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Plasmid Mapping

Restriction enzymes are proteins that separate a DNA molecule at a specific location (locus). Think of them as molecular scissors. The terms "cut," "digest," or "restrict" may be used to describe the action of a restriction enzyme. Whenever a DNA molecule is cut with a restriction enzyme, the resulting pieces often need to be reassembled in a map representing the relative locus where the restriction enzyme cut the DNA molecule.

This is because scientists are usually trying to determine where a specific gene is located in a certain piece of DNA. They start by using restriction enzymes that act close to either end of the gene of interest. Once the gene has been located on a piece of DNA, it is often useful to determine where the piece of DNA was originally located. To do this, scientists try to construct a map of the original piece of DNA using their experimental data.

Because plasmids are rings or circles of DNA, a restriction enzyme that cuts a plasmid once results in a linear piece of DNA that has the same number of base pairs as the original plasmid. A restriction enzyme that cuts a plasmid twice results in 2 linear pieces of DNA whose total number of base pairs equals the number of base pairs in the original plasmid. When 2 restriction enzymes cut the same plasmid, it is referred to as a double digest. It is usually necessary to use at least 2 restriction enzymes to map a plasmid. However, it is not uncommon for as many as 6 or 8 restriction enzymes to be used.

Plasmid maps normally take the form of a circle. The name of the restriction enzyme and the relative locus where the enzyme cuts the plasmid are shown on the map. The center of the map is labeled with the total number of base pairs in the plasmid. When teaching students how to create a plasmid map, it is helpful to use some type of 3-D circular object that you can cut up to demonstrate the process, e.g., string, rope, or rubber tubing

Mapping a plasmid is basically a game of logic. The key is to remember to account for all experimental data. Think of it as taking a clock apart and putting it back together again with no parts remaining. We have listed 13 plasmid map problems that we have collected over the years, most of which are not original. These exercises are a great way to get students to start thinking like scientists. Plasmid mapping can be done from at least 3 different views. You can present any one of the 3 views to your students, and they should be able to come up with the other 2.

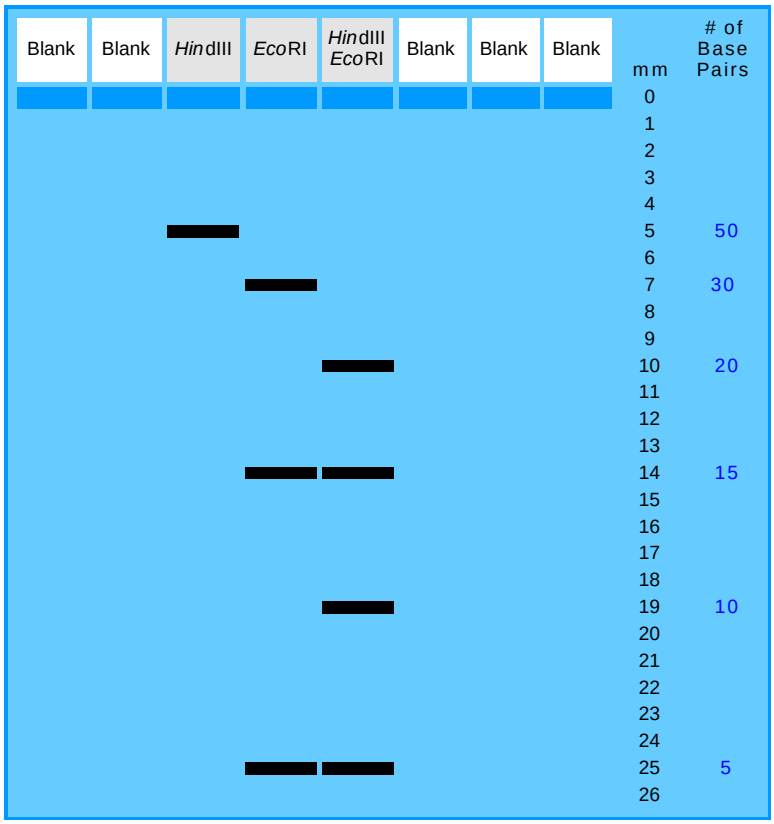
Start by showing your students how to solve one or 2 simple problems to give them a feel for the process. We have included a sample plasmid map problem (with a solution guide) for this purpose. Some students will demonstrate a knack for solving these problems while others will suffer and rue the day plasmid maps entered their classroom—remember, these are basically logic problems. We suggest that you let students work together in pairs if they wish.

There is no one certain way to do plasmid mapping. Just look for answers that account for all of the experimental data. It is a good idea for a few students to present their method of plasmid mapping to the whole class. This reinforces the process for the problem solvers and assists those having trouble. Perhaps the ultimate assessment is to have your students make up some plasmid mapping problems for their classmates to solve. Consider saving the best problems for reuse in subsequent classes. These make great activities for down time, e.g., when gels are setting up or during electrophoresis. Have fun with them.

After your students have had a chance at pen and paper problems, they will probably be ready for a more realistic DNA mapping problem. Carolina DNA mapping kits are a great way to make electrophoresis much more than just running a gel. These kits really get your students involved—hands on and minds on!

