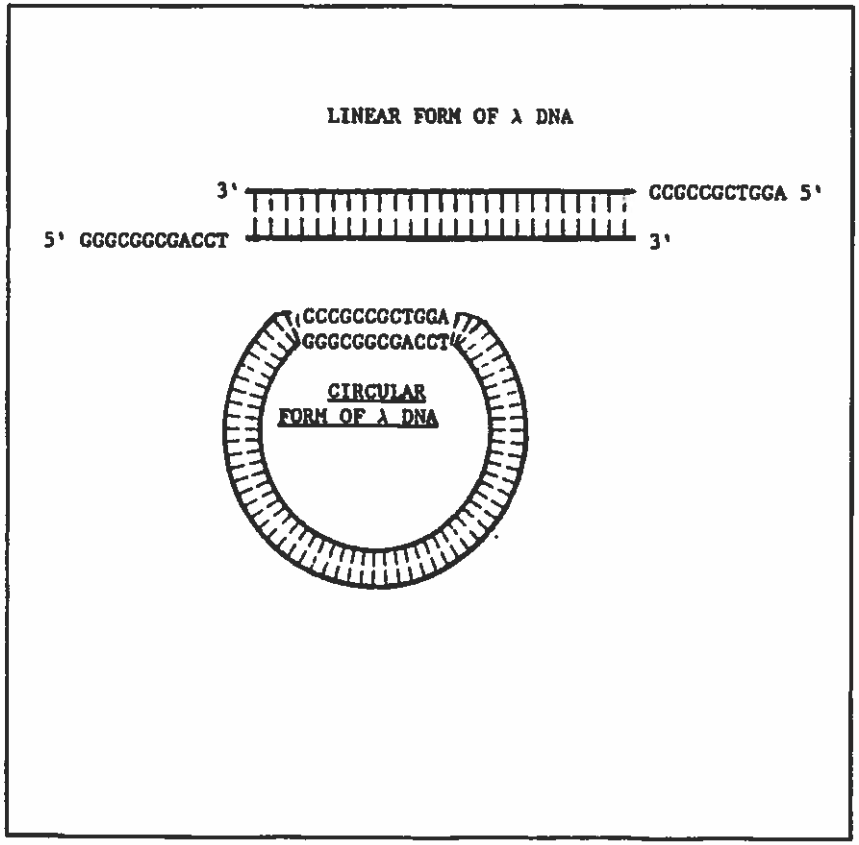
Experiment 302. Restriction Nuclease Mapping of DNA

Viruses are a unique group of parasitic organisms that grow only in the cells of bacteria, plants and animals. A virus that infects bacteria is called a bacteriophage, or simply, a phage. Viruses have proved to be invaluable tools for the study of molecular biology because they possess the essential properties of life yet are simpler than bacteria or eukaryotic cells in their structures and life cycles.

Bacteriophage lambda, which infects *E. coli*, is probably the best understood of the double-stranded DNA phages. The protein component of this phage consists of a protective coat that forms the tail assembly and the outer shell of the head. A single molecule of double-stranded DNA is located in the core of the phage head. The DNA molecule contains 48,502 base-pairs (molecular weight ≈ 3 x 10^7) that code for approximately 50 different phage proteins. The sequence of nucleotides along the entire lambda genome is known and the nucleotide sequences that comprise the major control regions for transcription and replication have been identified. Because of the vast amount of information about the biology of this phage, lambda has become a common cloning vector in genetic engineering.

Figure 2-1. A Diagram of a Lambda Phage DNA Molecule



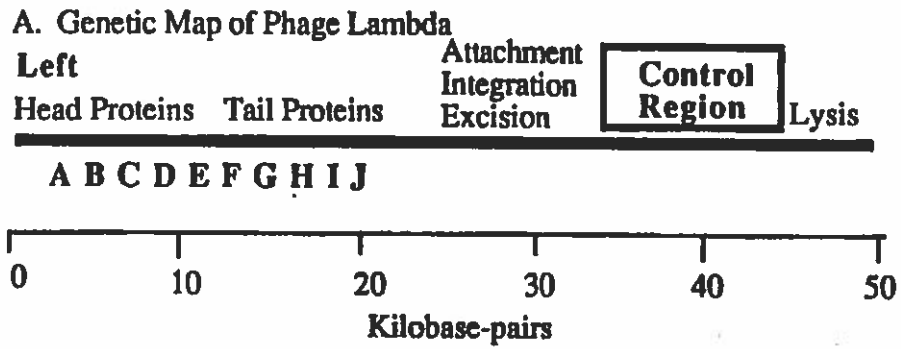
The lambda DNA molecule has an unusual structure. Single-stranded segments of DNA containing 12 nucleotides are found at both ends of the phage DNA molecule (Figure 2-1). The base sequence of these terminal regions, which are known as cohesive or sticky ends, are complementary to each other. Thus, by forming base pairs between the cohesive ends, the linear DNA molecule will circularize, as shown in Figure 2-1. This circularization is readily demonstrated in the laboratory. When lambda DNA is incubated at 37°C, the cohesive ends will anneal (bind) to each other by base-pairing and the circular DNA molecule will form. When this circular lambda molecule is heated to 75°C, the cohesive ends come apart and the DNA molecule is converted to a linear form. The disruption of base-pairing by heat treatment is known as denaturation.

