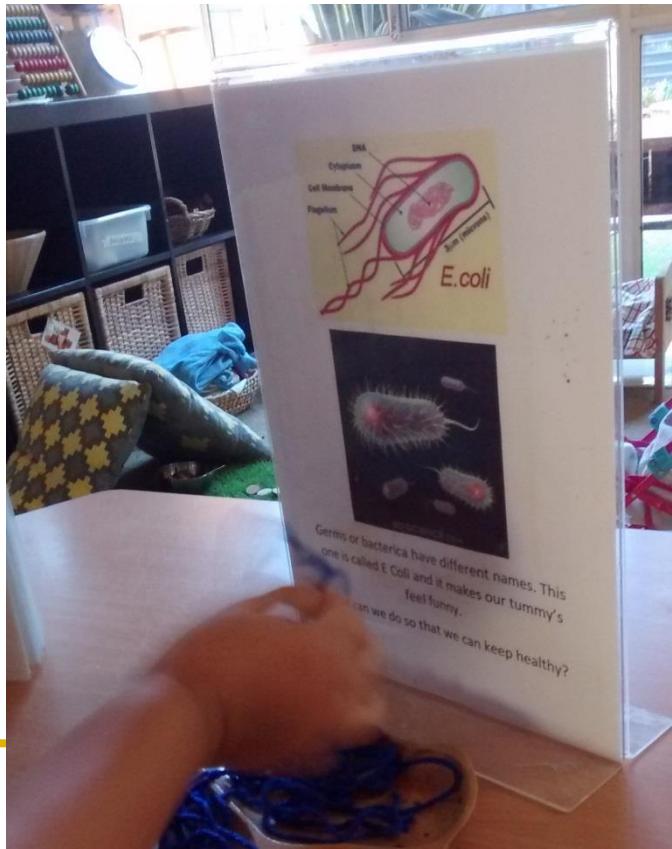


# **GENE2250 Prokaryotic Genetics I & II**



Lecturer: Dr. Heng Chooi

Suggested Reading:  
Pierce Genetics: A Conceptual Approach  
(2014) Chapters 9

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Slides adapted from Dr. Liz Quail's lecture

# Learning Outcomes

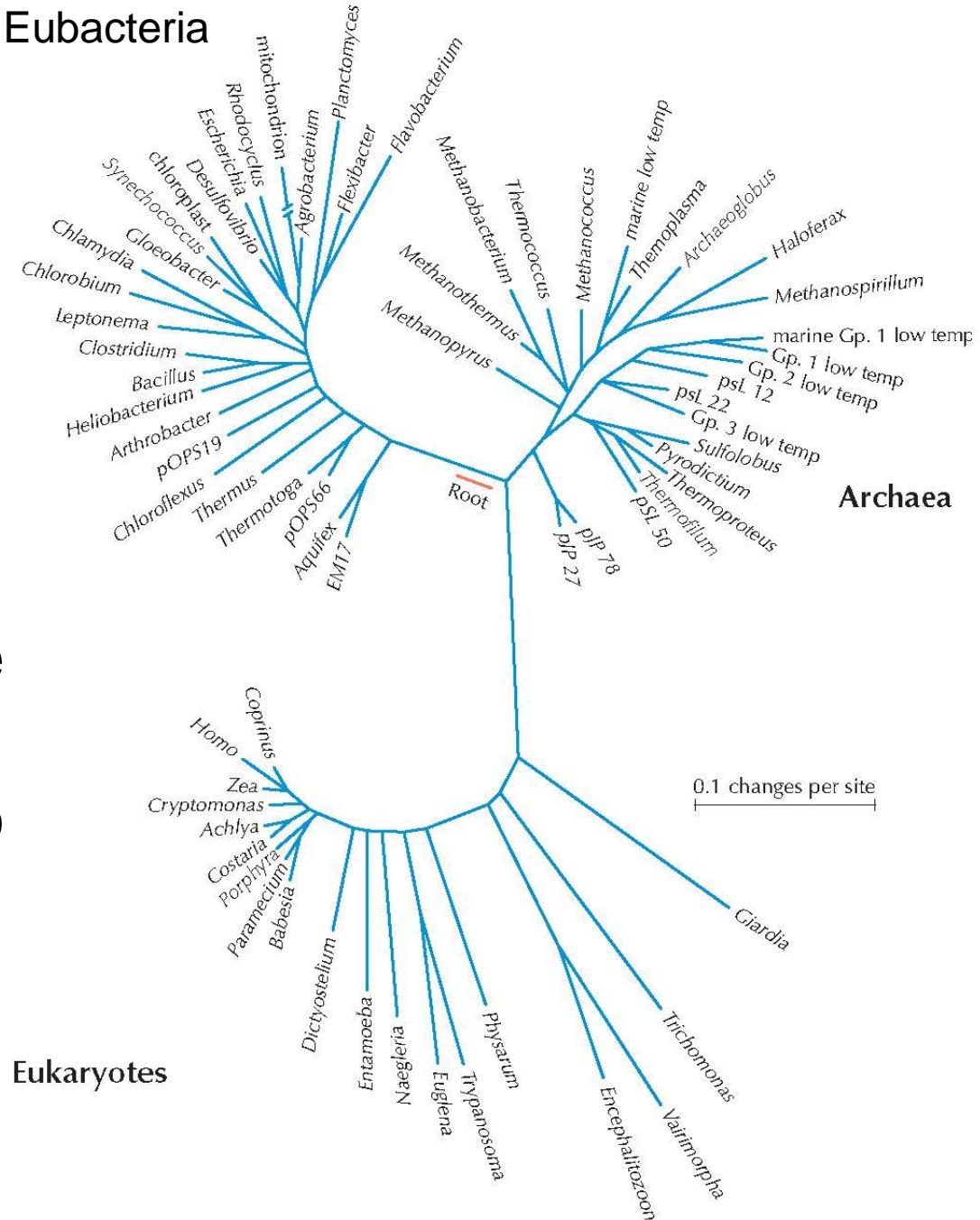
Following this lecture you should:

- ↳ Be able to define the characteristics of bacteria and bacteriophage.
- ↳ Understand how bacteria are grown and manipulated in the laboratory.
- ↳ Be able to define the bacterial genome and plasmids.
- ↳ Understand the mechanisms by which bacteria exchange genetic material (conjugation, transduction and transformation). Be able to describe each of these processes and distinguish between them.
- ↳ Understand how bacterial genomes can be mapped by determining transfer of genes.

# Domains of life

# Eubacteria

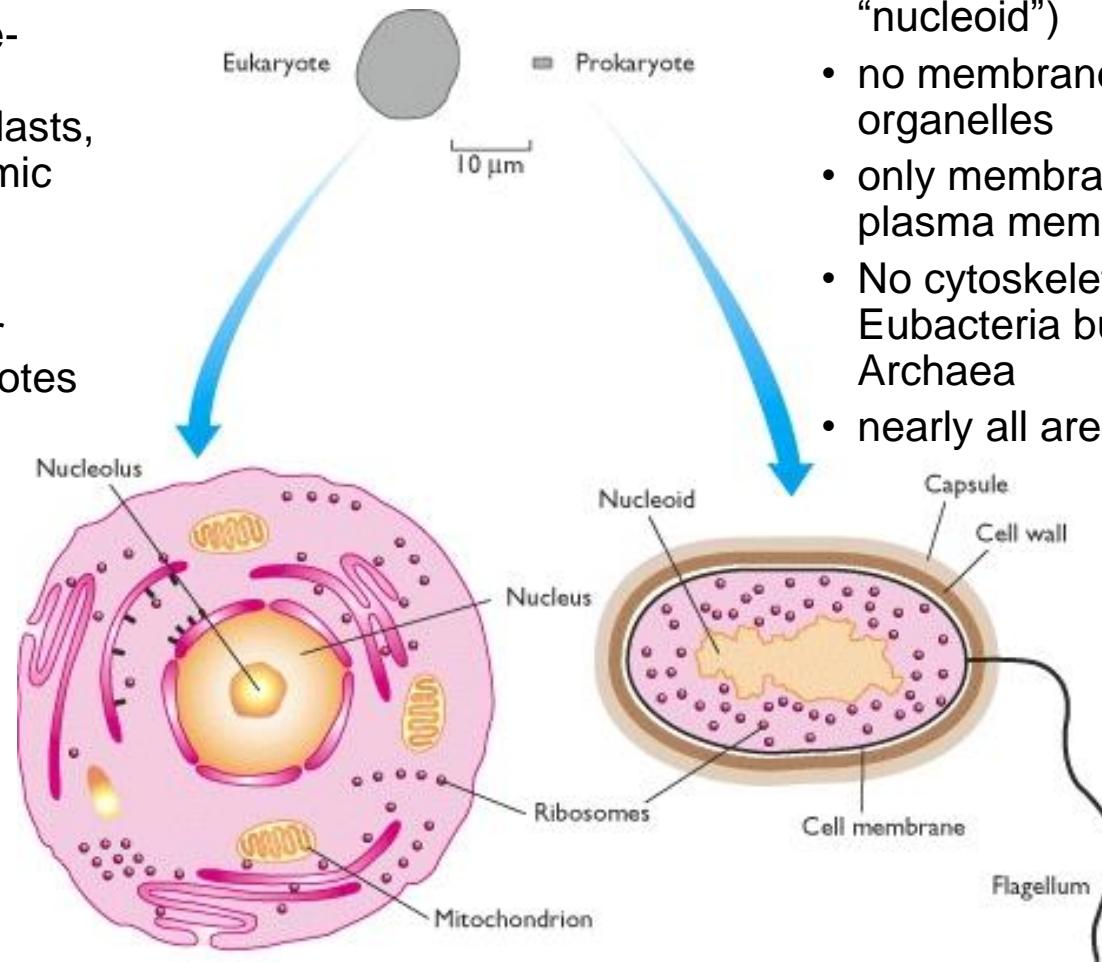
Will be considering the eubacteria, not archaea, which are as closely “related” to eukaryotes as to the eubacteria!



# Eukaryotes vs Prokaryotes

- DNA associated with histone proteins enclosed in membrane-bound nucleus
- have other membrane-bound organelles e.g. mitochondria, chloroplasts, lysosomes, endoplasmic reticulum
- have a cytoskeleton
- almost all multicellular organisms are eukaryotes

- genome located in the cytoplasm (sometimes confined to region called “nucleoid”)
- no membrane-bound organelles
- only membrane is the plasma membrane
- No cytoskeleton in Eubacteria but in some Archaea
- nearly all are unicellular



\*Ettema et al. An actin-based cytoskeleton in archaea. Mol. Microbiol. 2011 80(4):1052-61

# Eukaryote vs Prokaryote Genetics

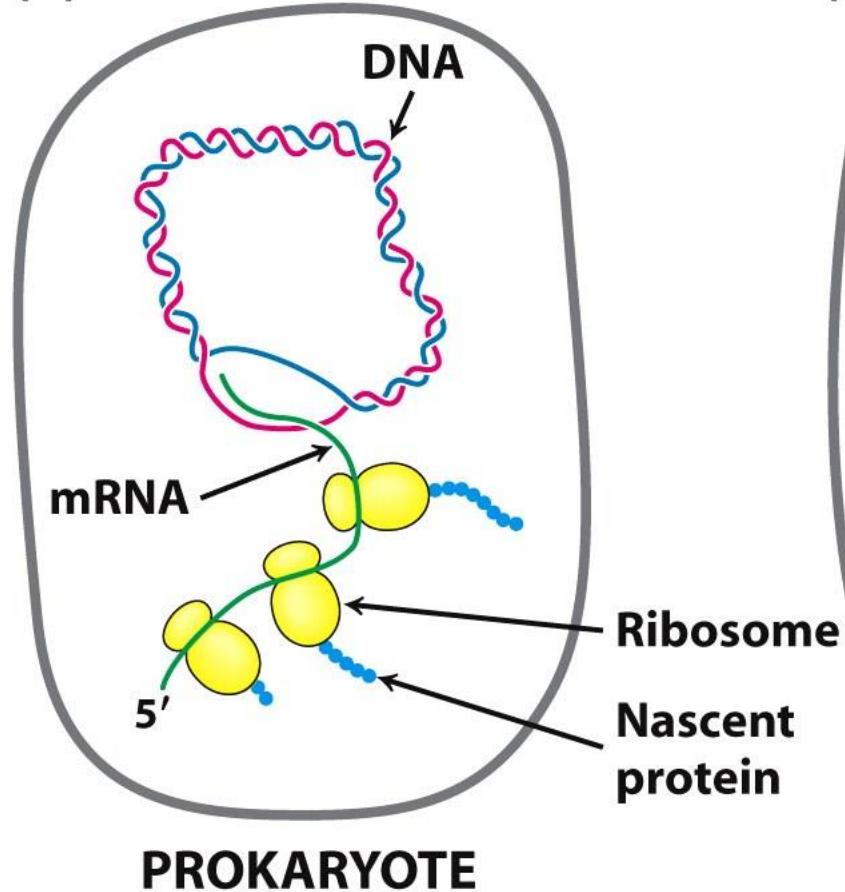
- Prokaryotes are haploid
- contain a single circular chromosome
- often contain small circular DNA molecules = “plasmids”, confer useful properties such as drug resistance
- translation coupled to transcription: translation of RNA starts before its transcription is finished
- Eukaryotes (esp. multicellular ones) are often diploid\*
- have linear chromosomes, usually more than 1
- transcription of genes occurs in nucleus
- protein translation occurs in cytoplasm

NOTE: many fungi (e.g. yeast), protozoans, algae and mosses can exist both as haploid and diploid



# Eukaryote vs Prokaryote Genetics

(A)



(B)

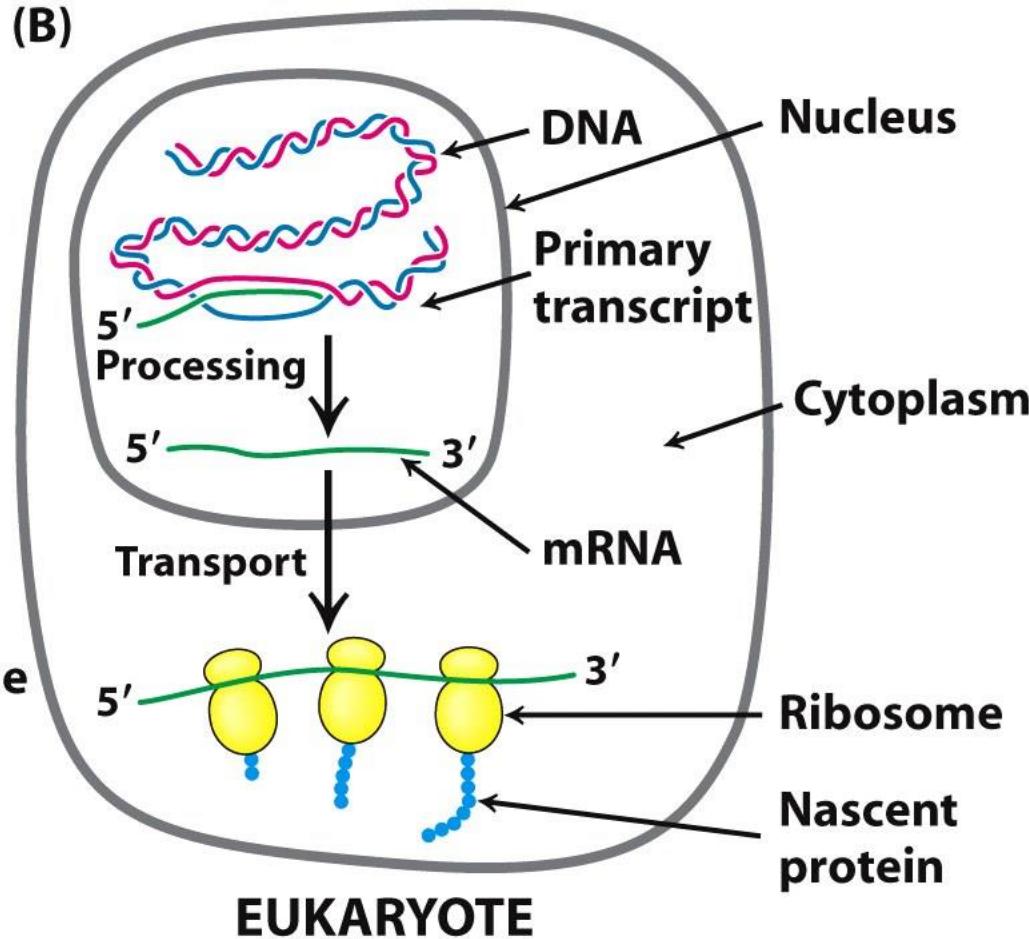


Figure 29.21

Biochemistry, Seventh Edition

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**Table 8.1 Advantages of using bacteria and viruses for genetic studies**

- 1. Reproduction is rapid.**
- 2. Many progeny are produced.**
- 3. The haploid genome allows all mutations to be expressed directly.**
- 4. Asexual reproduction simplifies the isolation of genetically pure strains.**
- 5. Growth in the laboratory is easy and requires little space.**
- 6. Genomes are small.**
- 7. Techniques are available for isolating and manipulating their genes.**
- 8. They have medical importance.**
- 9. They can be genetically engineered to produce substances of commercial value.**

Traits that make a good model organism

True for S.c yeast in Prac 1 too!