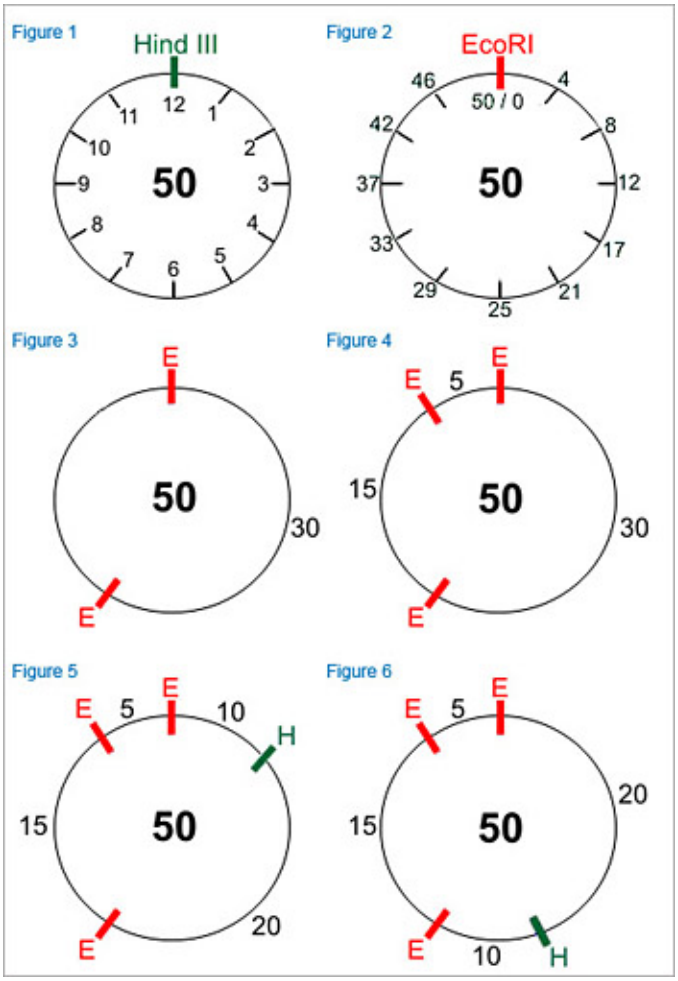
Sample Map Problem

Experimental data				
Number of base pairs per band				
***Eco*RI**	30	15	5	
***Hin*dIII**	50			
***Hin*dIII / *Eco*RI**	20	15	10	5



Here's our approach to solving this plasmid map sample problem

1. Determine the number of base pairs (bp) in the whole plasmid. Note that only one band results when the plasmid is cut by *Hin*dIII. This is a very good clue that *Hin*dIII has only one recognition site, and that when cut, the plasmid ring opens into a linear piece of DNA that is 50 bp long. This is confirmed by looking at the other combinations of restriction enzymes cutting the plasmid into pieces that, each time, add up to 50 bp.
2. Start drawing circles! (Note: Clock face references in the following instructions indicate cut sites or where lines are to be drawn.) The first circle should indicate one cut site for *Hin*dIII at 12:00. Label your circle with "*Hin*dIII" (or "H" for short) above a tick mark at 12:00 to indicate a single cut (a single digest) by this restriction enzyme. See figure 1. This accounts for your experimental data from the single digest with *Hin*dIII.
   a. Place the number 50 in the center of the circle to indicate the total number of bp.
3. Determine a scale for your circle. If the total number of bp going around the circle is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp. See figure 2.
4. Draw another circle to indicate where the *Eco*RI restriction sites are located. The data indicates 3 different-sized bands result when the plasmid is cut with just *Eco*RI (a single digest).
   a. Start by placing a tick mark at 12:00, as in figure 2, to indicate the first *Eco*RI cut site.
   b. Make another tick mark at about 7:00 to indicate a second *Eco*RI cut site 30 bp from the first site. See figure 3. This arrangement accounts for the 30 bp band in your experimental data. This leaves 20 bp between 7:00 and 12:00. The other 2 data results, 15 and 5, add up to 20 bp.
   c. Go to approximately 11:00 or 7:00 (either option accounts for the experimental data and is therefore acceptable because plasmid maps show relative locations) and make a third tick mark. See figure 4. For example, if you go to approximately 11:00 (a distance of 5 bp), the remaining distance to 7:00 represents 15 bp (for a total of 20 bp), which accounts for your experimental data.
5. Fit the 2 circles together to account for the experimental data from your double digest (*Hin*dIII/*Eco*RI). We recommend the use of 3-D plasmid models (loops) to help students visualize this process. You can make these loops out of inexpensive materials such as string, rubber tubing, paper strips, or Velcro strips.
   a. Mark one loop to indicate the *Hin*dIII digest.
   b, Mark another loop to indicate the *Eco*RI digests.
6. Place the *Hin*dIII loop on top of the *Eco*RI loop and align the *Hin*dIII tick mark at about 2:30 on the *Eco*RI loop, which indicates a site 10 bp from the *Eco*RI site at 12:00 and 20 bp from the *Eco*RI site at 7:00. See figure 5.



   a. Bear in mind, it cannot be determined from the data if the *Hin*dIII tick mark should be located 10 bp from the 12:00 site or 10 bp from the 7:00 site. Both locations would account for the experimental data. See figure 6.
   b. Remember that these plasmid maps give relative positions. For more accuracy, you must perform additional digests.
7. Have your students make a final “answer” plasmid map that indicates each *Eco*RI cut site, the *Hin*dIII cut site, and accounts for all experimental data.
8. Emphasize to your students that their circles show relative locations (loci) for the sites of the restriction enzymes. If they simply turn their paper over, flip it upside down, or spin it 90° the picture changes, but the relative positions remain the same.
9. Visit the Dolan DNA Learning Center at Cold Spring Harbor Laboratory's Web site for a look at a few plasmid maps that are used in some of our Carolina biotechnology kits.

Carolina DNA mapping kits

Restriction Mapping of Plasmid DNA

Electrophoresis of precut DNA with an analytical application. Students cast gels and perform electrophoresis of ready-to-load samples of predigested plasmid DNA. After staining with *Carolina*BLU™ stain, students determine the sizes of the plasmid DNA fragments in each sample and use the data to deduce a restriction map of the plasmid.
View Product

Restriction Mapping of Lambda DNA

This kit provides data that allow students to assemble a map of cutting sites of *Apa*I and *Eco*01091 on the genome of the bacterial virus lambda. This is accomplished by digesting lambda DNA with 2 restriction enzymes alone and in combination. The resulting restriction fragments are subjected to electrophoresis in an agarose gel along with lambda/*Hin*dIII size markers. The distances migrated by the lambda/*Hin*dIII fragments vs. their base pair sizes are plotted on semilog graph paper. The distances migrated by the unknown fragments are then used to extrapolate their base pair sizes. Accounting for the appearance and disappearance of bands in single vs. multiple digests allows students to position the restriction sites relative to one another, resulting in a restriction map.
View Product

