PM401
Basic Microbiology

Basic Microbial Genetics

(2015)

Basic Genetics

DNA Transfer & Genetic Variation

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5 May 2015

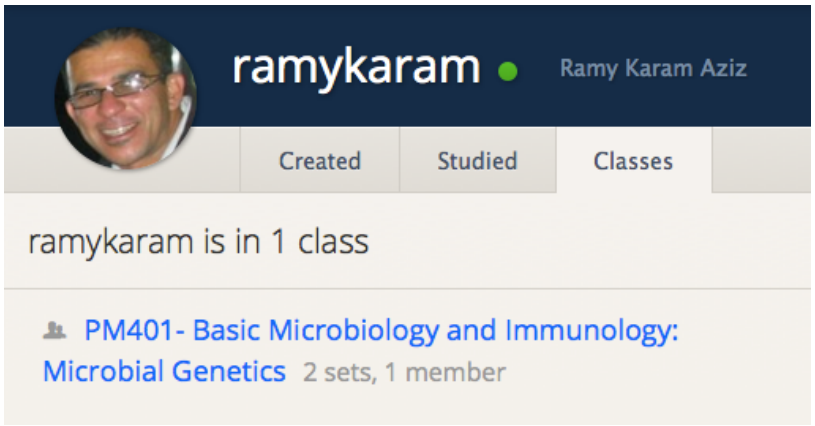
With slides from:
Dr. Aymen Yassin and Dr. Marwa Elrakaiby

“Variety is the spice of life”

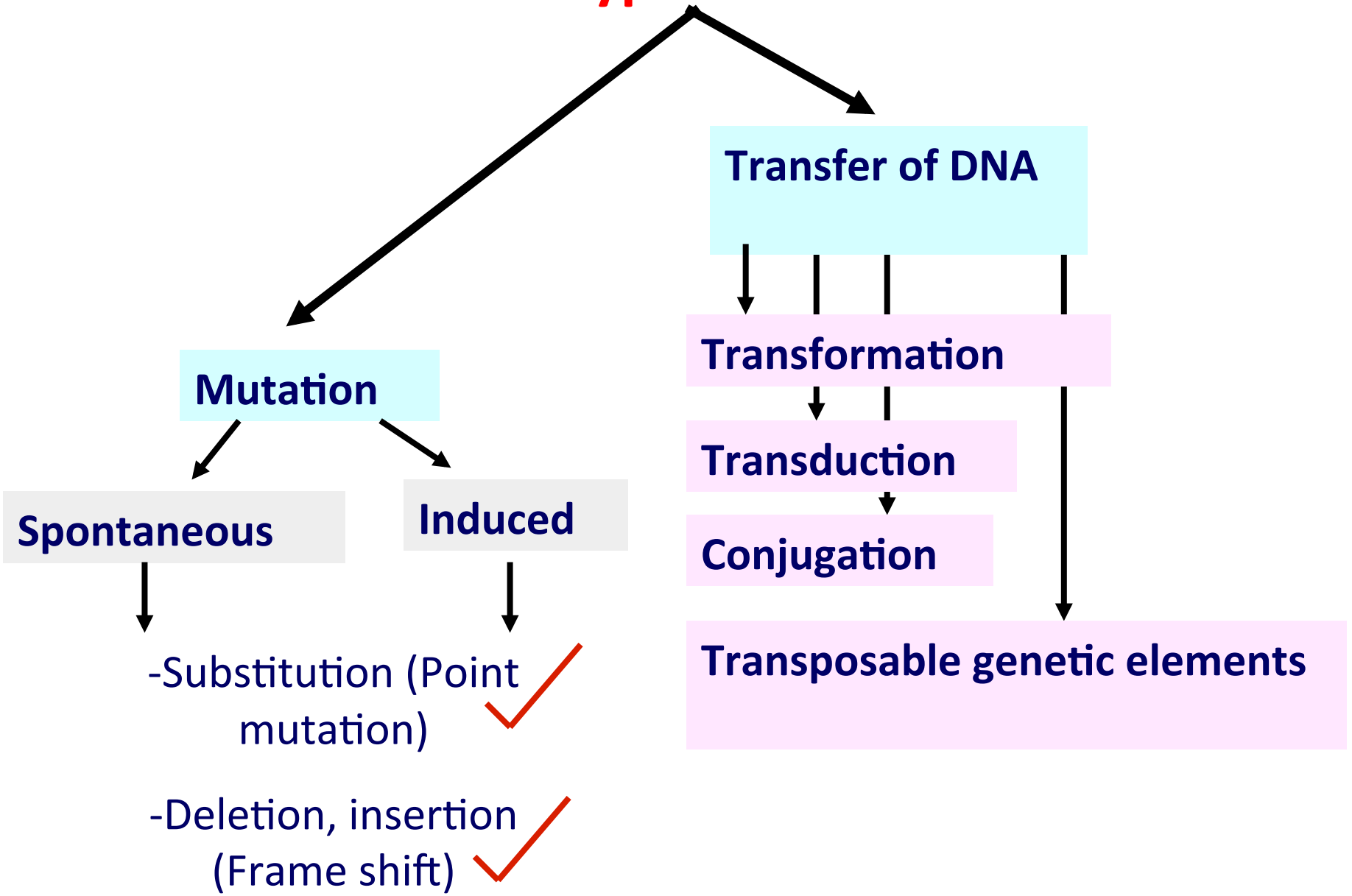
No variation = No life as we know it!

Announcements

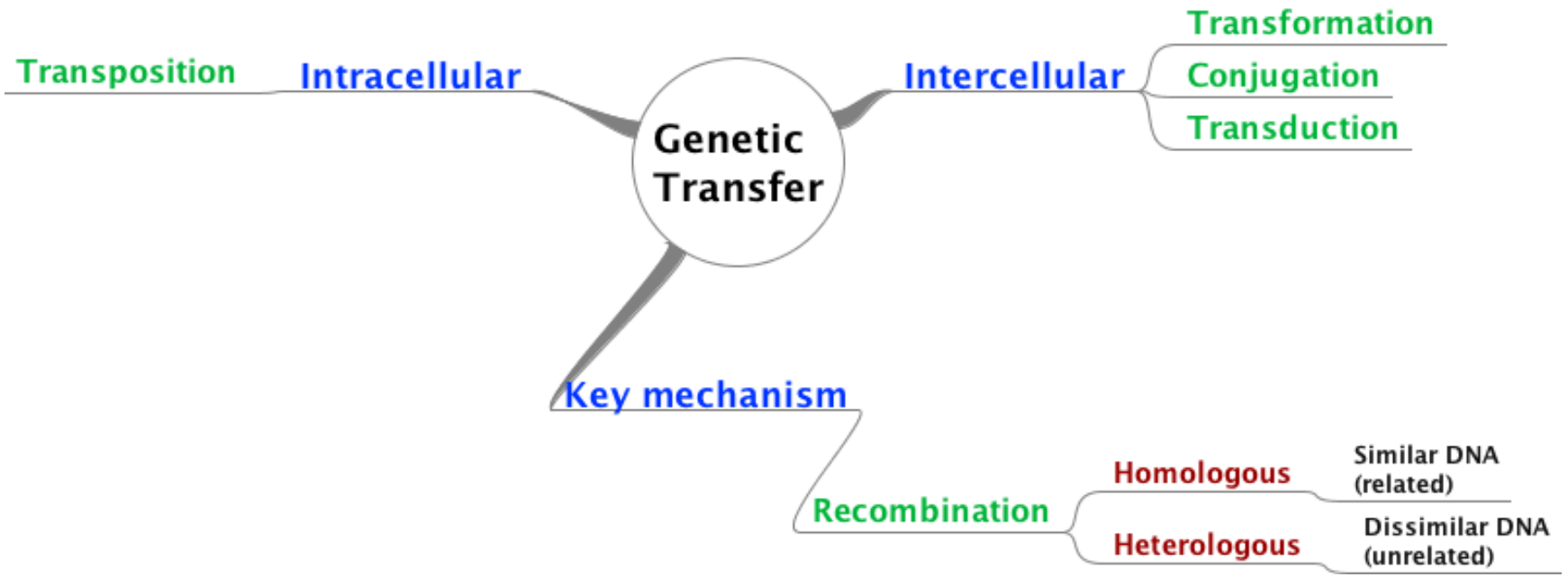
- Remember: Website
  - http://egybio.net/courses/FOPCU/pm401
    (questions/ videos/ assignments/ future: exam info)
- Study tool: Quizlet
  (web & cell phone App)
  - https://quizlet.com/ramykaram



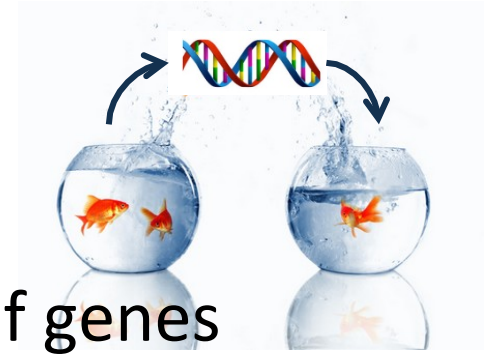
- Assignment:
  - Until midnight: only ONE student: Reem Yasser
    (PCR animation)
- Next week:
  No lecture, but I CAN be here for an hour of questions if you want.


