7.013 Recitation 9 -2013

Summary of lectures 14 & 15:

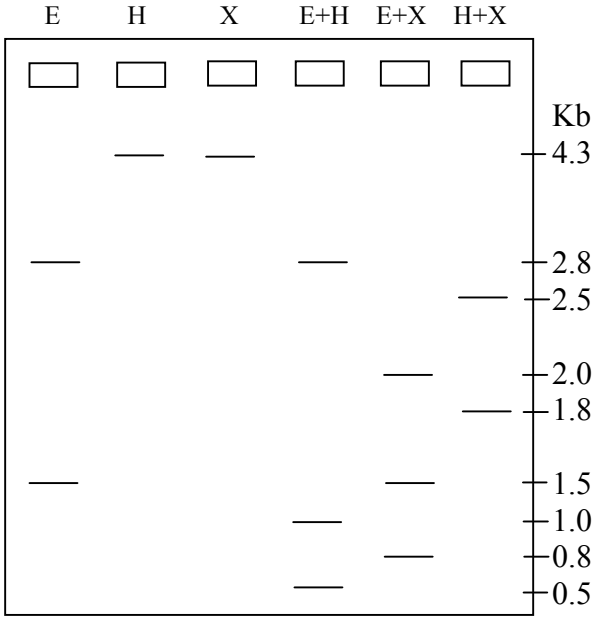
Cloning: Recombinant DNA is a DNA that contains fragments from two different organisms. The common reagents necessary for recombinant DNA technology are restriction enzymes, DNA ligases and vectors. **Restriction enzymes** occur naturally in bacteria where they serve as a form of immune system and have been co-opted by scientists for use in molecular biology. Restriction enzymes cut the DNA by recognizing and binding to a specific restriction sites. **DNA ligase** is used by the cell during DNA replication to join the fragments that get replicated on the lagging strand. They can also be used to join the pieces of DNA that have been cut by the same restriction enzymes. Vectors are the pieces of DNA that possess the qualities that allow any piece of DNA to be taken up by the cell and get replicated using the cell replication machinery.

The most common vector is a **plasmid**, which is a small circular double stranded DNA molecule. Plasmids used as vectors to carry DNA into bacterial cell should have the following features: a recognition sequence for a restriction enzyme such that you can cut the plasmid open with these enzymes and insert a piece of DNA into it, an origin of replication so that the plasmid can replicate once it is within the bacterial cell and a reporter gene such as an antibiotic resistant gene that allows you to select the bacterial cells that took up the plasmid. 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.

DNA gels: Gels are slabs of materials such as agarose and acrylamide that form gelatinous matrices when polymerized. DNA, RNA, and proteins can be inserted into such gels, the gels can be immersed in liquid, and then an electrical current can be applied to the gel such that these macromolecules move towards the pole to which they are attracted. Very long macromolecules will move slowly as they attempt to weave their way through the pores in the gel. Smaller macromolecules will move faster. This technique of gel electrophoresis allows macromolecules to be separated by size.

Questions:

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.



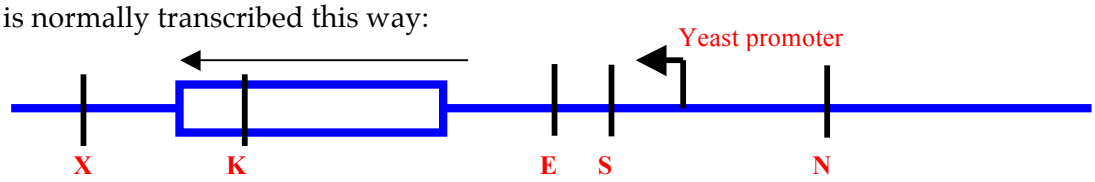
Draw a map of the plasmid indicating where each restriction enzyme cut site is, which restriction enzyme cuts at each site, and how far apart each cut site is.

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

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

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:

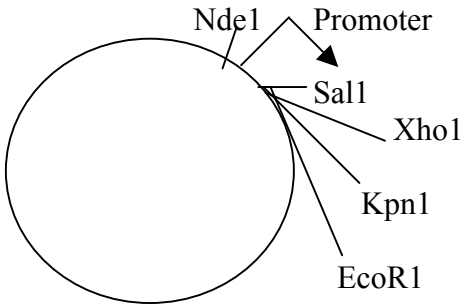


Below are the enzymes you can use, with their specific cut sites shown as

5′-XXXXXX-3′
3′-XXXXXX-5′

Xba I:	*Nde* I:	*Sal* I:	*EcoR* I:	*Xho* I:	*Kpn* I:
T|CTAGA AGATC|T	C|ATATG GTATA|C	G|TCGAC CAGC|TG	G|AATTC CTTAA|G	C|TCGAG GAGC|TC	G|GTACC CCATC|G

Below is the map of the plasmid



i. 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

ii. 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?

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

iv. Could the above sequence be cleaved by any of the 5 enzymes listed above?

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