Laboratory Exercise

RESTRICTION MAPPING OF PHAGE LAMBDA DNA

Before the lab: Read pages 419- 425 of Snustad & Simmons (fourth edition) for a description of restriction enzymes and their uses.

Among the most useful enzymes for the manipulation and study of DNA are *restriction enodonucleases*. Restriction enzymes are named for the bacterium from which they were isolated. The designation of an enzyme is derived by combining the first letter of the genus name with the first two letters of the species name. An additional letter may be used for a strain designation. The four restriction enzymes used in this exercise are *Bam*H I (from *Bacillus amyloliquefaciens* strain H), *Eco*R I (from *Escherichia coli* strain RY13), *Hae* III (from *Haemophilus aegypticus*). and *Hin*d III (from *Haemophilus influenzae* strain Rd). The roman numerals indicate whether the enzyme was the first, second, third, etc. isolated from that strain.

For molecular genetics the most useful restriction enzymes are the so-called type II enzymes, which recognize a short DNA sequence (4-10 bp in length) and then cleave the phosphodiester backbone of each strand within this sequence. Note that the recognition sequences are *palindromic* in that the sequence is the same on each strand relative to the 5' and 3' orientation of the strands. The recognition sequences for the enzymes used here are:

*Bam*H I:	5′ –GGATCC– 3′ 3′ –CCTAGG– 5′	*Hae* III:	5′ –GGCC– 3′ 3′ –CCGG– 5′
*Eco*R I:	5′ –GAATTC– 3′ 3′ –CTTAAG– 5′	*Hin*d III:	5′ –AAGCTT– 3′ 3′ –TTCGAA– 5′

Bacteriophage lambda has been the subject of genetic research for many decades (see Snustad & Simmons, Ch. 16, 17, and p. 487). The lambda genome, which comprises a double-stranded DNA molecule, contains 48,502 bp and has a molecular weight of 32 x 10^6 daltons. Because the recognition sites for each enzyme are each distributed throughout the genome, a digest of lambda DNA with a particular enzyme will yield a characteristic collection of fragments, each with a distinct size. To examine these fragments, agarose gel electrophoresis is used (see Snustad & Simmons, p. 498-499). Agarose is a disaccharide that, when heated and cooled, forms a matrix that can be used to resolve DNA fragments on the basis of their size. When DNA fragments are placed into this matrix and subjected to an electrical current, they will migrate in a manner proportional to their molecular weight. Small fragments will move through the matrix faster while large fragments will move more slowly. When the electrophoresis is finished, the DNA fragments can be visualized by staining the gel with the fluorescent dye ethidium bromide. For each restriction enzyme-DNA combination used, a characteristic pattern of fragments is

produced. This pattern is sometimes called a DNA fingerprint and can be used to identify a particular DNA (see Snustad & Simmons, p. 503-506).

Experimental Procedure

Four restriction enzyme digests will be performed by each group, one with each of the three enzymes used separately (single digests) and a fourth with either *Hae* III or with two of the enzymes used together (double digest). You will be assigned to a particular group in class.

1. Label four microcentrifuge tubes: B, E, H, and either HA or E+B.

2. For <u>each</u> of the single digests (B, E, H, and HA) add the following reagents to the appropriate tube:

   11.5 µl H_2O
   6.0 µl lambda DNA (50 ng/ µl)
   2.0 µl 10X buffer*
   0.5 µl restriction enzyme*

3. If you are doing the double digest (tube E+B) add the following reagents:

   11.5 µl H_2O
   6.0 µl lambda DNA (50 ng/ µl)
   2.0 µl 10X buffer*
   0.25 µl *Bam*H I*
   0.25 µl *Eco*R I*

*Because it is difficult to pipet a volume of less than 1 µl accurately, you may be given a premix containing enzyme and buffer in the correct proportions. Dispense 2.5 µl of this premix into the appropriate tube.

4. Spin the tubes briefly in a microcentrifuge and incubate at 37° C for three hours.

5. The samples will be electrophoresed in a 0.7% agarose gel and you will be given a photograph of the gel for examination and interpretation. For comparison, an ideal gel will be provided.

6. From the experimental photograph, measure the distance that each band migrated in the agarose matrix. Use the following fragment sizes for the *Hin*d III digest to construct a standard curve on semilog graph paper.

23, 130 bp
9,416
6,557
4,361
2,322
2,027
564 (may not be visible)
125 (may not be visible)

7. Plot the migration distances for the *Eco* RI and *Bam* HI digests on the standard curve to infer the sizes of these fragments. (It is not necessary to plot the *Hae* III or E+B digests.)