INQUIRY & INVESTIGATION

Designing Authentic Undergraduate Research Experiences in a Single-Semester Lab Course

RECOMMENDED FOR *AP Biology*

THOMAS A. MENNELLA

Abstract

The importance of a robust undergraduate research experience has been demonstrated time and again. However, too few undergraduates engage in genuine research and leverage this opportunity. Here, I present a laboratory course in cell and molecular biology that is designed to mimic a true research project. Students work through a 10-step experimental design culminating in the construction, expression, and visualization of microtubules fused to green fluorescent protein in baker's yeast. The steps of this project include the isolation of the tubulin gene from yeast genomic DNA, the cloning of that gene into an expression vector, the amplification of this plasmid in E. coli, and the expression of fluorescent tubulin in yeast. Controls and validation steps are embedded throughout the project, as they would be in a genuine research project. This laboratory course more closely resembles a one-semester undergraduate research experience than a typical lab course. However, because this course reaches a much larger number of students compared with undergraduate research opportunities, it provides students with a valuable research experience that remains confined to the scheduled time block of a typical lab course. In this way, many of the benefits of research are experienced by a large number of undergraduates.

Key Words: *Undergraduate research; cell biology; molecular biology; yeast; microtubules.*

○ The Need for Research Experience during Undergraduate Years

There is a wealth of information supporting the value of student research conducted during the undergraduate years. Such research experiences strengthen learning, improve retention, increase student satisfaction, and better prepare students for the needs and challenges of graduate school or postgraduate employment. In fact, one of the recommendations made by the *Vision and Change* report, jointly commissioned by the National Science Foundation, the Howard Hughes Medical Institute, and the National Institutes of Health, was to increase the opportunities for undergraduate research experiences (Brewer & Smith, 2011). This report states that "hands-on research also cultivates scientific thinking, allowing students to experience authentic activities of working scientists, including designing studies, interpreting unexpected outcomes, coping with experiments that fail, considering alternative approaches, and testing new techniques," but it goes on to state that these experiences are "difficult, if not impossible, to replicate in standard lecture or laboratory courses" (Brewer & Smith, 2011). Indeed, it is difficult to replicate research experiences that encompass all of these components in a standard laboratory course, given the time constraints of lab coursework coupled with fairly large student enrollments. Yet lab courses in general, and those in molecular and biochemical areas specifically, are indispensable (Costa, 2010). Although others have succeeded in creating lab courses that teach, promote, and cultivate student-centered, inquiry-based research, these courses fall short in training science majors in the lab skills and proficiencies they need in preparation for their postgraduate endeavors (Brownell et al., 2012; Kloser et al., 2013). This is undoubtedly due to the challenges of providing such training within the confines of a lab course while still remaining inquiry-based. It is possible to provide undergraduates with a much more realistic research experience than what current, traditional lab courses typically offer, while still training students in relevant lab skills and retaining some elements of an inquiry-based experience. Below, I outline a laboratory course that, I believe, nearly succeeds in replicating a genuine, bona fide research experience.

There is a wealth of information supporting the value of student research conducted during the undergraduate years.

The *Vision and Change* report also emphasizes the importance of undergraduate research by stating that "a growing body of literature has found a link between student research and lasting learning (National Research Council, 2000, 2003; Lopatto, 2003, 2007, 2009; Laursen et al., 2010)"

The American Biology Teacher, Vol. 77, No 7, pages. 526–531, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: 10.1525/abt.2015.77.7.7.

(Brewer & Smith, 2011). It is nearly irrefutable that undergraduates' research experiences improve their overall college education in a multifaceted way – but still we see a relatively small number of undergraduates engaging in research experiences.

○ Challenges Associated with Research Experience during Undergraduate Years

With so many apparent benefits of undergraduate research, why is it that we still see a relative minority of students seeking out, and engaging in, such opportunities? The reasons are many, I believe. First and foremost is the time commitment. Research takes time, and in the life sciences this is often time that does not conform to scheduled blocks throughout the day. With long incubations between steps, student often must "be on call," reporting back to the lab multiple times a day to push the experiment to the next step. Doing this within a schedule that includes class meeting times, extracurricular activities, work, and commuting can be challenging.

