Practice Problems for Recombinant DNA, Session 4: cDNA Libraries and Expression Libraries

Question 1
In a hypothetical scenario you wake up one morning to your roommate exclaiming about her sudden hair growth. She has been supplementing her diet with a strange new fungus purchased at the local farmer's market. You take samples of the fungus to your lab and you find that this fungus does indeed make a protein (the harE protein) that stimulates hair growth. You construct a fungal genomic DNA library in *E. Coli* with the hope of cloning the harE gene. If you succeed you will be a billionaire! You obtain DNA from the fungus, digest it with a restriction enzyme, and clone it into a vector.

a) What features must be present on your plasmid that will allow you to use this as a cloning vector to make fungal genomic DNA library.

b) You clone your digested genomic DNA into this vector. The *E. coli* (bacteria) cells that you will transform to create your library will have what phenotype prior to transformation?

c) How do you distinguish bacterial cells that carry a vector from those that do not?

d) How do you distinguish bacterial cells that carry a recombinant vector from those that carry the original cloning vector?

e) You could screen your library by hybridization with a probe.

- What information would you need to do this screen?

- What would you use as a probe?
  You would use a segment of SS DNA or denatured DS DNA complementary to the harE gene.

- Would the entire harE gene need to be present in a recombinant vector for your screen to work? Explain.

f) You screen your library by hybridization with a probe and identify a recombinant vector that contains the complete harE gene. In the mean time, you have developed an antibody to the harE protein. You use cells carrying the recombinant vector that contains the complete harE gene to test how well this antibody reacts with the harE protein. You do the experiment and find that the antibody does not react with the cells containing the recombinant vector. Does this result indicate that your antibody does not react to the harE protein? Explain.

Question 1, continued

g) You make a second library, a cDNA library. You plan to transform bacterial cells with this new library and then screen for a colony whose cells are making the harE protein using your antibody.

- Describe what cDNA is and how it differs from genomic DNA.

- What features must be present on your cloning vector that will allow you to use this to make fungal cDNA library and successfully identify a colony whose cells are making the harE protein?

Question 2

Folic acid (Vitamin B9) is often added to commercially produced breads to increase the nutritional value. You plan to create a strain of yeast that produces vast quantities of folic acid. Thus as the yeast cells leaven the bread, they also fortify the bread. To do this, you need to provide yeast cells with an enzyme, fol1 from the bacteria, Lactobacillus arabinosus.

First you create a genomic library from Lactobacillus arabinosus in E. coli. You then screen this library by hybridization with a probe to identify the cells carrying the desired recombinant vector. Once you have identified cells that carry the recombinant vector, you isolate it and cut the fol1 gene out of this vector. You then clone the fol1 gene into an expression vector that will allow expression of the fol1 protein in yeast cells.

a) The vector that you choose for making the genomic library from *Lactobacillus arabinosus* in *E. coli.* must have certain features for this experiment to work. List only the required features.

b) When you transform *E. coli* cells with the pool of recombinant library vectors, how do you distinguish between E. coli cells that received a vector and those that did not?

You identify cells that carry the desired recombinant vector. You purify recombinant vector DNA, cut it with an appropriate enzyme and isolate the fol1 gene. The sequence the fol1 gene that you obtain is shown below. Please note the promoter for the fol1 gene is not present on this sequence. The start and stop codons are indicated in bold. There are no Xba I, Sac I, Bam HI, Bgl II, or Nde I sites in the 1500 bp that are not shown.

```
      Xba I   Sac I     Bam HI    Bgl II    Nde I                                  Xba I   Sac I   Bam HI   Bgl II    Nde I
5′ GTCTAGAAGAGCTCCTTGGATCCAAAGATCTACCATATGTCTCGCCTA----------~ 1500 bp ----------GTCTAGAAGAGCTCTGGATCCAAGATCTACCATATGTAAGCG
3′ CAGATCTTCTCGAGGAACCTAGGTTTCTAGATGGTATACAGAGCGGAT----------~ 1500 bp ----------CAGATCTTCTCGAGACCTAGGTTCTAGATGGTATACATTCGC
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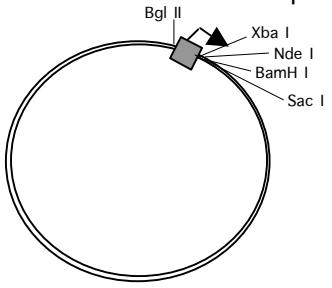
You purchase an appropriate vector for the second part of your experiment, the expression of the fol1 gene in yeast cells. Below is a schematic of the expression vector and included is the DNA sequence of the promoter and cloning region.

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      Bgl II                    Promoter                                                                    Xba I
5′  AGATCTCGATCCCCCGAAATTAATACGACTCACTATACGGGAATTGTCACCGCATAACAATTCCCCTCTAGAAATAATT
3′  TCTAGAGCTAGGGGGCTTTAATTATGCTGAGTGATATGCCCTTAACAGTGGCGTATTGTTAAGGGGAGATCTTTATTAA

                                        Nde I                                                    Bam HI
5′  TTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAA
3′  AACAAATTGAAATTCTTCCTCTATATGTATACCGATGCTACTGACCACCTGTCGTTTACCCAGCGCCTAGGCTT
                                    MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGlu
        Sac I
5′  TCGAGCTCCGTCGAC
3′  AGCTCGAGGCAGCTG
    LeuArgArgGlnAla
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Question 2, continued

c) For the expression vector to be useful, what specific protein (and from what organism) will bind to the promoter?

d) You cut the fol1 gene and the expression vector with the following enzymes and successfully form a recombinant expression plasmid. Assuming that extra amino acids on the amino terminus of the fol1 protein do not affect its function, answer the following questions.

i) If you created your recombinant vector by cutting the fol1 gene and the expression vector with Bam HI,

- would a yeast cell carrying this recombinant vector make an mRNA from the inserted DNA? Explain.

- would a yeast cell carrying this recombinant vector make functional fol1 protein? Explain.

ii) If you created your recombinant vector by cutting the fol1 gene and the expression vector with Sac I,

- would a yeast cell carrying this recombinant vector make an mRNA from the inserted DNA? Explain.

- would a yeast cell carrying this recombinant vector make functional fol1 protein? Explain.

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