E1. Because there were no colonies when 10^9 cells of the *bio*$^-$ *met*$^-$ *phe*$^+$ *thr*$^+$ or *bio*$^+$ *met*$^+$ *phe*$^-$ *thr*$^-$ strains were plated alone.

E2. Mix the two strains together and then put some of them on plates containing streptomycin and some of them on plates without streptomycin. If mated colonies are present on both types of plates, then the *phe*$^+$ and *thr*$^+$ genes were transferred to the *bio*$^+$ *met*$^+$ *phe*$^-$ *thr*$^-$ strain. If colonies are found only on the plates that lack streptomycin, then the *bio*$^+$ and *met*$^+$ genes are being transferred to the *bio*$^-$ *met*$^-$ *phe*$^+$ *thr*$^+$ strain. This answer assumes a one-way transfer of genes from a donor to a recipient strain.

E3. The U-tube can distinguish between conjugation and transduction because of the pore size. Since the pores are too small for the passage of bacteria, this prevents direct contact between the two bacterial strains. However, viruses can pass through the pores and infect cells on either side of the U-tube.

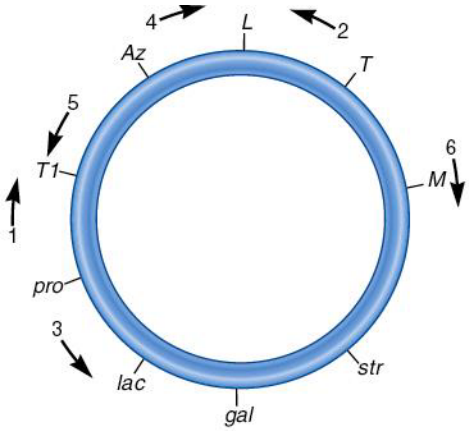
It might be possible to use a U-tube to distinguish between transduction and transformation if a filter was used that had a pore size that was too small for viruses but large enough to allow the passage of DNA fragments. However, this might be difficult since the difference in sizes between DNA fragments and phages are relatively small compared to the differences in sizes between bacteria and phages.

E4. An interrupted mating experiment is a procedure in which two bacterial strains are allowed to mate, and then the mating is interrupted at various time points. The interruption occurs by agitation of the solution in which the bacteria are found. This type of study is used to map the locations of genes. It is necessary to interrupt mating so that you can vary the time and obtain information about the order of transfer; which gene transferred first, second, etc.

E5. The time of entry is the time it takes for a gene to be initially transferred from one bacterium to another. To determine this time, we make many measurements at various lengths of time and then extrapolate these data back to the x-axis.

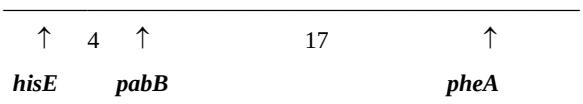
E6. Mate unknown strains *A* and *B* to the *F*$^-$ strain in your lab that is resistant to streptomycin and cannot use lactose. This is done in two separate tubes (i.e., strain *A* plus your *F*$^-$ strain in one tube, and strain *B* plus your *F*$^-$ strain in the other tube). Plate the mated cells on growth media containing lactose plates plus streptomycin. If you get growth of colonies, the unknown strain had to be the *F*$^+$ strain that had lactose utilization genes on its F factor.

E7.



E8. A. If we extrapolate these lines back to the x-axis, the *hisE* intersects at about 3 minutes and the *pheA* intersects at about 24 minutes. These are the values for the times of entry. Therefore, the distance between these two genes is 21 minutes (i.e., 24 minus 3).

B.



E9. First mate the streptomycin-resistant strain to the strain that has the genes that allow the cell to metabolize lactose. You can select for mated cells on growth media containing lactose and streptomycin. These cells will initially have an F factor with the streptomycin-resistant gene. Expose these cells to acridine orange in a media that also contains streptomycin. This will select for the survival of rare cells in which the F factor has become integrated into the chromosome to become Hfr cells.

E10. One possibility is that you could treat the P1 lysate with Dnase I, an enzyme that digests DNA. (Note: If DNA were digested with Dnase I, the function of any genes within the DNA would be destroyed.) If the DNA were within a P1 phage, it would be protected from Dnase I digestion. This would allow you to distinguish between transformation (which would be inhibited by Dnase I) versus transduction (which would not be inhibited by Dnase I). Another possibility is that you could try to fractionate the P1 lysate. Naked DNA would be smaller than a P1 phage carrying DNA. You could try to filter the lysate to remove naked DNA, or you could subject the lysate to centrifugation and remove the lighter fractions that contain naked DNA.

