Genetic Engineering Module

Challenge: "*E. coli* is often used as a host organism to produce recombinant proteins of interest. How do you genetically engineer bacteria, such as *E. coli*, to produce a desired recombinant protein?"

Generate Ideas: In class have the students write journal entries to answer the following three questions:

1. What are your initial ideas about how this question can be answered?
2. What background knowledge is needed?
3. What do you already know about genetically modifying *E. coli*?

Multiple Perspectives: In the whole-class setting, have students share ideas from their journals. Record their ideas on the board.

Break students up into groups and have each group review the ideas recorded on the board. Ask each group to categorize the ideas into about four categories. Have each group report and explain their categories. Work with the class to set up four main categories of necessary knowledge. If possible, have these four categories focus on:

1. Generation of DNA gene sequence fragments from donor
2. Joining fragments to a host vector
3. Introducing the vector to the bacteria
4. Selecting desired strain of *E. coli*

Research and Revise

Assign students to read specific sections of text book. (Bioprocess Engineering: Basic Concepts, 2nd Ed., by Shuler and Fikret, Prentice Hall, ISBN: 0-13-081908-5; 2002) These readings are intended to help the student review prerequisite material have additional prerequisite exposure to the workings of DNA and how it can be altered.

- Chapter 4: How Cells Work
  - 4.1 Introduction
  - 4.2 The Central Dogma
  - 4.3 DNA Replication
  - 4.4 Transcription
  - 4.5 Translation
- Chapter 8: How Cellular Information is Altered
  - 8.3 Natural Mechanisms for Gene Transfer and Rearrangement
  - 8.4 Genetically engineered cells

The students work through four main topics associated with the four categories outlined in Multiple Perspectives:

1. Genetic Engineering Lecture I: What tools of molecular biology are available for manipulating DNA?

This first lecture is essentially a review of prerequisite material, but focuses on topics directly related to the challenge. Ensure the students fully understand the central dogma of molecular biology. This will set the overall picture for not only this genetic engineering module, but for subsequent modules as well. Provide a review of genetic material and discuss the different tools of the trade used for manipulating DNA. Specifically discuss the cutting, modifying, and joining enzymes used for DNA manipulation.

2. Genetic Engineering Lecture II: How is the gene of interest isolated from donor DNA and how are copies of this gene generated?
   This lecture focuses on the polymerase chain reaction (PCR). Provide the students an overview of why it is important to isolate the gene of interest and generate millions of copies of the fragment of DNA coding for the protein of interest. Ensure the students appreciate the impact PCR has had and continues to have in the biological and health sciences. Discuss the biophysical and biochemical details of the three main steps of PCR: denaturation, annealing, and extension. Ensure the students are familiar with the terminology of PCR. Help the students make connections between the course prerequisite material and the molecular biology and organic chemistry mechanisms of PCR.

3. Genetic Engineering Lecture III: How are the billions of copies of the gene of interest generated by PCR inserted into a useable "context" or format in *E. coli* to propagate the expression of the gene?
   Discuss the concept of a vector and the importance of having a vehicle to insert foreign genes into a host. For this module we are interested in expressing the gene in *E. coli*. Introduce the bacterial plasmid. Discuss the four minimal requirements an expression plasmid must feature to successfully modify *E. coli* and express the gene of interest to produce the protein product. Ensure the students are familiar with the vocabulary of these features and the contributions for genetically modifying *E. coli*. Discuss the basics of promoter systems and how they are used to control and force the expression of the desired recombinant protein. Discuss how the multiple cloning site allows the gene of interest to be inserted into the plasmid. Ensure the students understand the importance in the design of the primers for PCR. The primers must match restriction sites on the polylinker of the plasmid and avoid sequences that exist as restriction sites in the gene of interest.

4. Genetic Engineering Lecture IV: How are bacterial plasmid constructs successfully inserted into *E. coli* and how does one confirm the bacteria have been transformed?
   Discuss the two most common methods for transforming bacteria with plasmid DNA: chemical transformation and electroporation transformation. Ensure the students understand the biophysical mechanisms and the trade offs of these two transformation methods. Discuss how the desired strain of *E. coli* is selected.

