Solution key - 7.012 Recitation 9 - 2010

Questions:

1. For each part, state how many bands you would see on a gel in a lane in which you loaded:

i. A circular piece of DNA that was cleaved with a restriction enzyme that cuts at one site on the circle. *This will give one band on a gel.*

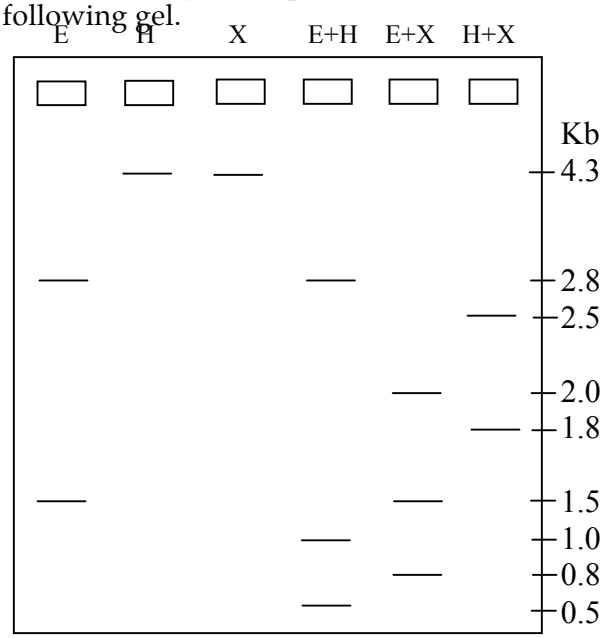
ii. A circular piece of DNA that was cleaved with a restriction enzyme that cuts at two sites on the circle those are equidistant from each other.

This will generate two bands, which are of equal size. Therefore they will migrate at the same rate and will be viewed as one single band on the DNA gel. However the intensity of this band will be much higher compared to the intensity you would expect for a single band.

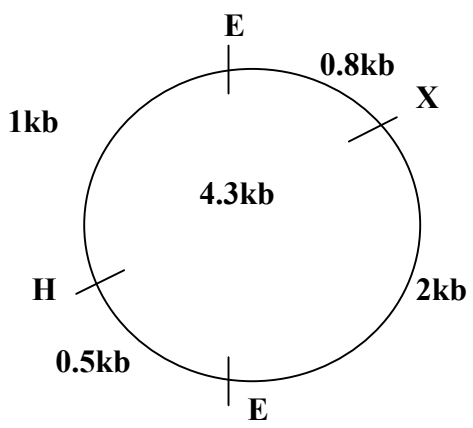
iii. A linear piece of DNA that was cleaved with a restriction enzyme that cuts at one site on the molecule.

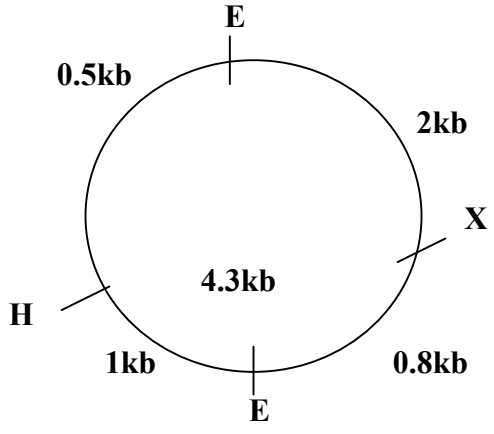
This will generate two bands. If these bands are of different sizes they will migrate differently and appear as two bands on gel. If however they are of the same size they will appear as one band, of higher intensity on a gel.

2. 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.



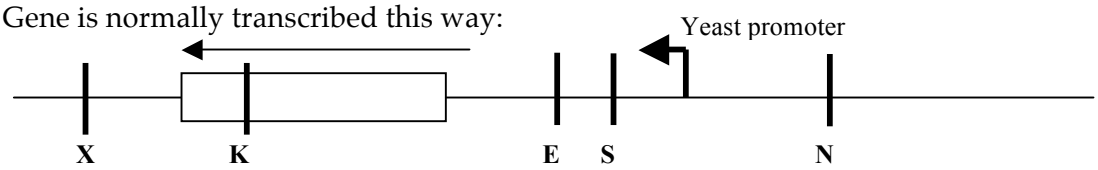


ii) What basic features should this plasmid have to serve as a vector?
To clone a gene in a plasmid, the plasmid should have an origin of replication, a site that can serve as the recognition sequence for restriction enzyme so that the plasmid can be cut open and used as a vector to clone the desired 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).

iii) 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 complemenatry 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

3. 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.

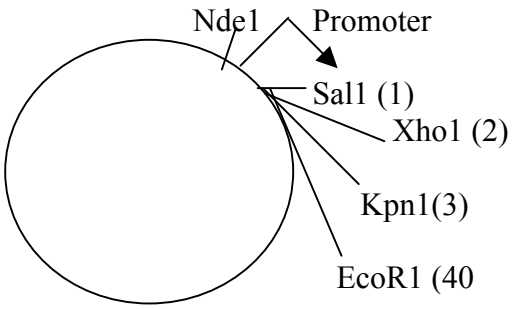
Gene is normally transcribed this way:



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:
T|CTAGA	C|ATATG	G|TCGAC	G|AATTC	C|TCGAG	G|GTACC
AGATC|T	GTATA|C	CAGC|TG	CTTA|AG	GAG|CTC	CCATG|G

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 and XhoI
ii. SalI and KpnI
iii. SalI and XhoI

iv. XhoI and EcoRI

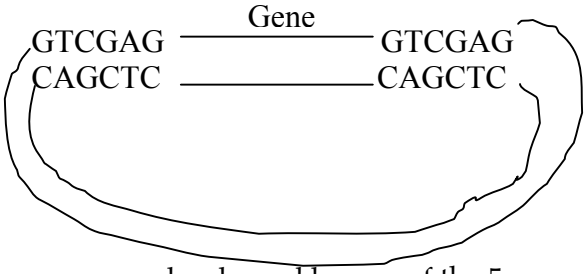
Cutting with this restriction enzyme pair will generate complementary ends and allow the insert to be ligated in the correct orientation.

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?

It can insert in two different ways;

5′TCGAC——————C3′ or 5′TCGAG——————C3′
3′G——————GAGCT5′ 3′C——————GAGCT5′

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



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