Sem- IV
MICROBIOLOGY CORE (MBIO CC408)

UNIT 3- Mechanism of Genetic Exchange

(Mapping of genome by conjugation, transduction and transformation)

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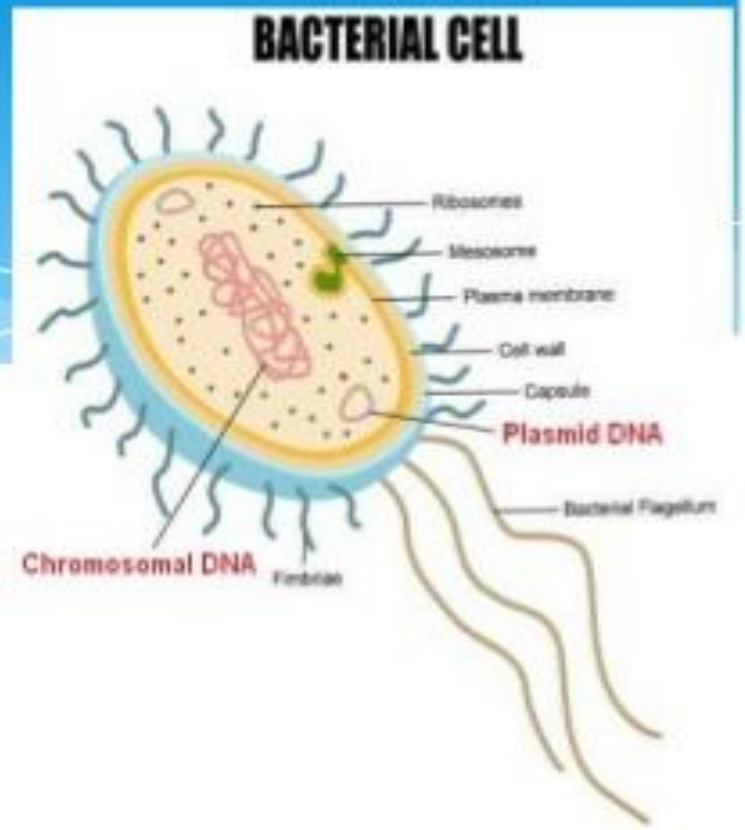
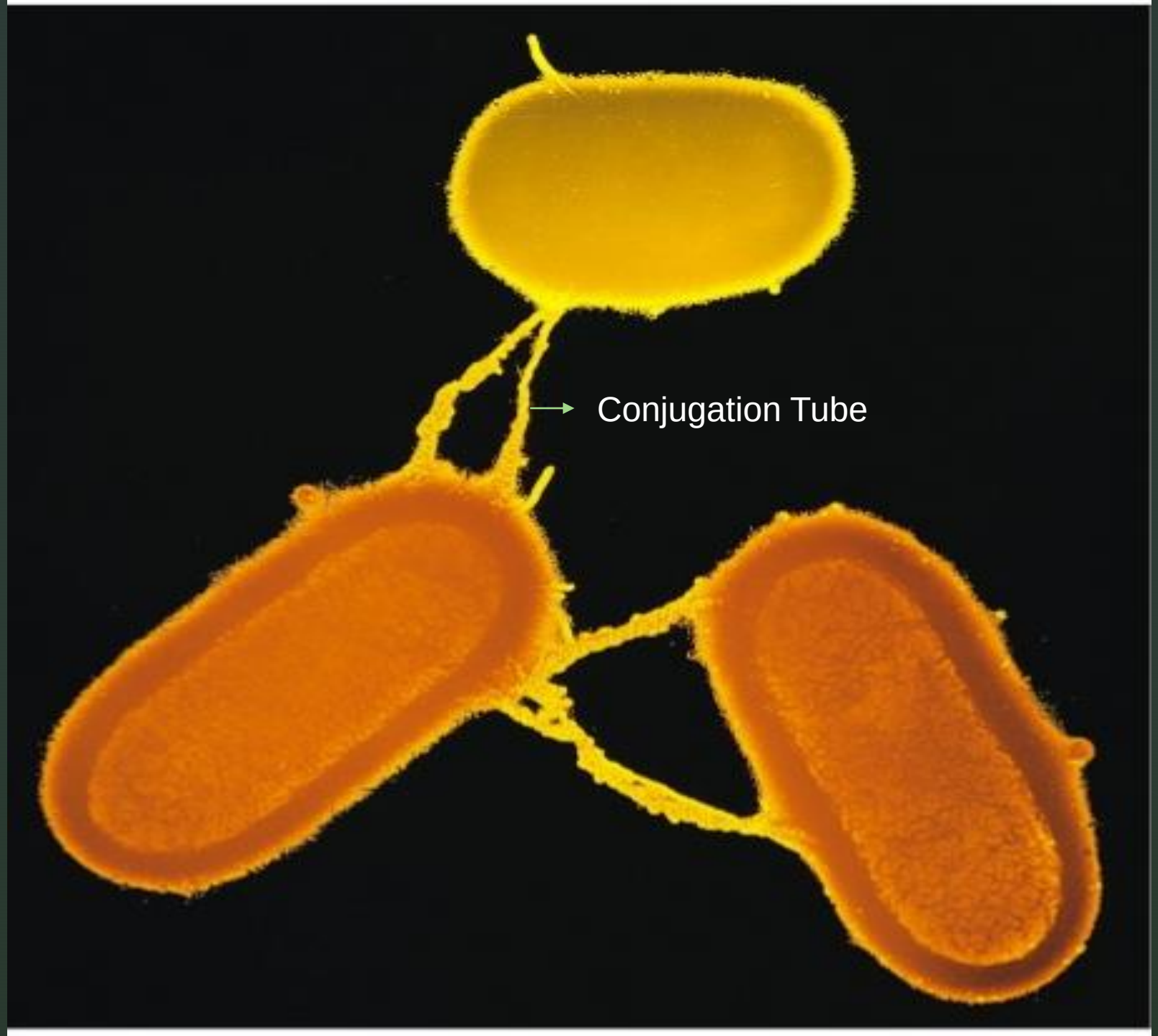
Genetic Transfer

Enhances genetic diversity

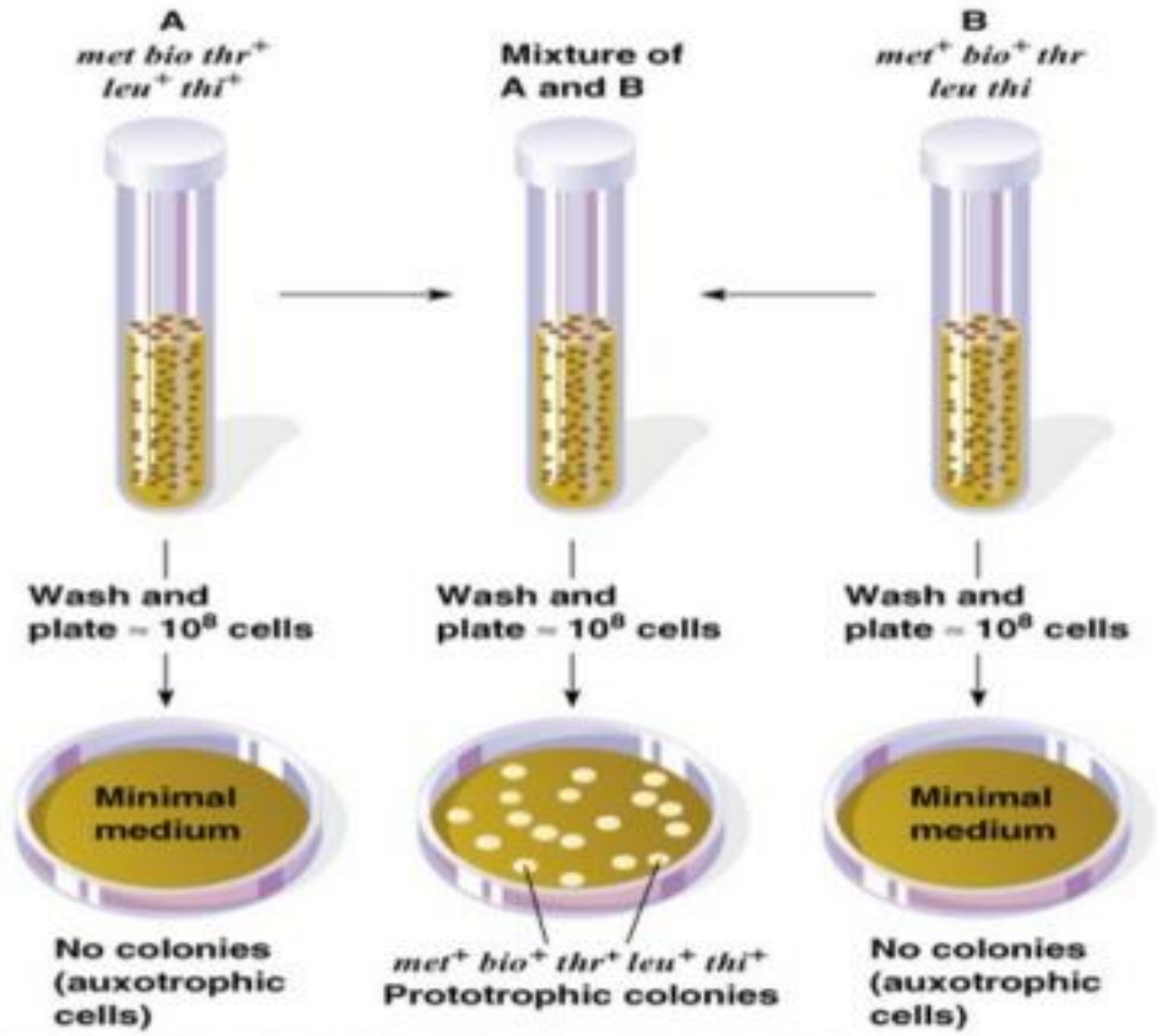
- Types of transfer
  - Conjugation
    - direct physical contact & exchange
  - Transduction
    - phage
  - Transformation
    - uptake from environment




Conjugation Tube

Lederberg & Tatum (1946)
Experiment demonstrating recombination in *E. coli.*

- Recombination of 2 complimentary auxotrophs gives rise to a strain that can synthesize all nutrients.



