Student Practice Problems

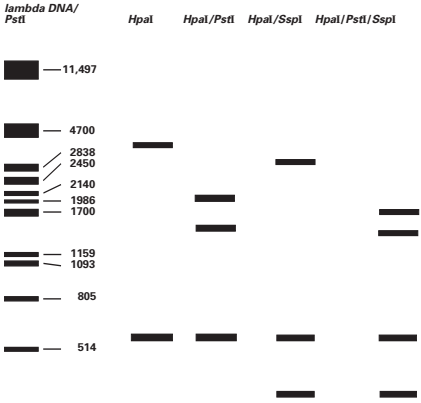
21-1175

Name _____

Date _____

Restriction Mapping of Plasmid DNA

Problem 1: Digested with *Hpa*I, *Hpa*I/*Pst*I, *Hpa*I/*Ssp*I, and *Hpa*I/*Pst*I/*Ssp*I



1. Estimate the sizes of the DNA fragments (in base pairs) by comparison to the lambda/*Pst*I size markers. These sizes do not have to be exact. Sizing of the smaller fragments will be more accurate than sizing of the larger fragments.

2. Determine the total size of the digested DNA by adding up the sizes of the fragments from each digest. You may take an average size from the 4 digests. The same DNA was digested in each sample so the fragment sizes from the different digests should always add up to the same total.

3. There are 2 *Hpa*I sites present. Based on the number of fragments obtained from the *Hpa*I digest, is this DNA linear or circular? Draw the DNA with the *Hpa*I sites present.

4. How many *Pst*I sites are present?

5. Where is the *Pst*I site? Draw the position of the *Pst*I site on the plasmid, relative to the *Hpa*I sites.

6. How many *Ssp*I sites are present?

7. Where is the *Ssp*I site? Draw the position of the *Ssp*I site on the plasmid, relative to the *Hpa*I sites. It might be best if this is done in a separate sketch from the *Pst*I site sketch, since we have not yet determined where the *Ssp*I and *Pst*I sites are relative to one another.

8. Will the 600-bp *Hpa*I fragment remain unchanged after digestion with either *Pst*I or *Ssp*I? (Check the gel.)

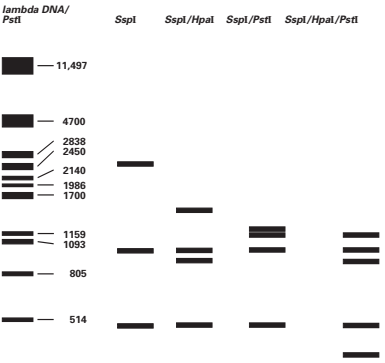
9. Which fragments are unchanged from the *Hpa*I/*Pst*I digest to the *Hpa*I/*Pst*I/*Ssp*I digest? Which fragments disappeared? Why did those fragments disappear?

10. Which fragments are unchanged from the *Hpa*I/*Ssp*I digest to the *Hpa*I/*Pst*I/*Ssp*I digest? Which fragments disappeared? Why did those fragments disappear?

11. Is there a fragment that appears only in the *Hpa*I/*Pst*I/*Ssp*I digest? What does this mean?

12. Draw the full plasmid map, with all restriction enzyme recognition sites present in their relative locations.

Problem 2: Digested with *Ssp*I, *Ssp*I/*Hpa*I, *Ssp*I/*Pst*I, and *Ssp*I/*Hpa*I/*Pst*I



1. Estimate the sizes of the DNA fragments (in base pairs) by comparison to the lambda/*Pst*I size markers. These sizes do not have to be exact. Sizing of the smaller fragments will be more accurate than sizing of the larger fragments.

2. Determine the total size of the digested DNA by adding up the sizes of the fragments from each digest. You may take an average size from the 4 digests. The same DNA was digested in each sample so the fragment sizes from the different digests should always add up to the same total.

3. This is plasmid DNA, which is circular. How many *Ssp*I sites are present? Draw the relative positions of the *Ssp*I restriction sites on the plasmid.

4. How many *Hpa*I sites are present?

5. Where is the *Hpa*I site? Draw the position of the *Hpa*I sites on the plasmid, relative to the *Ssp*I sites.

6. How many *Pst*I sites are present?

7. Where is the *Pst*I site? Draw the position of the *Pst*I site on the plasmid, relative to the *Ssp*I sites. It might be best if this is done in a separate sketch from the *Hpa*I site sketch, since we have not yet determined where the *Hpa*I and *Pst*I sites are relative to one another.

8. Will the 500- and 1000-bp *Ssp*I fragments remain unchanged after digestion with either *Pst*I or *Hpa*I? (Check the gel.)

9. Which fragments are unchanged from the *Ssp*I/*Hpa*I digest to the *Ssp*I/*Pst*I/*Hpa*I digest? Which fragment disappeared? Why did that fragment disappear?

10. Which fragments are unchanged from the *Ssp*I/*Pst*I digest to the *Ssp*I/*Hpa*I/*Pst*I digest? Which fragment disappeared? Why did that fragment disappear?

11. Which fragment appears only in the *Ssp*I/*Hpa*I/*Pst*I digest? Why is it present only in this digest?

12. Draw the full plasmid map with all restriction enzyme recognition sites present in their relative locations.