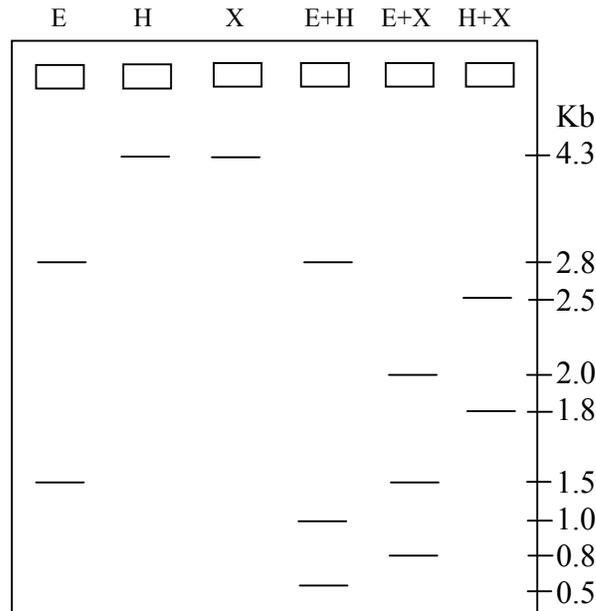
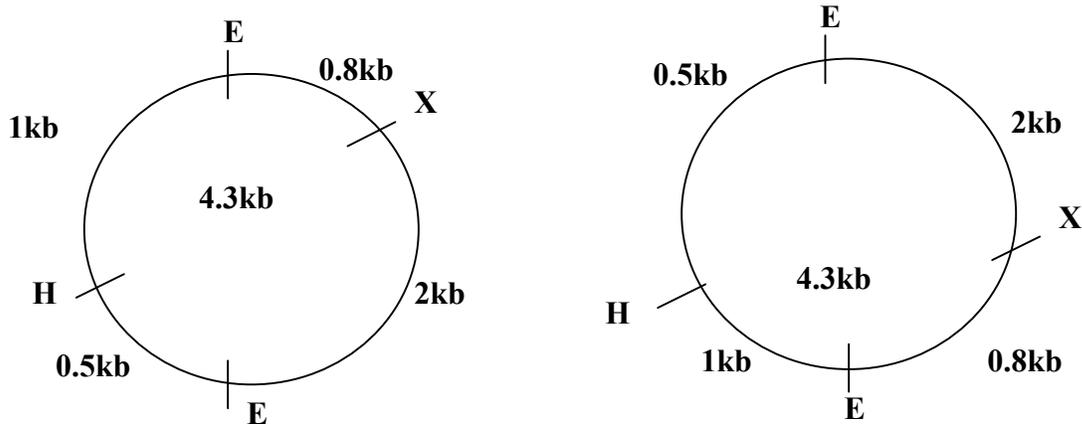


**SOLUTION KEY- SECTION 9**

1. Three restriction enzymes have recognition sites in a plasmid: EcoRI ("E"), HindIII ("H"), and XbaI ("X"). You digest the plasmid with each of the following combinations of enzymes and see the following gel.



i. Draw a map of the plasmid indicating where each restriction enzyme cut site is, which restriction enzyme cuts at each site, and how far apart each cut site is.



2. What basic features should this plasmid have to serve as a vector?

*To clone a gene in a plasmid, the plasmid should have an origin of replication, a site that can serve as the recognition sequence for restriction enzyme so that the plasmid can be cut open and used as a vector to clone the desired sequence and a reporter gene (i.e. antibiotic resistant gene) that can be used to differentiate between the untransformed host cells and the host cells that have obtained the plasmid. (Note: If you also want to express the gene you will need an appropriate promoter close to the 5' end of the cloned gene so that the gene will be expressed and a transcription termination sequence close to 3' end of the cloned gene to terminate transcription).*

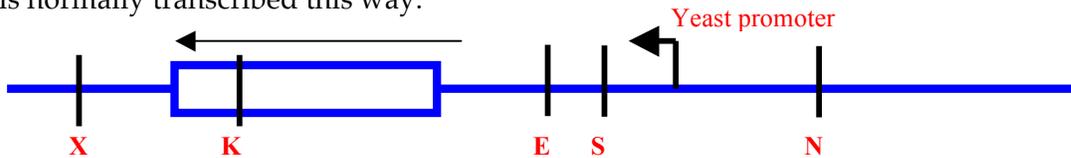
3. Outline the basic steps involved in cloning a gene.

