Restriction Mapping

At some point in a cloning project it will be necessary to construct a restriction map of a plasmid. This will involve manipulating restriction digest fragments into a circular map. Once you have done this a number of times and develop a "feel" for the process, mapping will be almost intuitive. However, there is a systematic approach to constructing a map, which is illustrated in the sample problem below.

Once you have mastered the sample problem, complete the remaining problems, plot the answers on polar coordinate graph paper, and hand them in. Be cautioned that working with actual restriction fragments from a gel is a bit messier than these problems because of experimental error in determining fragment sizes. Sometimes the mathematical approach used here needs to be supplemented with some logic.

Sample Problem

Plasmid pRIT450 is 7.0 kb in length and has single PstI, EcoRI, and BamHI sites. You have cut the plasmid with PstI and inserted a 4.0 kb fragment into the site. From the data below, determine the restriction map of the resulting plasmid.

PstI	7.0	4.0		
EcoRI	6.0	5.0		
BamHI	8.9	2.1		
PstI + EcoRI,	4.3	3.3	2.7	0.7
PstI + BamHI	6.1	2.8	1.2	0.9
EcoRI + BamHI	5.0	2.1	2.1	1.8

Solution to Sample Problem

1. If the new plasmid is cut with PstI, the vector and the insert will regenerated. We will use these as reference fragments as we build the map. Although it is convenient to think in terms of target and vector, any site can arbitrarily be used to generate reference fragments.

2. We will now arbitrarily begin by choosing PstI as our reference point, but we could have just as easily chosen to start with EcoRI or BamHI. It makes no difference. First, look at the PstI + BamHI digest. It helps to think of the double digest conceptually as a two-step process in which

the plasmid is first cut by PstI into the two reference fragments. BamHI then cuts the reference fragments further.

3.   In the PstI + BamHI double digest, we recover 4 fragments. Some combination of the fragments will add up to the vector and the remaining fragments will equal the insert. We see that 6.1+ 0.9 = 7.0 = vector and 2.8 + 2.1 = 4.0 = insert. Thus, we can organize the fragments as below:

```
 P                    B   P    P      B           P
┌────────────────────┬──────┐ ┌──────┬───────────┐
│        6.1         │ 0.9  │ │ 1.2  │    2.8    │
└────────────────────┴──────┘ └──────┴───────────┘
         7.0 Ref                    4.0 Ref
```

4.   The insert can be placed into the vector in two opposite orientations relative to the vector. We will arbitrarily one orientation for the vector and vary the insert. It we tried to vary both vector and insert, we would end up with identical maps, but in mirror image. There is, however, no absolutely correct orientation of a plasmid map, so it really makes no difference:

```
 P                    B   P    P      B           P
┌────────────────────┬──────┐ ┌──────┬───────────┐
│        6.1         │ 0.9  │ │ 1.2  │    2.8    │   Orientation A
└────────────────────┴──────┘ └──────┴───────────┘
         7.0 Ref                    4.0 Ref


                               P            B     P
                              ┌───────────┬──────┐
                              │    2.8    │ 1.2  │   Orientation B
                              └───────────┴──────┘
                                   4.0 Ref
```

