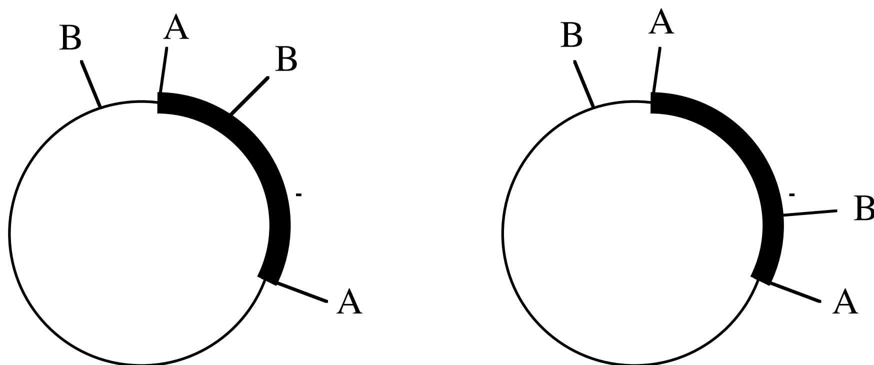
Experiment 1

Analysis of Plasmid DNA by Restriction Digestion and Agarose Gel Electrophoresis

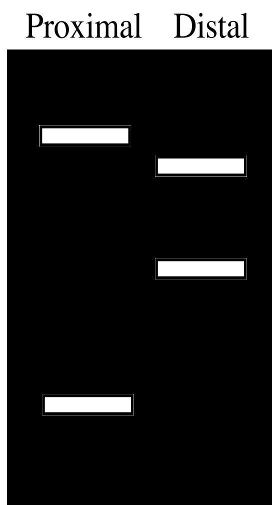
This experiment will provide an introduction to restriction enzyme digestion and gel electrophoresis, the two most fundamental techniques in recombinant DNA methodology. You will learn how to cut DNA with a restriction endonuclease and analyze the products on a gel. At the end of the experiment you should be able to construct a restriction map of a plasmid.

The plasmid pUC9 (pages 41 and 42) is 2.7 kb long and contains the gene for β-lactamase (*bla*), which confers resistance to ampicillin, the *lac* repressor, operator, and promoter, and the alpha peptide (containing the amino-terminal end) of the β-galactosidase (*lac*Z) gene. When in the appropriate host strain, one that contains the carboxyl end of *lac*Z, pUC9 produces functional β-galactosidase. Within the *lac*Z gene is a closely-spaced series of restriction sites, called the multiple cloning site, which may be used as insertion points for foreign DNA fragments. Insertion into *lac*Z disrupts the β-galactosidase and cells carrying this plasmid, therefore are lac^-.

When a DNA fragment is inserted into a plasmid, it can be found in either of two orientations In the diagram below, for example, the narrow line represents a plasmid into which a DNA fragment has been inserted into restriction site A. The plasmid and the insert each have a site for enzyme B. On the insert, B is asymmetric. In the left orientation, the two B sites are close to one another (proximal) and in the right orientation, they are far apart (distal).



If both of these plasmids are cut with enzyme A, the vector and insert fragments will be regenerated. Both orientations will produce exactly the same fragments. However, if the plasmids are cut with enzyme B, the resulting fragments will be different. The proximal orientation will produce a large and a small fragment while the distal orientation will produce two moderately sized fragments:



pRHR30 is a derivative of pUC9 in which a fragment of human DNA has been inserted into the *Eco*RI site in *lac*Z The fragment has an asymmetric *Hin*dIII site which is either proximal or distal to the *Hin*dIII site on pUC9. In this experiment, you will cut pUC9 and pRHR30 with EcoRI, HindIII, and BamHI and construct a plasmid map. The results of this experiment will be combined with Experiment 2 for your first formal lab report.

Note that in addition to the restriction digests done in this experiment, you will also perform several restriction digests that will be used for Experiment 3.

Procedure

1. Prepare the series of restriction digests described below according to the procedure on page 12. You will use 5 µl of DNA and 15 µl of reaction mix.

Tube	DNA	Enzyme	Buffer (2 µl)	Water	* Dye
1	pUC9 (3 µl)	none	none	17 µl	Orange
2	"	*Hind*III	Med	15 µl	"
3	"	*Bam*HI	NEB *Bam*HI	"	"
4	"	*Eco*RI	High	"	"
5	"	*Pst*I	NEB 3	"	"
6	1 kb ladder (3 µl)	none	none	17 µl	"
7	λ *Hind*III (3 µl)	none	none	17 µl	Blue
8	pRHR30 (5 µl)	none	none	15 µl	"
9	"	*Hind*III	Med	13 µl	"
10	"	*Bam*HI	NEB *Bam*HI	"	"
11	"	*Eco*RI	High	"	"
12	"	*Pst*I	NEB 3	"	"