Test Your Mettle: An effective way for the students to learn and apply this material is through integrated assignments covering the polymerase chain reaction, gene amplification, and insertion of gene in commercial vector. Have your students do the following activities:

PCR-Mediated Cloning for PixJ1 Protein
Plasmid Construction for Expressing PixJ1

Please see attached assignments.

Go Public: Create an informative and visually appealing booklet style brochure providing a basic technical introduction on the processes of genetically engineering *E. coli* to express a desired protein.

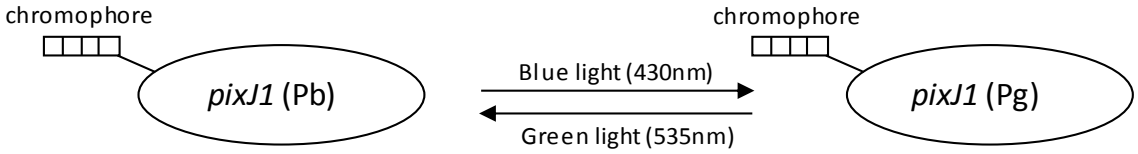
Please see the attached assignment.

Genetic Engineering Module

Test Your Mettle #1

PCR-mediated Cloning

The unicellular motile cyanobacterium *Synechocystis sp.* PCC 6803 (Pasteur Culture Collection) is a photosynthetic marine prokaryote. Investigators found a specific photoreceptive protein called PixJ1 that is responsible for the absorption of blue light and is thought to be involved with the signaling mechanism involving motility of the organism. Further investigations determined that this protein displays photoreversible states between blue and green absorbing forms.



You have been asked to amplify the PixJ1 gene sequence using PCR for subsequent cloning of the gene. Design the oligonucleotide primers (about 20 nucleotides) that you would use to amplify the PixJ1 gene sequence. Be sure to indicate the 5' and 3' ends of your primers. Below is the primary sequence for the PixJ1 gene:

ATGGCAGAGGCTTTTATAGCAGAAAATACCGCCGTGGAGGATGTTTCCCCTAACCCCAACCCCGCCATTGACACCGATGCCCTGGCCGCACTG
ACCCAATCGGCCGTAGAGTTGACCCCGCCGCCCCCGATCAATTTGCCCAAGGTGGAGTTACCCCCCATGCAACCCCTGGCTCCCCTCATGGCGA
TCGCCGACCCGGATAATTTGAGTCCGATGTCTACCTCAATCCAGGCACCTACCCAAAGTGGAGGACTTTCCCTCCGCAATAAAGCTGTGTTGAT
AGCCCTGTTAATTGGTTTGATCCCCGCTGGGGTGATTGGAGGGCTCAATCTCAGCAGTGTGGACAGGCTTCCAGTGCCCCAAACGGAACAACA
GGTCAAGGACTCCACCACTAAGCAAATCCGTGACCAAATTCTGATTGGGCTTTTGGTGACCGCGGTGGGGGCTGCCTTCGTTGCCTACTGGAT
GGTGGGGGAAAATACCAAAGCTCAAACCGCCCTGGCCTTGAAAGCTAAGCATTCCCACCGGAACCTCGACCAACCTCTAGCCGTGGCCGGTG
ATGAACTGGCGATCGCCGACCAAACCATTGATGCTCTCAGTGCCCAGGTGGAGAAATTGCGCCACCAGCAGGATTTATCCCTTAAACAAGCAG
AACTGCTGACCGAACTATCCCGGGCCAACCTTTCCGACATTGACGAAATTCAAGGCGTAATTCAGAAGAACCTCGACCAAGCCAGAGCTTTGT
TTGGTTGTGAGCGACTAGTGTTTTACTACCATCCCCGCTATCAGCCTGAAGCCATGGTAGTGCAAGCTTTGGACTTAACCACTCAAGGTTTAAT
TGACAGCAAAGATCCCCATCCCTGGGGCCAGGAAGATATGCCTTCCCAGATCGTTGCCATCAATGACACCAGCGGTGCCAGTATCAGTAACCC
CCATCGCCAATGGTTAGAGCAACATCAAGTCAAAGCGAGCTTGACCGTACCTCTACACCGGGATAACTACCCCCTCGGCCTGCTCATGGCCCAC
CATTGCCAACGTCCCCACCAGTGGGAAATGAGGGAAAGACAGTTTCTTCAGCAGTTGACCGAAGAACTACAAACCACCCTGGACCGGGCCAA
CCTGATCCAGGAAAGAAATGAAAGCGCCCAGCAAGCCCAAATCCTTAAAGAATTGACTCTGAAAATTTCCGCTGCCATCAACAGCGAGCAGGT
TTTTGACATCGCCGCCCAAGAAATTCGTCTGGCGCTCAAAGCAGACCGGGTCATTGTCTATCGGTTCGATGCCACCTGGGCCGGCACAGTAAT
TGTGGAATCGGTAGCAGAAGGATATCCCAAAGCCTTGGGGGCCACCATTGCCGACCCCTGCTTTGCTGATAGTTATGTAGAAAAATACCGTTC
TGGACGTATCCAAGCCACCCGAGATATTTACAACGCCGGCCTGACCCCCTGCCACATTGGCCAGCTCAAACCCTTTGAAGTCAAAGCCAACCTT
GTCGCCCCCATCAACTACAAAGGCAATCTGCTGGGACTACTCATCGCCCACCAGTGCTCCGGGCCGAGGGACTGGCACCAGAATGAAATTGAT
TTATTTGGCCAATTGACTGTGCAGGTGGGACTAGCTTTAGAACGCTCCGACCTCTTAGCCCAACAGAAAATCGCCGAAGTAGAACAACGACAA
ATGCGTGAAAAAATGCAAAAGCGGGCCTTGGAACTGCTGATGGAGGTAGACCCTGTCAGTCGAGGGGACTTGACCATCCGAGCCCACGTGAC
CGAGGACGAAATTGGCACGATCGCCGACTCCTACAACGCAACTATTGAAAGTCTGCGGCGCATTGTAACCCAAGTACAAACCGCTGCTAGTCA
ATTTACTGAGACCACCGACACCAACGAAGTGGCAGTGCGGCAACTAGCCCAGCAAGCCAATCGCCAGGCCTTGGATGTGGCAGAGGCCCTGG
AGCGGCTTCAGGCCATGAACAAGTCCATTCAAGCAGTGGCAGAAAATGCTGCCCAAGCAGAATCAGCAGTACAACGGGCGACCCAAACGGT
GGACCAAGGGGAAGATGCCATGAACCGCACCGTGGATGGCATTGTCGCAATTCGGGAAACGGTGGCCGCCACCGCCAAACAGGTGAAGCGA
TTGGGGGAATCATCCCAAAAGATCTCCAAAGTGGTGAACCTAATTGGCAGCTTTGCTGACCAAACCAACCTCCTAGCCCTAAATGCTGCCATTG
AAGCCGCCCATGCTGGTGAAGAAGGACGGGGATTTGCGGTGGTAGCCGATGAAGTACGTTCCCTGGCACGGCAATCAGCGGAAGCAACGGC
AGAAATTGCCCAATTGGTGGCGACAATTCAGGCGGAAACGAATGAAGTAGTGAATGCCATGGAAGCGGGCACCGAACAGGTAGTGGTGGGA
ACCAAATTGGTAGAAGAAACCAGGCGGAGCTTGAACCAAATTACGGCGGTGAGTGCCCAGATTAGTGGCTTGGTGGAAGCGATCACCTCTGC
GGCCATTGAGCAGTCCCAAACCAGTGAATCGGTGACCCAGACCATGGCTCTGGTGGCCCAGATTGCGGATAAAAACTCCAGTGAAGCGAGTG
GGGTATCTGCCACCTTTAAGGAACTGTTGGCAGTGGCCCAGTCATTGCAAGAGGCGGTTAAACAGTTCAAAGTGCAGTGA

Genetic Engineering Module
Test Your Mettle #2
Plasmid Construction for Expressing PixJ1

You have been asked to construct a plasmid that expresses the PixJ1 protein using Novagen's pET24a plasmid vector.

