Solutions for 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.
Your vector would certainly need to have a unique restriction enzyme site, a selectable marker such as the ampicillin resistance gene, and a bacterial origin of replication. Other features may be required depending upon how you plan to use this 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?
Prior to transformation, the E. coli cells that you will transform will be sensitive to antibiotic. This allows you to select for cells that obtained a plasmid.

c) How do you distinguish bacterial cells that carry a vector from those that do not?
Cells that obtained a vector will have obtained the selectable marker (one example is the ampicillin resistance gene). These cells can now express the selectable marker and will be able to grow in the presence of the antibiotic. Therefore, you can plate all the cells from your transformation on media containing the antibiotic, and those cells that have obtained a vector will grow.

d) How do you distinguish bacterial cells that carry a recombinant vector from those that carry the original cloning vector?
All cells with a vector, whether the original vector or a recombinant vector will have obtained the selectable marker. To distinguish bacterial cells that carry a recombinant vector from those that carry the original cloning vector, you would have to isolate the vector and determine it's size. You could do this by restriction mapping.

e) You could screen your library by hybridization with a probe.
   - What information would you need to do this screen?
     You would have to know some amount of the DNA sequence of the harE gene.

   - What would you use as a probe?
     You would use a segment of SS DNA or denatured DS DNA complementary to the harE gene. This DNA probe would be labeled in some way to allow visualization.

   - Would the entire harE gene need to be present in a recombinant vector for your screen to work? Explain.
     No. This screen would work as long as the part of the hare gene that is complementary to your probe is present.

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.
No. Screening with an antibody requires that the harE gene is expressed into protein. Your library used genomic fungal DNA and is contained in bacterial cells. Because of the difference in promoters and the presence of introns, the bacterial cells cannot express the harE protein. Thus you have no information about the ability of your antibody to bind.

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.
  cDNA is DNA that was made using mRNA as a template. cDNA does not contain any promoter sequences. In addition, because it was made from mRNA, all the introns have been removed.

- 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?
  The cloning vector would need to have a promoter that bacterial RNA polymerase recognizes and uses for transcription.

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.
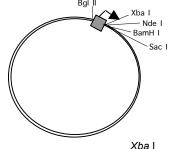
a) The vector that you choose for making the genomic library from *Lactobacillus arabinosus* in *E. coli.* must have certain features for this experiment to work. List only the required features.
A cloning site, a bacterial origin of replication, and a selectable marker (such as the ampicillin resistance gene).

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 would plate on media that contains ampicillin (or other appropriate antibiotic), and any cell that did not receive a plasmid will die.

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.

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    Xba I    Sac I    Bam HI      Bgl II    Nde I                          Xba I     Sac I    Bam HI    Bgl II     Nde I
5' GTCTAGAAGAGCTCCTTGGATCCAAAGATCTACCATATGTCTCGCCTA----------------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' AACAAATTGAAATTCTTCCTCTATATGTATACCGATCGTACTGACCACCTGTCGTTTACCCAGCGCCTAGGCTT
                                   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?
RNA polymerase from yeast would bind to this 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.
  You would expect an mRNA to be produced from the inserted DNA because the DNA inserted after the promoter.

- would a yeast cell carrying this recombinant vector make functional fol1 protein? Explain.
  You would NOT expect normal fol1 protein to be expressed. Although the insertion is after the promoter, and the first AUG of the mRNA encodes the MET of polylinker region. The frame set by this start codon does not correspond to the frame needed to properly translate fol1 inserted at the Bam HI site.

    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.
  You would expect an mRNA to be produced from the inserted DNA because the DNA inserted after the promoter.

- would a yeast cell carrying this recombinant vector make functional fol1 protein? Explain.
  You would expect normal fol1 protein to be expressed. The insertion is after the promoter, and the first AUG of the mRNA encodes the MET of polylinker region. The frame set by this start codon corresponds to the frame needed to properly translate fol1 if the insertion of the fol1 gene is at the Sac I site.

7.01SC Fundamentals of Biology
Fall 2011