LABORATORY

14

Genes and Chromosomes: Chromosome Mapping

OVERVIEW

Genes are located on chromosomes in particular positions called **loci.** Genes can be assigned to the sex chromosomes (*X* and *Y*) by studying inheritance patterns among family groups, but assigning genes to specific autosomes (non-sex chromosomes) is more difficult. Since segregation of alleles for linked genes is not independent, linkage studies make it possible to assign two genes to the same chromosome, but this information does not usually allow us to identify a particular gene as belonging to a specific chromosome. Several methods, including the use of restriction enzymes and recombinant DNA techniques (Laboratory 18), have made it possible not only to assign a gene to a particular chromosome, but to determine, or **map,** its location with respect to other genes on that chromosome.

In this laboratory, you will map the genes for spore color in the fungus *Sordaria.* Using linkage analysis, you will be able to determine the relative distance between the centromere and known mutant genes. You will also study the giant chromosomes of the fruit fly *Drosophila,* which will allow you to visualize genes on a eukaryotic chromosome. By using bacterial conjugation as a means of genetic recombination, you will be able to map several genes on the circular chromosome of the bacterium *Escherichia coli.* Finally, you will have the opportunity to use restriction enzymes and electrophoresis to compile a restriction map of the phage lambda (λ).

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency.

EXERCISE A Mapping the Genes of *Sordaria fimicola*

In *Sordaria fimicola,* ascus formation requires both meiotic and mitotic divisions to produce eight haploid ascospores. During the first nuclear division of meiosis (meiosis I, or MI), crossing-over often occurs between non-sister chromatids of homologous chromosomes, resulting in recombination of the genes on the two homologues (Figure 14A-1). The greater the distance between the two genes on the chromosome, the more likely it is that crossing-over will occur; the genes are "loosely" linked. If the genes are close together, then crossing-over is less likely; the genes are said to be "tightly" linked. Thus the frequency with which two genes recombine is related to how far apart the two genes are on the chromosome. By using recombination frequencies, it is possible to produce a *linkage map* for genes on a chromosome.

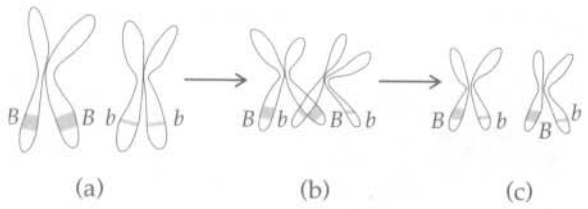


Figure 14A-1 *(a) Two homologous chromosomes carrying different alleles of a gene for a specific trait. The alleles are located at the same loci on the homologues. (b) Crossing-over occurs. (c) Alleles are exchanged, and each chromosome carries alleles that are different from those on the original chromosome.*

PART I Mapping *Sordaria* Chromosomes *(Week 2; see Laboratory 13, Exercise D)*

During ascus development, if no crossing-over occurs in meiosis I, a 4+4 pattern of ascospores is produced. These asci have an **MI pattern.** If crossing-over does occur, then the pattern of ascospores is referred to as **MII** and is either 2+2+2+2 or 2+4+2.

From the numbers of asci with MI and MII patterns following a cross, you can "map" the location of the gene for tan color (*t*) on the *Sordaria* chromosome. You will determine the distance of the tan gene locus from the centromere (centromere-to-locus distance). Note that this exercise considers just the gene for tan color, since the presence of a normal allele (t^+) or a mutant allele (t^-) will alter ascospore color. For this reason you will map the distance from the centromere, not the distance from another gene. (In more typical linkage mapping, recombination between two genes is studied to determine map distance between the genes.)

Review basic information about *Sordaria* genetics in Laboratory 13, Exercise D, before continuing. Be sure that you can recognize MI and MII ascus patterns.

Objectives

- ☐ Explain how meiosis and crossing-over result in different arrangements of ascospores within asci.
- ☐ Calculate the map distance between a gene for ascospore color and the centromere of the chromosome on which the gene is found.

Procedure

1. Your instructor will provide you with several brown paper bags used to simulate *Sordaria* perithecia from a black × tan cross. Each bag contains 10 asci. Remove the asci and determine whether they are nonhybrid or hybrid, MI or MII asci. Your instructor will ask you to report the number of MI and MII asci in your bag; your response should be verified (or corrected) by your laboratory partner. (You do *not* count the nonhybrid asci containing all like-color ascospores. *Why?*) After totaling the number of MI and MII asci for the entire class, you will be asked to determine the frequency of crossing-over and the map distance from the centromere to the tan gene, using steps 7 and 8 of this procedure. Record class data in Table 14A-1.

Table 14A-1 Class Data

Total asci counted	
Total MI asci	
Total MII asci	

a. What is the map distance of the tan gene from the centromere on the Sordaria *chromosome as simulated in this cross?* _____

2. Now, obtain your *Sordaria* cross (from Laboratory 13). Alternatively, your instructor has set up the *Sordaria* crosses and will provide you with a culture.
3. Where the mycelia of the two strains overlap and fuse, dark lines of tiny perithecia will be visible. Use a toothpick or spatula to gently scrape the surface of the agar to collect perithecia (see Figure 13D-4). It is usually best to collect perithecia toward the outer rim of the dish.
4. Place the perithecia in a drop of water on a slide. Cover with a coverslip and gently press on the coverslip (use a small cork) to rupture the perithecia. Be gentle so that the ascospores remain in the asci (see Figure 13D-2).
5. View the slide using the 10× objective and locate a group of hybrid asci (recall that asci produced by fusion of two identical strains, both black or both tan, will result in ascospores that are all of the same color within an ascus—disregard these asci). Hybrid asci contain both black and tan ascospores within each ascus.
6. Count at least 50 hybrid asci and score them as either MI asci (4+4 arrangement) in which alleles segregated in meiosis I, or MII asci (2+2+2+2 or 2+4+2) in which alleles segregated in meiosis II. (Remember, do *not* count the nonhybrid asci.) Record your results in Table 14A-2. Determine the number of M1 and M2 asci counted by all students and record class results in Table 14A-3.

Table 14A-2 Your Data

Number of MI Asci Showing No Crossover (4:4)		Number of MII Asci Showing Crossover (2:2:2:2) or (2:4:2)		Total MI + MII Asci	Percentage of Asci Showing Crossover	$\frac{\text{Frequency}}{2}$ (Map Units)
○○○○●●●●		○○●●○○●●				
●●●●○○○○		●●○○●●○○				
		○○●●●●○○				
		●●○○○○●●				

Recall that in this exercise, you are studying only one gene, and you will map its distance from the centromere by determining the frequency of crossover events involving that gene—crossovers that occur somewhere between the centromere and the gene and result in its recombination with the chromatid of a different chromosome.

The frequency of crossing-over between two genes is largely controlled by the distance between genes (or between gene and centromere, as in this case); the probability of a crossover occurring between two particular genes on the same chromosome increases as the distance between those genes increases. The frequency of crossing-over is, therefore, proportional to the distance between genes. An arbitrary unit of measure, the **map unit,** is generally used to describe distances between linked genes. A map unit is equal to a 1% frequency of crossovers. For instance, when there is a 30% frequency of crossing-over between two genes, these genes are said to be 30 map units apart.

7. Determine the frequency of crossing-over (percentage of crossovers) by dividing the number of MII crossover asci by the total number of asci counted, and multiplying by 100:

$$\text{Frequency of crossing-over} = \frac{\text{MII}}{\text{MI} + \text{MII}} \times 100$$

8. In *Sordaria,* since only 4 of the 8 ascospores carry recombinations, the frequency of recombination is one-half of the frequency of crossing-over.

b. Why do only half of the ascospores carry recombinant chromosomal strands?

_____

_____

The relationship between frequency of crossing-over and map distance is expressed as:

$$\text{Number of map units} = \frac{4 \times \text{number of recombinant (MII) asci}}{8 \times \text{total number of asci}} \times 100$$

or

$$\text{Number of map units} = \frac{\text{frequency of crossing-over}}{2}$$

9. Record the map distance of gene *t* or t^+ from the centromere. _____ Record this map distance in Table 14A-2. Published results indicate that the map distance of the tan spore-color gene from the centromere in *Sordaria fimicola* is 26 map units.

c. How closely do your data fit this measurement? _____

PART 2 The Chi-Square Test (*Optional*)

To test how closely your class crossover data agree with or "fit" the value for the map distance of the mutant tan gene obtained by others (26 map units), you can perform a chi-square test (see Appendix I). The chi-square test is a statistical test that allows you to determine if differences between your data (what was *observed*) and known or hypothetical values (what was *expected*) are significant.

Objectives

☐ Perform a chi-square test to determine the probability that your data "fit" the expected map distance.

Procedure

1. As you determine whether or not your observed data "fit" within the limits of what is expected, the chi-square analysis is actually testing the validity of your **null hypothesis** (Laboratory I). Your null hypothesis states: There will be no difference between the predicted (expected) map distance of 26 units and the observed map distance calculated from class data.

If there *is* a difference (your map distance is not 26 units), you want to know how significant this difference is. Is the difference due just to chance (perhaps sampling errors)? Or is it due to something real (a chromosomal rearrangement or deletion, etc.)? If the results of the chi-square test indicate that the observed data do *not* vary significantly from the expected, then you accept the null hypothesis: there is no difference. If, however, the results indicate that the

Table 14A-3 Class Data

Number of MI Asci		Number of MII Asci		Total MI + MII Asci	Percentage of Asci Showing Crossover	$\frac{\text{Frequency}}{2}$ (Map Units)
○○○○●●●●		○○●●○○●●				
●●●●○○○○		●●○○●●○○				
		○○●●●●○○				
		●●○○○○●●				

observed data vary significantly from the expected, then you *reject* the null hypothesis and accept your **alternative hypothesis** (see Laboratory I).