5.   We next must next determine which orientation, A or B, is correct. In orientation A, the two BamHI sites are close together (proximal) while in B, the sites are far apart (distal). Clearly, a BamHI single digest of each orientation will result in a different set of fragments and we can therefore determine expected fragment sizes for each orientation and then compare our expected values with the values observed:
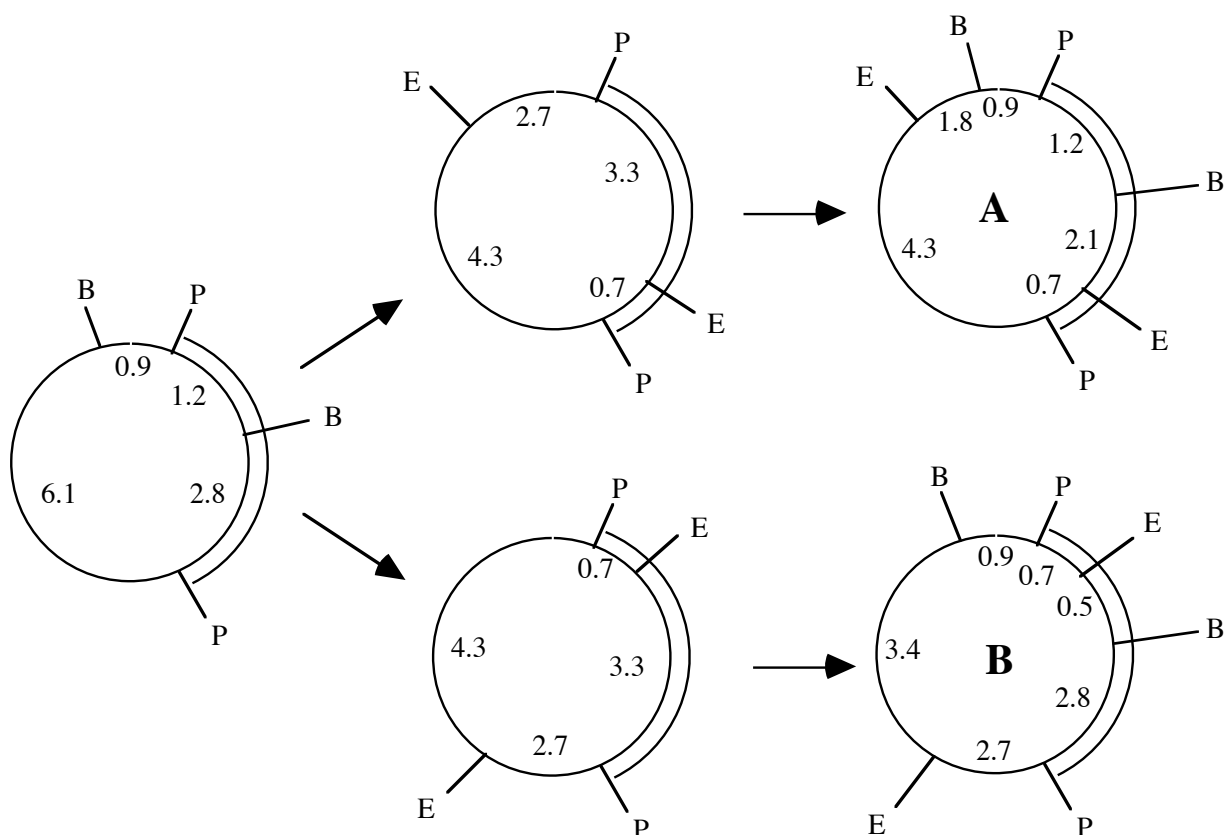
Orientation A				Orientation B				Observed
0.9	+ 1.2	=	2.1	0.9	+ 2.8	=	3.7	2.1
6.1	+ 2.8	=	8.9	6.1	+ 1.2	=	7.3	8.9
			11.0				11.0	11.0

6.   By comparing our observed and expected values, we find that orientation A is the correct orientation.

7.   Using exactly the same logic, we can now show that the correct orientation of the EcoRI sites is as below:

```
 P                    E        P    P            E     P
┌────────────────────┬────────┐ ┌────────────┬──────┐
│        4.3         │  2.7   │ │    3.3     │ 0.7  │
└────────────────────┴────────┘ └────────────┴──────┘
         7.0 Ref                    4.0 Ref
```

8.   To complete the map, we must now superimpose the BamHI and EcoRI maps. Let us arbitrarily
     assume that the orientation of the BamHI map is correct. We can now superimpose the EcoRI
     map on the BamHI map in either of two orientations, A or B. Using the alternate BamHI
     orientation would simply produce a mirror-image map.

