

Chapter Eight: Bacterial and Viral Genetic Systems

COMPREHENSION QUESTIONS

Section 8.1

1. Explain how auxotrophic bacteria are isolated.
Unlike prototrophic bacteria (wild-type) that can grow on minimal media, auxotrophic bacteria are mutant strains of bacteria that are unable to grow on minimal media. In other words, auxotrophs are not nutritionally self-sufficient. To isolate an auxotrophic bacterium from a culture of wild-type bacteria, first spread the bacterial culture out on a petri dish containing nutritionally complete growth medium allowing prototrophic and auxotrophic colonies to grow. Using the replica plating technique, transfer a few cells from each colony to replica plates. One of the replica plates should contain a selective medium that lacks a nutrient required by the auxotroph for growth, and the other replica plate should contain a nutritionally complete medium. The auxotrophic colonies should grow only on the nutritionally complete medium and not the selective medium. Prototrophic colonies should grow on both types of media.
2. Briefly explain the differences between F^+ , F^{\square} , Hfr, and F' cells.
An F^+ cell will contain the F factor as a circular plasmid separate from the chromosome. The Hfr cell has the F factor integrated into its chromosome. In F' strains, the F factor exists as a separate circular plasmid, but the plasmid carries bacterial genes that were originally part of the bacterial chromosome. The F^{\square} strain does not contain the F Factor and can receive DNA from cells that contain the F Factor (F^+ , Hfr, and F' cells).
- *3. What types of matings are possible between F^+ , F^{\square} , Hfr, and F' cells? What outcomes do these matings produce? What is the role of F factor in conjugation?

<i>Types of matings</i>	<i>Outcomes</i>
$F^+ \times F^{\square}$	<i>Two F^+ cells</i>
$Hfr \times F^{\square}$	<i>One F^+ cell and one F^{\square} cell</i>
$F' \times F^{\square}$	<i>Two F^{\square} cells</i>

The F factor contains a number of genes involved in the conjugation process, including genes necessary for the synthesis of the sex pilus. The F factor also has an origin of replication that allows for the factor to be replicated during the conjugation process, and genes for opening the plasmid and initiating the chromosome transfer..

- *4. Explain how interrupted conjugation, transformation, and transduction can be used to map bacterial genes. How are these methods similar and how are they different?
To map genes by conjugation, an interrupted mating procedure is used. During the conjugation process, an Hfr strain is mixed with an F^{\square} strain. The two strains must have different genotypes and must remain in physical contact for the transfer to occur. At regular intervals, the conjugation process is interrupted. The chromosomal

transfer from the Hfr strain always begins with a portion of the integrated F factor and proceeds in a linear fashion. To transfer the entire chromosome would require approximately 100 minutes. The time required for individual genes to be transferred is relative to their position on the chromosome and the direction of transfer initiated by the F factor. Gene distances are typically mapped in minutes of conjugation. The genes that are transferred by conjugation to the recipient must be incorporated into the recipient's chromosome by recombination to be expressed.

In transformation, the relative frequency at which pairs of genes are transferred or cotransformed indicates the distance between the two genes. Closer gene pairs are cotransformed more frequently. As was the case with conjugation, the donor DNA must recombine into the recipient cell's chromosome. Physical contact of the donor and recipient cells is not needed. The recipient cell uptakes the DNA directly from the environment. Therefore, the DNA from the donor strain has to be isolated and broken up before transformation can take place.

A viral vector is needed for the transfer of DNA by transduction. DNA from the donor cell is packaged into a viral protein coat. The viral particle containing the bacterial donor DNA then infects another bacterial cell or the recipient. The donor bacterial DNA is incorporated into the recipient cell's chromosome by recombination. Only genes that are close together on the bacterial chromosome can be cotransduced. Therefore, the rate of cotransduction, like the rate of cotransformation, gives an indication of the physical distances between genes on the chromosome.

These three processes are similar in that all involve the uptake by the recipient cell of a piece of the donor chromosome and the incorporation of some of that piece into the recipient chromosome by recombination. They also all calculate the mapping distance by measuring the frequency with which recipient cells are transformed. The process use different methods to get donor DNA incorporated into the recipient cell.

5. What is horizontal gene transfer and how might it occur?
Horizontal gene transfer occurs when a bacterial cell acquires genes from another species. Genome analysis experiments have shown that bacterial species have even acquired DNA from eukaryotic organisms. Three mechanisms that could lead to horizontal gene transfer are transduction, transformation, and conjugation.

Section 8.2

- *6. List some of the characteristics that make bacteria and viruses ideal organisms for many types of genetic studies.
 - (1) *Reproduction is rapid, asexual, and produces lots of progeny.*
 - (2) *Their genomes are small.*
 - (3) *They are easy to grow in the laboratory.*
 - (4) *Techniques are available for isolating and manipulating their genes.*
 - (5) *Mutant phenotypes, especially auxotrophic phenotypes, are easy to measure.*
7. What types of genomes do viruses have?
Viral genomes can consist of either DNA or RNA molecules. The viral nucleic acids can be either double-stranded or single-stranded, depending on the type of virus.