The circularization of the lambda DNA molecule also occurs in an infected bacterial cell. When lambda infects an *E. coli* host, the phage DNA is injected into the bacterium and the cohesive ends anneal or stick together to form a circular molecule. Transcription and replication of the phage DNA occurs and the newly formed DNA molecules are packaged into newly synthesized phage particles. About 100 lambda particles are then released into the medium when the bacterial cell is broken open (lysed). As a result of this lytic infection, the bacterium dies. Less commonly, the circular DNA phage molecule attaches and integrates into the *E. coli* DNA. After integration, the bacterial cell behaves normally and the lambda DNA is replicated as part of the bacterial chromosome. However, when the bacterium is subjected to an environmental insult, such as exposure to ultraviolet light, the lambda DNA is excised from the host chromosome and begins a normal cycle of viral replication.

Figure 2-2 shows a map of phage lambda DNA. The DNA of phage lambda may be divided into three regions. The left-hand region includes all the genes (A through J) whose products are necessary to produce phage head and tail proteins and to package the DNA into the virus. The central region contains elements involved in integration of the DNA into the *E. coli* chromosome. The remaining portion of the genome includes the major control region for transcription and replication and the genes necessary for cell lysis.

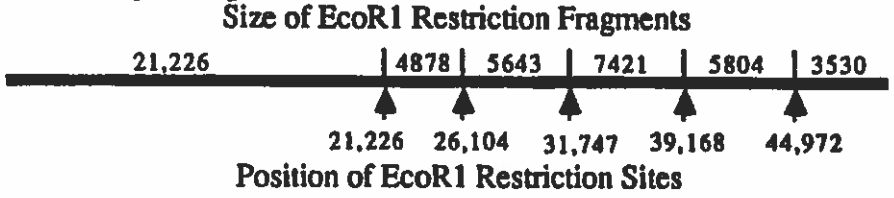
Restriction nucleases are enzymes that cut DNA at specific sites (See Part A-1). In this exercise, you will study phage lambda DNA using the restriction enzymes EcoR1 and Bam H1. The position of the restriction sites for these enzymes in lambda DNA is shown in Figure 2-2. Cleavage of lambda DNA by either EcoR1 or Bam H1 produces six DNA fragments, the sizes of which are indicated in the figure. The fragments derived from the termini contain the cohesive ends and will anneal together under the appropriate conditions.

Figure 2-2. Genetic and Restriction Map of Phage Lambda DNA



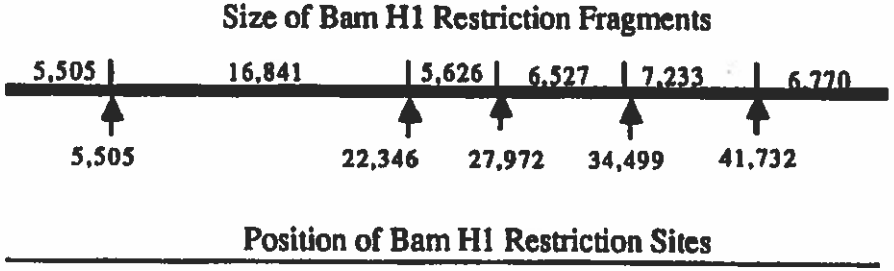
B. Restriction Map of Lambda DNA - EcoR1

Arrows indicate the position of restriction sites for EcoR1. Sites are in base-pairs from the left end of the phage DNA. Lambda DNA is 48,502 base-pairs long.



C. Restriction Map of DNA - Bam H1

Arrows indicate the position of restriction sites for Bam H1 Sites are in base-pairs from the left end of the phage DNA.



