EXERCISE 2: RESTRICTION MAPPING

Introduction

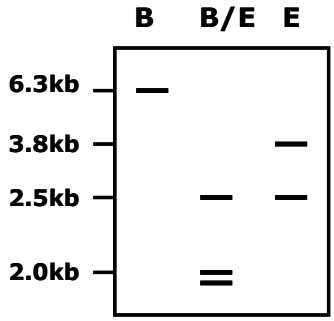
In the early days of molecular biology, scientists commonly used restriction mapping to understand the genes they were studying. In modern research labs, restriction mapping is not as widely used because sequencing has become more generally available and more economical than in the past. However, there are cases in which a restriction map may be adequate for a researcher to perform their investigations. The overall approach to restriction mapping involves the use of digestive enzymes to break down physically a sample of DNA. Once you measure the products of this digestion, you can "reassemble" the pieces and deduce the original sequence of DNA. Restriction mapping, therefore, allows you to determine where restriction sites are in your DNA sequence. If you have a look at appendix 4, you will see the pBluescript vector has many restriction sites annotated. Here, we will show you the basic principles of restriction analysis, then give a series of confidence-building exercises to give you practice. Please check Moodle for many more examples.

Basic principles

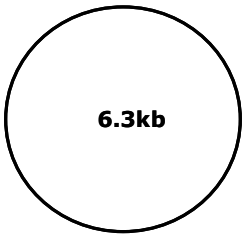
To build a restriction map, you need to estimate the sizes of DNA fragments produced on an agarose gel from a plasmid cut singly or doubly, using a range of restriction enzymes.

There are a few simple rules for mapping and the best way to explain is with the aid of an example.

Imagine in the lab you have taken a plasmid vector and digested this with single restriction enzymes, either BamHI (B) or EcoRI (E) alone and then performed a double digest with the two enzymes together. If you ran the resulting DNA fragments on an agarose gel, it might look like this:



This information can then be 'mapped' onto a circular plasmid (such as seen here) using some rules that will be described below:



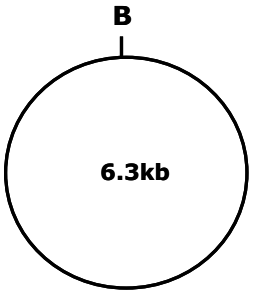
(a) **The sum total of the fragment sizes in every lane should always add up to the same value – the size of the uncut DNA.** In the example above, we know the size of the plasmid is 6.3kb, since the total of DNA fragments in each lane of the gel comes to this amount ie:

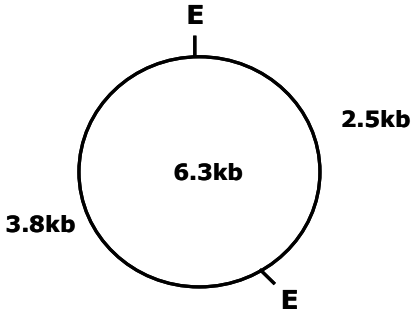
BamHI – 6.3

EcoRI - 3.8, 2.5

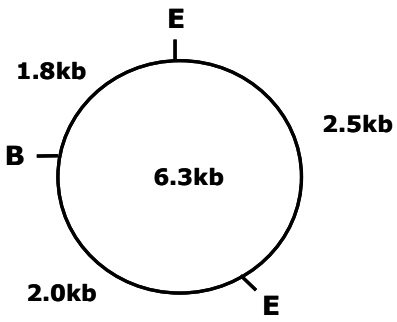
BamHI and EcoRI – 2.5, 2.0, 1.8

(b) **Digests using a single enzyme shows how many times a particular enzyme cuts the plasmid**. In the example above, when the plasmid is cut with BamHI this yields a single fragment, therefore, there must only be one BamHI site in this plasmid. On the other hand, when the plasmid is cut with EcoRI, this results in two DNA fragments being produced. These two pieces of information cannot be drawn on a single plasmid map until the result from the double digest is considered. Therefore, at the moment we can only place the single cuts on two separate maps as follows:

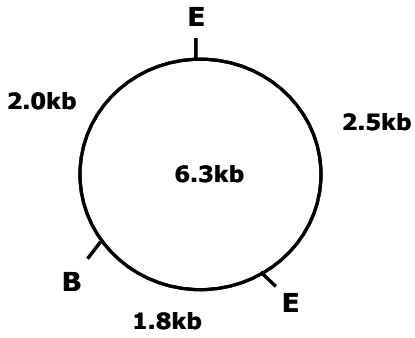




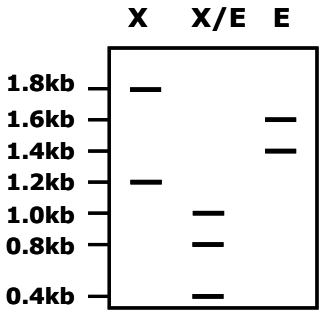
(c) **Cutting with two enzymes shows where the sites are relative to each other. The key to mapping is to identify the bands that change between the single digests and the double digests.** The double digest produced three fragments - 2.5, 2.0, and 1.8 kb long. A little bit of logic helps to order the sites relative to each other. Notice in the B/E double digest that the 2.5kb band seen in E alone is still present but the 3.8kb EcoRI band is gone and two new bands appear of 2kb and 1.8kb (=3.8kb). Therefore, there must be a BamHI site within the 3.8kb EcoRI fragment. The BamHI site is 1.8kb from one EcoR1 site and 2 kb from the other EcoR1 site. In this particular example, two possible maps could be drawn and both would be correct, since there is not yet enough information to place the enzymes exactly. The final maps could be one of the following:



OR



(d) **Watch out for DNA fragments that appear as the same size on an agarose gel!** Consider the following result from a plasmid digest using two restriction enzymes, XbaI (X) and EcoRI (E). The resulting gel might look like the following:



Using the rules set out previously, **can you first of all work out the size of the DNA fragments** in each lane of the gel and, hence, the total size of the plasmid:

XbaI –

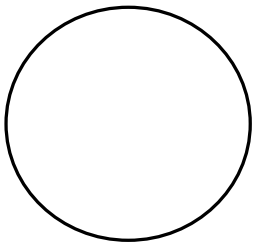
EcoRI -

XbaI and EcoRI –

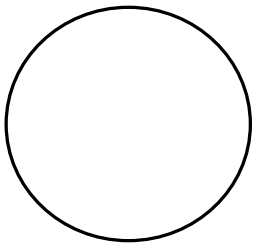
Does the size of the plasmid appear the same in each lane, and if not, why not?

What would the plasmid maps look like from a digest with each single enzyme?

XbaI:

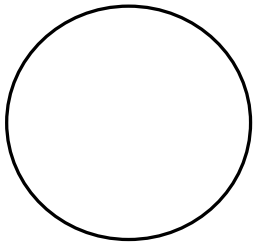


EcoRI:



What would the final restriction map look like when all the information is brought together (Hint: there may be more than one orientation so your answer may not appear the same as your neighbour!)?

XbaI and EcoRI:



There are videos accessible through the Molecular Methods app, which explain restriction mapping:

https://www.youtube.com/watch?v=yR_heZ4n4Gc

https://www.youtube.com/watch?v=MeTWD8ECeiQ

Restriction mapping confidence builders

Below are a few examples of restriction mapping problems. Draw restriction maps alongside, for each of the following examples (of increasing complexity). We have not given you the gel images for these digests, but provided you with the length of fragments once digested.

Question 1

Uncut plasmid (circular)	7.0kb
EcoRI	7.0 kb
XhoI	5.5, 1.5 kb
EcoRI+XhoI	3.0, 2.5, 1.5 kb

Question 2

Uncut plasmid (circular)	6kb
EcoRI	1.0, 2.0, 3.0 kb
XhoI	6.0 kb
EcoRI + XhoI	1.0, 1.2, 1.8, 2 kb

Question 3

Uncut plasmid (circular)	7.0 kb
EcoRI	7.0 kb
XhoI	6.0, 1.0 kb
EcoRI + XhoI	3.0, 1.0 kb

Question 4

Uncut plasmid (circular)	7.0 kb
EcoRI	5.0, 2.0 kb
XhoI	6.0, 1.0 kb
EcoRI + XhoI	3.0, 2.0, 1.0 kb

Question 5

Uncut plasmid (circular)	6.0 kb
EcoRI	2.8, 3.2 kb
XhoI	1.3, 4.7 kb
EcoRI + XhoI	0.6, 0.7, 2.1, 2.6 kb

Question 6

Uncut plasmid (circular)	7.0 kb
EcoRI	5.0, 1.2, 0.8 kb
XhoI	4.0, 3.0 kb
EcoRI+XhoI	3.0, 2.0, 1.0, 0.2, 0.8 kb

Question 7

A PCR product was prepared by digestion with PstI and XhoI and was cloned into pBluescript, the vector shown in Appendix 4 of this manual. The following bands were obtained after a single and double digestion of the recombinant plasmid. Draw the restriction map and indicate where the pBluescript vector sequence is (the vector is 3kb in size).

PstI	3.5, 1.8, 1.7
XhoI	3.6, 3.4
PstI + XhoI	3.0, 1.8, 1.1, 0.6, 0.5

Post-Lab Online restriction mapping resources

If you want further examples of restriction mapping then have a look at the Molecular Methods Moodle site – go to 'Other Supporting Resources' and then 'Restriction Mapping Examples'.

There are now many online tools freely available for you to identify restriction sites you are interested in within a particular sequence. Say for example, you wanted to find out if the BamHI and KpnI restriction enzymes were going to cut your cloned *Nrg* gene in half, making it impossible to clone using these enzymes. How would you find out?

See the following exercise on our Molecular Methods Moodle site – Restriction Mapping Examples - Restriction Mapping Online Resource Exercise.