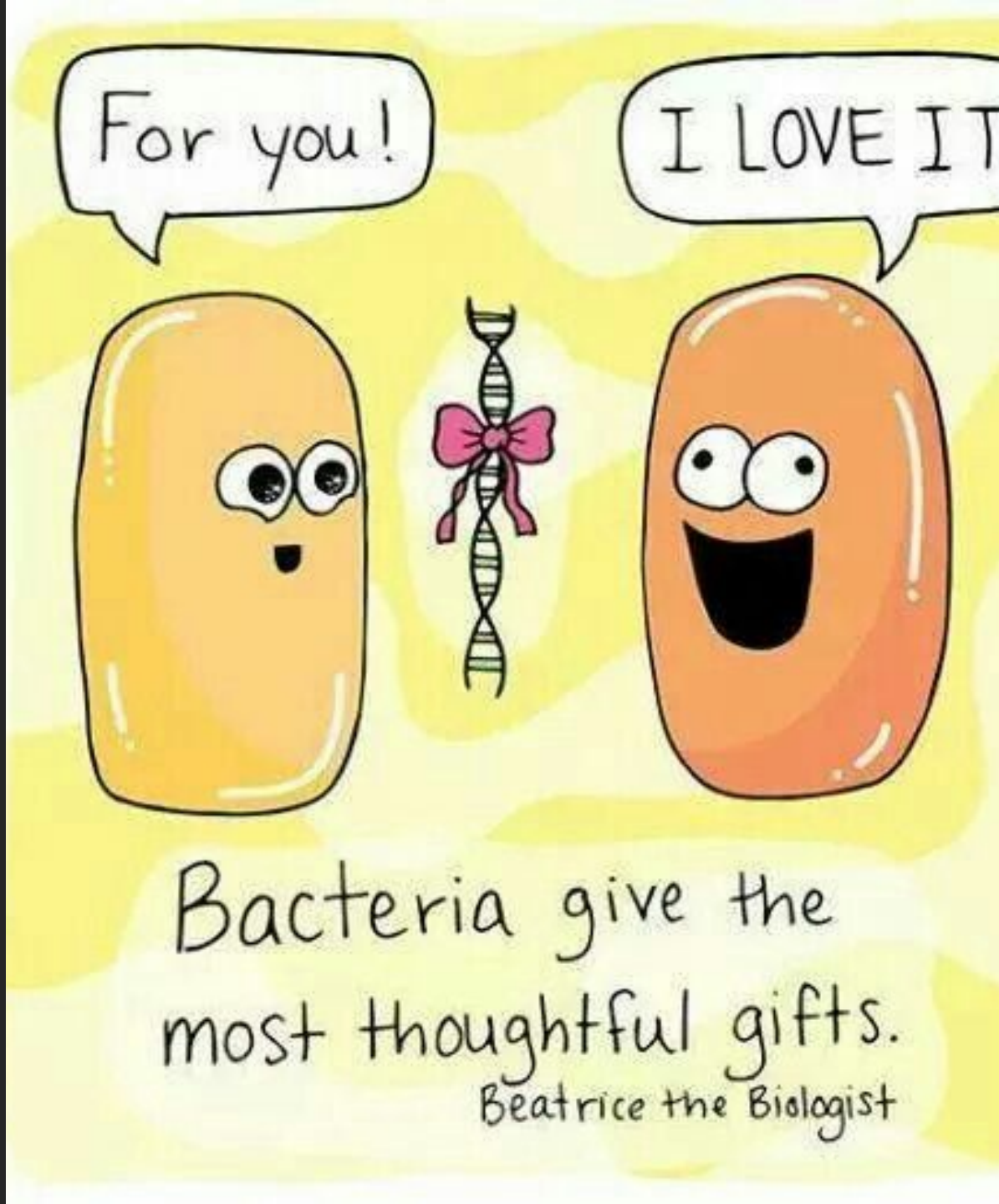
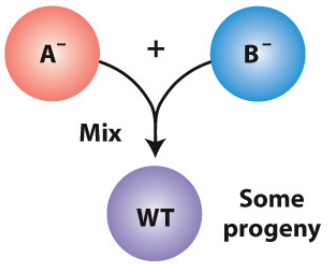
Chapter 5: Bacterial Genetics



