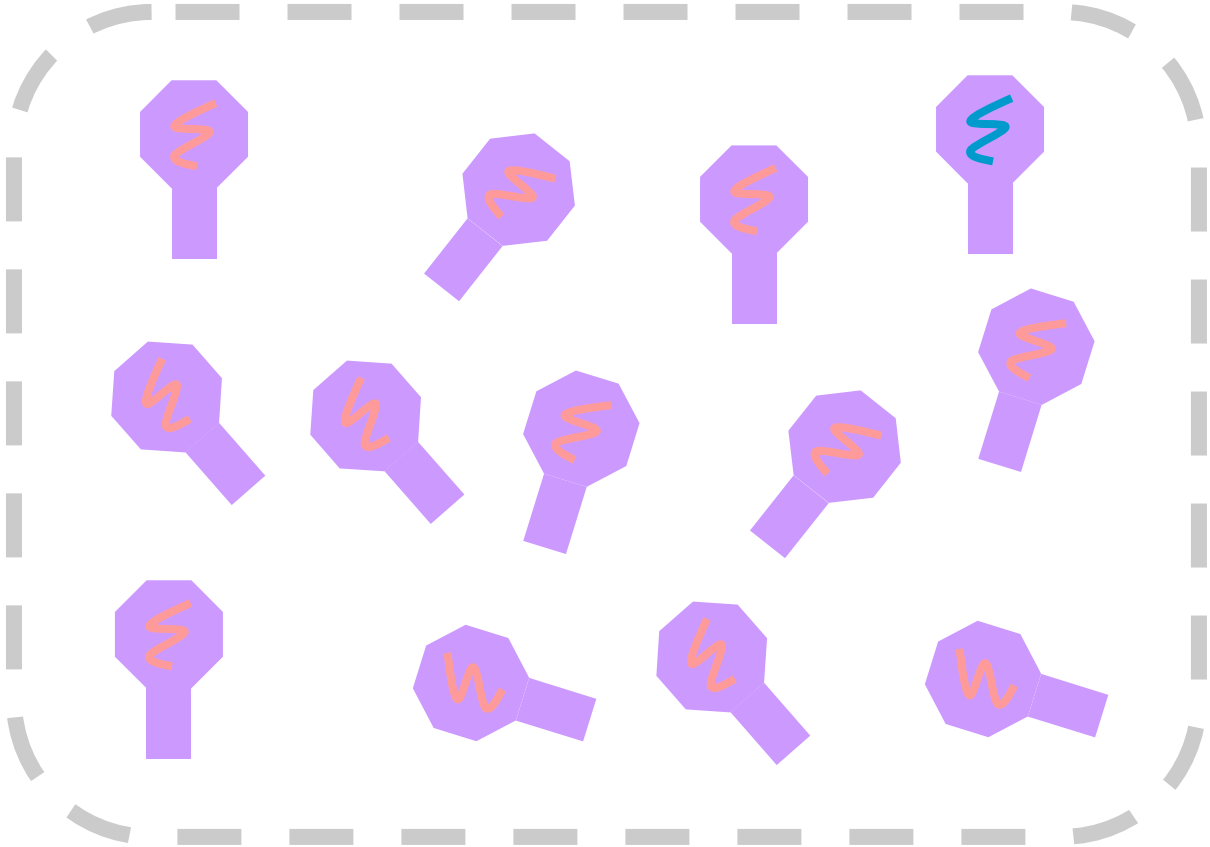
Replication



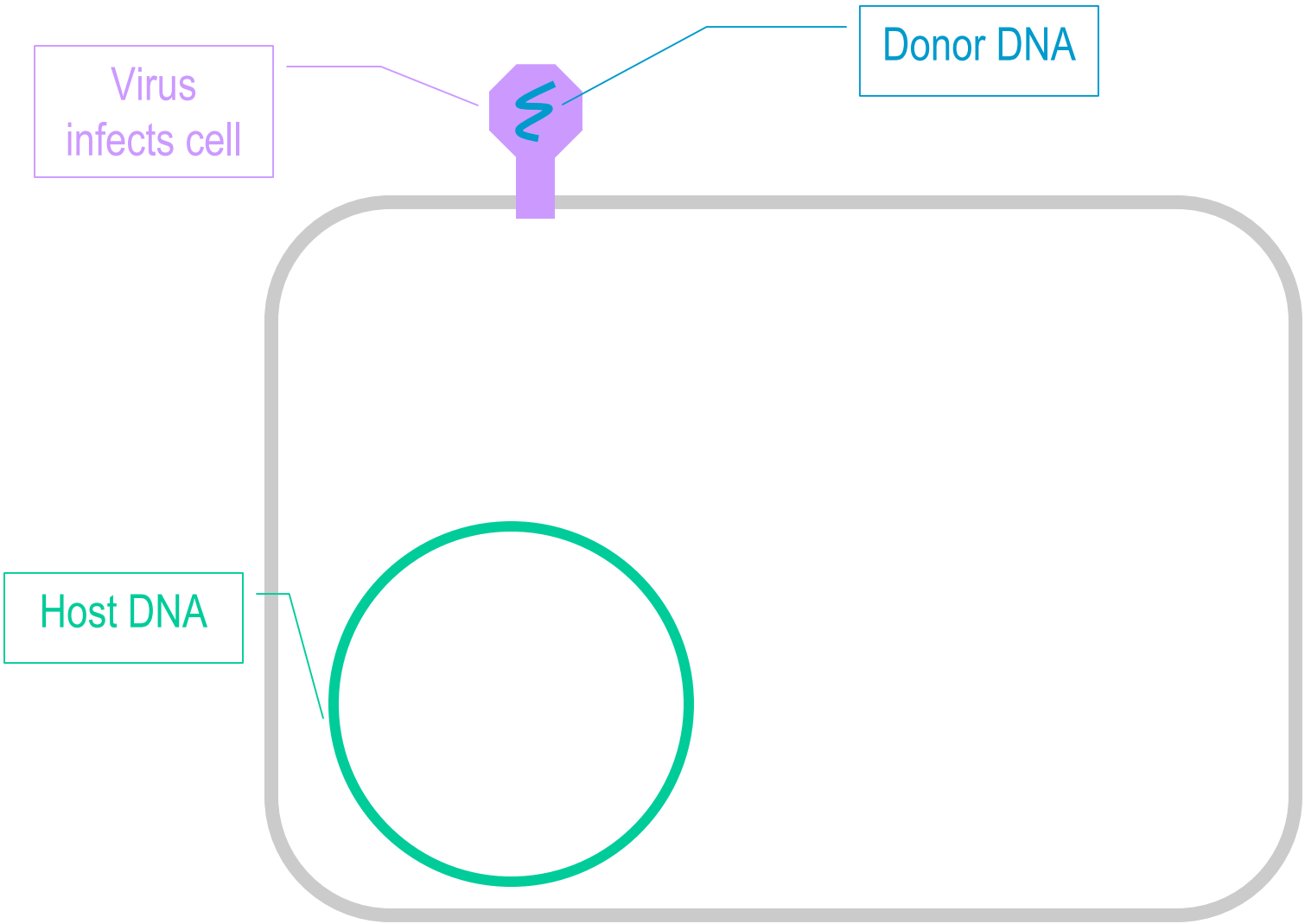
Assembly



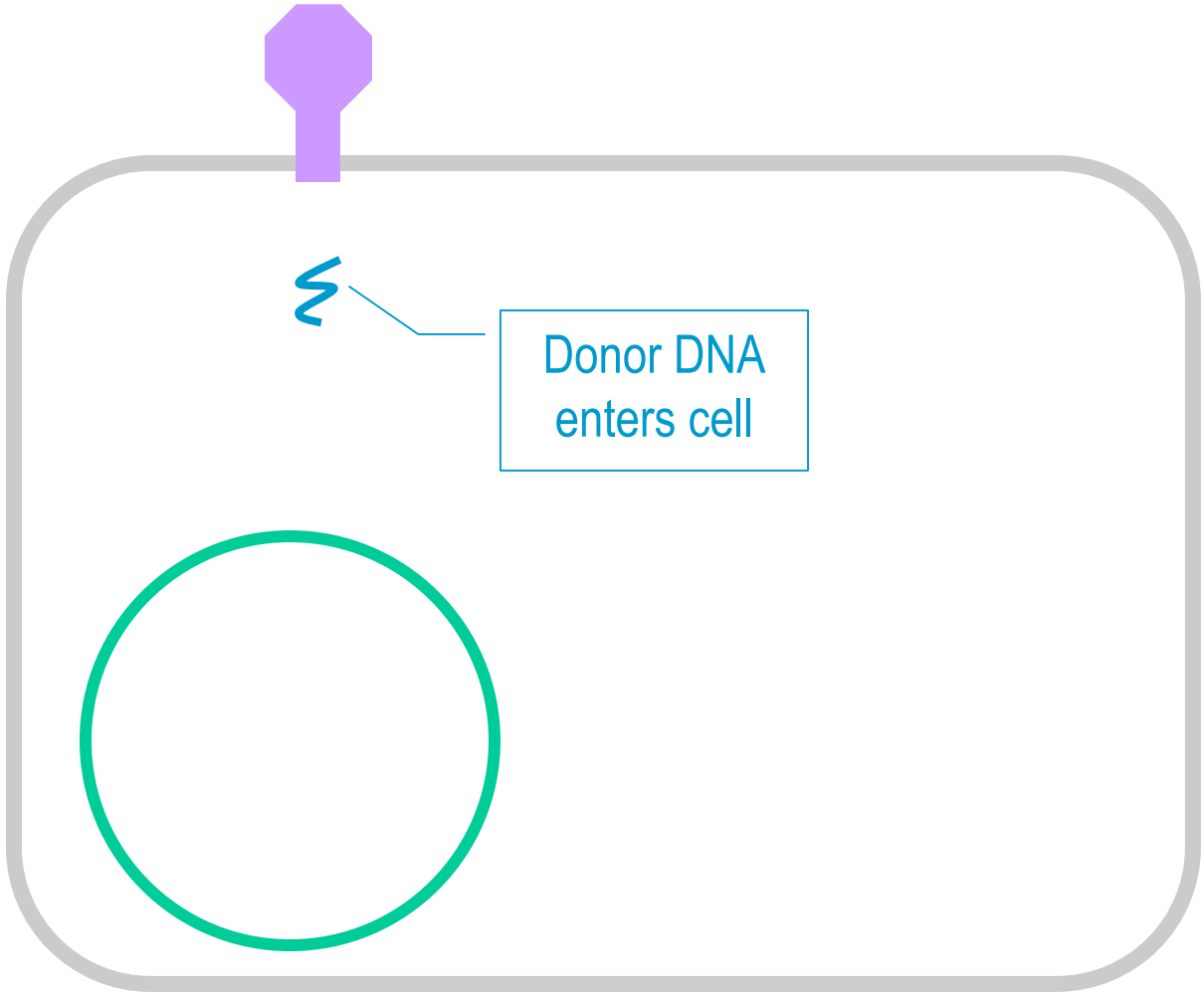
Lysis



Infection by Transducing Phage

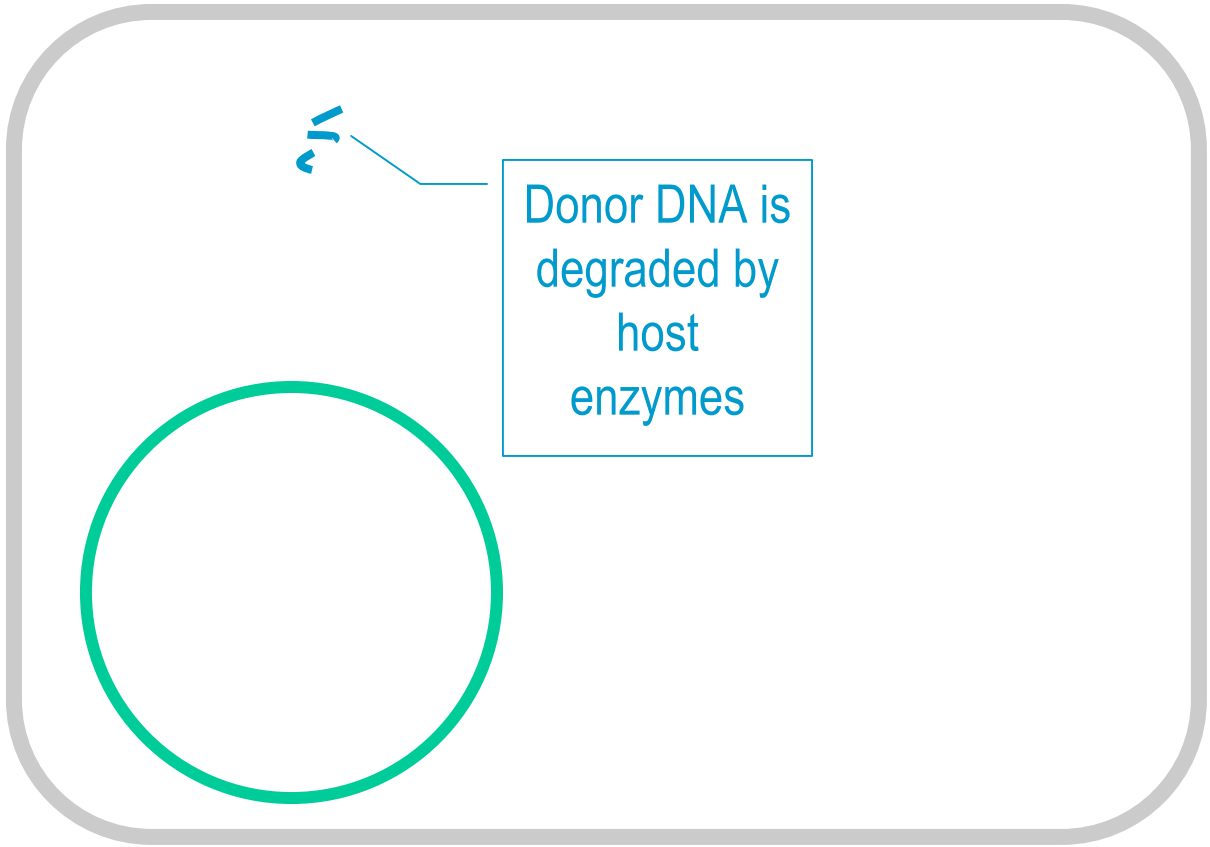


Infection



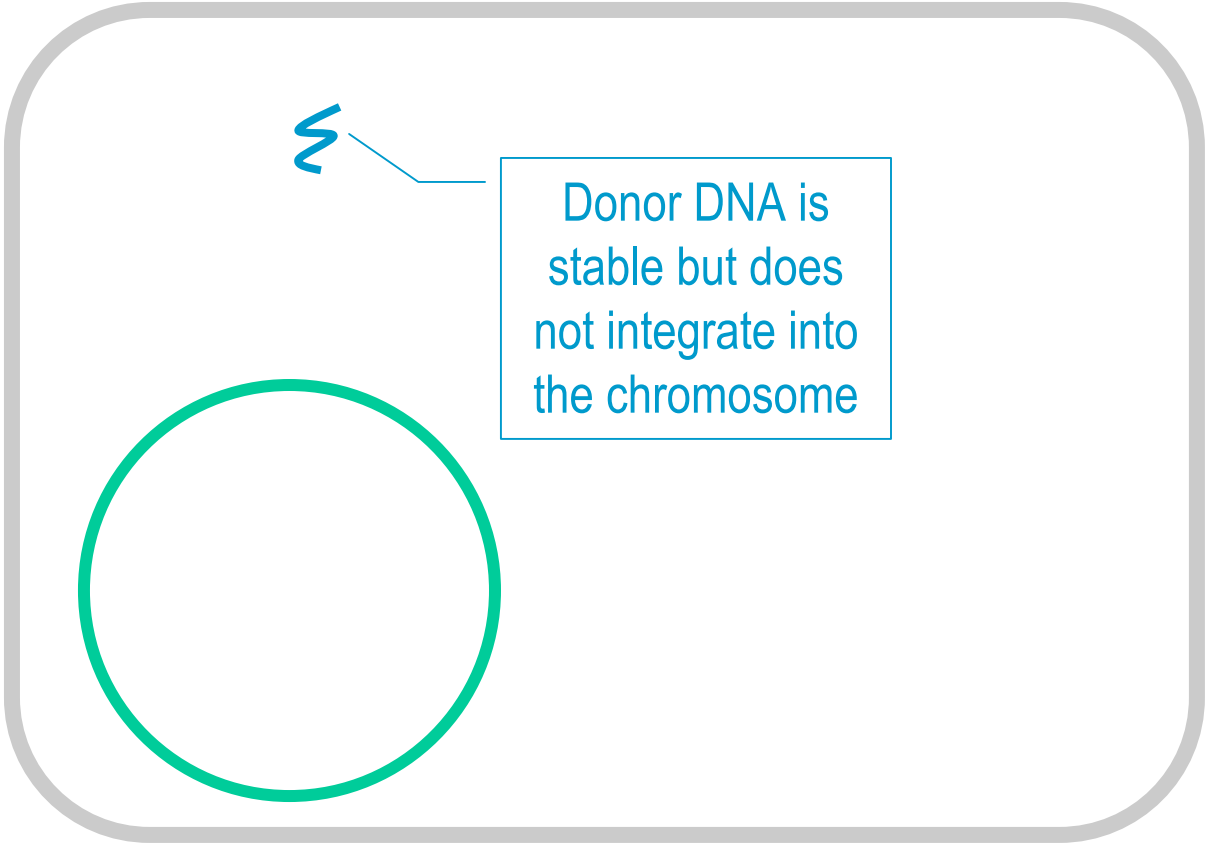
Fate 1: Degradation

X Unsuccessful gene transfer X