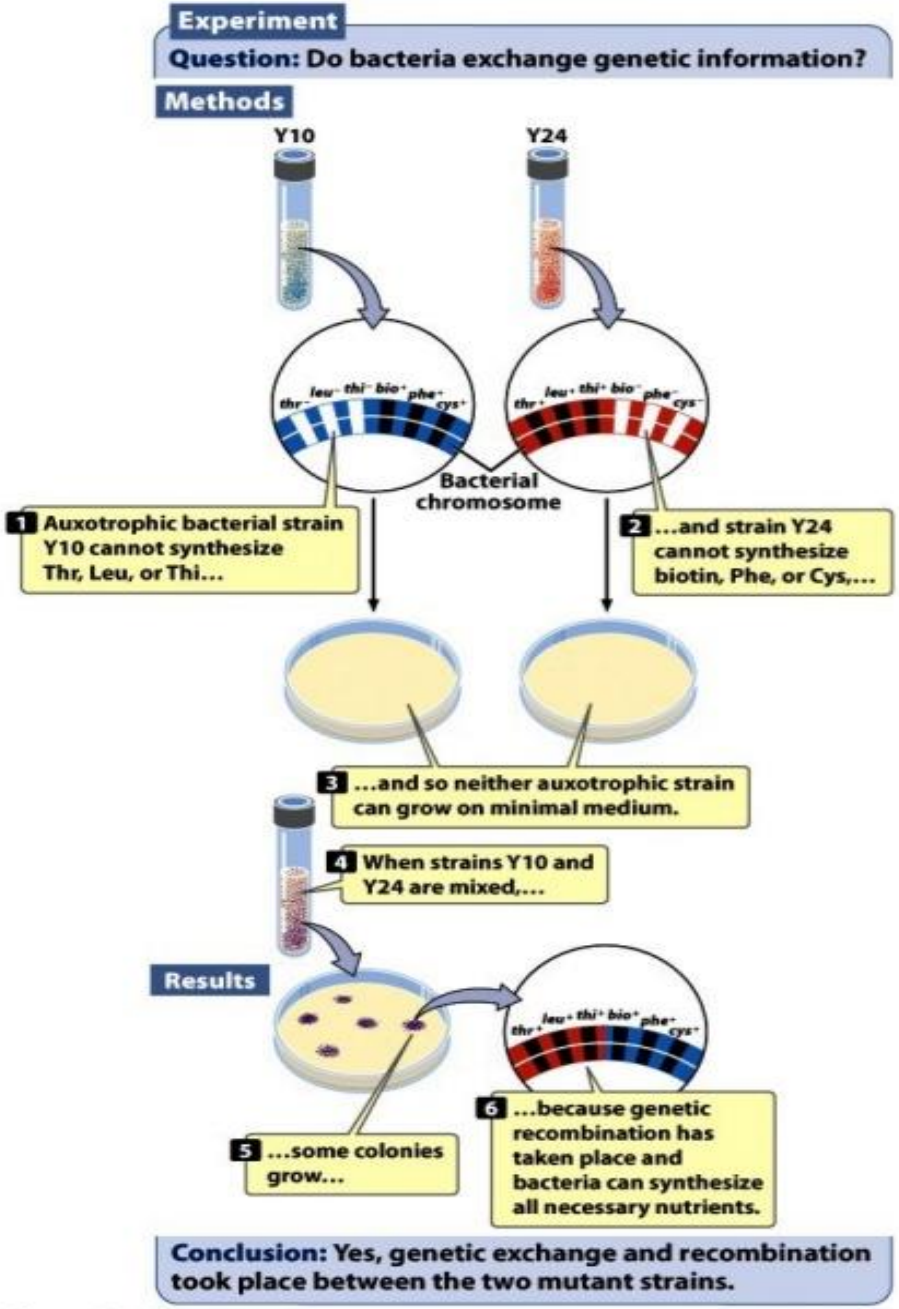


Figure 8-10
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Conjugation-transfer of the sex factor *F*:

1. William Hayes (1953) demonstrated that genetic exchange in *E. coli* occurs in only one direction.

2. Genetic transfer is mediated by sex factor *F*.

3. Donor is F^+ and recipient is F^-.

4. *F* is a self-replicating, circular DNA plasmid (1/40 the size of the main chromosome).

5. *F* plasmid contains an origin sequence (*O*), which initiates DNA transfer. It also contains genes for hair-like cell surface (*F*-pili or sex-pili), which aid in contact between cells.

6. No conjugation can occur between cells of the same mating type.

7. Conjugation begins when the *F* plasmid is nicked at the origin, and a single strand is transferred using the rolling circle mechanism.

8. When transfer is complete, both cells are F^+ double-stranded

25 tra genes
Determines expression of sex pilli, synthesis, and transfer of DNA .

***Tra* genes are two types**:
Mating pair formation (MPF) also include Type IV secretion system by which DNA and proteins pass and

Dtr (involved in DNA transfer)

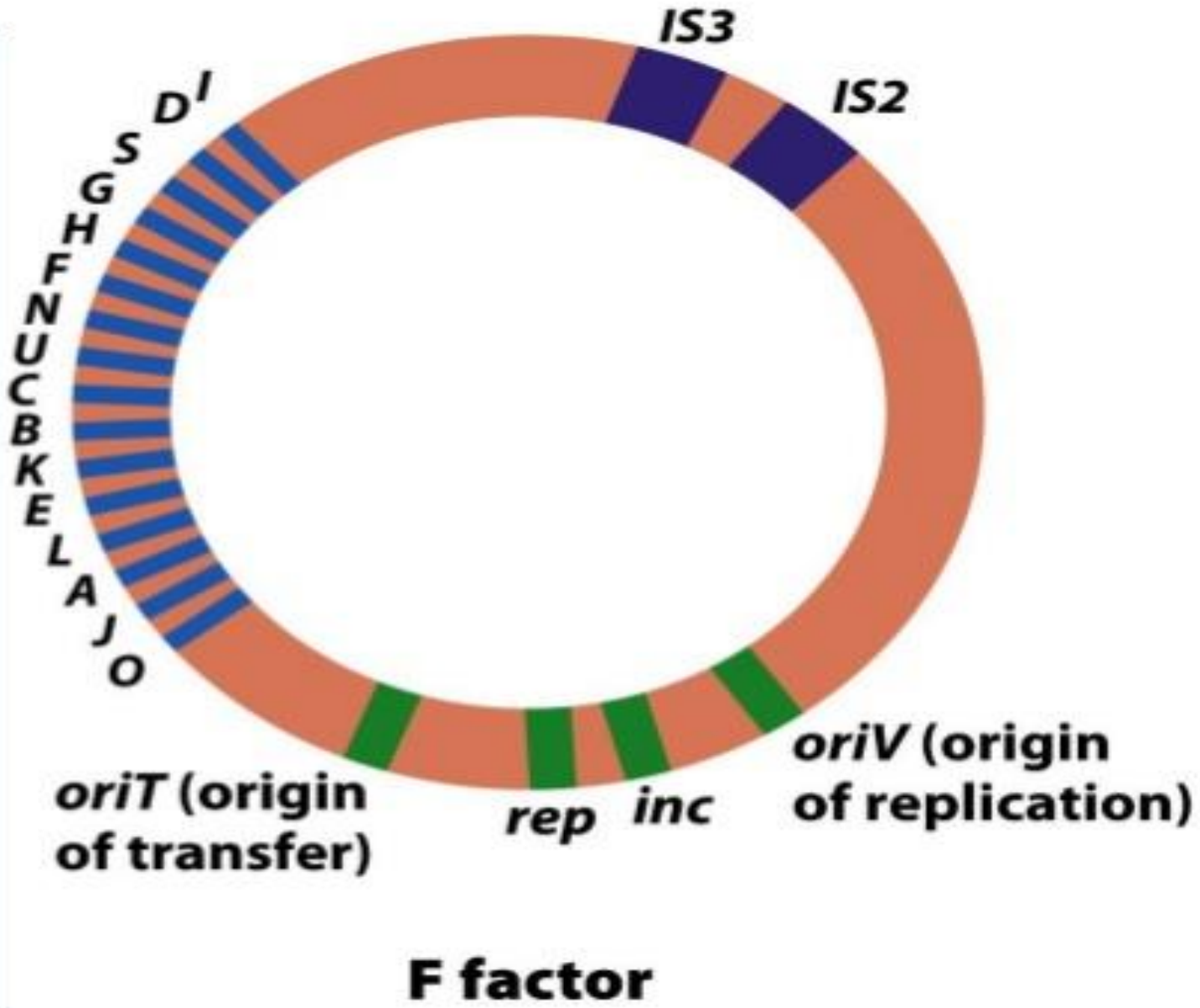

Figure 8-8
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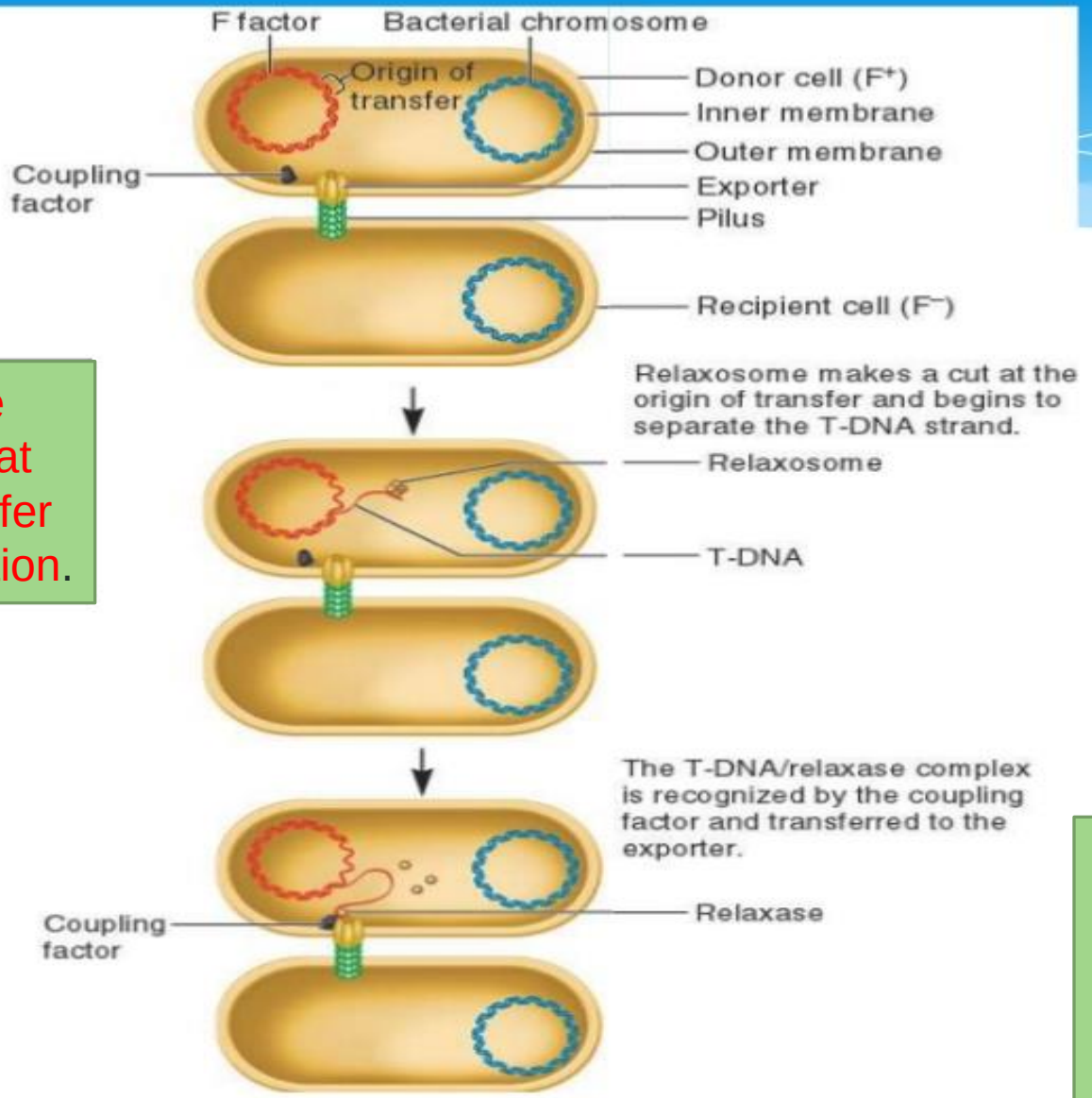
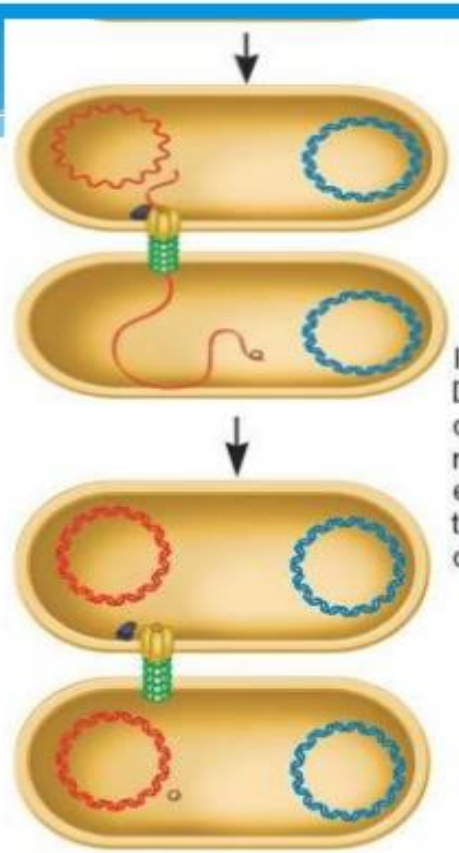
Conjugation