Your instructor will use the data from the *Sordaria* simulation to demonstrate how to perform the chi-square test following the steps outlined below.

a. How well do the data from the class simulation fit the expected result of 26 map units?

---

2. Use the class data on *Sordaria* cross plates from Table 14A-3.

Chi-square is calculated as shown in the following example.

Example If 26 map units is the expected locus-to-centromere distance for the tan gene on the *Sordaria* chromosome, you should expect 52% of all asci you observe to be crossover asci. (Recall that you divided the percentage of crossovers by 2 to calculate map distance; 26% × 2 = 52%.) Suppose 1,000 asci are counted by the class; 52% of 1,000 = 520. Thus, 520 of the asci should be crossovers and 480 should be non-crossovers. This is what is *expected*. What was actually observed by the class was

Crossovers = 510

Non-crossovers = 490

Using the formula

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

for both crossovers and non-crossovers, the expected (or hypothetical values) are filled in as follows:

$$\chi^2 = \frac{(\text{observed crossovers} - 520)^2}{520} + \frac{(\text{observed non-crossovers} - 480)^2}{480}$$

Since 510 crossovers and 490 non-crossovers were observed in class,

$$\chi^2 = \frac{(510 - 520)^2}{520} + \frac{(490 - 480)^2}{480}$$

$$\chi^2 = \frac{(-10)^2}{520} + \frac{(10)^2}{480} = \frac{100}{520} + \frac{100}{480} = 0.192 + 0.208 = 0.400$$

Do observed and expected results differ in this case, where $\chi^2 = 0.400$? To decide, you must refer to a Critical Values of χ^2 Table (Appendix I). Table 14A-4 is an abbreviated table for use in this laboratory.

Table 14A-4 Critical Values of Chi-Square

Degrees of Freedom (*df*)	Probability (*p*)				
	0.9 (9 in 10)	0.5 (1 in 2)	0.2 (1 in 5)	0.05 (1 in 20)	0.001 (1 in 100)
1	0.016	0.46	1.64	**3.84**	6.64
2	0.21	1.39	3.22	5.99	9.21
3	0.58	2.37	4.64	7.82	11.35

First, you must determine the **degrees of freedom** (*df*) for your experiment. In this example, the degrees of freedom are 1 less ($n - 1$) than the number of attributes being observed ($n = 2$, since crossover and non-crossover are the only attributes being studied). Next, you determine a **probability value** (p value). For most scientific studies, the minimum probability for rejecting a null hypothesis is usually $p = 0.05$. In selecting a probability of $p = 0.05$, you set a "level of rejection" for your null hypothesis. If you

reject your null hypothesis, you have a 1 in 20 (or 5%) chance of being wrong in doing so (a fairly low probability of making the wrong decision!). Having determined the *df* and *p* values, you next find the **critical value** from the chi-square table, in this case 3.84.

- *If the calculated chi-square value is* ***greater than or equal to*** *the critical value, then you* ***reject*** *the null hypothesis (and accept the alternative hypothesis).* You conclude that deviations from the expected are sufficiently large to be meaningful (significant), so there must actually be a difference (you reject the null or no-difference statement).
- *If the calculated chi-square value is* ***less than*** *the critical value, then you* ***accept*** *the null hypothesis.* You conclude that deviations from the expected are sufficiently small that there is no difference (you accept the null or no-difference statement).

The chi-square value for this example ($\chi^2 = 0.40$) is much smaller than 3.84. This means that you *accept* the null hypothesis: that class observations agree with (there is no difference from) the published or known value of 26 map units for the distance of the tan gene from the centromere on the *Sordaria* chromosome. [*Note:* If you rejected the null hypothesis based on $\chi^2 = 0.40$, you would have a *greater* chance of being wrong in making this decision—almost a 1 in 2 chance (see $p = 0.5$ for a critical value of 0.46), and this is not an acceptable degree of error.]

3. Now, apply the chi-square test to your class results recorded in Table 14A-3. Perform your calculations in the space below.

b. What is your null hypothesis for this investigation? _____

What is your alternative hypothesis? _____

c. How well do your class data fit the expected value of 26 map units? _____

d. Do you accept or reject your null hypothesis? _____

e. What do you conclude about the distance of the tan gene from the centromere on the Sordaria *chromosome?* _____

_____

✓ EXERCISE B Examining the Giant Chromosomes of *Drosophila*

During development, cells of the *Drosophila* salivary glands pass through the cell cycle many times, their DNA replicating in preparation for division. However, the cells do not divide and as a result, the number of DNA strands comprising each chromosome continues to increase; the chromosomes become multistranded and are called *polytene* chromosomes. The DNA content of polytene chromosomes is approximately 1,000 times greater than the normal DNA content of chromosomes containing a single helical strand of DNA.

The many DNA strands of a polytene chromosome condense and fold in the same manner as the single helical strand of DNA in other chromosomes. Highly condensed or folded areas stain darkly and give chromosomes a banded appearance. Since all of the DNA strands in the giant polytene chromosome are duplicates of one another, the folded portions are in register with one another and the bands appear to stretch across the entire chromosome, giving the chromosomes a very dramatic "striped" appearance (Figure 14B-1).

During transcription of messenger RNA, some of the banded regions of the polytene chromosomes uncoil and expand to form "puffs." Thus, it is possible to visualize genes on these giant chromosomes. If the protein product synthesized by the gene can be identified, so can the gene. Proof of its location can also be obtained by isolating the mRNA produced by the "puff" region, labeling it in some way, and

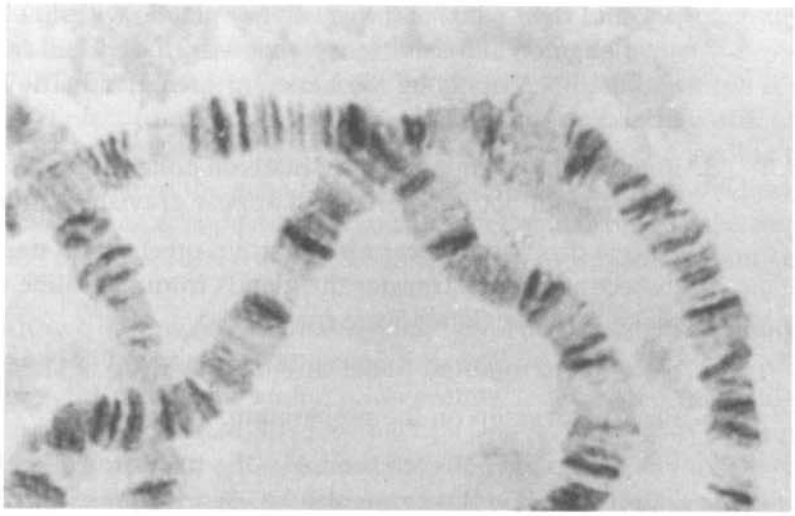


Figure 14B-1 *The polytene chromosomes of* Drosophila melanogaster.

hybridizing this mRNA with the chromosome. Where the bases are complementary to the DNA, they bind, allowing the genes to be identified by autoradiographic or immunofluorescent techniques.

In this exercise you will prepare chromosome "squashes" of *Drosophila* polytene chromosomes and observe their banded nature.

Objectives

- ☐ Describe how the banded pattern of the giant chromosomes of the *Drosophila* salivary gland is related to the arrangement of genes on the chromosome.

Procedure

1. Use the dissecting microscope. If your microscope does not have a built-in illuminator, use a black background or a mirror and a transparent glass plate. Obtain a clean slide and place a drop of 0.7% saline toward one end.
2. Place a *Drosophila* larva in the saline, and with two dissecting needles decapitate the larva. Place one needle at the middle of the larva and the other just behind the head. Often you can see the two salivary glands separating from one another in a **V** at this point, and it is best to place the point of the needle in the middle of the **V.** Pull the needle at the head with a quick

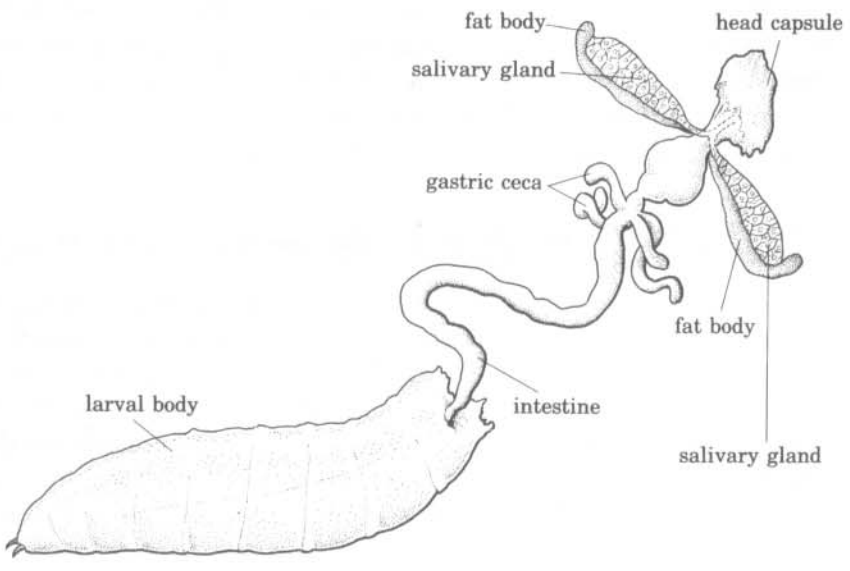


Figure 14B-2 *Dissection of the salivary glands of a* Drosophila melanogaster *larva.*

jerk and then relax, and the salivary glands will slowly be pushed out of the body. The glands are elongated and semitransparent—the individual cells are large (grapelike in appearance) and are lined up along the lumen of each gland. The lumen connects to the digestive tract through a duct (Figure 14B-2).

