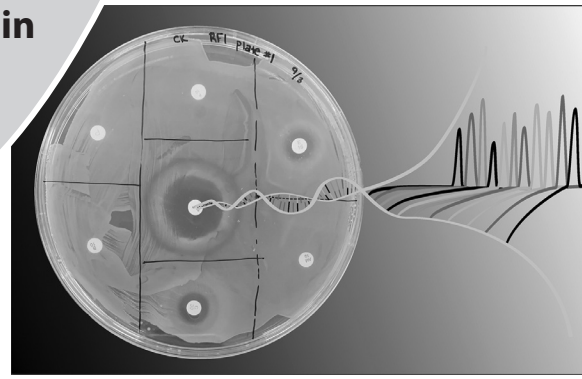
INQUIRY & INVESTIGATION

Linking Genetics, Microbiology & Molecular Biology Courses to Provide Research Experiences in Undergraduate Biology

BETHANY LUCAS, GENA NICHOLS, MAX BOECK



Abstract

Course-based undergraduate research experiences (CUREs) can have benefits for many students, especially those who lack access to traditional apprenticeships for research. As part of an effort to create more opportunities for students to have access to primary research and move away from traditional cookie-cutter labs, we have created a multicourse CURE spanning three undergraduate teaching labs in which students can pick and choose to take any of the courses that most interest them. This CURE explores the essential understanding of the emergence of antibiotic-resistant bacteria as well as high-throughput sequencing and mutagenesis screens. These low-cost modular labs are designed to be flexible and integrated into any single teaching lab to increase exposure to both fundamental lab skills and primary research.

Key Words: *antibiotic resistance; genetics; genomics; interdisciplinary; microbiology; mutagenesis; sequencing; undergraduate.*

○ Introduction

Overall, research experiences for undergraduates are invaluable for their development as scientists and individuals, in addition to increasing retention in the sciences (Lopatto, 2004, 2007; Seymour et al., 2004; Hunter et al., 2007; Russell et al., 2007; Jones et al., 2010; Stanford et al., 2017). The traditional "apprenticeship" method, while important (Hunter et al., 2007; Stanford et al., 2017), can be difficult for some students to engage in because of budgetary and time constraints for those students (Bangera & Brownell, 2014) and financial and resource constraints for faculty (Wei & Woodin, 2011; Rodenbusch et al., 2016; Shortlidge et al., 2017; Corwin et al., 2018). Additionally, research opportunities tend to be undertaken by students with high GPAs and later in their undergraduate careers (Russell et al., 2007), suggesting that research opportunities need to be made available for a broader range of students (Jones et al., 2010; Harrison et al., 2011; Wei & Woodin, 2011). Therefore, course-based undergraduate research experiences (CUREs) provide a valuable opportunity for students who would not otherwise be exposed to research at this level (Bangera & Brownell, 2014).

CUREs have the benefit of increasing access to research opportunities while also moving away from traditional cookie-cutter labs, which may not promote students' development as scientists and as professionals to the same extent that more authentic research experiences do (Brownell et al., 2012; Olimpo et al., 2016). While there are a vast number of ways a CURE can be run (Wei & Woodin, 2011), iterative laboratory activities increase student resilience and understanding of the scientific method (Gin et al., 2018; Light et al., 2019), while also encouraging feelings of student ownership of the project (Corwin et al., 2018). It is important to remember, however, that increased feelings of ownership are also seen in undergraduate research more broadly (Hanauer & Dolan, 2014; Cooper et al., 2019; Esparza et al., 2020).