8. Briefly describe the differences between the lytic cycle of virulent phages and the lysogenic cycle of temperate phages.
Virulent phages reproduce strictly by the lytic cycle and ultimately result in the death of the host bacterial cell. During the lytic cycle, a virus injects its genome into the host cell. The genome directs production and assembly of new viral particles. A viral enzyme is produced and breaks open the cell, releasing new viral particles into the environment.
Temperate phages can utilize either the lytic or lysogenic cycle. The infection cycle begins when a viral particle injects its genome into the host cell. In the lysogenic cycle, the viral genome integrates into the host chromosome as a prophage. The inactive prophage can remain part of the bacterial chromosome for an extended period and is replicated along with the bacterial chromosome prior to cell division. Certain environmental stimuli can trigger the prophage to exit the lysogenic cycle and enter the lytic cycle.
9. Briefly explain how genes in phages are mapped.
To map genes in phages, bacterial cells are doubly infected with phage particles that differ in two or more genes. During the production of new phage progeny, the phage DNAs can undergo recombination, thus resulting in the formation of recombinant plaques. The rate of recombination is used to determine the linear order and relative distances between genes. The farther apart two genes are on the chromosome, the more frequently they will recombine.
- *10. How does specialized transduction differ from generalized transduction?
In generalized transduction, randomly selected bacterial genes are transferred from one bacterial cell to another by a virus. In specialized transduction, only genes from a particular region of the bacterial chromosome are transferred to another bacterium. The process of specialized transduction requires lysogenic phages that integrate into specific locations on the host cell's chromosome. When the phage DNA excises from the host chromosome and the excision process is imprecise, the phage DNA will contain a small part of the bacterial DNA that was adjacent to the viral insertion site. The hybrid DNA must be injected by the phage into another bacterial cell during another round of infection.
Transfer of DNA by generalized transduction requires that the host DNA be broken down into smaller pieces and that a piece of the host DNA is packaged into a phage coat instead of phage DNA. The defective phage cannot produce new phage particles upon a subsequent infection, but it can inject the bacterial DNA into another bacterium. Through a double crossover event, the donor DNA can become incorporated into the bacterial recipient's chromosome.
- *11. Briefly explain the method used by Benzer to determine whether two different mutations occurred at the same locus.
Benzer conducted complementation tests by infecting cells of E. coli K with large numbers of the two mutant phage types. For successful infection to occur on the E. coli K strains, each mutant phage needed to supply the gene product or protein missing in the other. Complementation will happen only if the mutations are at separate loci. If the two mutations are at the same locus, then complementation of gene products will not occur and no plaques will be produced on the E. coli K lawns.

- *12. Explain how a retrovirus, which has an RNA genome, is able to integrate its genetic material into that of a host having a DNA genome.
Retroviruses are able to integrate their genomes into the host cell's DNA genome through the action of the enzyme reverse transcriptase. Reverse transcriptase can synthesize complementary DNA from either a RNA or DNA template. The retrovirus enzyme synthesizes a double-stranded copy of DNA using the retroviral single-stranded RNA as the template. The newly synthesized DNA molecule can then integrate into the host chromosome to form a provirus.
13. Briefly describe the genetic structure of a typical retrovirus.
Retroviral genomes all have three genes in common: gag, pol, and env. Proteins that make up the viral capsid are encoded by the gag gene. Reverse transcriptase and an enzyme called integrase are encoded by the pol gene. While reverse transcriptase synthesizes double-stranded viral DNA from an RNA template, integrase results in the insertion of the viral DNA into the host chromosome. Finally, the env gene encodes for proteins found on the viral envelope.
14. What are the evolutionary origins of HIV-1 and HIV-2?
Both HIV-1 and HIV-2 are related to simian immunodeficiency viruses (SIV). Analysis of DNA sequences indicates that HIV-1 is related to simian immunodeficiency virus found in chimpanzees (SIVcpz). DNA sequence analysis also reveals that SIVcpz is a hybrid virus created by recombination between a retrovirus in red-capped mangabey and a retrovirus found in the great spot monkey. HIV-2 sequence analysis shows that it evolved from a simian immunodeficiency virus found in sooty mangabeys (SIVsm).

APPLICATION QUESTIONS AND PROBLEMS

Section 8.1

- *15. John Smith is a pig farmer. For the past five years, Smith has been adding vitamins and low doses of antibiotics to his pig food; he says that these supplements enhance the growth of the pigs. Within the past year, however, several of his pigs have died from infections of common bacteria, which failed to respond to large doses of antibiotics. Can you offer an explanation for the increased rate of mortality due to infection in Smith's pigs? What advice might you offer Smith to prevent this problem in the future?
Over the past five years, Farmer Smith, by treating his pigs with low doses of antibiotics, has been selecting for bacteria that are resistant to the antibiotics. The doses used killed sensitive bacteria, but not those bacteria that were moderately sensitive or slightly resistant. Over time, only resistant bacteria will be present in his pigs because any sensitive bacteria will have been eliminated by the low doses of

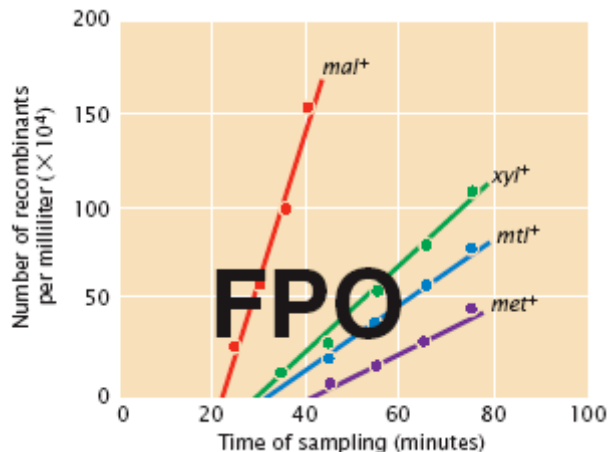
antibiotics. The pigs that died this past year were infected by bacteria that had become so resistant they were not killed by even high doses of antibiotics.

In the future, Farmer Smith can continue to use the vitamins, but he should use the antibiotics only when a sick pig requires them. In this manner, he will not be selecting for antibiotic-resistant bacteria, and the chances of the antibiotic therapy successfully treating his sick pigs will be greater.

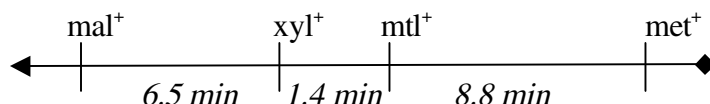
16. Rarely, conjugation of Hfr and F^{\square} cells produces two Hfr cells. Explain how this occurs.