Mixing bacterial genotypes produces rare recombinants



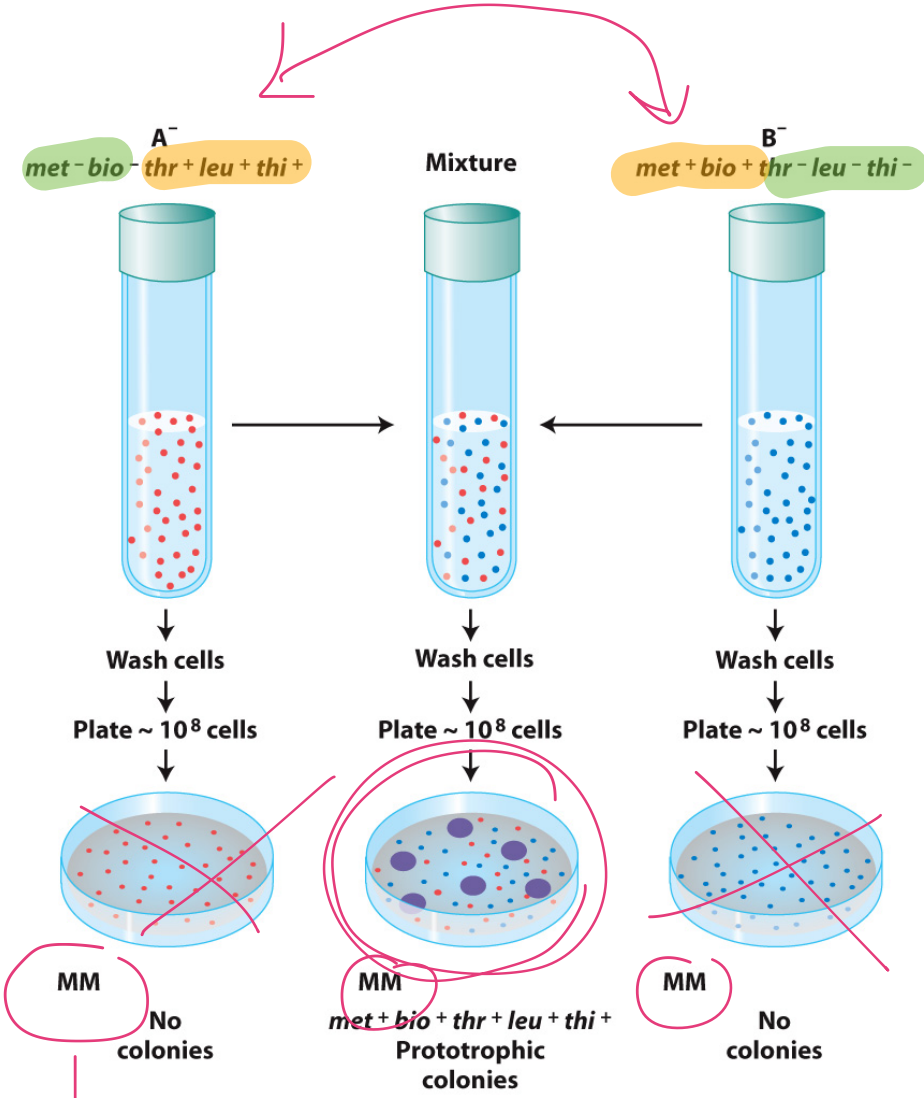


Figure 5-5
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Bacteria exchange DNA by several processes – horizontal transmission

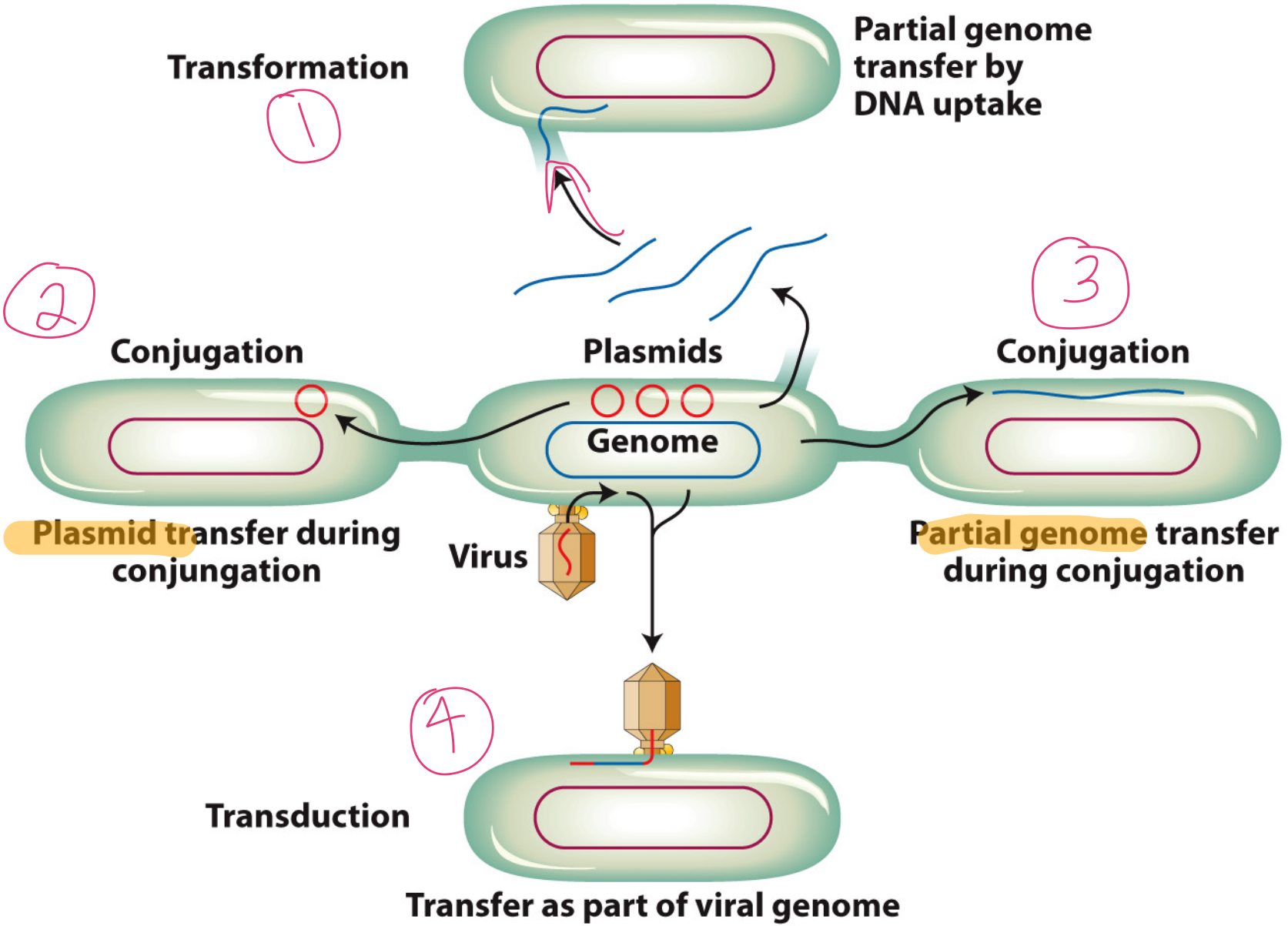

Figure 5-2
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F plasmids transfer during conjugation