Relaxosomes are the complex of proteins that facilitates plasmid transfer during bacterial conjugation.

Coupling factors- group of protein which helps in the coupling of main components of the conjugation and actively mediate DNA transport.

Conjugation contd…..



The exporter pumps the T-DNA/relaxase complex into the recipient cell.

In the donor cell, the F factor DNA is replicated to become double stranded. In the recipient cell, relaxase joins the ends of the T-DNA strand. It is then replicated to become double stranded.

F^+ cell

F^+ cell

High-frequency recombination

1. No chromosomal DNA is transferred by standard sex factor *F*.

2. Transfer of chromosome DNA is facilitated by special strains of F^+ integrated into the bacteria chromosome by crossing over.

3. *Hfr* strains = high frequency recombination strains.

4. Discovered by William Hayes and Luca Cavalli-Sforza.

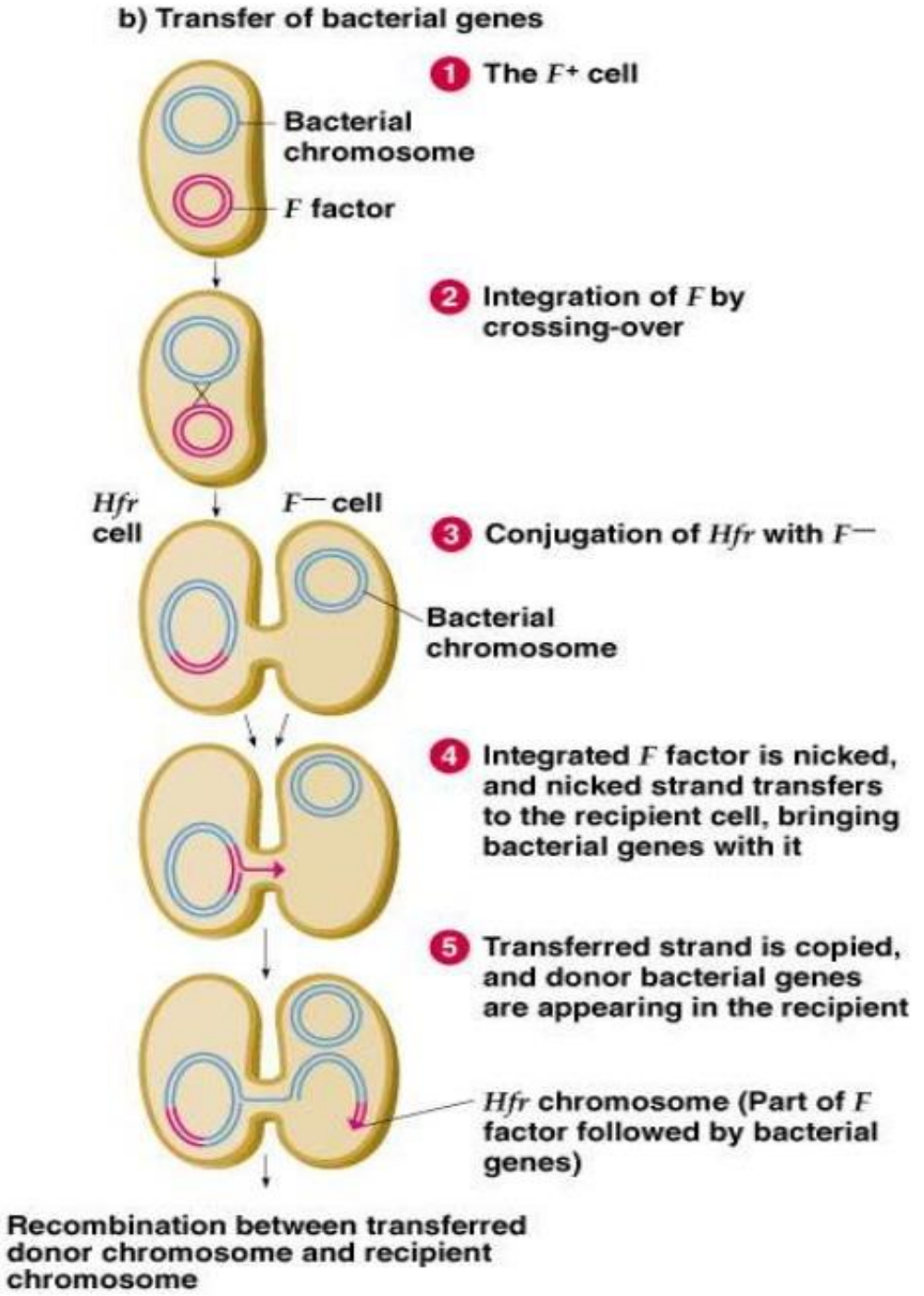
5. *Hfr* strains replicate *F* factor as part of their main chromosome.

6. Conjugation in *Hfr* strains begins when F^+ is nicked at the origin, and F^+ and bacteria chromosomal DNA are transferred using the rolling circle mechanism.

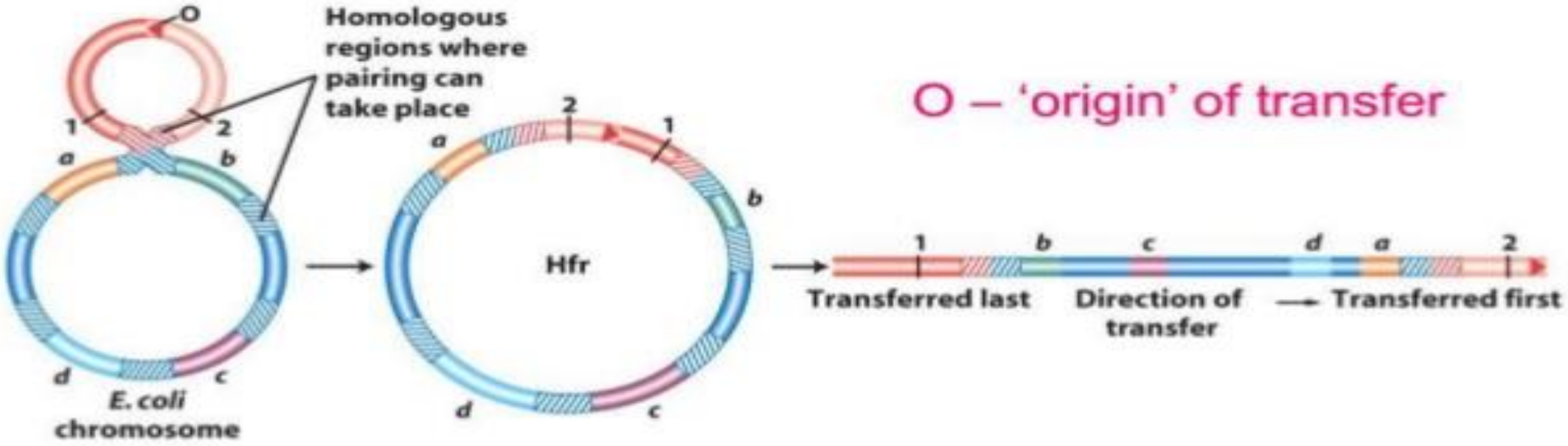
7. Complete F^+ sequence (or complete chromosomal DNA) is rarely transferred (1/10,000) because bacteria separate randomly before DNA synthesis completes.

8. Recombinants are produced by crossover of the recipient chromosome and donor DNA containing F^+

Transfer of the *Hfr* F^+ factor



Insertion of F factor into bacterial chromosome

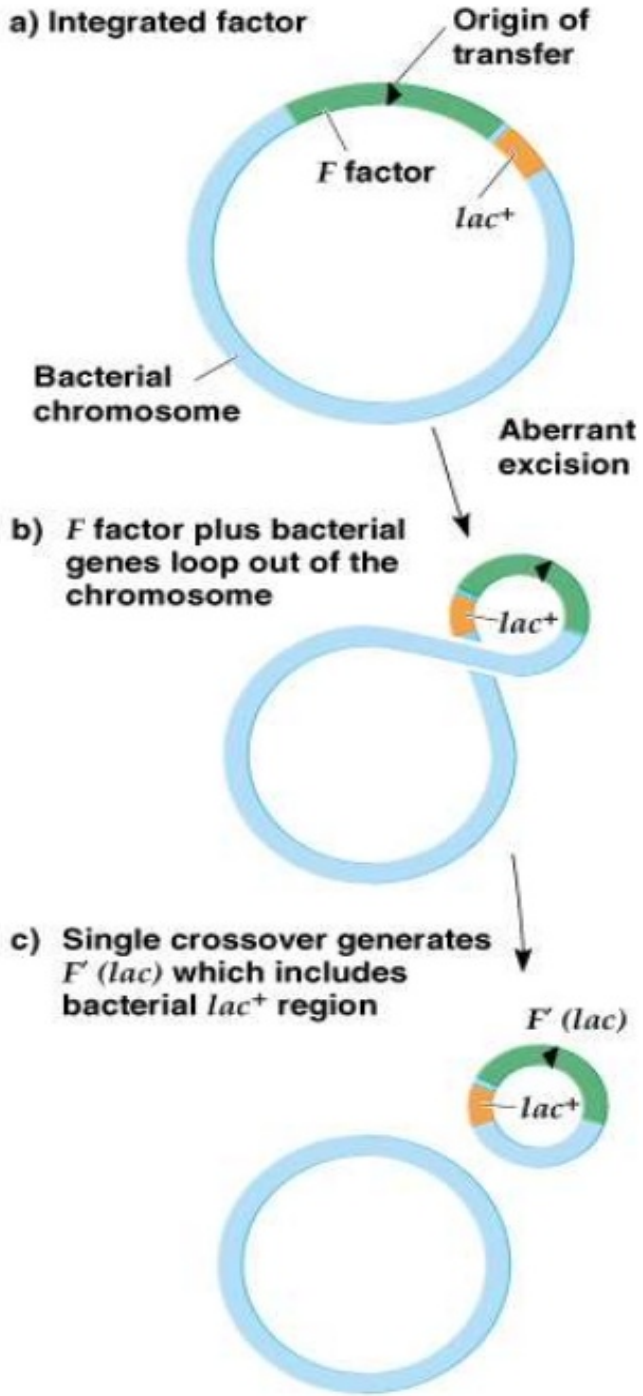


Excision of the F^+ factor also occurs spontaneously at low frequency.