Additionally, it is usually the student's responsibility to initiate an undergraduate research project: he or she must select an undergraduate research advisor, approach that faculty member regarding the possibilities of doing research, and so forth. This can be a tall order for an undergraduate to fulfill, and it requires a certain level of motivation and self-confidence that perhaps a good number of undergraduates lack. So, we are left in a situation where undergraduate research greatly enhances a student's overall undergraduate training, yet many obstacles lie in the way of seizing this opportunity.

○ Traditional Laboratory Courses: A Poor Substitute

For many years, it has been the role of the lab course component to fill this void in undergraduate research experience. The lab component was created and designed to provide all students with "hands-on" experience directly relevant to the topics being covered by the companion lecture course. And, although well-designed lab courses are fun, engaging, and provide students with hands-on learning that serves to fortify the course material, lab courses are not research. Research projects are long, multistep, goal-oriented endeavors that can often take weeks or months (or even years) to complete. They are fraught with failures, and validation steps are embedded in the research design as checkpoints along the way to ensure that the project is progressing as planned. On the other hand, lab course exercises are typically "canned" experiments that are designed to succeed at almost all costs. They can typically be completed in a single 3-hour block. And students leave the class period with data in hand. While it is clear that these traditional lab courses do not mimic genuine research, the lack of efficacy of this approach is difficult to quantify. Publications espousing the lack of effectiveness of traditional, stalwart pedagogies are not common. Empirically, however, most college educators can appreciate the limitations of these antiquated approaches, and the calls for change in designing lab courses far outweigh calls for the status quo.

There are positive attributes, however, that laboratory courses offer. The primary advantage of traditional undergraduate lab courses are that they are mandatory (at least for students taking such courses required by their major), reaching a much larger number of students than undergraduate research opportunities. What is needed is a lab course that reaches nearly all majors, but which also provides students with a genuine research experience. While only true research is true research, I believe that the lab course described below optimally strikes the balance of providing hands-on experience and activities in the lab that train students in needed skills, while still capturing the multistep, comprehensive, and unpredictable qualities of a genuine research program.

○ A New Breed of Laboratory Course: A Reasonable Substitute for Undergraduate Research

For this lab course to truly replicate an undergraduate research experience, there were four criteria that I felt must be met: (1) The experimental project must require a number of steps to be completed over a significant period of time. (2) Experimental failures need to be expected and planned for. (3) Experimental tests, controls, and/or validations must be embedded into the overall project to verify that the project is progressing as intended and planned. This includes experimental steps whose sole purpose is to check on the progression of the overall project as a whole. (4) All lab students/groups must be synchronized so that all students are working on the same steps during the same class period.

I designed a lab course that is a companion for a typical, one-semester undergraduate course in cell and molecular biology (BIO-320) at Bay Path University. This is a 300-level course typically taken by juniors, but sophomores and seniors are also often enrolled in the course, though they make up a minority. This course is required for all majors in our Biochemistry, Biotechnology, Neuroscience, and Forensic Science programs. Standard biology majors must take either Cell and Molecular Biology (and the lab that accompanies it, described here) or Biochemistry; however, the majority of biology majors elect to take both courses. An undergraduate course in genetics is a prerequisite for this lab course. In Genetics, students are introduced to basic laboratory skills, including micropipetting, PCR, and calculating concentrations and dilutions, but many students enrolled in the Cell and Molecular Biology lab have no prior experience with more advanced laboratory techniques (e.g., gel electrophoresis, genomic DNA isolation, protein isolation).