3. Remove the opaque fat body material adhering to the glands. If you are using transmitted light, the fat body material will appear grayish. With reflected light, it will appear white.
4. Place a drop of acetocarmine or aceto-orcein stain next to the drop of saline and, with a dissecting needle, transfer the glands from the saline to the stain. Examine the drop of stain to make sure the glands were transferred.
5. Stain for 10 minutes. Make sure that the drop of stain does not dry up.
6. Place a coverslip on the preparation.
7. Place the slide between the folds of a paper towel and press down on the coverslip firmly. The eraser end of a pencil can also be used for pressing.
8. Examine the slide using low power (10×) to locate the chromosomes. Examine the chromosomes to observe the banded pattern.

   a. Do you see any bulges along the length of the chromosome? _____ *What do they represent?*

   _____

   b. How many chromosomes do you see? (Note: *Homologous chromosomes are synapsed along their entire length, so what appears as one chromosome is actually two.)* _____

Note: The chromosome studies in Exercises A and B have introduced you to some of the basic, and more classical, techniques for locating genes on eukaryotic chromosomes. Rapid advances in biotechnology have made it possible to map eukaryotic genes more quickly and more accurately. To do this, special enzymes, **restriction endonucleases** (see Exercise D), are used to chop DNA into small fragments. These enzymes recognize specific nucleotide sequences in the DNA and always cut the DNA at the same sites. (Mutations in the DNA may alter the nucleotide sequence of a site so that the restriction endonuclease no longer cuts the DNA at the original position: a fragment with a "new length" is produced.)

Fragments produced by restriction endonucleases are called RFLPs (pronounced "rif-lips"). (See Laboratory 15, Exercise F.) By studying how frequently characteristic RFLPs appear in several generations of families that exhibit a particular genetic trait or disorder, geneticists can determine the approximate location of the gene for that disorder on a particular chromosome. Just as in linkage studies, where two genes are said to be linked if they constantly appear together (they are so close together on the chromosome that no crossing-over can occur), the RFLP and a particular trait are assumed to be "linked" and, thus, in close proximity to one another on the chromosome. If the RFLP is hybridized to the DNA of the chromosome, the physical location of the gene can be determined. In addition, the RFLP can serve as a "marker" for a specific trait. If the DNA of a patient contains the RFLP associated with a particular disorder, it is likely that the person will manifest that disorder. This is especially important as a diagnostic tool for disorders that are characterized by late onset.

EXERCISE C Mapping the Chromosome of *Escherichia coli*

Reproduction in bacteria is primarily an asexual process involving fission, but a type of sexual reproduction known as **conjugation** can also occur. During this process, genetic material is transferred from one bacterium (the + or donor strain) to another bacterium (the − or recipient strain). Donor cells contain a fertility factor, or *F* factor, carried by a plasmid (a small extrachromosomal, circular piece of DNA), which can be transferred during conjugation. These donors (males) are designated as F^+. If the *F* factor becomes integrated into the bacterial chromosome of the donor, the chromosome (although usually not the entire chromosome) can be transferred to the recipient bacterial cell. If recombination takes place, the recipient

may express characteristics originally unique to the donor. Bacteria containing the *F* factor integrated into the chromosome (as an **episome**) are called **Hfr** (high frequency of recombination) **cells.**

The amount of chromosomal DNA that can be transferred from an Hfr cell to a recipient cell is determined by how long the cells remain in contact (during conjugation, the cells are attached by **pili;** DNA transfer occurs through cytoplasmic "bridges"). The farther away a given gene is from the leading point of the chromosome being transferred, the less chance the gene has of being transferred before the bridge is broken. By interrupting mating at specific times, it is possible to construct a circular map of the bacterial chromosome. The map distances are in "minutes," referring to the time it took to transfer certain genes (Figure 14C-1).

In this exercise, you will work with two strains of *E. coli*. The donor, the Hfr strain, is streptomycin-sensitive: it does not carry the gene for streptomycin resistance and thus will be killed by this antibiotic. This "wild type" also carries alleles for synthesizing the amino acids proline, leucine, and threonine, and the vitamin thiamine. Thus this strain is designated $Str^s\ pro^+\ leu^+\ thi^+\ thr^+$. The wild-type strain can live on minimal medium that contains only glucose, ammonia, and inorganic salts, because it can make all of the amino acids needed for growth. (Remember: if streptomycin is present in the medium, these cells will die.)

The mutant strain, which is the recipient, carries the gene for streptomycin resistance, but does not carry the alleles for synthesizing proline, leucine, threonine, and thiamine. This mutant strain is designated $Str^r\ pro^-\ leu^-\ thi^-\ thr^-$. For the recipient to survive (prior to recombination), the minimal medium must be supplemented with the amino acids proline, leucine, and threonine and the vitamin thiamine; streptomycin in the medium will not harm the recipient strain.

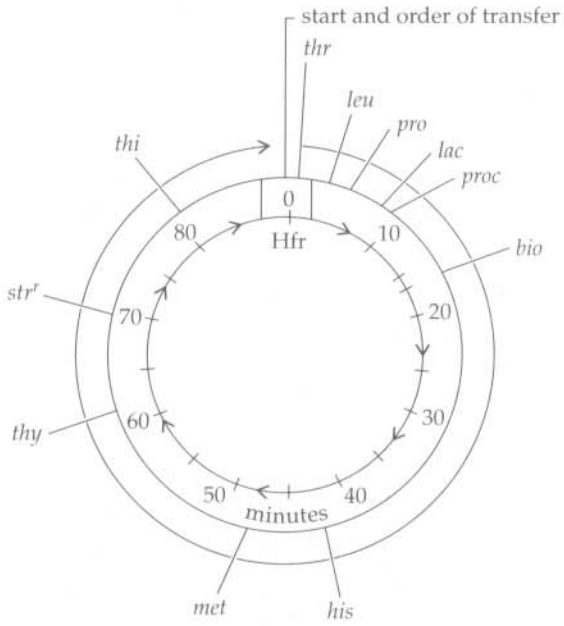


Figure 14C-1 *Genetic map of* Escherichia coli *showing several genes.*

You can determine whether particular genes have been transferred from the donor to the recipient by testing to see if the recipient has acquired the ability to synthesize any of the nutrients that had previously been synthesized only by the donor strain. For example, the transfer of the thr^+ gene can be detected in the recipient by the fact that the recipient, previously thr^- (unable to synthesie threonine), is now thr^+, due to recombination. [Since this gene is close to the origin of replication and transfer (Figure 14C-1), it is the most likely gene to be transferred to the recipient.] The recipient can now synthesize its own threonine and no longer requires a medium containing threonine for its survival.

a. If you had unlabeled cultures of Str^s pro^+ leu^+ thi^+ thr^+ *and* Str^r pro^- leu^- thi^- thr^-, *what could you do to tell them apart?* _____

_____

_____

b. What kind of test could you devise to tell whether recombination had occurred between Str^s pro^+ leu^+ thi^+ thr^+ *and* Str^r pro^- leu^- thi^- thr^-*?* _____

_____

_____

Procedure

1. Work in pairs. Review Appendix III, Preparing Serial Dilutions. Obtain a broth culture of *E. coli* strain Str^s pro^+ leu^+ thi^+ thr^+ labeled "D" for "donor strain."

2. Obtain a broth culture of *E. coli* strain Str^r pro^- leu^- thi^- thr^- labeled "R" for "recipient strain."

3. Also obtain from your instructor four agar plates containing minimal medium supplemented with streptomycin and thiamine (STR/THI) and two agar plates containing minimal medium (M) supplemented with the amino acids proline, leucine, threonine, and the vitamin thiamine, but without streptomycin (M/PLTT).

   Notice that both types of plates you are using are supplemented with thiamine. Examine Figure 14C-1.

   c. Where is the thiamine (thi) *gene located?* _____ *What is the chance that it will be transferred from donor to recipient during conjugation?* _____ *Why would you supplement plates with this vitamin?* _____

4. Before proceeding, gather the following equipment and set up your work area on a clean surface:

   1 sterile, *empty* capped test tube (the "conjugation" tube)

   5 tubes containing 9.0 ml of sterile distilled water

   1 beaker containing 95% ethyl alcohol

   1 glass spreading rod

   10 sterile 1-ml pipettes

5. Using aseptic technique, first flaming the mouth of the tube and using a sterile pipette, transfer 1.0 ml of "D" suspension into the conjugation tube. Aseptically transfer 1.0 ml of "R" suspension into the conjugation tube. *Note the time* of addition of culture "R" to culture "D" in the conjugation tube: _____ Gently agitate the mixture by rotating the tube between the palms of your hands. Allow the culture to incubate at 37°C for 30 minutes. Be sure that the mating mixture is *not* disturbed during this time.

6. Aseptically pipette 1.0 ml of "D" culture into a 9.0-ml sterile water blank and mix thoroughly. Note that the donor culture is now diluted to 1:10. Label the tube "D 1:10."

7. Using the diluted donor culture, prepare a spread-plate (see Laboratory 6, Exercise C). Use a sterile 1-ml pipette to remove 0.1 ml of dilute culture and transfer it to the center of the STR/THI plate. Dip a glass spreading rod in 95% ethyl alcohol and flame it. Let it cool for a

few seconds and touch it to the outer edge of the agar plate—if it sizzles, it is still too hot to use. When the rod has cooled, spread the inoculum over the surface of the plate. To avoid contamination, hold the lid above the plate as you are working with the bacteria.

8. Use a sterile 1-ml pipette to remove 0.1 ml of dilute "D" culture and transfer it to the center of a M/PLTT plate. Spread the bacteria as described in step 7.

d. Do you expect the donor strain to grow on the STR/THI plate? _____ *Why or why not?*

_____

e. Do you expect the donor strain to grow on the M/PLTT plate? _____ *Why or why not?*

_____

f. Why did you prepare both the STR/THI and the M/PLTT plates using only donor cells?

