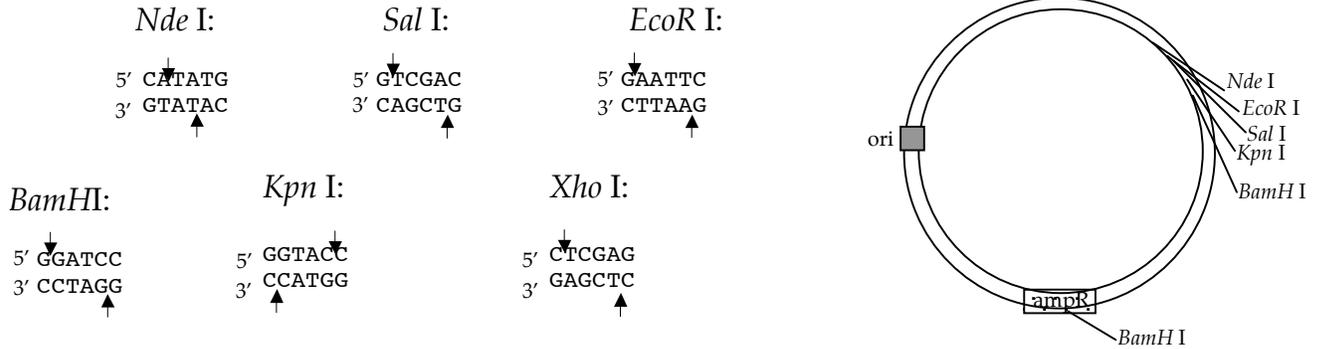


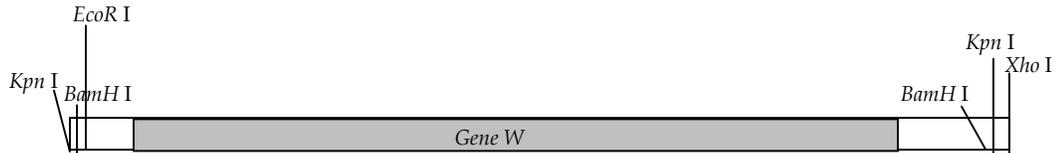
Practice Problems for Recombinant DNA, Session 2: Basic Mechanism of Cloning

Question 1

A schematic of the vector p7012 is shown. The restriction enzymes listed cut only where indicated; they do not cut anywhere else in the vector or insert.



a) A schematic of gene W is below. You want to clone all of gene W DNA into the p7012 vector. Give three different strategies that you could use to clone gene W into p7012, and obtain colonies that contain a recombinant plasmid.



- Strategy 1 uses the restriction enzyme(s) _____ to cut the vector and restriction enzyme(s) _____ to cut Gene W
- Strategy 2 uses the restriction enzyme(s) _____ to cut the vector and restriction enzyme(s) _____ to cut Gene W
- Strategy 3 uses the restriction enzyme(s) _____ to cut the vector and restriction enzyme(s) _____ to cut Gene W

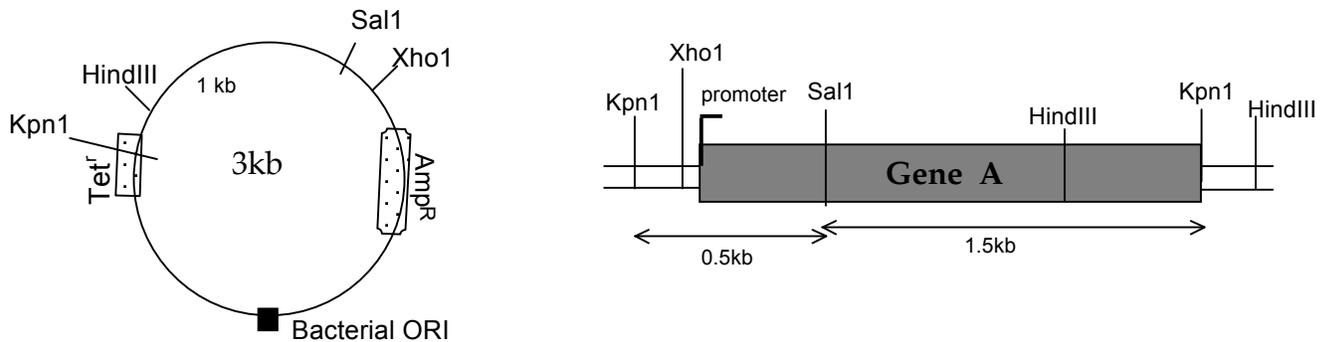
b) In which of the strategies would Gene W be inserted into the vector in only one direction?

Question 2

You purify a protein from a plant cell that can act as a potential appetite suppressant. Owing to its possible commercial application you decide to clone the gene, Gene A, that encodes this protein. You isolate this gene from the plant cell, clone it into a plasmid vector and **amplify** it in the bacterial cells.

a) List three features that a plasmid **must** have to allow the cloning and amplification of Gene A in bacterial cells.

b) You decide to use the following plasmid to clone Gene A. To achieve this you digest both the genomic DNA and plasmid DNA using a restriction enzyme. You then ligate the Gene A DNA into the cut plasmids. Finally, you transform the *E. coli* bacterial cells with the ligation mix (the recombinant plasmids). **Note: The recognition sites for *Kpn* 1 and *Sal*1 on plasmid are 1 kb apart.**



- Which restriction enzyme (*Kpn* I, *Hind* III, *Sal* I or *Xho* I) did you use to digest Gene A for insertion in to the plasmid?
- Which restriction enzyme (*Kpn* I, *Hind* III, *Sal* I or *Xho* I) did you use to digest the plasmid before insertion of Gene A? Briefly explain why.

c) You then plate these transformed bacterial cells onto media that will allow you to distinguish between bacterial cells that obtained the plasmid and those that did not. Onto what type of growth medium will you plate your transformation mix? Explain your answer.

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