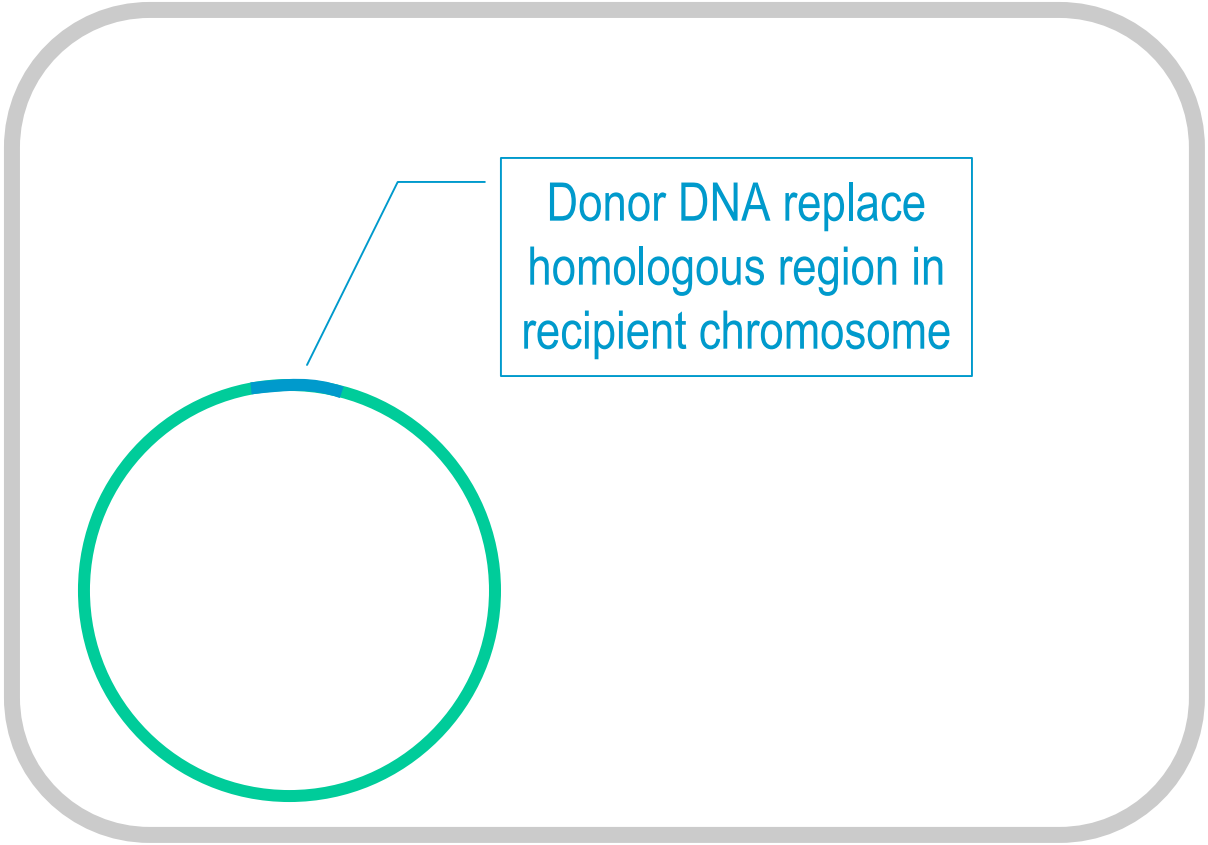
Fate 2: Not Replicated

X Abortive transduction X



Fate 3: Integration

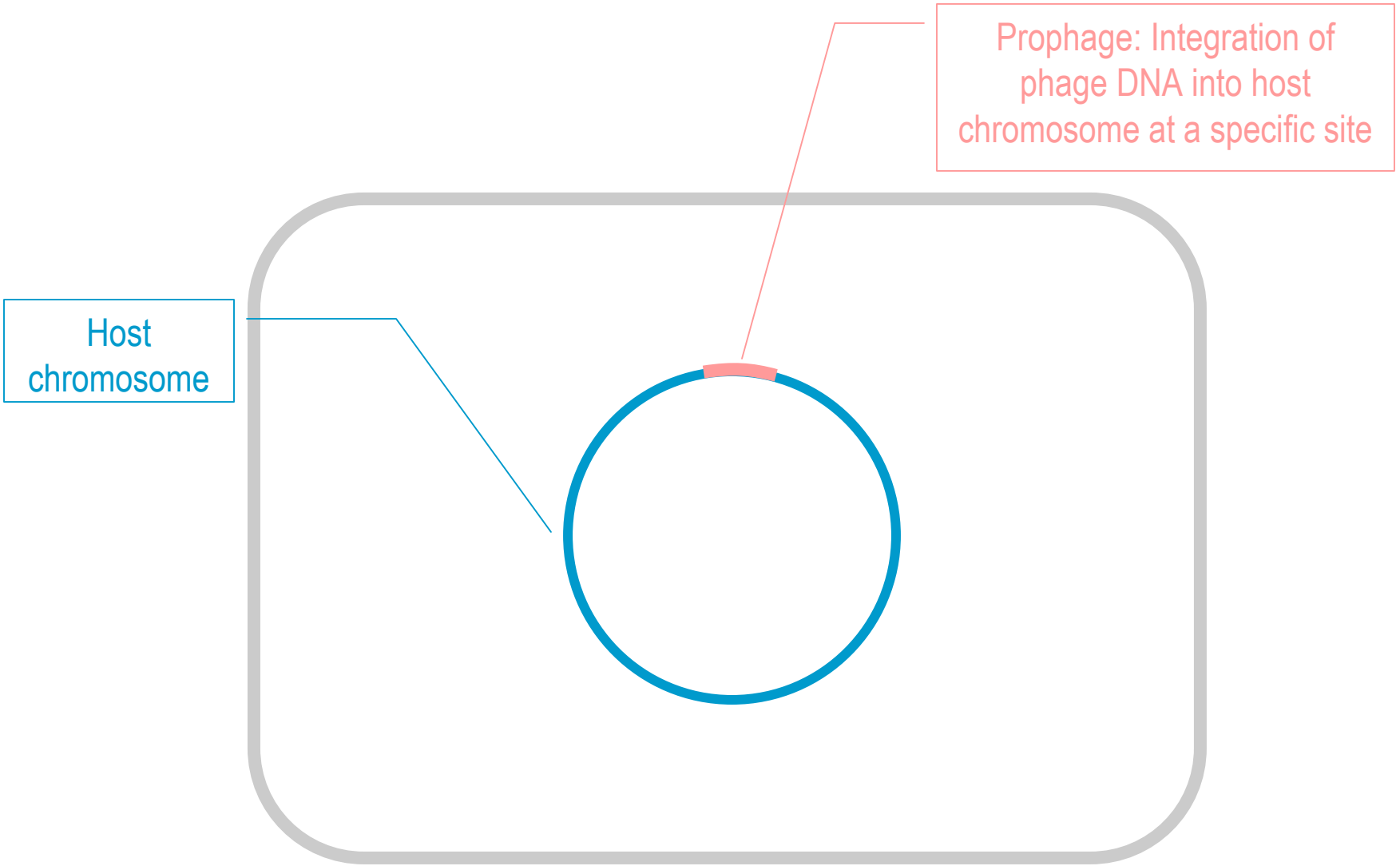
✓ Stable gene transfer ✓



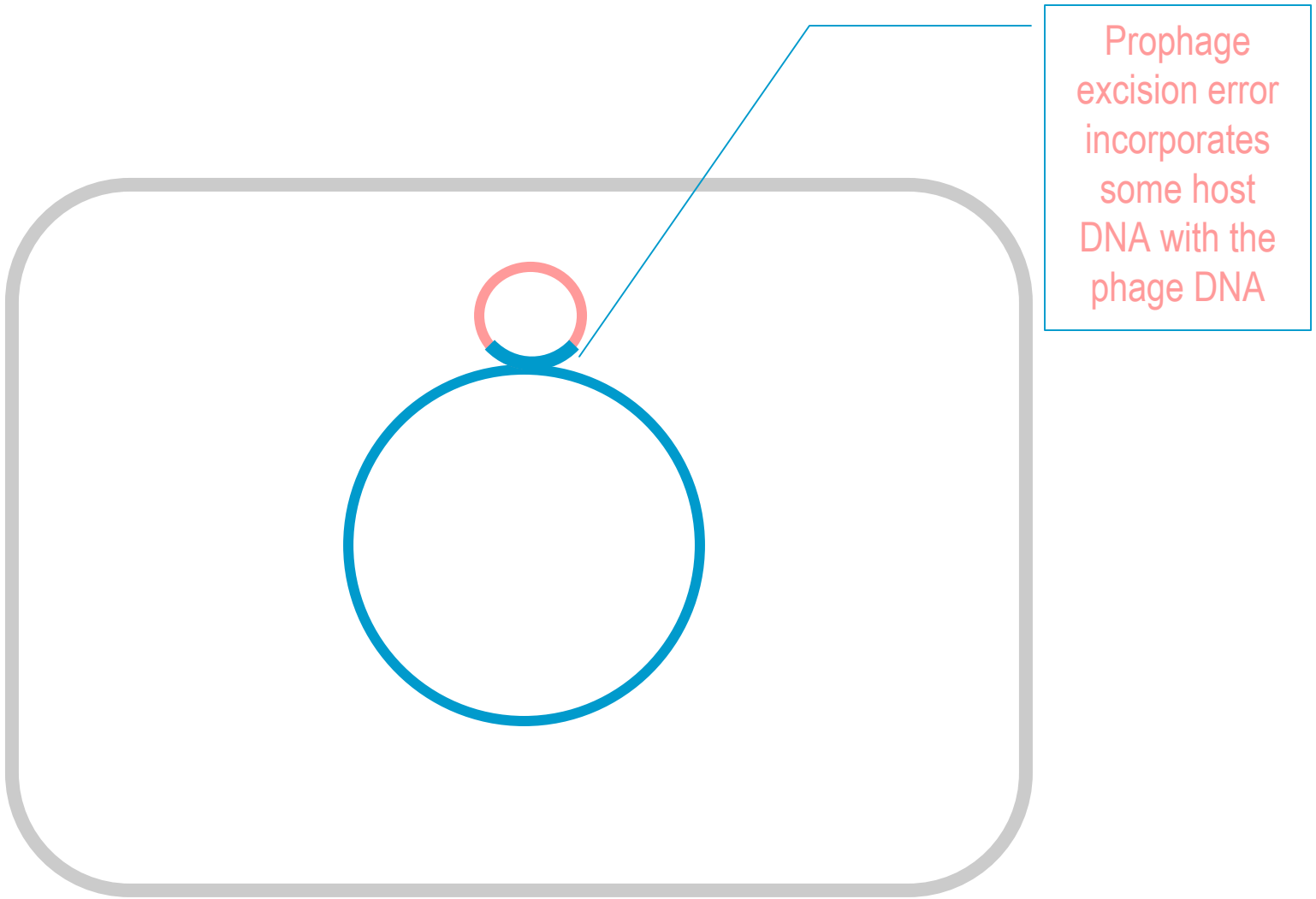
Specialised Transduction

- Utilises the lysogenic cycle of some bacteriophage
  - eg. bacteriophage λ
- Phage DNA is propagated along with the cell's chromosome