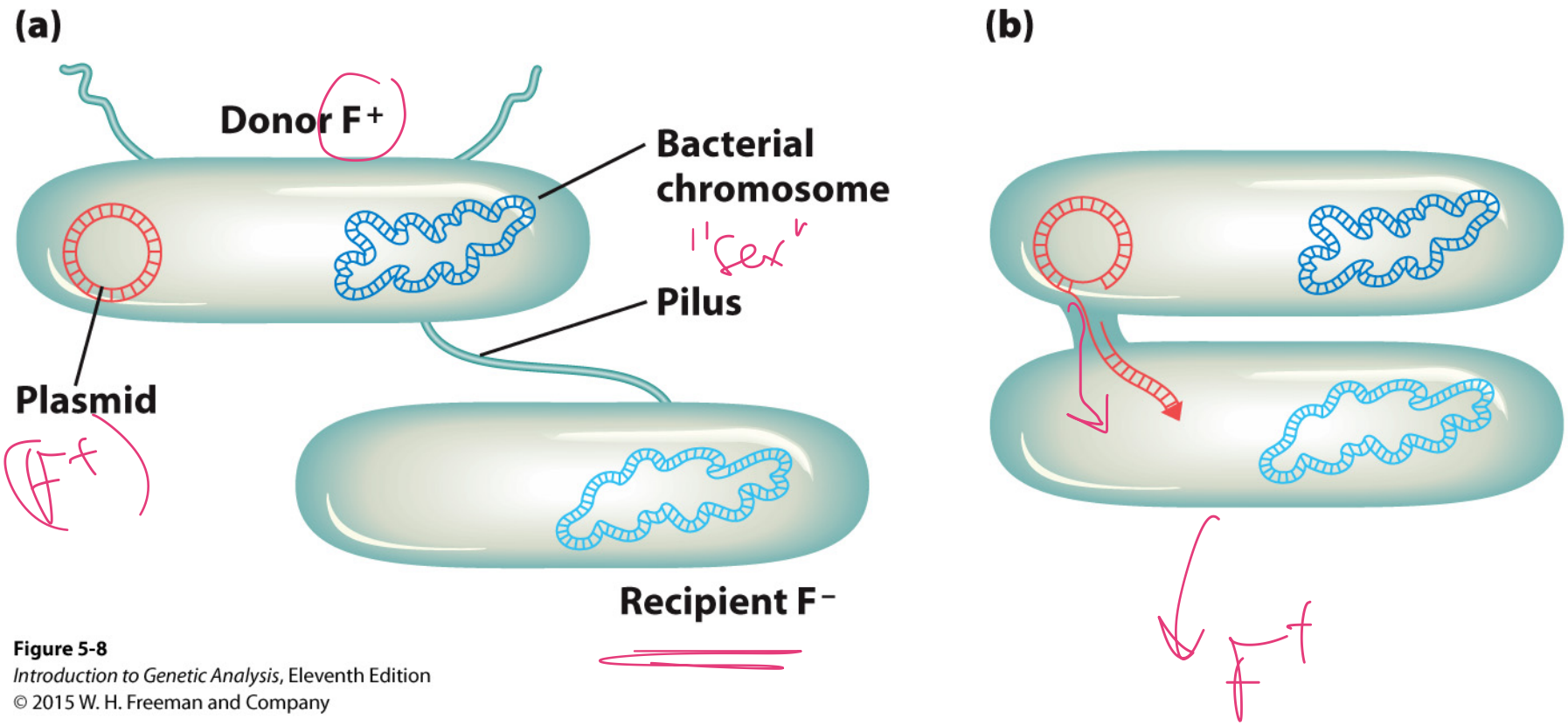

Figure 5-8
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Integration of the F plasmid creates an Hfr strain

F incorporates → Hfr

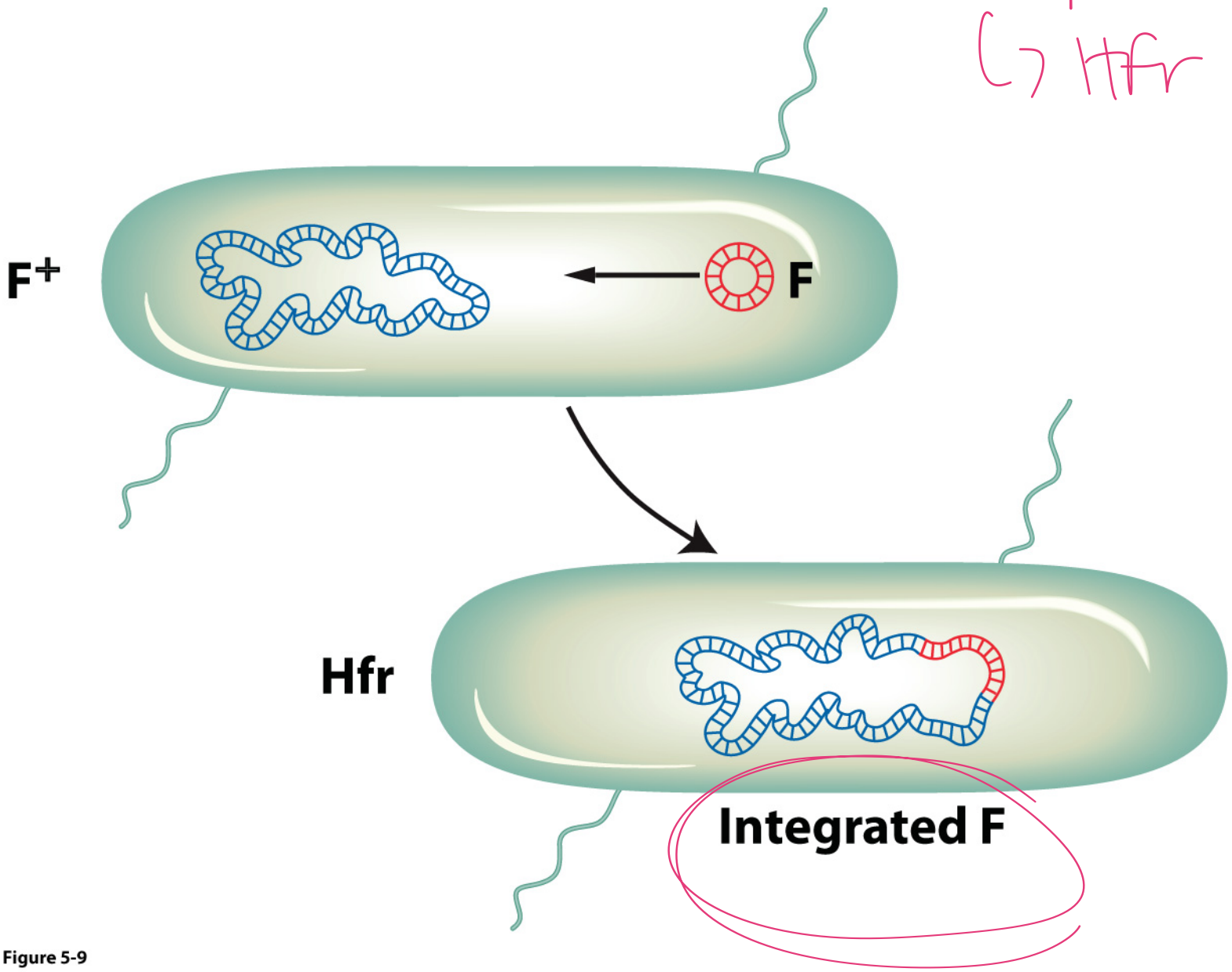

Figure 5-9
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Crossovers integrate parts of the transferred donor fragment