Hfr strains contain an F factor integrated into the bacterial chromosome. The F factor mediates transfer of the bacterial chromosome. During conjugation of an Hfr strain with an F^{\square} strain, the transfer process begins within the F factor. So literally, part of the F factor is the first to arrive in F^{\square} cell. However, the remaining part of the F factor is transferred last. Because nearly 100 minutes are required to completely transfer the donor chromosome, the two cells must remain in contact for the entire 100 minutes. So, if the donor and recipient cell are not disturbed and the transfer process is not interrupted, then the entire Hfr strain's chromosome, including the F factor, can be donated to the F^{\square} cell. Following recombination between the donor and recipient chromosomes, this results in the production of a second Hfr strain.

17. Austin Taylor and Edward Adelberg isolated some new strains of Hfr cells that they then used to map several genes in *E. coli* by using interrupted conjugation (A. L. Taylor and E. A. Adelberg, 1960. *Genetics* 45:1233–1243). In one experiment, they mixed cells of Hfr strain AB-312, which were $xyl^+ mtl^+ mal^+ met^+$ and sensitive to phage T6, with F^- strain AB-531, which was $xyl^{\square} mtl^{\square} mal^{\square} met^{\square}$ and resistant to phage T6. The cells were allowed to undergo conjugation. At regular intervals, the researchers removed a sample of cells and interrupted conjugation by killing the Hfr cells with phage T6. The F^- cells, which were resistant to phage T6, survived and were then tested for the presence of genes transferred from the Hfr strain. The results of this experiment are shown in the accompanying graph. On the basis of these data, give the order of the *xyl*, *mtl*, *mal*, and *met* genes on the bacterial chromosome and indicate the minimum distances between them.



The closer genes are to the F factor, the more quickly they will be transferred and more recombinants will be produced. The transfer process will occur in a linear fashion. By interrupting the mating process, the transfer will stop and the F^{\square} strain will have received only genes carried on the piece of the Hfr strain's chromosome that entered the F^{\square} cell prior to the disruption. From the graph, we can determine when the first recombinants for each marker were first identified and subsequently approximate the minutes that separate the different genetic markers.



- *18. A series of Hfr strains that have genotype $m^+ n^+ o^+ p^+ q^+ r^+$ are mixed with an F^{\square} strain that has genotype $m^{\square} n^{\square} o^{\square} p^{\square} q^{\square} r^{\square}$. Conjugation is interrupted at regular intervals and the order of appearance of genes from the Hfr strain is determined in the recipient cells. The order of gene transfer for each Hfr strain is:

Hfr 5 $m^+ q^+ p^+ n^+ r^+ o^+$

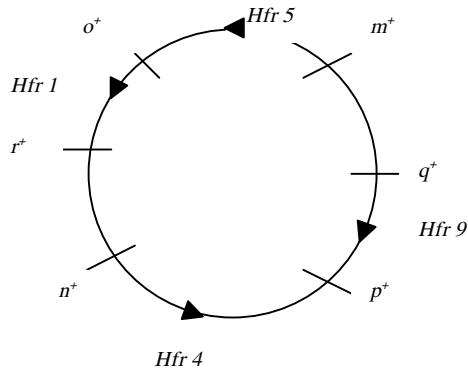
Hfr 4 $n^+ r^+ o^+ m^+ q^+ p^+$

Hfr 1 $o^+ m^+ q^+ p^+ n^+ r^+$

Hfr 9 $q^+ m^+ o^+ r^+ n^+ p^+$

What is the order of genes on the circular bacterial chromosome? For each Hfr strain, give the location of the F factor in the chromosome and its polarity.

In each of the Hfr strains, the F factor has been inserted at a different location in the chromosome. The orientation of the F factor in the strains varies as well.

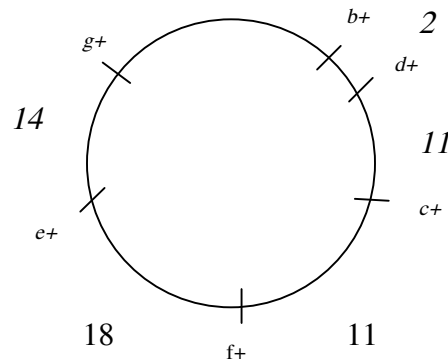


- *19. Crosses of three different Hfr strains with separate samples of an F⁺ strain are carried out, and the following mapping data are provided from studies of interrupted conjugation:

		Appearance of genes in F ⁺ cells				
Hfr1:	Genes	b ⁺	d ⁺	c ⁺	f ⁺	g ⁺
	Time	3	5	16	27	59
Hfr2:	Genes	e ⁺	f ⁺	c ⁺	d ⁺	b ⁺
	Time	6	24	35	46	48
Hfr3:	Genes	d ⁺	c ⁺	f ⁺	e ⁺	g ⁺
	Time	4	15	26	44	58

Construct a genetic map for these genes, indicating their order on the bacterial chromosome and the distances between them.

The F factor for each Hfr strain has been inserted into a different location on the chromosome, and the orientation of the F factor varies in the different strains. Although most of the selective markers transferred from each Hfr strain to the F strain are the same, some of the markers for a given Hfr strain are not transferred due to the mating being disrupted prior to the transfer of that selective marker. The relative position of the genes to each other in minutes does not vary. So, for the different Hfr strains, the distance in minutes between each gene remains constant. The genes and their relative positions are shown below. Times are in minutes of conjugation.