1. Design the oligonucleotide primers (about 20 nucleotides plus the restriction site sequences) that you would use to amplify the PixJ1 gene sequence with appropriate flanking restriction sequences for insertion into the multiple cloning site of the pET24a vector. You'll need to do the following:
    a. Choose the appropriate restriction sequences that are included in the MCS and do not cut the PixJ1 gene. (hint: use the NEBcutter tool: http://tools.neb.com/NEBcutter2/index.php)
    b. Ensure that after the PCR fragments of the PixJ1 gene are digested with your chosen restriction enzymes and inserted into the vector that the correct reading frame is maintained for expression. (hint: study the cloning/expression region of the pET-24a vector http://www.emdbiosciences.com/docs/docs/PROT/TB070.pdf)
    c. Indicate the 5' and 3' ends of your primers and clearly indicate the restriction sequence in your primers.

Note that the pET24a plasmid fuses a hexa-histidine tag (6 histidine residues in a row) at the C-terminus of the expressed protein. This is used as handle for protein purification purposes and downstream processing. You will cover this in BIOE 4010.

5'—ATGGCAGAGGCTTTTATAGCAGAAAATACCGCCGTGGAGGATGTTTCCCCTAACCCCAACCCCGCCATTGACACCGATGCCCTGGCCGCA
CTGACCCAATCGGCCGTAGAGTTGACCCCGCCGCCCCCGATCAATTTGCCCAAGGTGGAGTTACCCCCCATGCAACCCCTGGCTCCCCTCATGG
CGATCGCCGACCCGGATAATTTGAGTCCGATGTCTACCTCAATCCAGGCACCTACCCAAAGTGGAGGACTTTCCCTCCGCAATAAAGCTGTGTT
GATAGCCCTGTTAATTGGTTTGATCCCCGCTGGGGTGATTGGAGGGCTCAATCTCAGCAGTGTGGACAGGCTTCCAGTGCCCCAAACGGAACA
ACAGGTCAAGGACTCCACCACTAAGCAAATCCGTGACCAAATTCTGATTGGGCTTTTGGTGACCGCGGTGGGGGCTGCCTTCGTTGCCTACTG
GATGGTGGGGGAAAATACCAAAGCTCAAACCGCCCTGGCCTTGAAAGCTAAGCATTCCCACCGGAACCTCGACCAACCTCTAGCCGTGGCCG
GTGATGAACTGGCGATCGCCGACCAAACCATTGATGCTCTCAGTGCCCAGGTGGAGAAATTGCGCCACCAGCAGGATTTATCCCTTAAACAAG
CAGAACTGCTGACCGAACTATCCCGGGCCAACCTTTCCGACATTGACGAAATTCAAGGCGTAATTCAGAAGAACCTCGACCAAGCCAGAGCTT
TGTTTGGTTGTGAGCGACTAGTGTTTTACTACCATCCCCGCTATCAGCCTGAAGCCATGGTAGTGCAAGCTTTGGACTTAACCACTCAAGGTTT
AATTGACAGCAAAGATCCCCATCCCTGGGGCCAGGAAGATATGCCTTCCCAGATCGTTGCCATCAATGACACCAGCGGTGCCAGTATCAGTAA
CCCCCATCGCCAATGGTTAGAGCAACATCAAGTCAAAGCGAGCTTGACCGTACCTCTACACCGGGATAACTACCCCCTCGGCCTGCTCATGGCC
CACCATTGCCAACGTCCCCACCAGTGGGAAATGAGGGAAAGACAGTTTCTTCAGCAGTTGACCGAAGAACTACAAACCACCCTGGACCGGGC