Genotypic variation
Transfer of DNA
Mutation
Transformation
Transduction
Conjugation
Spontaneous
Induced
Transposable genetic elements
-Substitution (Point mutation)
-Deletion, insertion (Frame shift)

Intercellular genetic transfer

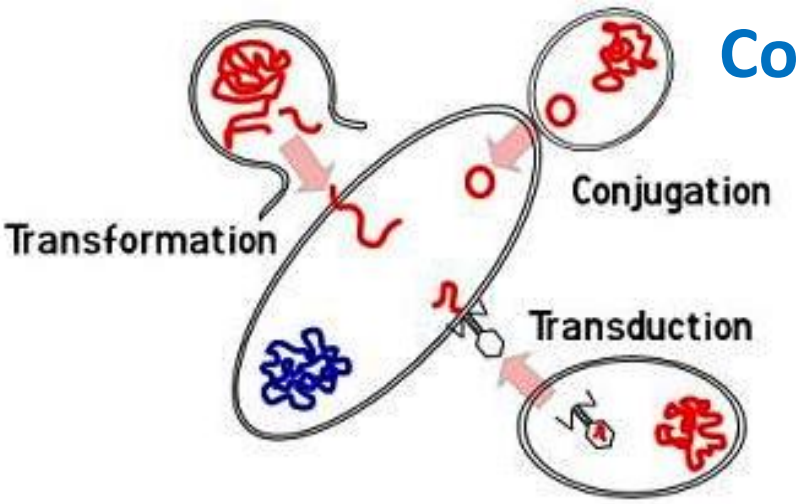


Genetic transfer & recombination



- Genetic Recombination is the **exchange** of genes between two DNA molecules to form **new combinations or variants** of genes

- In prokaryotes, recombination occurs via **gene transfer** between cells or within cell by



Conjugation

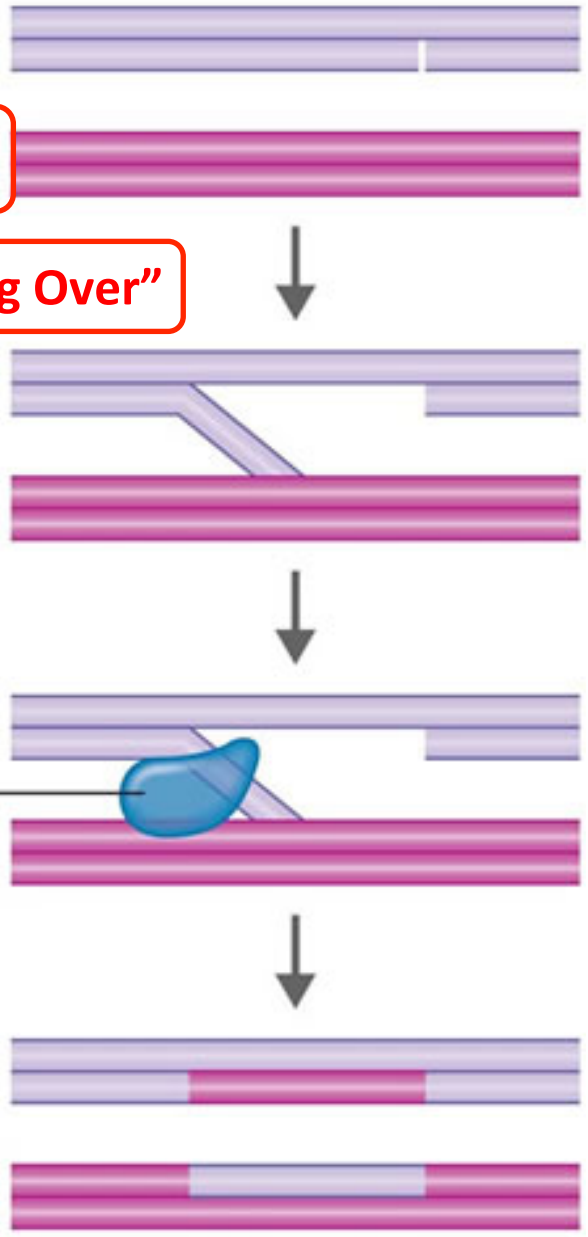
Transposition

Transformation

Transduction

Genetic recombination

1. DNA from one cell aligns with DNA in the recipient cell. Notice that there is a nick in the donor DNA.
2. DNA from the donor aligns with complementary base pairs in the recipient's chromosome. This can involve thousands of base pairs.
3. RecA protein catalyzes the joining of the two strands.
4. The result is that the recipient's chromosome contains new DNA. Complementary base pairs between the two strands will be resolved by DNA polymerase and ligase. The donor DNA will be destroyed. The recipient may now have one or more new genes.



DO NOT MEMORIZE

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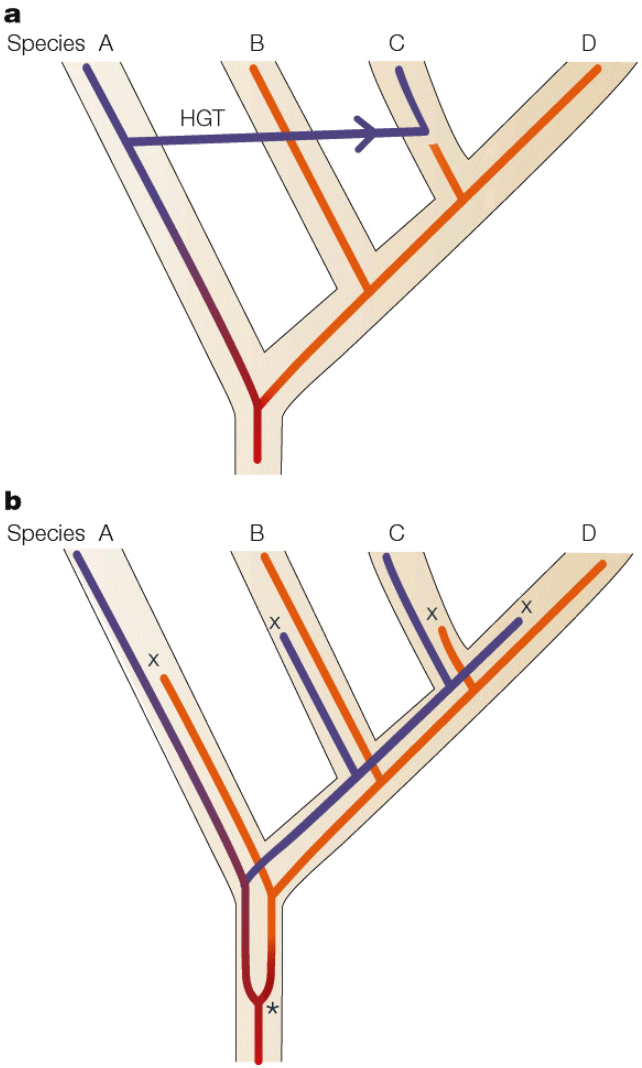
Vertical vs. horizontal gene transfer

HGT: Transmission of genes to **neighboring** cells of the **same** generation

VGT : Transmission of genes from the **parental** generation to **offspring**



HOW DO CELLS PROTECT THEIR DNA (IDENTITY)?

Ali Baba has the answer!

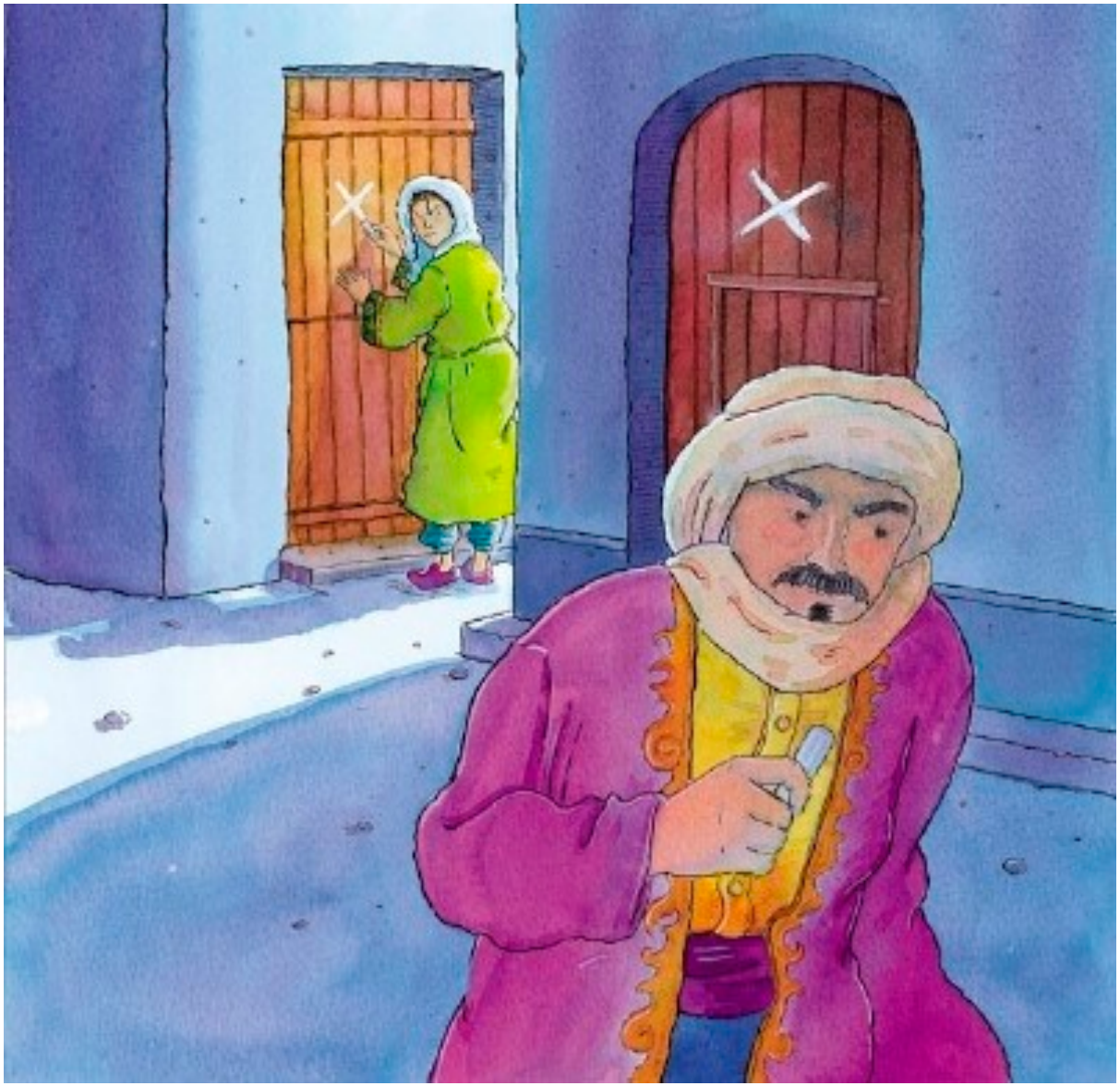


Image source:
http://www.howstuffworks.com/ali-baba-story.htm

Restriction/modification systems

Two enzymes, closely related in their specificity, **modify** and **protect** DNA of a given bacterial species against foreign DNA (which they will **degrade**):

A) **Methyl transferase**: adds methyl groups to Adenine and Cytosine residues in the same target sequence that constitutes the restriction enzyme binding site. (Methylation renders the target site **resistant** to restriction)

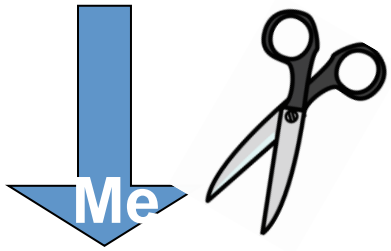
B) **Restriction Endonuclease**: degrade non-methylated DNA at specific positions. The specific position is called **palindrome** which consist of 6 - 8 bases pairs having the same sequence on both strands.

Palindrome?