*Tracking dye (5 µl) is added **AFTER** the incubation period

2. Prepare the restriction digests and heat inactivations for steps 1 and 2 of Experiment 3.

3. Place samples 4, 5, 6, 8, 9, and 10 in a 37° water bath for 40 minutes.

4. While you are waiting for your DNA's to digest, pour a 1% agarose gel according to the procedure on page 17.

5. At the end of 40 minutes, remove the samples and add 5 µl of tracking dye to each sample.

6. Load the entire 25 µl onto the gel and run at 120 volts for about 90 minutes.

7. Stain your gel with ethidium bromide and photograph.

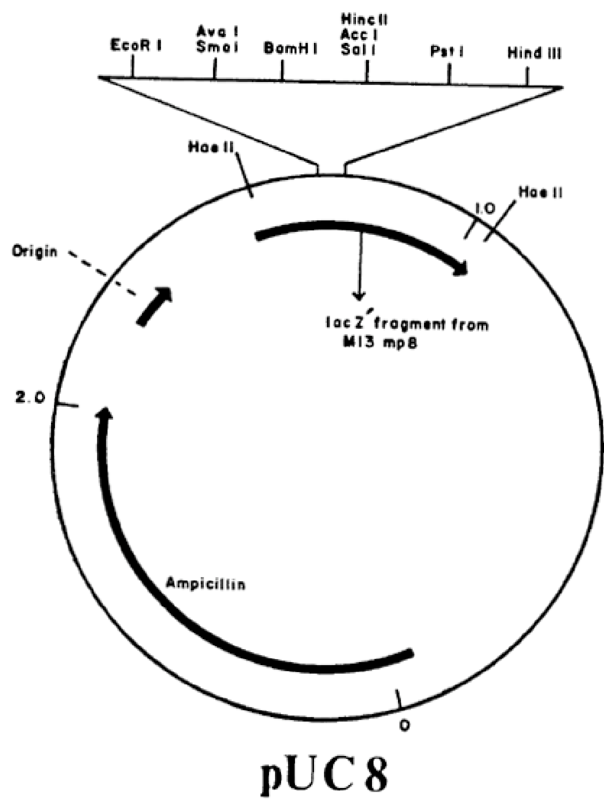
Analysis

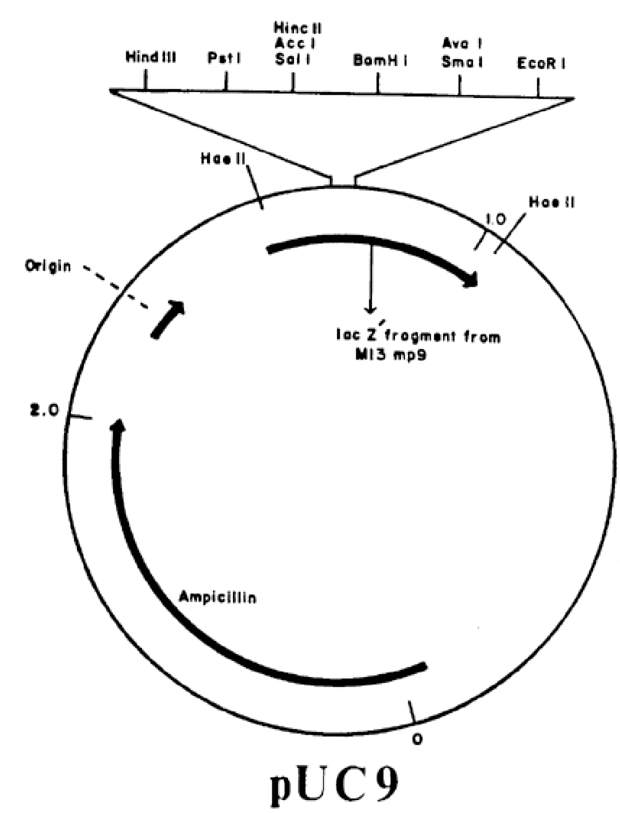
1. Measure the distance that each band migrated from the origin and record the results in a table.

2. Graph the migration of your molecular weight standards (samples 1, 2, 11, & 12) on semi-log paper. Plot log kb versus distance. This will be your standard curve. I will examine your standard curves to make sure that they are plotted correctly.

3. When your standard curve is correct, plot the distances of your cut samples on the curve and determine the size of each fragment. Record the migration distances and molecular weights in a table.

4. Construct a map for pRHR30


EcoR I
Ava I
Sma I
BamH I
Hinc II
Acc I
Sal I
Pst I
Hind III
Hae II
Hae II
1.0
Origin
lac Z′ fragment from
M13 mp8
2.0
Ampicillin
0
pUC8


Hind III
Pst I
Hinc II
Acc I
Sal I
BamH I
Ava I
Sma I
EcoR I
Hae II
Hae II
1.0
Origin
lac Z′ fragment from
M13 mp9
2.0
Ampicillin
0
pUC9