On E. coli insulin:

<https://www.gene.com/stories/cloning-insulin>

# Studying Bacteria

Bacteria require nutrients to grow and divide

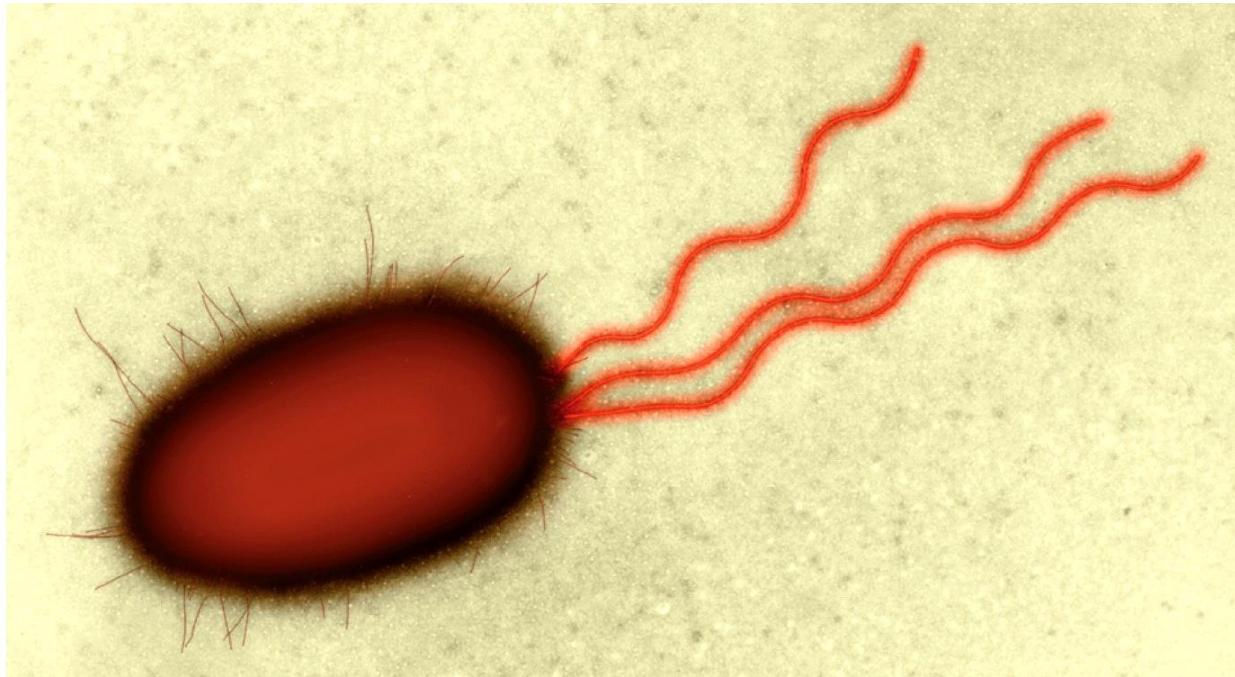
Media for culture usually contain:

- Carbon source
- Essential elements, N, P, etc
- Vitamins
- Trace metals, ions, etc
- Oxygen if aerobic
- Warmth for most common bacteria (accelerate growth)

Minimal medium contains inorganic N, P, mineral (Na, K, Mg, Ca), plus a carbon source (glucose or glycerol). Another important feature of minimal medium, also known as synthetic medium, is that it is defined.

# Studying Bacteria

*Escherichia coli* (*E. coli*), a common GI tract bacteria, grows on partially digested extracts made from yeast & animal products, at 37°C in a normal atmosphere.



# Bacterial Cultures

Liquid “broth” culture

Nutrients allows rapid growth, high density. Is easy and cheap.

Solid media (individual colonies)

Use same nutrient broth as liquid culture, solidifying with agar = polysaccharide from seaweed, most bacteria can't digest.

Can isolate individual bacterial cells, then grow each cell up into a colony, standard way to create pure culture of bacteria. All cells of a colony are closely related to the original cell, with only a small amount of genetic variation possible.

Can count number of bacteria that were in a culture tube.



# Bacterial genetics terminology

**Wild-type** bacteria can grow on media containing simple ingredients (listed previously) = minimal media, and these bacteria are known as **prototrophs**. Prototrophs can use simple molecules to build more complex ones.  
Most wild-type bacteria are sensitive to antibiotics.

**Auxotrophs** require some extra nutrient/s in their media.

WHY?

We will be using yeast adenine auxotroph in Prac 1

Eg, a leucine auxotroph ( $leu^-$ ) needs Leu to be added to the growth media (minimal medium with Leu supplement or complete medium); whereas a prototroph is  $leu^+$  and does not need any Leu supplementation (on minimal medium).

NOTE: The development of defined minimal medium is very important for studying the genetics of biochemical pathways.

# Bacterial genetics terminology

Chemoauxotrophs are mutants that can't use some nutrient (usually a sugar, amino acid or vitamin) that prototrophs can

eg lac<sup>-</sup> mutants can't grow on lactose, but lac<sup>+</sup> prototrophs can.



Figure 5-4  
*Introduction to Genetic Analysis*, Tenth Edition  
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EXAMPLE: Red cells are lac<sup>+</sup> and can metabolise lactose, grow much faster and change indicator dye (call ONPG) to red.  
lac<sup>-</sup> mutant do not have b-galactosidase activity and therefore cannot metabolise lactose and cannot convert the indicator dye to red.

# Bacterial mutants

Random mutagenesis - Bacteria mutants are generated in the lab by exposing them to mutagens, e.g. UV, ethyl methanesulfonate (EMS) - often used in forward genetics

Targeted mutagenesis - by genetic transformation – often used in reverse genetics

Resistance mutants confer resistance to some environmental toxin: drugs, heavy metals, bacteriophage (bacterial viruses, later), etc.

$\text{amp}^r$  = resistant to the antibiotic penicillin/ampicillin

**Table 5-1 Some Genotypic Symbols Used in Bacterial Genetics**

Symbol	Character or phenotype associated with symbol
<i>bio</i> <sup>r</sup>	Requires biotin added as a supplement to minimal medium
<i>arg</i> <sup>r</sup>	Requires arginine added as a supplement to minimal medium
<i>met</i> <sup>r</sup>	Requires methionine added as a supplement to minimal medium
<i>lac</i> <sup>r</sup>	Cannot utilize lactose as a carbon source
<i>gal</i> <sup>r</sup>	Cannot utilize galactose as a carbon source
<i>str</i> <sup>r</sup>	Resistant to the antibiotic streptomycin
<i>str</i> <sup>s</sup>	Sensitive to the antibiotic streptomycin

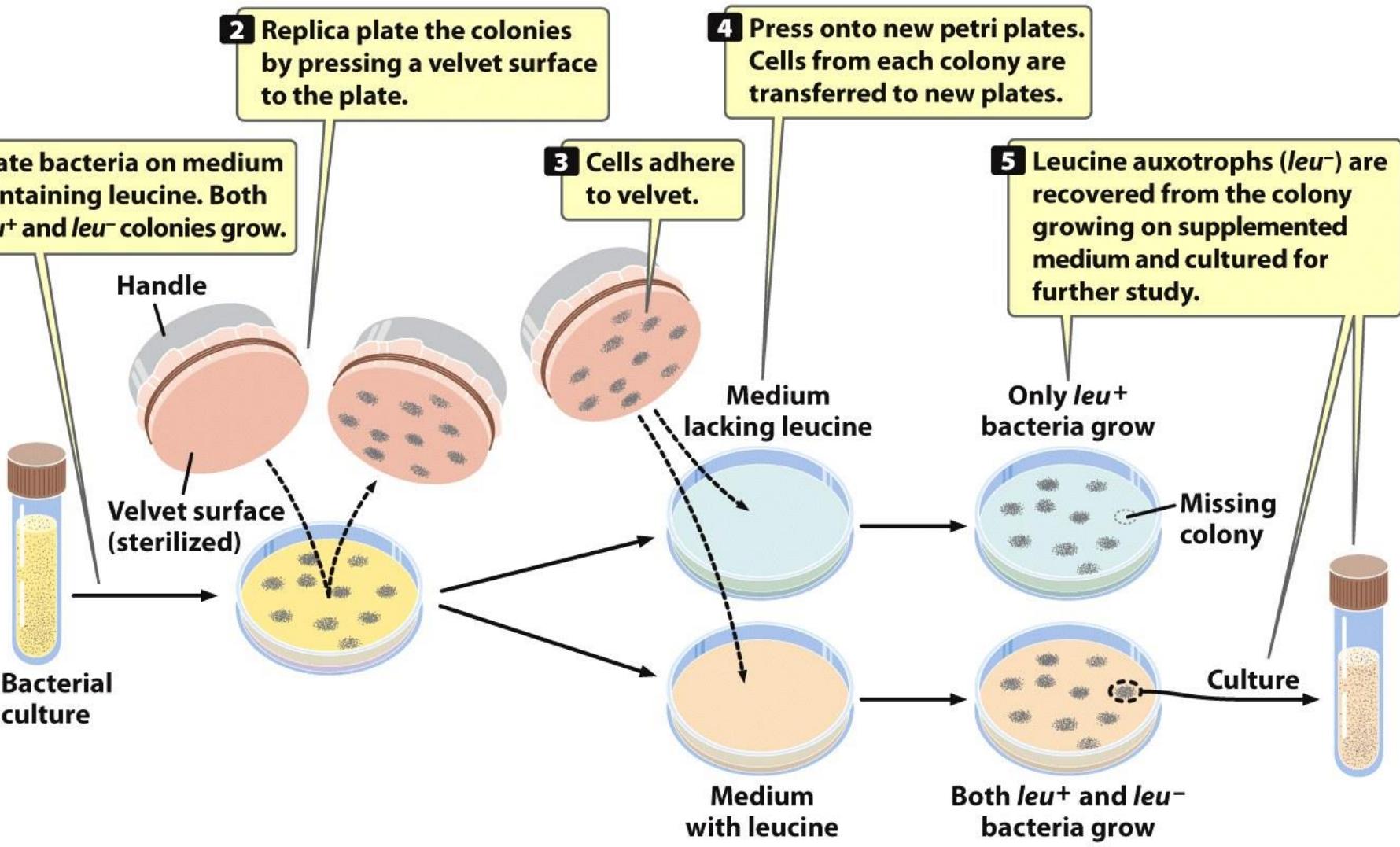
**Note:** Minimal medium is the basic synthetic medium for bacterial growth without nutrient supplements.

# Replica plating

A common way to find bacterial mutants is replica plating – making identical copies of the colonies on a petri dish under different conditions.

Eg, to find  $leu^-$  (auxotrophs), one plate would contain added Leu and the other plate would not.

Bacteria first plated on permissive plate, (allows both mutants and wild type to grow) i.e. has Leu added. After growth, a copy of the plate is made by pressing a piece of velvet onto the surface, then moving it to a fresh plate with the restrictive condition (no Leu). The velvet transfers some cells from each colony to an identical position on the restrictive plate.



**Conclusion:** A colony that grows only on the supplemented medium has a mutation in a gene that encodes the synthesis of an essential nutrient.

Figure 8.3

Genetics: A Conceptual Approach, Fourth Edition

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# Replica plating

Colonies that grow on the permissive plate, but not the restrictive plate are leu<sup>-</sup> (auxotrophs), because they can only grow if leucine is supplied.

The auxotrophs can be selected and studied – this is how many biochemical pathways were elucidated...

## Supplementary reading (for your interest)

Beadle-Tatum experiment (Nobel Prize, 1958) : George Beadle and Edward Tatum used auxotrophs of Neurospora bread mold to demonstrate the “**one gene-one enzyme**” hypothesis.

See animations:

1. <https://www.dnalc.org/view/16360-animation-16-one-gene-makes-one-protein-.html>
2. [http://wps.prenhall.com/wps/media/objects/1552/1589869/web\\_tut/21\\_04/21\\_04\\_01a.swf](http://wps.prenhall.com/wps/media/objects/1552/1589869/web_tut/21_04/21_04_01a.swf)

# Bacterial Genome

Most have one circular chromosome – a single DNA molecule, several million bp.

*E. coli* has 4.6 million bp, 90% of which encodes proteins (only <2% in humans!).

Some bacteria have multiple chromosomes, some have linear chromosomes.

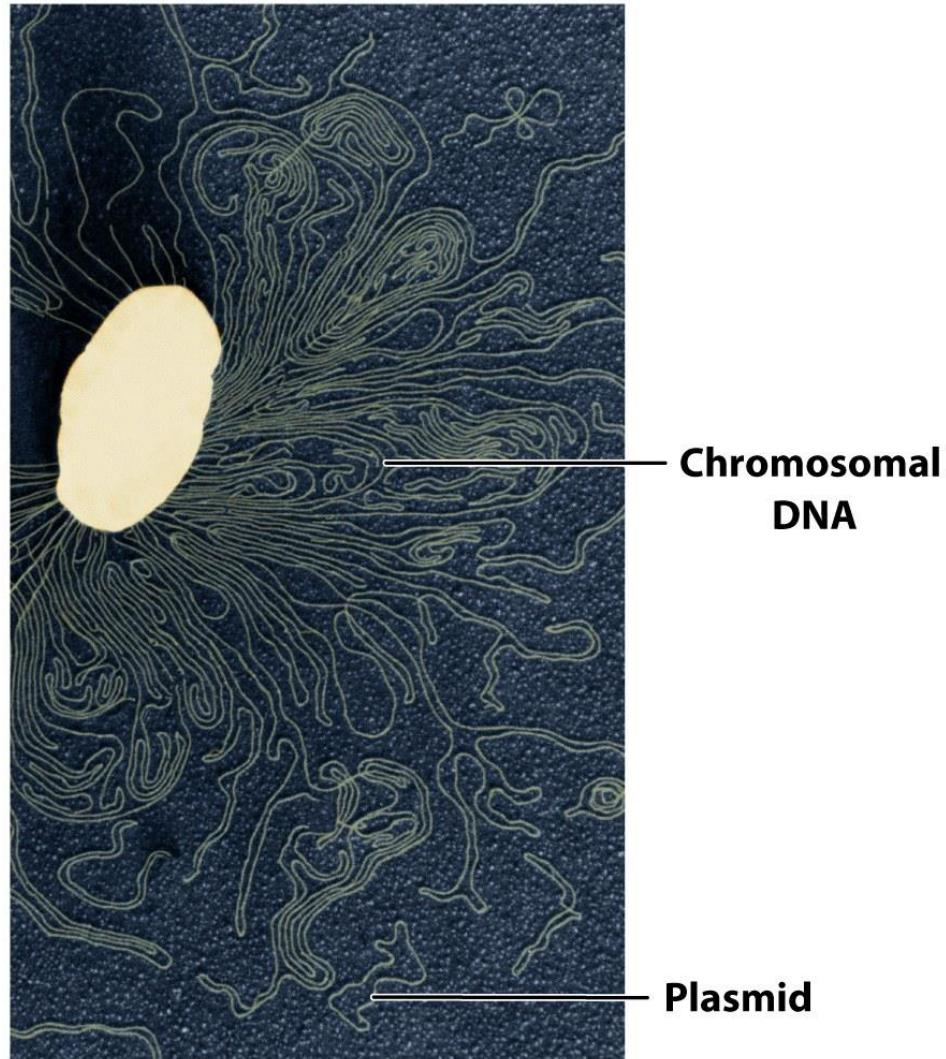


Figure 8.4

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# Bacterial Plasmids

- Many bacteria also have small, circular DNA molecules (plasmids), usually several thousand bp long. Numbers vary - can have one, two or many copies per cell.
- Plasmids contain genes which are not essential to function, but play a role in life cycle or growth of the bacteria or in specific environmental/ecological niches.
- Plasmids have own origin of replication and can replicate independently of chromosome.
- Some plasmids contain genes for antibiotic resistance and are used in genetic engineering (later).