In this Cell and Molecular Biology lab course, students work in pairs so that each student gets frequent opportunities to engage directly in the experimental process. The overall goal of the semester-long research project is that each lab group (i.e., pair of students) must create and visualize a fluorescently labeled cytoskeletal component in a eukaryotic cell. They accomplish this by fusing the *TUB1* gene from baker's yeast (this gene encodes the α subunit of tubulin in *Saccharomyces cerevisiae*) to the gene for green fluorescent protein (GFP). This results in a yeast cell that expresses a Tub1-GFP recombinant protein, giving rise to green-glowing microtubules when visualized under fluorescent microscopy. It should be noted that this is not a hypothesis-driven project. The creation of fluorescently labeled microtubules is the end-point and goal of the semester. However, this remains a realistic research endeavor. The creation of a new reagent or resource for further experimentation is often a goal of a research laboratory, and such goal-oriented (as compared to hypothesis-driven) research is commonplace in most research settings.

Each week, the lab course meets for a single, 3-hour session, and during that time a portion of this experimental project is completed. The weeks are organized as outlined in Table 1. The semester begins with an in-depth lecture and overview of the entire semester's activity schedule. Students are guided to understand the overall "big picture" of the course while also seeing how each week's activity fits into this project. Then, each subsequent week begins with a 20- to 30-minute mini-lecture in which that week's activity is reviewed in detail and placed in the context of the overall, semester-long project. This review is both procedural (showing students where equipment can be found, how equipment is properly used, etc.) as well as conceptual (explaining to students what will be occurring "in the tube"; reviewing the underlying concepts governing the reactions and techniques to be used). Elements of this information can be found for each exercise in the laboratory manual as part of the "Background" section and in the italicized descriptors that accompany some steps. This manual is being made available as part of the supplemental materials associated with this publication. This mini-lecture is highly interactive, and students are prompted to contribute their suppositions for the purpose of specific controls, steps, samples, etc. Students are asked to imagine causes of potential failures and describe the predicted outcomes for the relevant controls that would indicate the nature of such failures. In this way, students are engaged in the "thinking portion" of experimental design and planning.

Fortifying the critical-thinking components of this course are the writing assignments. Students write four summary lab reports throughout the semester: one each after weeks 3, 6, 8, and 11. Each summary report begins with background information for the overall, semester-long project. Students must describe how the two or three exercises being highlighted in that particular report fit into the overall scheme of the entire semester's project. Students must also provide any relevant background on the techniques and controls used in those exercises. Students are encouraged to use the lab manual as a guide (focusing on those background and italicized sections), but they must expand on this information using outside sources. The methods and results sections of these summary reports focus exclusively on the experiments conducted in the intervening weeks since the last report was submitted. For the

Table 1. A weekly outline of laboratory activities for Cell and Molecular Biology Lab.

WEEK 1	Organizational meeting where the overall, semester-long project is outlined and explained to the students in detail. This serves as the informational foundation for the rest of the semester.
WEEK 2	Genomic DNA is prepared and isolated from yeast to be used as template for the subsequent polymerase chain reaction (PCR) experiment.
WEEK 3	PCR amplification of the TUB1 gene from yeast genomic DNA and agarose gel electrophoresis of that PCR product.
WEEK 4	Purification of the PCR product and digestion of that PCR product with restriction enzymes in preparation for directional cloning. During this week, a cloning vector (or plasmid) already encoding the GFP gene is also digested with the same restriction enzymes. This is a shuttle vector that is capable of being maintained and amplified both in yeast and *E. coli*. However, the TUB1-GFP gene being constructed can only be expressed in yeast because it is controlled by a eukaryotic promoter (i.e., a TATA box).
WEEK 5	Agarose gel electrophoresis and purification of the TUB1 and vector restriction digests; these two fragments are also used in a ligation reaction during this week's exercise.
WEEK 6	Transformation of the ligated TUB1-GFP-containing vector into *E. coli* for plasmid amplification.
WEEK 7	Mini-prep of the plasmid from *E. coli*; the plasmid is also used in a sequencing reaction during this week. This is our first validation step, where we try to ensure that we have made the correct fusion gene on our plasmid.
WEEK 8	The sequencing data is reviewed, interpreted, and discussed. The plasmid is then transformed into yeast.
WEEK 9	Total protein is prepared from yeast expressing TUB1-GFP, using the trichloroacetic acid (TCA) method. This protein preparation is done as part of our second validation step. Before proceeding to fluorescent microscopy to observe their glowing microtubules, students verify that the plasmid-transformed yeast cells are indeed expressing Tub1-GFP protein.
WEEK 10	SDS-PAGE (polyacrylamide gel electrophoresis) of the yeast protein preparation and electroblotting that gel onto nitrocellulose membrane (i.e., part 1 of a western blot).
WEEK 11	The western blot is finished with primary antibodies specific to GFP, secondary antibodies fused to alkaline phosphatase, and an AP-dependent chromogenic reaction to specifically visualize the Tub1-GFP protein band on the western blot.
WEEK 12	Living yeast cells are observed under fluorescent microscopy to visualize Tub1-GFP-containing microtubules.

