A Laboratory-Based Approach for an Introduction to Biomolecular Engineering

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Abstract

Many chemical engineering departments have expanded into the field of biomolecular engineering or created concentrations in the field. But for many students entering the engineering program, their most recent course in biology may have been during their freshman year of high school. To effectively bridge this knowledge gap and motivate students in applications of molecular biology within engineering, a laboratory-based course has been developed that demonstrates protein engineering. The term laboratory-based learning is used because the majority of the course takes place in a lab setting, but the more widely used term of project-based learning also describes the general methodology. Specifically, this course introduces students to bench-scale bacterial culturing, isolation of plasmid DNA, the polymerase chain reaction, mutagenesis, bacterial cloning, protein expression, protein purification, gel electrophoresis, protein quantification methods, and software needed to analyze plasmid sequences and model protein structure. The aim of the course is to engage the students in exciting laboratory activities and introduce them to the basic principles of molecular biology and biochemistry.

Keywords

Biomolecular engineering, project-based learning, laboratory-based, peer-to-peer learning

Introduction

To effectively motivate, engage and educate students in the field of biomolecular engineering, a laboratory-based class was developed to introduce chemical engineering students to this biologically oriented field of engineering. Traditionally, chemical engineering curriculum contained no biology-based courses, but over the past couple decades, many chemical engineering programs have broadened the already comprehensive curriculum to include concentrations in biomolecular engineering. Some programs have even broadened the name of the department to include biological, biochemical, or biomolecular engineering.[1] The author found through analysis of department names, that roughly 35% of chemical engineering departments now include a bio-related title in the name. To educate students in biomolecular engineering or bioengineering, students generally take traditional lecture-based courses in biology, biochemistry, and bioengineering, but acquire minimal laboratory experience. The lack of exposure to a hands-on experience and ability to see the biomolecular concepts in action can make much of the course content seem abstract, especially since some chemical engineering students', who are not pursuing a concentration in bio-based chemical engineering, last course in biology may be in their freshman year of high school.

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To expedite the acquisition of practical knowledge and make future lecture-style courses in the biological area more effective, it is proposed that a laboratory-based course would be an effective introduction to biomolecular engineering. The described laboratory-based learning method is similar to the project-based learning practices that have become common in the field of engineering education and undergraduate education in general.[2-5] This method also falls into the broad pedagogical practice of active-learning, which has been shown to be extremely effective in engaging the student in the course content.[6-8]

Within the course, students learn the basics of bench-scale bacterial culturing, isolation of plasmid DNA, the polymerase chain reaction, mutagenesis, bacterial cloning, protein expression, protein purification, gel electrophoresis, and protein quantification methods (Figure 1). Initially within the course, students choose a protein of interest (green fluorescent protein, gene-3-protein of phage, and epidermal growth factor-like domains) to study and apply the various molecular biological techniques to engineer their protein within the semester long laboratory course. Because of the breadth of the course and the student's initial level of understanding of biomolecular engineering, the depth of biochemistry and biology content is shallow. From this introductory course, which uses a laboratory-based learning approach, a general foundation is formed expediting more effective attainment of deeper understanding from future biochemistry and biology courses in the curriculum.

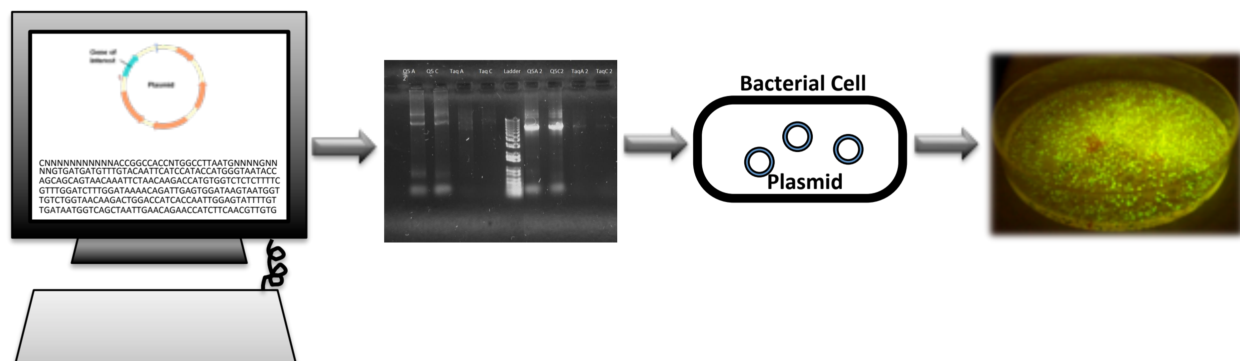

Figure 1. A depiction of the experimental sequence of the laboratory-based approach for an introduction to biomolecular engineering to educate students in applied molecular biological techniques. Initially, students use software packages to study the gene and protein sequences, then molecular biological techniques to modify the genetic sequence and construct a DNA plasmid, followed by bacterial transformation and protein expression.