_____

_____

9. Now repeat the procedure outlined in steps 6 through 8, but this time use the recipient strain and fresh STR/THI and M/PLTT plates. (Label the diluted recipient strain culture "R 1:10.")

g. Do you expect the recipient strain to grow on the STR/THI plate? _____ *Why or why not?*

_____

h. Do you expect the recipient strain to grow on the M/PLTT plate? _____ *Why or why not?*

_____

i. Why did you prepare both the STR/THI and M/PLTT plates using only recipient cells?

_____

_____

10. After conjugation has proceeded for 30 minutes (step 5), remove the culture from the 37°C water bath or incubator and vigorously agitate the mixture by rotating the tube between the palms of your hands (create a vortex, if possible).

11. Aseptically transfer 1.0 ml of the conjugation mixture to a sterile water blank (9.0 ml). Mix thoroughly (roll between your palsm) and label the tube "D × R 1:10."

12. Aseptically pipette 1.0 ml of the D × R 1:10 suspension into a second sterile water blank (9 ml). Mix thoroughly (roll between your palms) and label the tube "D × R 1:100."

13. Aseptically transfer 0.1 ml of the D × R 1:10 dilution to the surface of one of the two unused STR/THI plates. Mark the plate "STR/THI 1:10" and label it with your name and the date.

14. Similarly, transfer 0.1 ml of the D × R 1:100 dilution to the surface of the other STR/THI plate. Mark the plate "STR/THI 1:100" and label it with your name and the date.

15. Sterilize a glass spreading rod by dipping it in alcohol and passing it quickly through a flame. Spread the bacteria on the STR/THI 1:10 plate as in step 7. Resterilize the spreading rod and spread the bacteria on the STR/THI 1:100 plate.

16. Be sure that all six of your plates are clearly labeled. Tape them together. Invert them and place them in an area designated by your instructor. They will be incubated for 2 days at 37°C and then refrigerated until the next laboratory period.

17. During the next laboratory period, complete Table 14C-1 using ✔ to indicate growth and 0 to indicate no growth. Interpret your results.

j. Did conjugation occur? _____ *How do you know?* _____

_____

Table 14C-1 Record of Growth (✓) and No Growth (0) of *E. coli*

	Donor (1:10)	Recipient (1:10)	D × R 1:10	D × R 1:100
STR/THI				
M/PLTT				

k. With reference to the circular map of E. coli, *which genes$^{(+)}$ are found in the recombinant cells that were mutant$^{(-)}$ in the recipient cells?* _____

_____

l. It is unusual for the entire E. coli *chromosome to be transferred during the process of conjugation. What is the consequence of this fact for genes that are farther away from the origin of replication and transfer?* _____

_____

m. Why was streptomycin included in the agar used for plating recombinant cells?

_____

If the recipient bacterial strain had also been *met*$^-$ (lacking the ability to synthesize the amino acid methionine) and recombination with the same wild-type donor cell occurred, what would have happened under the following conditions? (Refer to Figure 14C-1.)

- Conjugation is interrupted after 10 minutes and recombinants are plated onto minimal medium containing streptomycin. *n. Would recombinants grow?* _____

  o. Would these recombinants grow on medium containing both streptomycin and methionine?

  _____

- Conjugation is allowed to continue for 60 minutes and recombinants are plated onto minimal medium containing streptomycin. *p. Would these recombinants grow?* _____

  q. What does this indicate about the relative positions of the genes for proline, leucine, threonine, methionine, and thiamine? _____

_____

✓ EXERCISE D Restriction Endonucleases: Mapping Bacteriophage Lambda

Restriction endonucleases are essential tools in recombinant DNA methodology. Several hundred have been isolated from a variety of prokaryotic organisms. In the nomenclature of restriction endonucleases, the letters refer to the organism from which the endonuclease was isolated. The first letter of the name stands for the genus name of the organism. The next two letters represent the initial letters of the second word of the species name. The fourth letter (if there is one) represents the strain of the organism. Roman numerals indicate whether the particular endonuclease was the first isolated, the second, and so on. For example:

*Eco*RI
- *E* = genus *Escherichia*
- *co* = *coli*
- R = strain RY 13
- I = first endonuclease isolated

*Hin*dIII
- *H* = genus *Haemophilus*
- *in* = *influenza*
- d = strain Rd
- III = third endonuclease isolated

Each restriction endonuclease "recognizes" a specific DNA sequence (usually a 4- to 6-base-pair sequence of nucleotides) in double-stranded DNA and digests phosphodiester bonds at specific sites in the sequence. For example, the restriction endonuclease *Eco*RI cuts double-stranded DNA as follows:

```
      ↓
–G–A–A–T–T–C–
–C–T–T–A–A–G–
            ↑
```

Since this endonuclease cuts at specific sites on each strand, "**sticky ends**" are produced. (Sticky ends make it possible to insert DNA pieces, usually containing a gene of interest, into plasmids or viral vectors that have been cut with the same endonuclease, providing complementary sites for attachment—see Laboratory 18.) Other restriction endonucleases cut DNA in the same position on both strands to produce fragments with "**blunt ends.**"

Fragments of DNA produced by restriction enzyme cleavage can be separated by gel electrophoresis. When any molecule enters an electrical field, its mobility, or the speed at which it will move, is influenced by the charge on the molecule, the strength of the electrical field, the size and shape of the molecule, and the density of the medium (gel) through which it is migrating. Consequently, it is possible to separate heterogeneous populations of molecules (such as fragments of DNA). When all molecules are positioned at a uniform starting site on a gel, the gel is placed in a chamber containing a buffer solution and electrodes, and an electric current is applied, the molecules will migrate and form bands (concentrations of homogeneous molecules). Since the phosphates in the DNA backbone are negatively charged at neutral pH, DNA fragments will migrate through the gel toward the positive electrode.

After electrophoretic separation and staining of the gels with colored or fluorescent dyes, the sizes of DNA fragments can be determined by comparing them with markers of known molecular weight or other fragments of known sizes. Once this information is available, it is possible to construct a **restriction map** of the DNA in question.

✓ PART I Estimating DNA Fragment Size Using Gel Electrophoresis

In this exercise, you will use an agarose gel to separate a mixture of bacteriophage lambda (λ) DNA fragments produced using two restriction enzymes, *Eco*RI and *Hin*dIII. (The density of an agarose gel can be varied to improve the resolution of similar-sized molecules.) In agarose, the migration rate of linear fragments of DNA is inversely proportional to their size; the smaller the DNA fragment, the faster it migrates through the gel. The size of the fragments is measured in number of base pairs (bp).

The size of fragments produced by a specific endonuclease (*Eco*RI in this exercise) can be determined by using *standard* fragments of known size (fragments produced by *Hin*dIII in this case). These fragments are electrophoresed together and, after visualizing the bands by staining with dyes such as methylene blue or ethidium bromide (a fluorescent dye), a standard curve can be plotted for *Hin*dIII bands of known size. The sizes of the standard fragments are plotted on the *Y*-axis and the distances they migrated are plotted on the *X*-axis. By plotting the migration distances of the fragments of unknown size on the *X*-axis, you will be able to determine the size of the *Eco*RI fragments from the standard curve.

Objectives

- ☐ Explain the principles of electrophoresis as they pertain to separating and identifying DNA fragments.
- ☐ Determine DNA restriction fragment sizes using a standard curve.

Procedure

Work in groups of four.

A. Casting Agarose Gels

1. Tightly tape the ends of the gel bed with masking tape to make a good seal (Figure 14D-1).
2. Place the gel comb across the gel bed, putting the ends of the comb into the notches on the side of the bed nearest the end of the bed. (The comb creates "wells" in the gel into which you will load various samples for testing.)
3. Obtain a bottle of 1% agarose (50 ml) from your instructor. ***Caution:*** The bottle will be hot since it has just been heated to melt the agarose. (Add 2 drops of Carolina Blu stain if required by your instructor.)

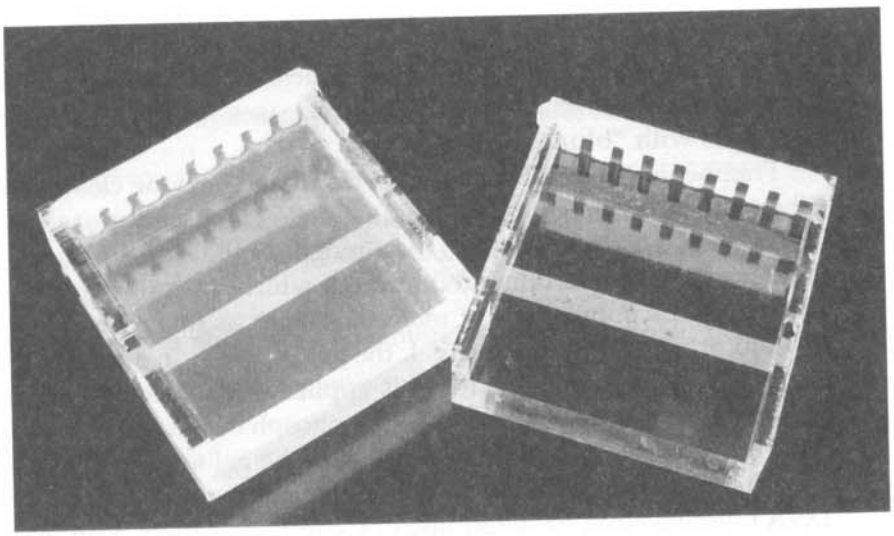


Figure 14D-1 *Preparation of the gel bed. The bed on the left has been taped and the agarose gel has been poured into it.*

4. Use a Pasteur pipette to transfer some agarose solution into the gel bed at the edges of the tape to make sure there are no leaks, but do this quickly and do not let it solidify. Now, carefully pour the remaining agarose into the gel bed. The agarose should cover only about one-third the height of the teeth (about 5 to 7 mm thick; you do not want your gel to be too thick, or too thin). If bubbles appear around the teeth of the comb, remove the comb and reinsert it. If bubbles appear in the gel, use a Pasteur pipette to draw them off to the sides.
5. Keep the gel bed completely immobile while the gel is setting (approximately 15 minutes). The final appearance of the gel will be cloudy.
6. When ready, remove the tape from the ends of the gel bed.
7. Carefully lower the gel bed into the chamber with the comb nearest to the *negative* (black) electrode (Figure 14D-2). Make sure that the gel bed is properly seated and centered.
8. Fill the electrophoresis chamber with running TBE buffer (tris-borate-EDTA) until the buffer covers the surface of the gel. (Make sure the 10× stock buffer has been diluted 1:10.) (Add 12 drops of Carolina Blu stain to 1 liter of buffer, if required by your instructor.)
9. Carefully remove the comb (pull it straight up). Make sure that the sample wells are filled with buffer. Remove any bubbles by using a Pasteur pipette to blow gently into the buffer above the wells. *Do not stick the end of the pipette into the wells*—you might puncture the gel!

