Restriction analysis of Plasmid DNA

In this exercise, you will digest the plasmid pBR322 with 5 different restriction enzymes and resolve the fragments by agarose gel electrophoresis. pBR322 is a small cloning vector with several unique restriction sites; it forms the backbone of many larger, more sophisticated plasmids in use today.

Procedure

1. Set up the following 6 restriction digests in 1.5 mL microcentrifuge (eppendorf) tubes A-F. All reagents and tubes should be stored on ice.

The reactions should be set up as follows: First, add water to each tube (you may use the same tip); next, add 2x buffer; then DNA. Always add enzymes last.

All volumes are in **microliters (μL).**

Tube	DNA	2X restriction buffer	BglII	DraI	EcoRI	HaeIII	HindIII	H2O (distilled)
A	1	5	1	--	--	--	--	3
B	1	5	--	1	--	--	--	3
C	1	5	--	--	1	--	--	3
D	1	5	--	--	--	1	--	3
E	1	5	--	--	--	--	1	3
F	1	5	--	--	--	--	--	4

Total reaction volume: 10 μL
Note DNA concentration.

2. Tightly close the tubes and mix reactants by tapping. Spin for 1-2 seconds in microcentrifuge to mix & collect at the bottom.

3. Incubate in 37^{o}C water bath for 35-45 minutes. Make sure lids are tightly closed to prevent evaporation (a problem with such tiny volumes).

4. Prepare 1% agarose gel.

5. Remove the tubes from water bath and spin 1-2 seconds to collect condensate. Add loading dye to each tube (if 10X loading dye, 1 μL; if 6X loading dye, 2 μL). Be careful to change tips each time!

6. Tap to mix & spin.

7. Load entire contents of each sample in wells from left to right, A-F.

8. Obtain a DNA size marker/standard (e.g., λHindIII). Load appropriate amount in next well. (Instructor will tell you.)

9. Electrophorese at 100-150V until the bromophenol blue has migrated to within 3 cm of the end of the gel. Make sure wells are nearest to the cathode (black, negative).

10. Stain, view, and photograph gel.

pBR322

4,361 base pairs
GenBank Accession #: J01749
See page 141 for ordering information.

pBR322 is an *E. coli* plasmid cloning vector containing the origin of replication from pMB1 (a plasmid in the ColE1 compatibility group; 1–3). The *rop* gene product, which regulates plasmid replication by stabilizing the interaction between RNAI and RNAII transcripts, maintains the copy number at about 20 per cell. However, pBR322 can be amplified with chloramphenicol or spectinomycin (4).

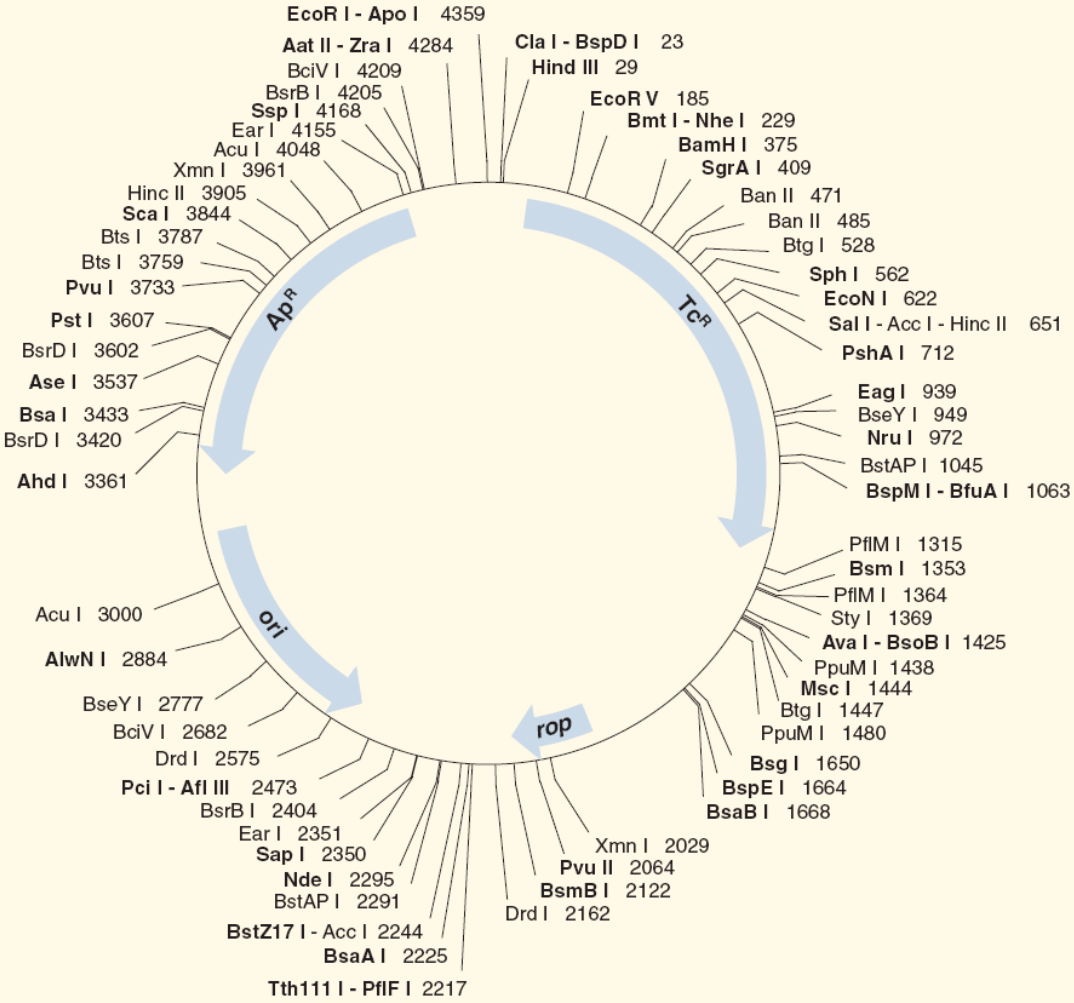
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. The accompanying table shows restriction sites of those enzymes that cut a moderate number of times. Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
tet (Tc^R)	86-1276	pSC101
bla (Ap^R)	4153-3293	*Tn3*
rop	1915-2106	pMB1
origin	3122-2534	pMB1

ori = origin of replication
Ap = ampicillin, Tc = tetracycline



References

1. Bolivar, F. et al. (1977) *Gene* 2, 95–113.
2. Sutcliffe, J.G. (1979) *Cold Spring Harb. Symp. Quant. Biol.* 43, 77–90.
3. Watson, N. (1988) *Gene* 70, 399–403.
4. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Map of pBR322, courtesy of neb.com (New England Biolabs)
Important features: ampicillin & tetracycline resistance genes; origin of replication

For your restriction digestion experiment:

The enzymes you used cut pBR322 at the following **locations**:

Bgl II	no sites
DraI	3230, 3249, 3941
EcoRI	4359
HindIII	29
HaeIII	more than 6 cut sites

In your lab notebook, be sure to include the following elements:

1. Draw a simple pBR322 restriction map showing the sites for enzymes used, in relative locations;
2. Predict the expected number of linear DNA bands following complete digestion with each enzyme;
3. Predict the expected sizes of those bands and use the size marker to help identify them on your gel;
4. Explain what you see when pBR322 is digested with BglII;
5. Explain why HaeIII is expected to have many more restriction sites in pBR322 than the other enzymes;
6. Comment on any unexpected bands in your gel.