Student Composition

The current implementation of the laboratory-based course in biomolecular engineering is co-listed (4000 and 6000 level course), allowing both graduate and undergraduate students to participate. It is recommended that students have completed a biochemistry course, but this prerequisite can be waived for properly motivated students. Typically these students have had a significant disadvantage in the course.

There are two motivations for the co-listing. First, there is significant interest from both graduate and undergraduate students within the Chemical Engineering Program. Second, it is

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beneficial for lab group dynamics. The groups in the initial course were composed of a graduate student group coordinator and two undergraduate students. The graduate students tended to have slightly more exposure to molecular biology concepts, which allowed them to help direct the experiments.

The initial implementation of the course could only support a total of 12 students in the section. This is partly due to the equipment requirement, but also the amount of instructor attention needed to properly guide the students through the experiments. The number of sections offered and the number of students per section of the course will be able to grow with the future training of graduate student TAs, who can effectively proctor the lab and troubleshoot experimental issues.

Course Objectives

The overall objective of the biomolecular engineering course is to introduce students to the modern molecular biological techniques used to engineer proteins in order to enhance and modify the properties of the protein. Protein engineering is a rapidly growing field in engineering with many new techniques constantly being developed. This course is designed to give a generalized overview of the modern molecular biological methods, recombinant DNA techniques, computations packages, and practical applications for protein engineering, thus allowing students to better comprehend future biochemistry and bioengineering material.

The course is a combination of a lecture-style and a laboratory course, with a greater emphasis on the laboratory component to fully utilize modern methods of molecular biology to engineer proteins. The applications of the engineered proteins can range from molecular therapeutics, energy production, chemical production, or material science applications for the next generation of bioengineering technologies. This course uses software packages to model proteins and design genes for the production of engineered proteins in bacterial or mammalian cell hosts. The students acquire the necessary molecular biological techniques required to create the DNA constructs and express proteins in host organisms, as depicted in Figure 1. Weekly updates of group projects are shared between groups to discuss lessons learned from the previous week. There is also extensive discussions of current research efforts in the field of protein engineering.

By the end of the course, students should be able to demonstrate the ability to:

- Design expression vectors using a chosen vector mapping software
- Isolate DNA from bacterial cells
- Read and verify DNA sequencing data
- Prepare sterile bacterial growth media
- Use proper sterile techniques for handling bacteria
- Perform the polymerase chain reaction
- Perform DNA and protein gel electrophoresis
- Isolate bacterial clones and culture bacteria
- Express and purify protein from bacterial cultures
- Quantify protein concentration and function

Course Structure

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The biomolecular engineering course is structured to include four contact hours with students. One hour a week is for lecture and student updates. Three hours a week is reserved for laboratory time, though additional hours of lab time may be needed later into the semester. During the first two weeks of the semester, the laboratory hours are used for lecture, introduction to the required software, creation of the groups, and selection of the protein to be engineered. The general course schedule is shown in Table 1.

Assessment of the student's performance is based on lab notebooks (20%), group final presentation (20%), individual term paper and protocol summaries (20%), class participation (20%), and final exam (20%). During the written portion of the exam the students are able to use the materials they generated during the semester. A portion of the exam test their ability to use the computational tools to analyze DNA sequences and construct primers for specific changes to a gene sequence.