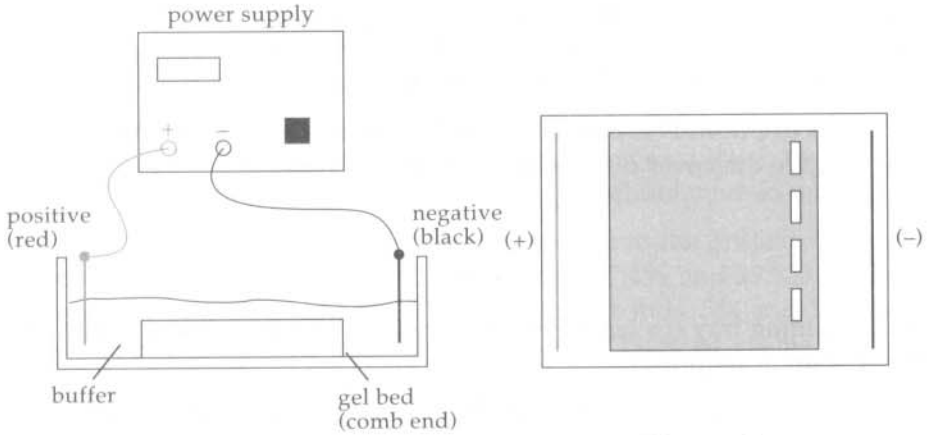


Figure 14D-2 *Setup for the electrophoresis system.*

B. Loading Samples

1. If a practice gel is available, practice loading the samples using the gel-loading solution only.
   a. To load practice samples on the gel, use a small micropipette or plastic transfer pipette. Pull a small amount of the practice gel-loading solution into the end of the pipette. (Do not allow the solution to move up into the body of the pipette or bubbles will be introduced into the well of the agarose gel during loading.)
   b. Dip the pipette into the buffer and hold the tip of the pipette slightly *above* the well in the gel. Gently dispense the solution. The loading dye is denser than the buffer and will move into the well. (Do *not* place the tip of the pipette into the well or you might puncture the gel.)
2. After practicing, you are ready to load the gels. Obtain a microtest tube containing phage lambda DNA digested with *Eco*RI endonuclease.* FIll one well of the electrophoresis apparatus with approximately 20 μl of this solution. The DNA is mixed with a solution containing tracking dye that will make it possible to trace the process of the DNA migration in the agarose gel.
3. Obtain a microtest tube containing phage lambda DNA digested with *Hin*dIII endonuclease. Follow your instructor's directions and fill a second well with 20 μl of this *Hin*dIII digest. The DNA fragments from this digest are of known size and will serve as a "standard" for measuring the size of the *Eco*RI fragments from step 2.
4. Load 20 μl of undiluted phage lambda DNA (control) into a third well.

C. Electrophoresis

1. Place the top on the electrophoresis chamber and connect the electrical leads (black to black and red to red). If using an Edvotek chamber, set the voltage to 50 volts. If using a Cabisco apparatus, set to 80 volts and check for a current reading of 50 to 100 milliamperes. When the current is flowing, you should see bubbles on the electrodes.
2. Allow electrophoresis to continue for a minimum of 1½ hours, or until the loading dye has moved at least 5 to 7 cm from the wells. The tracking dye will eventually form two bands of color. A purplish band (bromophenol blue) will be seen farthest from the wells. A slower-moving aqua-colored band (xylene cyanol) will migrate through the gel at a rate

**DNA for this procedure has been predigested to save time. However, your instructor may wish to have you perform the digests rather than use predigested material. If this is the case, your instructor will give you separate directions.*

equivalent to that of a DNA fragment approximately 2,000 bp long. This aqua band will be migrating just in front of your smallest DNA fragments. Turn off the electrophoresis apparatus when the bromophenol blue band (purple band) reaches the opposite end of the gel.

3. After electrophoresis is completed and the power supply is turned off, disconnect the leads and remove the cover of the electrophoresis chamber.

D. Staining

Wear gloves!

1. Fill a staining tray (or large Petri dish) with methylene blue staining solution (or Carolina Blu Final Stain, if required).
2. Carefully remove the gel bed from the chamber and gently transfer the gel to a staining tray. Use the scooper provided with your kit or keep your hands under the gel during the transfer. You may wish to remove a small piece of gel from the upper right-hand corner to keep track of the gel's orientation. *Do not stain in the electrophoresis apparatus.*
3. Stain for 30 minutes.
4. Carefully transfer the gel to a tray containing approximately 500 ml of distilled water to destain. Rinse several times and then let the gel destain for 1 to 24 hours. *Do not* change the water during this time or the bands will fade.
5. Transfer the gel to a visible-light box or overhead projector for examination.

E. Determining Fragment Size

1. After observing the gel on the light box, carefully wrap the gel in plastic wrap and smooth out all the wrinkles, or overlay with a transparency sheet.
2. Use a permanent marking pen to trace the outlines of the sample wells and the location of the bands.
3. Remove the plastic wrap and flatten it out on a white piece of paper on the laboratory bench. Save the gel in a plastic bag. Add several drops of the water used for destaining. Close the bag tightly and store at 4°C.
4. If the exercise was done as a demonstration, your instructor will transfer the marks onto an overhead transparency and will make copies of the transparency for each student. If you have run your own gel, you can make measurements directly from the plastic wrap.
5. For the *Hin*dIII fragments, measure the migration distance in centimeters (to the nearest millimeter). Measure from the front edge of the sample well to the front edge of each band on your gel.

Table 14D-1

*Hin*dIII Fragment (bp)	Distance Traveled (cm)
23,130	
9,416	
6,557	
4,361	
2,322	
2,027	

The distance a fragment migrates is related to its molecular weight. The greater its molecular weight, the shorter the distance the fragment will travel through the gel. For simplicity, we will use base-pair length instead of molecular weight. The known *Hin*dIII fragment lengths are given in Table 14D-1. Indicate the distance in centimeters that each has traveled. You will identify each fragment by the distance traveled: the shortest fragment will have traveled farthest, the next shortest will be just behind, and so on.

Note: You will observe six bands (Figure 14D-3). The band closest to the origin may appear to be diffuse—it is actually composed of pieces of DNA of two different sizes, 27,491 and 23,130 bp. For graphing purposes, you will use a base-pair size of 23,130. Two additional bands, 564 and 125 bp, are usually not observed. The larger (564 bp) usually does not contain enough DNA to be visible using methylene blue stain; the smaller (125 bp) usually runs off the end of the gel.

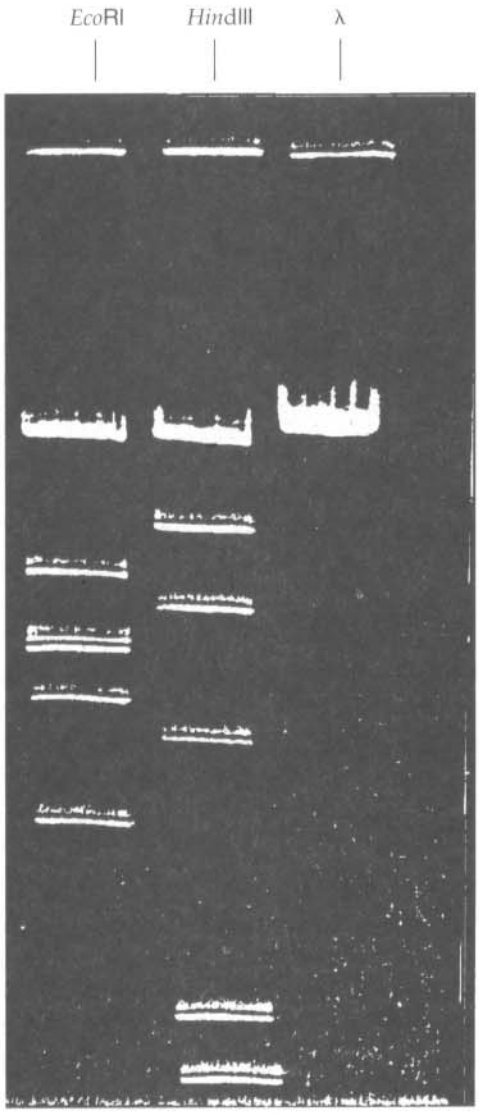


Figure 14D-3 *Agarose gel (photo not to scale) from electrophoresis of fragments produced by restriction endonuclease digestion of lambda DNA. Lane 1, digestion with* EcoRI*; lane 2, digestion with* HindIII*; lane 3, undigested lambda DNA.*

6. Use Figure 14D-4b at the end of the lab to graph your results. [The horizontal (*X*) axis of semilog paper is divided into a linear scale; the vertical (*Y*) axis is divided into a logarithmic scale.] Mark the *X*-axis at 1 cm, 2 cm, 3 cm, 4 cm, and 5 cm. Label this axis "migration distance."