# Bacterial Plasmids

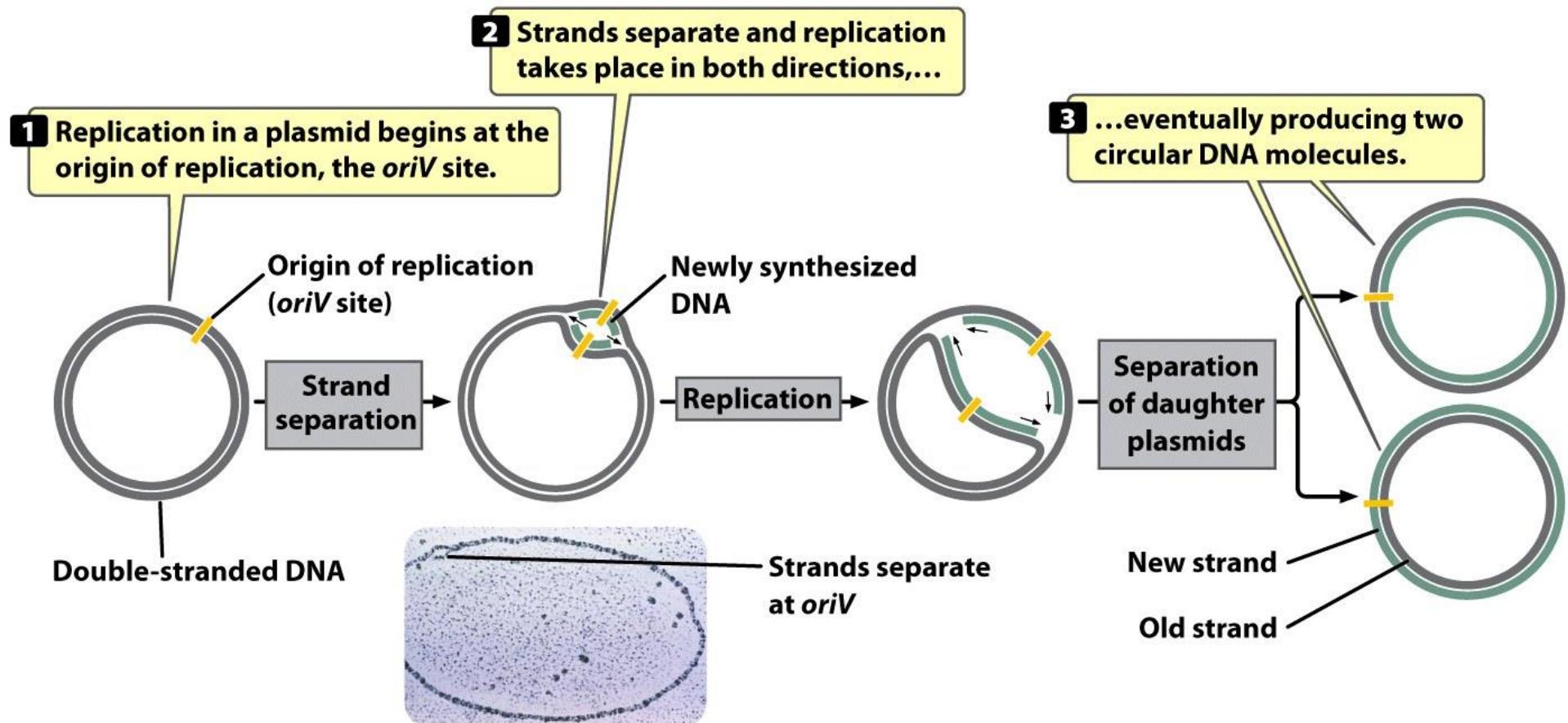


Figure 8.5

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# Plasmids

Episome = plasmid which can freely replicate and integrate into bacterial chromosome.

F (fertility) factor – an E. coli episome which regulates transfer, replication and insertion

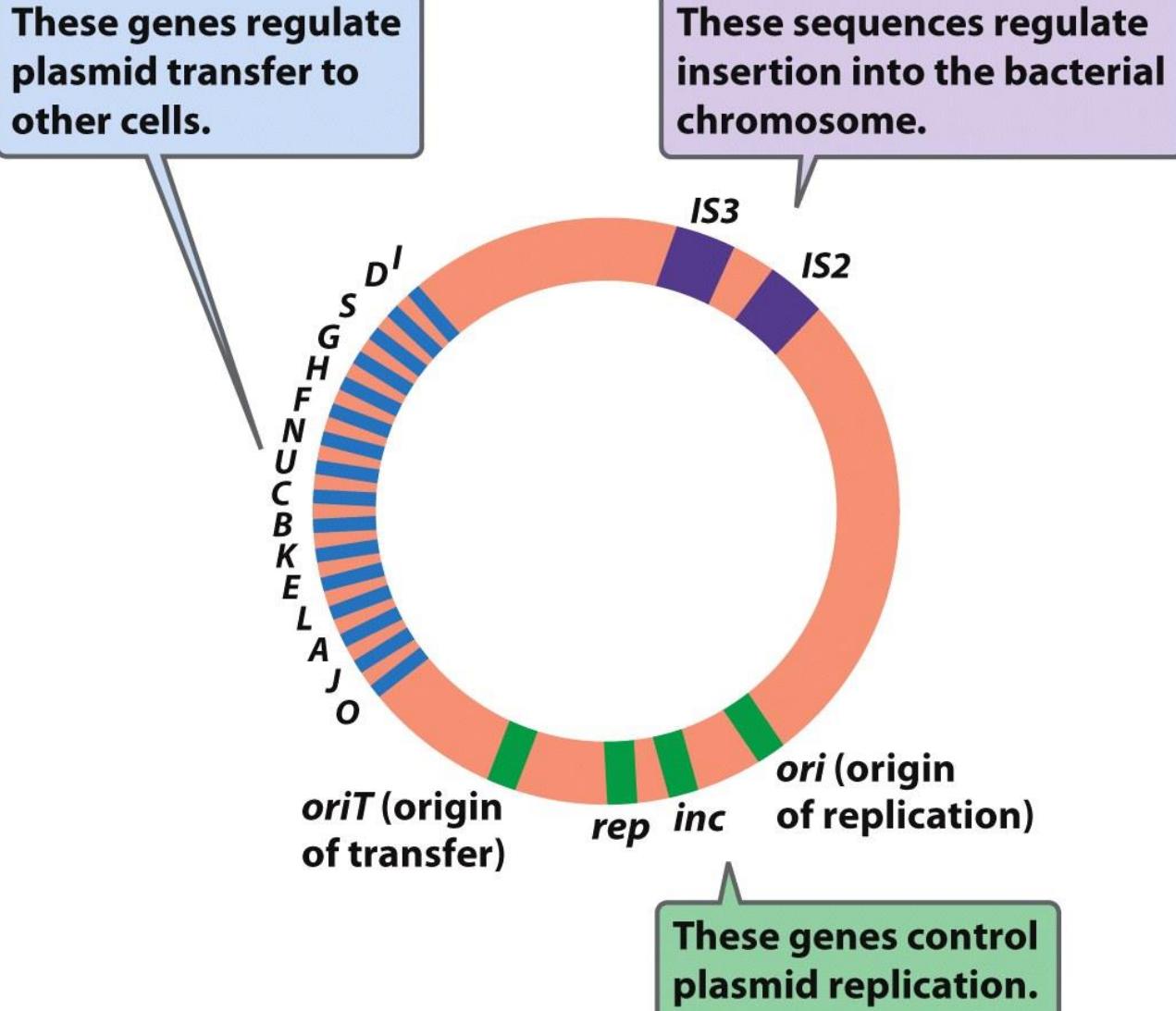
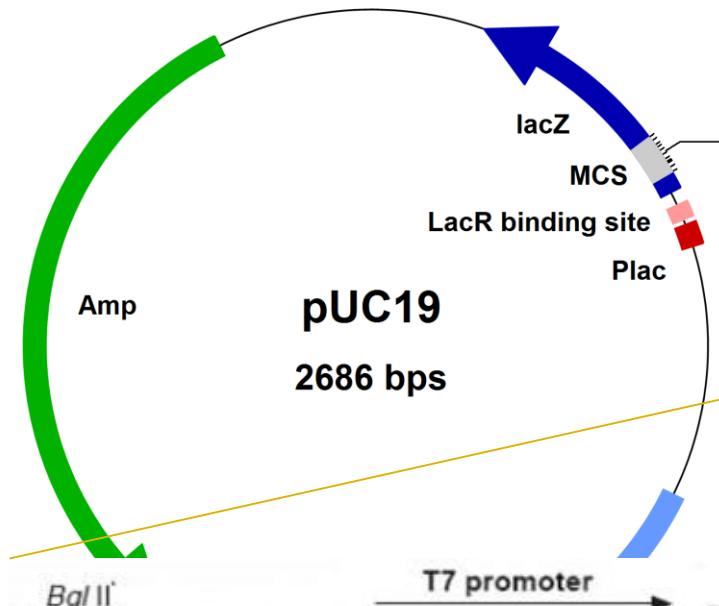


Figure 8.6  
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**F factor**

# Commonly used plasmid in lab

## **Cloning plasmid e.g. pUC19**



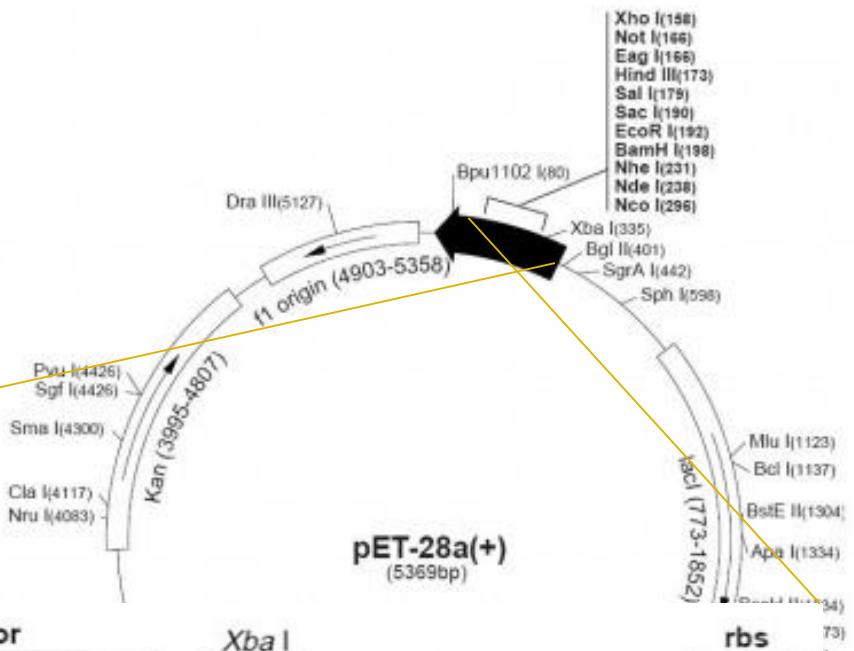
Nco I                    His-Tag                    Nde I    Nhe I            T7-Tag

TATACCATGGCAGCACCGATCATCATCATCACAGCAGGGCTGGTGCCTGGGAGCCATGGCTAGCATGACTGGGACAGCAA  
 Met-Glu-Ser-Ser-His-Lys-His-His-His-Ser-Ser-Cys-Ser-His-Met-Ala-Ser-Met-The-Cys-Cys-Cys

*Bpu1102 I* **T7 terminator**

---

## **Expression plasmid e.g. pET28a**



# Exchange of Genetic Material



In eukaryotes, meiosis reduces diploids to haploids, and fertilisation returns cells to the diploid state. Bacterial processes are not so regular; however, they serve the same aim: to mix the genes from two different organisms together.

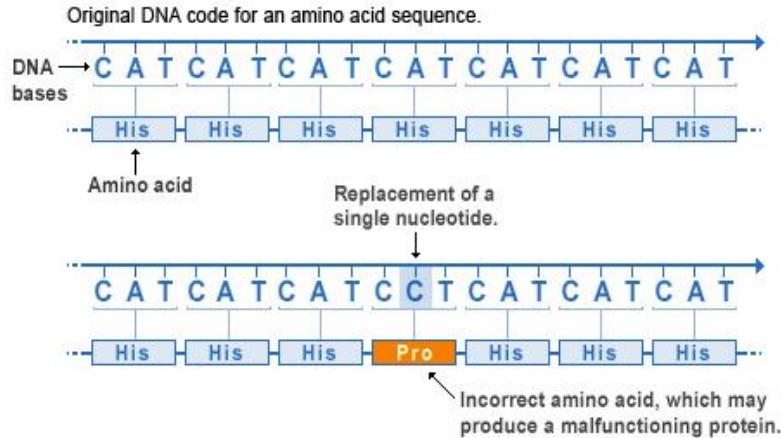
Are there three bacterial genetic exchange processes:

1. **conjugation**: direct transfer of DNA from one bacterial cell to another (an animation posted on LMS)
2. **transduction**: use of a bacteriophage to transfer DNA between cells.
3. **transformation**: naked DNA is taken up from the environment by bacteria.