20. DNA from a strain of *Bacillus subtilis* with the genotype $trp^+ tyr^+$ is used to transform a recipient strain with the genotype $trp^{\Delta} tyr^{\Delta}$. The following numbers of transformed cells were recovered:

<u>Genotype</u>	<u>Number of transformed cells</u>
$trp^+ tyr^{\Delta}$	154
$trp^{\Delta} tyr^+$	312
$trp^+ tyr^+$	354

What do these results suggest about the linkage of the *trp* and *tyr* genes?

During transformation, only genes that are closely linked or located near each other on the donor's chromosome will be transformed together. In other words, a higher cotransformation frequency indicates a shorter distance between the two genes on the donor's chromosome.

*To calculate the cotransformation frequency of the trp^+ and tyr^+ genes from *Bacillus subtilis*, divide the number of transformed cells with the genotype $trp^+ tyr^+$ by the total number of transformed cells (354/820). The frequency of cotransformation is 0.43, or 43%. The high level of cotransformation indicates that these two genes are closely linked.*

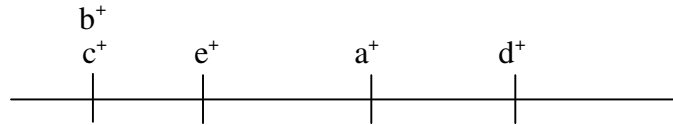
21. DNA from a strain of *Bacillus subtilis* with genotype $a^+ b^+ c^+ d^+ e^+$ is used to transform a strain with genotype $a^{\Delta} b^{\Delta} c^{\Delta} d^{\Delta} e^{\Delta}$. Pairs of genes are checked for cotransformation and the following results are obtained:

<u>Pair of genes</u>	<u>Cotransformation</u>
a^+ and b^+	no
a^+ and c^+	no
a^+ and d^+	yes
a^+ and e^+	yes
b^+ and c^+	yes
b^+ and d^+	no
b^+ and e^+	yes
c^+ and d^+	no
c^+ and e^+	yes
d^+ and e^+	no

On the basis of these results, what is the order of the genes on the bacterial chromosome?

Only genes located near each other on the bacterial chromosome will be cotransformed together. However, by performing transformation experiments and screening for different pairs of cotransforming genes, a map of the gene order can be determined. Gene pairs that never result in cotransformation must be farther apart on the chromosome, while gene pairs that result in cotransformation are more closely linked. From the data, we see that gene a^+ cotransforms with both e^+ and d^+ . However, genes d^+ and e^+ do not exhibit cotransformation, indicating that a^+ and e^+ are more closely linked than d^+ and e^+ . Gene a^+ does not exhibit cotransformation with either gene b^+ or c^+ , yet gene e^+ does. This indicates that gene e^+ is more closely

linked to genes b^+ and c^+ than is gene a^+ . The orientation of genes b^+ and c^+ relative to e^+ cannot be determined from the data provided.

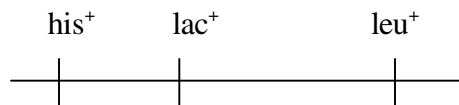


22. DNA from a bacterial strain that is $his^+ leu^+ lac^+$ is used to transform a strain that is $his^- leu^- lac^-$. The following percentages of cells were transformed:

Donor strain	Recipient strain	Genotype of transformed cells	Percent
$his^+ leu^+ lac^+$	$his^\square leu^\square lac^\square$	$his^+ leu^+ lac^+$	0.02%
		$his^+ leu^+ lac^\square$	0.00%
		$his^+ leu^\square lac^+$	2.00%
		$his^+ leu^\square lac^\square$	4.00%
		$his^\square leu^+ lac^+$	0.12%
		$his^\square leu^- lac^+$	3.00%
		$his^\square leu^+ lac^\square$	1.50%

- a. What conclusions can you make about the order of these three genes on the chromosome?

The percentages of cotransformation between his^+ , leu^+ , and lac^+ loci must be examined. Genes that cotransform more frequently will be closer together on the donor chromosome. Cotransformation between lac^+ and his^+ occurs in 2.02% of the transformed cells. By comparing this value with the cotransformation of lac^+ and leu^+ at 0.12% and the cotransformation of his^+ and leu^+ at .02%, we can see that lac^+ and his^+ cotransform more frequently. Therefore, lac^+ and his^+ must be more closely linked than the other gene pairs combinations. Because lac^+ and leu^+ cotransform more frequently than his^+ and leu^+ , leu^+ must be located closer to lac^+ than it is to his^+ .



- b. Which two genes are closest?

From the cotransformation frequencies, we can predict that lac^+ and his^+ are the closest two genes.

23. Rollin Hotchkiss and Julius Marmur studied transformation in the bacterium *Streptococcus pneumoniae* (R. D. Hotchkiss and J. Marmur. 1954. *Proceedings of the National Academy of Sciences* 40:55–60). They examined four mutations in this bacterium: penicillin resistance (P), streptomycin resistance (S), sulfanilamide resistance (F), and the ability to utilize mannitol (M). They extracted DNA from strains of bacteria with different combinations of different mutations and used this DNA to

transform wild-type bacterial cells ($P^+ S^+ F^+ M^+$). The results from one of their transformation experiments are shown here.

Donor DNA	Recipient DNA	Transformants	Percentage of all cells
$M S F$	$M^+ S^+ F^+$	$M^+ S F^+$	4.0
		$M^+ S^+ F$	4.0
		$M S^+ F^+$	2.6
		$M S F^+$	0.41
		$M^+ S F$	0.22
		$M S^+ F$	0.0058
		$M S F$	0.0071

- a. Hotchkiss and Marmur noted that the percentage of cotransformation was higher than would be expected on a random basis. For example, the results show that the 2.6% of the cells were transformed into M and 4% were transformed into S . If the M and S traits were inherited independently, the expected probability of cotransformation of M and S ($M S$) would be $0.026 \times 0.04 = 0.001$, or 0.1%. However, they observed 0.41% $M S$ cotransformants, four times more than they expected. What accounts for the relatively high frequency of cotransformation of the traits they observed?