1. Begin with *Hfr* cell containing F^+.

2. Small section of host chromosome also may be excised, creating an *F'* plasmid.

3. F' plasmid is named for the gene it carries, e.g., *F' (lac)*



Mapping By conjugation

Commonly used genetic markers

- Prototrophic markers: wild-type bacteria are prototrophs (grow on minimal medium)
- Auxotrophic markers: mutants that require additional nutrient (fail to grow on minimal medium)
- Antibiotic-sensitivity: wild-type bacteria are susceptible (fail to grow on antibiotic-containing medium)
- Antibiotic-resistance: mutants that grow in presence of antibiotic (grow on antibiotic-containing medium)

Using conjugation to map bacterial genes

1. Begin with appropriate *Hfr* strains selected from F^+ x F^- crosses and perform an interrupted mating experiment.

2. HfrH= thr+ leu+ azi^R ton^R lac+ gal+ str^R
   F^- = thr leu azi^S ton^s lac gal str^S

3. Mix 2 cell types in medium at 37°C.

Using conjugation to map bacterial genes

4. Remove at experimental time points and agitate to separate conjugating pairs.

5. Analyze recombinants with selective media.

6. Order in which genes are transferred reflects linear sequence on chromosomes and time in media.

7. Frequency of recombinants declines as donor gene enters recipient later.

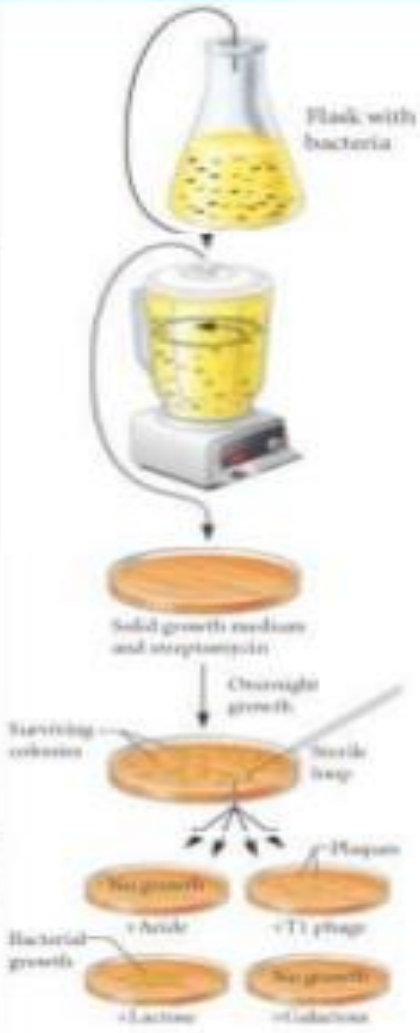
Interrupted Mating Technique

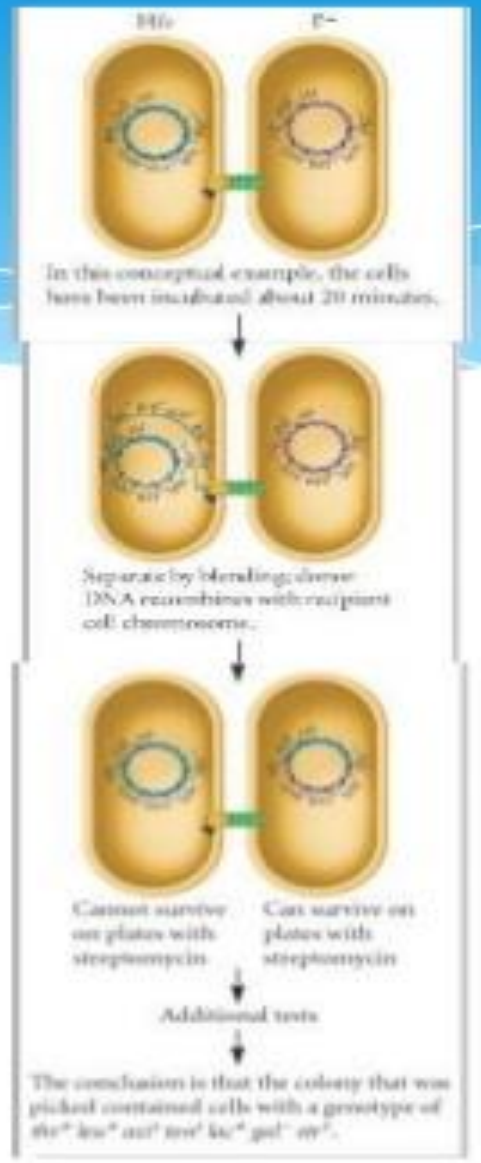
1. Mix together a large number of Hfr donor and F⁻ recipient cells.

2. After different periods of time, take a sample of cells and interrupt conjugation in a blender.

3. Plate the cells on growth media lacking threonine and leucine but containing streptomycin. Note: The general methods for growing bacteria in a laboratory are described in the appendix.

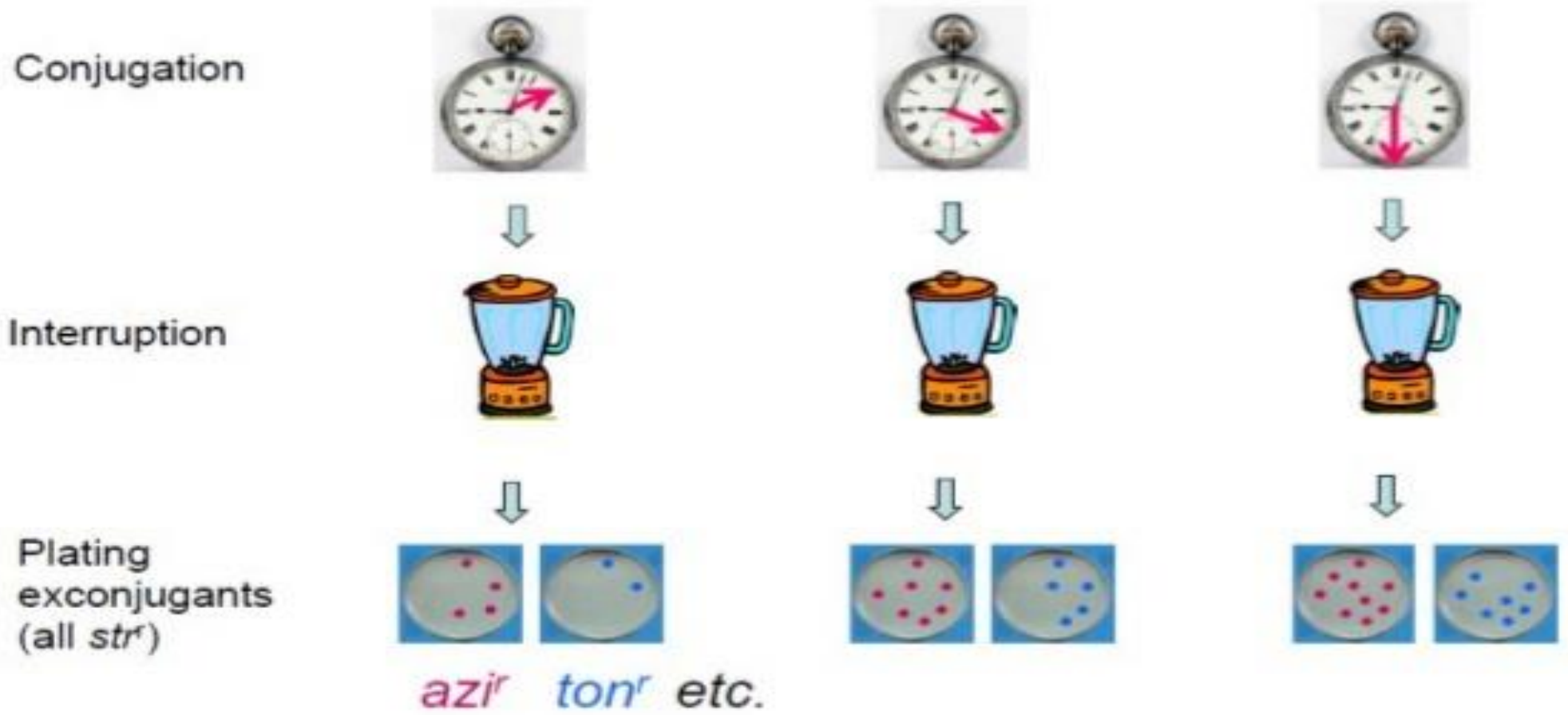
4. Pick each surviving colony, which would have to be thr^+ leu^+ str^r, and test to see if it is sensitive to killing by acids, sensitive to infection by T1 bacteriophage, and able to metabolize lactose or galactose.