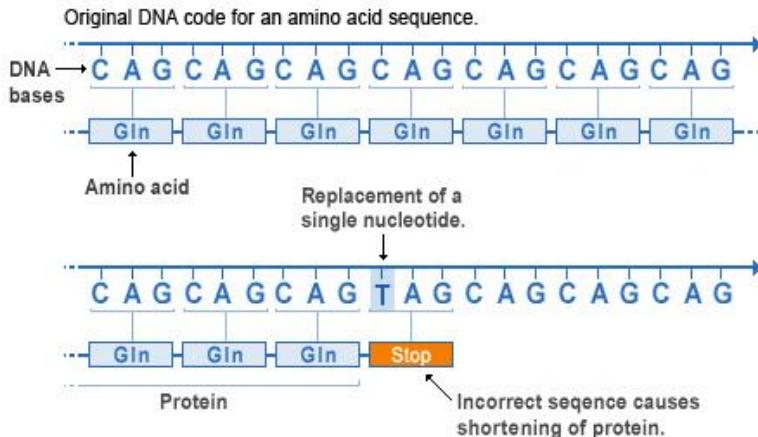
# Common types of DNA mutations at single nucleotide level

## substitution

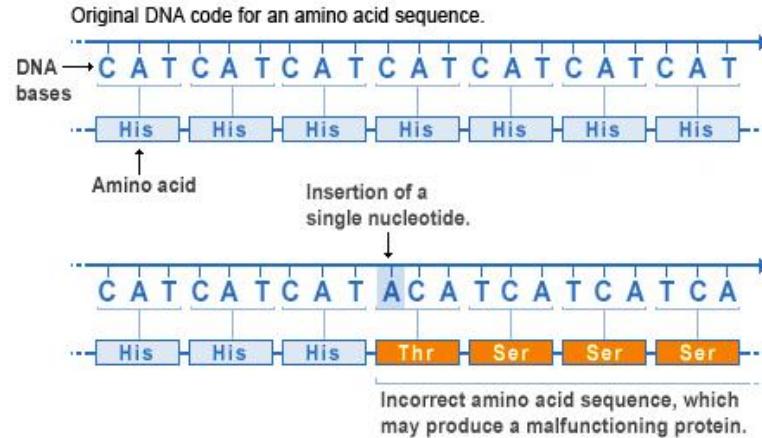
### Missense mutation



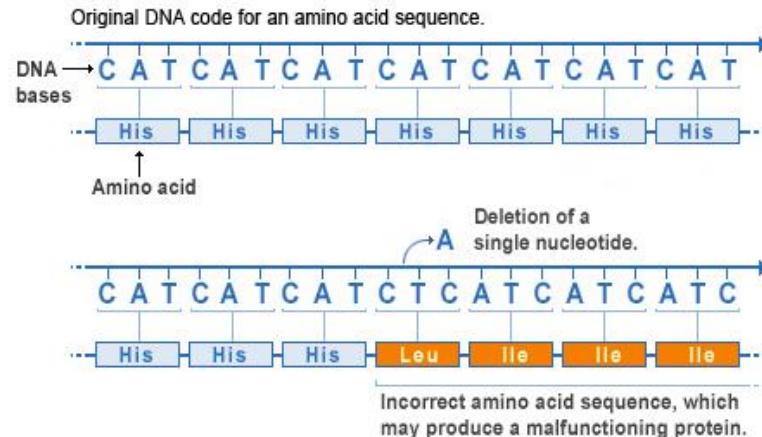
### Nonsense mutation



### Insertion mutation

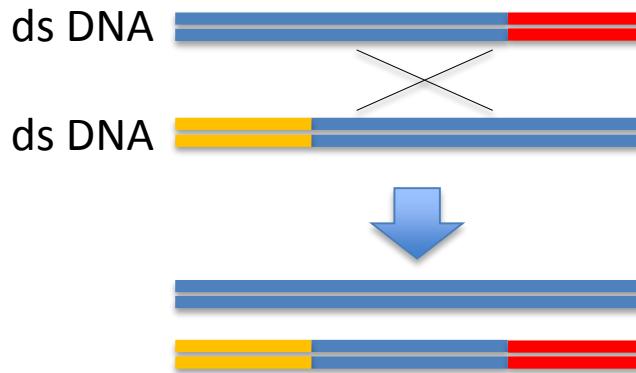


### Deletion mutation

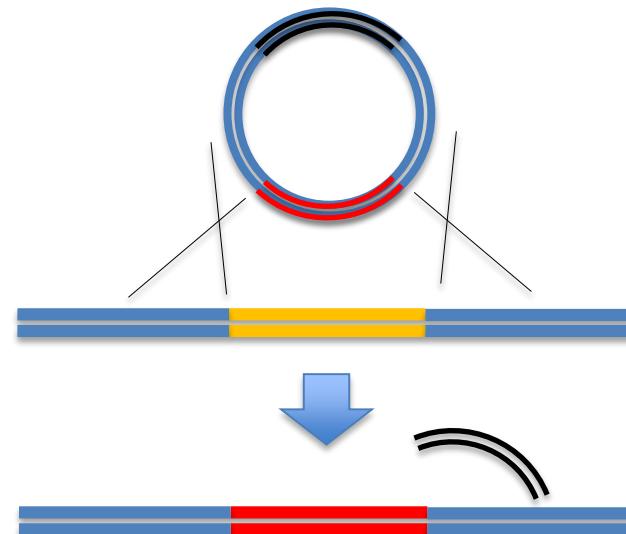
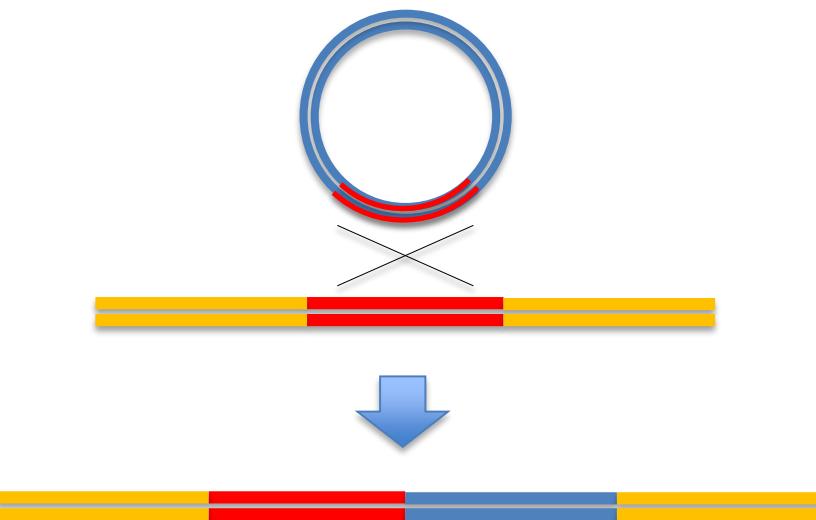
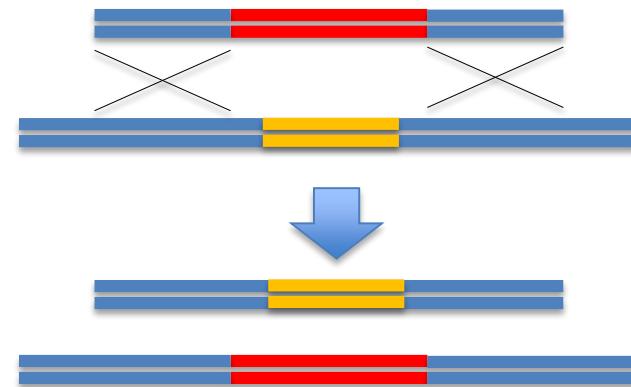


# Homologous DNA recombination: A DNA repair mechanism

Single crossover



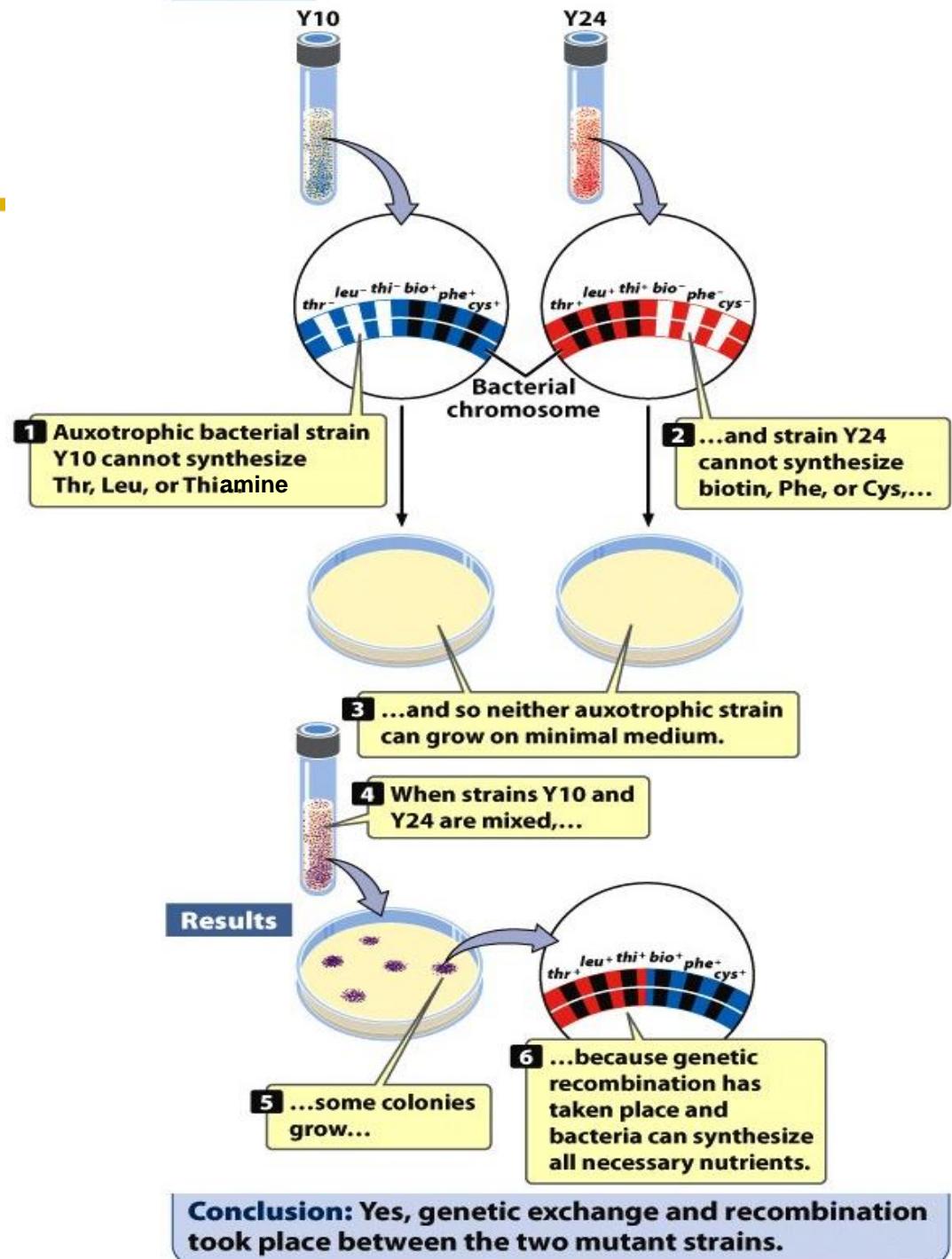
Double crossover



Involves complementary DNA hybridisation, Holliday junction, enzymes and double strand DNA breaks

# Conjugation

In 1946 Lederberg and Tatum showed that bacteria can transfer and recombine genetic material.

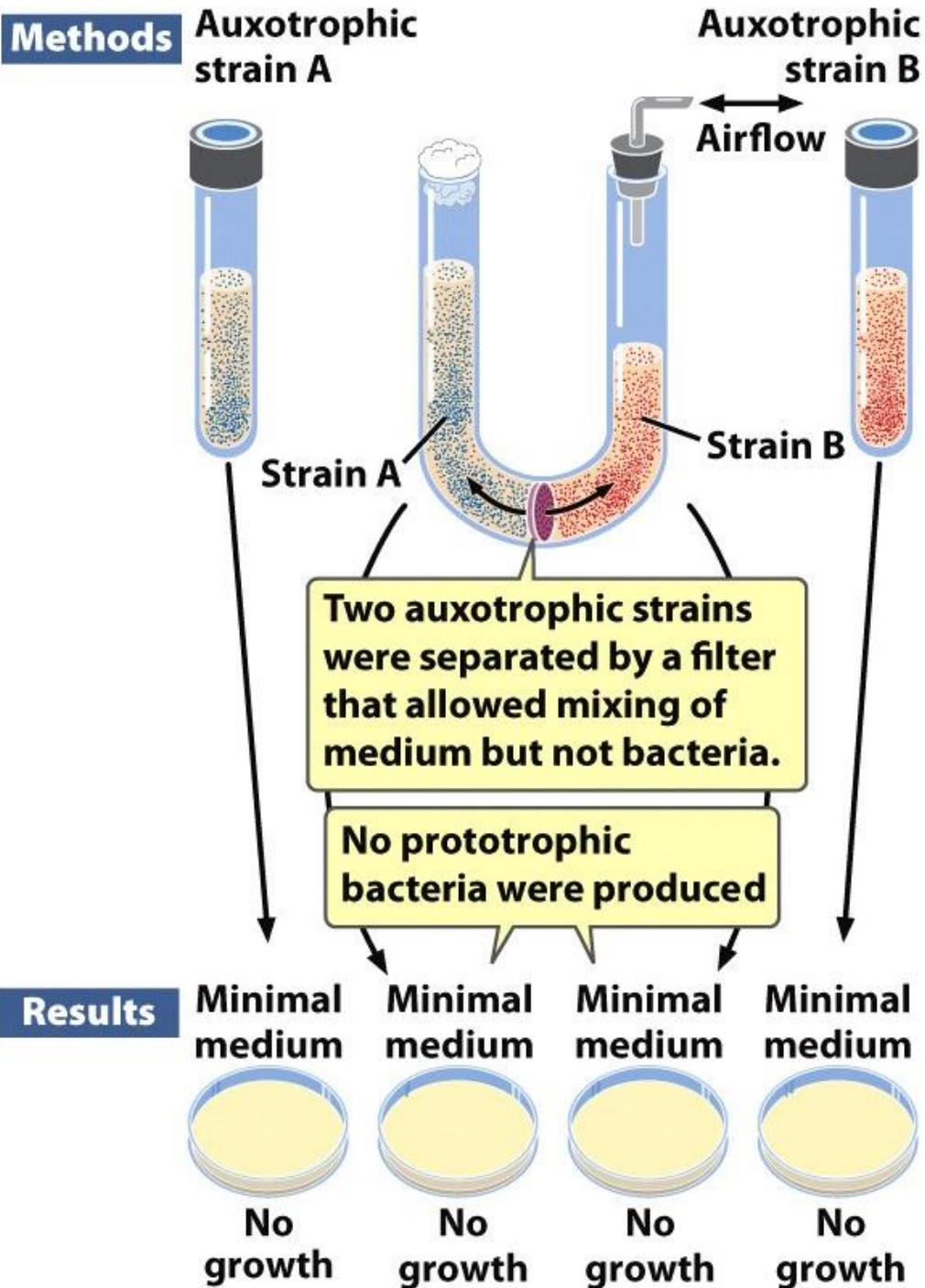


# Conjugation

Davis showed that contact between the bacterial cells was required for genetic exchange.