E11. You could infect *E. coli* with lambda and then grow under conditions that promote the lysogenic cycle (i.e., on minimal media). You could then infect the lysogenic *E. coli* strain with P1. On occasion, the P1 phage packages a piece of the bacterial chromosome and therefore it could package the lambda DNA that has been integrated into the bacterial chromosome. (Note: The size of the lambda genome is small enough to fit inside of P1.) The P1 phages isolated from the lysogenic *E. coli* strain could then be used to infect a nonlysogenic strain.

E12. Cotransduction frequency = $(1 - d/L)^3$

For the normal strain:

Cotransduction frequency = $(1 - 0.7/2)^3 = 0.275$, or 27.5%

For the new strain:

Cotransduction frequency = $(1 - 0.7/5)^3 = 0.64$, or 64%

The experimental advantage is that you could map genes that are farther than 2 minutes apart. You could map genes that are up to 5 minutes apart.

E13. Cotransduction frequency = $(1 - d/L)^3$

Cotransduction frequency = $(1 - 0.6 \text{ minutes}/2 \text{ minutes})^3$

Cotransduction frequency = 0.34, or 34%

E14. Cotransduction frequency = $(1 - d/L)^3$

$$0.53 = (1 - d / 2 \text{ minutes})^3$$

$$(1 - d / 2 \text{ minutes}) = \sqrt[3]{0.53}$$

$$(1 - d / 2 \text{ minutes}) = 0.81$$

$$d = 0.38 \text{ minutes}$$

E15. You would conclude that the two genes are farther apart than the length of 2% of the bacterial chromosome.

E16. A. We first need to calculate the cotransformation frequency, which equals 2/70, or 0.029.

Cotransformation frequency = $(1\text{-}d / L)^3$

$$0.029 = (1 - d / 2 \text{ minutes})^3$$

$$d = 1.4 \text{ minutes}$$

B.

$$\text{Cotransformation frequency} = (1 - d/L)^3$$
$$= (1 - 1.4/4)^3$$
$$= 0.27$$

As you may have expected, the cotransformation frequency is much higher when the transformation involves larger pieces of DNA.

E17. A P1 plaque mostly contains P1 bacteriophages that have a phage coat and P1 DNA. On occasion, however, a phage coat contains a segment of the bacterial chromosome. It would also contain material from the *E. coli* cells that had been lysed.

E18. Benzer could use this observation as a way to evaluate if intragenic recombination had occurred. If two *rII* mutations recombined to make a wild-type gene, the phage would produce plaques in this *E. coli K12(λ)* strain.

E19. In *E. coli B,* both *rII* mutants and wild-type strains could produce plaques. In *E. coli K12(λ)* only the rare recombinants that produced the wild-type gene would be able to form plaques. If the phage preparation was not diluted enough, the entire bacterial lawn would be lysed so it would be impossible to count the number of plaques.

E20. *rIIA:* L47, L92

rIIB: L33, L40, L51, L62, L65, and L91

E21. You would basically follow the strategy of Benzer (see Figure 6.18) except that you would not need to use different *E. coli* strains. Instead, you would grow the cells at different temperatures. You would coinfect an *E. coli* strain with two of the temperature-sensitive phages and then grow at 32°C. You would then reisolate phages as shown in step 4 of Figure 6.18. You would take some of the phage preparation, dilute it greatly (10^{-8}), infect *E. coli,* and grow at 32°C. This would tell you how many total phages you had. You would also take some of the phage preparation, dilute it somewhat (10^{-6}), infect *E. coli,* and then grow at 37°C. At 37°C, only intragenic recombinants that produced wild-type phages would form plaques.

E22. Benzer first determined the individual nature of each gene by showing that mutations within the same gene did not complement each other. He then could map the distance between two mutations within the same gene. The map distances defined each gene as a linear, divisible unit. In this regard, the gene is divisible due to crossing over.

E23. You can first narrow down a mutation to one of several different regions by doing pairwise crosses with just a few deletion strains. After that, you then make pairwise crosses only with strains that have also been narrowed down to the same region. You do not have to make pairwise crosses between all the mutant strains.