

BUILDING MOLECULAR BIOLOGY LABORATORY SKILLS IN ChE STUDENTS

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Historically, chemical engineering graduates have been hired predominantly by the chemical process industry and petrochemical industry, a fact indicated by the focus of applications in the chemical engineering curriculum.^[1] Nowadays, however, chemical engineering graduates are also heavily recruited for jobs in the pharmaceutical, semiconductor, and environmental industries.^[2] This diversity of job opportunities is expected to increase as new technologies, such as nanotechnology, smart drug design, and bioinformatics, continue to evolve.

Since the 1980s, chemical engineering educators have been encouraged to modify the curriculum to include new technologies, such as biotechnology and semiconductor processing.^[3-4] In particular, the biotechnology area has been receiving increased attention since many of the high-tech applications of biotechnology (such as drug engineering, drug discovery and pharmaceutical production based on recombinant DNA processes) have become established in the marketplace. As these processes have been scaled up for production, the participation of chemical engineers has become a necessity.

Many underlying chemical engineering principles, such as reactor design and mass transfer, can be transferred to the biotechnology industry, but educators have begun to realize that biology itself must be incorporated into the chemical engineering curriculum in order for chemical engineering graduates to be competitive in industry.^[2,5,6] This situation is similar to a prior shift to focus on chemistry in the curriculum when chemical engineering graduates were predominantly hired in the chemical process and petrochemical industries. In fact, a number of chemical engineering departments have changed their names to the “Chemical and Biology Engineering Department”^[7-11] or similar designations, in recognition of the increased importance of biology in many of the jobs their graduates will one day be hired for. However, constraints such as ABET requirements, significant General Education requirements (along with a push to keep the units required for the degree as low as possible), and even the current appli-

cations focused on in many popular chemical engineering textbooks, have posed challenges to increasing the biology content to acceptable levels.^[12]

Although some chemical engineering departments have introduced basic biology into the curriculum, it has become increasingly important for chemical engineers hired by biotechnology companies to have some understanding of molecular biology, an advanced topic. One of the best ways to help students achieve this understanding is by successfully completing molecular biology experiments. Not only do students gain the hands-on skills required for successful molecular biology protocols, but they also must explain what they did and what their results mean in laboratory reports.

Chemical engineering students at San Jose State University are exposed to these experiments in a biochemical engineering laboratory course developed by Drs. Komives (ChE), McNeil (ChE), and Rech (Biological Sciences). The course is a senior-level course open to chemical engineering students, biochemistry students, and biology students. Chemical engineering students are required to have a biochemistry course and biochemical engineering lecture course prior to

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enrolling in the laboratory. The first half of this laboratory course is focused on molecular biology experiments such as polymerase chain reaction, ligation, and bacterial transformation, while the latter half is focused on enzyme kinetics, fermentation, and protein purification.

The nature of molecular biology is that small volumes (microliter levels) are used and the results of a given experiment are often not known until two or three experiments later, corresponding to two or three weeks later in a laboratory course. Thus, it is imperative to quickly and effectively train chemical engineers in basic biological techniques such as micropipetting, sterile techniques, streaking and spreading samples on agar plates, and loading DNA-containing samples on an agarose gel.

In addition, our laboratory course attracts a multidisciplinary population of students from biology, biochemistry, and chemical engineering. A number of the science students have some or most of the required laboratory skills. In order to avoid redundancy for the experienced science students, and because the laboratory curriculum does not allow multiple days to train chemical engineering students, we have developed a one-day Biology Laboratory Skills-Building session for students. On the first day of class, students answer a questionnaire regarding their prior laboratory experience and are given protocols for each procedure they will complete during the skills-building session that is held during the first day of the laboratory. They are arranged into two- or three-member teams and are instructed to meet with their team prior to the first laboratory session so they will arrive prepared to complete the activities. Every attempt is made to include at least one student experienced in the necessary laboratory techniques on each team.

BIOLOGY LABORATORY SKILLS-BUILDING SESSION

The course is set up as two lecture hours directly followed by a three-hour laboratory session. Due to the nature of some of the experiments,^[13] however, the laboratory portion often takes the entire five hours. This is the case for the Biology Laboratory Skills-Building Session. Table 1 lists the skills-building stations that students complete during the first official laboratory session of the semester. It is not enough for the students to blindly perform each activity. Instead, they are allowed to make mistakes at the various stations so they can figure out what the most common mistakes might be and

TABLE 1
Biology Laboratory Skills-Building Stations

1. Micropipetting	All groups at once
2. Autoclaving	Two groups at a time, one at each autoclave
3. Gel loading	Two groups at a time, one at each gel
4. Material data safety sheets (MSDSs)	One group at each computer
5. Agarose gel preparation	Two groups at a time
6. Sterile techniques and agar plate streaking	Each group separately
7. Station clean up	Each group separately

how to identify them. The students must also answer a set of questions associated with each station—questions that have been written to help the students identify common errors or common pitfalls associated with a given protocol. The activities and questions associated with each station are described in Table 1. The answers to most of the protocol questions can be answered by the students with reference to the protocol sheet they were given on the first day of class.