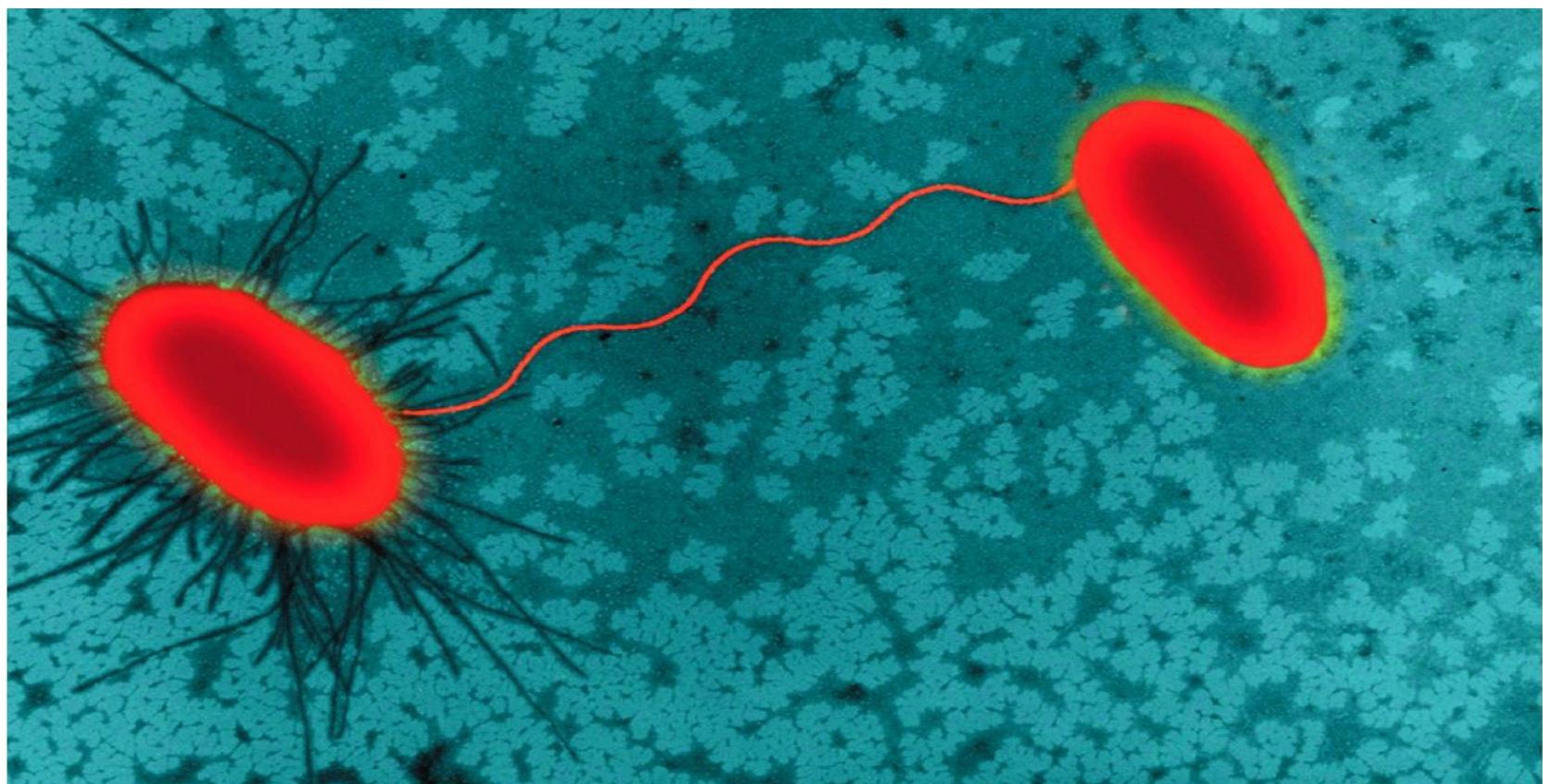
This direct process of gene transfer was termed conjugation.

## Methods



# Conjugation

In 1953, Hayes determined that the transfer of genetic material in *E. coli* is not reciprocal, i.e. a donor cell transfers part of its genome to another (recipient) cell. Donors have the fertility factor (F), designated F<sup>+</sup>, strains which lack F are recipients (F<sup>-</sup>).

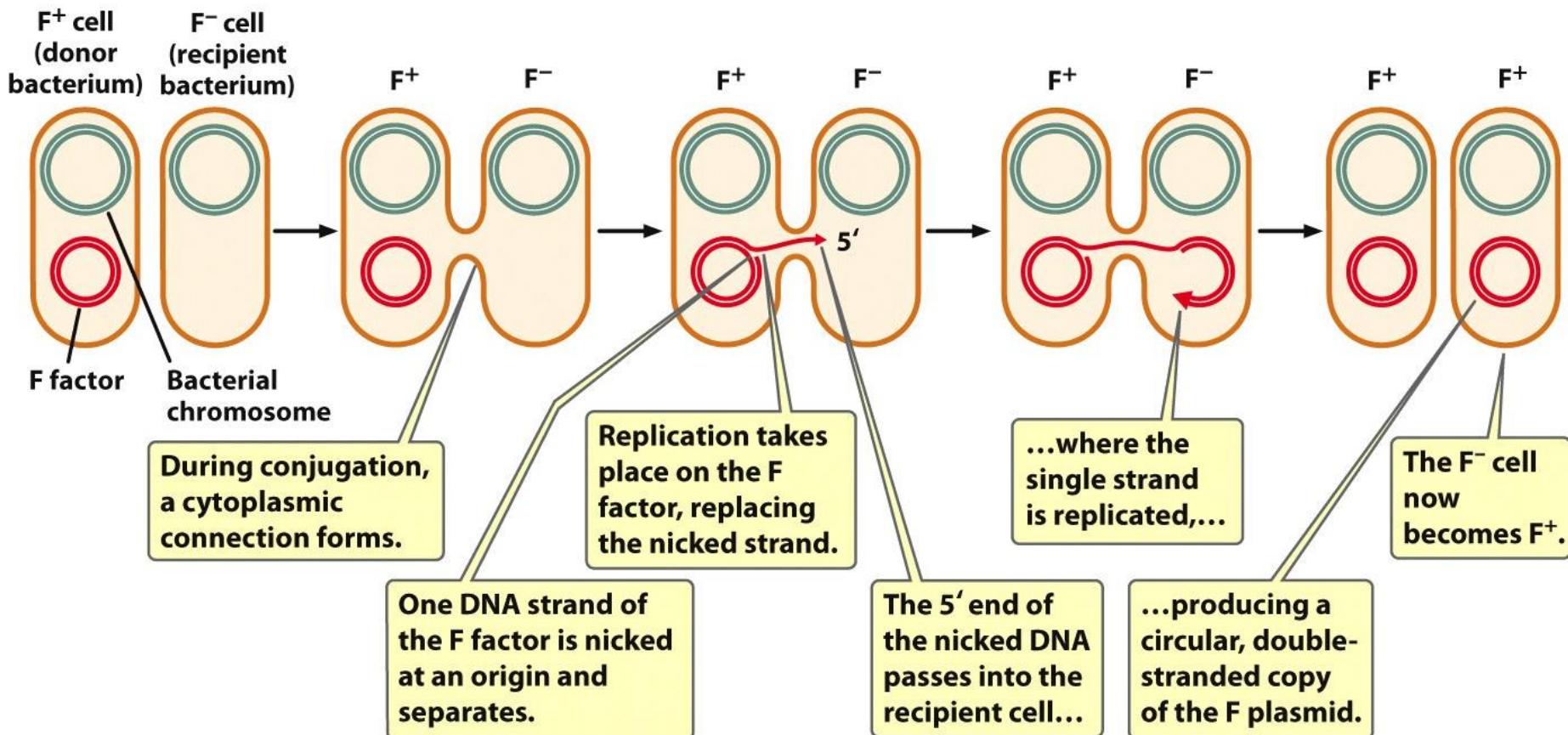


# Conjugation

- F gene is on an episome (F factor) - directs synthesis of pili that initiates contact with the recipient and draws it closer.
- F factor contains an origin of replication and genes required for conjugation, eg the sex pili (extension of cell membrane) which makes contact with a receptor on F- cell and pulls the cells together.
- Conjugation only occurs between F<sup>+</sup> and F<sup>-</sup> cells. Usually the only genes exchanged are on the F factor. Transfer is initiated by nicking a strand on F (at origin, oriT), which separates from the plasmid and moves into the recipient cell.

# Conjugation

- The F DNA in the donor cell makes a single-stranded copy of itself = rolling circle replication. The SS copy is cast out through a pore into the recipient cell where the 2<sup>nd</sup> strand is made to give DS DNA. So a copy of F remains in donor and now the recipient cell also has F.



# Conjugation

- Conjugation only explains transfer of F genes, not of chromosomal genes. Hfr (high frequency) strains have the F factor integrated into the bacterial chromosome.

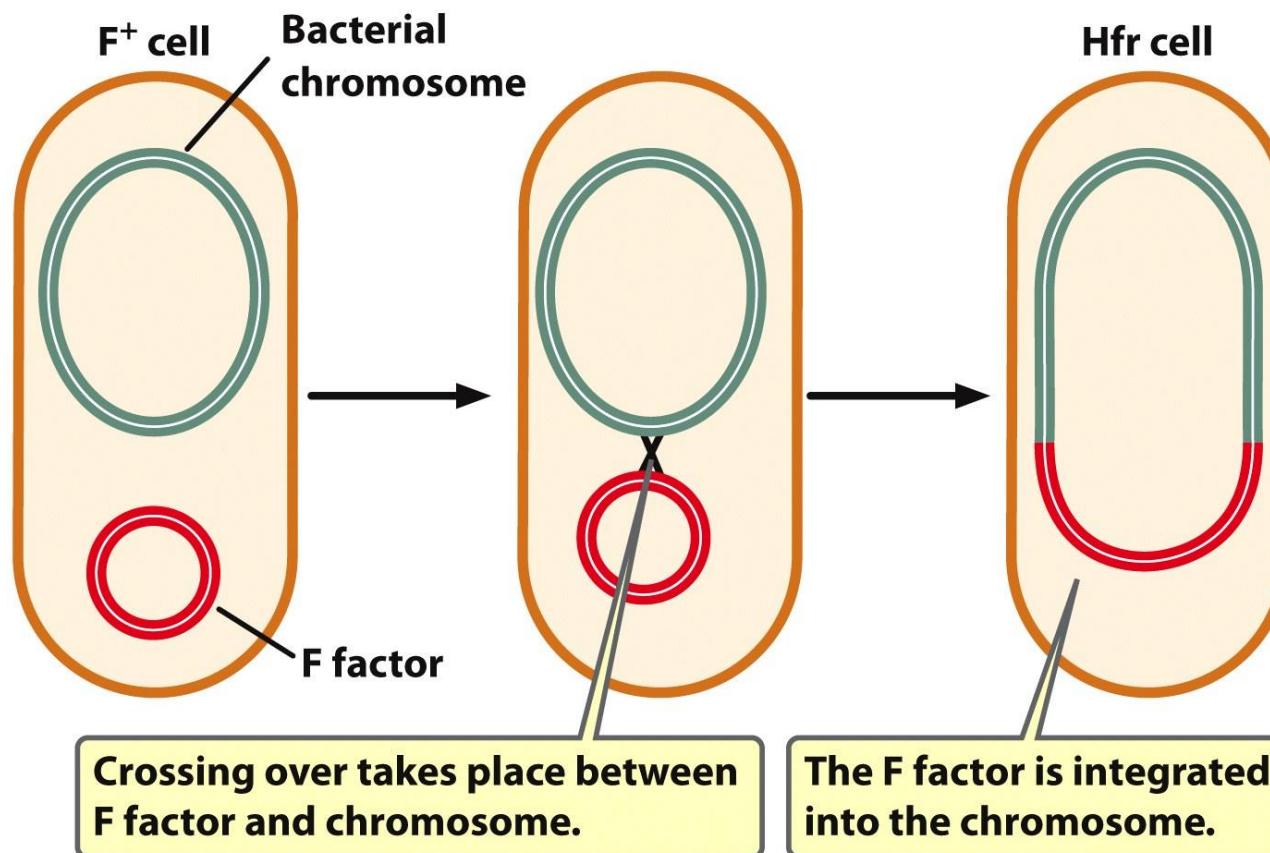


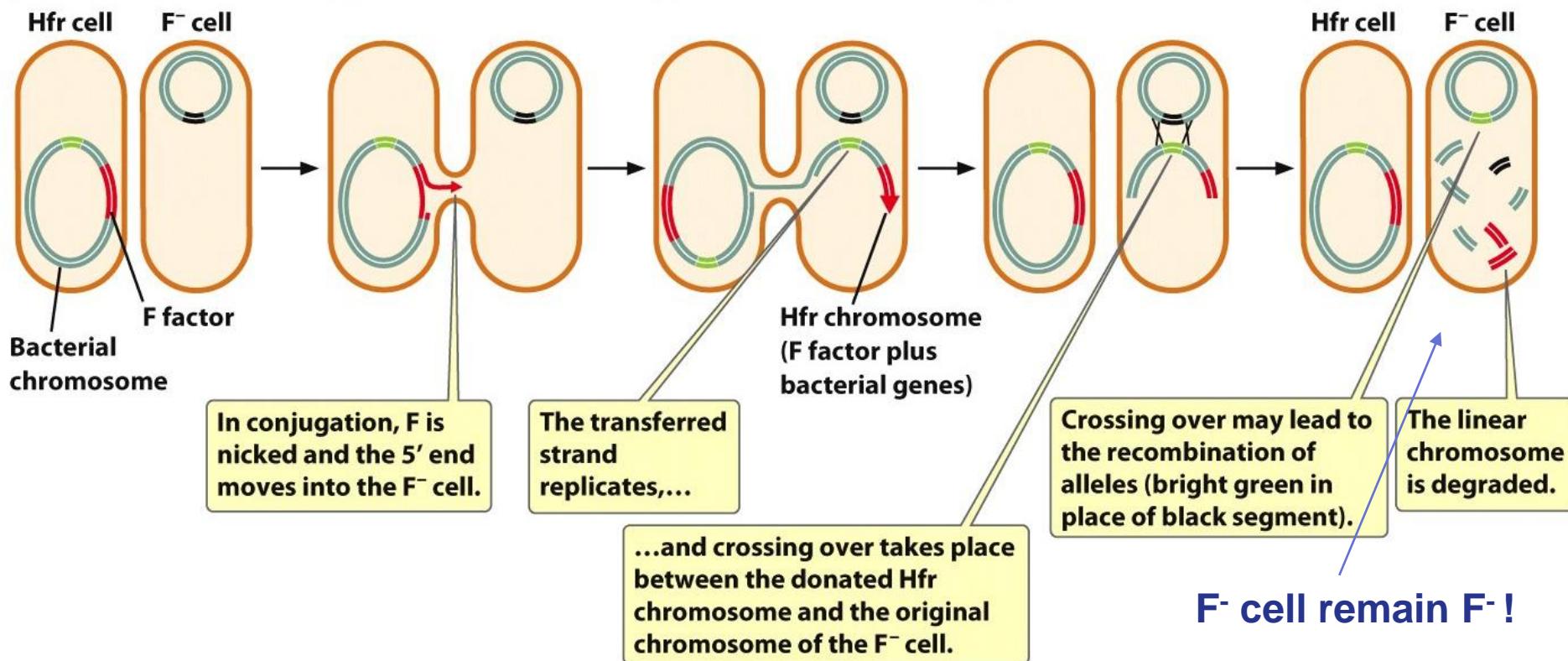
Figure 8.12

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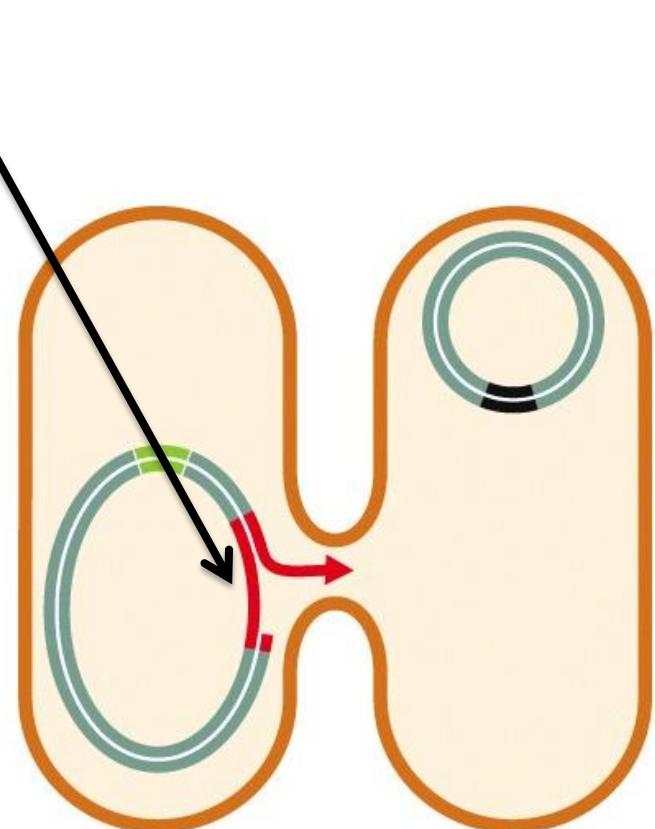
# Conjugation

If get conjugation between Hfr and F<sup>-</sup>, then the chromosome follows the F factor into the recipient cell... the amount of transfer depends on the time the cells are joined. In the recipient cell, can get crossing over with the inserted DNA.