It is likely that the M and S traits are linked or in other words they are located very close to each other on the Streptococcus pneumoniae chromosome. By being located close together on the chromosome, these markers are more likely to be cotransformed on a single fragment of DNA.

- b. On the basis of the results, what conclusion can you make about the order of the M , S , and F genes on the bacterial chromosome?

Because M and S cotransform quite frequently, they are likely close to each other on the chromosome as indicated above. S and F cotransform more frequently (.22) than do M and F (0.0058). The transformation data suggests that S and F are located closer together than are M and F.



- c. Why is the rate of cotransformation for all three genes ($M S F$) almost the same as the cotransformation of $M F$ alone?

Genes M and F cotransform infrequently, which is likely due to the physical distance between them. Genes M S are more closely linked on chromosome and the relative positions of M, S, and F make it likely that, if M and F are cotransformed on the same DNA molecule, then S will be cotransformed as well.

24. In the course of a study on the effects of the mechanical shearing of DNA, Eugene Nester, A. T. Ganesan, and Joshua Lederberg studied the transfer, by transformation, of sheared DNA from a wild type strain of *Bacillus subtilis* ($his_2^+ aro_3^+ try_2^+ aro_1^+ tyr_1^+ aro_2^+$) to strains of bacteria carrying a series of mutations (E. W. Nester, A. T. Ganesan, and J. Lederberg. 1963.

Proceedings of the National Academy of Sciences 49:61–68). They reported the following rates of cotransformation between his_2^+ and the other genes (expressed as cotransfer rate), shown here.

Genes	Rate of cotransfer
his_2^+ and aro_3^+	0.015
his_2^+ and try_2^+	0.10
his_2^+ and aro_1^+	0.12
his_2^+ and tyr_1^+	0.23
his_2^+ and aro_2^+	0.05

On the basis of these data, which gene is farthest from his_2^+ ? Which gene is closest?

Genes that are more closely linked or located physically nearer each other on the chromosome will cotransform more frequently than those genes that are not. Genes that are farther apart will cotransform less frequently with each other. From the above data, we can see that his_2^+ and tyr_1^+ cotransform the most frequently together (0.23), which indicates tyr_1^+ is the closest gene to the his_2^+ marker. Also from the above data, we can see that his_2^+ and aro_3^+ cotransform less frequently than the other pairs (0.015), which indicates that aro_3^+ is the farthest gene from his_2^+ .

- *25. Anagnostopoulos and I. P. Crawford isolated and studied a series of mutations that affected several steps in the biochemical pathway leading to tryptophan in the bacterium *Bacillus subtilis* (C. Anagnostopoulos and I. P. Crawford. 1961. *Proceedings of the National Academy of Sciences* 47:378–390). Seven of the strains that they used in their study are listed here, along with the mutation found in that strain.

Strain	Mutation
T3	T^-
168	I^-
168PT	I^-
TI	I^-
TII	I^-
T8	A^-
H25	H^-

To map the genes for tryptophan synthesis, they carried out a series of transformation experiments on strains having different mutations and determined the percentage of recombinants among the transformed bacteria. Their results were as follows:

Recipient	Donor	Percent recombinants
T3	168PT	12.7
T3	T11	11.8
T3	T8	43.5
T3	H25	28.6

168	H25	44.9
TII	H25	41.4
TI	H25	31.3
T8	H25	67.4
H25	T3	19.0
H25	TII	26.3
H25	TI	13.4
H25	T8	45.0

On the basis of these two-point recombination frequencies, determine the order of the genes and the distances between them. Where more than one cross was completed for a pair of genes, average the recombination rates from the different crosses. Draw a map of the genes on the chromosome.

Although this likely is not the actual case for the tryptophan operon, we will assume that each mutation listed (T, I, A, and H) represents a single gene and not just a selectable phenotype. The percent recombination can be used to determine the map distances. Some gene pairs have multiple crosses so we will have to average the percentages of recombination to determine the map distances.

T---I: 12.7% (T3 \square 168PT) and 11.8% (T3 \square TII)

T---A: 43.5% (T3 \square T8)

T---H: 28.6% (T3 \square H25) and 19.0 (H25 \square T3)

I---H: 44.9% (168 \square H25), 41.4% (TII \square H25), 31.3% (TI \square H25), 26.3% (H25 \square TII), and 13.4% (H25 \square TI)

A---H: 67.4% (T8 \square H25) and 45.0% (H25 \square T8)

From the above crosses, we can determine the map distances by taking the averages of the crosses for each gene.

$$I - T = 12.3$$

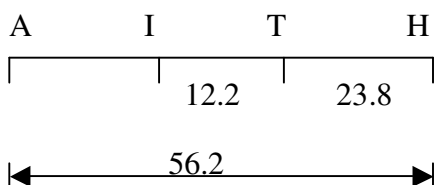
$$T - A = 43.5$$

$$T - H = 23.8$$

$$I - H = 31.5$$

$$H - A = 56.2$$

From these results, we can see that A and H are the furthest apart and that T is between I and H. This gives a map of:



We have no direct experimental measure of the distance between A and I. However, since map distances are additive, we can calculate that this distance by subtraction. If we use the A to H distance, this is: $56.2 - (12.2 + 23.8) = 20.2$. If we use the A to T distance, this is: $43.5 - 12.2 = 31.3$. The true distance between A and I is probably close to these estimates. Using the average $(20.2 + 31.3) / 2 = 25.8$, we can complete the map.