9.   From the alternative orientations, A and B, we can predict the outcome of an EcoRI + BamHI
     double digest:

Orientation A				Orientation B				Observed
4.3	+	0.7	= 5.0	2.7	+	2.8	= 5.5	5.0
			2.1				0.5	2.1
1.2	+	0.9	= 2.1	0.7	+	0.9	= 1.6	2.1
			1.8				3.4	1.8
			11.0				11.0	11.0

10. By comparing the results of the EcoRI + BamHI double digest with our predictions, we see that
     orientation A is correct and the plasmid is now mapped. In step 8, we arbitrarily chose one of
     the two possible orientations for the BamHI map. Using the other, as noted, would have given
     the mirror image. Try it. Neither mirror image is more correct than the other. However, once
     the map is published, then, by convention, the one orientation published would then become the

"correct" version. If the plasmid was meant to be a new cloning vector, then one would map all plasmids subsequently derived from it in the same orientation.

11. The final step is to plot the map on polar coordinate graph paper. On polar coordinate graph paper, you plot degrees. Thus an entire circle, irrespective of total kilobases, will be 360°. Since the map in this case is 11 kb long , then:

$$360^O/11kb = 32.7^O/kilobase.$$

Now we multiply each kb value by 32.7 to obtain the degrees below:

kilobases	degrees
1.2	39.2
2.1	68.7
0.7	22.9
4.3	140.6
1.8	58.9
0.9	29.4
11.0	359.9

The last step is to decide on the circumference of the circle. If you are mapping several plasmids, you would want to draw them to scale. You can adjust the total size of your map by altering the circumference. Thus, for example, if you wanted to draw your map to the scale of 1 inch = 1 kb, then circumference = 11 kb x 1 inch/kb = 11 inches. You can determine the radius of a circle with a circumference of 11 inches by applying the formula:
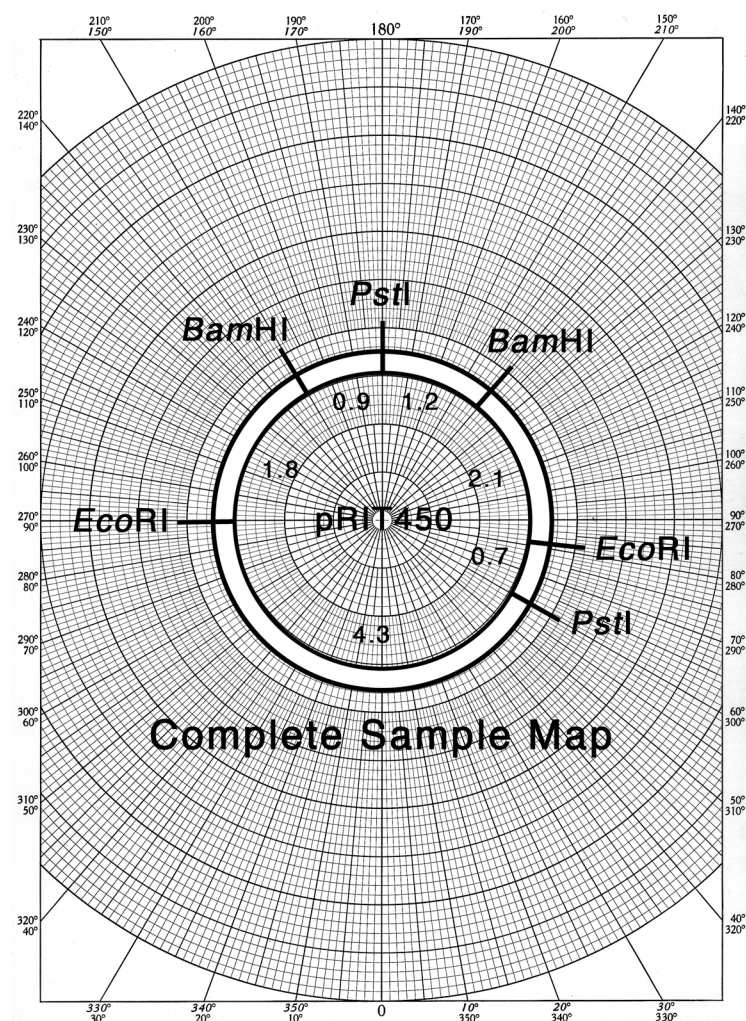
$$2 \pi r = C$$