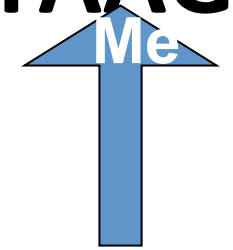
- Words:
  - radar, level, madam
- Phrases:
  - Nurses run
    - n u r s E s r u n
  - Madam In Eden, I’m Adam
    - m a d a m i n e d e n i m a d a m
  - Was it a rat I saw
    - w a s i t a r a t i s a w

Restriction/modification systems

Modifying enzyme

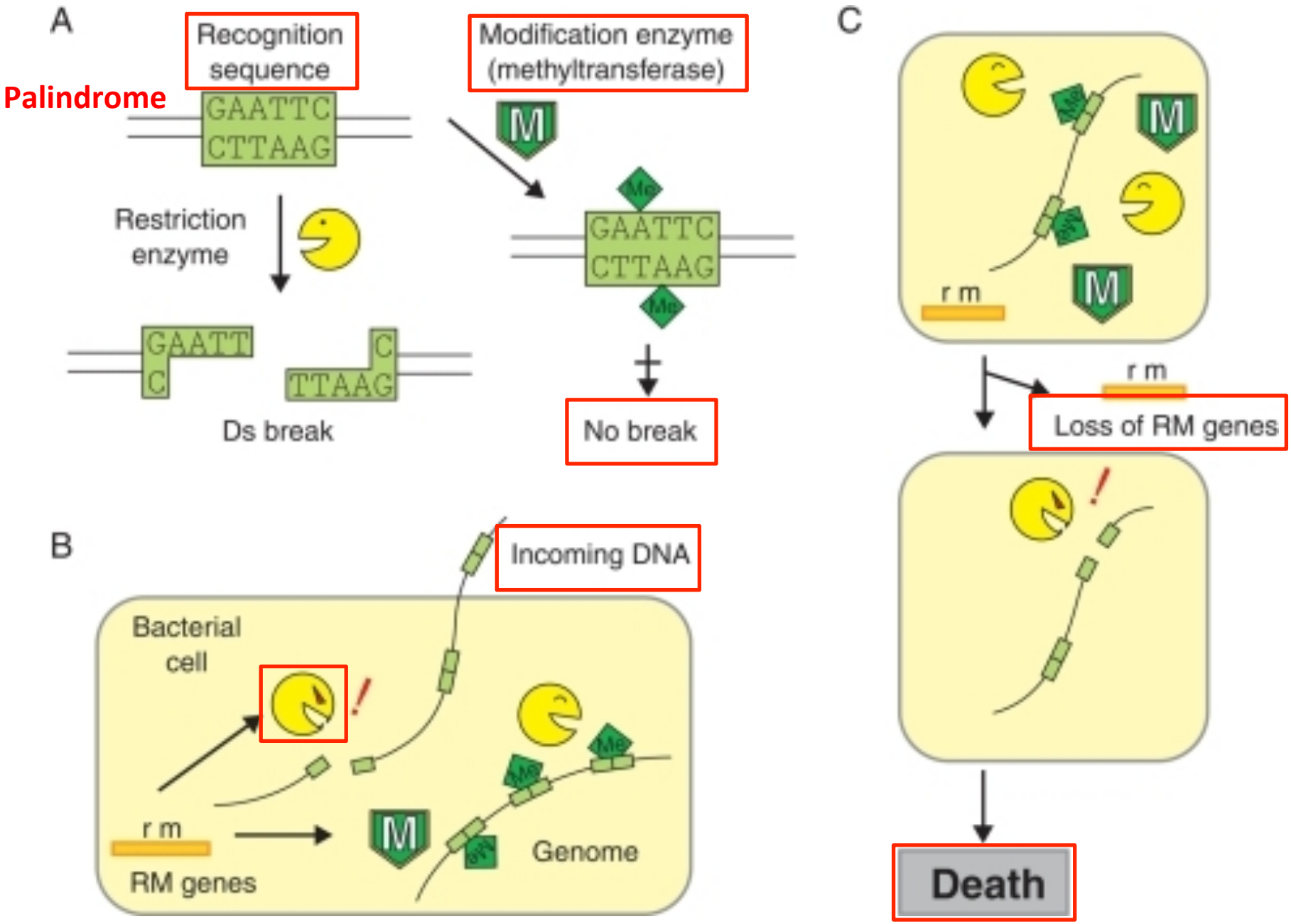


- GAATTC
  CTTAAG



Restriction enzyme

- GAATTC
  CTTAAG


A
Recognition sequence
Palindrome
GAATTC
CTTAAG
Modification enzyme (methyltransferase)
M
Restriction enzyme
GAATT
C
C
TTAAG
Ds break
Me
GAATTC
CTTAAG
Me
No break
B
Incoming DNA
Bacterial cell
!
r m
RM genes
M
Me
Me
Genome
C
M
r m
M
r m
Loss of RM genes
!
Death

a. Transformation

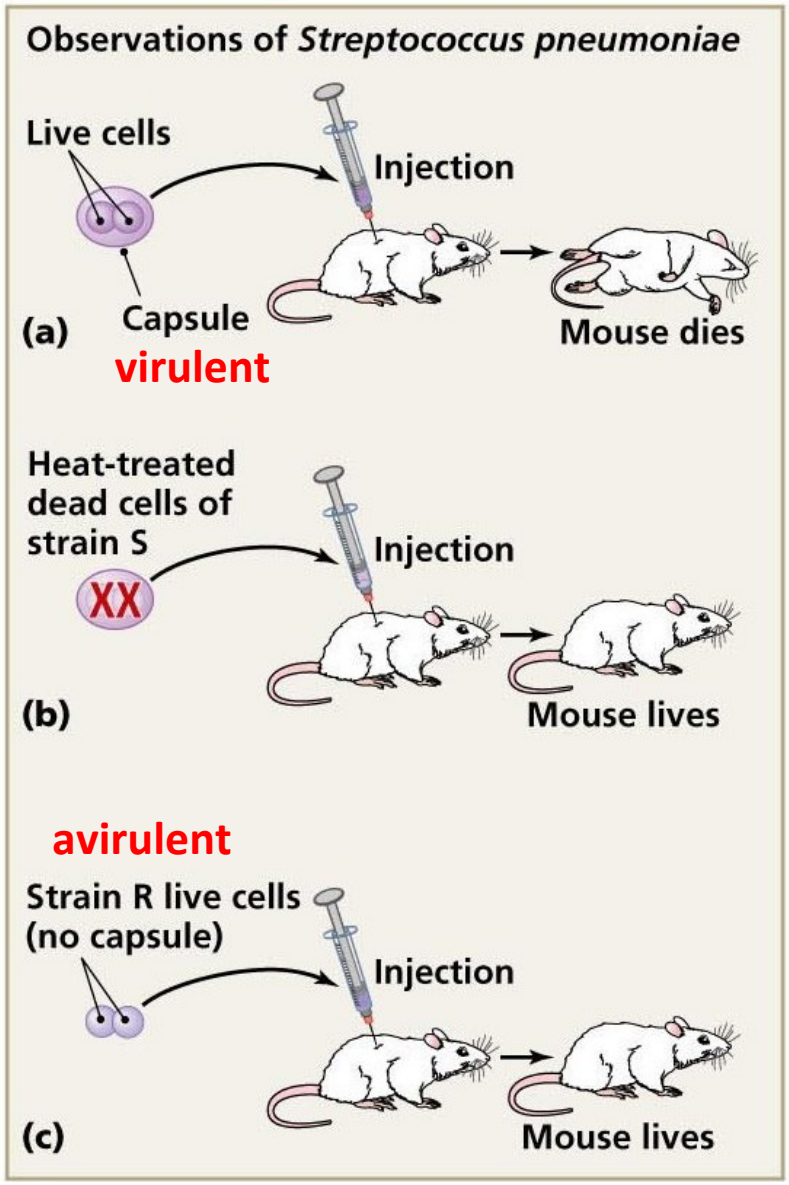
- Gene transfer by uptake of **naked/soluble DNA** fragments from the surrounding environment and the expression of the encoded genetic information in the recipient cell.

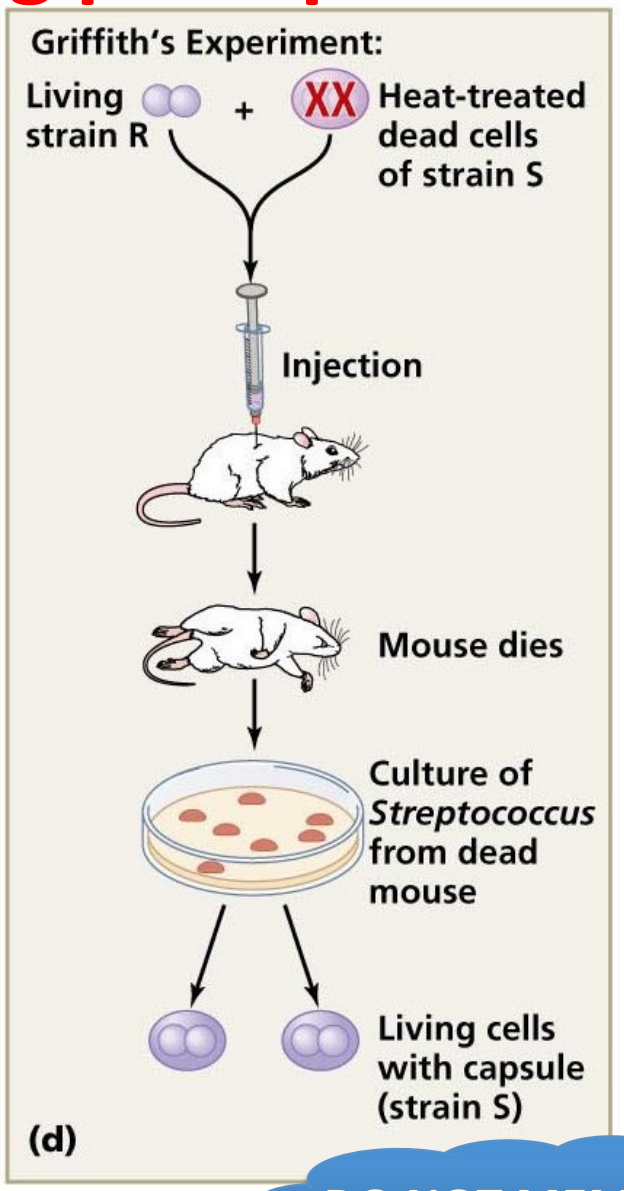
- It works best with DNA from **closely related** species

- **Competent** cells are those able to pick naked DNA and incorporate it into its genome by recombination

The transforming principle:

Griffith Experiment (1928)