A	I	T	H				
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25.8	12.2	23.8					

Section 8.2

26. Two mutations that affect plaque morphology in phages (a^{\square} and b^{\square}) have been isolated. Phages carrying both mutations ($a^{\square} b^{\square}$) are mixed with wild-type phages ($a^{+} b^{+}$) and added to a culture of bacterial cells. Subsequent to infection and lysis, samples of the phage lysate are collected and cultured on bacterial cells. The following numbers of plaques are observed:

Plaque phenotype	Number
$a^{+} b^{+}$	2043
$a^{+} b^{\square}$	320
$a^{\square} b^{+}$	357
$a^{\square} b^{\square}$	2134

What is the frequency of recombination between the a and b genes?

First, we must identify the progeny phage whose plaque phenotype is different from either of the infecting phage. The original infecting phages were wild-type ($a^{+} b^{+}$) and doubly mutant ($a^{\square} b^{\square}$). Any phages that give rise to the $a^{+} b^{\square}$ plaque phenotype or the $a^{\square} b^{+}$ plaque phenotype were produced by recombination between the two types of infecting phage particles.

<i>Plaque phenotype</i>	<i>Number</i>
$a^{+} b^{+}$	2043
$a^{+} b^{\square}$	320 (<i>recombinant</i>)
$a^{\square} b^{+}$	357 (<i>recombinant</i>)
$a^{\square} b^{\square}$	2134
<i>Total plaques</i>	4854

The frequency of recombination is calculated by dividing the total number of recombinant plaques by the total number of plaques ($677/4854$), which gives a frequency of 0.14, or 14%.

27. T. Miyake and M. Demerec examined proline-requiring mutations in the bacterium *Salmonella typhimurium* (T. Miyake and M. Demerec. 1960. *Genetics* 45:755–762). On the basis of complementation studies, they found four proline auxotrophs: *proA*, *proB*, *proC*, and *proD*. To determine if *proA*, *proB*, *proC*, and *proD* loci were

located close together on the bacterial chromosome, they conducted a transduction experiment. Bacterial strains that were $proC^+$ and had mutations at $proA$, $proB$, or $proD$ were used as donors. The donors were infected with bacteriophages, and progeny phages were allowed to infect recipient bacteria with genotype $proC^+ proA^+ proB^+ proD^+$. The bacteria were then plated on a selective medium that allowed only $proC^+$ bacteria to grow. The following results were obtained:

Donor genotype Number	Transductant genotype	
$proC^+ proA^- proB^+ proD^+$	$proC^+ proA^+ proB^+ proD^+$	2765
	$proC^+ proA^- proB^+ proD^+$	3
$proC^+ proA^+ proB^- proD^+$	$proC^+ proA^+ proB^+ proD^+$	1838
	$proC^+ proA^+ proB^- proD^+$	2
$proC^+ proA^+ proB^+ proD^-$	$proC^+ proA^+ proB^+ proD^+$	1166
	$proC^+ proA^+ proB^+ proD^-$	0

- Why are there no $proC^-$ genotypes among the transductants?
Transductants were initially screened for the presence of $proC^+$. Thus, only $proC^+$ transductants were identified.
 - Which genotypes represent single transductants and which represent cotransductants?
The wild-type genotypes ($proC^+ proA^+ proB^+ proD^+$) represent single transductants of $proC^+$. Both the $proC^+ proA^- proB^+ proD^+$ and $proC^+ proA^+ proB^- proD^+$ genotypes represent cotransductants of $proC^+$, $proA^-$ and $proC^+$, $proB^-$.
 - Is there evidence that $proA$, $proB$, and $proD$ are located close to $proC$? Explain your answer.
From the data, it appears that both $proA$ and $proB$ are located close to $proC$. Both are capable of being cotransduced along with $proC$. The $proD$ marker may be located at distance from $proC$ so that it cannot cotransduce with $proC$. However, the data is not conclusive.
- *28. A geneticist isolates two mutations in bacteriophage. One mutation causes the clear plaques (c) and the other produces minute plaques (m). Previous mapping experiments have established that the genes responsible for these two mutations are 8 map units apart. The geneticist mixes phages with genotype $c^+ m^+$ and genotype $c^\square m^\square$ and uses the mixture to infect bacterial cells. She collects the progeny phages and cultures a sample of them on plated bacteria. A total of 1000 plaques are observed. What numbers of the different types of plaques ($c^+ m^+$, $c^\square m^\square$, $c^+ m^\square$, $c^\square m^+$) should she expect to see?
We know that the two genes are 8 map units apart. These 8 map units correspond to a percent recombination between the two genes of 8%. When the geneticist mixes the two phages ($m^+ c^+ \square m^\square c^\square$), creating a double infection of the bacterial cell, she should expect the two types of recombinant plaque phenotypes, $m^+ c^\square$ and $m^\square c^+$, to

comprise 8% of the progeny phage. The remaining 92% will be a combination of the wild-type phage and the doubly mutant phage.

<i>Plaque phenotype</i>	<i>Expected number</i>
$c^+ m^+$	460
$c^\square m^\square$	460
$c^+ m^\square$	40 (recombinant)
$c^\square m^+$	40 (recombinant)
<i>Total plaques</i>	1000

29. The geneticist carries out the same experiment described in Problem 23, but this time she mixes phages with genotypes $c^+ m^\square$ and $c^\square m^+$. What results are expected with this cross?

We know that the two genes are 8 map units apart, corresponding to 8% recombination. The phage used by the geneticist in this experiment will produce recombinants with different phenotypes from her previous experiment, but the number of recombinants will remain the same.