Incorporating aspects of a research experience into multiple laboratory courses may increase the likelihood that students will take one of these courses earlier in their undergraduate career, thereby leveling student access to research (Bangera & Brownell, 2014). When students are exposed to these research experiences earlier in their education, they graduate at a higher rate than their peers; likewise, they are more apt to finish a degree in science (Graham et al., 2013; Rodenbusch et al., 2016; Indorf et al., 2019). Ultimately, even when students cannot take more than one of the associated courses, they benefit from the cross-curriculum nature of expansive CUREs by allowing them to encounter meaningful research experiences in courses that interest them. Providing multiple CURE offerings around the same general focus has had success at Georgia State University, though their project required an increased laboratory budget overall (Parks et al., 2020), which may be less feasible at other institutions.

Here, we present a series of related research experiences that were implemented across the biological sciences curriculum at a small liberal arts college, Regis University. By threading a line of inquiry throughout a variety of undergraduate research courses, we were able to make real headway on a research question from multiple vantage points using novel methods, and students had an opportunity to feel true ownership of the project by encountering it in multiple courses. Students in microbiology, genetics and genomics, molecular and cellular biology, and research methods laboratory courses all worked on this research project in some way. Additionally, students participating in independent research with

The American Biology Teacher, Vol. 83, No. 7, pp. 474–478, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2021.83.7.474.

the associated professors also had the opportunity to work on this project in a more specialized, apprenticeship fashion.

○ Coursework

Microbiology

This multicourse CURE began in the microbiology lab, an upper-level elective composed of a mix of sophomores, juniors, and seniors. Generally, we have two lab sections enrolling around 20 students each, taught by a single professor. For this module, students self-select into groups of three to five, depending on the number of students in the course section. For any given section, there are four or five groups, each using soil samples from a different location.

The module used in this lab followed the previous well-designed and implemented project "Assessing the Prevalence of Antibiotic Resistance in the Environment," often referred to as PARE (Genné-Bacon & Bascom-Slack, 2018). This particular CURE is accessible to students and instructors at large public institutions as well as two-year colleges (Genné-Bacon et al., 2020) and can take approximately three two-hour lab sessions to complete.

The goal of the microbiology module is to identify multidrug-resistant bacteria from environmental soil samples, while introducing students to the actions of antibiotics and mechanisms of resistance. Once isolated, broth cultures of antibiotic-resistant bacterial colonies were grown for use in the molecular biology portion of the CURE. Any or all of the bacterial isolates identified in this portion could be used in the downstream modules described below, depending on class size and interest. For our purposes, three isolates were used in the other courses; these isolates had the greatest level of antibiotic resistance from all microbiology labs in the particular semester.

The occurrence of antibiotic-resistant bacteria in soil samples is variable and depends on a variety of factors. Generally, each group finds at least one antibiotic-resistant colony after the first week of the module. If a group does not have resistant bacteria, they share samples with another group that had success. This allows all students to continue the process without starting over.

Molecular Biology

The molecular biology lab is an elective course that is cross-listed with our neurobiology and biochemistry majors as an optional lecture and lab. The course generally enrolls eight to 12 students and consists almost entirely of seniors, with one or two juniors every year. The lab students are separated into pairs and each pair is given one of the three bacterial isolates.

The stated learning objective of the lab is to familiarize students with basic techniques involving DNA, RNA, and proteins. In this course, as part of the DNA module, students worked to extract the genome of the three bacterial species isolated from local soil, prepare the DNA for high-throughput genomic sequencing, and analyze the results. This allowed students to understand the connection between fundamental molecular biology techniques like DNA extraction and modern approaches to molecular biology like genomics, sequencing, and bioinformatics.

Genomics and high-throughput sequencing can seem unapproachable to both professors and students at the undergraduate levels (Whitley et al., 2020). However, as the cost of sequencing decreases, the need for scientists who are comfortable generating and analyzing high-throughput sequencing is increasing. Further, the rapid decrease in cost has made high-throughput sequencing affordable in undergraduate labs. As an example, we spent a total of $200 on actual sequencing and $200 on reagents, for a total of $40 per student for a five-week lab module. To date, there has been a small number of larger, collaborative genomic projects at the undergraduate level (Jordan et al., 2014; Hanauer et al., 2017), but our project aims to demonstrate the ability to integrate genomics at a smaller, modular level.