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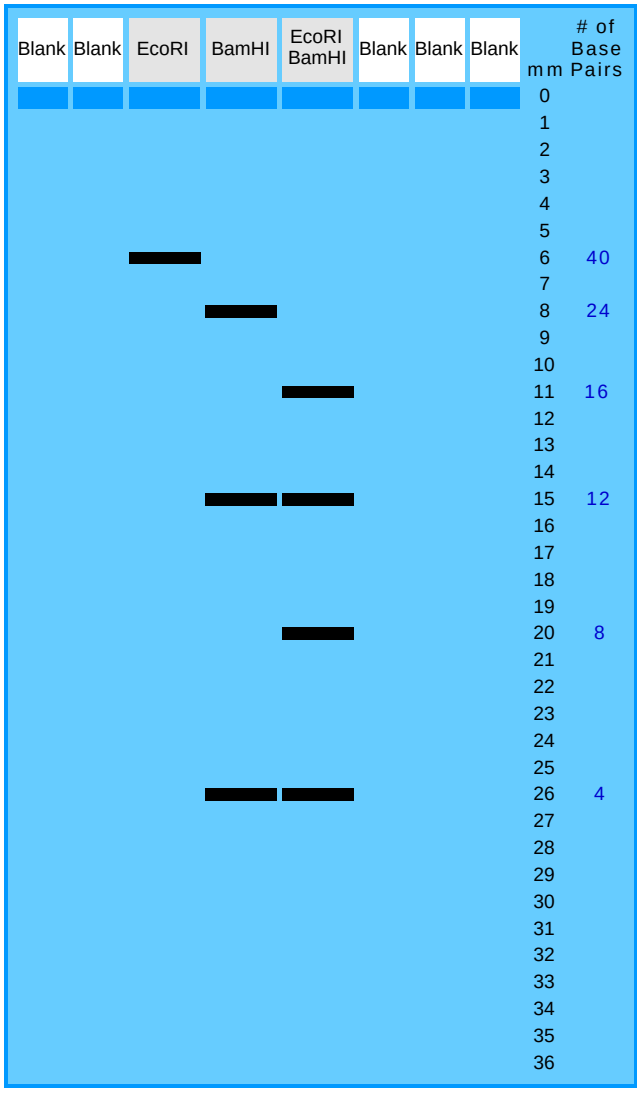
Plasmid mapping: Exercise # 1

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data				
Number of base pairs per band				
BamHI	24.0	12.0	4.0	
EcoRI	40.0			
EcoRI / BamHI	16.0	12.0	8.0	4.0



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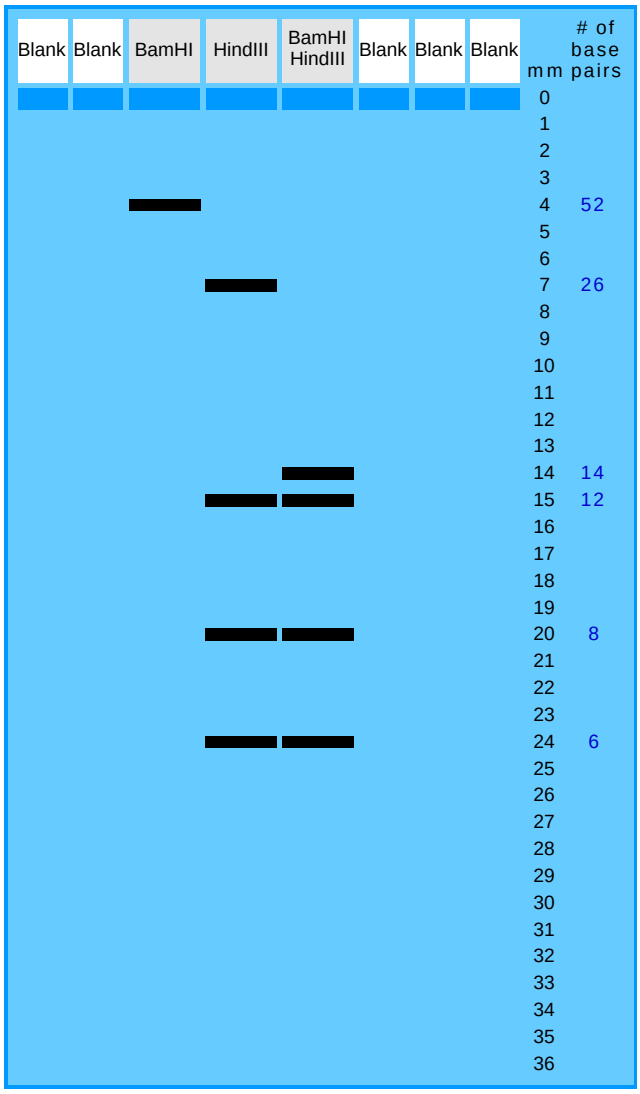
Plasmid mapping: Exercise # 2

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data				
Number of base pairs per band				
BamHI	52.0			
HindIII	26.0	12.0	8.0	6.0
BamHI / HindIII	14.0	12.0	8.0	6.0



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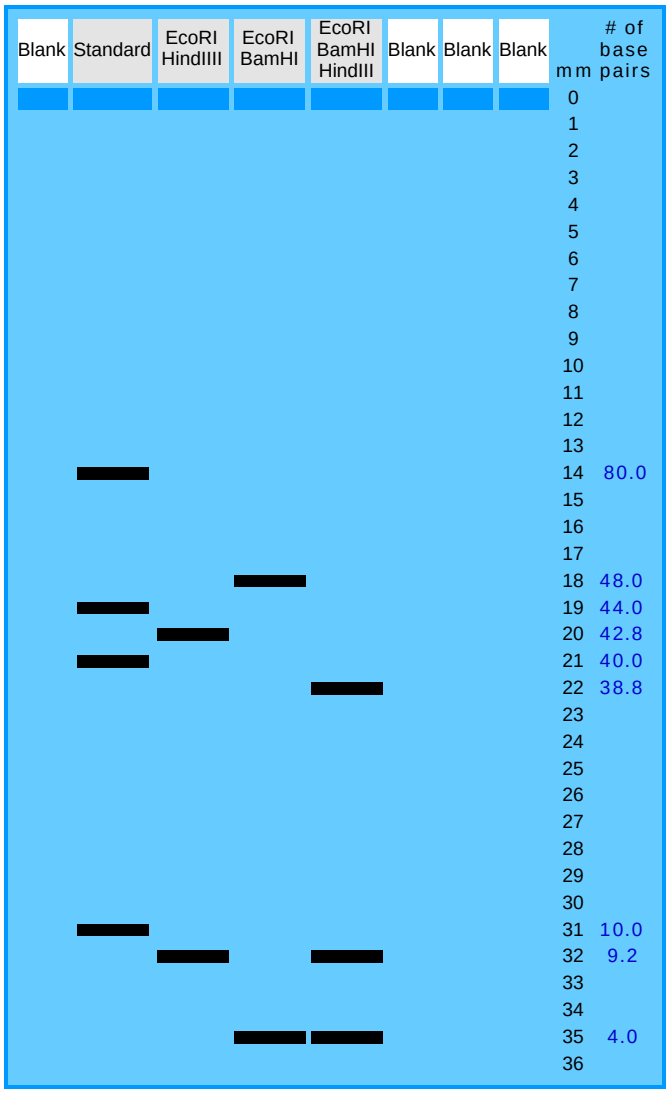
Plasmid mapping: Exercise # 3