- Integration into the chromosome is a site-specific recombinational event
- DNA transfer is the result of errors in the bacteriophage lifecycle
- Only DNA around the site of insertion can be transferred

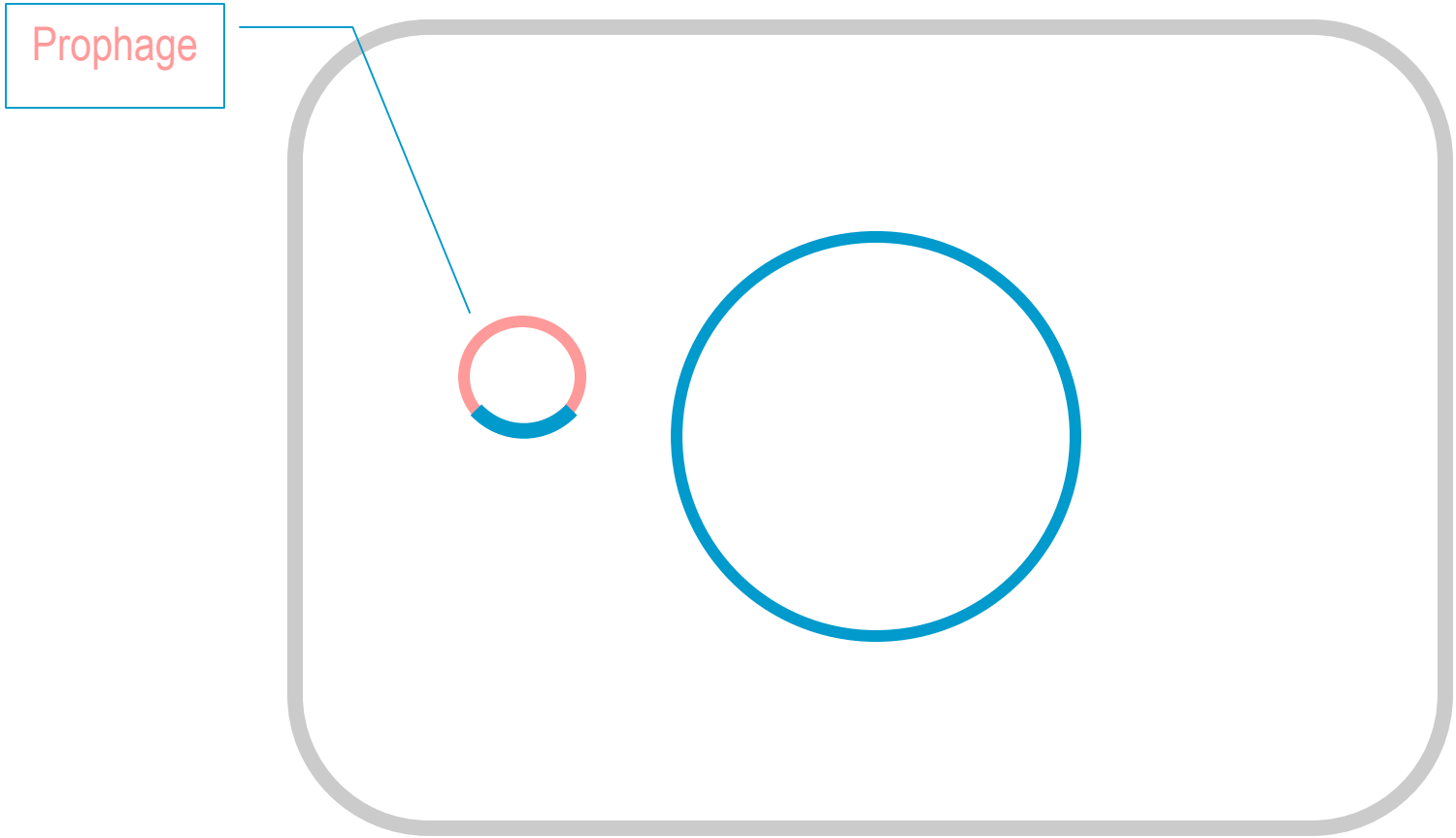
Prophage


Prophage: Integration of phage DNA into host chromosome at a specific site
Host chromosome