<i>Plaque phenotype</i>	<i>Expected number</i>
$c^+ m^+$	40 (recombinant)
$c^\square m^\square$	40 (recombinant)
$c^\square m^+$	460
$c^+ m^\square$	460
<i>Total plaques</i>	1000

- *30. A geneticist isolates two r mutants (r_{13} and r_2) that cause rapid lysis. He carries out the following crosses and counts the number of plaques listed here:

Genotype of parental phage	Progeny	Number of plaques
$h^+ r_{13}^\square \times h^\square r_{13}^+$	$h^+ r_{13}^+$	1
	$h^\square r_{13}^+$	104
	$h^+ r_{13}^\square$	110

	$h^{\square} r_{13}^{\square}$	2
	Total	216
$h^+ r_2^{\square} \times h^{\square} r_2^+$	$h^+ r_2^+$	6
	$h^{\square} r_2^+$	86
	$h^+ r_2^{\square}$	81
	$h^{\square} r_2^{\square}$	7
	Total	180

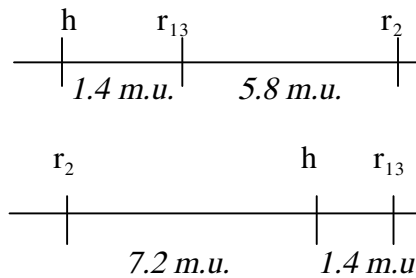
- a. Calculate the recombination frequencies between r_2 and h and between r_{13} and h .

To determine the recombination frequencies, the recombinant offspring must be identified. The recombination frequency is calculated by dividing the total number of recombinant plaques by the total number of plaques.

<i>Genotype of parents</i>	<i>Progeny</i>	<i>Number of plaques</i>
$h^+ r_{13}^{\square} \times h^{\square} r_{13}^+$	$h^+ r_{13}^+$ (recombinant)	1
	$h^{\square} r_{13}^{\square}$	104
	$h^+ r_{13}^{\square}$	110
	$h^{\square} r_{13}^+$ (recombinant)	2
	<i>total</i>	216
$h^+ r_2^{\square} \times h^{\square} r_2^+$	$h^+ r_2^+$ (recombinant)	6
	$h^{\square} r_2^+$	86
	$h^+ r_2^{\square}$	81
	$h^{\square} r_2^{\square}$ (recombinant)	7
	<i>total</i>	180

The recombination frequency between r_2 and h is $13/180 = .072$, or 7.2%. The RF between r_{13} and h is $3/216 = 0.014$, or 1.4%.

- b. Draw all possible linkage maps for these three genes.

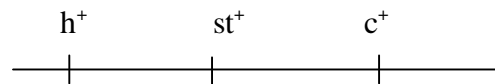


- *31. *E. coli* cells are simultaneously infected with two strains of phage \square . One strain has a mutant host range, is temperature sensitive, and produces clear plaques (genotype = $h st c$); another strain carries the wild-type alleles (genotype = $h^+ st^+ c^+$). Progeny phages are collected from the lysed cells and plated on bacteria. These genotypes of the progeny phage are:

Progeny phage genotype	Number of plaques
$h^+ c^+ st^+$	321
$h c st$	338
$h^+ c st$	26
$h c^+ st^+$	30
$h^+ c st^+$	106
$h c^+ st$	110
$h^+ c^+ st$	5
$h c st^+$	6

- a. Determine the order of the three genes on the phage chromosome.
First, we need to identify the progeny phages that have genotypes similar to the parents and the progeny phages that have genotypes that differ from the parents. The parental genotypes are $h^+ c^+ st^+$ and $h c st$. Any genotype that differs from these two genotypes had to be generated by recombination. By comparing the genotype of the double-recombinant phage progeny with the nonrecombinants, we can predict the gene order.

Phage genotype	Number of progeny	Type
$h^+ c^+ st^+$	321	Parental
$h c st$	338	Parental
$h^+ c st$	26	Recombinant
$h c^+ st^+$	30	Recombinant
$h^+ c st^+$	106	Recombinant
$h c^+ st$	110	Recombinant
$h^+ c^+ st$	5	Double-recombinant
$h c st^+$	6	Double-recombinant
Total	942	

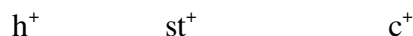


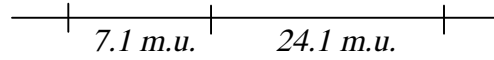
- b. Determine the map distances between the genes.
The map distances can be calculated by determining the percent recombination between each gene pair. The double-recombinant progeny, $h^+ c^+$ and $h c$, appear to be parentals. However, this genotype was generated by a double-crossover event. To consider the double-crossover events, multiply the number of double-recombinant progeny by two.

$$h^+ st^+: [(26+30+5+6)/942] \times 100 \% = 7.1\%, \text{ or } 7.1 \text{ m.u.}$$

$$h^+ c^+: [(26+30+106+110+10+12)/942] \times 100\% = 31.2\%, \text{ or } 31.2 \text{ m.u.}$$

$$st^+ c^+: [(106+110+5+6)/942] \times 100 \% = 24.1\% \text{ or } 24.1 \text{ m.u.}$$





- c. Determine the coefficient of coincidence and the interference.

$$COC = \frac{(\text{observed number of double recombinants})}{(\text{expected number of double recombinants})}$$

$$COC = (6 + 5) / (.071 \times .241 \times 942) = 0.68$$

$$\text{Interference} = 1 - COC = 1 - 0.68 = 0.32$$

32. A donor strain of bacteria with genes $a^+ b^+ c^+$ is infected with phages to map the donor chromosome with generalized transduction. The phage lysate from the bacterial cells is collected and used to infect a second strain of bacteria that are $a^\square b^\square c^\square$. Bacteria with the a^+ gene are selected and the percentage of cells with cotransduced b^+ and c^+ genes are recorded.

Donor	Recipient	Selected gene	Cells with cotransduced gene (%)
$a^+ b^+ c^+$	$a^\square b^\square c^\square$	a^+	25 b^+
		a^+	3 c^+

Is the b or c gene closer to a ? Explain your reasoning.

The gene b^+ cotransduces more frequently with a^+ , the selective marker, than does c^+ . Because genes that are closer together on the donor bacterial chromosome cotransduce more frequently, we can see that b^+ is closer to a^+ .