DO NOT MEMORIZE

Transformation Mechanism

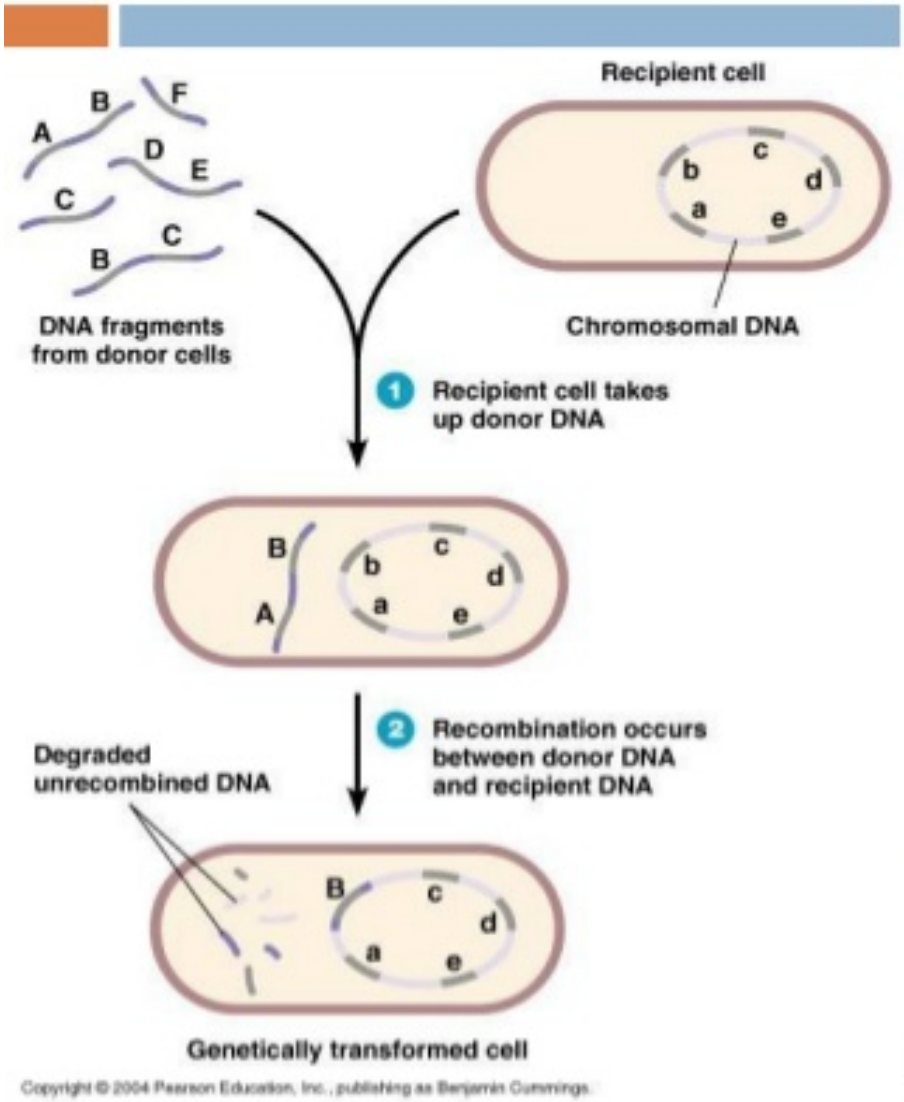
Gram positive

- In general, Gram-positive cells **degrade one strand** of the DNA as it is being transferred, **the other strand enters** and can associate with a similar region in the chromosome and replace a similar sequence.

Gram negative

- In Gram-negative bacteria, **both strands** enter the cell then one strand is digested before incorporation into the bacterial chromosome.

Transformation



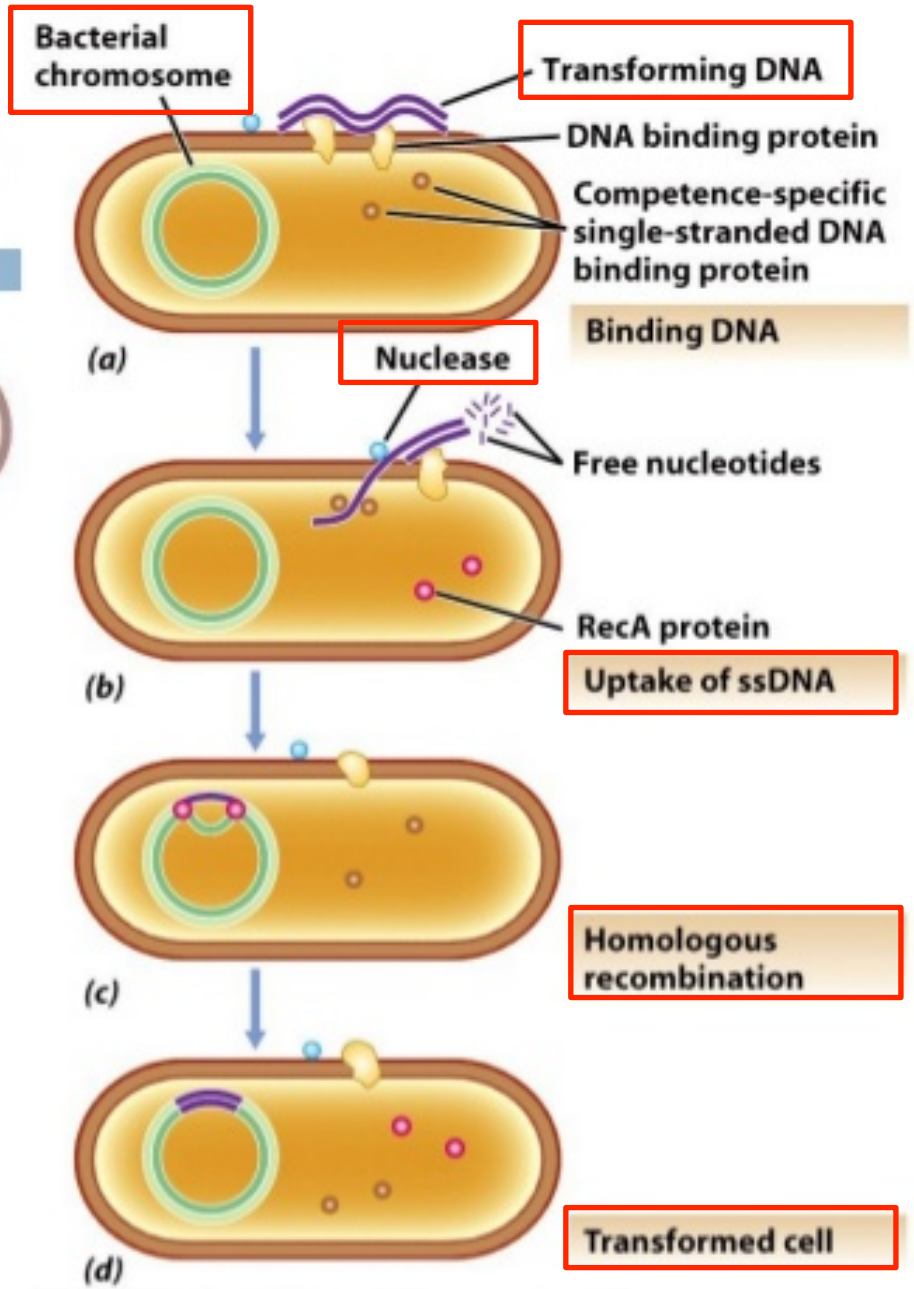


Figure 10-14 Brock Biology of Microorganisms 11/e

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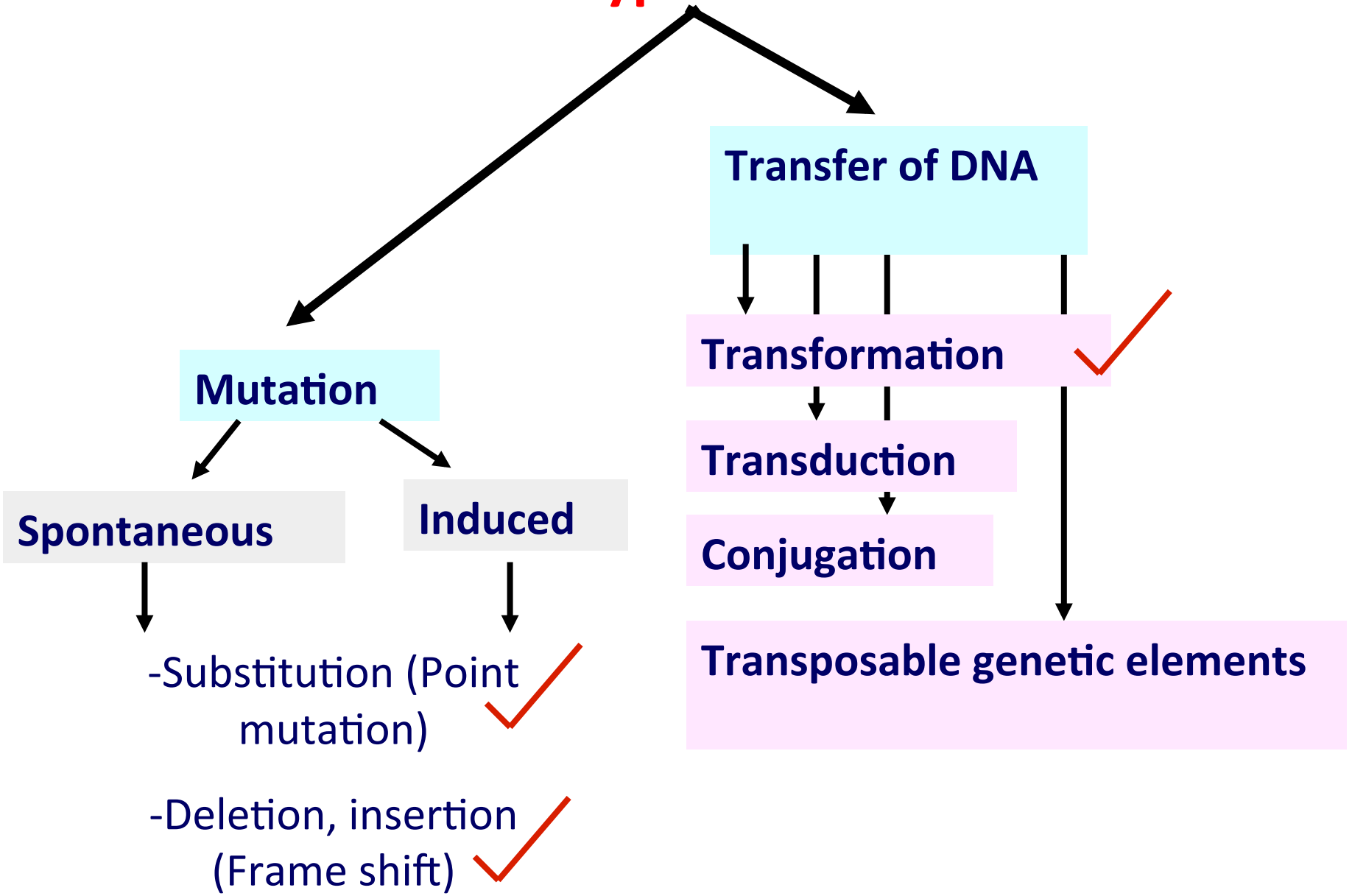
How to make cells competent?