# Conjugation

- F<sup>-</sup> cell virtually never converted to a F<sup>+</sup> or HFr (when mate with a HFr cell), as the F factor is nicked in the middle when transfer is initiated.
- To become F<sup>+</sup> the entire chromosome must be transferred, which hardly ever happens, as it would mean the conjugating cells staying together for a long time.
- Hfr cells produced via F plasmid integration only occurs in 1/10000 cells.

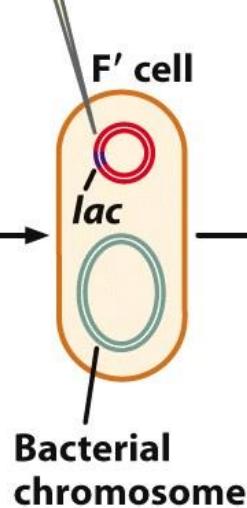
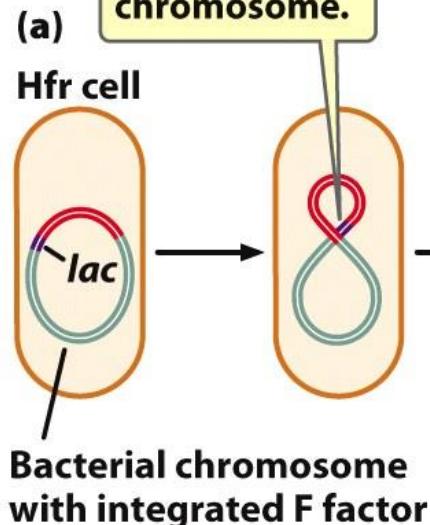


# Conjugation

F' cells contain the F factor plus some bacterial genes that were transferred with it when the F factor was excised from an Hfr cell's chromosome...

When the F factor excises from the bacterial chromosome, it may carry some bacterial genes (in this case, *lac*) with it.

Crossing over takes place within the Hfr chromosome.

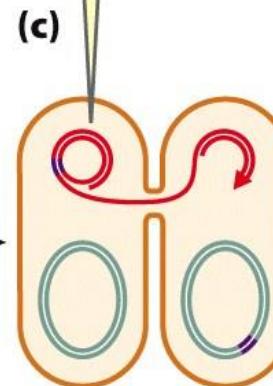
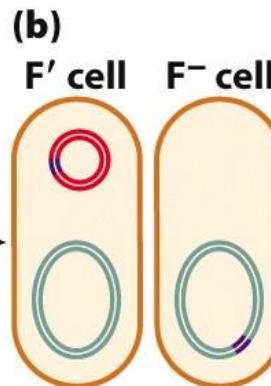


Excision is uncommon, about 1 in 10000 cells, but does happen to get F' cells.

This is sexduction

During conjugation, the F factor with the *lac* gene is transferred to the F<sup>-</sup> cell,...

...producing a partial diploid with two copies of the *lac* gene.

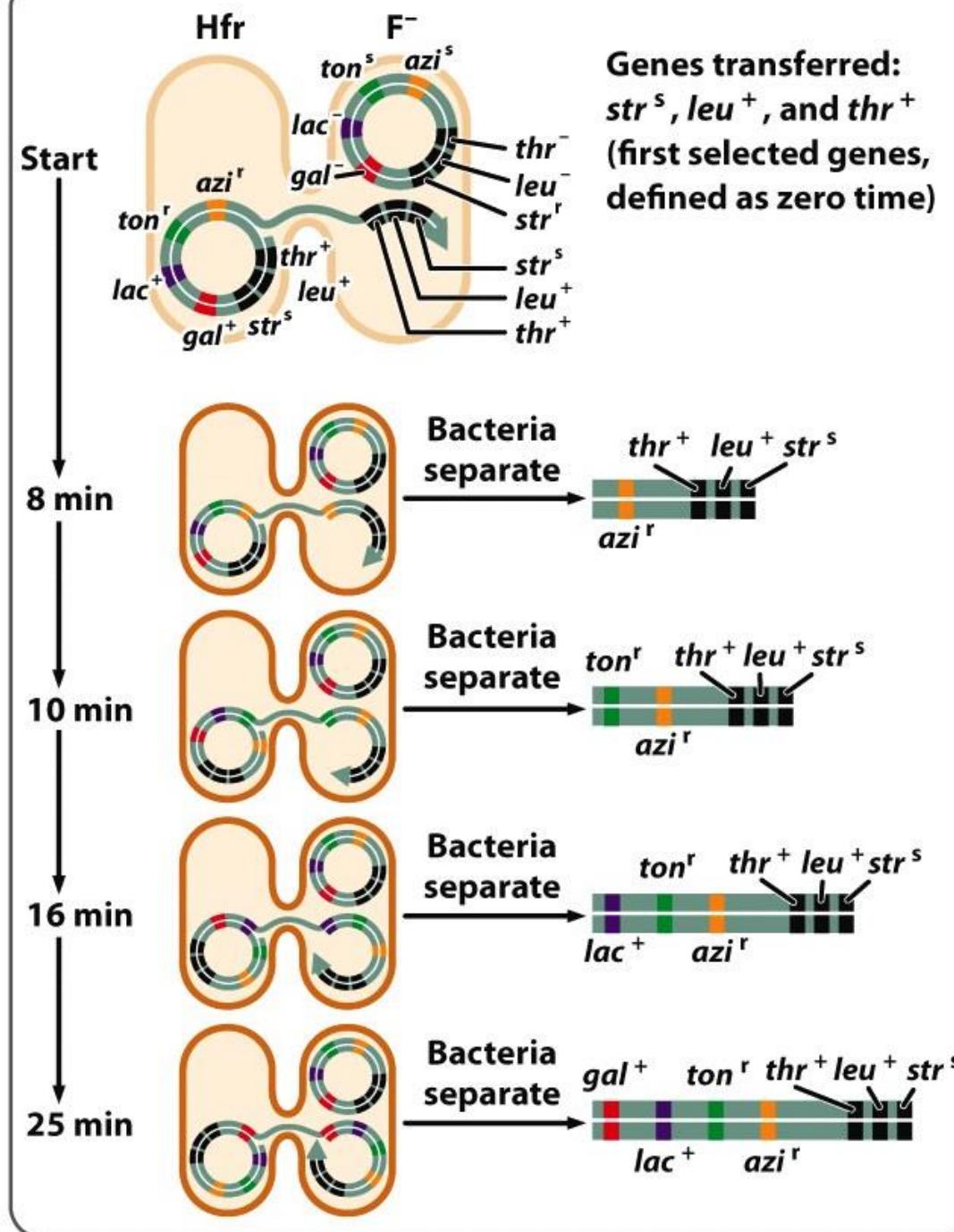


partial diploids = merozygotes

F' cells can conjugate with F<sup>-</sup> cells.

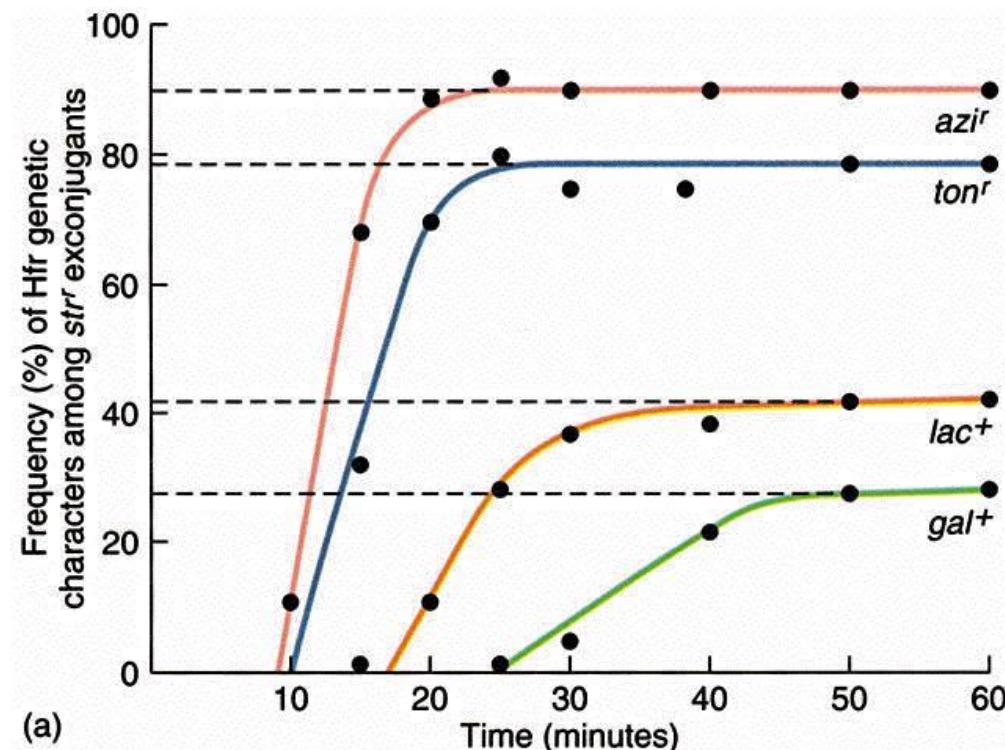
- Interrupted conjugation can be used in mapping. To transfer entire E. coli chromosome = 100min.
- If transfer interrupted then not all the chrom will be in recipient. Chrom transfer starts with F factor and proceeds in one direction, so time indicates relative distances, and is measured in minutes.

azi – sodium azide (S/R)  
ton – T1 phage (S/R)

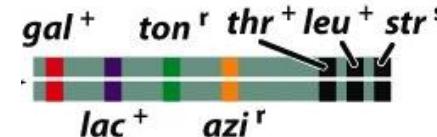


# Interrupted Conjugation Mapping

- Chromosome transfer from the Hfr into the F<sup>-</sup> is slow: about 100 minutes to transfer entire chromosome.
- The conjugation process can be interrupted by agitation (using a kitchen blender).
- By interrupting the mating at various times, can determine the proportion of F<sup>-</sup> cells that have received a given marker.

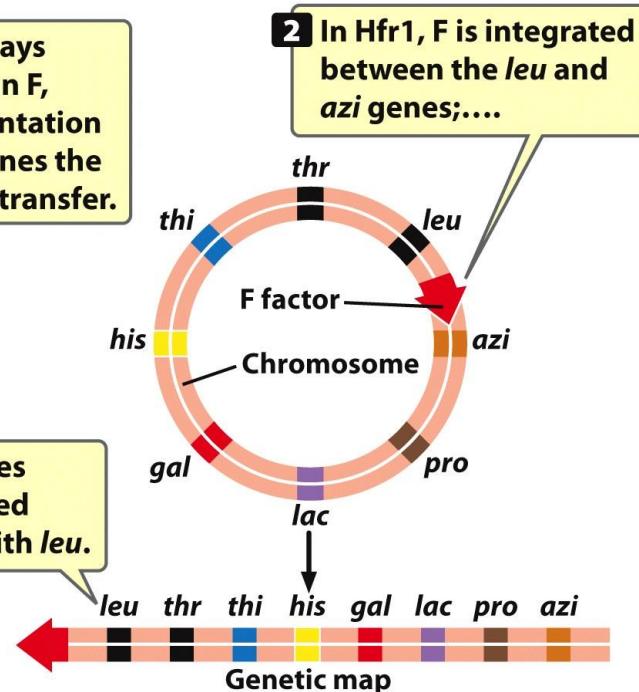


This technique can be used to make a map of the circular bacterial chromosome.



## Hfr1

1 Transfer always begins within F, and the orientation of F determines the direction of transfer.



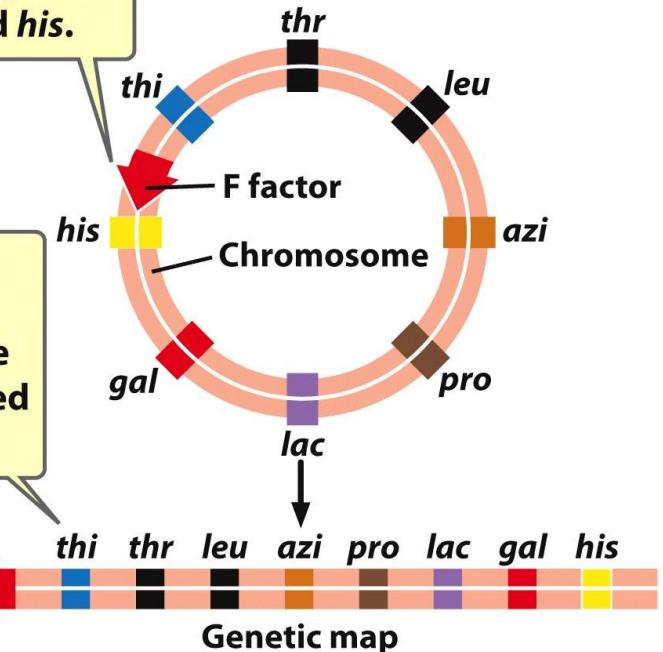
2 In Hfr1, F is integrated between the *leu* and *azi* genes;....

3 ...so the genes are transferred beginning with *leu*.

F factor orientation determines direction of gene transfer, site & orientation differs between Hfr strains

## Hfr5

4 In Hfr5, F is integrated between *thi* and *his*.



5 F has the opposite orientation in this chromosome; so the genes are transferred beginning with *thi*.

Still get the same relative distances between genes and same order!

First evidence that bacterial chromosome is circular!

Figure 8.16a  
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**Table 8.2****Characteristics of *E. coli* cells with different types of F factor**

Type	F Factor Characteristics	Role in Conjugation
F <sup>+</sup>	Present as separate circular DNA	Donor
F <sup>-</sup>	Absent	Recipient
Hfr	Present, integrated into bacterial chromosome	High-frequency donor
F'	Present as separate circular DNA, carrying some bacterial genes	Donor

**Table 8.2**

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**Table 8.3****Results of conjugation between cells with different F factors**

Conjugating	Cell Types Present after Conjugation
$F^+ \times F^-$	<b>Two <math>F^+</math> cells (<math>F^-</math> cell becomes <math>F^+</math>)</b>
$Hfr \times F^-$	<b>One Hfr cell and one <math>F^-</math> (no change)*</b>
$F' \times F^-$	<b>Two <math>F'</math> cells (<math>F^-</math> cell becomes <math>F'</math>)</b>

\*Rarely, the  $F^-$  cell becomes  $F^+$  in an  $Hfr \times F^-$  conjugation if the entire chromosome is transferred during conjugation.