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data				
# of base pairs per band				
EcoRI / HindIII	42.8	9.2		
EcoRI / BamHI	48.0	4.0		
EcoRI / BamHI / HindIII	38.8	9.2	4.0	
Size Standard	80.0	44.0	40.0	10.0



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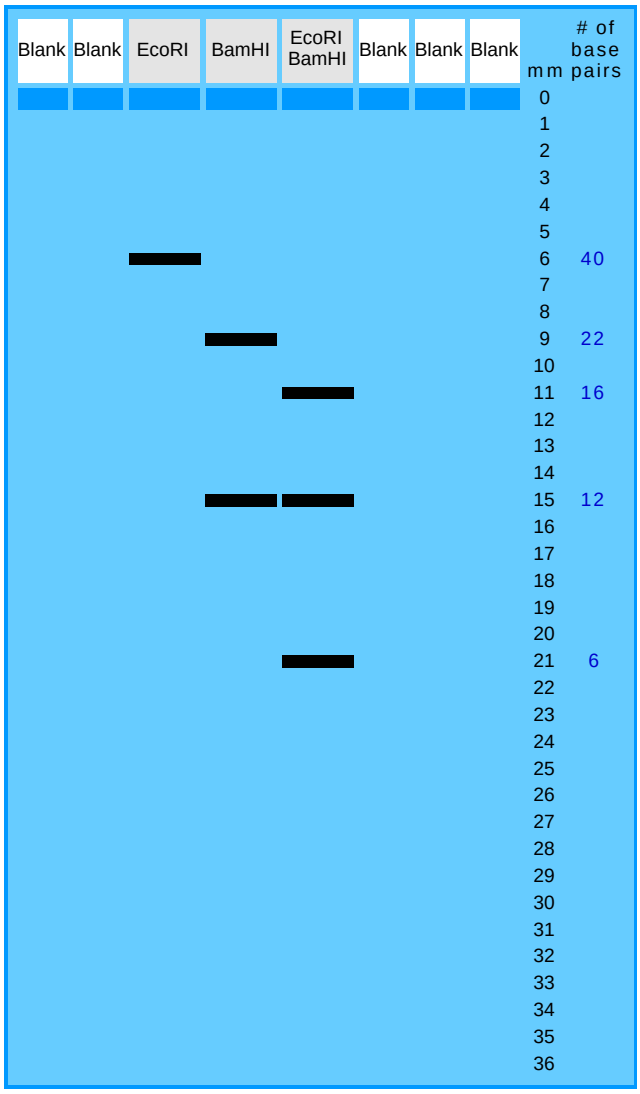
Plasmid mapping: Exercise # 4

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data				
Number of base pairs per band				
EcoRI	40.0			
BamHI	22.0	12.0		
EcoRI / BamHI	16.0	12.0	6.0	



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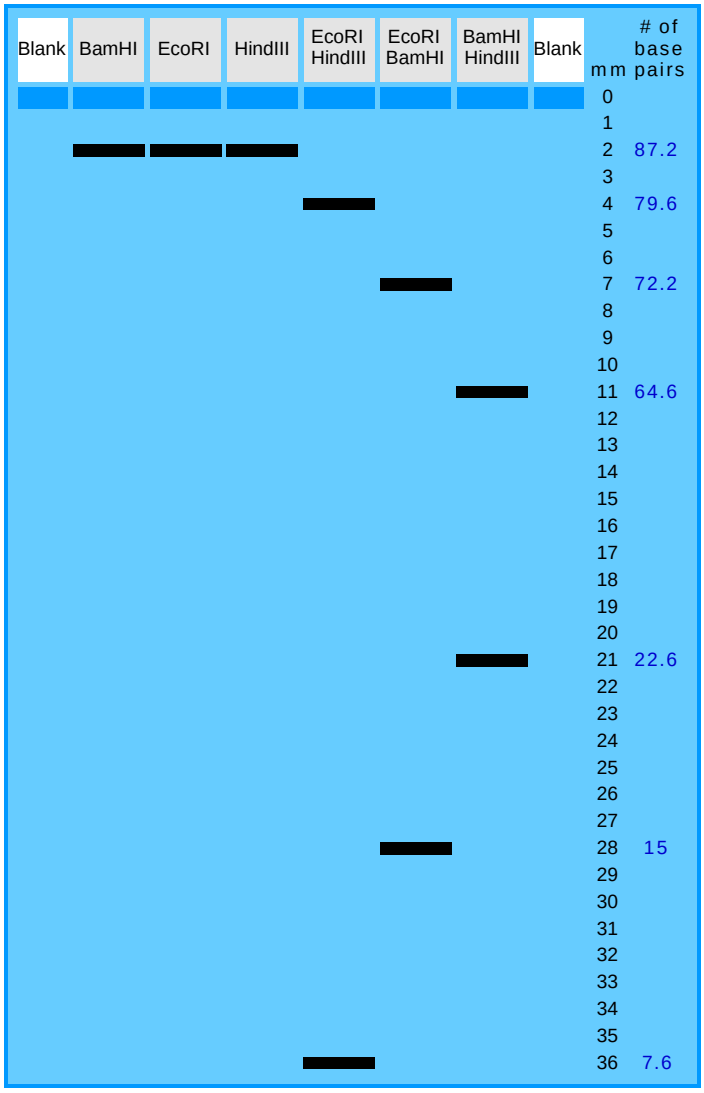
Plasmid mapping: Exercise # 5

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data				
Number of base pairs per band				
BamHI	87.2			
EcoRI	87.2			
HindIII	87.2			
EcoRI / HindIII	79.6	7.6		
EcoRI / BamHI	72.2	15.0		
BamHI / HindIII	64.6	22.6		



