Building a Restriction Map

As we have learned, a restriction enzyme is an enzyme that cuts DNA at a specific sequence. Restriction enzymes are named for the bacteria they are found in. For example, EcoRI was isolated from *E. coli*. The Roman numeral tells us it was the first one isolated from that species. Remember, that a typical restriction site is 4-6 bp in length and are palindromic. Because DNA contains only four different nucleotides, any sequence of four or six nucleotides will be randomly repeated every few hundred to few thousand bases. When a segment of DNA is digested by a restriction enzyme, the sizes of the resulting fragments correspond to the distances between the restriction sites. Of course, each time the same piece of DNA is digested with the same restriction enzyme, the same fragments result. As we know, restriction enzymes play an important role in our manipulation in a variety of DNA technology techniques.

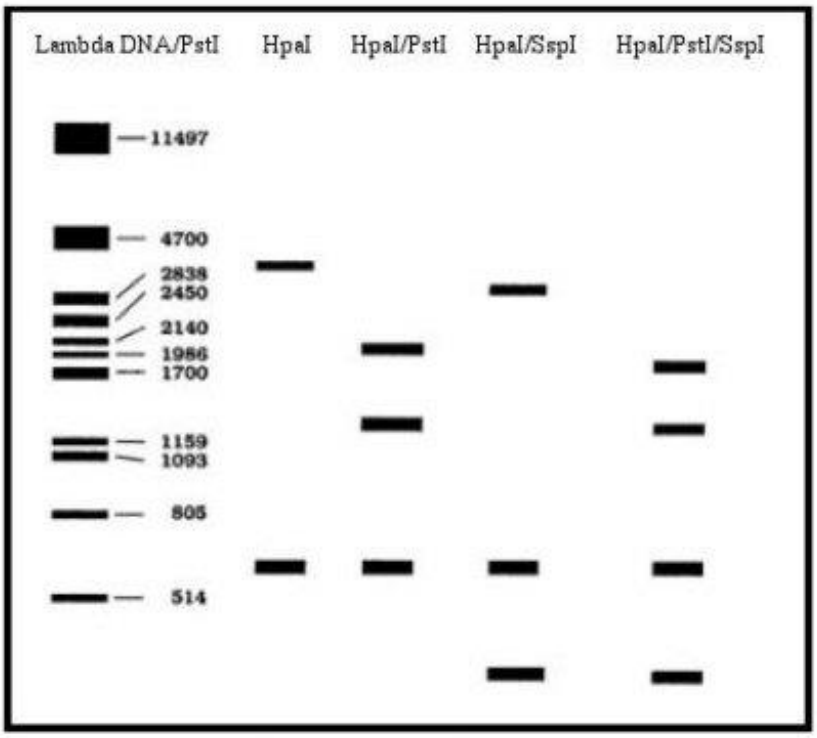

Figure 1 Gel of Lambda DNA digest

In this activity we will simulate using restriction enzymes to help us make a restriction map of an unknown piece of DNA. We can do this by comparing the fragments obtained from a digest to fragments of known size. Figure 1 shows DNA from the bacteriophage lambda (the sequence of which is known) that has been digested with the restriction enzyme PstI (lane 1). The size of each fragment is marked on the gel. The gel also shows the fragments from an unknown DNA sample in four separate restriction digests. By comparing the fragment sizes in the digests using more than one restriction enzyme, we will be able to determine the position of the fragments relative to one another.

Questions

1. Estimate the sizes of all fragments.
2. Determine the total length of the DNA segment digested. Do your estimates from all four digests agree?
3. There are two HpaI restriction sites present in the sample. Is the piece of DNA circular or linear? Draw the DNA showing the HpaI restriction sites.
4. How many PstI restriction sites are there? Add the PstI site(s) to your drawing.
5. How many SspI restriction sites are there? Add the SspI site(s) to your drawing.
6. Will the 600 bp HpaI fragment remain unchanged after digestion with either PstI or SspI?
7. Which fragments are unchanged from the HpaI/PstI digest to the HpaI/PstI/SspI digest? Which fragments disappear? Why did those fragments disappear?
8. Which fragments are unchanged from the HpaI/SspI digest to the HpaI/PstI/SspI digest? Which fragments disappear? Why did those fragments disappear?