methods section, students are specifically instructed to focus on the "why," not the "how." In other words, these are not traditional methods sections that describe the steps of the experiment. Instead, students must describe, in their own words, what is transpiring in each step, what each step of each exercise is accomplishing, and why each step was performed. Finally, the results are reported, with figures where appropriate, and interpreted. In the interpretation of the results, students are required to explain any failures that they encountered, using the results of the relevant controls to guide that interpretation. If a conclusive interpretation of failure is not possible, students must provide a reasonable possible cause for the failed step(s) and explain how that cause for failure would have resulted in the data/results they obtained. Finally, students end each summary report with a return to the overall project. Students describe how the steps completed in the exercises covered by that report moved them closer to achieving their goal of GFP-tagging tubulin, and they state what steps are upcoming to continue advancing toward that goal. These summary reports allow me to determine which students are "getting it" – understanding the comprehensive aspects of this course – and which students are not. It is also where students think critically about, and analyze, their activities in the course. Just as is true for genuine research, the synthesis of knowledge and integration of data and results for this course occurs mostly upon reflection of work done, not during the doing of work. Lastly, these summary reports serve to scaffold the writing of a much larger, and manuscript-type, comprehensive lab report (described below).

To summarize, in this laboratory course students see an entire research project through, from beginning to end. They gain an appreciation for the complexity of such a project, for the controls that are involved along the way, for the need of a true research design, and for validation steps to ensure progress throughout the project. Also, since students are working on a single project all semester, they begin to own their project and develop a thorough understanding of the methods being used, the purpose of those methods, and the overall experimental objectives and goals. Finally, the lecture course, of which the lab is a companion, delves deeply into topics such as transcription, translation, model organisms, and cytoskeletal components as well as experimental techniques such as DNA sequencing and PCR. And, wherever possible, these topics are reiterated in the lab during the same weeks they are being presented in lecture, providing students with fortification and increased mastery of this course content. For example, during weeks 4 and 5 of the semester, the lab course is isolating the *TUB1* gene and cloning it into the GFP expression vector. During these same weeks in lecture, we are focusing on the Central Dogma of Molecular Biology (i.e., transcription and translation of a gene to synthesize a protein) and introducing the concepts underlying gene regulation. In other words, as we are learning about expressing and regulating genes in lecture, we are manipulating the expression and regulation of a gene in lab. As another example, we discuss the cytoskeleton during week 11 of the lecture course, just as we are isolating and identifying tubulin protein from microtubules in the western blot portion of the lab course. In this way, the lab course not only serves to mimic a true research project, but also complements the lecture course's content in the way that all successful undergraduate lab courses should.