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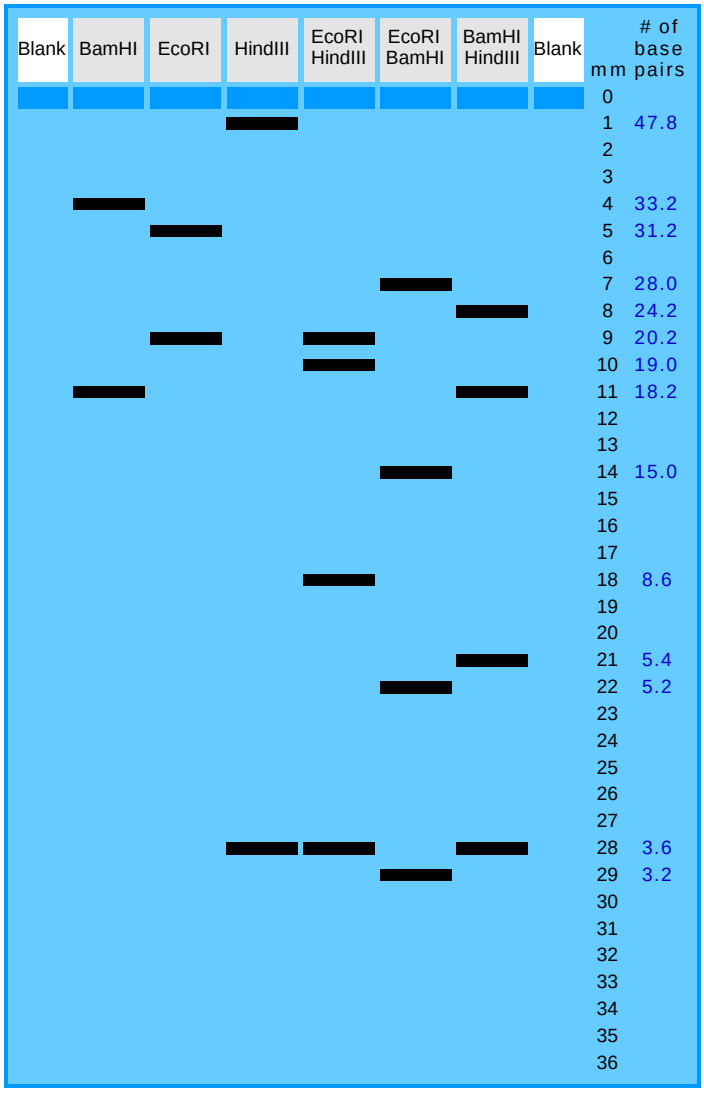
Plasmid mapping: Exercise # 6

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data				
Number of base pairs per band				
BamHI	33.2	18.2		
EcoRI	31.2	20.2		
HindIII	47.8	3.6		
EcoRI / HindIII	20.2	19.0	8.6	3.6
EcoRI / BamHI	28.0	15.0	5.2	3.2
BamHI / HindIII	24.2	18.2	5.4	3.6



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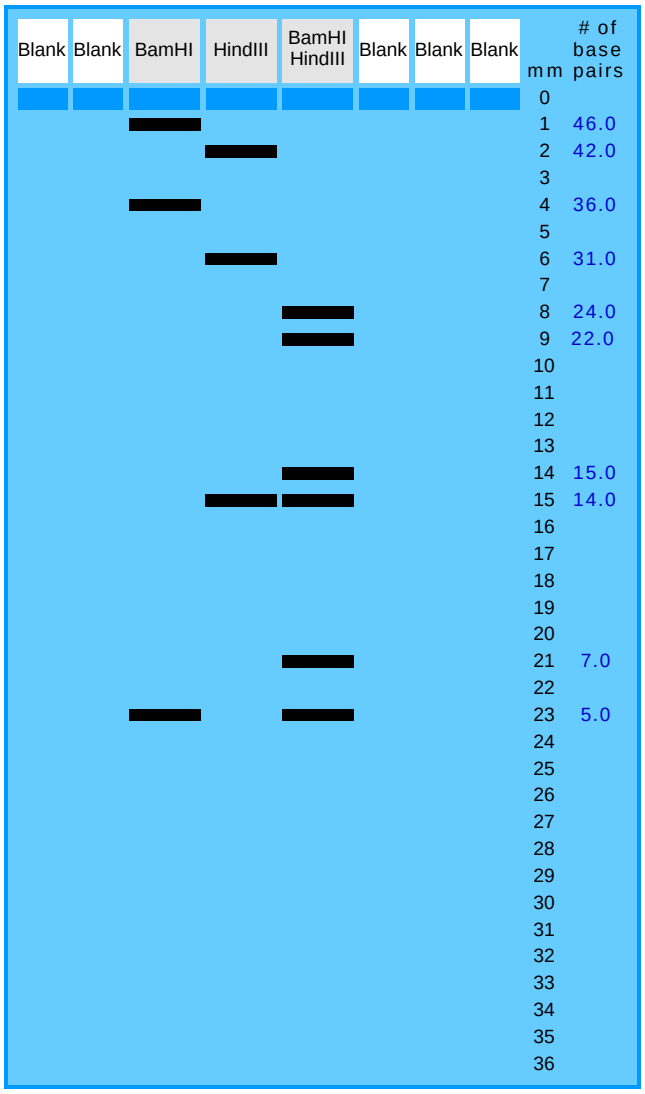
Plasmid mapping: Exercise # 7

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data						
Number of base pairs per band						
HindIII	42.0	31.0	14.0			
BamHI	46.0	36.0	5.0			
BamHI / HindIII	24.0	22.0	15.0	14.0	7.0	5.0