33. A donor strain of bacteria with genotype $leu^+ gal^\square pro^+$ is infected with phages. The phage lysate from the bacterial cells is collected and used to infect a second strain of bacteria that are $leu^\square gal^+ pro^\square$. The second strain is selected for leu^+ , and the following cotransduction data obtained:

Donor	Recipient	Selected gene	Cells with cotransduced gene (%)
$leu^+ gal^\square pro^+$	$leu^\square gal^+ pro^\square$	leu^+	47 pro^+
		leu^+	26 gal^\square

Which genes are closest, leu and gal or leu and pro ?

Because leu and pro cotransduce together more frequently, they must be the closest.

34. A geneticist isolates two new mutations from the rII region of bacteriophage T4, called rII_x and rII_y . *E. coli* B cells are simultaneously infected with phages carrying the rII_x mutation and with phages carrying the rII_y mutation. After the cells have lysed, samples of the phage lysate are collected. One sample is grown on *E. coli* K cells and a second sample on *E. coli* B cells. There are 8322 plaques on the *E. coli* B and 3 plaques on *E. coli* K. What is the recombination frequency between these two mutations?

$$\text{The recombination frequency} = \frac{(2 \times \text{plaques on K})}{\dots}$$

(Total number of plaques)

$$\text{Recombination frequency} = (2 \times 3)/8322 = 7.2 \times 10^4, \text{ or } 0.072\%$$

35. A geneticist is working with a new bacteriophage called phage Y3 that infects *E. coli*. He has isolated eight mutant phages that fail to produce plaques when grown on *E. coli* strain K. To determine whether these mutations occur at the same functional gene, he simultaneously infects *E. coli* K cells with paired combinations of the mutants and looks to see whether plaques formed. He obtains the results at the top of the following page. (A plus sign means that plaques were formed on *E. coli* K; a minus sign means no plaques were formed on *E. coli* K).

Mutant	1	2	3	4	5	6	7	8
1								
2	+							
3	+	+						
4	+	-	+					
5	-	+	+	+				
6	-	+	+	+	-			
7	+	-	+	-	+	+		
8	-	+	+	+	-	-	+	

- a. To how many functional genes (cistrons) do these mutations belong?
The geneticist is essentially conducting the “trans” portion of the cis–trans test. If complementation occurs between the different phage mutants that are infecting the E. coli K cells, then plaques will form on the lawn of E. coli K. Complementation can occur only when the mutations of the different phages are located on different cistrons or functional genes. Phage mutants that do not complement each other have mutations that lie on the same cistrons.

From the formation of plaques on E. coli K, we can see three groups of phages that failed to complement with other phages within their group but did complement the phages in the other groups. Because there are three groups, we can infer the presence of three cistrons or functional genes.

- b. Which mutations belong to the same functional gene?
We will identify the groups as group 1, group 2 and group 3.
Group 1: Mutants 1, 5, 6, and 8
Group 2: Mutants 2, 4, and 7
Group 3: Mutant 3

CHALLENGE QUESTIONS

Section 8.1

36. As a summer project, a microbiology student independently isolates two mutations in *E. coli* that are auxotrophic for glycine (*gly*⁰). The student wants to know whether these two mutants occur at the same cistron. Outline a procedure that the student

could use to determine whether these two gly^{\square} mutations occur within the same cistron.

To determine if the two gly^{\square} are with the same cistrons, a strain of bacteria will have to be constructed that contains both genes, but on different DNA molecules within the strain. Only by both mutations being present in the same cell can complementation of the two mutations be tested. The student will need to create a merodiploid or partial diploid strain. A method for doing this is to create an F^{\square} that contains one of the gly^{\square} markers. Because gly^{\square} results in an auxotrophic mutant, it would be difficult to use as an initial screen for an F^{\square} that contains it. Therefore, the student will need to use $Hfr \square F^{\square}$ matings to map the location of gly^{\square} marker and identify other protrophic markers that are nearby. By screening for F^{\square} strains that have the nearby protrophic markers, an F^{\square} strain that contains the gly^{\square} marker should be identified.

Next, the F^{\square} strain having the gly^{\square} marker should be mated to a F^{\square} that contains the other gly^{\square} mutation. The identified exconjugants should contain both gly^{\square} mutations on separate DNA molecules within the cell. If the exconjugant can grow on minimal media not supplemented with glycine, then complementation has occurred and the two gly^{\square} mutations are located on different cistrons.

37. A group of genetics students mixes two auxotrophic strains of bacteria: one is $leu^+ trp^+ his^{\square} met^{\square}$ and the other is $leu^{\square} trp^- his^+ met^+$. After mixing the two strains, they plate the bacteria on minimal medium and observe a few prototrophic colonies ($leu^+ trp^+ his^+ met^+$). They assume that some gene transfer has occurred between the two strains. How can they determine whether the transfer of genes is due to conjugation, transduction, or transformation?

Conjugation requires the direct contact of the donor bacterial strain and the recipient. If the transfer does not occur when the bacteria are kept physically separate, then conjugation is not the likely pathway. Another test would be to conduct interrupted mating experiments (assuming that one of the bacterial strains is an Hfr strain) to see if the transfer of the different markers is time dependent, which is also indicative of conjugation.

If the transfer occurs by transformation, then extraction of DNA from either strain and exposure of the other strain to the extracted DNA should result in the transfer of the DNA molecules. By selecting one of the mutations as a selective marker and measuring cotransformation frequencies between the selective marker and the other genes individually, the frequency of the transfer will hint toward the mechanism of transfer.

Finally, if the transfer is by transduction, then by exposing the one cell type to extracted DNA from the other cell type, transfer of the genes would not be expected. Potential cotransduction frequencies could be measured similarly to the cotransformation frequency. Also, the presence of plaques might be evident.