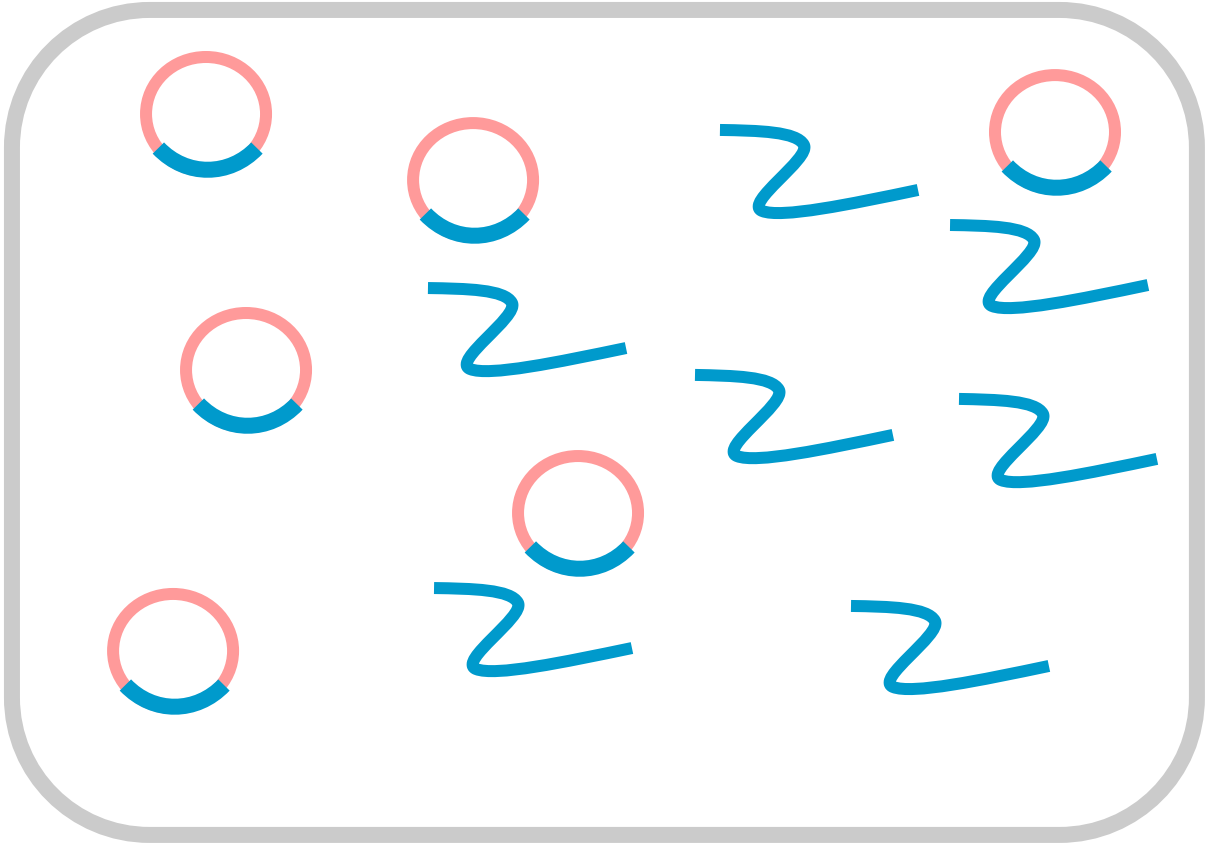
Induction


Prophage excision error incorporates some host DNA with the phage DNA

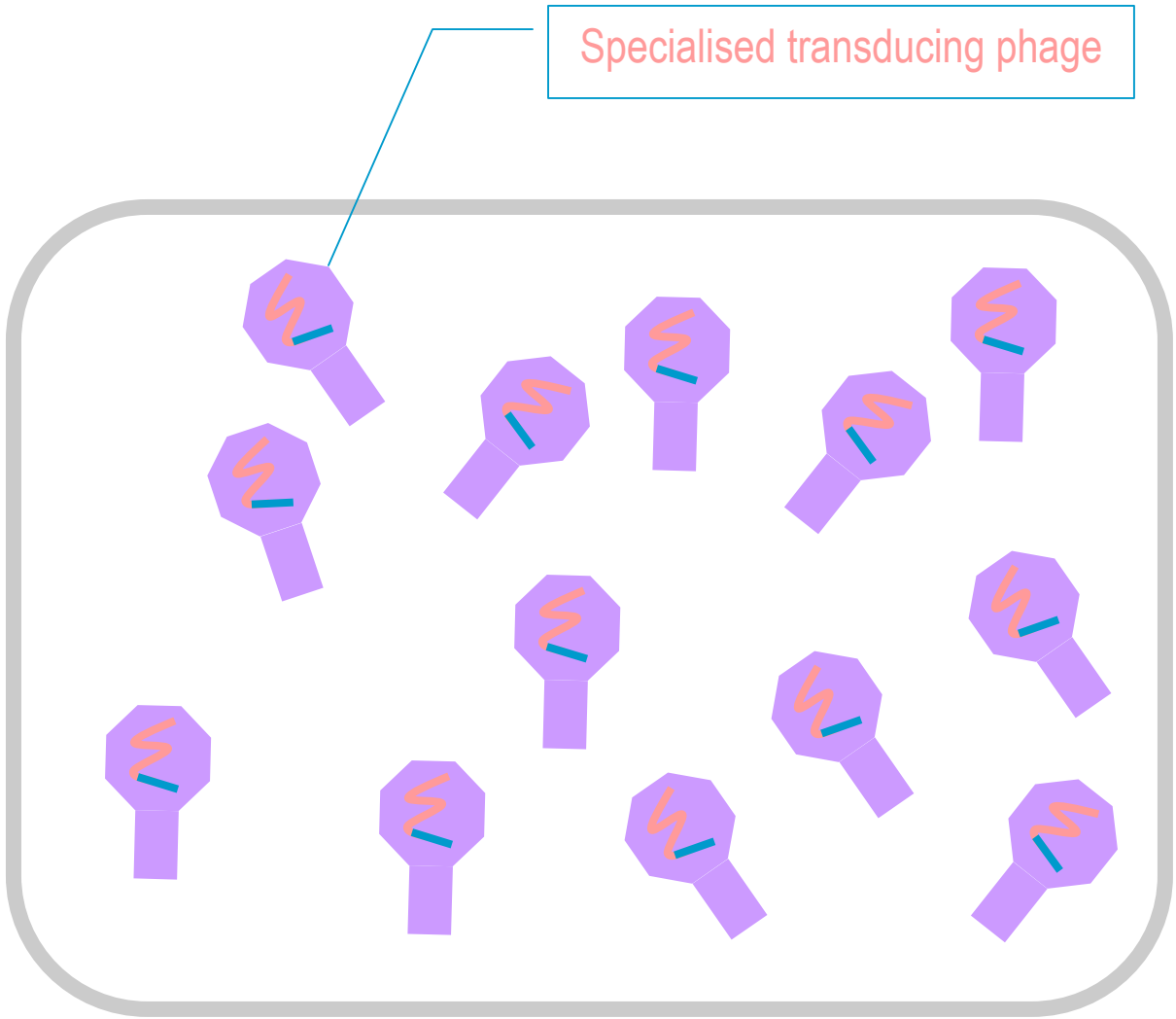
Initiation of the Lytic Cycle



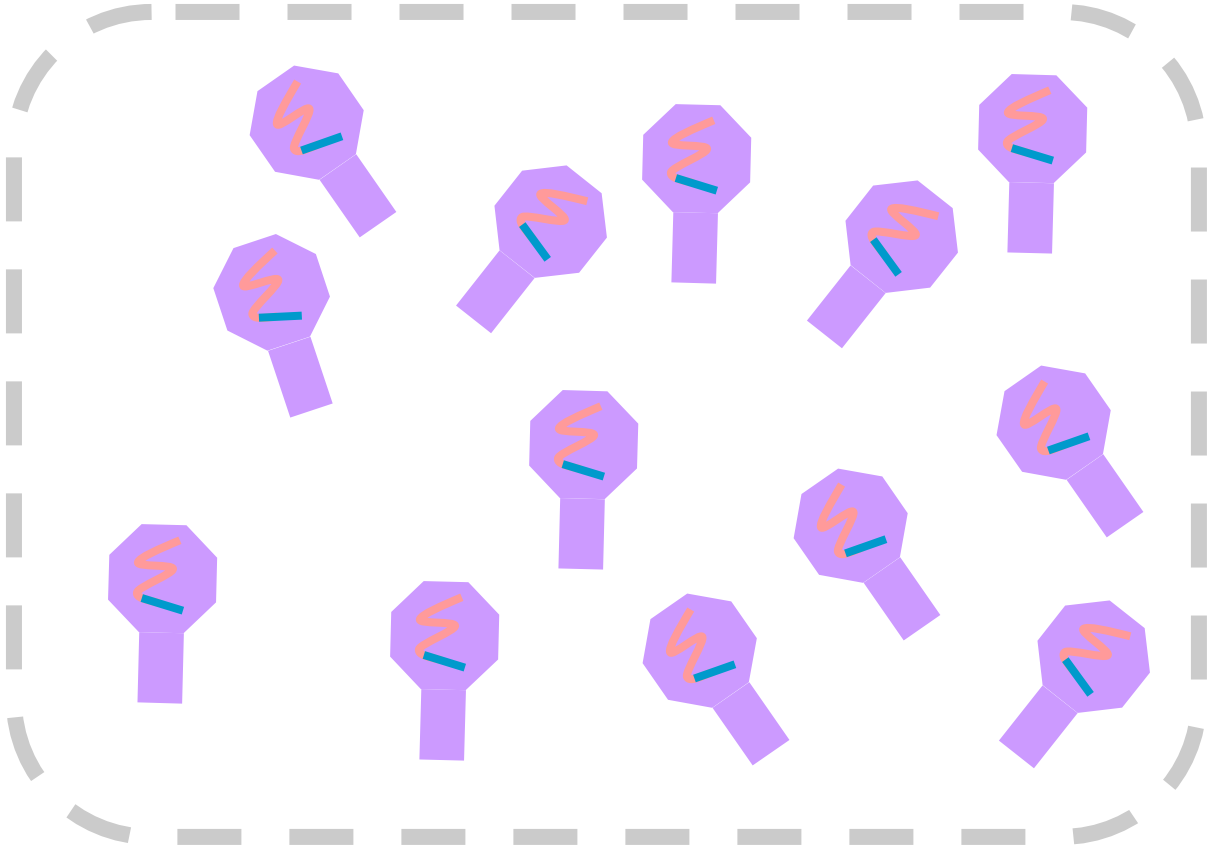
