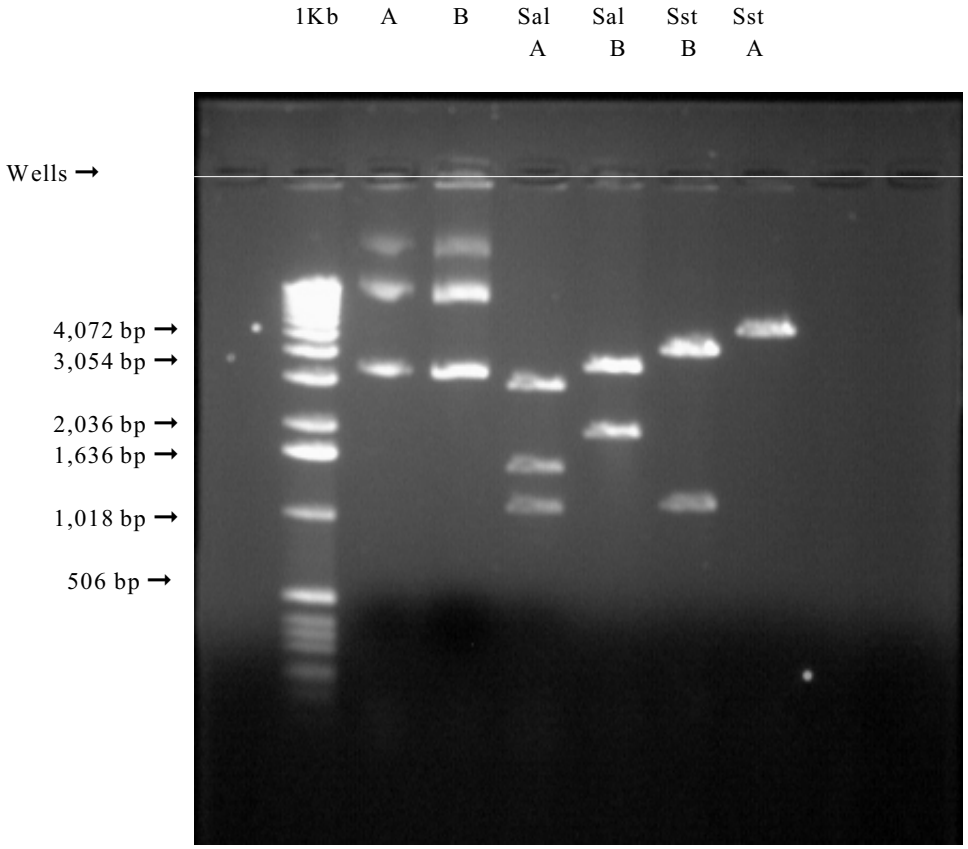
Analysis of SalI and SST Restriction Digests

Use the information provided below can be used to create a standard curve. From this curve you can and determine the size of the restriction fragments for the plasmid you are studying.

Figure 1. Digests of unknown plasmids A and B using SalI and SST. The source of the bands is indicated by the headings at the top of the gel. A or B indicates no restriction enzyme was used.



1kb ladder - fragment sizes	Distance migrated (mm)
1,018 bp	_____
1,636 bp	_____
2,036 bp	_____
3,054 bp	_____
4,072 bp	_____

Plasmid_____

Distance migrated (mm): SalI _____ SST _____

_____ _____

_____

You are now ready to graph the data and determine the size of the unknown fragments.

Using Minitab to create a standard curve to relate the distance DNA migrates to fragment size.

A standard curve can be created using a modification of the regression analysis with a fitted line plot.

Enter the data for the 1 Kb ladder

C1 = # base pairs for each fragment
C2 = distance migrated by fragments

Stat ➤Regression ➤Fitted line plot ➤Y (C1) ➤ X (C2) ➤Options (✓ $\log_{10}$Y, ✓ display logscale for Y)

Print the Graph - then by hand

- Indicate where the unknown fragments would be on the regression line
- Make a figure caption that provides enough information that the figure stands alone.

Note: the $\log_{10}$ base pairs for the unknown fragments can be calculated from the above regression equation.

C3 = distance migrated for unknown Sal and SST fragments
C4 -Use the *Calc menu* and the regression equation calculated above to determine the $\log_{10}$ base pairs for the unknown fragments in C3.
C5 - Use the *Calc menu* to convert from $\log_{10}$ base pairs to base pairs by taking the antilog of the data in C4.

Ask for help if you have questions about this analysis.

Record your results below:

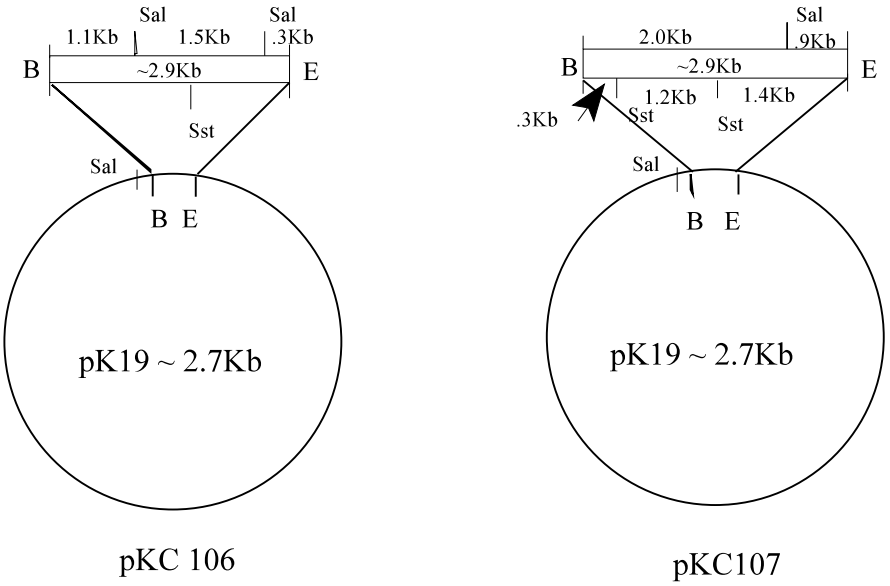
Plasmid_____

Fragment size: SalI _____ SST _____
_____ _____
_____

Plasmid Maps

You are now ready to see if the fragments isolated in the gel are the size you would have expected from Sal and SST digests of pKC106 and pKC107. The plasmid you are working with this week harbors a DNA insert from *Rhodobacter sphaeroides* (Figure 1). By determining the size of fragments you would expect from each restriction digest, you will be able to determine which plasmid corresponds to your unknown. Solving this puzzle, you will come to appreciate the utility of restriction enzyme analysis in mapping DNA.

In the chart below indicate the expected fragment sizes from SalI and Sst digests.



pKC 106 pKC107

Figure 2 Restriction maps of pKC106 and pKC107. In the above diagram the ring represents the plasmid. The line above the plasmid is the2.9 Kb *R. sphaeroides* insert. The unpaired ends of the *R. sphaeroides* DNA insert into the pK19 plasmid following the rules of base pairing. The BamHI (B) end inserts at the BamHI site on the plasmid and the EcoRI end inserts at the EcoRI (E) site on the plasmid. Restriction sites for SalI and Sst are also shown. The SalI site in the ring is very close to the EcoRI site and its distance cannot be detected using electrophoresis . The lengths of the pieces are given in kilobase pairs (Kb).

pKC106

SalI _____

_____

_____ Sst _____

pKC107

SalI _____

_____

Sst _____

_____

Based on your analysis of the gel and the expected fragment sizes which plasmid did you have?