Table 8.3

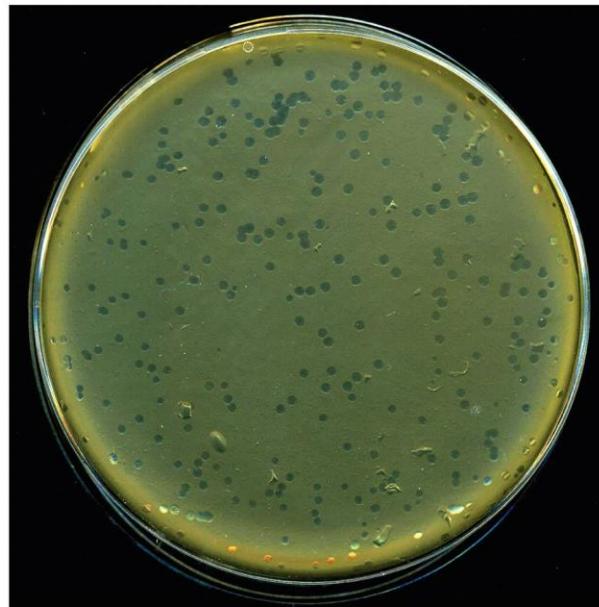
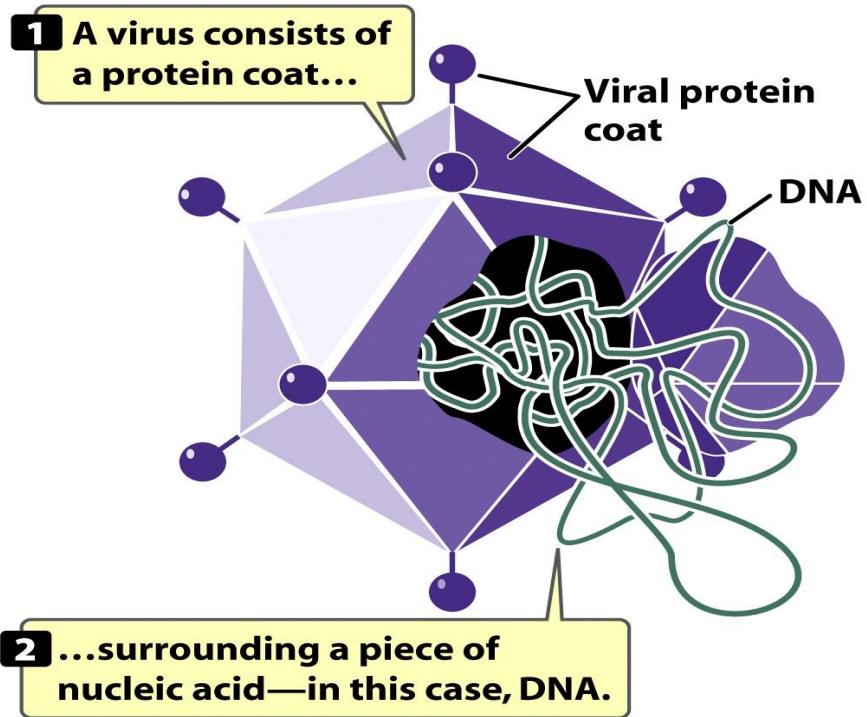
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R plasmids can also be transferred by conjugation and genes on these can confer antibiotic resistance.

# Bacteriophage

- Viruses can infect all organisms. Comprise protein coat and internal nucleic acid. Bacterial viruses = bacteriophage or phage. Protein coat binds to bacterial surface, then injects the phage NA.
- Phage only reproduce inside host cell. When plate bacteria with phage, will get infection and cell death resulting in plaques on lawn of bacteria...



# Phage “life” cycles: lytic or lysogenic

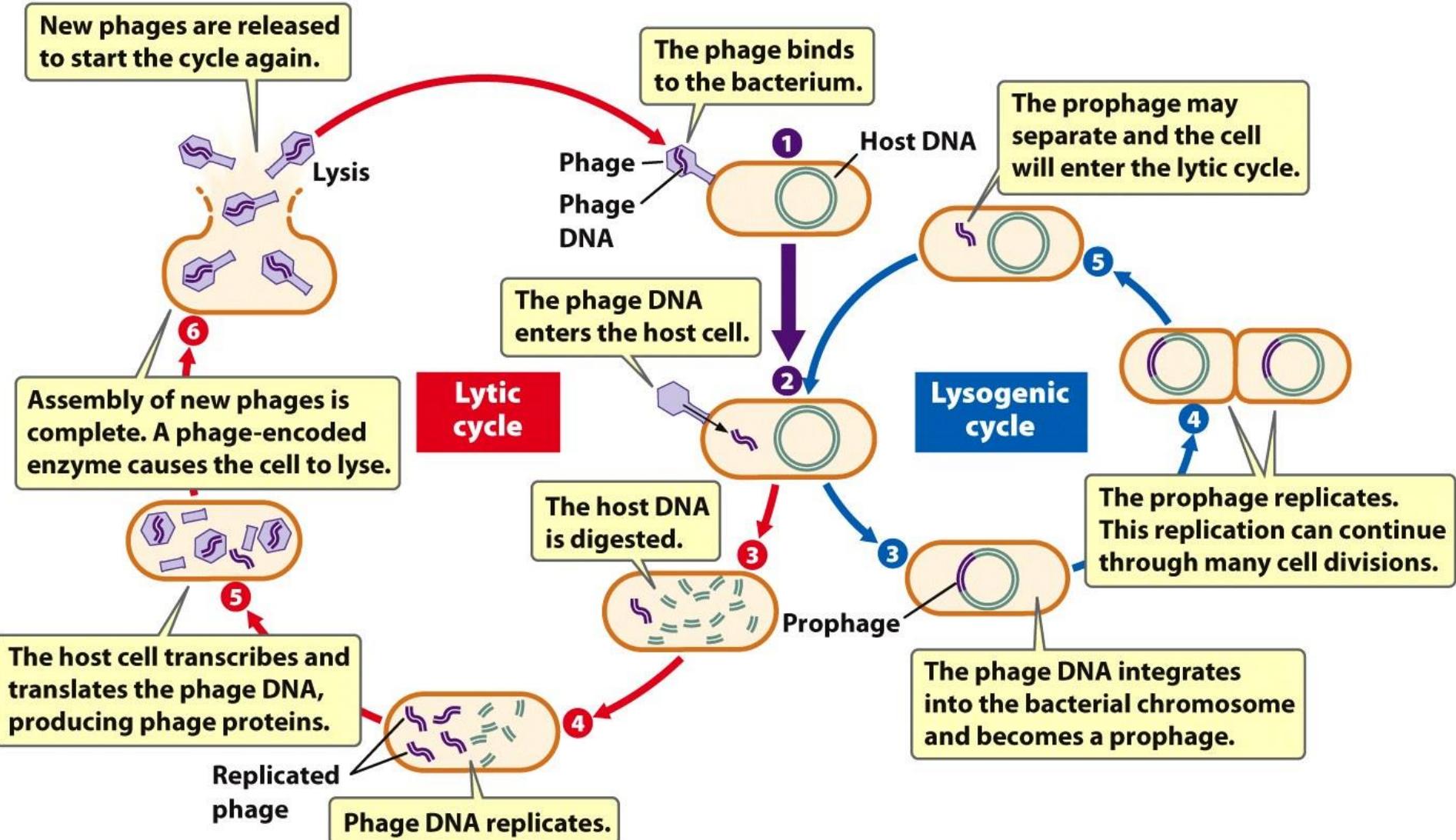


Figure 8.21

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# Transduction

Process of moving bacterial DNA from one cell to another using phage.

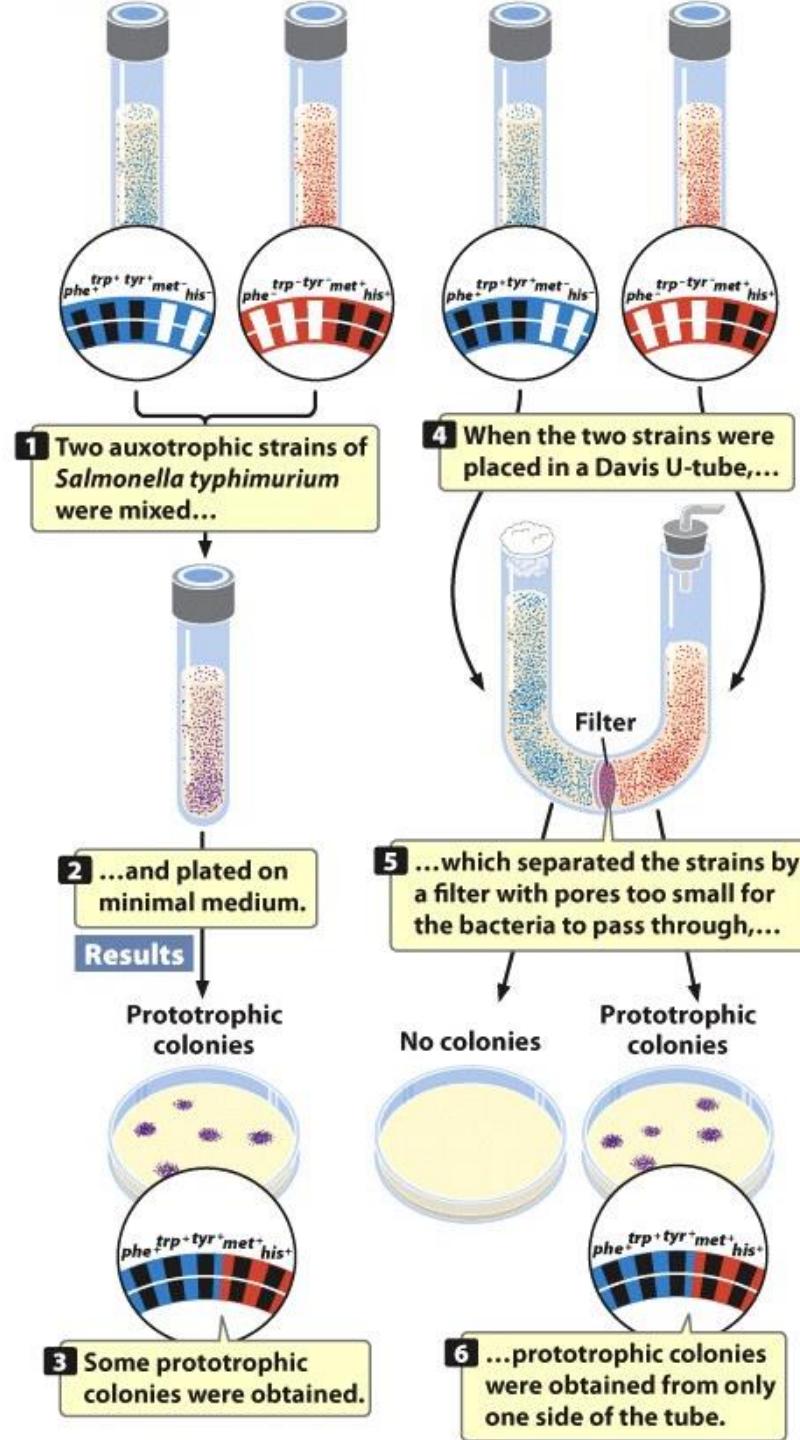
Two forms of transduction:

1. generalised: any piece of the bacterial genome can be transferred
2. specialised: only specific pieces of the chromosome can be transferred

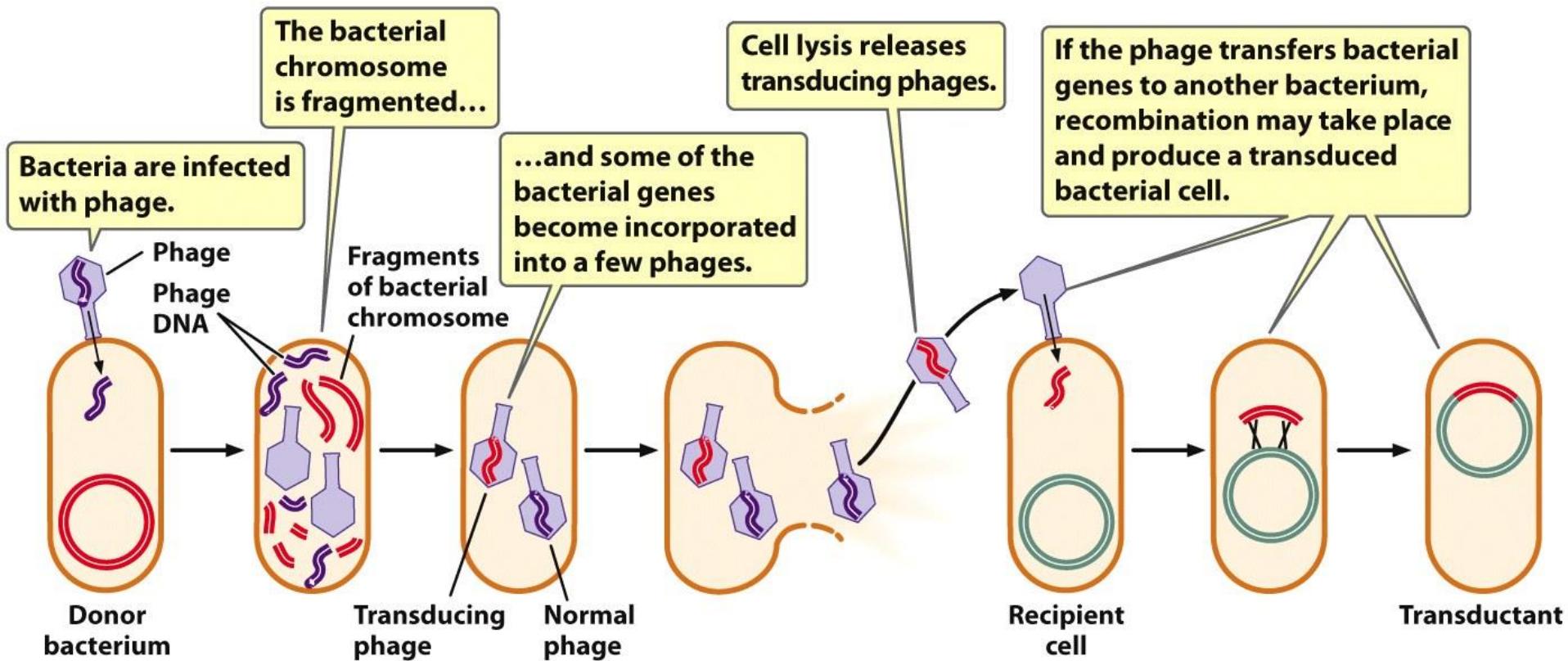
Most bacteriophage have a limited host range, so transduction is normally between bacteria of the same or closely-related species.

# Generalised Transduction

- 1952 – Lederberg and Zinder: Can bacterial genes exchange without cell-cell contact?
- Genetic exchange did NOT take place through conjugation. A phage was the agent of transfer = transducing phage, and the recombinant bacteria are transductants.



# Generalised Transduction



In lytic cycle, bacterial chromosome broken into fragments and some phage will take up a piece of this DNA into phage coat. The phage then infects another cell and by double crossover the genes integrate.