LIST OF M13mp VECTORS

M13mp1

	1	2	3	4	5	6
	THR	MET	ILE	THR	ASP	SER
ATG	ACC	ATG	ATT	ACG	GAT	TCA

M13mp2

	1	2	3	4	5	6
	THR	MET	ILE	THR	ASN	SER
ATG	ACC	ATG	ATT	ACG	AAT	TCA

Sites: *EcoR* I

M13mp7/pUC7

	1	2	3	4	5	(1	2	3	4	5	6	7	8	9	10	11	12	13	14)	6
	THR	MET	ILE	THR	ASN	ser	pro	asp	pro	ser	thr	cys	arg	ser	thr	asp	pro	gly	asn	SER
ATG	ACC	ATG	ATT	ACG	AAT	TCC	CCG	GAT	CCG	TCG	ACC	TGC	AGG	TCG	ACG	GAT	CCG	GGG	AAT	TCA

Sites: *EcoR* I; *BamH* I; *Sal* I / *Acc* I / *Hinc* II; *Pst* I; *Sal* I / *Acc* I / *Hinc* II; *BamH* I; *EcoR* I

M13mp8/pUC8

	1	2	3	4	5	6	(1	2	3	4	5	6	7	8	9	10	11)	7	8
	THR	MET	ILE	THR	ASN	SER	arg	gly	ser	val	asp	leu	gln	pro	ser	leu	ala	LEU	ALA
ATG	ACC	ATG	ATT	ACG	AAT	TCC	CGG	GGA	TCC	GTC	GAC	CTG	CAG	CCA	AGC	TTG	GCA	CTG	GCC

Sites: *EcoR* I; *Sma* I / *Xma* I; *BamH* I; *Sal* I / *Acc* I / *Hinc* II; *Pst* I; *Hind* III

M13mp9/pUC9

	1	2	3	4	(1	2	3	4	5	6	7	8	9	10	11)	5	6	7	8
	THR	MET	ILE	THR	pro	ser	leu	ala	ala	gly	arg	arg	ile	pro	gly	ASN	SER	LEU	ALA
ATG	ACC	ATG	ATT	ACG	CCA	AGC	TTG	GCT	GCA	GGT	CGA	CGG	ATC	CCC	GGG	AAT	TCA	CTG	GCC

Sites: *Hind* III; *Pst* I; *Sal* I / *Acc* I / *Hinc* II; *BamH* I; *Sma* I / *Xma* I; *EcoR* I

M13mp10/pUC12

1	2	3	4	5	6	(1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16)	7	8
THR	MET	ILE	THR	ASN	SER	ser	ser	pro	gly	asp	pro	leu	glu	ser	thr	cys	ser	pro	ser	leu	ala	LEU	ALA
ACC	ATG	ATT	ACG	AAT	TCG	AGC	TCG	CCC	GGG	GAT	CCT	CTA	GAG	TCG	ACC	TGC	AGC	CCA	AGC	TTG	GCA	CTG	GCC

Sites: *EcoR* I; *Sst* I; *Sma* I / *Xma* I; *BamH* I; *Xba* I; *Sal* I / *Acc* I / *Hinc* II; *Pst* I; *Hind* III

M13mp11/pUC13

1	2	3	4	(1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16)	5	6	7	8
THR	MET	ILE	THR	pro	ser	leu	gly	cys	arg	ser	thr	leu	glu	asp	pro	arg	ala	ser	ser	ASN	SER	LEU	ALA
ACC	ATG	ATT	ACG	CCA	AGC	TTG	GGC	TGC	AGG	TCG	ACT	CTA	GAG	GAT	CCC	CGG	GCG	AGC	TCG	AAT	TCA	CTG	GCC

Sites: *Hind* III; *Pst* I; *Sal* I / *Acc* I / *Hinc* II; *Xba* I; *BamH* I; *Sma* I / *Xma* I; *Sst* I; *EcoR* I

M13mp18/pUC18

	1	2	3	4	5	6	(1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18)	7	8
	THR	MET	ILE	THR	ASN	SER	ser	ser	val	pro	gly	asp	pro	leu	glu	ser	thr	cys	arg	his	ala	ser	leu	ala	LEU	ALA
ATG	ACC	ATG	ATT	ACG	AAT	TCG	AGC	TCG	GTA	CCC	GGG	GAT	CCT	CTA	GAG	TCG	ACC	TGC	AGG	CAT	GCA	AGC	TTG	GCA	CTG	GCC

Sites: *EcoR* I; *Sst* I; *Kpn* I; *Sma* I / *Xma* I; *BamH* I; *Xba* I; *Sal* I / *Acc* I / *Hinc* II; *Pst* I; *Sph* I; *Hind* III

M13mp19/pUC19

	1	2	3	4	(1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18)	5	6	7	8
	THR	MET	ILE	THR	pro	ser	leu	his	ala	cys	arg	ser	thr	leu	glu	asp	pro	arg	val	pro	ser	ser	ASN	SER	LEU	ALA
ATG	ACC	ATG	ATT	ACG	CCA	AGC	TTG	CAT	GCC	TGC	AGG	TCG	ACT	CTA	GAG	GAT	CCC	CGG	GTA	CCG	AGC	TCG	AAT	TCA	CTG	GCC

Sites: *Hind* III; *Sph* I; *Pst* I; *Sal* I / *Acc* I / *Hinc* II; *Xba* I; *BamH* I; *Sma* I / *Xma* I; *Kpn* I; *Sst* I; EcoR I