Table 1. Weekly Schedule for the Protein Engineering Course

Week 1	Review syllabus, brief presentation of last year's projects, familiarization of software needed for molecular biology, installation of required software, creation of groups of 3-4 members each.
Week 2	Discussion of possible protein engineering projects, groups choose two projects and write a brief summary of each project to then be presented in class, review of basic DNA evaluation software.
Week 3	Final selection of group project. Design of gene construct using vector mapping and cloning software. Design of primers for modification of genes. Describe the process of PCR.
Week 4	Work in groups to learn experimental methods to isolate DNA templates for future modification.
Week 5	Use PCR to modify gene. Learn about gel electrophoresis. Run PCR product on agarose gels.
Week 6	Learn about DNA digestion and ligation. Digest expression plasmid and gene insert.
Week 7	Review topics covered and repeat any failed experiments so all groups are at the same stage of the project.
Week 8	Run agarose gels to determine quality of DNA insert and prepared plasmid. Ligation of gene constructs. Transformation of DNA into bacterial host.
Week 9	Isolation of DNA from bacterial clones. Sequencing of DNA to determine accuracy of the modified gene sequence.
Week 10	Learn about protein purification techniques. Review topics covered and repeat any failed experiments so all groups are at the same stage of the project.
Week 11	Transfer positive DNA constructs to appropriate expression cell line. Express a small-scale batch of proteins.
Week 12	Learn about polyacrylamide gel electrophoresis (PAGE) for protein analysis, then analyze the crude protein expression.
Week 13	Use the chosen protein purification techniques for small batch protein purification.
Week 14	Protein functionality test, such as ELISA, enzyme inhibition, cell signaling, cell adhesion, or fluorescence measurements.

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Week 15 Project term papers due, lab notebook examination, and group presentation of project.

Laboratory Design

The laboratory design proposed here is atypical, in that it continuously builds off the previous week's activity. A traditional lab course has pre-defined experiments that are independent from one week to the next or spanning an additional week. Because of the nature of this course, the laboratory design is challenging and contingency plans need to be in place if unanticipated complications arise which may hinder progress through the semester. The proposed laboratory process is outlined with appropriate plans. An hour of lecture a day prior to the lab section allows for an introduction to the experimental methods that are used during the week's laboratory.

At the beginning of the course, a majority of the students may enter with very little experimental biological background and some only having high school biology. This knowledge gap may seem daunting at first, with effective learning and successful course outcomes seeming unlikely, but properly implemented, the course can motivate students, thus leading to improved student learning. Because of this knowledge gap, it is imperative to have the laboratory experiments designed in a way to enable a high chance of success. To achieve this, at each check point of the course, groups who were not able to obtain proper results, even after repeated attempts, are given samples that allow them to progress to the next set of experiments or share samples with another group who successfully completed the outcome. Experiments in molecular biology can often lead to unexpected results. The cause of a student group to not meet the end goal of the experiment will have to be assessed by the instructor or TA to determine if the failure is due to poor laboratory techniques, poor understanding, or simply experimental stochasticity.

During the first three weeks of the course, lab time is dedicated to computer applications to analyze DNA sequences and protein structure. Students tryout multiple DNA and vector analysis software, including Geneious[9], Ape[10], and Gene Designer[11]. During this time students are introduced to the general concepts of DNA plasmids, gene regulation, restriction enzyme digestion sites, primer design, PCR, and mutagenesis. These first few weeks give students enough knowledge to generally understand the overall process that takes place during the laboratory-based course and enough detail to perform the initial experiments. During this time the students also select the protein they are interested in engineering. The protein should have a known crystal structure or at least a closely related homolog. The structure is used to identify locations to mutate the protein or add an affinity tag for purification. Students download and install DeepView (Swiss Pdb Viewer)[12] to become familiar with the 3D structure of the protein. Primers are then designed and ordered to insert the desired mutations for the week five experiments. During week four, students learn sterile techniques to isolate single clones of bacteria and grow cultures for DNA plasmid purification. The major challenge of this step is the sterile technique and pipetting skills the student must acquire. The students have a pipetting challenge during this time, where each student pipettes various volumes of water onto a scale to observe the volume pipetted as well as the accuracy of the pipette, which familiarize the students with the small volumes used in molecular biology. Overnight cultures of bacteria are already

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prepared for students to be able to extract plasmid DNA, but students also prepare overnight cultures to learn the proper sterile techniques and media preparation required for such cultures.

During week five, students preform their first PCR and agarose gel electrophoresis to determine their success. If successful, the PCR product is excised from the gel and stored in the refrigerator for next week. If not successful, we discuss what went wrong and determine the proper troubleshooting steps to perform next week to address the issues.