- *E.coli* does not develop competence in normal growth; however, competence can be induced in the lab:
  - **Chemically:** by chilling the cells at 4°C after treating with $CaCl_2$ then heating the cells at 42°C for ~ one minute.
  - **Electrically:** by growing cells to mid-log phase, chilling them at 4˚C, then treating them with an electric pulse (high voltage for very short time), a process known as **electroporation**.
- This treatment apparently alters the membrane and allow the passage of DNA.
- In lab and in Industry, competent cells play an important role in genetic engineering and cloning.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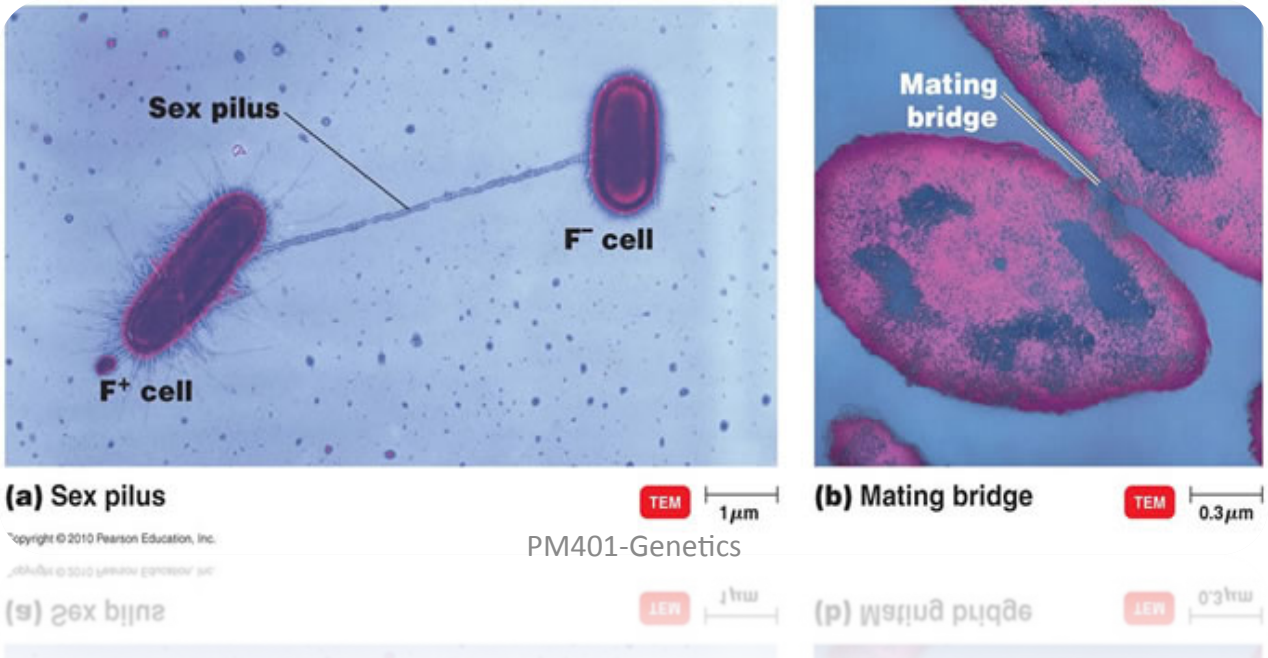
-Deletion, insertion (Frame shift)
Transposable genetic elements

b. Conjugation

- Gene transfer by direct contact between cells via **sex pilus (G-)** or **mating bridge (G+)**
- Transfer mediated by a **plasmid** which has genes for its own transfer
- Conjugation requires cell to cell contact between two cells of **opposite mating type** (donor: F^+ and recipient: F^-)



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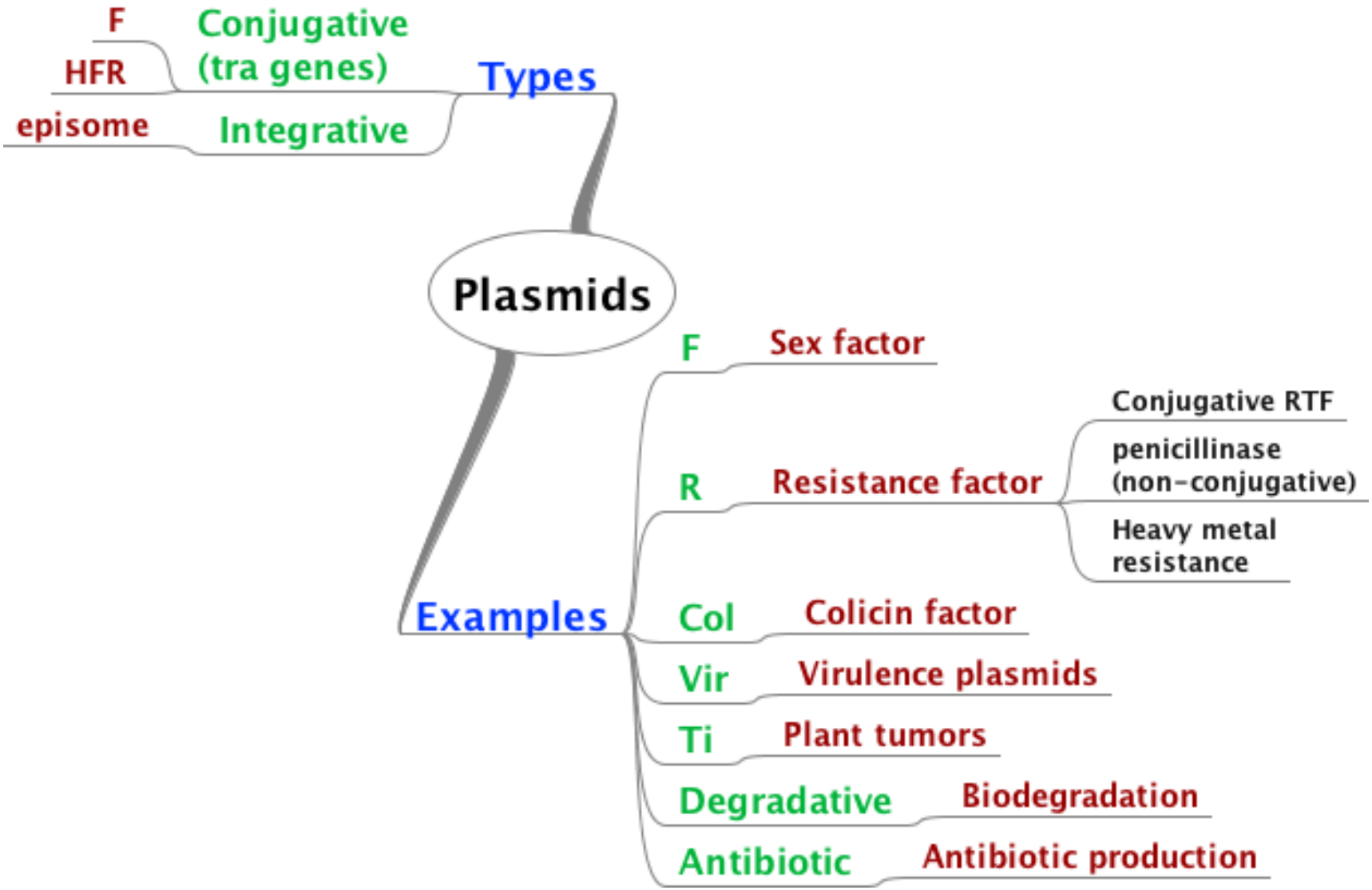
Plasmids

Plasmids are small circular double stranded DNA extrachromosomal genetic element. They contain few genes controlling non essential functions. They are capable of autonomous replication (**Self replication**)

Copy number: Ratio of plasmid copies to the chromosome copies in a cell. It is higher for small non conjugative plasmids and low approaching one for large conjugative plasmids.

Plasmid curing: Acridine dye and UV inhibit plasmid replication and at certain doses could get rid of it from the cell