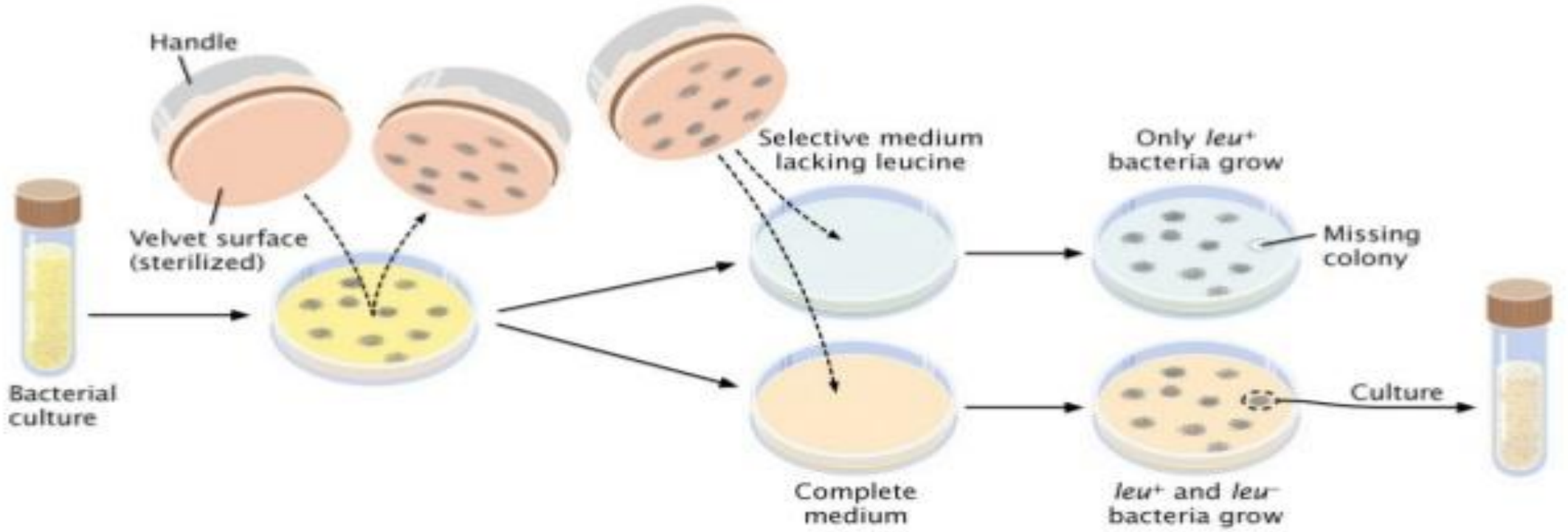


Interrupted-conjugation experiment of Jacob and Wollman (1957)

Hfr: *str*^{s} *azi*^{r} *ton*^{r} *lac*$^{+}$ *gal*$^{+}$ x F^{-}: *str*^{r} *azi*^{s} *ton*^{s} *lac*$^{-}$ *gal*$^{-}$



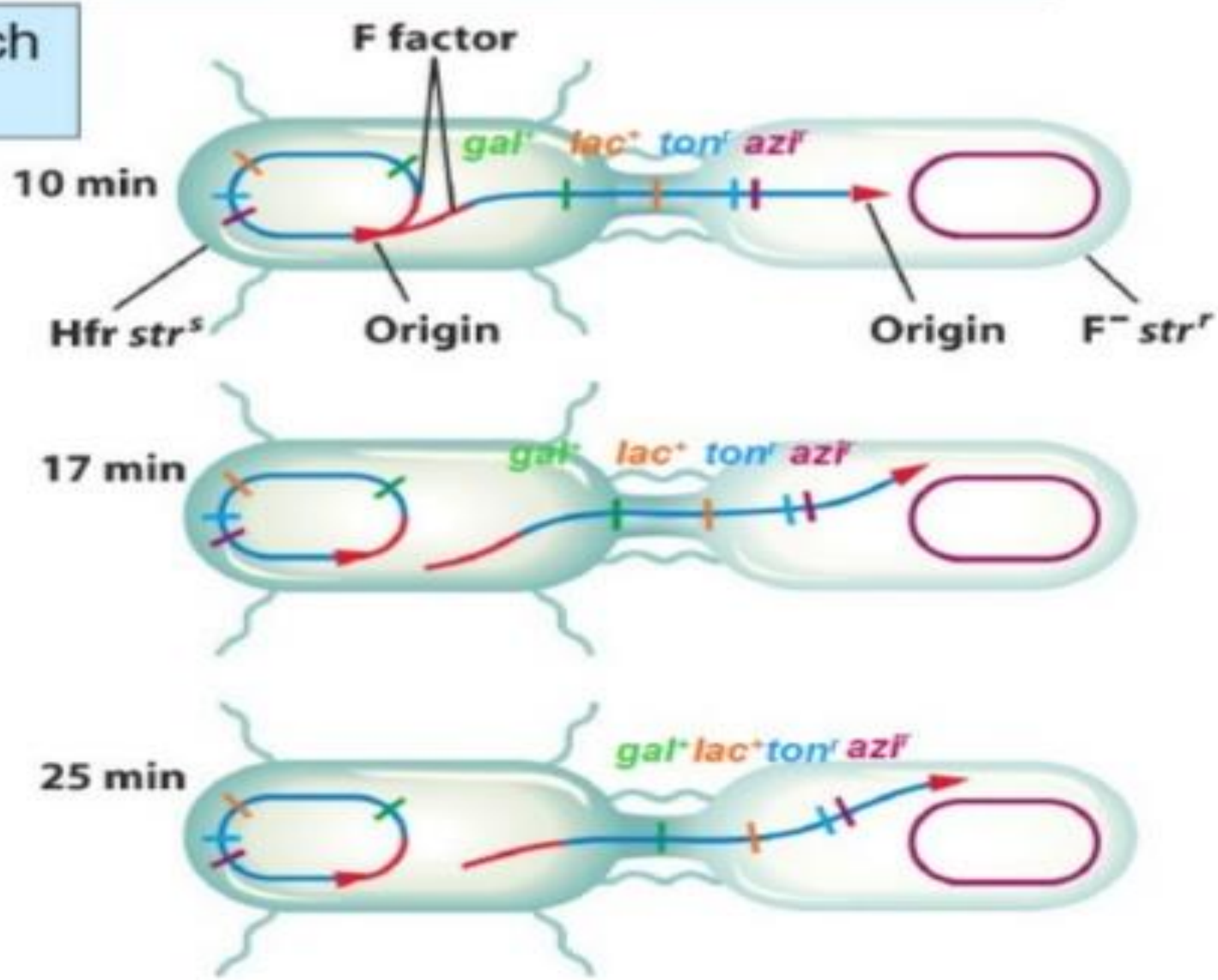
Testing for Nutritional Requirements



Replica plating transfers the pattern of bacterial colonies to test plates.

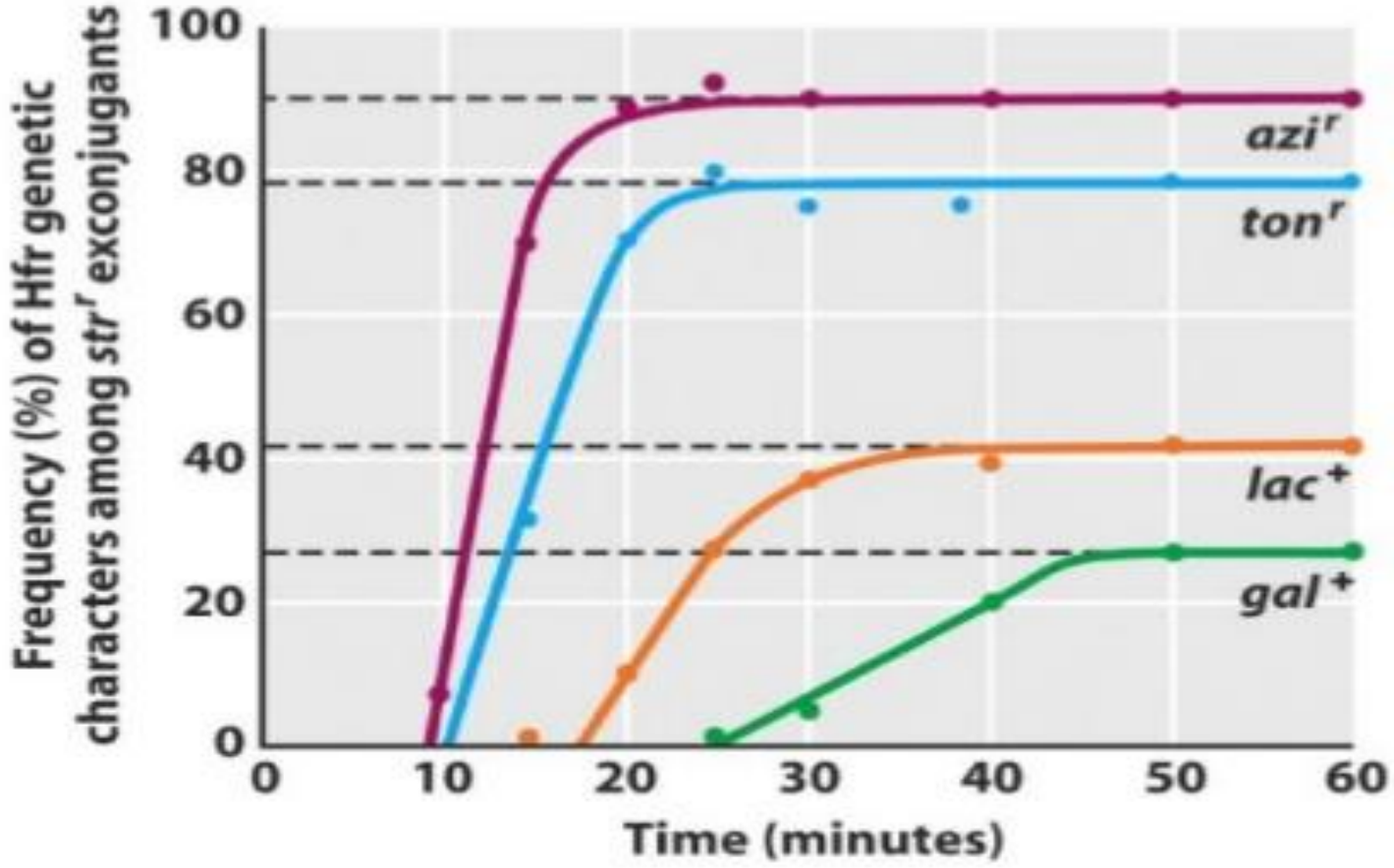
Transfer of the chromosome by Hfr during conjugation