STATION ACTIVITIES

The instructions for activities and questions associated with each station are as follows:

1. Micropipetting

Each group should check each other out to confirm that each member understands the use of micropipettes. Be able to demonstrate the answer to the following questions and record your answers in your laboratory notebook:

- How do you set the volume required?
- Which tips go on which pipettes for which range of volumes?
- How do you pipette a certain aliquot of solution? Note how you might get aerosols, suction against the tube bottom, or air bubbles—none of which you want.
- How do you release the aliquot?
- How should the micropipettes be stored?
- In which direction should you NOT hold loaded micropipettes (*our students have often been observed holding the micropipette in directions such that the chamber can become contaminated*)?
- What aspects of sterile technique should you keep in mind while micropipetting?

2. Autoclaving

Each group should autoclave 500 ml of DI water, in a labeled capped bottle (label should include contents, composition, date, one team member's initials, and course number).

- Which settings should you use?
- How do you program the autoclave?
- How do you add the water used for steam?
- How long should you autoclave?
- How should your container of water be autoclaved e.g. with a lid?
- How do you remove items after autoclaving?
- How do you know if the autoclave has reached sterilization temperature?
- How do you know if the contents are sterile?
- What aspects of sterile technique should you keep in mind while autoclaving?

3. Gel Loading

Each gel contains two sets of 16-well lanes. Each group can use ONE set of 16, although you don't have to use them all if all group members are confident on their loading technique sooner.

- Pipette 150 μ L of sterilized water into an Eppendorf tube using a STERILE pipette tip.
- Add 10 μ L of loading dye.

- Fingertip flick to mix.
- Load 10 μL at a time into each gel.
- Make sure your tip is in the well.
- The dye is heavy so it will sink.
- If you haven't loaded before, hit the bottom of one of the lanes just to see what it feels like.
 - a. What is best technique for you to steady your hand when you load?
 - b. Are you satisfied with the way your sample loaded in the well?
 - c. Where are the tips disposed?
 - d. What aspects of sterile technique should you keep in mind while loading the gel?

4. Material Data Safety Sheets (MSDSs)

Each group will download two MSDSs from the Internet and will give a brief report on any safety hazards associated with that chemical at the end of the lab period.

- Group 1 - Ethidium bromide, Tris
- Group 2 - EDTA, agarose
- Group 3 - Glacial acetic acid, LB medium
- Group 4 - DMSO, glucose

5. Agarose Gel Preparation

The instructor will demonstrate preparation of the agarose gel. *Note:* Some glassware is reserved for making the gel since residual amounts of ethidium bromide may contaminate the glassware.

- a. How do you tell when the gel solution has been microwaved long enough?
- b. How do you tell when to pour the gel?
- c. How do you tape the gel plate?
- d. When is the ethidium bromide added and in what amount?
- e. Where are the ethidium bromide-contaminated tips etc. collected?

6. Sterile technique and agar plate streaking

- Each group will get four LB agar Petri dishes.
- Label the dishes so they can be identified (content, date, one member's initials, and course number)
- Where should you label, top, bottom, and/or side? TELL THE INSTRUCTOR YOUR ANSWER BEFORE LABELING.
- Each member should practice streaking using the technique shown on the handout given in the first class.
- One member should streak with unsterilized tap water, one with unsterilized DI water and one with the water you just sterilized in this lab. REMEMBER to use sterile techniques when pouring your sterilized water. Review your sterile techniques protocol. Put your dishes upside down in a 37 °C incubator, after they have been streaked. Use the Internet to find out the difference between streaking and spreading techniques.
- The fourth dish is to show you why you need to use sterile techniques.

Group 1 - open up the dish and talk over it, then close it up and incubate it.

Group 2 - swab the doorknob with a cotton tip, then lightly streak your dish, and then incubate it.

Group 3 - have each member swab their hand, each with their own cotton swab, then lightly streak each swab on your dish, and then incubate the dish.

Group 4 - swab the computer keyboard, then lightly streak your dish, and then incubate it.

- These dishes should incubate for 24-36 hours. If someone in your group cannot come in to put the dishes in the refrigerator, e-mail the instructor and she will take care of that step.
- All groups should review all 4 of these plates after incubation. Label them well.

7. Station Clean-up

When you are done, clean up your station. Review the clean-up protocol you were given on the first day.