*You cut the DNA fragment that you plan to clone by using a specific restriction enzyme. You also select an appropriate plasmid that has been cut either by the same restriction enzyme or another enzyme that generates complementary ends. You ligate the digested DNA fragments together by using a ligase enzyme. Once you have the recombinant plasmid with the DNA sequence of interest, you transform the plasmid into the bacterial cell (i.e. change the growth conditions to encourage the bacterial cells to take up recombinant plasmid). You then grow the*

transformed bacteria on plates that contain specific antibiotic to which the genes on the plasmid confers resistance. Any cell that took up the plasmid will grow on medium containing this antibiotic and those that did not take in the plasmid will die in the medium. (Note; you may want to look at <http://www.youtube.com/watch?v=acKWdNj936o&feature=related>)

4. You want to insert a specific yeast gene into a specific bacterial plasmid such that the yeast gene will be transcribed in the bacterial cell. Below is a restriction map of a portion of yeast chromosome that contains the yeast gene in which you are interested. The box indicates the open reading frame of this gene.

Gene is normally transcribed this way:

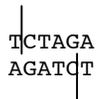


Below are the enzymes you can use, with their specific cut sites shown as

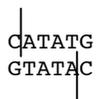
5' -XXXXXX-3'

3' -XXXXXX-5'

Xba I:



Nde I:



Sal I:



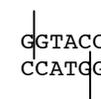
EcoR I:



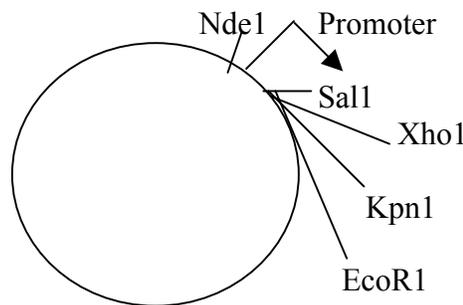
Xho I:



Kpn I:



Below is the map of the plasmid



a) Your task is to design a strategy to insert the yeast gene into the bacterial plasmid. With which one set of enzymes would you choose to cut the yeast genomic DNA and the plasmid, out of the following choices? **Explain** why you selected that pair.

- (i) NdeI & XhoI    (ii) SalI & KpnI    (iii) SalI & XhoI    (iv) XhoI & EcoRI

Choice (i): Since you want to transcribe and express the yeast gene in the bacterial cells, you will need a bacterial promoter (as shown on the plasmid). However if you use the restriction enzyme NdeI and XhoI pair you will end up deleting this promoter (which you don't want to do). So this is not a good choice.

Choice (ii): You will not use this pair since kpnI restriction enzyme cuts within/ disrupts the open reading frame of the yeast gene which you want to insert in the plasmid and express in the bacterial cells.

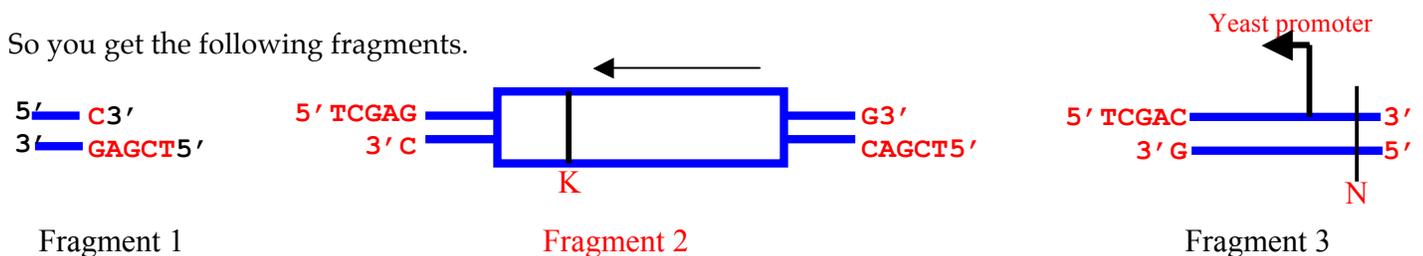
Choice (iii): This is a good choice for the reasons below.

If you cut the yeast genomic DNA with XhoI and SalI restriction enzyme you will have the following fragments.

b) If you did the digestion and ligation with the two enzymes you chose above, in how many ways could the insert be inserted into the vector?

You cut this portion of yeast genomic DNA with the XhoI and SalI that have the following restriction enzyme sites:

So you get the following fragments.

