# Activity 6.1.1: Constructing a Plasmid Map

A gene must be inserted into an appropriate location of a plasmid to be expressed. For example, if the gene is placed within the promoter region, RNA polymerase will not be able to bind and the gene will never be transcribed. In addition, placing it after an operator allows the new gene can be turned on or off using the chemical that induces or represses the expression of the operon genes. In this activity, you will construct plasmid maps using information gathered from restriction enzyme digestions of plasmids.

The first step in building a plasmid restriction map is to determine which restriction enzymes digest the plasmid and where the digestion sites are located. Molecular biologists incubate plasmid DNA with restriction enzymes one at a time and then in conjunction with each other. The digestions are run on a gel and, once the fragment sizes are determined, the information can be used to build a plasmid map.

## Materials

calculator pair of compasses

## Procedure

1. With a compass and pencil, draw a circle on a piece of paper to represent plasmid A.
2. Using the information from **Table 4**, indicate where the restriction enzyme cuts are located on the plasmid. (*Hint:* Notice how all the fragments add up to the same total.) Check your answer with your teacher.

**Table 4** Results of Restriction Fragment Digestion of Plasmid A

| ***Eco*RI (base pairs)** | ***Bam*HI (base pairs)** | ***Eco*RI + *Bam*HI (base pairs)** |
|---|---|---|
| 1800 | 1200<br>600 | 200<br>600<br>1000 |

*(continued)*

3. Using the information provided in the next three digestions (**Tables 5–7**), draw the maps for plasmids B, C, and D.

**Table 5** Results of Restriction Fragment Digestion of Plasmid B

| *Eco*RI (base pairs) | *Bam*HI (base pairs) | *Eco*RI + *Bam*HI (base pairs) |
|---|---|---|
| 1200 | 1200 | 200 |
| 600 | 600 | 200 |
| | | 400 |
| | | 1000 |

**Table 6** Results of Restriction Fragment Digestion of Plasmid C

| *Bam*HI (base pairs) | *Hin*dIII (base pairs) | *Bam*HI + *Hin*dIII (base pairs) |
|---|---|---|
| 900 | 500 | 300 |
| 1500 | 1600 | 400 |
| 3000 | 3300 | 500 |
| | | 1200 |
| | | 3000 |

**Table 7** Results of Restriction Fragment Digestion of Plasmid D

| *Eco*RI (base pairs) | *Bam*HI (base pairs) | *Sma*I (base pairs) | *Eco*RI + *Bam*HI (base pairs) | *Eco*RI and *Sma*I (base pairs) | *Bam*HI + *Sma*I (base pairs) | *Eco*RI + *Bam*HI + *Sma*I (base pairs) |
|---|---|---|---|---|---|---|
| 800 | 250 | 900 | 200 | 350 | 150 | 150 |
| 1500 | 700 | 1400 | 250 | 450 | 250 | 200 |
| | 1350 | | 300 | 450 | 550 | 250 |
| | | | 500 | 1050 | 600 | 300 |
| | | | 1050 | | 750 | 350 |
| | | | | | | 450 |
| | | | | | | 600 |

*(continued)*

## Analysis

(a) Why is it necessary to digest a plasmid with individual restriction enzymes first, then follow with a combination of digestions?

(b) How is the number of fragments produced associated with the number of cut-sites available to a restriction enzyme?

(c) Why do all the fragment sizes for each restriction enzyme add up to the same total?

(d) Sketch the pattern that would be found on a gel after the digestions in **Table 6**, on the previous page, are completed.

(e) Explain how a molecular biologist could determine whether a cut-site was in the middle of an antibiotic-resistance gene.

(f) Explain how restriction enzyme digestion results would change if a foreign gene were inserted into a plasmid.