CAACCTGATCCAGGAAAGAAATGAAAGCGCCCAGCAAGCCCAAATCCTTAAAGAATTGACTCTGAAAATTTCCGCTGCCATCAACAGCGAGCA
GGTTTTTGACATCGCCGCCCAAGAAATTCGTCTGGCGCTCAAAGCAGACCGGGTCATTGTCTATCGGTTCGATGCCACCTGGGCCGGCACAGT
AATTGTGGAATCGGTAGCAGAAGGATATCCCAAAGCCTTGGGGGCCACCATTGCCGACCCCTGCTTTGCTGATAGTTATGTAGAAAAATACCG
TTCTGGACGTATCCAAGCCACCCGAGATATTTACAACGCCGGCCTGACCCCCTGCCACATTGGCCAGCTCAAACCCTTTGAAGTCAAAGCCAAC
CTTGTCGCCCCCATCAACTACAAAGGCAATCTGCTGGGACTACTCATCGCCCACCAGTGCTCCGGGCCGAGGGACTGGCACCAGAATGAAATT
GATTTATTTGGCCAATTGACTGTGCAGGTGGGACTAGCTTTAGAACGCTCCGACCTCTTAGCCCAACAGAAAATCGCCGAAGTAGAACAACGA
CAAATGCGTGAAAAAATGCAAAAGCGGGCCTTGGAACTGCTGATGGAGGTAGACCCTGTCAGTCGAGGGGACTTGACCATCCGAGCCCACGT
GACCGAGGACGAAATTGGCACGATCGCCGACTCCTACAACGCAACTATTGAAAGTCTGCGGCGCATTGTAACCCAAGTACAAACCGCTGCTAG
TCAATTTACTGAGACCACCGACACCAACGAAGTGGCAGTGCGGCAACTAGCCCAGCAAGCCAATCGCCAGGCCTTGGATGTGGCAGAGGCCC
TGGAGCGGCTTCAGGCCATGAACAAGTCCATTCAAGCAGTGGCAGAAAATGCTGCCCAAGCAGAATCAGCAGTACAACGGGCGACCCAAAC
GGTGGACCAAGGGGAAGATGCCATGAACCGCACCGTGGATGGCATTGTCGCAATTCGGGAAACGGTGGCCGCCACCGCCAAACAGGTGAAG
CGATTGGGGGAATCATCCCAAAAGATCTCCAAAGTGGTGAACCTAATTGGCAGCTTTGCTGACCAAACCAACCTCCTAGCCCTAAATGCTGCC
ATTGAAGCCGCCCATGCTGGTGAAGAAGGACGGGGATTTGCGGTGGTAGCCGATGAAGTACGTTCCCTGGCACGGCAATCAGCGGAAGCAA
CGGCAGAAATTGCCCAATTGGTGGCGACAATTCAGGCGGAAACGAATGAAGTAGTGAATGCCATGGAAGCGGGCACCGAACAGGTAGTGGT
GGGAACCAAATTGGTAGAAGAAACCAGGCGGAGCTTGAACCAAATTACGGCGGTGAGTGCCCAGATTAGTGGCTTGGTGGAAGCGATCACC
TCTGCGGCCATTGAGCAGTCCCAAACCAGTGAATCGGTGACCCAGACCATGGCTCTGGTGGCCCAGATTGCGGATAAAAACTCCAGTGAAGC
GAGTGGGGTATCTGCCACCTTTAAGGAACTGTTGGCAGTGGCCCAGTCATTGCAAGAGGCGGTTAAACAGTTCAAAGTGCAGTGA—3'

Genetic Engineering Module
Go Public

(100 pts.)

Create an informative and visually appealing booklet style brochure providing a basic technical introduction on the processes of genetically engineering *E. coli* to express a desired protein.

Starting with the source DNA and finishing with the genetically engineered organism, your brochure must include the following topics:

- Generation of DNA gene sequence fragments from donor
- Joining fragments to a host vector
- Introducing the vector to the bacteria
- Selecting desired strain of *E. coli*

Guidelines:

- Assume your audience understands the central dogma of molecular biology.
- You must cite any references you use on the back of the brochure (last page).