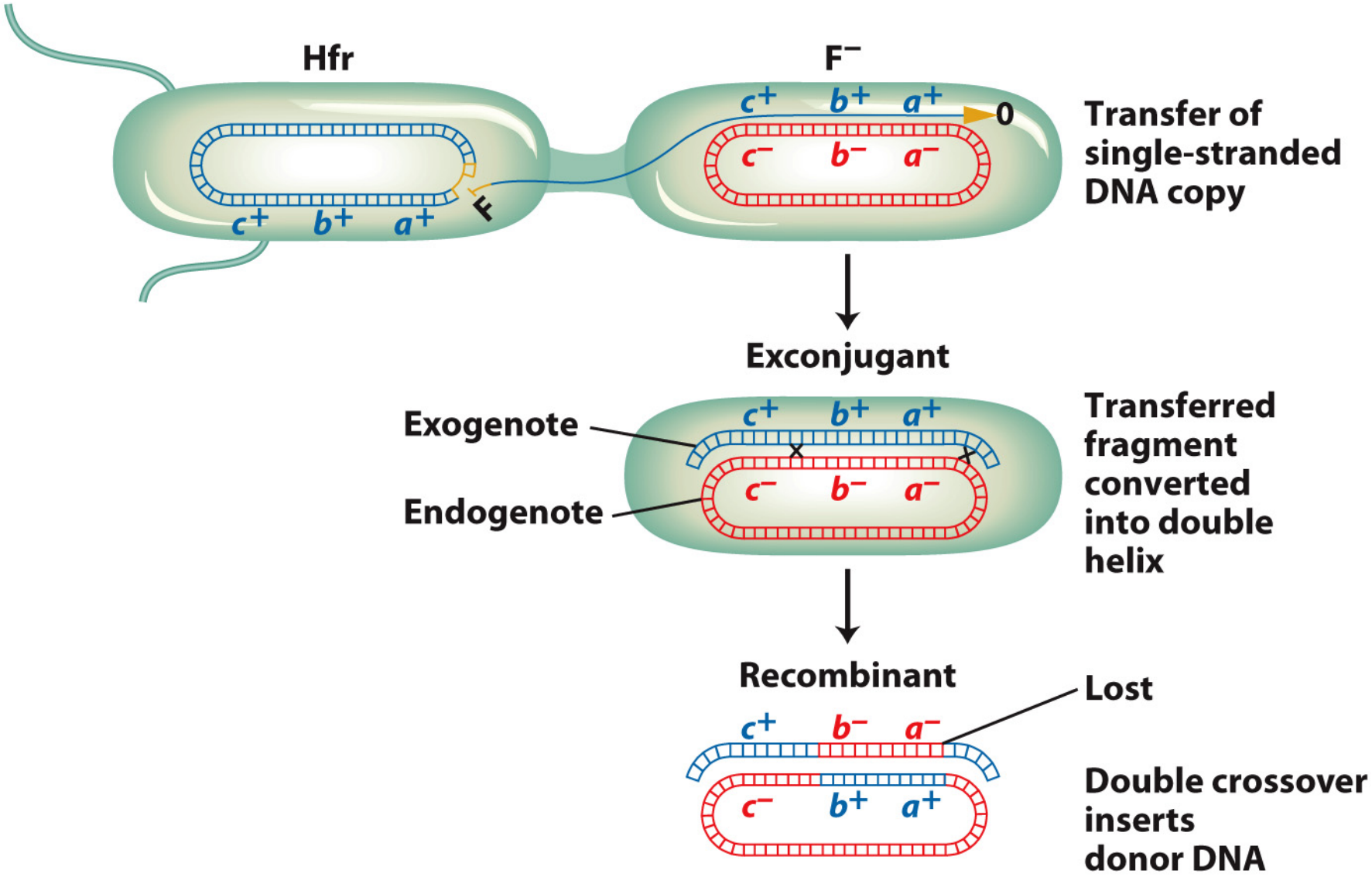

Figure 5-11
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5. In Figure 5-11, which donor alleles become part of the recombinant genome produced?