During weeks six, seven, and eight, groups perform the gel extraction and any troubleshooting experiments needed to try and resolve the PCR issues. If the experimental issues cannot be resolved by the group, the PCR products of successful groups are shared to allow the project to continue. If no students are successful, plasmid DNA is digested to obtain a gene insert to then ligate into a digested vector, in lieu of the PCR products. Groups then use the proper restriction enzymes to digest the PCR products and plasmid DNA. Agarose gel electrophoresis is carried out to separate the proper fragments for the proceeding ligation step. Ligation of the gene insert with the plasmid backbone followed by the subsequent transformation into the bacterial host completes the cloning experimental sequence by the end of week 8.

Clonal isolation, plasmid DNA extraction, and sequencing is performed during weeks nine and ten. These weeks also allow time for any groups who experienced hurdles during the previous weeks to catch up. Separate lab time can be scheduled if necessary for all groups to obtain clones and sequences. Three to five clones are isolated per group to give significant likelihood that a correct sequence is obtained (Molecular Cloning Laboratory[13] offers Sanger sequencing for $2 per sequence, minimizing the cost of sequencing the clones of every group). During these weeks, students are introduced to polyacrylamide gel electrophoresis (PAGE) and prepare the SDS buffer involved. The students also learn about protein purification techniques.

In weeks eleven, twelve, and thirteen, students perform a small-scale expression of the protein using the clone with the correct sequence and then lyse the cells. Then the groups purify the protein using benchtop purification with nickel-NTA agarose. This is where the students begin to see the fruits of their labor, especially the groups working with fluorescent proteins. Until now, most of the experimental techniques have mostly involved students pipetting small volumes of clear liquids. This sequence of events should also allow for some extra time for groups to catch-up and troubleshoot any minor issues. If major issues arise, materials between groups can be shared so each group has a cell-line that expresses a protein that can be purified.

Depending on the protein chosen, a functional assay is performed during the final week of experiments. Previously, students worked with fluorescent proteins, phage proteins, and domains of an extracellular matrix (ECM) protein. The fluorescent protein has straightforward functional assays that can be performed. The phage proteins that were mutated were coat proteins that the group fused an antibody epitope tag. ELISA can be performed as a functional assay to detected the mutated region. The ECM proteins can be tested using ELISA and the appropriate detection antibody. The ECM domains can also be tested by an adhesion assay using tissue culture cell-lines, but this will need to be performed by the TA or professor with the students observing.

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During the last two weeks of the course, students prepare a term paper summarizing their experimental procedures and results, which is very similar to an introduction, material and methods, and results section of a journal paper. The groups also prepare a final presentation in which the class and students outside the course are the audience, allowing all students to share what they have learned from the projects.

Lessons Learned

The overall student response from the initial course was positive. Students felt they gained a lot of knowledge and understanding from the extensive use of laboratory activities. However, it will take a longer-term study and control groups to determine if the course aids the students to better understand concepts of future lecture-based biologically oriented courses. From communication with students, they felt this course greatly improved their knowledge in the subject area even if they had previously taken courses with molecular biology content.

The groups consisting of a graduate student and two undergraduate students had a very good dynamic. The graduate student was not necessarily the leader of the group, but the interactions of the different levels of student were very fruitful and led to effective knowledge transfer and transformative experiences within the class.

One of the major challenges of the course was that different proteins were chosen by each group for their project. This required a lot of effort on the part of the instructor and made some group projects more difficult for the students. In the future versions of the course, it is proposed to have all groups engineer the same protein, but target different locations within the protein and use different amino acids substitutions. By constraining the possible proteins, the groups can then better compare their data and aid in solving any problems that might arise. In future iterations of the course, green fluorescent protein will be the targeted protein, this also allows for a visually appealing end product if students are successful in the lab.

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Jeffrey J. Rice received his Ph.D. in Chemical Engineering from the University of California, Santa Barbara in 2007 where he engineered protein display techniques to discover novel therapeutic peptides and played a major role in transitioning the technology for industrial use at CytomX Therapeutics, Inc. Currently, he is an assistant professor at the Tennessee Technological University carrying out research in the area of protein and biological engineering. Before beginning his faculty position, he was a Whitaker post-doctoral research fellow at the École Polytechnique Fédérale de Lausanne. His research focuses on applying protein engineering techniques to modify extracellular matrix proteins and growth factors.

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