Replication



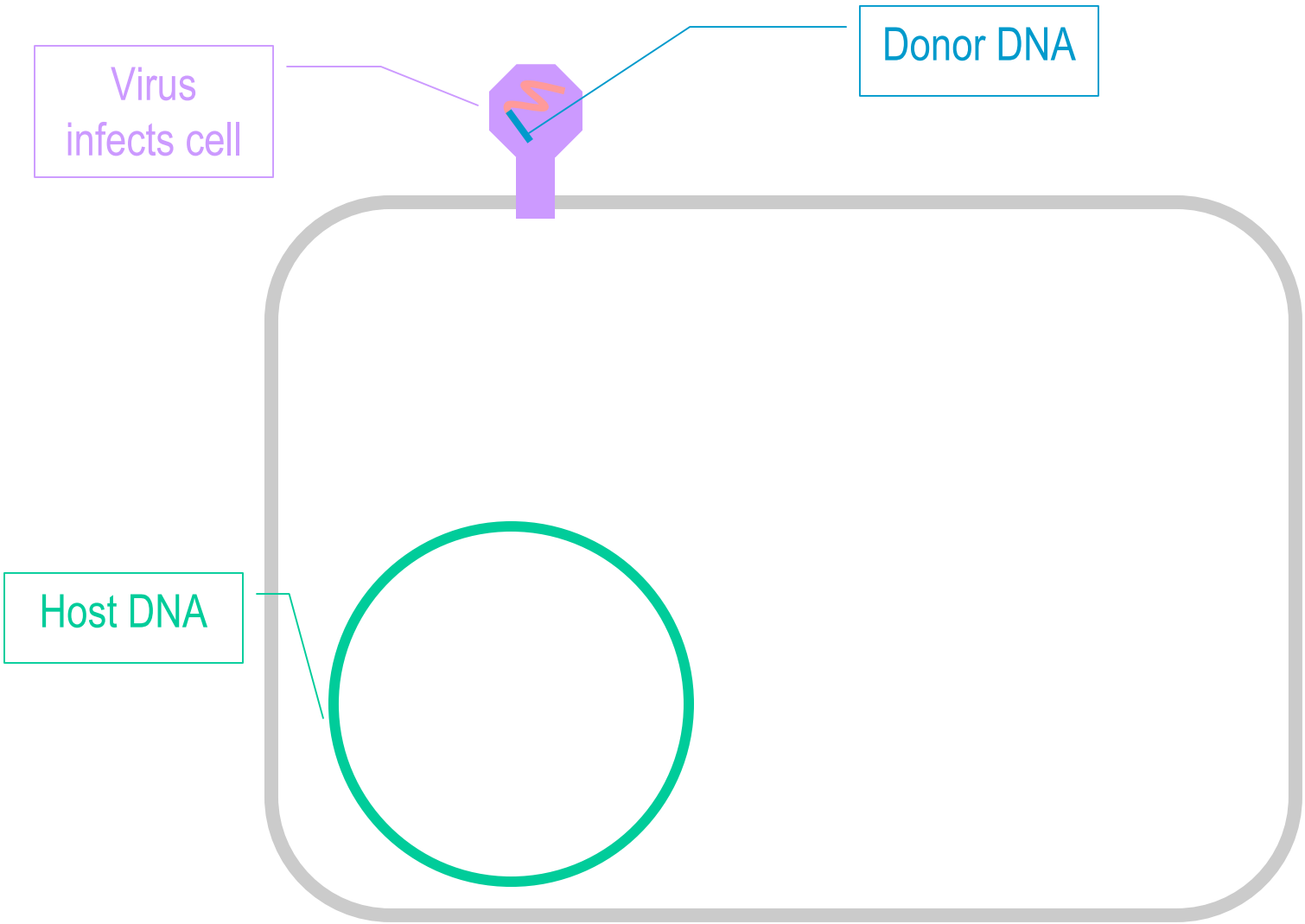
Assembly



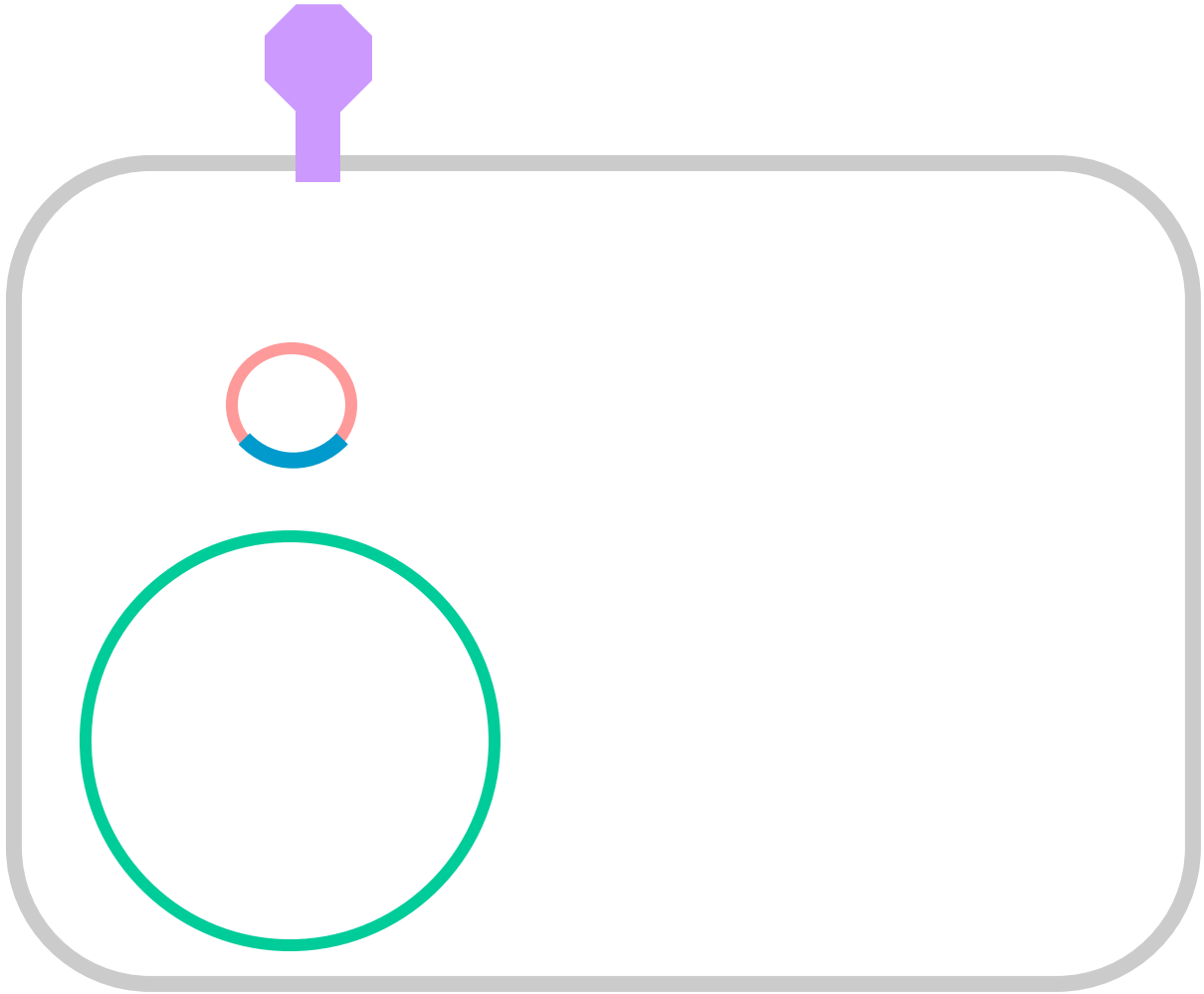
Lysis



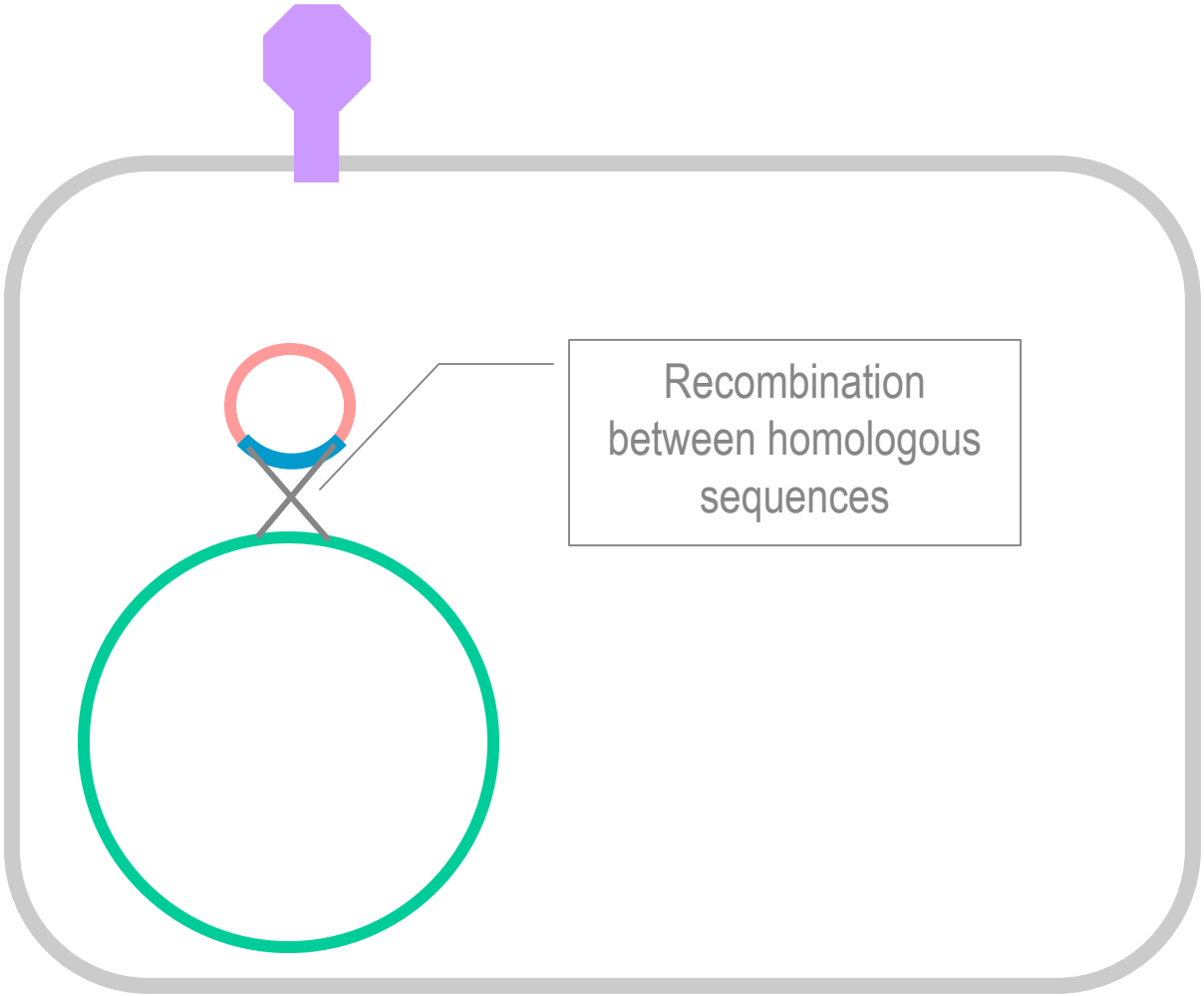
Infection by Transducing Phage



Infection