○ Conclusions

The primary challenge that I faced in redesigning and implementing this lab course was mimicking true research while also keeping all students synchronized, doing the same steps on the same days each week. You can easily imagine that as steps fail, students would need to repeat those steps in order to see the project through, but then they would fall behind in the class schedule and never complete the final objective of the course. To address this possible pitfall, I chose to prepare working samples for each group at the beginning of each lab session. For example, if a pair of students sees that their PCR failed at the conclusion of the agarose gel run during week 3, I have a tube containing a known, working *TUB1* PCR product for that group. Those students simply swap their failed sample for my working one, and they are back on track and synchronized with the other groups, ready for week 4. Additionally, these known working samples can also be used as positive controls where appropriate. Without this accommodation, students might get a much more genuine research experience, but at the risk of not reaching their objective, thus leaving them with a negative opinion of what may be their first research experience. While true research is hard and requires patience – and failed days far outnumber successful ones – I have chosen to strip away as much of the frustration of research as I could for this course to enhance the positives, namely the excitement and thrills that come with success and the objective reached after a long and laborious road.

In my experience, students tolerate their failures well. Initially, they are warned of how sensitive many steps are to small deviations of precision and accuracy, and they are told of the unpredictability in dealing with living systems. In lieu of having students repeat failed steps, I will often sit with a group that experienced a recent failure and discuss with them what might have gone wrong. Together, we brainstorm possible troubleshooting experiments that could be done to address their failure if time permitted. If possible, I share some stories of personal experience with similar failures and tell the students what was eventually found to be the culprit in those cases. After this failure "debrief," all students I have encountered prefer to move ahead with the working samples I provide rather than fall behind repeating a failed step.

I have encountered no other major challenges in the logistics of running my lab course as outlined above. However, I have had to implement some smaller, time-saving experimental shortcuts in order to make specific steps fit into a 3-hour time block. These short cuts are briefly described at the beginning, and/or in the "Instructor's Notes," of each lab exercise as part of the lab course manual.

All assessment of this course is done through the writing assignments described above and by a comprehensive report covering the entire project, which students are required to submit just prior to final exam week. While this final, comprehensive report is long and thorough, the summary reports serve to scaffold the writing. In reality, each summary report is a partial rough draft of the final comprehensive report. Students are strongly encouraged to use their summary reports as a starting point for the comprehensive report, to integrate my corrections and comments into their final report as a means for improvement of that report, and to edit and revise their

Table 2. A sampling of student evaluation comments from the Spring 2013 and 2014 semesters (these have not been edited or corrected).

"i liked that the labs all lead to one big project"
"I loved the idea of a continuous laboratory project. It was great to see the progression through the semester. It also allowed for thorough understanding of what was going on and why. It was also great that the lab went along with the class."
"I liked the entire set up and pacing of the course. I also liked the multi-step experiment."
"I loved being able to conduct an undergraduate research project for the entire semester. We used all the techniques I would need to use/know in a future laboratory setting."
"The experiment done over the semester was interesting"
"I really enjoyed that we had one large experiment to do rather than a bunch of little ones. there was also a lot of focus on making sure we understood what we were doing and why we were doing it. this helped me a lot when it came to lab. also i really appreciated the lab summaries rather than doing a lab report. it was a nice change of pace as well as i believe i was able to retain and understand more information that way. . . . I also really like the way his lab write ups were constructed. each step is explained and that also helps me to understand what im doing."
"I liked how the class was one big experiment. It helped me fell like we actually accomplished something in class. Often there is a new lab every week and by that time I have forgotten what we had done. The lab summaries also helped reinforce what we had been doing."
"I liked doing the one big lab with small parts, it was great watching everything fit it and it fit in with what we were learning. I also LOVED how everything thing in the lab was explained I feel like a lot of teachers just say put this in there and do this but now I understand why I'm putting the things in."
"I liked that this was a semester long experiment, instead of several different experiments that don't tie together."
"I liked that this lab was a string of continuous experiments as opposed to random unconnected labs that wouldn't stick in my head like other science course."
"I loved the semester long experiment. It is more efficient than doing random labs throughout the semester."