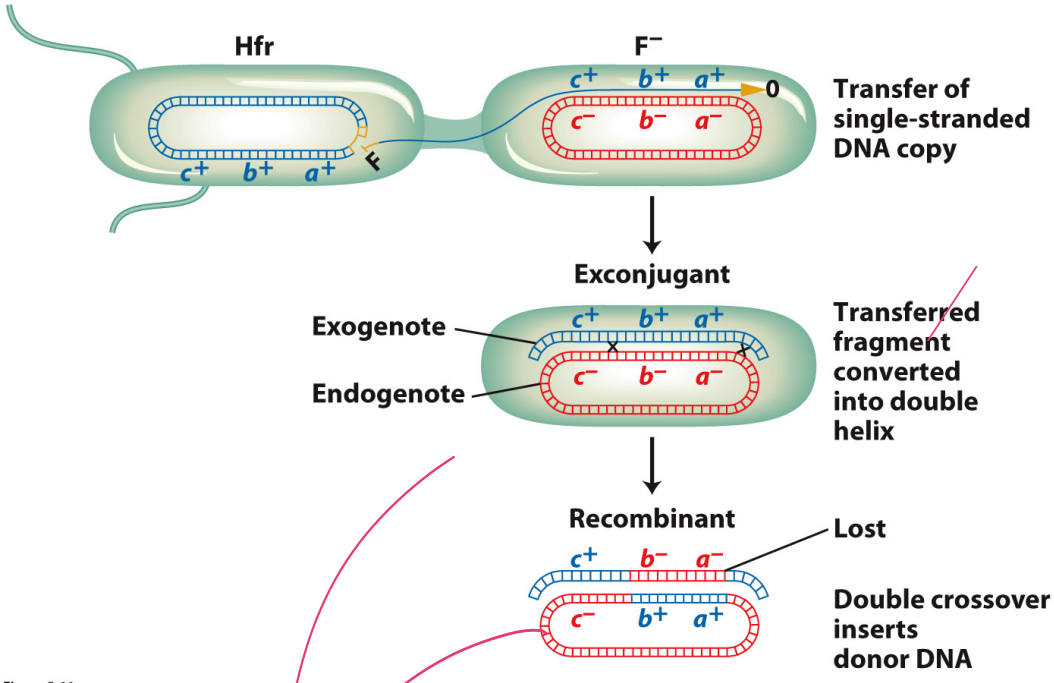

Figure 5-11
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→ dd genotype $c^- b^- a^-$

→ new $c^- b^+ a^+$

The generation of various recombinants by crossing over in different regions

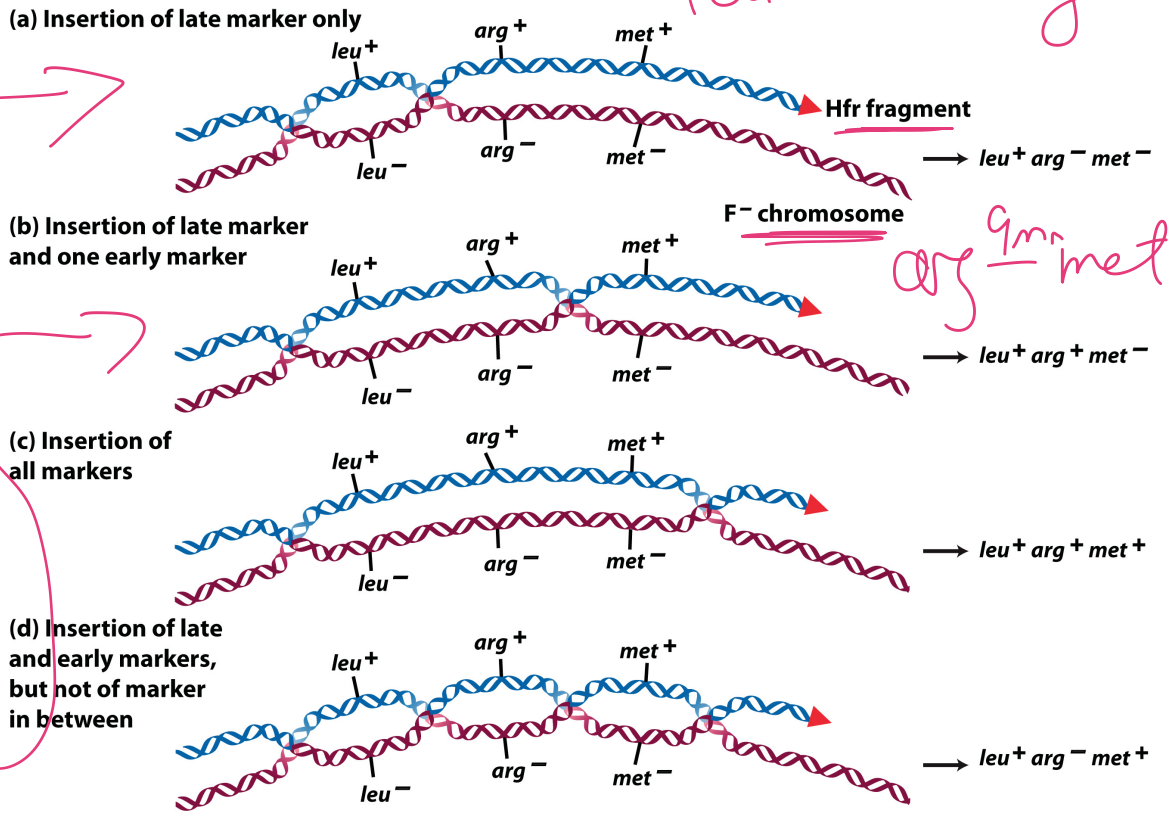

Figure 5-17
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Prototrophic → can grow / divide on MM
Auxotrophic → mutants that need one or more
cells using
minimal media (MM) – inorganic salts, C source, H_2O

TABLE 5-1 Some Genotypic Symbols Used in Bacterial Genetics

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

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

Table 5-1
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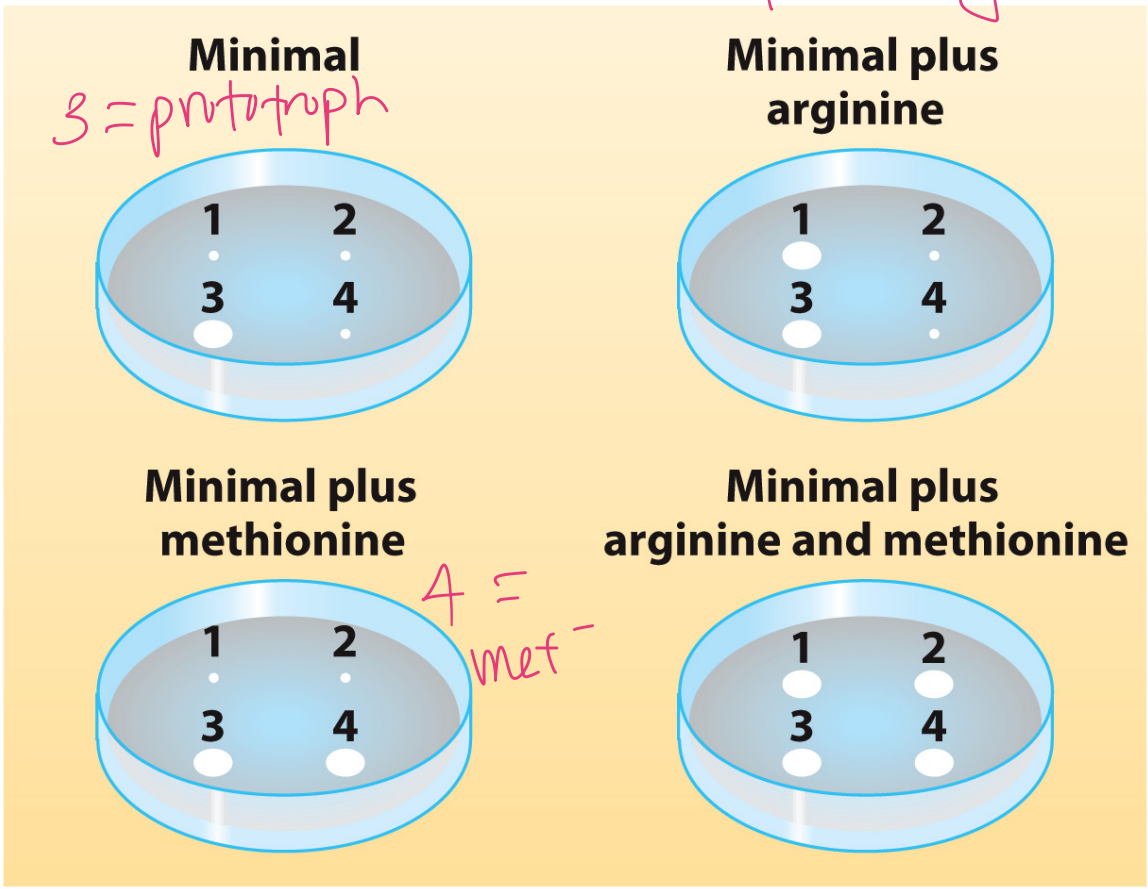
auxotrophs
cannot use a particular C source
resistant / sensitive to inhibitor