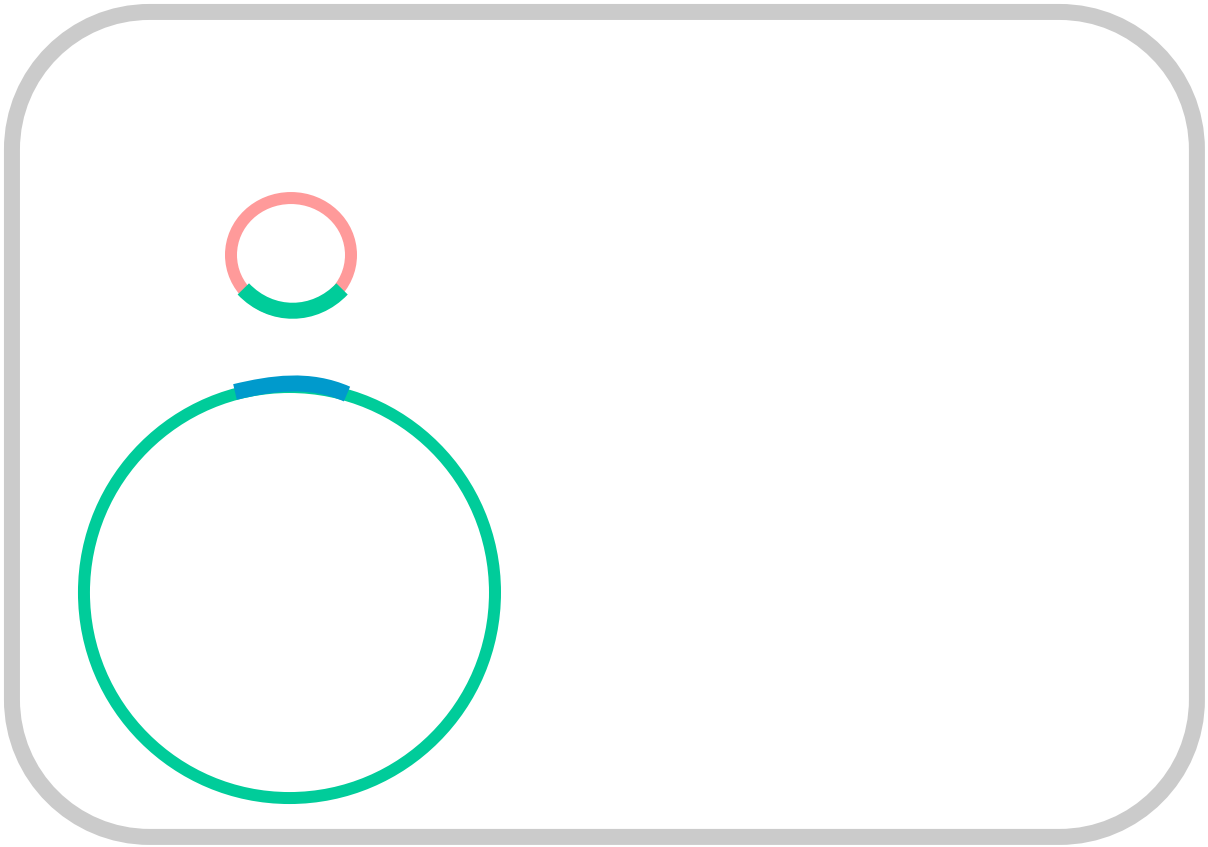


Fate 1: Recombination

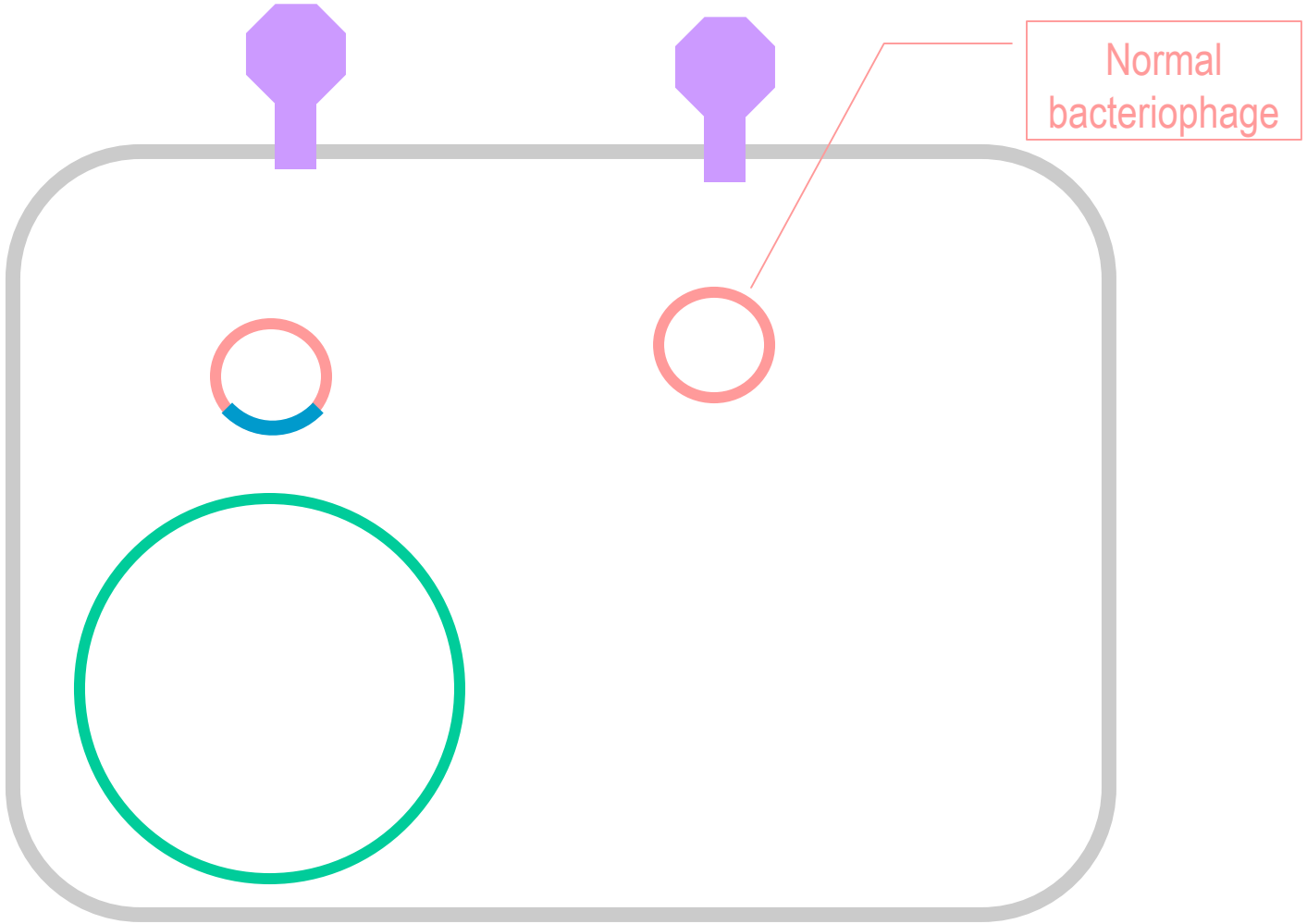


Fate 1: Recombination

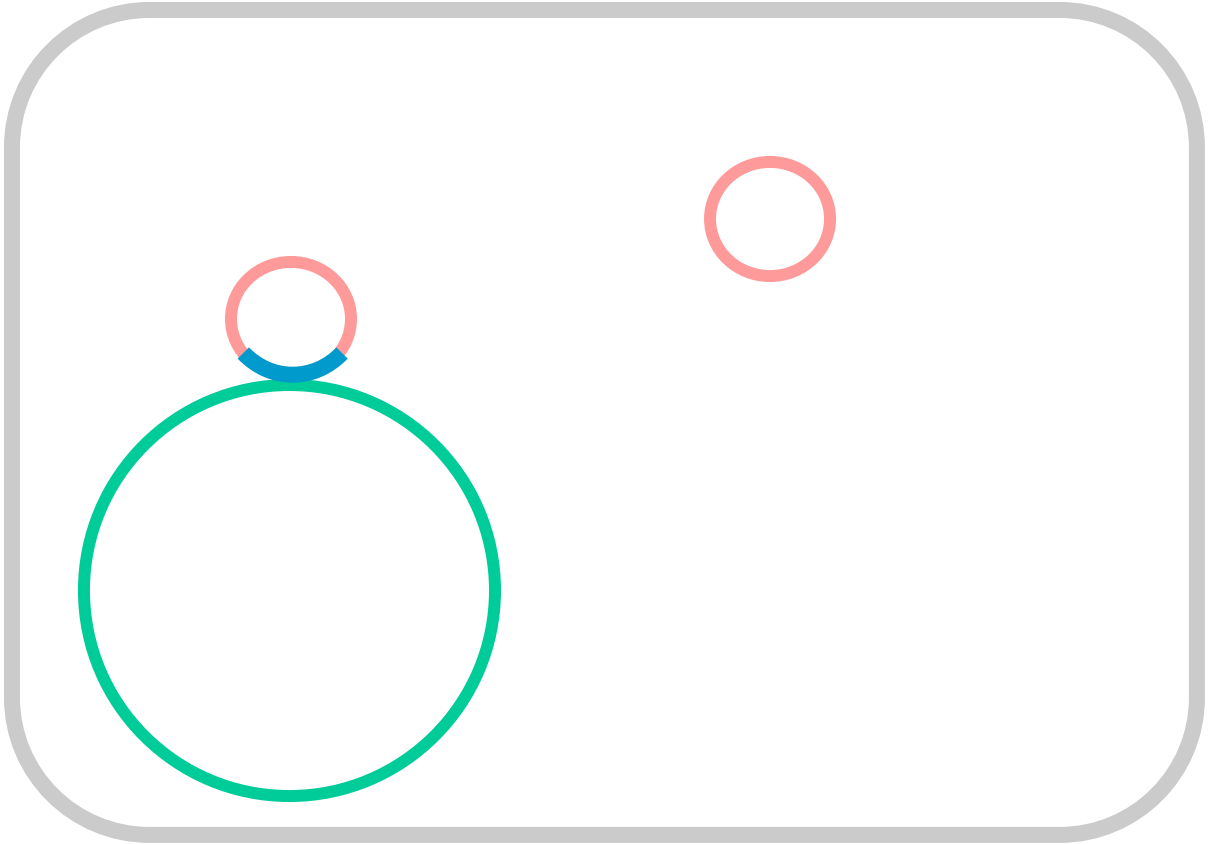
- ✓ Bacterial chromosome contains donor DNA