freq. 10^{-4} per each Hfr cell

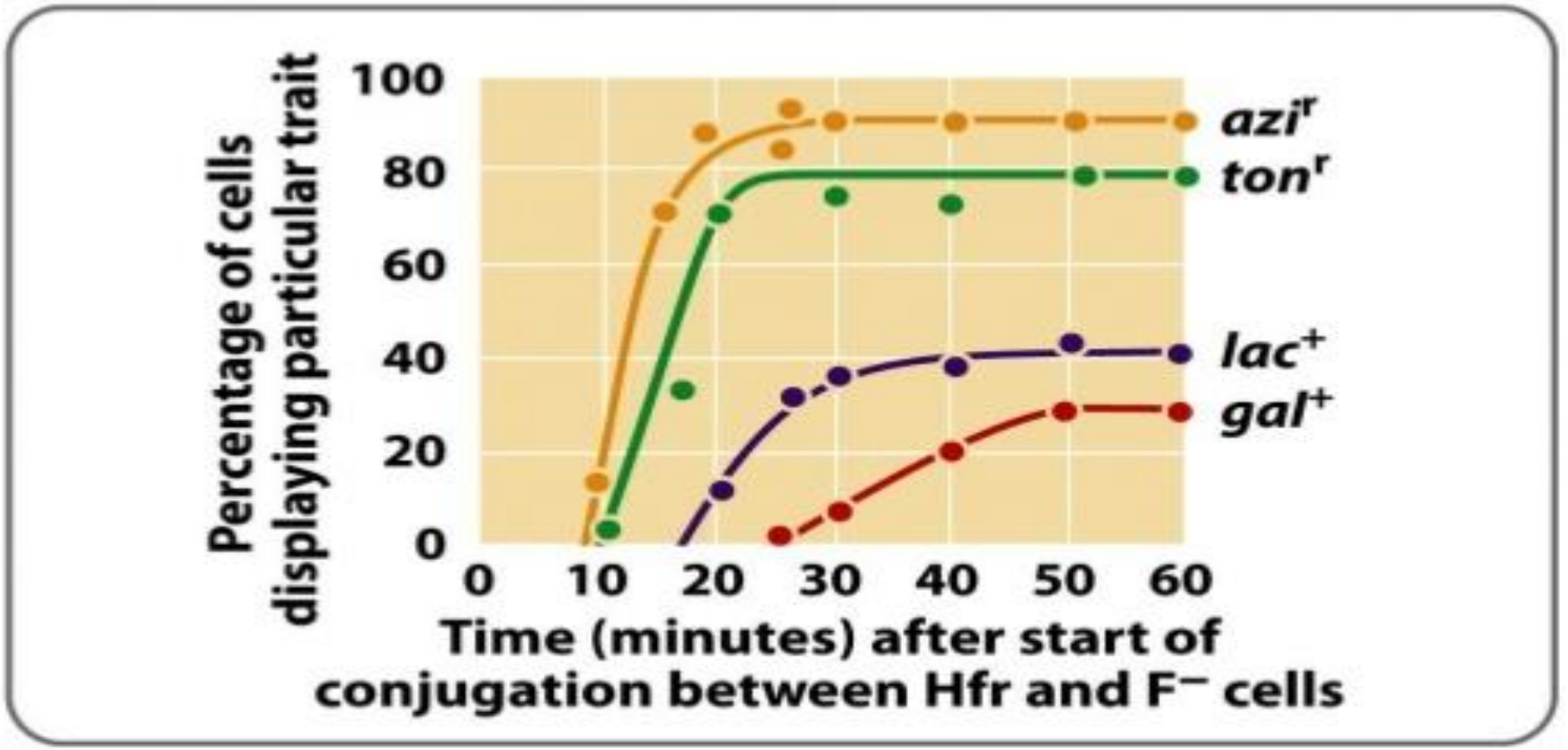


Interrupted-conjugation experiment of Jacob and Wollman (1957)

Hfr: str^s azi^r ton^r lac^+ gal^+ x F⁻: str^r azi^s ton^s lac^- gal^-



Results



Conclusion: The transfer times indicate the order and relative distances between genes and can be used to construct a genetic map.

Figure 8-17 part 2
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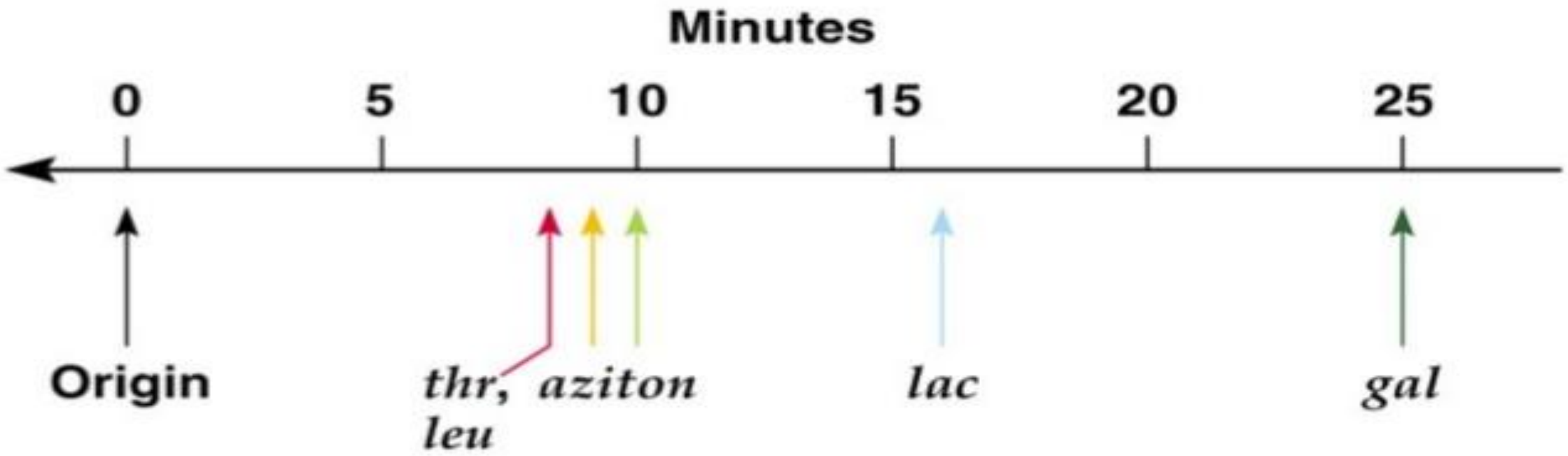
Genetic map-results of interrupted *E. coli* mating experiment.



Generating a map for all of *E. coli*

1. Location and orientation of the *Hfr* F^+ in the circular chromosome varies from strain to strain.

2. Overlap in transfer maps from different strains allow generation of a complete chromosomal map.

a) Orders of gene transfer

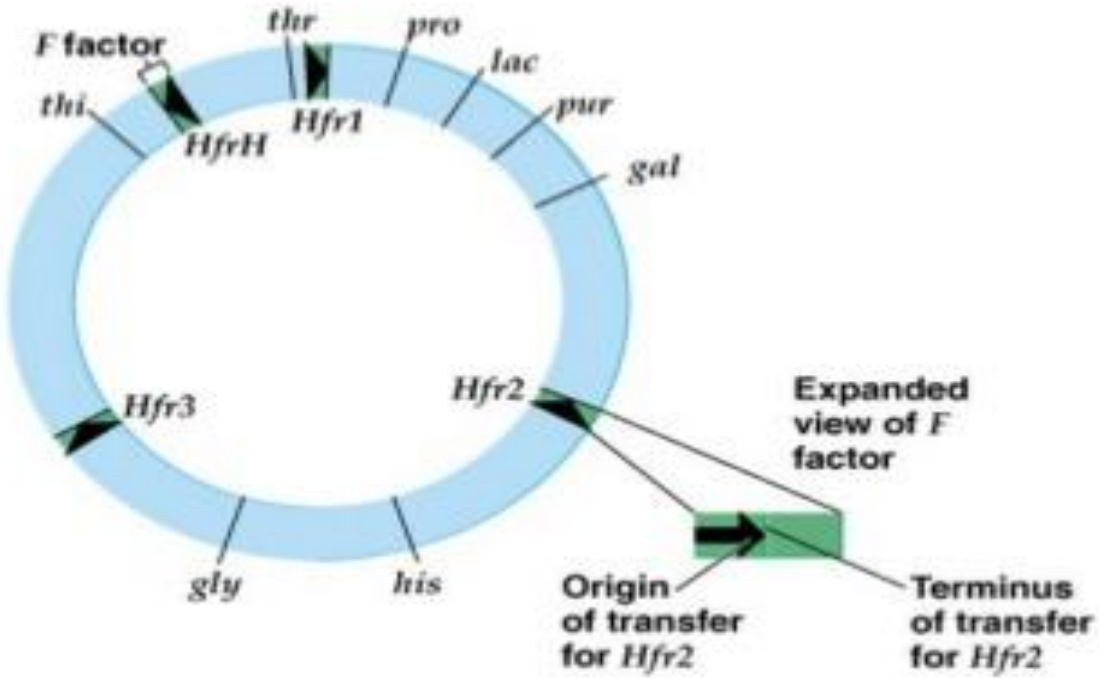
***Hfr* strains:**

H origin—thr–pro–lac–pur–gal
1 origin—thr–thi–gly–his
2 origin—his–gly–thi–thr–pro–lac
3 origin—gly–his–gal–pur–lac–pro

b) Alignment of gene transfer for the *Hfr* strains

H thr–pro–lac–pur–gal
1 his–gly–thi–thr
2 his–gly–thi–thr–pro–lac
3 pro–lac–pur–gal–his–gly

c) Circular *E. coli* chromosome map derived from *Hfr* gene transfer data



Problems on Interrupted mating

Problem no.1).

In *E. coli*, four Hfr strains donate the following genetic markers shown in the order donated:

Strain 1:	Q	W	D	M	T
Strain 2:	A	X	P	T	M
Strain 3:	B	N	C	A	X
Strain 4:	B	Q	W	D	M

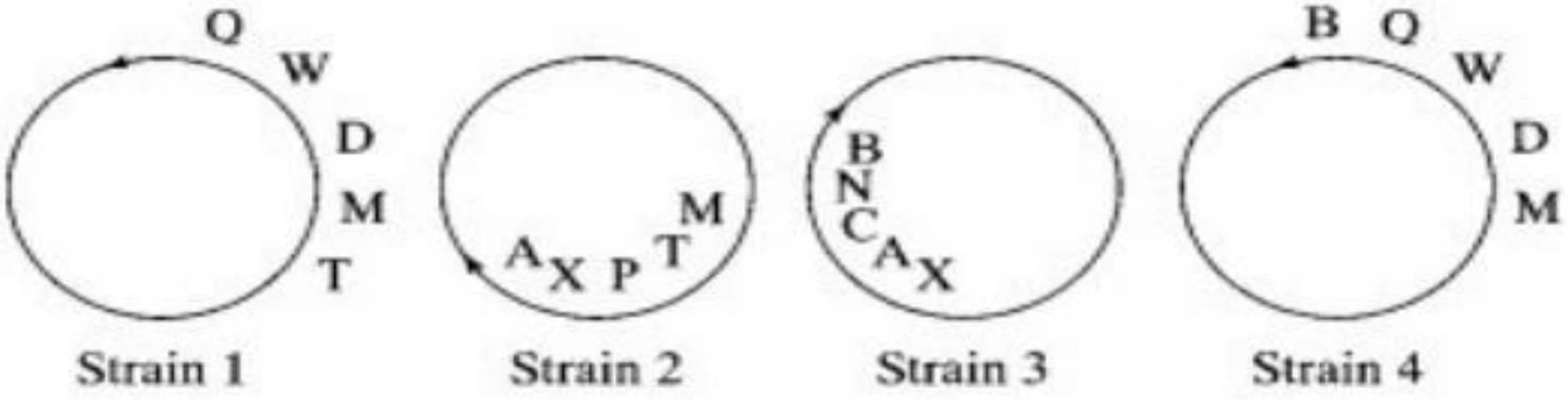
All of these Hfr strains are derived from the same F^+ strain. What is the order of these markers on the circular chromosome of the original F^+?