<u>Grading Guidelines:</u>

Generation of DNA gene sequence fragments from donor	
– Polymerase Chain Reaction (PCR)	15 pts.
Joining fragments to a host vector	
– *E. coli* expression vector requirements	10 pts.
– Restriction mapping	10 pts.
– Primer design	15 pts.
– Sequential steps for inserting fragments into vector	15 pts.
Introducing the vector to the bacteria	
– Transformation techniques	10 pts.
Selection of the desired strain of E. coli	
– Requirements and conditions of selection	10 pts.
Creativity	5 pts.
Readability	5 pts.
Clarity	5 pts.
Total:	**100 pts.**

Below is the suggested time line for the Genetic Engineering Legacy Cycle. After using it as an overview to plan out lessons, you can also make a copy to mark up and use as a checklist to keep track of what things your class has completed. This can be especially useful if you have multiple classes working through the challenge, as multiple classes are seldom at the same place at the same time in a series of lesson.

✓	*page(s)*	**LECTURE DAY ONE**
		• Introduce the Challenge Question.
		• Students independently work in their journals to answer the Generate Ideas questions. In the whole-class setting, have students share ideas from their journals. Record their ideas on the board.
		• Break students up into groups and have each group review the ideas recorded on the board. Ask each group to categorize the ideas into about four categories.
		• Have each group report and explain their categories. Work with the class to set up the four main categories of necessary knowledge as listed in the Multiple Perspectives .
		• Before starting the series of lectures and discussions, ask the students to define in their own words "genetic engineering". Ask the students to work together to see if they can generate a comprehensive answer.
		LECTURE DAY TWO
		• Start of the lecture by reviewing the overall goal of the module by repeating the challenge question and listing the 4 categories developed in the Multiple Perspectives: 1) Generation of DNA gene sequence fragments from donor; 2) Joining fragments to a host vector; 3) Introducing the vector to the bacteria; 4) Selecting desired strain of *E. coli*.
		• Introduce the lecture session by providing the motivation for a bioprocess engineer or bioengineer for learning, understanding, and applying genetic engineering. The overall motivation is to alter the cell's content of genetic information to improve bioprocesses.
		• Ask the students to complete a very simple, linear concept map that describes the central dogma of molecular biology. Ensure the students understand the flow of information from DNA through to the protein product.
		• Provide a brief review of genetic material in terms of DNA, RNA, and genes. This should be an elaboration of the central dogma concept map.
		• Discuss the genetic engineering tools of the trade and how they are applied to this particular challenge: Restriction enzymes or endonucleases (5' and 3' overhangs and blunt ends); Exonucleases; Polymerases (point out *Taq* DNA polymerase and include reverse transcriptase); and Ligases.
		• Discuss the importance of manipulating and modifying DNA into a workable form for genetically transforming *E. coli*.
		• Assign readings from the textbook listed in Research and Revise.

		LECTURE DAY THREE
		• Start of the lecture by reviewing the overall goal of the module by repeating the challenge question and listing the 4 categories developed in the Multiple Perspectives: 1) Generation of DNA gene sequence fragments from donor; 2) Joining fragments to a host vector; 3) Introducing the vector to the bacteria; 4) Selecting desired strain of *E. coli*.
		• The next big topic is the Polymerase Chain Reaction (PCR). Help the students understand that the gene of interest needs to be isolated from the donor DNA and copied. Ask the students about their initial ideas on how this can be done. Ask the students if it is easier if the genetic sequence is known. Ask why it is necessary to generate many copies.
		• Provide a brief history of PCR and how it has revolutionized biotechnology.
		• Introduce the three main steps of PCR: Denaturation; Annealing; and Extension. Briefly provide an overview of each step and how it fits together for an overall biochemical reaction system for amplifying a specific gene sequence from a DNA template (donor).
		• Show several diagrams of the PCR process and emphasize the importance and role of each step. Ensure the students understand the concept of using two different primers for isolating the gene of interest. Describe the biophysical mechanisms occurring during PCR to help the students understand each step.
		• Show a short video of PCR that depicts the process graphically.
		• Use Peer Instruction Techniques to ensure the students understand the various concepts of PCR and the importance of this process for answering the challenge question.
		• Assign Test Your Mettle #1: PCR-Mediated Cloning. If time allows, have the students work this assignment in class. This will give them the opportunity to receive immediate feedback on any misconceptions and to help solidify the concepts and process of PCR.
		LECTURE DAY FOUR
		• Start of the lecture by reviewing the overall goal of the module by repeating the challenge question and listing the 4 categories developed in the Multiple Perspectives: 1) Generation of DNA gene sequence fragments from donor; 2) Joining fragments to a host vector; 3) Introducing the vector to the bacteria; 4) Selecting desired strain of *E. coli*.
		• Discuss Test Your Mettle #1: PCR-Mediated Cloning assignment with the class. Clarify any misconceptions and ensure the students clearly understand how to design primers for isolating a specific gene sequence of interest.
		• Discuss the next step of inserting copies of the desired genetic sequence, generated by PCR, into a useable "context" or format in *E. coli* to propagate the expression of the gene. Provide a clear definition of a vector.
		• Introduce and discuss plasmids (bacterial vector). Ensure the students understand the original symbiotic role of plasmids and how they are now used to introduce