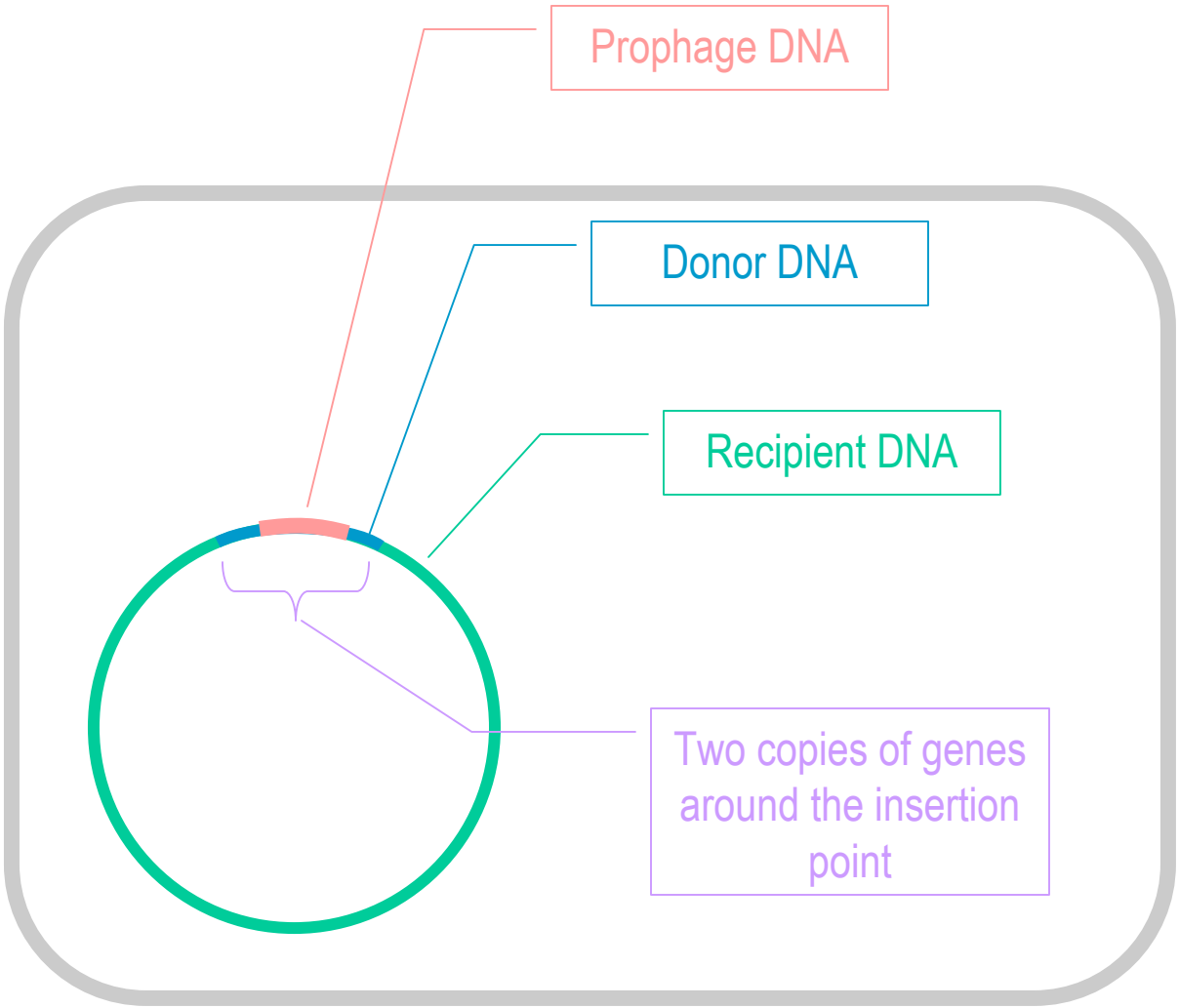
Fate 2: Integration



Fate 2: Integration



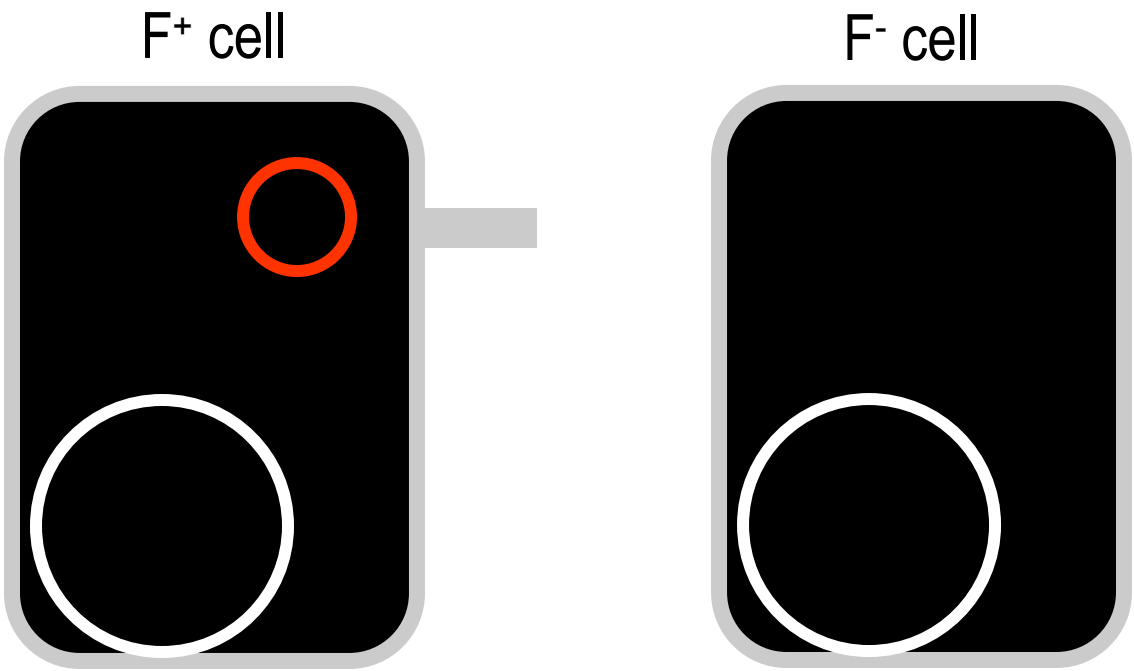
Fate 2: Integration



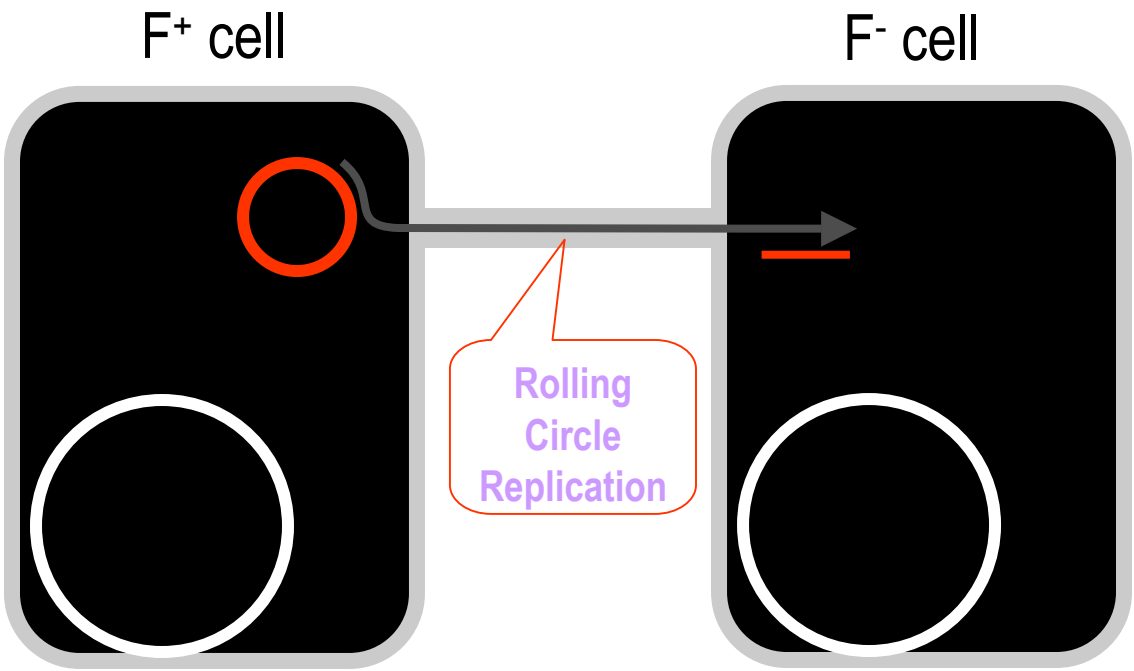
Conjugation

- Sex in bacteria
- Direct transfer of DNA from one cell to another
  - across a protein bridge
    - pilus
  - rolling-circle replication
- Requires an F-plasmid
  - transfer of plasmid DNA from F^+ to F^- cell
  - when an episome…
    - can get Hfr
    - transfer of chromosomal DNA