The two-step approach that works for solution

(1)deter-mine the underlying principle, and

(2) draw a diagram. Here the principle is clearly that each Hfr strain donates genetic markers from a fixed point on the circular chromosome and that the earliest markers are donated with the highest frequency.

Because not all markers are donated by each Hfr, only the early markers must be donated for each Hfr. Each strain allows us to draw the following circles:



From this information, we can consolidate each circle into one circular linkage map of the order

Q, W, D, M, T, P, X, A, C, N, B, Q.

Problem no. 2.

In an Hfr × F^- cross, *leu*$^+$ enters as the first marker, but the order of the other markers is unknown. If the Hfr is wild type and the F^- is auxotrophic for each marker in question, what is the order of the markers in a cross where *leu*$^+$ recombinants are selected if 27 percent are *ile*$^+$, 13 percent are *mal*$^+$, 82 percent are *thr*$^+$, and 1 percent are *trp*$^+$?

We have selected for the earliest marker in this cross, the frequency of recombinants is a function of the order of entry for each marker. Therefore, we can immediately determine the order of the genetic markers simply by looking at the percentage of recombinants for any marker among the *leu*$^+$ recombinants. Because the inheritance of *thr*$^+$ is the highest, this must be the first marker to enter after *leu*.

The complete order is *leu, thr, ile, mal, trp.*

Two bacterial strains were obtained with the following genotypes:

Hfr: *ala*$^{-}$ *leu*$^{+}$*azi*^{S}*Str*^{S}

F^{-}: *ala*$^{+}$ *leu*$^{-}$*azi*^{R}*Str*^{R}

After an uninterrupted conjugation, you want to select F^{-} recombinants that are *ala*$^{+}$ *leu*$^{+}$. Which of the following media would you use for this selection?

A) Complete medium containing streptomycin
B) Minimal medium containing streptomycin
C) Minimal medium containing leucine and streptomycin
D) Minimal medium containing sodium azide and leucine
E) Minimal medium containing alanine, leucine, and streptomycin

If a *met-thr-* Hfr strain is mated with an F- of genotype *leu- thi-*, the prototrophic recombinants can be detected by plating the mixture on

A) leucine and methionine.
B) threonine and thiamine.
C) leucine and thiamine.
D) methionine, threonine, leucine, and thiamine.
E) minimal medium.

.From one F^+ strain the following three Hfr strains were derived, each shown with the first three markers transferred during an Hfr × F^- cross:

Hfr 1 . . . *D A F* →

Hfr 2 . . . *E B F* →

Hfr 3 . . . *E C D* →

The order of the genes on the bacterial chromosomal circle must be which of the following? (*A* is shown at both ends to represent circularity. Assume that the Hfr picks up all intermediates between any two represented genes.)

A) *A D C E B F A*

B) *A B C D F E A*

C) *A C D F E B A*

D) *A E F B C D A*

E) *A F B D E C A*

An Hfr strain of *E. coli* with the genotype *gly+ azi*R *Str*S is mated with an F- strain of *E. coli* of genotype *gly- azi* S *Str*R. *Gly* refers to the amino acid glycine, *azi* refers to sodium azide, and *Str* refers to the antibiotic streptomycin, where S is sensitive and R is resistant. Conjugation occurs and the progeny are screened on a selective medium to detect recombinants. If you wanted to select for the F- recombinant genotype *gly+azi*R *Str*R, you should use a minimal medium containing

A) streptomycin.

B) sodium azide.

C) glycine.

D) glycine and streptomycin.

E) streptomycin and sodium azide.

F) Glycine, sodium azide, and streptomycin.

Mapping by recombination and co- transduction of markers

Genetic Mapping in bacteria by Transformation

- Bacterial transformation is used to map the genes of certain bacterial species in which mapping by other methods (conjugation and transduction) was not possible.
- DNA from donor bacterial strain is extracted, purified and broken into small fragments.
- This DNA is then added to recipient bacteria with a different genotype.
- If the donor DNA is taken up by a recipient cell and recombines with the homologous parts of the recipient chromosome, a recombinant chromosome is produced and can be detected phenotypically (these will be called as transformants).

Figure 18.9

Transformation in *Bacillus subtilis*. (a) The linear donor double-stranded bacterial DNA fragment carries the a^+ allele, and the recipient bacterium carries the a allele. (b) One donor DNA strand enters the recipient. (c) The single linear DNA strand pairs with the homologous region of the recipient's chromosome, forming a triple-stranded structure. (d) A double crossover produces a recombinant a^+/a recipient chromosome and a linear a DNA fragment. The linear fragment is degraded, and by replication, one-half of the progeny are a^+ transformants and one-half are a nontransformants.

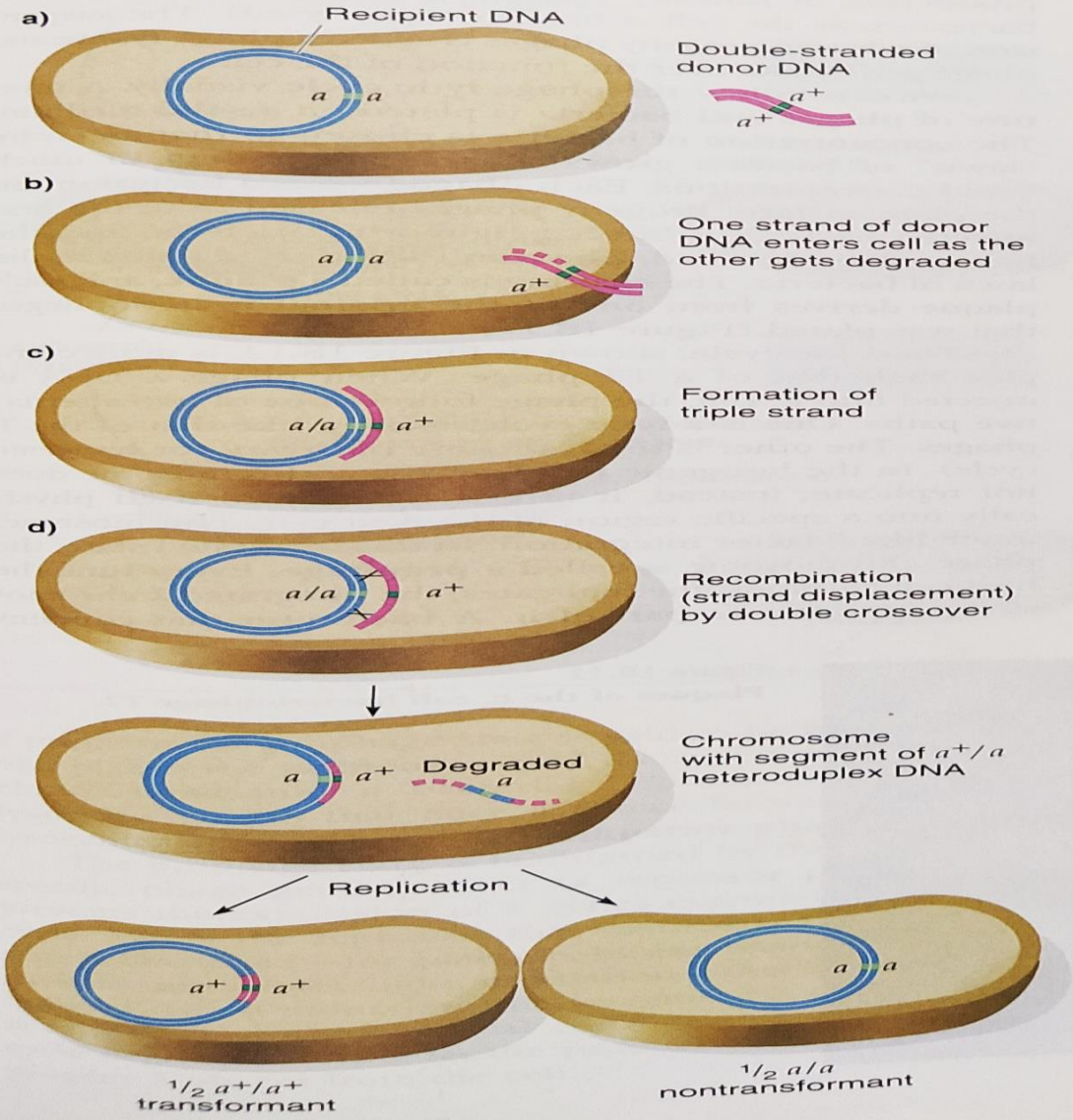
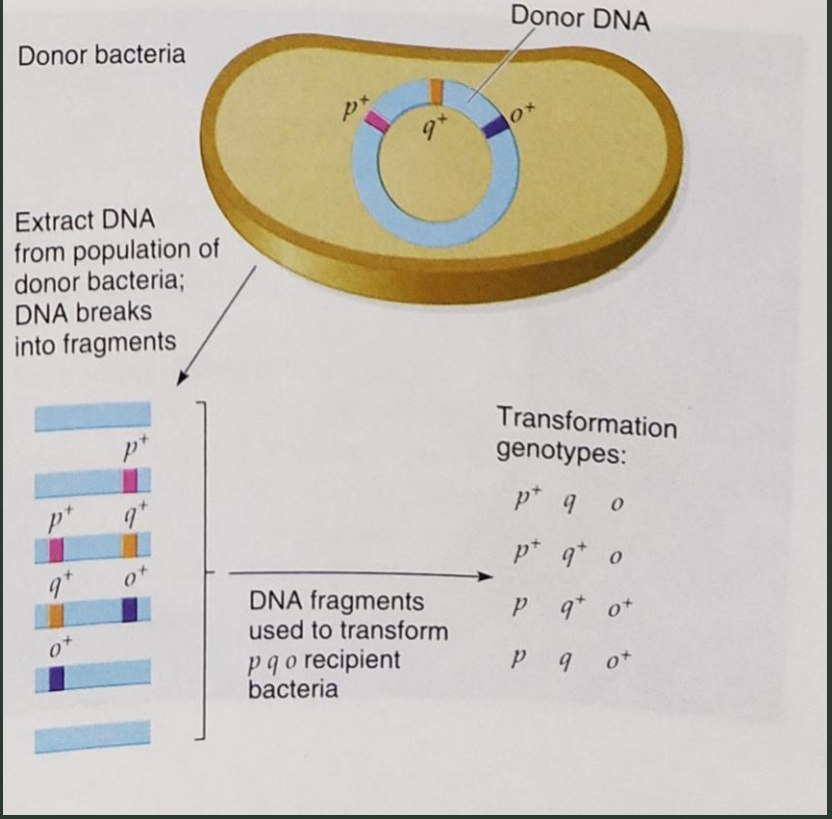
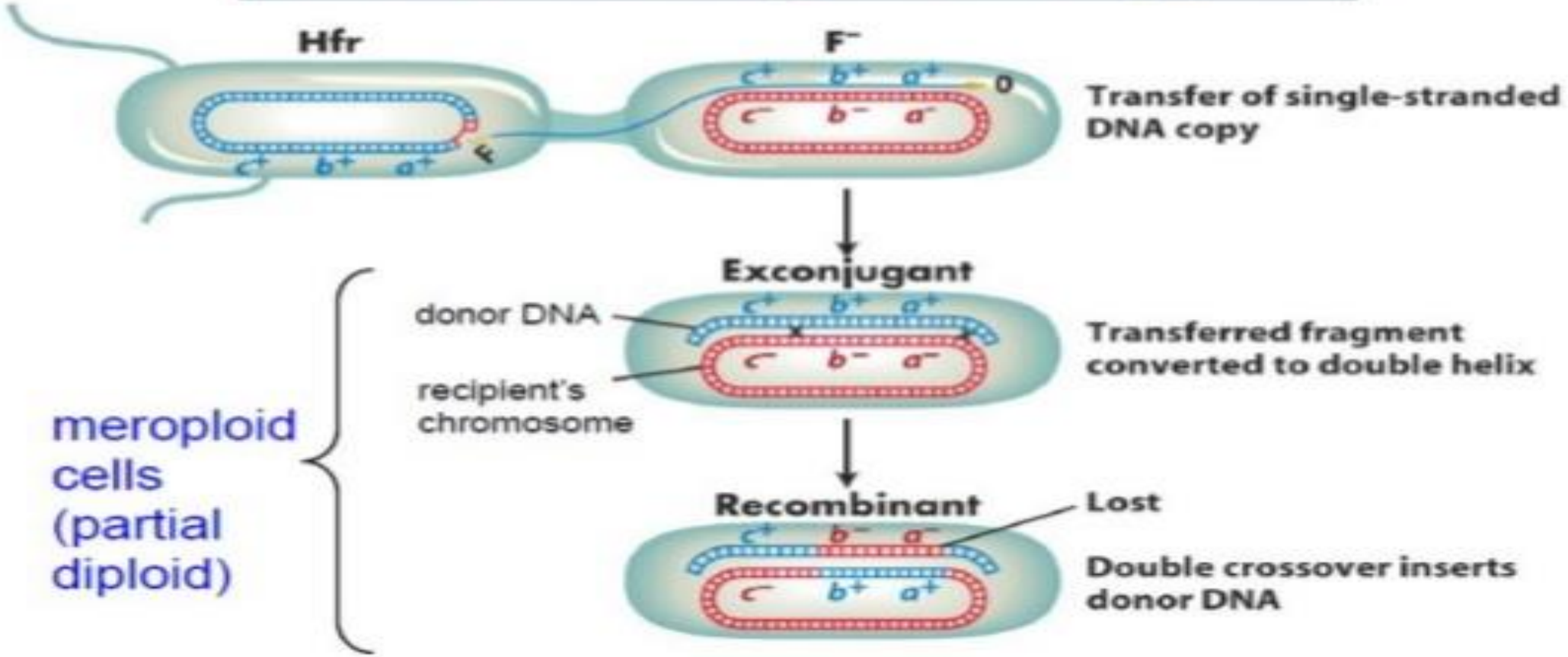