7. Size in base pairs is plotted along the *Y*-axis. Assume that the first section or cycle of semilog paper represents 0 to 1,000 bp, the second represents 1,000 to 10,000 bp (see Figure 14D-4a). (On semilog paper, each section along the vertical axis is used to represent an increase by the power of 10.) On the *Y*-axis, mark the approximate position of each of the phage lambda *Hin*dIII standard fragment sizes in base pairs.

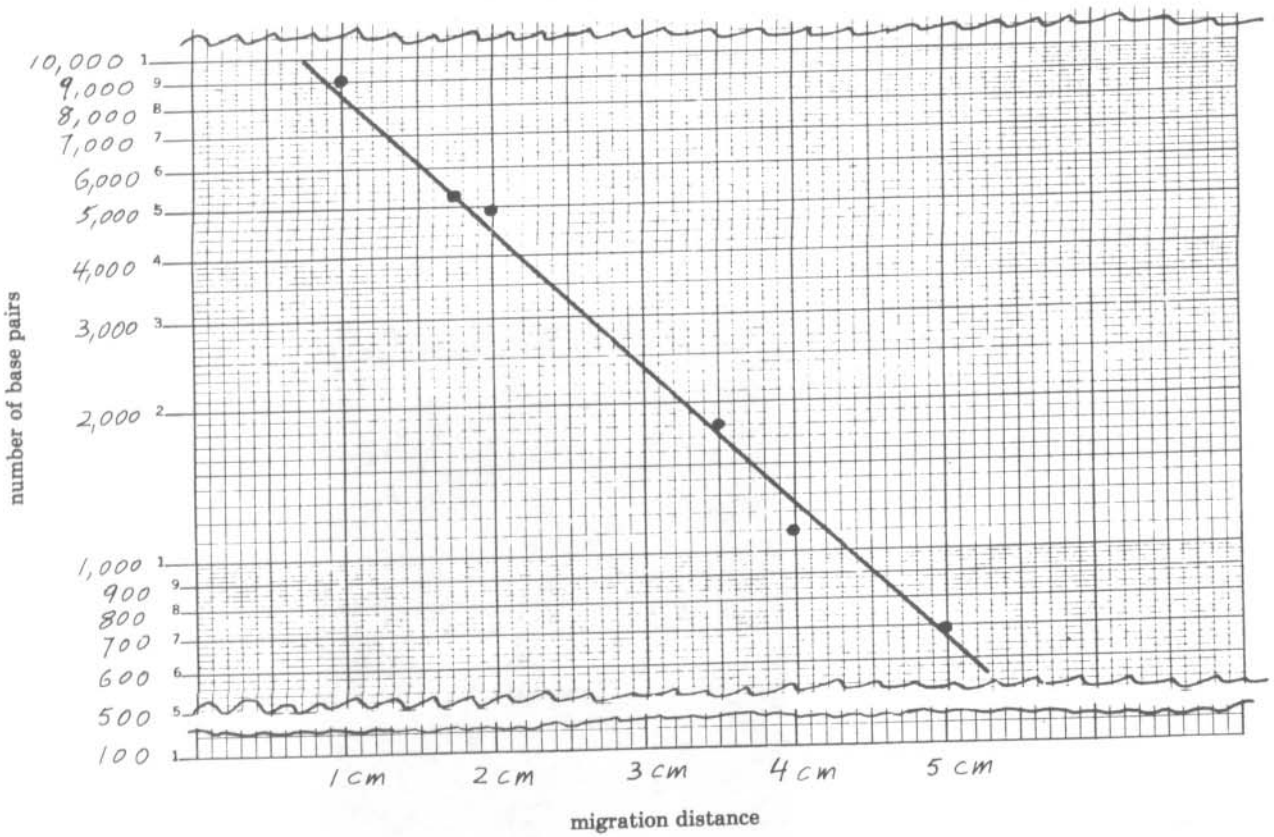


Figure 14D-4a *Example of a standard curve used to determine DNA fragment size. Note: The electrophoresis running time specified in your experiment is different from the running time used to generate the example standard curve shown here, so your standard curve will differ from this one.*

8. Each band on your gel of the *Hin*dIII digest should correlate with one of the fragment sizes in Table 14D-1. To plot your curve, locate the base-pair length you marked for each fragment on the *Y*-axis, then move horizontally along the *X*-axis according to the distance the fragment has traveled. When you have plotted all your points, draw a straight line that fits as close as possible to all the points (although it will not intersect all of them). This line describes the trend of the data and will serve as what is called a *standard curve,* similar, but not identical, to the one shown in Figure 14D-3.
9. Use this standard curve to determine the sizes of the fragments of phage lambda DNA digested with *Eco*RI. You should observe five bands (Figure 14D-3). Measure the migration distance for each *Eco*RI fragment. Locate that distance on the *X*-axis of your graph and use a ruler to extend a line upward until it crosses your standard curve. Mark the point where the lines cross, and use a ruler or the edge of a piece of paper to find where this point lies on the *Y*-axis, which gives you the number of base pairs.

a. What is the relationship between DNA fragment size and rate of travel through the agarose gel?

Expected *Eco*RI fragment sizes in base pairs are listed in Table 14D-2. Compare your observed results with the expected sizes by entering the base-pair sizes you observed beside the corresponding expected fragment size. *Note:* This technique is not exact—you should expect as much as a 10% to 15% error.

Table 14D-2 *Eco*RI Fragment Sizes for Phage Lambda DNA

Expected	Observed
21,226	
7,421	
5,643	
4,878	
3,530	

PART 2 Constructing Restriction Maps

A **restriction map** shows the location of each restriction site (place where the restriction endonuclease "cuts" the DNA) in relation to other sites. A restriction map of a viral or bacterial chromosome can be constructed by comparing the sizes of DNA fragments produced when the chromosomal DNA is digested by a combination of restriction enzymes. First, individual enzymes are used to cut the DNA into fragments of a certain size. A mixture of the same enzymes is then used to cut the DNA into fragments of different sizes (Figure 14D-5a). By determining fragment size and sequencing (establishing the order of) the overlapping fragments, the restriction sites on the DNA can be mapped in relation to the linear sequence of the DNA fragments (Figure 14D-5b). Note that "kb" indicates kilobase pairs; 1,000 base pairs.

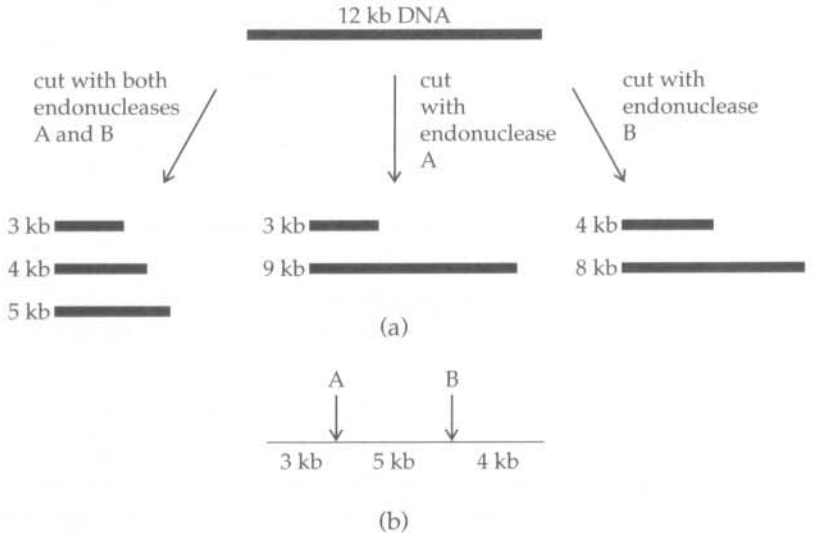


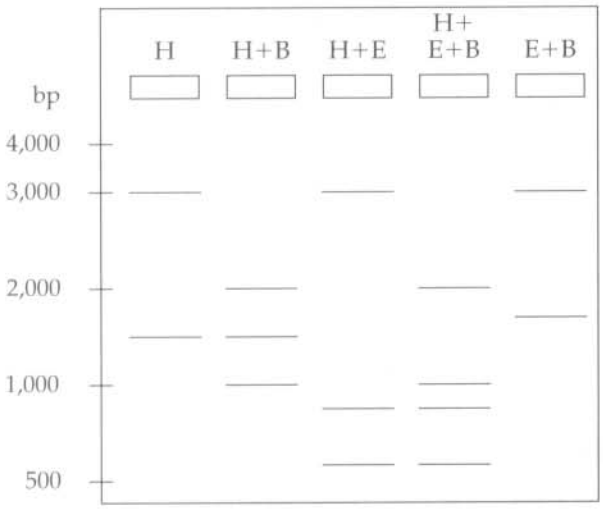
Figure 14D-5 *(a) Enzyme A cuts at one end of the DNA molecule while enzyme B cuts at the other end. The fragments could NOT be arranged in sequence as 3/4/5 kb because enzyme B produced two pieces of 4 kb and 8 kb and there is no way to get an 8-kb piece unless the 3-kb piece is next to the 5-kb piece. (b) For this reason the fragments must be arranged as 3/5/4 kb.*

Objectives

- ☐ Construct restriction maps of circular plasmid DNAs.
- ☐ Construct restriction maps of linear DNA molecules.

Procedure

1. A circular plasmid has been cut with restriction enzymes H, B, and E alone and in combination. Electrophoresis of restriction fragments produces the gel shown below. Base pair (bp) sizes are shown on the scale to the left of the gel. Estimate the size of each fragment using this scale. Start with enzyme H and assume that it produces two fragments.