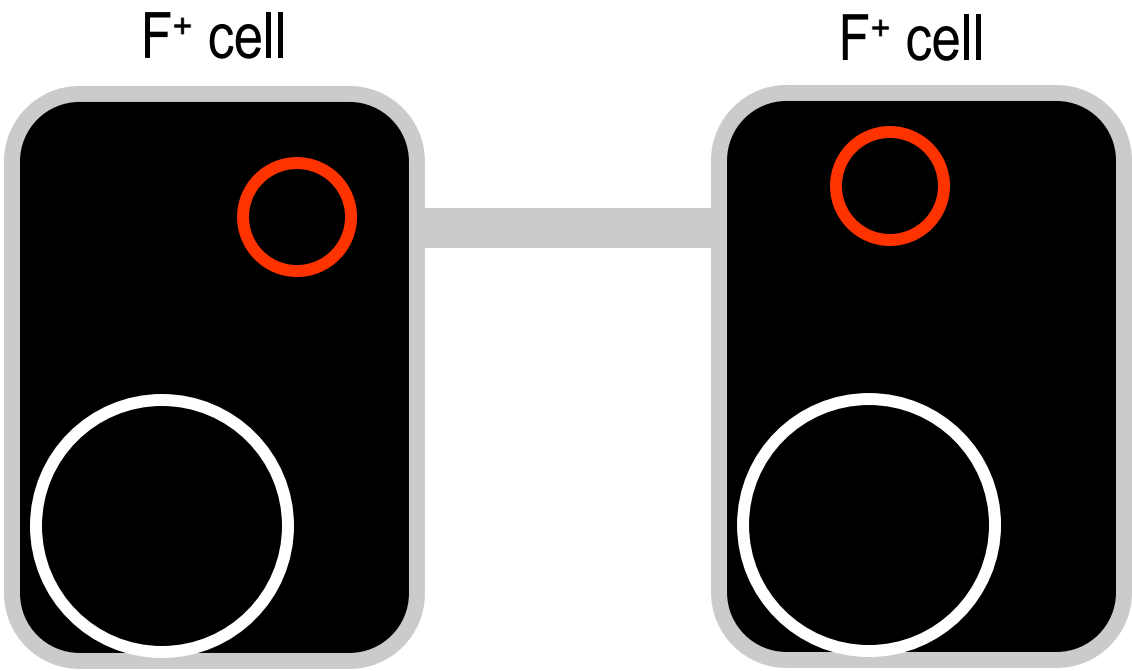
Bacterial Mating



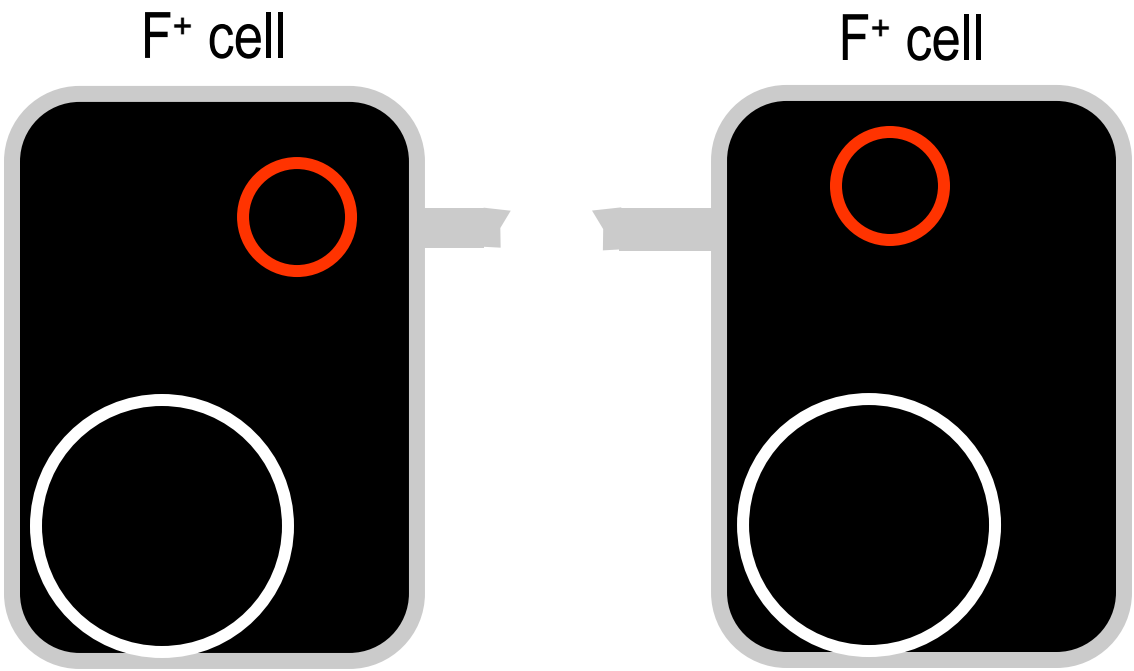
Pilus Forms and Replication



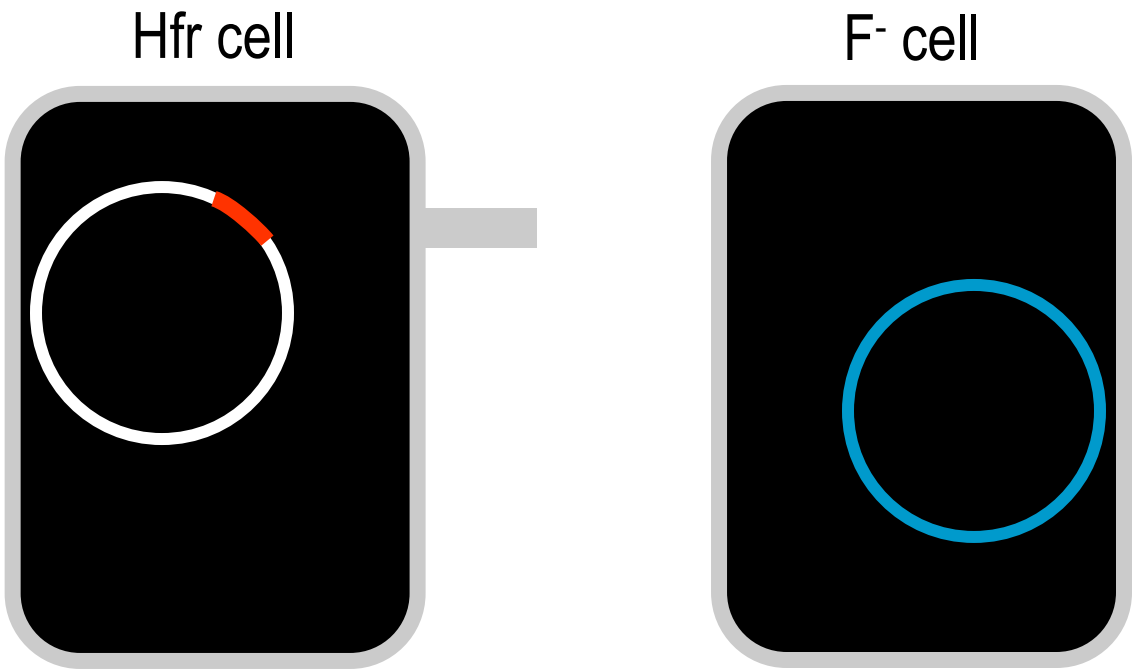
Plasmid Transferred



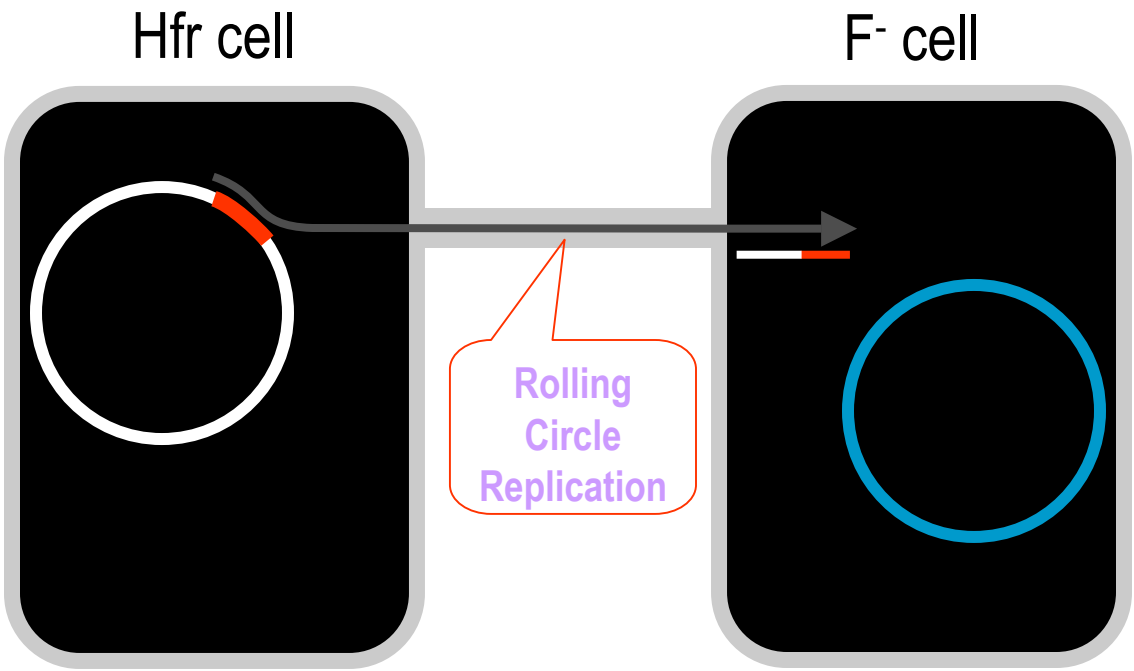
Disrupt Mating



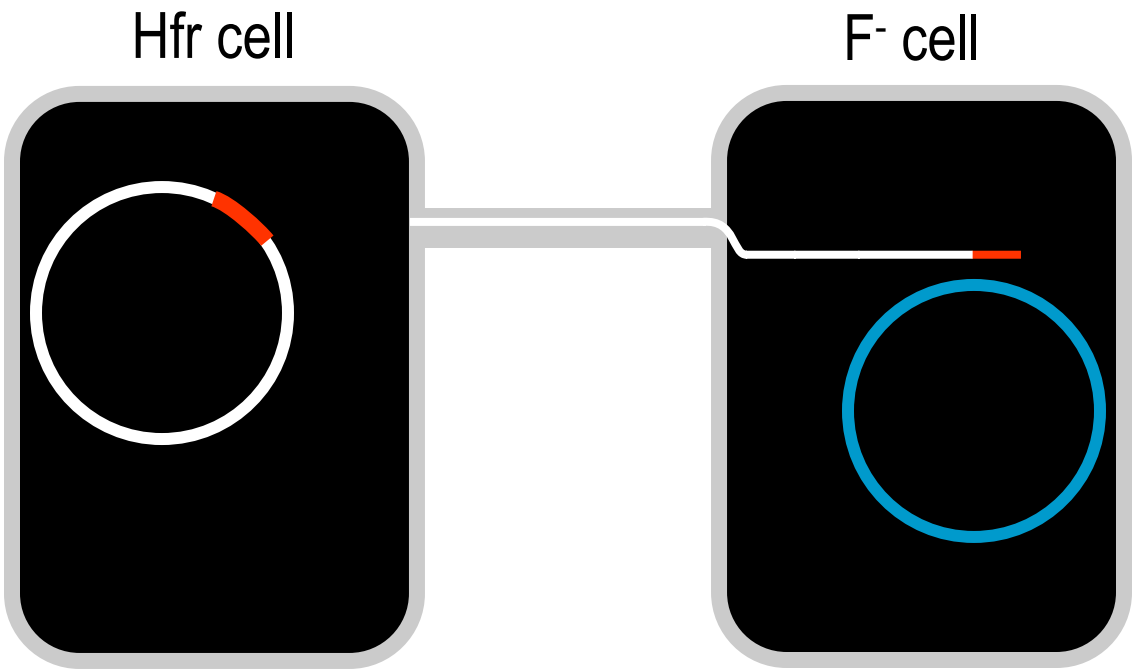
Higher Frequency Recombinants



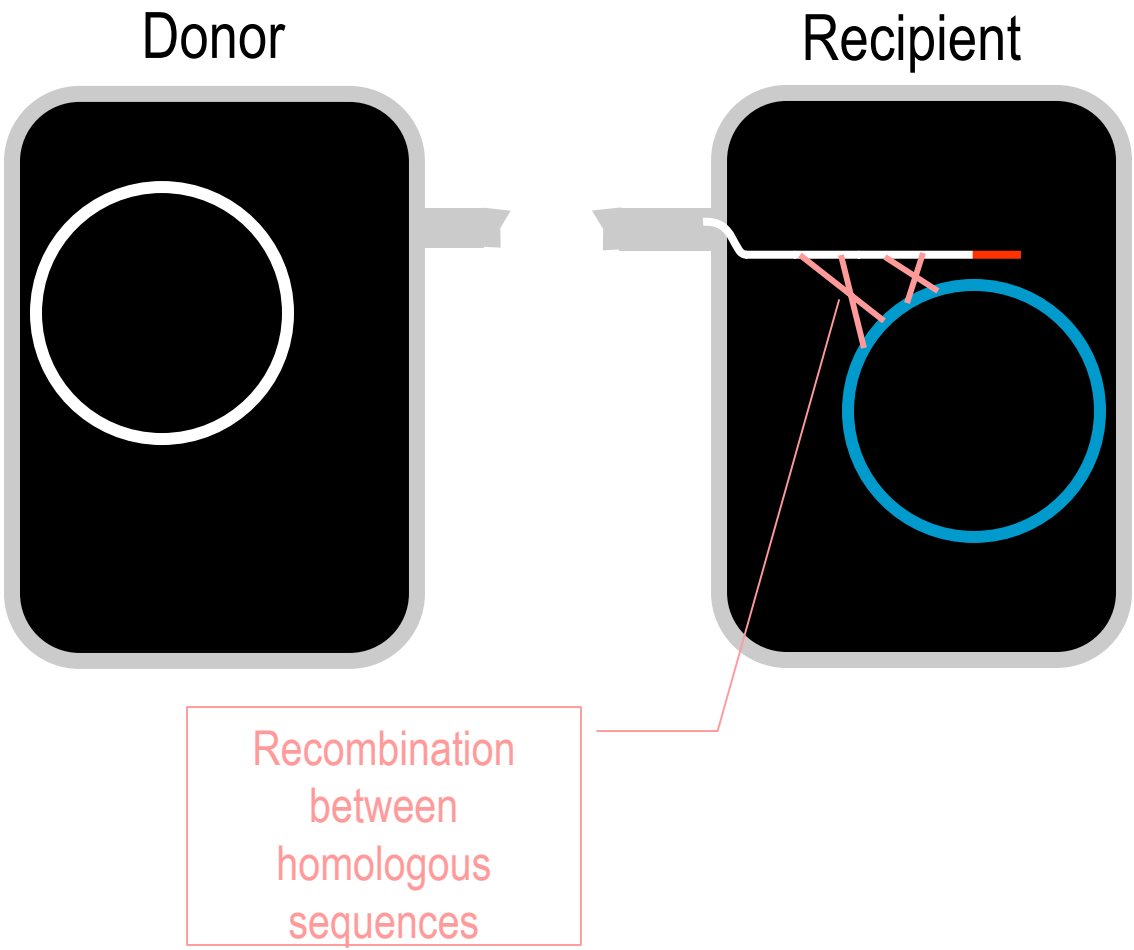
Rolling-Circle Replication



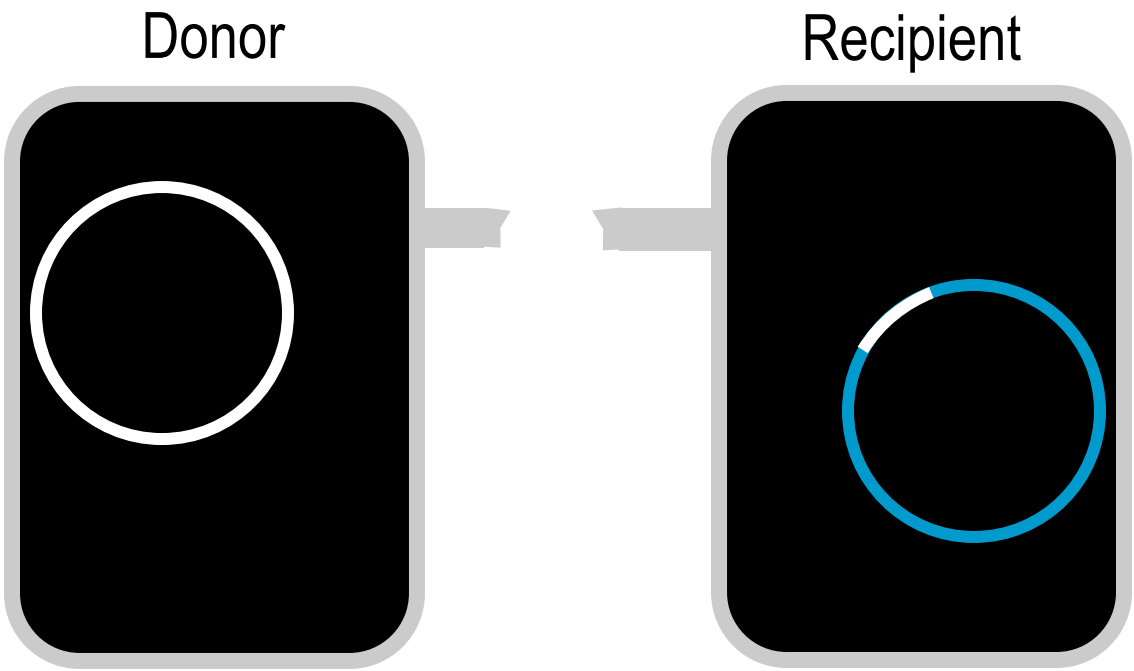
Transfer Continues…



Disrupt Mating



Successful Recombinant

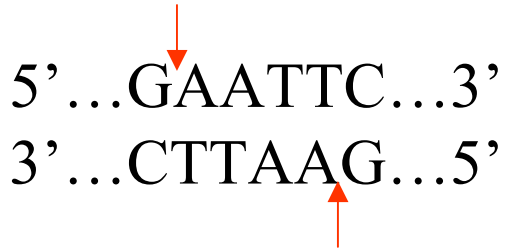


Distinguishing between Mechanisms of DNA Transfer

Transfer Process	Cell-Cell contact required?	Sensitive to Dnase?
Transformation	No	Yes
Transduction	No	No
Conjugation	Yes	No

Topic 5a: Protection Against Foreign DNA

- Repair mechanisms protect against mutation
- Mechanism exists to protect cells against foreign DNA
- Restriction endonucleases
  - cleave DNA at specific sequences
    - eg. *Eco*RI from *Escherichia coli* RY13 cuts at the sequence GAATTC



Restriction Enzymes and the Bacterial Immune System

- Any DNA sequence that matches the recognition sequence of the enzyme will be cleaved
  - eg. *Eco*R I cuts λ DNA 5 times
- What protects the cell from it's own REs?
  - Methylation
  - Specific sequences that would be recognised by the host RE are methylated
  - RE can't digest methylated DNA
  - bacterial immune system

Suggested Reading and Learning Exercises

- Atlas: Chapter 7, Sections 7-2 to 7-4
- Learning Exercises
  - Rolling-circle replication
  - Bacteriophage Mu
  - Module 3 question sheet

Next Week…

Module 4

Physiological Adaptation