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.

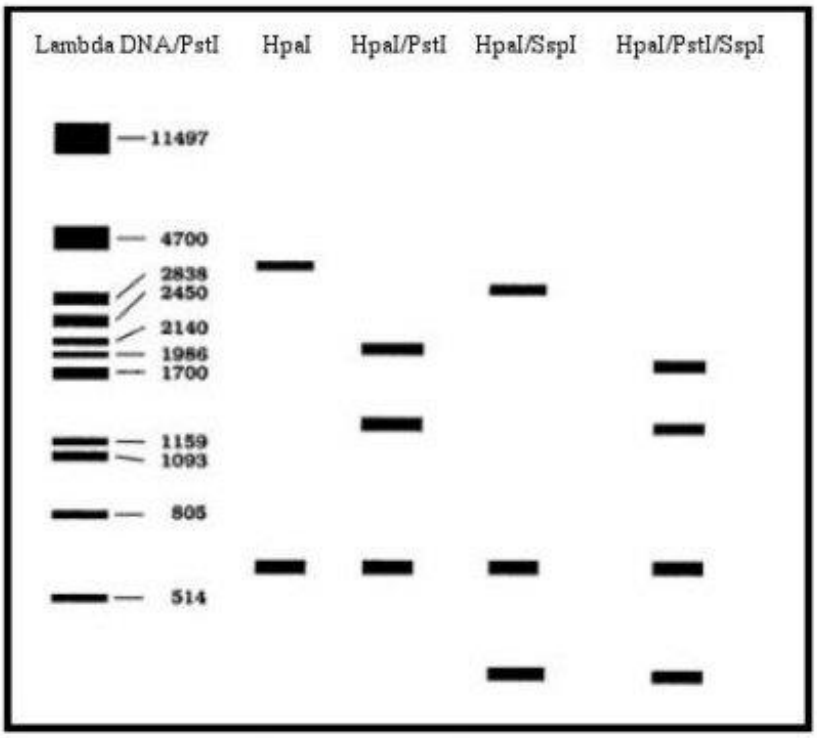

Figure 1 Gel of Lambda DNA digest

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. [SP2, SP4] Estimate the sizes of all fragments.

2. [SP4,SP5] Determine the total length of the DNA segment digested. State whether your estimates from all four digests agree.
3. [SP2, SP6] There are two HpaI restriction sites present in the sample. Identify the piece of DNA as circular or linear. Justify your response. Draw the DNA showing the HpaI restriction sites.
4. [SP2, SP6] State the number of PstI restriction sites on the DNA. Add the PstI site(s) to your drawing.
5. [SP2, SP6] State the number of SspI restriction sites on the DNA. Add the SspI site(s) to your drawing.
6. [SP2, SP6] The 600 bp HpaI fragment remains unchanged after digestion with both PstI or SspI. Provide reasoning for this observation.
7. [SP2, SP6] Identify the fragments that are unchanged from the HpaI/PstI digest to the HpaI/PstI/SspI digest. Identify the fragments that disappear. Provide reasoning to explain why those fragments disappeared.

8. [SP2, SP6] Identify the fragments that are unchanged from the HpaI/SspI digest to the HpaI/PstI/SspI digest. Identify which fragments disappear. Provide reasoning to explain why those fragments disappeared.