Plasmids

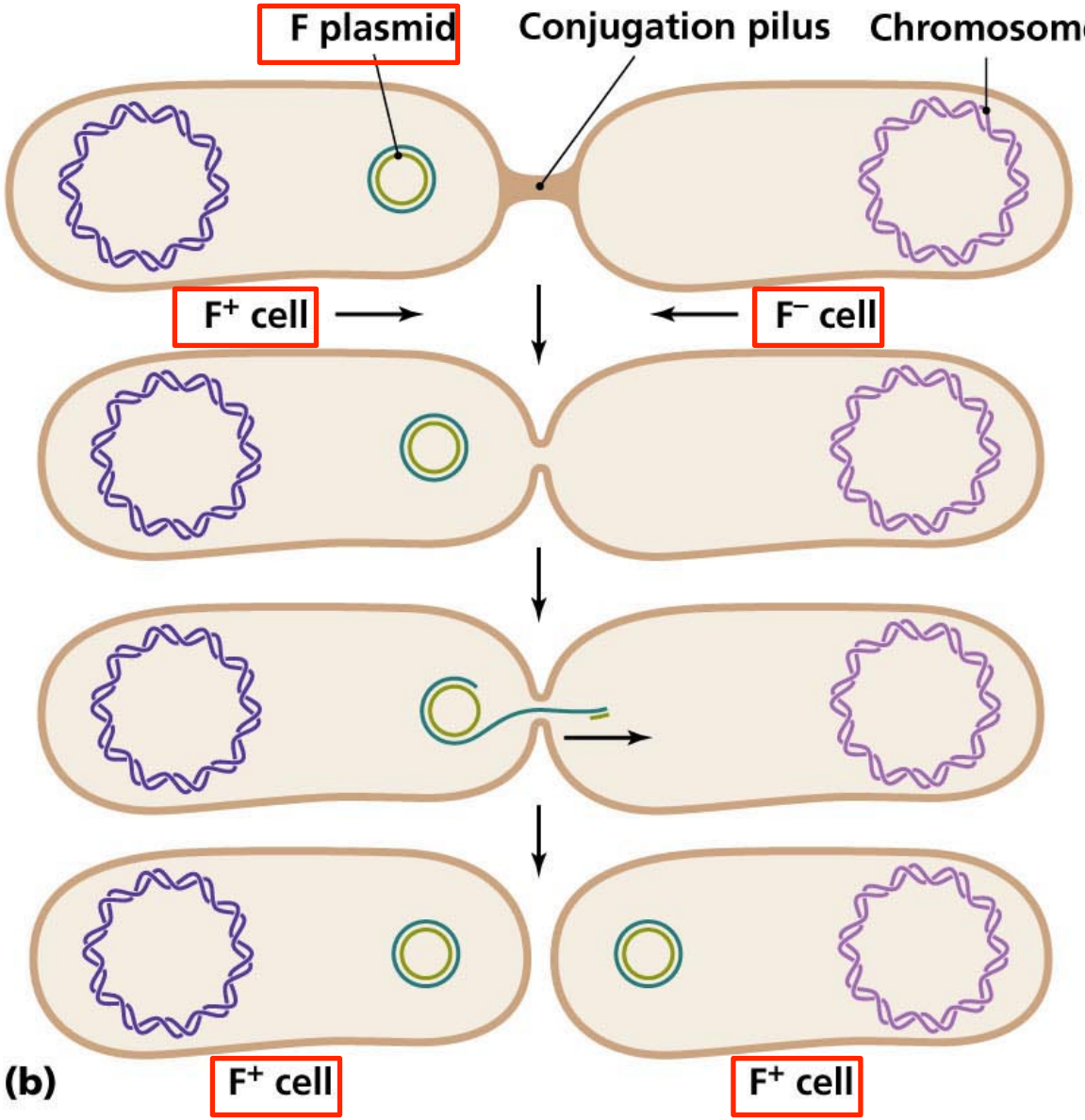

Plasmids
Types
Conjugative (tra genes)
F
HFR
Integrative
episome
Examples
F
Sex factor
R
Resistance factor
Conjugative RTF
penicillinase (non-conjugative)
Heavy metal resistance
Col
Colicin factor
Vir
Virulence plasmids
Ti
Plant tumors
Degradative
Biodegradation
Antibiotic
Antibiotic production

Examples of plasmids

1- Sex Factor: (**F plasmid**) Fertility plasmid mediating transfer of gene from one cell to the other through sex pilus (**conjugative plasmid**).

2- Resistance Factor: (**R plasmid**) plasmid mediating resistance to antimicrobial agents. In G-, R plasmids are conjugative and called **resistance transfer factor** (RTF) i.e. : carry F and R genes. Many of the "R" or "RTF" plasmids could be integrated into the bacterial chromosome at different positions acting as **transposons** or **jumping genes**

Conjugation



1. Donor cell attaches to a recipient cell with its pilus. The pilus draws the cells together.
2. The cells contact one another.
3. One strand of plasmid DNA transfers to the recipient.
4. The recipient synthesizes a complementary strand to become an F^+ cell; the donor synthesizes a complementary strand, restoring its complete plasmid.

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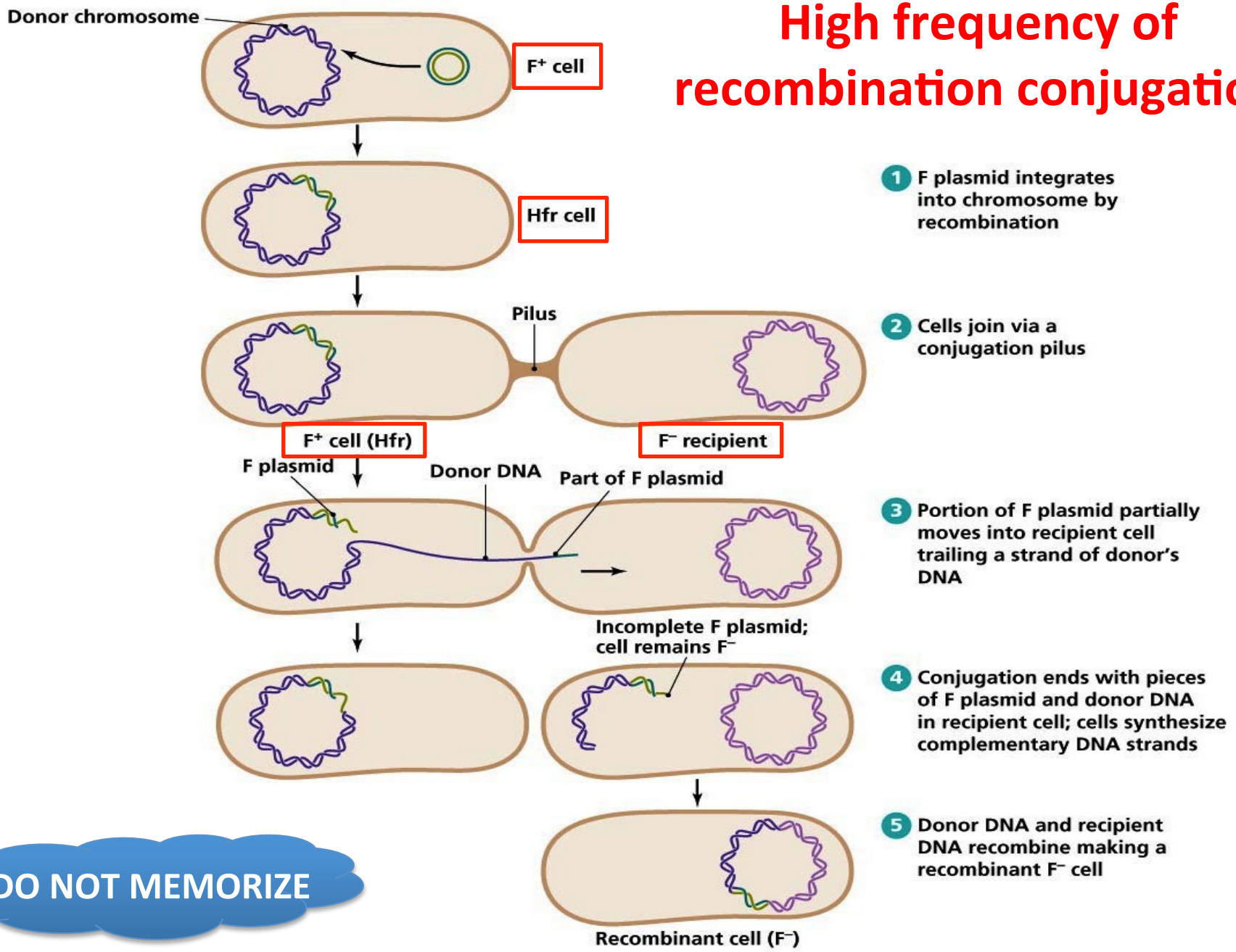
High frequency of recombination conjugation



