

## *Experiment 9*

# Construction of a Restriction Map of Plasmids pRIT4501 and pRIT4502

Now that you have constructed, purified, and verified two recombinant plasmids, you need to characterize them by constructing a detailed restriction map. At this point you should be able to do this without supervision and guidance. We will devote one week to this project and in the second week, we will continue with other work, although there will still be time to run additional gels.

You need to map only one of the plasmids - you may choose which. Once that is done, you can easily calculate the map of the other. For the second map, you must run a confirmation gel to verify that the two maps are consistent. You may ask all the questions you like. I will be helpful on technical matters but I will be purposefully vague about giving direction. The intent of this experiment is to think and work independently.

With the conclusion of this experiment you will prepare a comprehensive lab report on all work from Experiments 3 through 9. Since this includes so much time and work during the quarter, this lab report will be worth twice the other lab reports.

### **Construction of the Primary Map**

1. You may choose either of your plasmids to map the following sites:

<i>EcoRI</i>	<i>HindIII</i>
<i>PstI</i>	<i>XbaI</i>
<i>BamHI</i>	<i>BglII</i>

2. You may do any series or combinations of restriction digests that you deem appropriate.
3. You may run any concentration(s) of gels that you deem appropriate.  
*Some things to think about:*
  - Bear in mind that you are working with real data, not idealized gels as in your problem set, so don't expect all of your digests to add up to the same molecular weight.
  - You can do a lot of mapping using common sense by comparing single and double digest bands and trying to figure out what changes. In this way you can learn a lot about where sites are relative to other sites without ever calculating a fragment size.
  - Don't think that you can perform all of the digests and then at the very end sit down the night before the project is due and calculate the map. Rather, analyze each experiment as you complete it, make the best map you can. Then try to determine what additional information you need and design an experiment to get it.
  - Be wary of partial digests.

### The Confirmation Map

1. Once one plasmid has been mapped, you can automatically work out the map of the other. For the second plasmid, you must provide such a map and run a gel that confirms this map.
2. For the confirmation gel, you do not need to perform all of the digestions. Rather, choose two digestion conditions, (single or double) and predict what the fragment sizes would be if you did such an experiment.
3. Do the digestion and show that you indeed do obtain the fragments that you predicted. You have then confirmed your map.

You may use any restriction digest you like for your confirmation *except* for *HindIII*. This is because you used *HindIII* to originally characterize the two plasmids and you already know what to expect.