Problem 5-40
Deduce the genotypes of the following *E. coli* strains:



Problem 5-40
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19. Describe the state of the F factor in an Hfr, F^+, and F^- strain.

Hfr → F plasmid is integrated into chromosome

F^+ → in cytoplasm

F^- → has no fertility factor

F^+ → Hfr

F'

21. With respect to gene transfer and the integration of the transferred gene into the recipient genome, compare:
    a. Hfr crosses by conjugation and generalized transduction.
    b. F' derivatives such as F' lac and specialized transduction.

(a) Hfr → genes enter in linear fashion
- depends on time & strain

GT → random pieces of bacterial DNA packaged in phage
- any gene

(b) only certain genes can be transferred

"Interrupted mating"

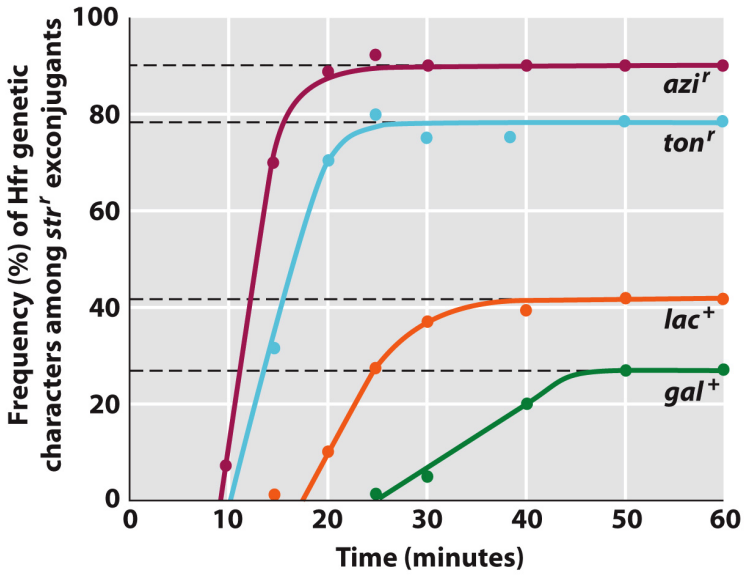

Figure 5-12a
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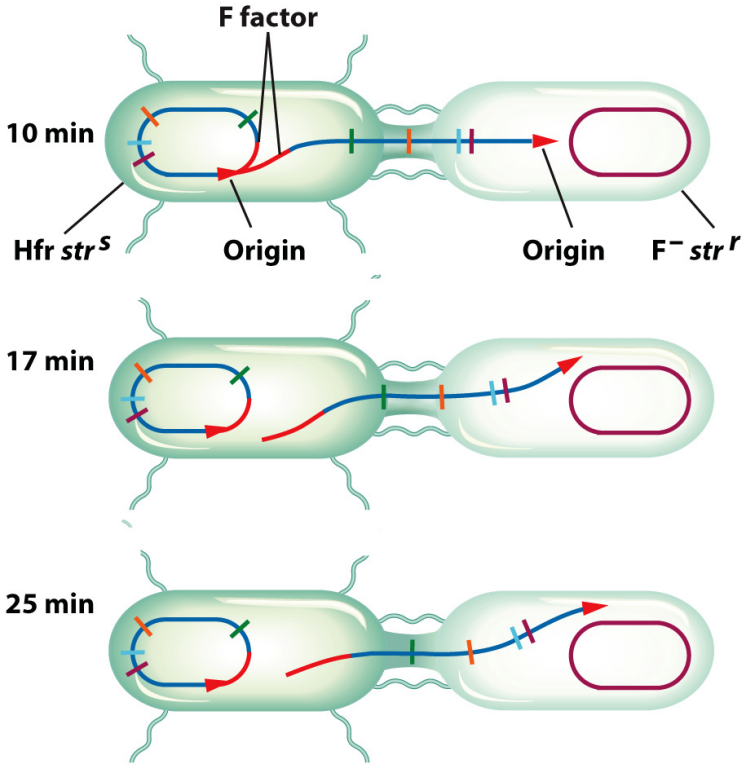

Figure 5-12b
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24. *In E. coli*, four Hfr strains donate the following markers, shown in the order donated:

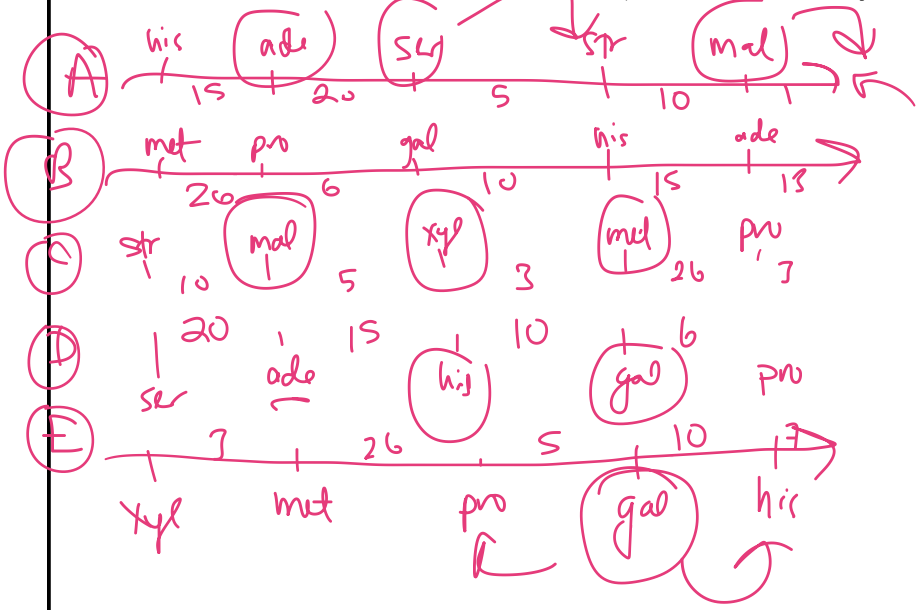
Strain 1: M Z X W C
Strain 2: L A N C W
Strain 3: A L B R U
Strain 4: Z M U R B

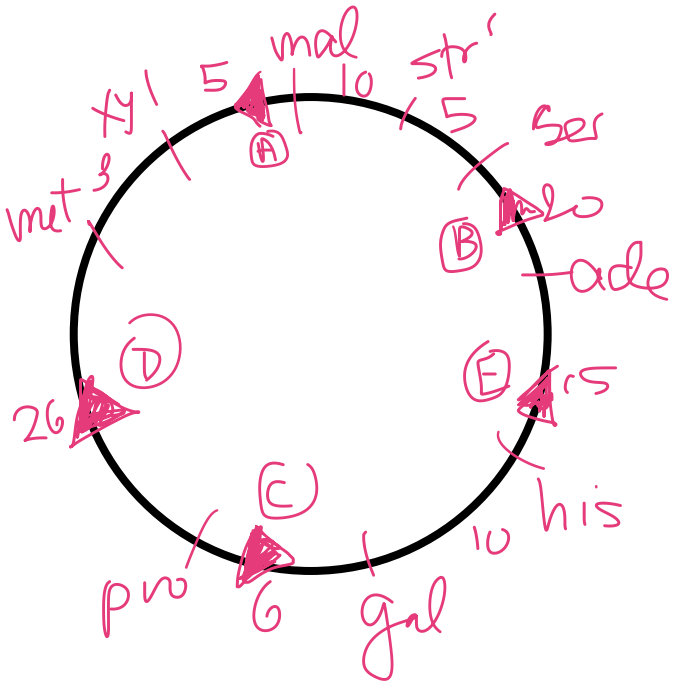
All these Hfr strains are derived from the same F^- strain. What is the order of these markers on the circular chromosome of the original F^+?

31. Five Hfr strains A through E are derived from a single F^+ strain of *E. coli*. The following chart shows the entry times of the first five markers into an F^- strain when each is used in an interrupted-conjugation experiment:

A		B		C		D		E	
mal$^+$	(1)	*ade*$^+$	(13)	*pro*$^+$	(3)	*pro*$^+$	(10)	*his*$^+$	(7)
str^s	(11)	*his*$^+$	(28)	*met*$^+$	(29)	*gal*$^+$	(16)	*gal*$^+$	(17)
ser$^+$	(16)	*gal*$^+$	(38)	*xyl*$^+$	(32)	*his*$^+$	(26)	*pro*$^+$	(23)
ade$^+$	(36)	*pro*$^+$	(44)	*mal*$^+$	(37)	*ade*$^+$	(41)	*met*$^+$	(49)
his$^+$	(51)	*met*$^+$	(70)	*str*^{s}	(47)	*ser*$^+$	(61)	*xyl*$^+$	(52)

a. Draw a map of the F+ strain, indicating the positions of all genes and their distances apart in minutes.
b. Show the insertion point and orientation of the F plasmid in each Hfr strain.
c. In the use of each of these Hfr strains, state which allele you would select to obtain the highest proportion of Hfr exconjugants.





34. *E. coli* cells were infected with two strains of T4 virus. One strain is minute (m), rapid lysis (r), and turbid (t); the other is wild type for all three markers. The lytic products of this infection were plated and classified. The resulting 10,343 plaques were distributed among eight genotypes as follows:

Genotypes	Plaques
m r t	3469
+ + +	3727
m r +	854
m + t	163
m + +	521
+ r t	475
+ r +	171
+ + t	963

a. What are the linkage distances between m and r, between r and t, and between m and t?
b. Determine the linkage order for the three genes.
c. What is the coefficient of coincidence (see Chapter 4) in this cross? What does it signify?

47. A bacterial transformation is performed with a donor strain that is resistant to four drugs: A, B, C, and D, and a recipient strain that is sensitive to all four drugs. The resulting recipient cell population is divided and plated on media containing various combinations of the drugs. The following table shows the results.

Drugs added	Number of colonies	Drugs added	Number of colonies
None	10,000	BC	50
A	1155	BD	48
B	1147	CD	785
C	1162	BC	31
D	1140	ABD	43
AB	47	ACD	631
AC	641	BCD	35
AD	941	ABCD	29

a. One of the genes is distant from the other three, which appear to be closely linked. Which is the distant gene?
b. What is the likely order of the three closely linked genes?