final report instead of writing it "from scratch." Not only does this allow students to spread out the work of writing the final report over the entire semester, but it also best mimics the genuine process of scientific writing, in which drafts are shared with colleagues, often in pieces, and then comments and suggestions are used in editing the final product. This comprehensive report does, however, contain one element that is not present in any of the previous summary reports: students are required to offer ideas of how the techniques and strategies that they have engaged in all semester can be used to specifically address some current need in science. These needs can be focused on human health, the environment, agriculture, etc., and students must demonstrate literary research beyond the standard course materials and cite these sources. In this way, the course comes full circle, closing the loop of an inquiry-based experience. The students explore and decide for themselves how a fluorescently labeled protein, made recombinantly, might be used beyond the scope of the limited project in which they have just been engaged. This, too, mimics a genuine experience as part of a typical research program.

Student reaction to this lab course has been overwhelming positive. The consistency of the overall project allows students to become very comfortable with what they are doing, why they are doing it, and so on. Students appreciate the wide array of techniques they use, but more importantly, they gain a greater understanding of *why* these methods and techniques are used (i.e., what specific purpose each method serves in the greater context of the overall project). And many students express that they wish more lab courses were designed in this way; perhaps this is the strongest endorsement that the students can give. A sample of student comments from course evaluations is provided as Table 2.

It's been about 2 years since I set out to design a laboratory course that met weekly for 3 hours a session but that also mimicked a true research project, as one might be conducted in a true research environment. While not perfect, I believe that the design presented here comes close to realizing this goal and provides students with a genuine and bona fide research experience as part of their regular and scheduled course load. This design likely cannot be adopted for all lab courses, but it does have its potential in many. As long as an effort is made by all laboratory instructors to provide undergraduates with genuine research experiences – research experiences that truly resemble a day in the life of a scientist – students will be better prepared for postgraduate training in such a setting and will be provided with a rich educational experience through which they can flourish.

References

Brewer, C.A. & Smith, D. (Eds.) (2011). *Vision and Change in Undergraduate Biology Education: A Call to Action*. Washington, DC: AAAS.

Brownell, S.E., Kloser, M.J., Fukami, T. & Shavelson, R. (2012). Undergraduate biology lab courses: comparing the impact of traditionally based "cookbook" and authentic research-based courses on student lab experiences. *Journal of College Science Teaching*, *41*, 36–45.

Costa, M.J. (2010). Commentary: why abandoning undergraduate laboratories is not an option. *Biochemistry and Molecular Biology Education*, *38*, 335–336.

Kloser, M.J., Brownell, S.E., Shavelson, R. & Fukami, T. (2013). Effects of a research-based ecology lab course: a study of nonvolunteer achievement, self-confidence, and perception of lab course purpose. *Journal of College Science Teaching*, *42*, 72–81.

Laursen, S., Hunter, A.-B., Seymour, E., Thiry, H. & Melton, G. (2010). *Undergraduate Research in the Sciences: Engaging Students in Real Science*. San Francisco, CA: Jossey-Bass.

Lopatto, D. (2003). The essential features of undergraduate research. *Council on Undergraduate Research Quarterly*, *24*, 139–142.

Lopatto, D. (2007). Undergraduate research experiences support science career decisions and active learning. *CBE Life Sciences Education*, *6*, 297–306.

Lopatto, D. (2009). *Science in Solution: The Impact of Undergraduate Research on Student Learning*. Tucson, AZ: Research Corporation for Science Advancement.

National Research Council (2000). *How People Learn: Brain, Mind, Experience, and School: Expanded Edition*. Washington, DC: National Academies Press.

National Research Council (2003). *Improving Undergraduate Instruction in Science, Technology, Engineering, and Mathematics: Report of a Workshop*. Washington, DC: National Academies Press.

THOMAS A. MENNELLA is an Associate Professor of Biology at Bay Path University, 588 Longmeadow St., Longmeadow, MA 01106; e-mail: tmennella@baypath.edu.