Restriction Mapping of a pAK Simple Recombinant

1. Use miniprep plasmid DNA from pAK you made in labs 7-10. Choose either M1 or M2. Add 10 µl water to the miniprep so you have enough volume.

2. Set up the following restriction digests:

Tube	DNA	5X RB/RNase	EcoRI	BamHI	HindIII	ddH_2O
1	5 µl	2 µl	1 µl	---	---	2 µl
2	5 µl	2 µl	1 µl	1 µl	---	1 µl
3	5 µl	2 µl	1 µl	---	1 µl	1 µl
4	5 µl	2 µl	----	1 µl	1 µl	1 µl
5	5 µl	2 µl	1 µl	1 µl	1 µl	---

3. Incubate the tubes at 37 ^{o}C for at least 45 min.

4. Pour a 1.2 % agarose gel.

5. Following digestion add 2 µl of 6X loading dye to each tube.

6. Load each sample onto the gel along with 1 kb ladder size marker.

7. Run your gel at about 100 V; stain, wash, view, photograph.

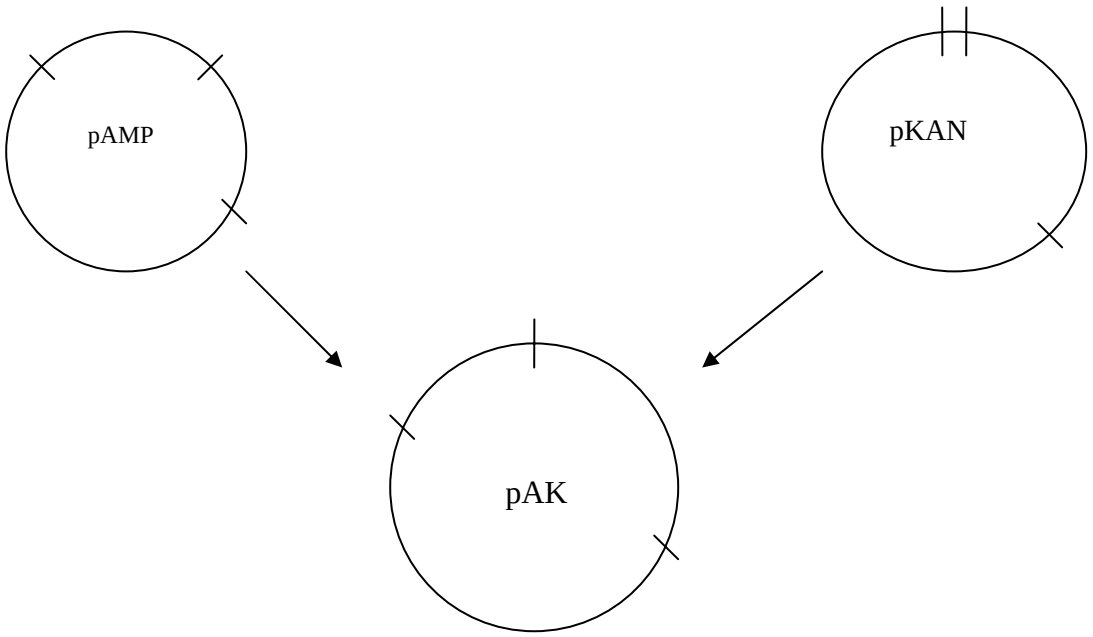
8. Determine the sizes of the fragments in each lane. Properly label all bands in the photo in your lab notebook.

9. Compare your results to the expected results (see next page).

Predicted results of pAK restriction mapping experiment

Refer to plasmid maps pAMP & pKAN at the end of your textbook.

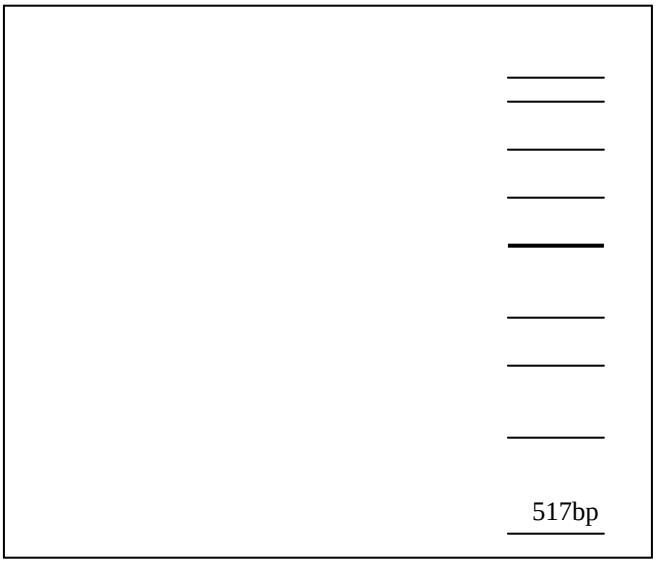
1. Label cut sites (BamHI, EcoRI, HindIII) in the plasmid maps below (include "addresses"):



2. For pAK only, list predicted fragment sizes for the digests listed by the gel below.

3. Draw correct bands on the gel.

1. EcoRI only
2. EcoRI + BamHI
3. EcoRI + HindIII
4. BamHI + HindIII
5. Eco+Bam+Hind



(approximate illustration)