(a) B distant (ACD)

(b) AD > AC

A – D – C

Accompanying Markers in Specific P1 Transductions

TABLE 5-3 Accompanying Markers in Specific P1 Transductions

Experiment	Selected marker	Unselected markers
1	*leu*$^+$	50% are *azi*^{r}; 2% are *thr*$^+$
2	*thr*$^+$	3% are *leu*$^+$; 0% are *ari*^{r}
3	*leu*$^+$ and *thr*$^+$	0% are *azi*^{r}

Table 5-3
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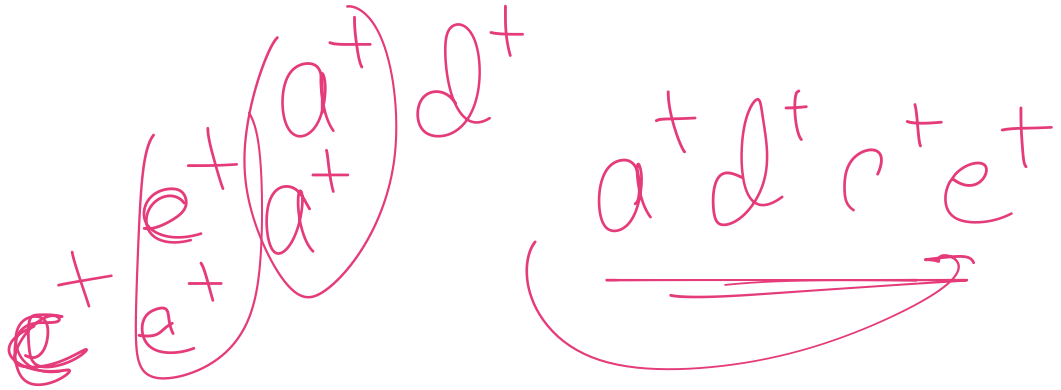
50. A generalized transducing phage is used to transduce an $a^-b^-c^-d^-e^-$ recipient strain of *E. coli* with an $a^+b^+c^+d^+e^+$ donor. The recipient culture is plated on various media with the results shown in the following table. (Note that a^- indicates a requirement for A as a nutrient, and so forth.) What can you conclude about the linkage and order of the genes?

Compounds added to minimal medium	**Presence (+) or absence (–) of colonies**
CDE	–
BDE	–
BCE	+
BCD	+
ADE	–
ACE	–
ACD	–
ABE	–
ABD	+
ABC	–



17. In Figure 5-33, which is the rarest genotype produced in the initial lysate?

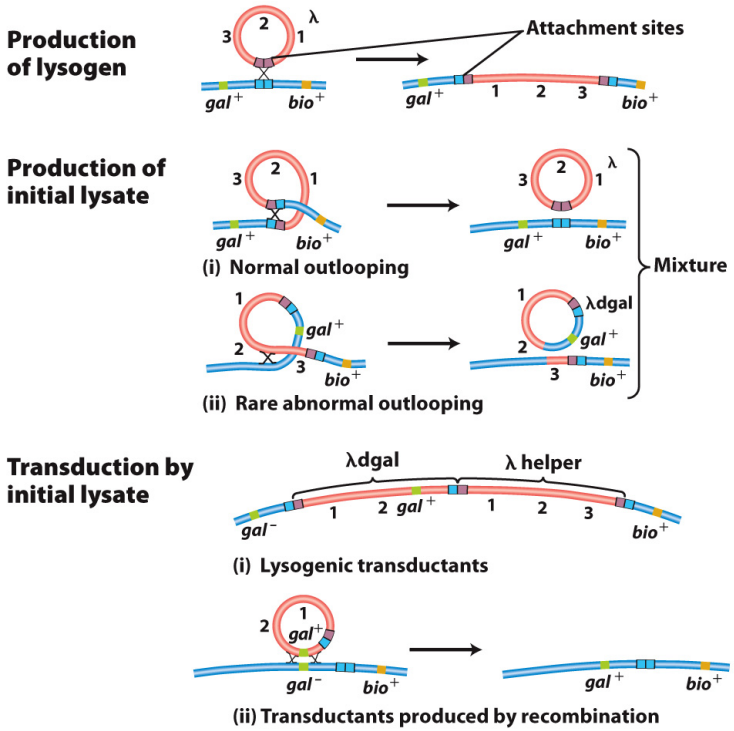


Figure 5-33
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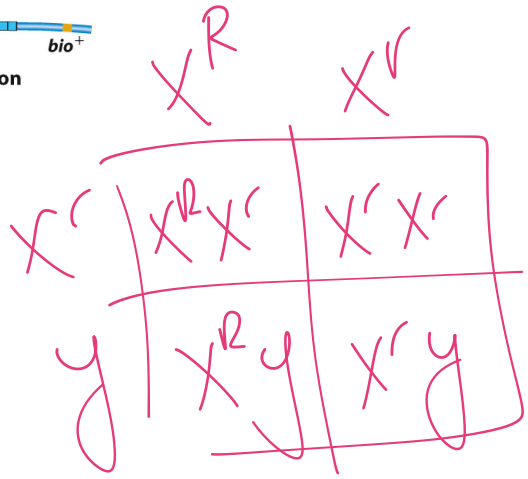

(7)

25. You are given two strains of *E. coli*. The Hfr strain is $arg^+ala^+glu^+pro^+leu^+T^s$; the F^- strain is $arg^-ala^-glu^-pro^-leu^-T^r$. All the markers are nutritional except T, which determines sensitivity or resistance to phage T1. The order of entry is as given, with arg^+ entering the recipient first and T^s last. You find that the F^- strain dies when exposed to penicillin (pen^s), but the Hfr strain does not (pen^r). How would you locate the locus for *pen* on the bacterial chromosome with respect to *arg, ala, glu, pro*, and *leu*? Formulate your answer in logical, well-explained steps and draw explicit diagrams where possible.

Hfr arg^+ ala^- glu^+ pro^+ leu^+ T^S

↳ pen^r ?

(1) arg^+ T^S or leu^+

(2) look pen^r

(3) which genes appear w/ pen^r the most?
↳ highest linkage (pro^+ leu^+)

(4) select leu^+ → pro^+ pen^r pro^- pen^r

(5) Recombination frequencies ↳ recombinants

28. A particular Hfr strain normally transmits the pro^+ marker as the last one in conjugation. In a cross of this strain with an F^- strain, some pro^+ recombinants are recovered early in the mating process. When these pro^+ cells are mixed with F^- cells, the majority of the F^- cells are converted into pro^+ cells that also carry the F factor. Explain these results.