		foreign DNA from another organism in to functional genes in *E. coli*.
		• Discuss how plasmids have been engineered (available commercially) to optimize their use as vectors for DNA cloning and recombinant protein expression.
		• Discuss the minimal structural features expression vectors must contain: Replication origin; Selectable antibiotic resistance gene; Promoter system; and Multiple cloning site.
		• Discuss the *lac* promoter system as an example of how promoter systems are used to force and control the expression of the desired recombinant protein. Ensure the students understand the necessary components for the expression vector and strain of *E. coli* to support this type of system.
		• Discuss the use of the multiple cloning site on a commercial vector and ensure the students understand the implications of having a unique set of restriction sites.
		• Provide an example of a commercial expression vector (such as the Novagen pET24a plasmid) and point out the four requirements for an expression vector. Discuss how to read a vector map and to look for key features.
		• Ask the students to consider how the PCR-generated PixJ1 DNA gene fragments (from Test Your Mettle #1) can be inserted into the pET24a plasmid. Help them understand the initial problem of not having bookended restriction sites on the fragments. Once they understand this need have them form groups to discuss ways of including restriction sites at the termini of their DNA fragments. Ask each group to discuss their solution.
		LECTURE DAY FIVE
		• Continue with the discussion of using PCR to not only copy desired segments of DNA, but to use PCR to append restriction sites to the termini of the DNA fragments.
		• Discuss the process for designing PCR primers for inserting DNA fragments into the plasmid MCS. Ensure the students understand the primers must be properly designed to maintain reading frame alignment and the restriction sites are not present in the gene of interest. Introduce the concept of restriction mapping. Provide the students with an example of generating a restriction map of the PixJ1 gene using free software such as New England Biolabs Cutter.
		• Assign Test Your Mettle #2: Plasmid Construction for Expressing PixJ1. Allow time for the students to work on this assignment in class. Explain how to read a commercial vector MCS and reading frame sequence and the design requirements for their PCR primers.
		LECTURE DAY SIX
		• Discuss Test Your Mettle #2: Plasmid Construction for Expressing PixJ1 assignment with the class. Clarify any misconceptions and ensure the students clearly understand how to design primers for isolating a specific gene sequence of interest and inserting the gene into a commercial vector.
		• Ask the students to consider how the plasmid construct will be inserted into the E. coli cells? Have the students form groups to discuss ways of transforming *E. coli* with commercial vectors. Have the different groups report their ideas to the entire

		class.
		• Discuss the two common techniques for bacterial transformations: Chemical transformation and Electroporation transformation. Point out the specific biophysical and biochemical mechanisms of these two transformation processes.
		• Discuss how the desired strain of E. coli that expresses the foreign protein of interest is selected. Ensure the students understand the concept of selection and the necessity of including antibiotics to the growth medium.
		• Review the entire Genetic Engineering Module.
		• Ask the students to write down their "muddiest point" about the module on a piece of paper. Have the students form groups to discuss their "muddiest points" with their classmates. Have each group report their points and discuss these points and clarify any misconceptions.
		• Assign the Go Public: Create an informative and visually appealing booklet style brochure providing a basic technical introduction on the processes of genetically engineering E. coli to express a desired protein.