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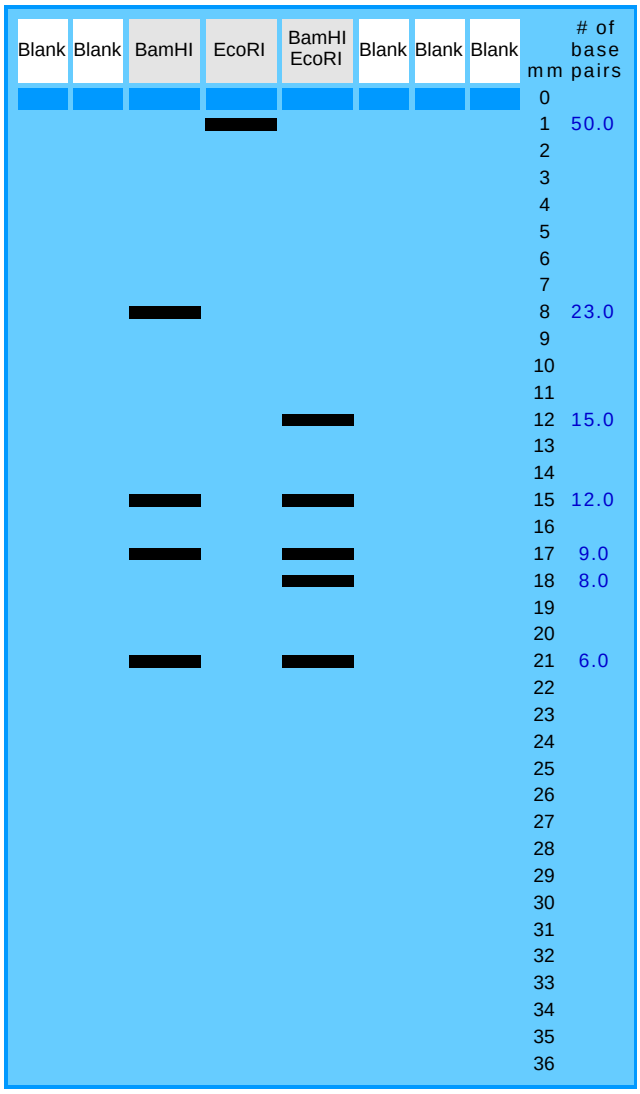
Plasmid mapping: Exercise # 8

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data						
Number of base pairs per band						
BamHI	23.0	12.0	9.0	6.0		
EcoRI	50.0					
BamHI / EcoRI	15.0	12.0	9.0	8.0	6.0	



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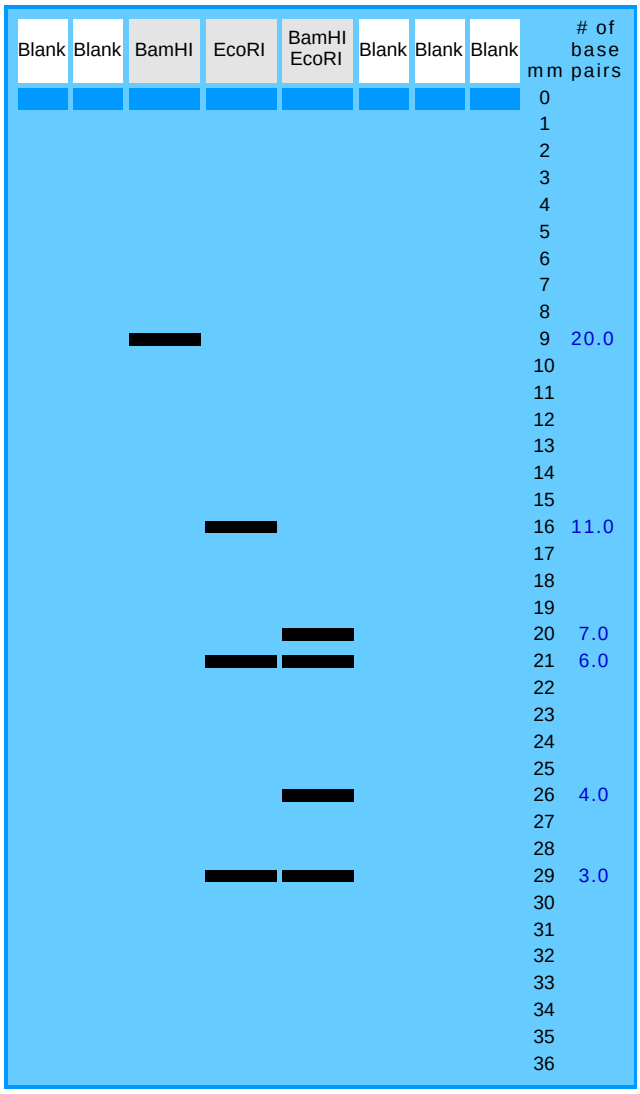
Plasmid mapping: Exercise # 9

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data					
Number of base pairs per band					
BamHI	20.0				
EcoRI	11.0	6.0	3.0		
BamHI / EcoRI	7.0	6.0	4.0	3.0	



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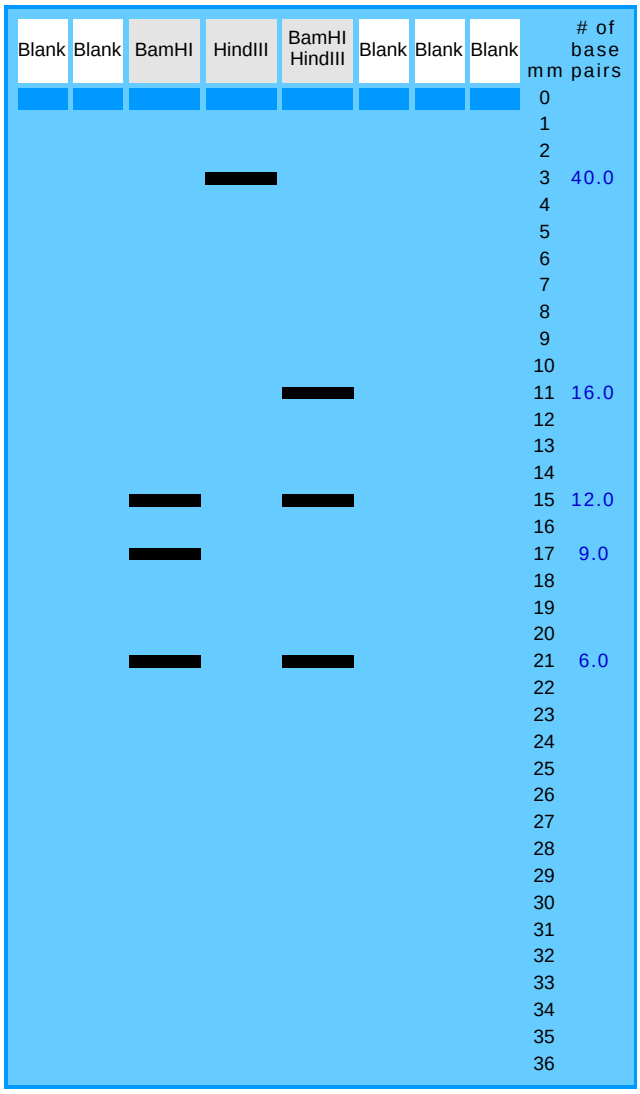
Plasmid mapping: Exercise # 10