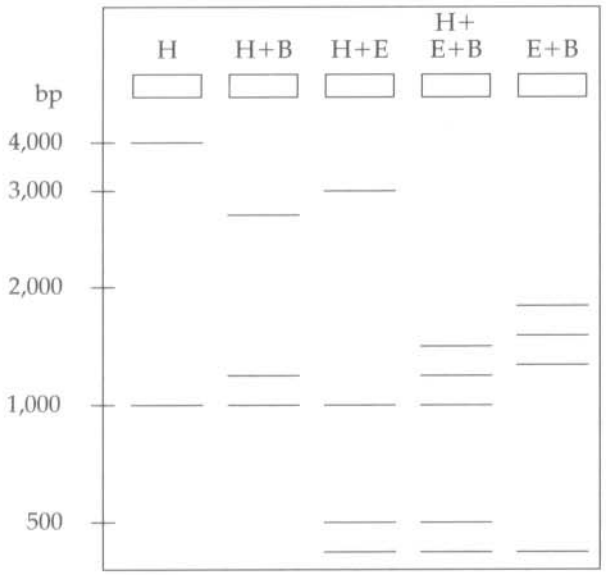


a. How many restriction sites were present for enzyme H? _____

2. Draw a circle in the space below and locate the enzyme H restriction sites in relation to approximate sizes of the restriction fragments.

3. Continue to add restriction sites at the appropriate locations by determining the approximate sizes of the restriction fragments in the H+B lane on the gel. Follow with each additional set of fragments until you have established a complete restriction map; a restriction map for which the correct fragment sizes would be produced by digestion with restriction endonucleases H, E, and B.

4. Next, a linear piece of DNA is cut using enzymes H, B, and E alone and in combination. Electrophoresis of restriction fragments produces the gel shown on page 14-21. Base-pair sizes are shown on the scale to the left of the gel. Estimate the sizes of each fragment using this scale. Start with enzyme H and assume that it produces two fragments.



b. How many restriction sites were present for enzyme H? _____

5. Draw a line in the space below and indicate the position of the restriction site(s) for enzyme H.

6. Now continue to add restriction sites at the appropriate places along the line by estimating the fragment sizes produced by the other enzymatic digestions represented on the gel. By sequencing the overlapping fragments, you will create a complete restriction map for the linear DNA molecule.

PART 3 Mapping the Bacteriophage Lambda (λ) Chromosome

Bacteriophage lambda (λ) is a temperate phage; it can replicate autonomously or can convert *E. coli* to the **lysogenic** cycle by inserting into the *E. coli* chromosome as a prophage. Phage λ exists as a double-stranded DNA molecule of 48,502 base pairs. It can be either a linear or circular molecule because each end of the chromosome has a single-stranded tail (called the COS site). The tails are complementary (like sticky ends), allowing the linear molecule to easily convert to a circle. Because of its relatively small size, restriction enzyme digestion of phage λ DNA can be used to construct a restriction map.

Objectives

- ☐ Construct a restriction map of phage λ DNA from restriction digest fragment sizes.
- ☐ Use electrophoretic data to construct a map of phage λ DNA.

Procedure

1. Lambda DNA (48,502 bp) is cut using restriction enzymes *Afl*II (from *Anabaena flos-aquae*) and *Apa*I (from *Acetobacter pasteurianus*), as shown on the following page.

*Afl*III –C–T–T–A–A–G
–G–A–A–T–T–C–

*Apa*I –G–G–G–C–C–C–
–C–C–C–G–G–G–

The enzymes are used both alone and in combination, and fragments of the following approximate sizes (in bp) are produced:

*Afl*III	*Apa*I	*Afl*III + *Apa*I
5,872	10,086	2,532
6,078	38,416	3,546
6,540		5,872
30,012		6,540
		30,012

Use these data to establish the sequence of fragments and the restriction sites of *Afl*III and *Apa*I on λ DNA. Record this sequence as a linear restriction map (see Figure 14D-5) of bacteriophage λ on the line below.

42,502 bp

2. In Part 1 of the exercise you determined the restriction fragment sizes for lambda DNA digested by *Hin*dIII and *Eco*RI. Using the *Hin*dIII map (Figure 14D-6) for practice, determine the sizes of the known fragments (the length of DNA in bp between two restriction sites) by subtracting the restriction site bp designation at the left of the fragment from the bp designation on the right. For example, the fragment marked (*) is 25,157 bp − 23,130 bp = 2,027 bp in length. Record this known size below the *Hin*dIII map on the lines provided (____ bp).

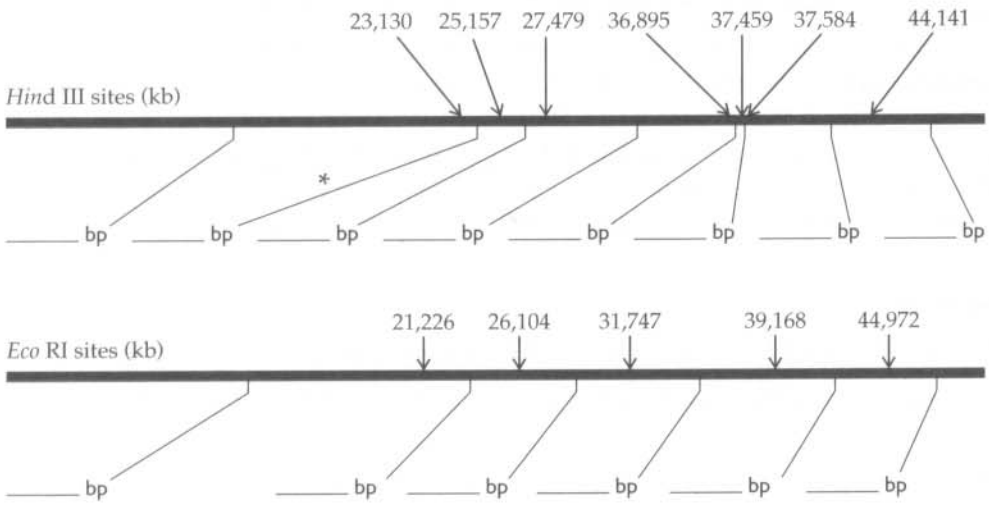


Figure 14D-6 *Lambda DNA restriction enzyme sites. The locations of "cuts" (arrows) are indicated in base pairs (bp) from the origin.*

Using your data from Part 1, compare your restriction fragment sizes determined by electrophoresis (see Tables 14D-1 and 14D-2) with those in the restriction-site maps of λ DNA in Figure 14D-6.

a. Are there any fragments shown on the map that are missing from your gel? _____

How could this be the case? _____

3. Now, using your own electrophoresis data for *Eco*RI fragments (Part 1), compare the restriction fragment sizes you determined by electrophoresis to the actual *Eco*RI fragment sizes recorded on the bacteriophage λ restriction map in Figure 14D-6.

b. How do the fragment sizes for EcoRI *that you determined from your electrophoresis standard curve compare to those of known length as determined from the* EcoRI *map in Figure 14D-6?*

_____

4. Use your *Eco*RI data from Part 1 to construct your own λ DNA map. By matching your fragment sizes (as determined after electrophoresis from your standard curve) to the known data for *Eco*RI (Figure 14D-6), you should be able to establish the correct order of the fragments. Some bands of similar size may migrate together.

(λDNA)

Recall that the base sequences of the fragments of DNA produced by restriction enzyme digests can be determined by several means. This makes it possible to develop a complete genetic sequence of the chromosome. For larger pieces of DNA, a procedure called **"chromosome walking"** can be used. Two different restriction digests are used to cut identical pieces of DNA into fragments. Each of these DNA fragments is introduced into a bacterium via a plasmid vector. Each bacterium then clones its fragment as it replicates and forms a colony. Thus, each bacterial colony contains many copies (clones) of the same fragment, and

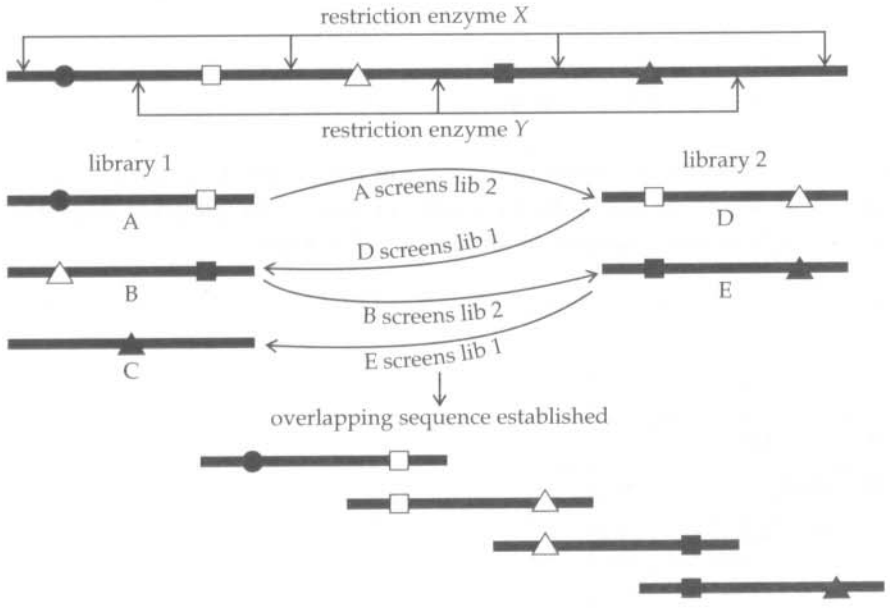


Figure 14D-7 *Chromosome walking. Restriction enzyme fragments are introduced into plasmids for cloning. All fragments formed by digesting DNA with Enzyme* X *form a library 1. All fragments formed by digesting DNA with Enzyme* Y *form library 2. By searching for complementary ends, hybridizing probes from library 1 clones with library 2 clones, it is possible to determine the linear sequence of DNA on a chromosome.*

there are many colonies, each containing a different "clone" fragment. The group of cloned colonies that develops from the fragments created by one restriction enzyme represents a **library.** The two resulting libraries can be used as probes to screen each other for overlapping complementary sequences; the right-hand end of one piece complements the left-hand end of the next piece and the right-hand end of the second piece complements the left-hand end of the next piece as if "walking" down the chromosome (Figure 14D-7).