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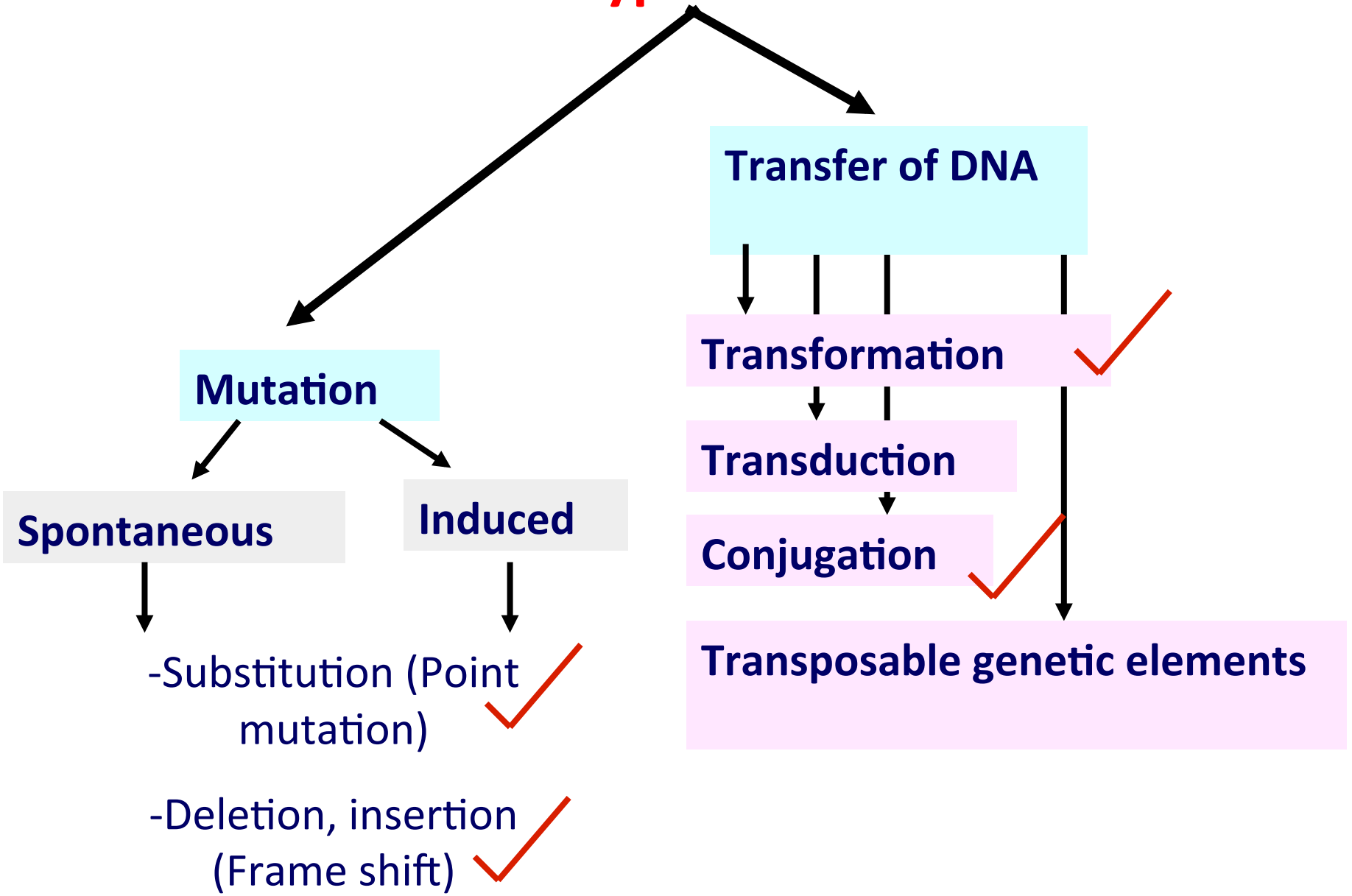
Genotypic variation
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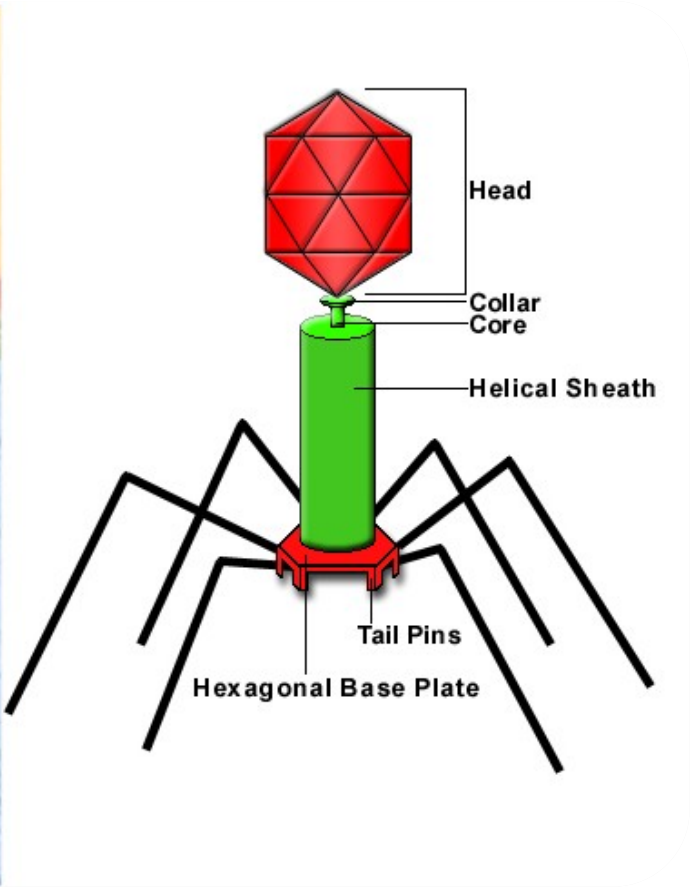
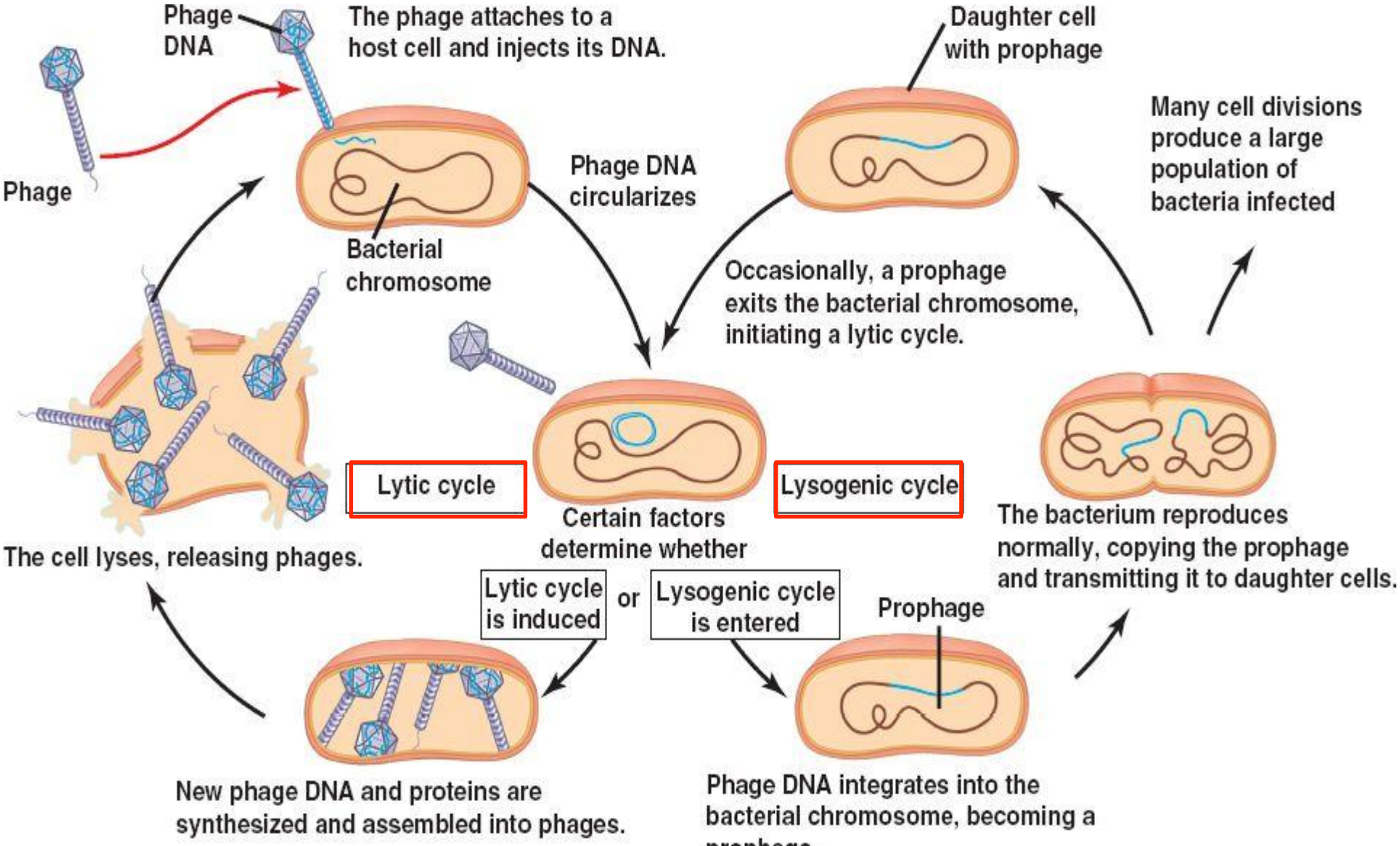
Transposable genetic elements