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data						
# of base pairs per band						
BamHI	12.0	9.0	6.0			
HindIII	40.0					
BamHI / HindIII	16.0	12.0	6.0			



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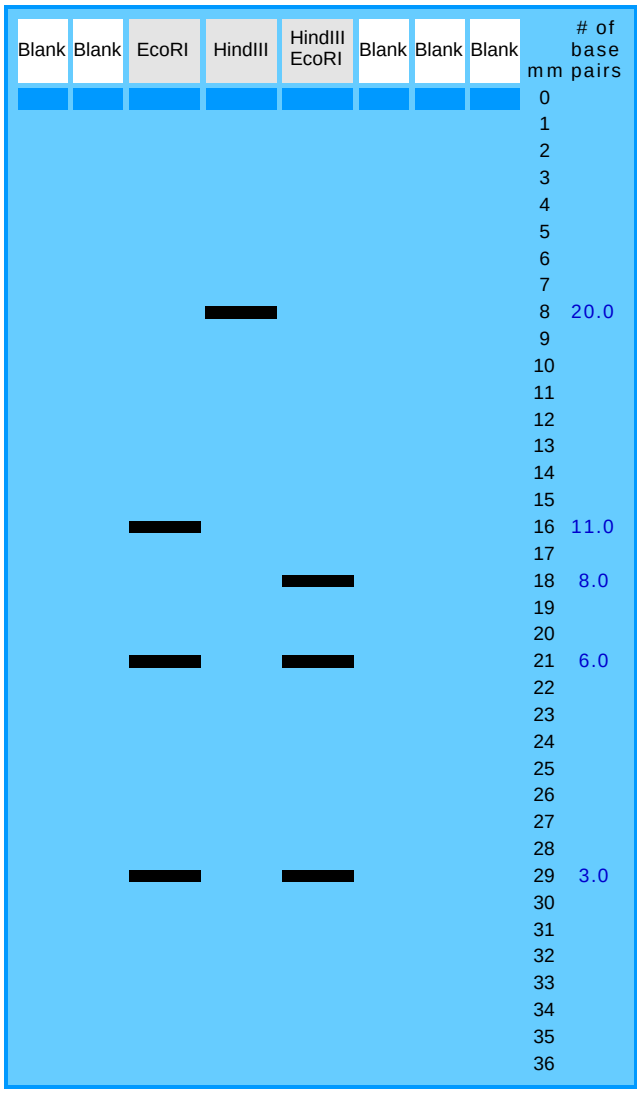
Plasmid mapping: Exercise # 11

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data					
Number of base pairs per band					
EcoRI	11.0	6.0	3.0		
HindIII	20.0				
HindIII/ EcoRI	8.0	6.0	3.0		



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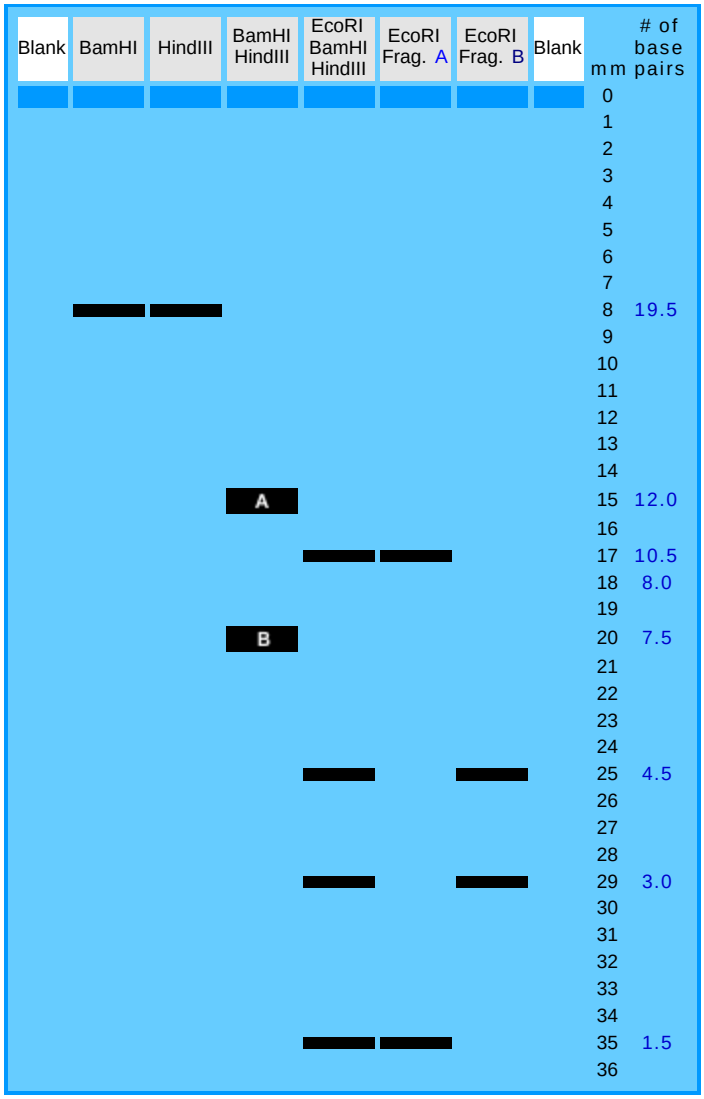
Plasmid mapping: Exercise # 12

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data					
Number of base pairs per band					
BamHI	19.5				
HindIII	19.5				
BamHI / HindIII	12.0	7.5			
EcoRI / BamHI / HindIII	10.5	4.5	3.0	1.5	
EcoRI (Fragment A)	10.5	1.5			
EcoRI (Fragment B)	4.5	3.0			