where C = circumference and r = radius, and solving for r:

$$2 \pi r = 11 \text{ in}$$

$$r = 11 \text{ in}/2 \pi$$

$$r = 1.75 \text{ in}$$

The map of the sample plasmid on the following page is graphed in just this way. The position of the restriction sites are plotted according to the table above and the circumference of the outer circle is 11 inches, with a radius of 1.75 inches.

210°   200°   190°   180°   170°   160°   150°
150°   160°   170°   190°   200°   210°

*Pst*I

*Bam*HI   *Bam*HI

0.9   1.2

1.8   2.1

*Eco*RI   pRIT450   *Eco*RI

0.7

*Pst*I

4.3

Complete Sample Map

12.   Now, determine the restriction maps for the remaining plasmids and hand them in as homework.

Mapping Problems

Plasmid pRIT451 was cut with SmaI, BglII, and AvaI. From the data below, determine the map.

SmaI	5.9	4.3		
BglII	8.2	2.0		
AvaI	5.3	4.9		
SmaI + BglII	5.4	2.8	1.5	0.5
SmaI + AvaI	3.3	2.6	2.3	2.0
AvaI + BglII	5.3	2.1	2.0	0.8

Plasmid pRIT452 was cut with PstI, HindIII, and EcoRI. From the data below, determine the map.

PstI	6.8	5.9		
HindIII	6.5	6.2		
EcoRI	9.2	3.5		
PstI + HindIII	4.8	4.2	2.0	1.7
PstI + EcoRI	5.4	3.8	3.0	0.5
EcoRI + HindIII	6.2	3.5	1.8	1.2

Plasmid pRIT453 was cut with SmaI, HindIII, and EcoRI. From the data below, determine the map.

EcoRI	7.7	1.6		
HindIII	7.4	1.9		
SmaI	6.6	2.7		
EcoRI + HindIII	4.5	1.9	1.6	1.3
EcoRI + SmaI	5.7	2.0	0.9	0.7
SmaI + HindIII	2.7	2.5	2.2	1.9

Plasmid pRIT454 was cut with PstI, HindIII, and EcoRI. From the data below, determine the map.

PstI	6.0	5.3			
HindIII	5.8	5.5			
EcoRI	6.5	3.0	1.8		
PstI + HindIII	4.0	3.8	2.0	1.5	
PstI + EcoRI	3.5	3.0	2.5	1.8	0.5
EcoRI + HindIII	5.0	3.0	1.5	1.0	0.8

Plasmid pRIT455 was cut with BamHI, HindIII, and EcoRI. From the data below, determine the map.

EcoRI	8.5	2.0	0.5		
HindIII	5.6	5.4			
BamHI	6.5	4.5			
EcoRI + BamHI	4.5	2.2	2.0	1.8	0.5
EcoRI + HindIII	4.3	4.2	1.2	0.8	0.5
BamHI + HindIII	3.4	3.1	2.5	2.0	

Plasmid pRIT456 was cut with PstI, HindIII, and EcoRI. From the data below, determine the map.

EcoRI	5.8	3.3	2.9		
PstI	7.4	4.6			
HindIII	6.8	5.2			
PstI + EcoRI	3.3	3.3	2.5	2.1	0.8
PstI + HindIII	6.3	4.1	1.1	0.5	
EcoRI + HindIII	3.6	3.3	2.2	1.6	1.3

Plasmid pRIT457 was cut with BamHI, HindIII, and PstI. From the data below, determine the map.

BamHI	5.1	4.5	3.8		
HindIII	8.1	5.3			
PstI	13.4				
BamHI + HindIII	5.1	3.3	2.5	2.0	0.5
BamHI + PstI	5.1	4.5	2.5	1.3	
HindIII + PstI	8.1	3.3	2.0		