c. Transduction

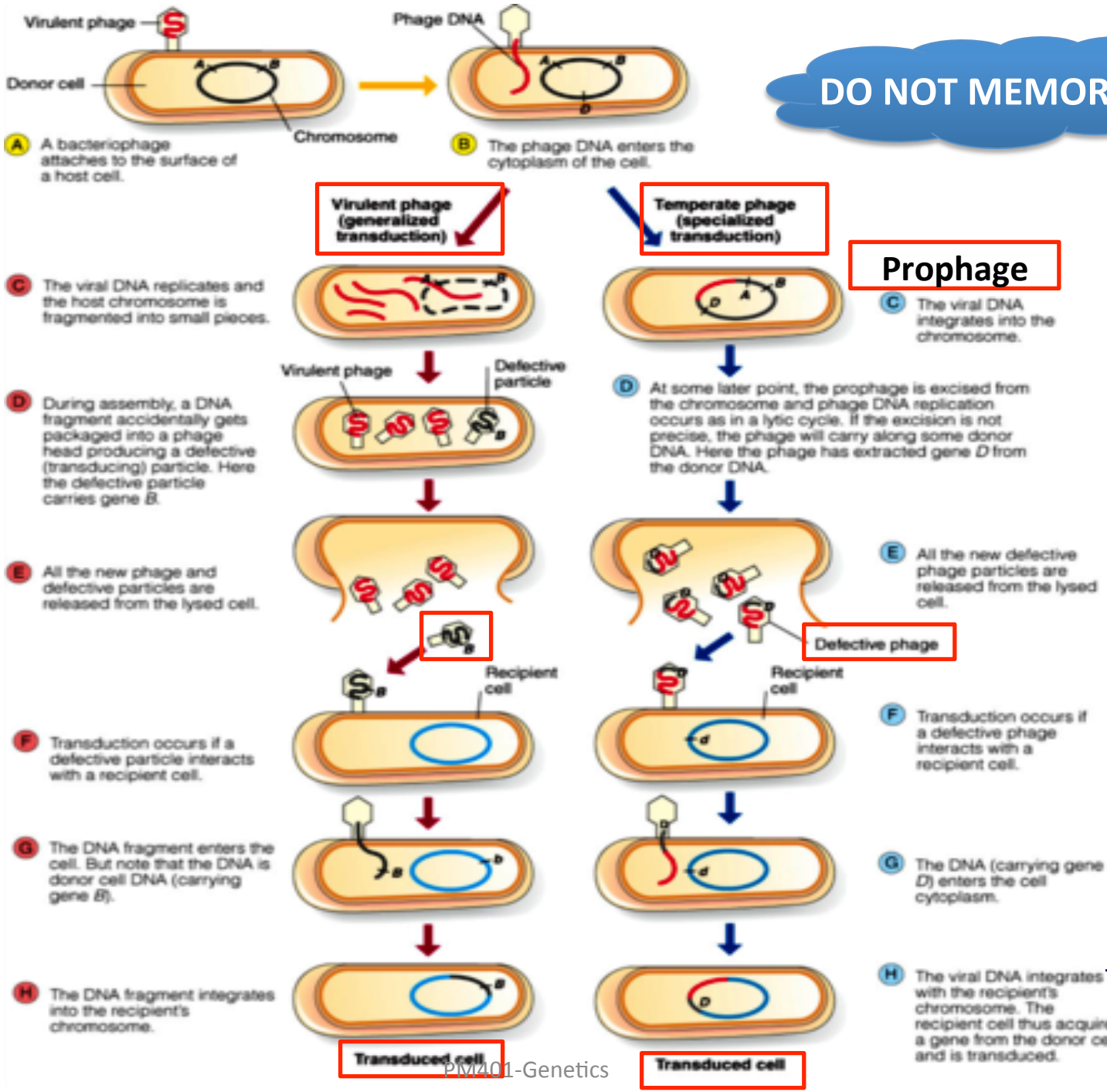
Gene transfer by bacteriophage



Lytic Cycle vs. Lysogenic Cycle



Generalized Transduction



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Specialized Transduction

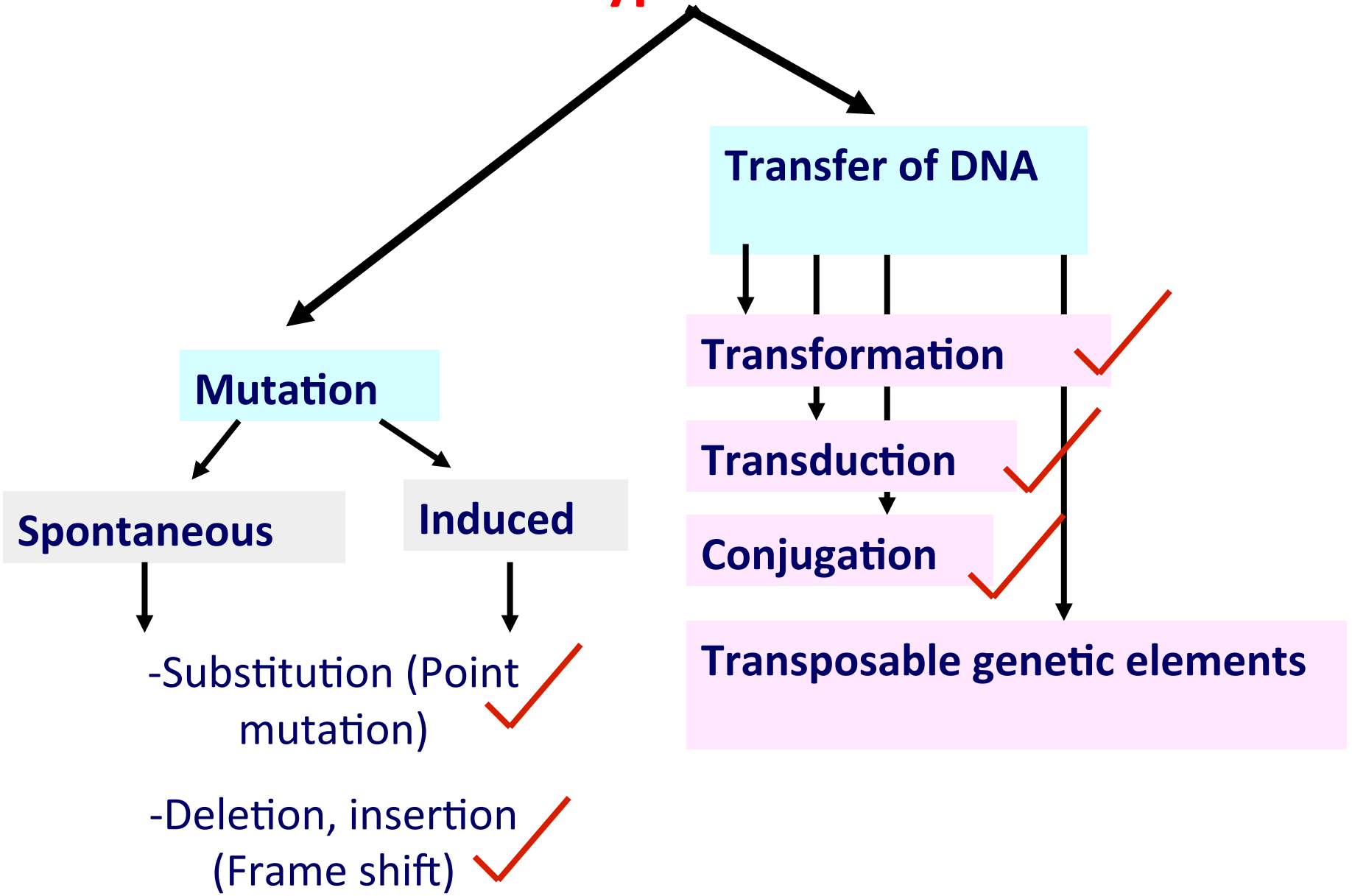
Rare:
10^{-5}-10^{-6}

Rare too:
10^{-5}-10^{-6}

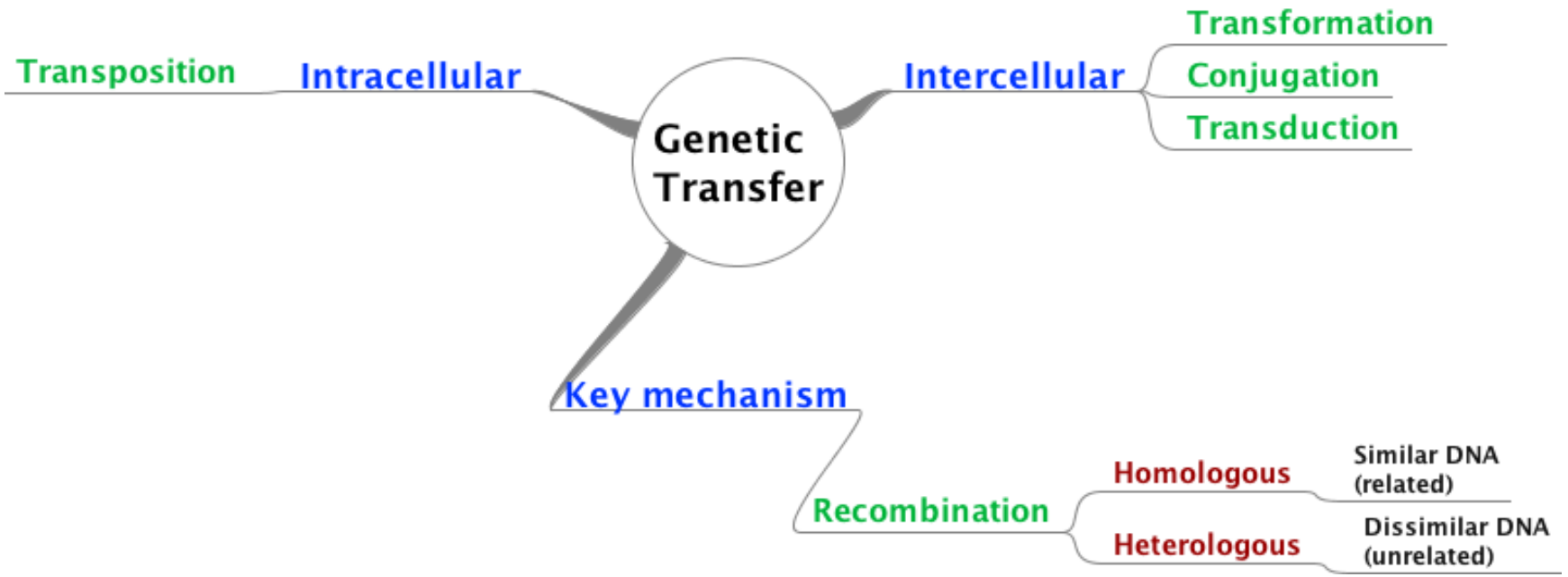
Transduction

1. Generalized
2. Restricted
3. Co-transduction:
   - Used for genetic mapping (no longer common with DNA sequencing made easy)
4. Abortive
   - A special case of generalized transduction, where the exogenote fails to be integrated for several generations.

Genotypic variation



Intracellular genetic transfer



Transposable genetic elements

Insertion sequences

- Found at one or more site at the bacterial chromosome.
- No genetic information other than the ability to insert copies of themselves into the bacterial chromosome.
- IS form copies of themselves and the copies move into other areas of the chromosome.
- They can interrupt the coding sequence of a gene resulting in the production of a wrong protein or no protein at all.

Transposons

- Are pieces of DNA that move readily from one site to another, either within or between the DNAs of bacteria, plasmids or bacteriophages.
- They are named "jumping genes".
- They can code for drug resistance, enzymes or toxins and are larger than insertion sequences.
- When they "move" or "jump" they leave their original location empty (in IS the copies move)
- They can either cause mutations in the gene in which they insert or alter the expression of the nearby genes.

Summary: Mobile genetic elements

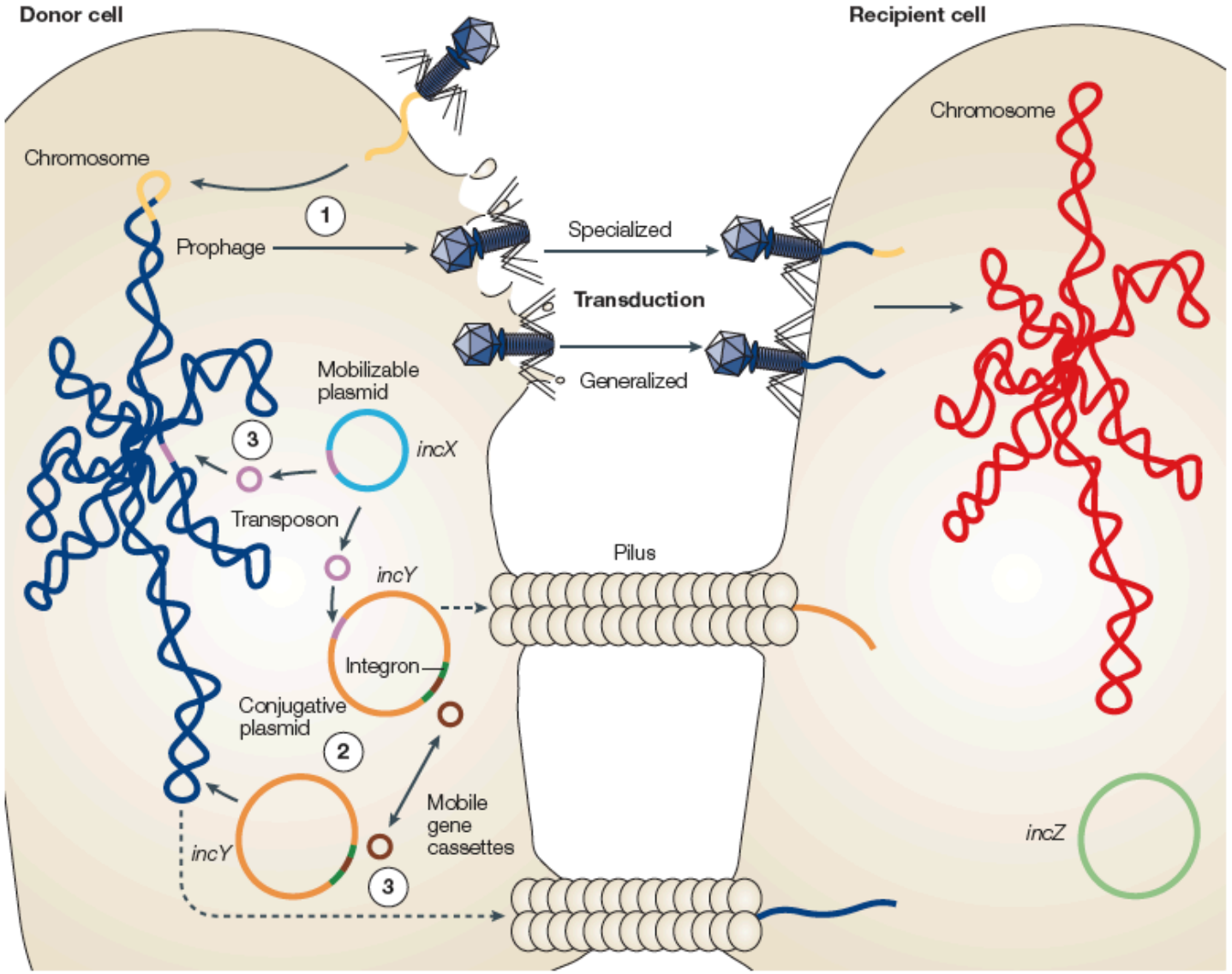


Figure 1 | **Transfer of DNA between bacterial cells.** Transduction (1). The DNA genome (yellow) of a temperate phage inserts

Source: Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol. 2005 Sep;3(9):722-32.

What's taking part of the exam?

- The genetic code
- Possible diagrams

- PCR principle and simple steps
- The 3 methods of intercellular gene transfer + intracellular gene transfer (transposition)

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