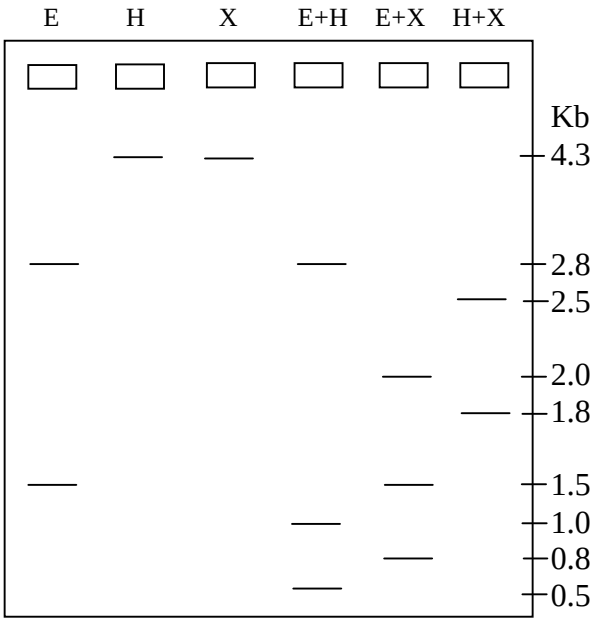
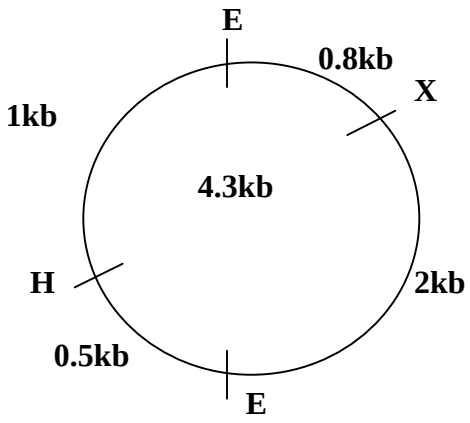
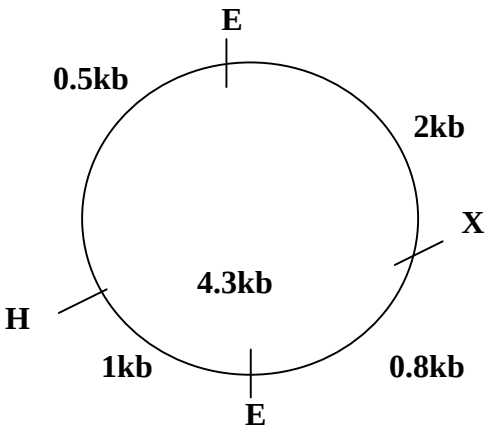
The key: 7.013 Recitation 8 – Spring 2018

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.



a) 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.

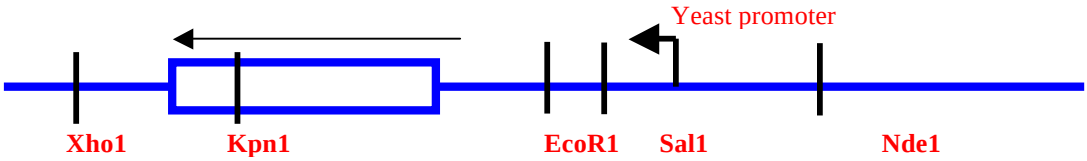




b) What basic features should this plasmid have to serve as a vector for cloning and expressing a cDNA copy of a human gene in bacteria?

*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 a species specific promoter prior to the transcription start site of the gene).*

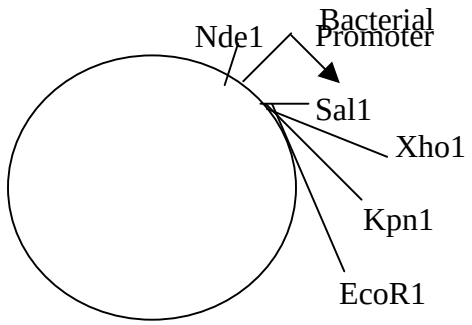
2. You want to insert a specific yeast gene (Gene A) into a specific bacterial plasmid such that the yeast gene will be transcribed in the bacterial cell. Below is a restriction map of a portion of yeast chromosome that contains Gene A in which you are interested. The box indicates the **open reading frame** of this gene. Gene is normally transcribed this way as shown by the arrow:



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

***Nde* I:**	***Sal* I:**	***EcoR* I:**	***Xho* I:**	***Kpn* I:**
C/ATATG	G/TCGAC	G/AATTC	C/TCGAG	G/GTACC
GTATA/C	CAGCT/G	CTTAA/G	GAGCT/C	CCATG/G

On the right is the restriction map of the plasmid.



a) Your task is to design a strategy to insert the yeast gene into the bacterial plasmid. Which of the following pair of enzymes would you choose to cut the yeast genomic DNA and the plasmid.

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 Nde1 and Xho 1 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 kpn1 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 Xho1 and Sal1 restriction enzyme you will have the following fragments.
Choice (iv): This is not a good choice since the gene will be inserted in an orientation that is opposite to the orientation of the promoter.

3. You plan to clone a PKX fusion gene. You start by fusing the DNA encoding GFP (green fluorescent protein) to the DNA encoding the C terminus of PKX gene. The following is the partial **cDNA sequence** encoding the C terminus of the PKX gene. The sequence encoding the stop codon is shown in bold. The bars above the sequence show restriction enzyme recognition sites.

```
          Z                    Y
5′-TCAAGAGGATCCCCGCGGTACCGAATTCCATGTTA TAG CAAGCTCGGAATTAACCCTCAC-3′
3′-AGTTCTCCTAGGGGCGCCATGGCTTAAGGTACAAT ATC GTTCGAGCCTTAATTGGGAGTG-5′
```

The following is the partial cDNA sequence encoding the N terminus of GFP. The codons are underlined. The bars above the sequence show the restriction enzyme recognition sites.

```
                Z          Y
5′-TCTAGAGGTACCGGGATCCGAATTCCC GTG CCA AGC GGC-3′
3′-AGATCTCCATGGCCCTAGGCTTAAGGG CAC GGT TCG CCG-5′
```

The recognition sequences and the cleavage sites (indicated by /) for each enzyme are given below.

Enzyme Z	*Enzyme Y*
5′ G/GATC C 3′	5′ G/AATT C 3′
3′ C CTAG/G 5′	3′ C TTAA/G 5′

Choose the restriction enzyme that you will use to cut the two genes before ligating them to make a fusion gene. **Explain** why you chose this restriction enzyme.

You will use restriction enzyme Z since it keeps the codons of the fusion gene in frame.

5′-TCAAGAGGATCCGAATTCCCGTGCCAAGCGGC-3′
3′-AGTTCTCCTAGGCTTAAGGGCACGGTTCGCCG-5′

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