Objective

To study the effects of EcoR1 and Bam H1 on phage lambda DNA and to identify restriction fragments that contain the cohesive ends.

Materials

A. The solutions and materials required for electrophoresis, sample handling and gel staining (see Appendices 1 and 2).

B. The samples below are provided in the container marked **Experiment 302**:

1. Phage Lambda DNA.

2. EcoR1: The restriction enzyme to be used in this laboratory should be made up immediately before the laboratory session as described in Appendix 2 of the Instructor Manual and stored in the refrigerator before use. The solution contains the enzyme suspended in a nuclease digestion buffer. The solution of enzyme and DNA must not be contaminated, so use a fresh micropipet whenever you remove the enzyme and DNA from the stocks.

3. EcoR1 + Bam H1: This mixture contains both restriction enzymes suspended in a nuclease digestion buffer and should be made up as described in Appendix 2 of the Instructor Manual.

4. Electrophoresis sample buffer.

Materials Not Provided

1. Water baths for tube incubation maintained at 37°C and 70°C: A beaker containing water heated to 70°C with a Bunsen burner can serve as the latter bath.

2. Ice bath: Ice chips in a beaker can be used.

Procedure

The experiment was designed for 8 students working individually or 16 students working in teams of two.

A. Preparing the DNA samples

1. Number four small tubes 1to4 with a water-proof marking pen.

2. Place 10 μl of distilled water into tube 1, 10 μl of the EcoR1-buffer solution into tube 2 and 10 μl of the EcoR1 + Bam H1 mixture into tubes 3 and 4.

3. Add 5 μl of lambda phage DNA to each tube. Gently tap the tubes with the tip of your index finger to mix the solutions. Incubate the tubes for 50 minutes at 37°C. During this incubation, the fragments containing the cohesive ends (the fragments derived from the termini) should anneal together.

4. While the tubes are incubating, prepare 1.2% agarose gels as described in Section IV.

5. At the end of the 50 minute incubation period, add 5µl of electrophoresis sample buffer to each of the four tubes.

6. Transfer tube #4 to the 70°C water bath and, after 5 minutes, place this tube in an ice bath. This heat treatment will separate the two fragments containing the cohesive ends. Thus, in tube #4, the cohesive end fragments should be separated while they should be together in tubes 1 to 3.

B. Electrophoresis

1. Load 15µl of the following samples from the above section into the sample wells.

Sample Well	Sample
1	Tube 1
2	Tube 2
3	Tube 3
4	Tube 4
5	Tube 1
6	Tube 2
7	Tube 3
8	Tube 4

2. Seal the wells with agarose and electrophorese until the bromophenol blue in the samples has migrated to within 2 mm of the positive electrode end of the gel.

3. Remove the gels from the unit and stain them as described in Section IV.

4. Measure the distance of the DNA bands (in cm) from the sample wells and draw a picture of the DNA bands in each gel lane. Note the relative intensity and positions of the DNA bands in lane 3 and lane 4. Note especially the intensity of the next to the largest DNA band.

Study Questions

1. The sizes of the DNA bands in lanes 2 and 6 should be 21.2, 7.4, 5.8, 5.6, 4.8 and 3.5 kilobase pairs (see Figure 2-2). From the map of phage lambda given in Figure 2-2, identify the DNA bands that contain the genes for the head proteins and the genes that control lysis of the host cell.

2. Maps of the restriction sites for EcoR1 and Bam H1 in lambda DNA are given in Figure 2-2. With this information:

A. Calculate the length of DNA fragments that should have been produced when lambda DNA was digested with both EcoR1 and BamH1. List these values below and indicate the two fragments that contain the cohesive ends.

DNA Fragment Length
(base-pairs)

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____
11. _____

B. Identify the above fragments on lanes 3 and 4 of your gels.

3. A comparison of the results of lanes 3 and4 on your gels can be used to identify the DNA terminal fragments of phage lambda without a knowledge of the map shown in Figure 2-2. Explain.

4. In DNA of uniform composition, a specific hexanucleotide occurs by chance once in every 4016 base-pairs. The recognition sequence for EcoR1 is a hexanucleotide (see Part A-1, Table 1). How many restriction sites for EcoR1 would you expect to exist in lambda DNA if the phage DNA were of uniform composition? There are few restriction sites for a number of enzymes including EcoR1 and HpaI in the left 20 kilobase of lambda DNA. Explain. (Hint: The left region of lambda DNA is rich in G+C base pairs.)