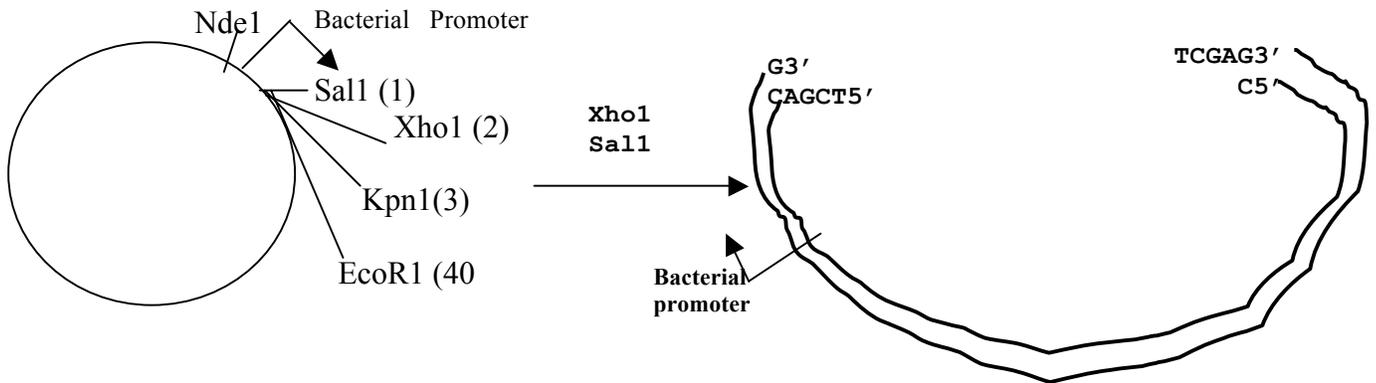


Fragment 1

Fragment 2

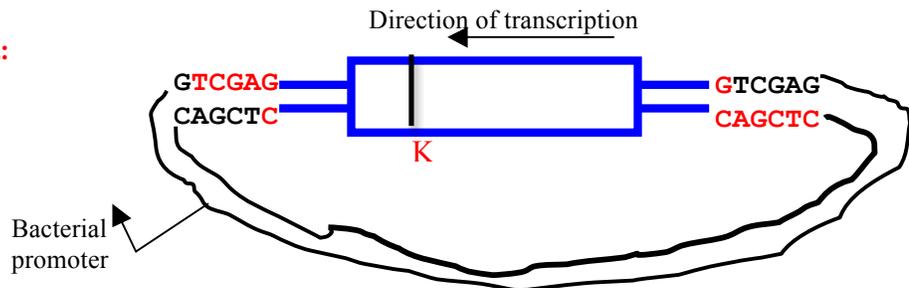
Fragment 3

Now you cut your plasmid with the same restriction enzyme pair (Sal1 and Xho1) to get the following ends as shown below



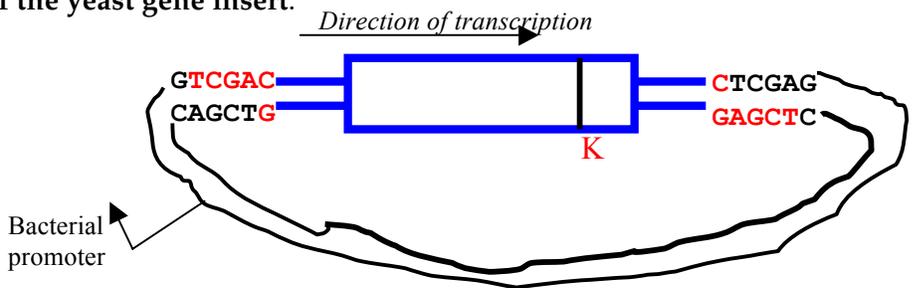
**Fragment 2** can insert in the **Xho1 and Sal1 digested plasmid vector** in two different orientations as shown below:

**1st orientation of fragment 2:**



Please look at the direction of transcription and the position of orientation. This will help you figure out that this is the **reverse orientation of the yeast gene insert**.

**2nd orientation of fragment 2:**



Please look at the direction of transcription and the position of orientation. This will help you figure out that this is the **correct orientation of the yeast gene insert** and this orientation it can be transcribed.

c) If the insert is inserted backwards, what would the DNA sequences be at the two sites where ligation happened?

*It is the recombinant plasmid with the insert that is shown in the first orientation.*

d) Could the above sequence be cleaved by any of the 5 enzymes listed above? *No*

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