# Generalised Transduction

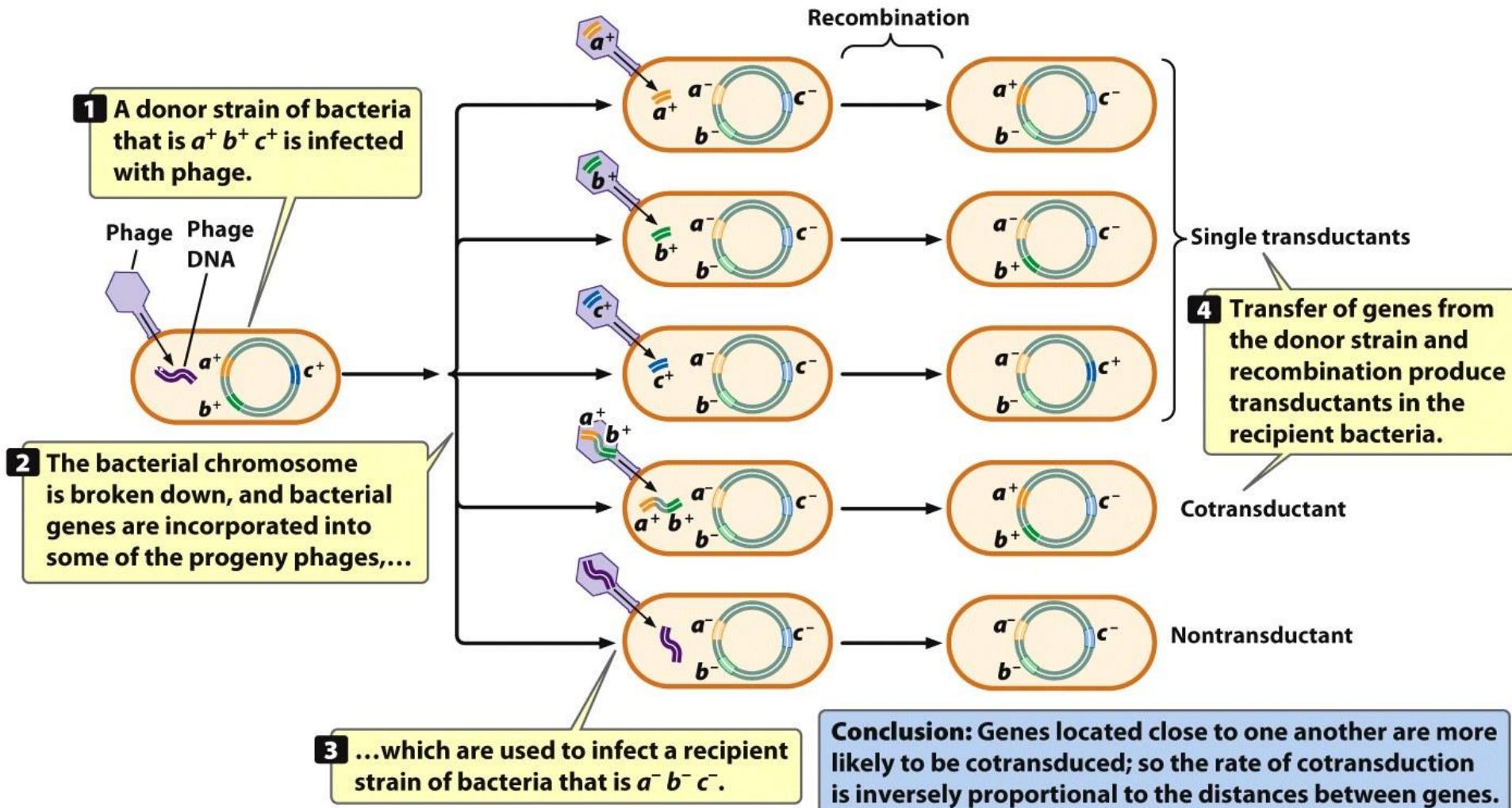
Transduction is rare (1/100,000 to 1/1,000,000)  
and requires:

phage degrades the bacterial chromosome  
phage packages this bacterial DNA  
transferred bacterial genes recombine with

recipient cell's chromosome

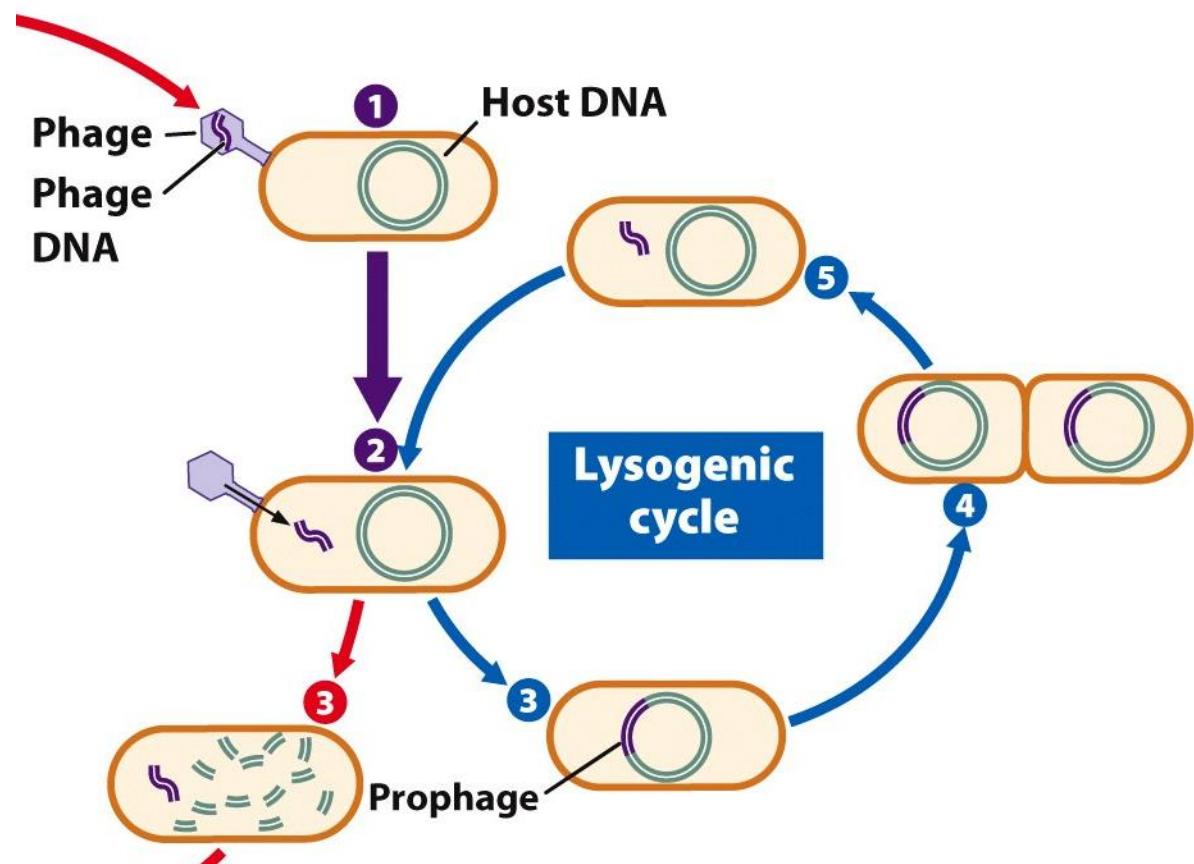
- Due to phage size, only  $\pm 1\%$  of bacterial chromosome can be transduced, thus only genes close together on chromosome transferred together = cotransduced.
- Very unlikely to get two separate transductions together. SO, the rate of cotransduction indicates physical distances between bacterial genes...

# Generalised Transduction to Map Genes

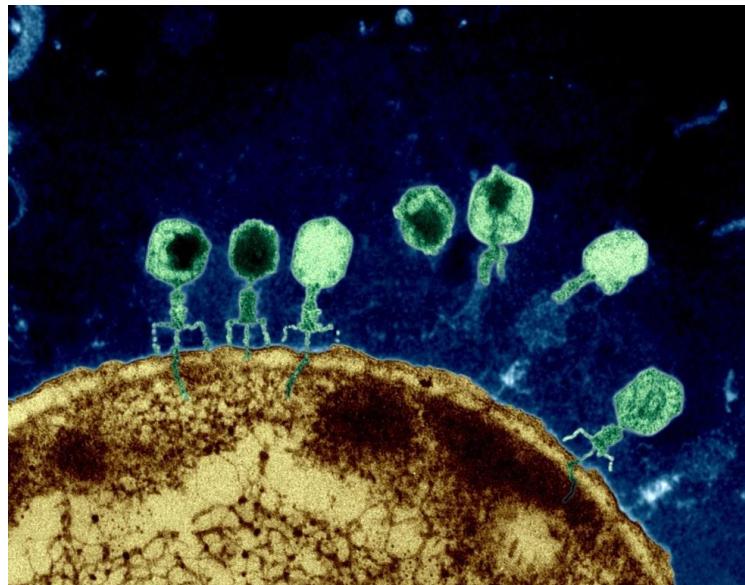


# Specialised Transduction

- Only genes near particular sites on chromosome are transferred, requires lysogenic bacteria.
- When prophage excised, takes some bacterial chromosome with it, which can then be injected into another bacterium ... similar to F' cells where F plasmid carries genes from one cell to another.



# Medicine turns to bacteriophage therapy to beat superbugs



<http://www.smh.com.au/national/health/medicine-turns-to-bacteriophage-therapy-to-beat-superbugs-20160123-gmcjfl.html>

# Transformation

Requires the uptake of DNA from outside the cell and its integration into the bacterial chromosome (or plasmid).

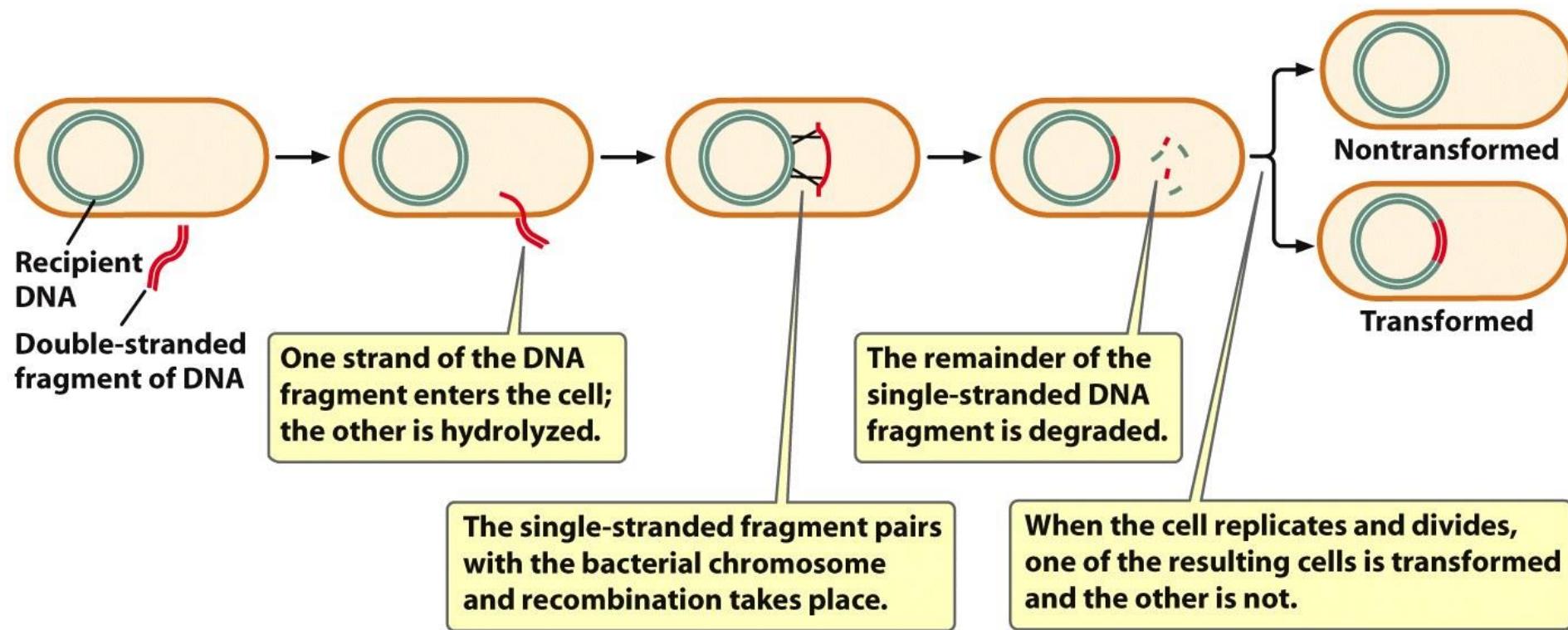


Figure 8.18

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# Transformation

- Competent cells can take up DNA through their cell membrane.
- Level of competence differs:
  - species
  - growth stage
  - [DNA] in environment
  - environmental factors
- Virtually any type of DNA (not just bacterial) can be taken up. When the DNA enters the cell one strand is broken up, while the other strand moves across the membrane where it can be integrated by homologous recombination into the chromosome.
- Any remaining SS DNA is degraded.
- In the lab, transformation of DS DNA plasmid is often done

# Transformation

Used in laboratory to introduce DNA into bacteria. Strains developed which are more competent.

**Chemical transformation:** Treatment with  $\text{CaCl}_2$ , heat or electrical field increases the permeability of the cell membrane to enhance DNA uptake. Enable usually incompetent cells (such as *E. coli*) to be transformed.

**Electroporation:** electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing DNA to be introduced into the cell.



# Transformation Mapping

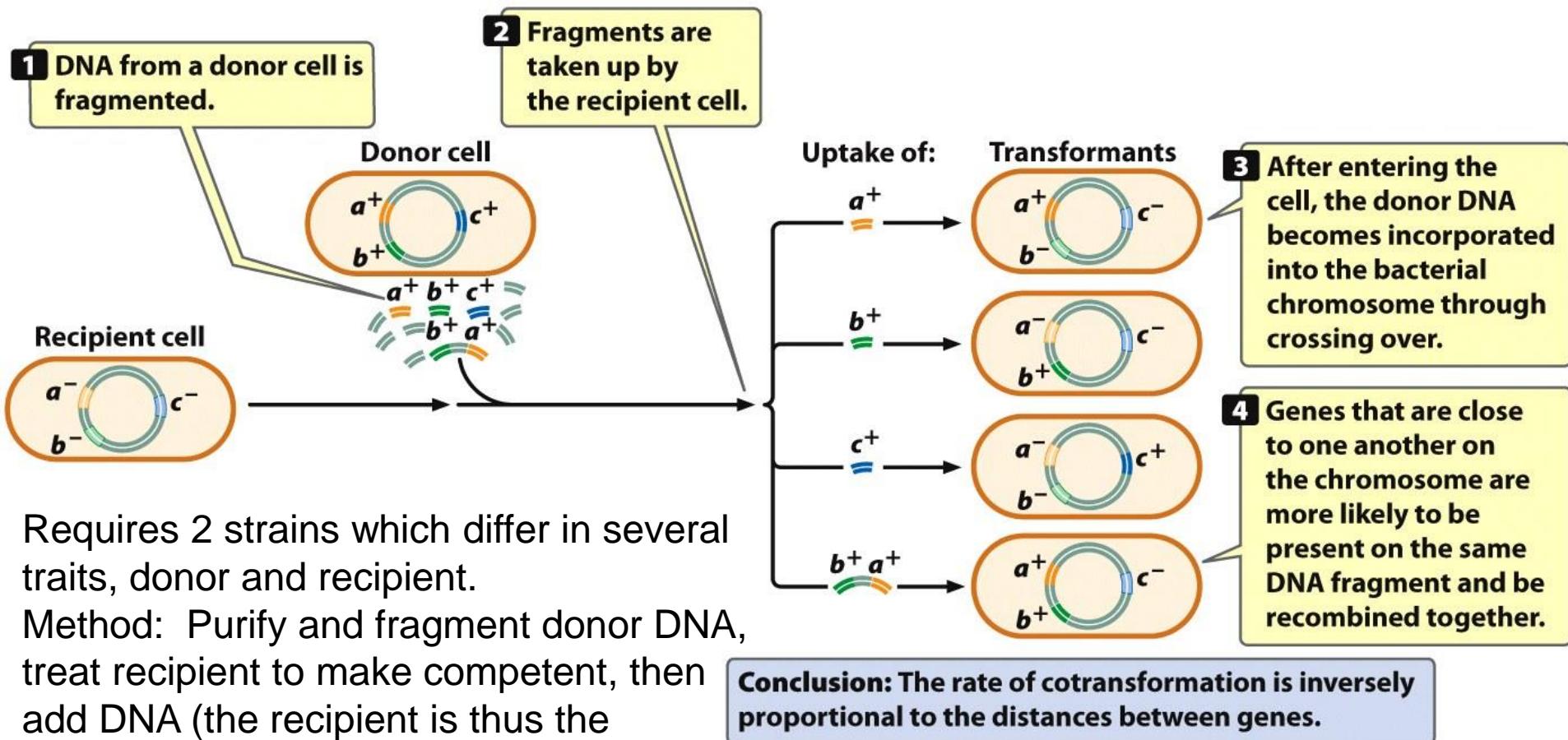


Figure 8.19  
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Transformation - recombinant DNA...

# Transformation

Scientists use bacterial transformation to introduce genes of interest and make multiple copies of these quickly and inexpensively.

Plasmids have been modified to enable insertion of genes in specific positions using restriction enzymes and DNA ligase (multiple cloning sites)

Lab class:

Transform E. coli and look to see if a protein encoded by a gene of interest (GFP) is expressed.

Listen to prelab video and learn how plasmid DNA is transferred via transformation