- a. What clean-up protocol do you need to follow?
- b. Where do the waste chemicals go?
- c. Where do the waste plastic supplies go?

DISCUSSION AND CONCLUSIONS

The activities included in the Biology Laboratory Skills-Building session were designed to serve several purposes. We recognized that each subsequent molecular biology-related laboratory would occupy essentially all of the student's concentration due to the detailed nature of the protocols and the number of samples, controls, and calibration standards that would be tested. It has been our experience that students can concentrate on one new activity at a time. All of the molecular biology experiments were new, and thus areas such as safety or sterile techniques tend to be ignored if they were first introduced at the same time as the new molecular biology protocol.

The Biology Laboratory Skills-Building session was designed to introduce students to safety, sterile techniques, and basic protocols (preparing an agar gel, micropipetting, autoclaving) before their concentration was focused on the new molecular biology protocols (ligation, digestion, transformation, etc.). For instance, the MSDSs that they had to download were selected for chemicals that had the most safety hazards to consider and for chemicals that were used most often. The questions assigned to each activity were designed to have the students address important issues—for instance, how to dispose of ethidium bromide-contaminated items or what not to do with a loaded micropipette.

The inclusion of this skills-building session has not eliminated all problems associated with the lack of molecular biology skills common to chemical engineering students without prior experience. For instance, during the transformation experiment later in the semester, some students gouged their agar as they roughly spread their transformed bacteria. This resulted in zero colony growth. Also, sterile techniques were often treated with less diligence than was optimal. Students were very appreciative of the initial training session, however. All students were visibly impressed with the colonies

that grew on their agar after the first laboratory session. The colonies obtained off swabs from their skin, the doorknob, and the computer keyboard were multicolored and profuse, making a vivid impression on the students. During the semester, anytime they relaxed their attention, one reference back to these colonies inspired them to renew their sterile techniques.

It should be noted that it takes a fairly long period of time for many students to gain an appreciation and mastery of biology laboratory skills such as sterile techniques, micropipetting, and culturing. When SJSU science and engineering faculty compared the skill levels of students in their laboratory courses, the general consensus was that microbiology students have higher skills than biochemistry students, who have higher skills than biochemical engineering students. Not surprisingly, the higher skill level corresponds to the greater amount of time these topics are focused on in the typical curriculum (lecture and laboratory) in microbiology, biochemistry, and chemical engineering. Other universities may be able to offer one, two, or three biology-related courses in their chemical engineering curriculum, but even that number will not be sufficient to build up skills to the level required in industry. It is an acceptable start, however—especially given the constraints (ABET, unit load, textbook examples, etc.) chemical engineering departments face as they try to incorporate additional biology into the curriculum.^[6,7,12]

We have found that the experienced science students were invaluable during the skills-building session. It would be hard to imagine one instructor and one graduate student assistant being able to work with every group at every station in enough depth to make sure the students were being properly trained. With at least one experienced student on every team, there was enough experience and attention to make sure the less-experienced students were adequately trained. If experienced students are not available at other universities (for instance if such a class was open only to chemical engineering students), it might be more productive to take two sessions to make sure all the students had enough time to gain adequate competency in these critical basic skills. Molecular biology-related experiments, such as ligation and transformation, are complex and time intensive. Lack of the basic biology laboratory skills can be a major reason for the failure in obtaining desired results (*e.g.*, failed transformation) in molecular biology-related experiments. Students tend to be very disappointed if they spend a few weeks on an experiment only to find out it has failed. Thus, it is worthwhile to spend time at the beginning to build the basic biology laboratory skills so students can focus on the multitude of steps needed to successfully complete their subsequent molecular biology-related experiments.

Since it can be difficult for a chemical engineering department to have all the equipment and supplies necessary to run in-depth molecular biology experiments, we thought it might

be useful to mention that the Bay Area Biotechnology Education Consortium (BABEC) has developed some kit experiments that are sold through Bio-Rad. The experiments are described on the BABEC website at <<http://www.babec.org/curricula.htm>>. Incorporating the Biology Laboratory Skills-Building session along with one or more of these kit experiments (several of which are designed to be done in sequence, if desired) would be a low-cost means of giving chemical engineering students hands-on exposure to important molecular biology skills.

In conclusion, incorporating a Biology Laboratory Skills-Building session prior to the start of molecular biology experiments has resulted in student teams, predominantly populated with students having low biology laboratory skills, successfully completing complex molecular biology experiments. Issues such as safety, sterile techniques, and basic biology laboratory skills (making an agarose gel, micropipetting, autoclaving) were emphasized, allowing these skills to be developed early so the students could then concentrate on the new concepts introduced by each molecular biology protocol introduced in subsequent experiments (ligation, transformation, etc.).

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