Figure 18.10

Demonstration of the determination of gene order by cotransformation.



Recombination during bacterial conjugation



Single crossover would make the chromosome linear and the cell would die

Mapping using transformation:

1. Recombination frequencies are used to infer gene order.

p+ q+ o+ x p q o

2. If p+ and q+ frequently cotransform, order is p-q-o.

3. If p+ and o+ frequently cotransform, order is p-o-q.

Genetic Mapping in bacteria by transduction

- **Transductants** - once the donor genetic material has been introduced into the recipient. It may undergo genetic recombination with a homologous region of the recipient chromosome. The recombinant recipient is called transductants.
- **Phage Lysate-** the suspension of released progeny phages is called as a phage lysate.
- **Plaque**
- **Prophage**
- **Lysogenic**

- **Lysogeny**
- **Temperate Phages**
- **Generalised transduction**
- **Specialised transduction**
- **In generalised transduction <u>any gene</u> can be transferred between bacteria whereas in specialised transduction <u>only specific genes</u> are transferred.**
- **Prototrophs** grows on **Minimal media**
- **Auxotrophs** grows on **Auxotrophic media**

- Generalized transduction can be used to make linkage map and also to find gene order and map distance between co-transduced genes.
- **Selected markers -** a marker gene which help in the selection of transduced cells among the population of transduced and non transduced cells.
- **Unselected markers-** rest other markers are considered as unselected markers.

Leu+, thr+, aziR

Donor (grow on minimal media and resistant to sodium azide)

Leu. Thr, aziS

Recipient (requires leucine and threonine supplement and sensitive to sodium azide

P1 phage was grown on the bacterial donor cells

Phage lysate is used to infect the recipient bacterial cells.

Transductants are selected for any of the SELECTED MARKER (leu+) and analysed for the presence of the other UNSELECTED MARKER (thr+ and azi R)

If leu+ is transferred to the recipient then look for the other unselected marker which could have been COTRANSDUCED

Cotransduction can be possible in two ways

If two genes are close enough so that they can be packaged physically into a cell by a single phage

ONLY WAY POSSIBLE

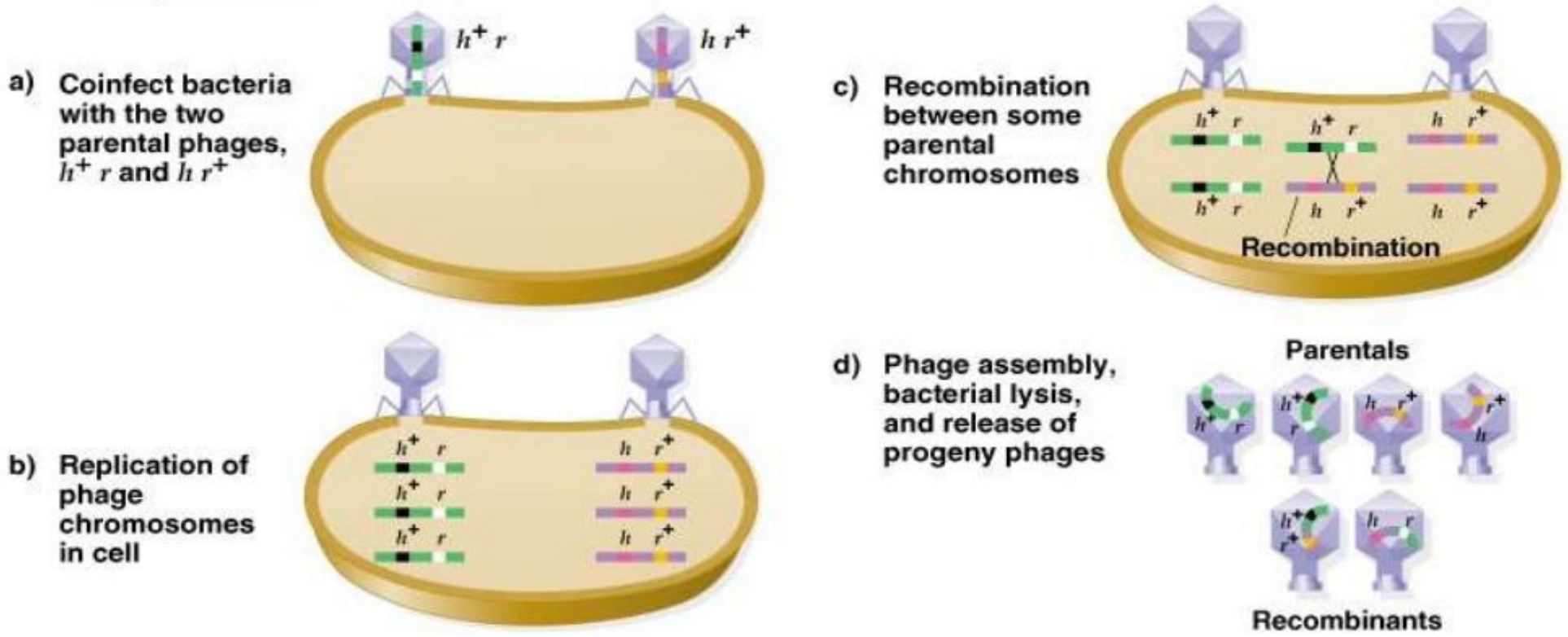
If two genes are not closely linked and are introduced into the same bacterium by simultaneous infection with two different phages

Mapping genes using bacteriophages

1. Infect bacteria with phages of different genotypes using two-, three-, or four-gene crosses ⇒ crossover.

2. Count recombinant phage phenotypes by determining differences in cleared areas (no bacteria growth) on a bacterial lawn.

3. Different phage genes induce different types of clearing (small/large clearings with fuzzy/distinct borders).



Constructing a Linkage map

- Two markers located in close proximity on the same chromosome tend to be linked
- Frequency of such occurrence leads to distance estimation between markers

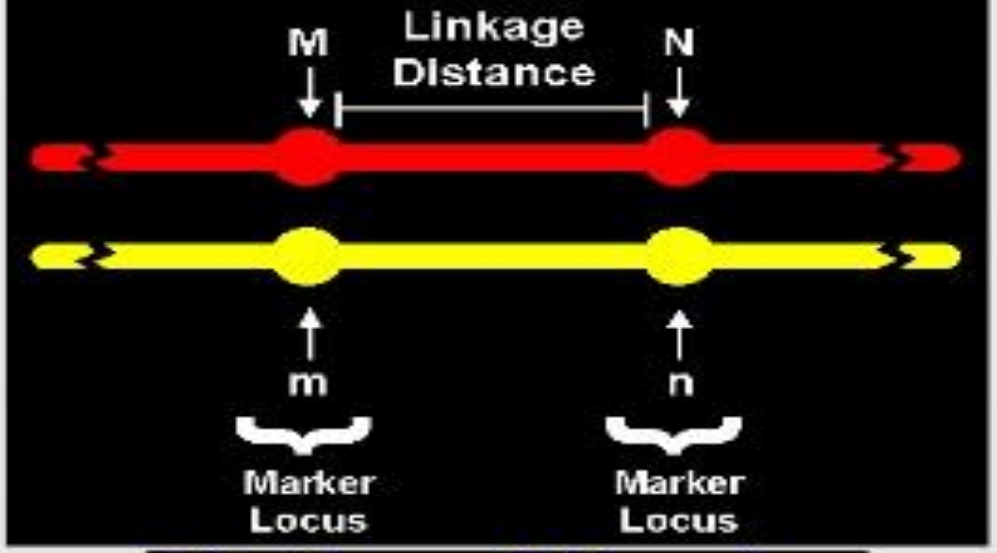
Thus the greater the frequency of recombination (segregation, localization of different chromosomes) between two genetic markers, the farther apart they are assumed to be. Conversely, the higher the frequency of association between the markers, the smaller the physical distance between them.

- **Recombination Frequency**

$$\frac{\text{\# of recombinants}}{\text{Total progeny}} = \frac{r}{p + r}$$

- 0% < recombination frequency < 50%
- centimorgans (cM)

$$= \frac{r}{p + r} \times 100$$



Linkage Mapping