Laboratory Review Questions and Problems

1. Ascospores of the fungus *Sordaria* are haploid. Why is this an advantage in studying the genetics of the organism?

2. Two genes for shell color, genes *A* and *B*, are on the same arm of a chromosome in a rare species of clam. Mating yellow-shelled individuals produced 800 yellow-shelled clams and 225 orange-shelled clams. Orange shells result from recombination of alleles in crossing-over events that occurred during meiosis and gamete production. What is the map distance between genes *A* and *B*?

3. There are four genes, *A*, *B*, *C*, and *D*, on a chromosome that you wish to map. These are the recombination frequencies among these genes: $B \times D = 4\%$, $B \times C = 10\%$, $D \times A = 2\%$, $C \times A = 16\%$, $C \times D = 14\%$. Map the chromosome.

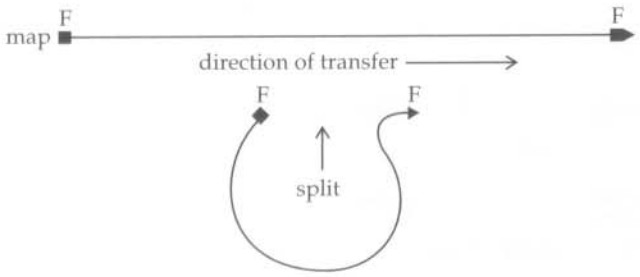
4. In a series of breeding experiments among frogs, a linkage group composed of genes *A*, *B*, *C*, and *D* was found to show the following crossover frequencies. Map the chromosome. (Use the matrix like a Punnett square to show recombinations.)

	A	*B*	*C*	*D*	
A	—	10%	4%	9%	
B	10%	—	6%	19%	
C	4%	6%	—	13%	
D	9%	19%	13%	—	

5. You are trying to map a newly isolated bacterial chromosome. After allowing different mutant strains to conjugate for different lengths of time, you test whether a certain gene (for example, leu^-) that was previously nonfunctional in the mutant is now functional (leu^+) due to transfer

of the gene from the donor strain and subsequent recombination. Only recombinants will grow on minimal medium. Use these data to map the *leu*, *pro*, *bio*, and *thi* genes on the chromosome. (*Note:* When the bacterial chromosome breaks open for replication and transfer, the *F* factor is split. The leading edge of the chromosome being transferred is on the *right* in the diagram below.)

		Growth on Minimal Medium			
Donor	Recipient	5 min	10 min	30 min	45 min
$leu^+\ bio^-$	× $leu^-\ bio^+$	Yes	Yes	Yes	Yes
$bio^+\ pro^-$	× $bio^-\ pro^+$	No	Yes	Yes	Yes
$pro^+\ leu^-$	× $pro^-\ leu^+$	No	No	Yes	Yes
$pro^-\ leu^+$	× $pro^+\ leu^-$	Yes	Yes	Yes	Yes
$bio^-\ thi^+$	× $bio^+\ thi^-$	No	No	No	Yes



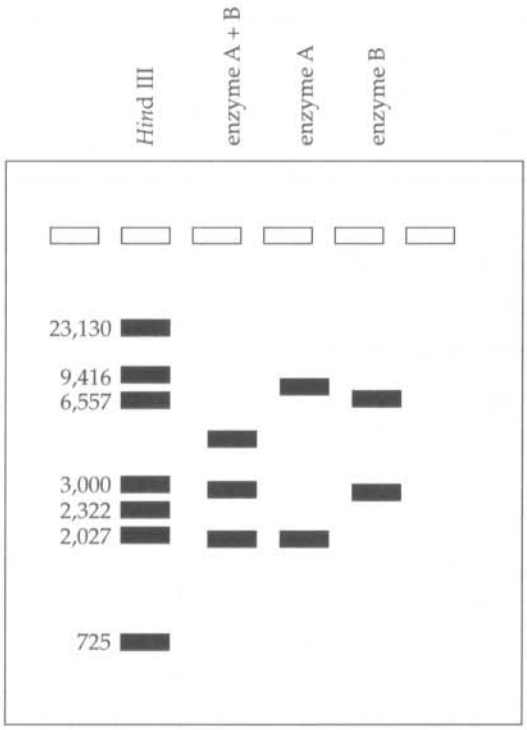
6. When samples of DNA are subjected to electrophoresis, why should the samples be loaded onto the gel at the negative pole of the electrophoresis apparatus?

7. Restriction endonucleases are used by bacteria as a form of "protection." Explain how this occurs and why the endonucleases do not destroy the bacterial cells themselves.

8. A circular bacterial DNA plasmid is cut using two restriction endonucleases. Restriction enzyme A yields a single linear molecule of 45,000 base pairs (4.5 kilobase pairs, or 4.5 kb). Enzyme B produces two restriction fragments of 1.2 kb and 3.3 kb. A combination of enzymes A and B produces three restriction fragments of 1.2 kb, 1.3 kb, and 2.0 kb. Map the plasmid, showing restriction sites for A and B and relative fragment lengths.

How many recognition sites are present for each restriction enzyme?

9. A length of human DNA from chromosome 2 has been cut by two restriction enzymes, A and B. Electrophoresis is carried out using *Hind*III fragment markers for a standard. The resulting gel is shown below. Note that enzyme A cuts the DNA into two pieces, and enzyme B cuts the DNA into two pieces, but of different sizes. When A and B are used together as a double digest, three pieces are produced.



Use the *Hind*III fragments to make a standard curve, using Figure 14D-4b. Then determine the lengths of the fragments produced by enzymes A and B alone and A and B in combination.

To determine the base sequence of the fragments, you first need to know their order in the single piece of human DNA (this is like knowing the letters in three words but not knowing the order of the words in a sentence). In trying to find the order, you are mapping the gene. This is called a restriction map. How are the pieces of DNA ordered in the original piece of human DNA?

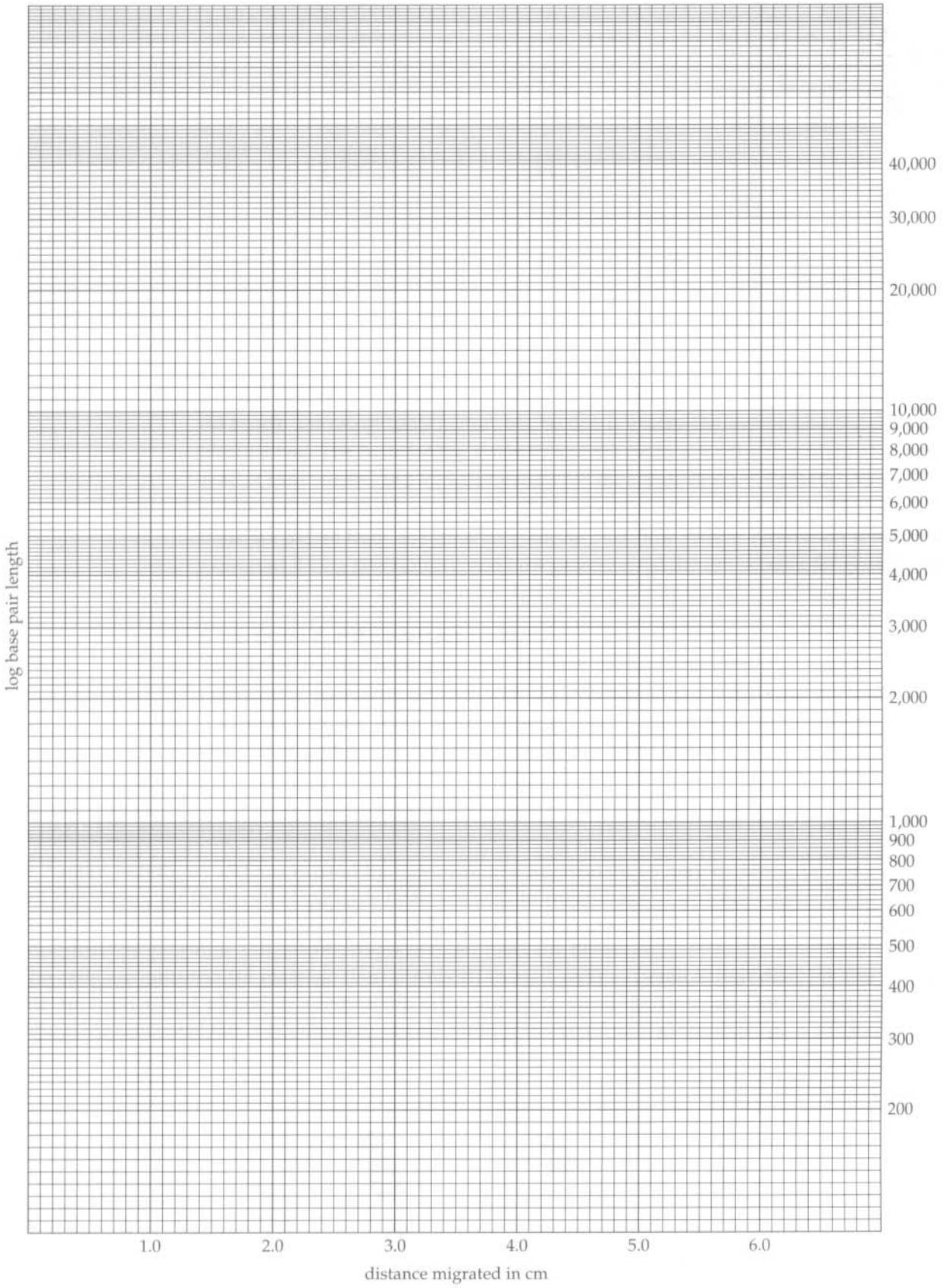


Figure 14D-4b *Semilog graph paper.*