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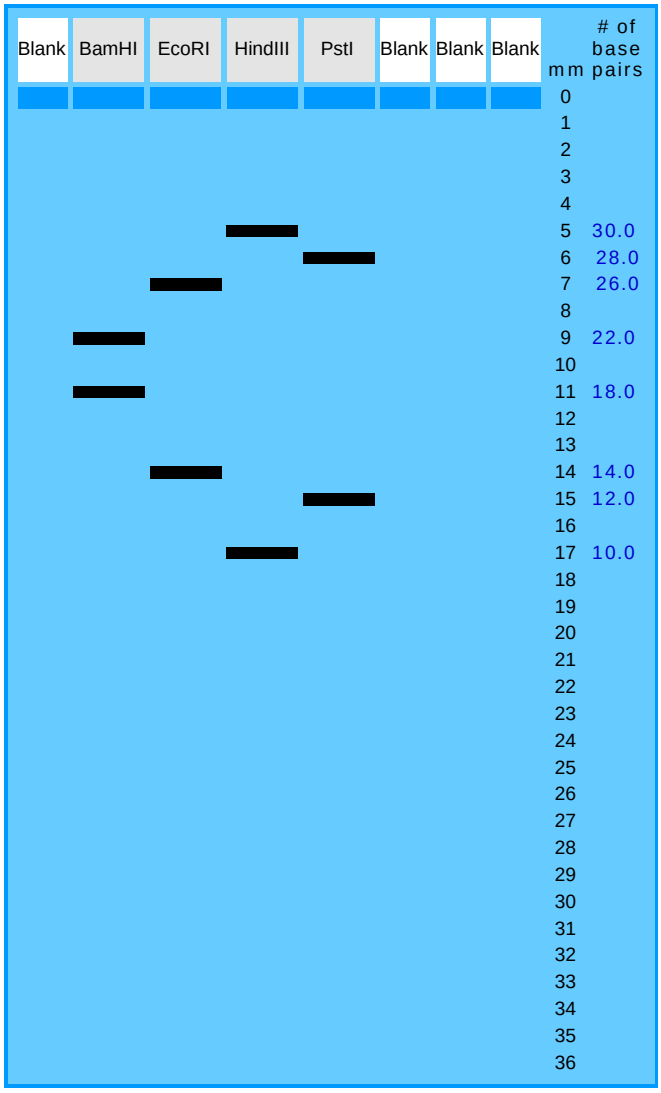
Plasmid mapping: Exercise # 13-A

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data						
Number of base pairs per band						
BamHI	22.0	18.0				
EcoRI	26.0	14.0				
HindIII	30.0	10.0				
PstI	28.0	12.0				



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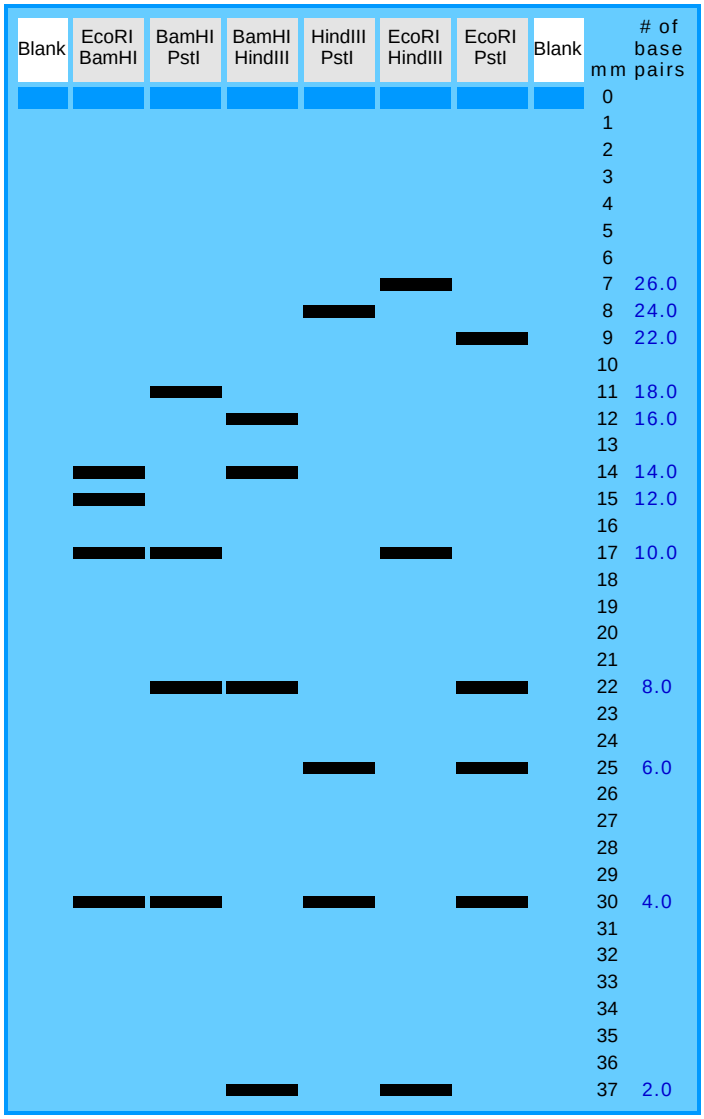
Plasmid mapping: Exercise # 13-B

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data						
Number of base pairs per band						
EcoRI / BamHI	14.0	12.0	10.0	4.0		
BamHI / PstI	18.0	10.0	8.0	4.0		
BamHI / HindIII	16.0	14.0	8.0	2.0		
HindIII / PstI	24.0	6.0	4.0			
EcoRI / HindIII	26.0	10.0	2.0			
EcoRI / PstI	22.0	8.0	6.0	4.0		



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Plasmid mapping: Exercise # 13-C

Instructions

Determine the number of base pairs (bp) in the whole plasmid, and then determine a scale for your plasmid map. Visualizing the map as a clock face is helpful. For example, if the total number of base pairs going around the map is 50, then 6:00 represents 25 bp, 3:00 represents about 12–13 bp, and 9:00 represents about 37–38 bp.

Experimental data						
Number of base pairs per band						
BamHI / EcoRI / HindIII	14.0	12.0	8.0	2.0		
BamHI / EcoRI / PstI	12.0	10.0	6.0	4.0		
EcoRI / HindIII / PstI	22.0	6.0	4.0	2.0		
BamHI / HindIII / PstI	14.0	10.0	6.0	4.0	2.0	
BamHI / EcoRI / HindIII / PstI	12.0	10.0	4.0	2.0