Below is a week-by-week breakdown of the laboratory activities (Table 2). As part of the lecture portion of the course, we had already gone over the theory behind DNA isolation, ligation, and a basic introduction to library preparation. Students first isolated genomic DNA, then fragmented the genome for high-throughput library preparation, ligated adapters with barcodes followed by submission for sequencing at a core facility, and then analyzed the output using a bioinformatics pipeline. For a more detailed protocol, see the Supplemental Material available with the online version of this article.

Table 1. Microbiology module schedule.

Week	Activity	Description & References
1	Soil dilutions and spread plates	Serial dilutions of soil samples were prepared and plated on agar with or without Tetracycline (Genné-Bacon & Bascom-Slack, 2018).
2	Kirby-Bauer disk diffusion assay	Percentage of Tet-resistant bacteria was calculated; resistant colonies were selected for further multidrug testing using a disk diffusion assay.
3	Determination of multidrug resistance	The zones of inhibition were measured and antibiotic resistance was determined.

Table 2. Molecular biology module schedule.

Week	Activity	Description & References
1	Genomic isolation	DNA was isolated from bacteria using the DNeasy PowerSoil Kit (Qiagen).
2	DNA fragmentation	DNA was fragmented for library preparation.
3	Library prep day 1	Fragmented DNA was end-prepped and A-tailed in preparation for adapter ligation, followed by cleanup using magnetic beads.
4	Library prep day 2	Adapters were ligated and final PCR was performed, followed by cleanup using magnetic beads.
5	Bioinformatics and poster presentation	Once the sequencing results returned, students used BLAST (Altschul et al., 1990) and InterPro (Mitchell et al., 2018) to annotate function of antibiotic resistance genes.

Genetics & Genomics

The genetics and genomics laboratory course is required for all biology majors, meaning that all graduates will have some introduction to this research question by the time they graduate. The course is generally a mix of sophomores, juniors, and seniors. Typically, there are two sections, with 24 students each. For this module, students self-select into pairs at the beginning of the project. Each week, if pairs fail to get results, they are absorbed by another pair group. By the end of the module, groups tend to be four to six students; because of the collapsing of groups, the groups naturally end up being a mix of stronger and weaker students. Because this course covers both genetics and genomics, it is taught by two instructors in rotation.

In this course, the students worked to identify bacteria that had lost their antibiotic resistance – and to identify the genes behind that resistance – through transposition mutagenesis, tagging, and Sanger sequencing. This allowed the students to learn multiple valuable genetics and genomics techniques, such as sequence analysis, DNA ligation, and transposon insertion, while also contributing to the research field.

Importantly, by using transposon mutagenesis and sequencing, we were able to have students mutagenize their bacteria without using potentially dangerous methods. The materials were relatively cheap, and the safety of this protocol makes it highly desirable for an undergraduate lab. Transposon mutagenesis has been used successfully in the undergraduate classroom (White et al., 2011; Davis et al., 2017). However, to the best of our knowledge, we are the first to report using the *MmeI* restriction digest-based transposon-sequencing (Tn-seq) method (van Opijnen et al., 2009; van Opijnen & Camilli, 2013) in the undergraduate lab, though we modified the procedure to use Sanger sequencing because of our very focused questions and limited budget. Additionally, because Tn-seq does not require a separate cloning step into a bacterial vector, the time required for the CURE was drastically reduced.

Table 3 provides a week-by-week breakdown of the laboratory activities. Students had already learned pipetting and the biology behind transposons in earlier weeks of the course. Very broadly, the students, working in groups of two or three, performed transposon mutagenesis as previously described (Wiles et al., 2013) and sequenced the interrupted antibiotic resistance genes using an *MmeI* restriction digest-based method (van Opijnen et al., 2009). The final column of Table 3 lists papers that we had students read and analyze during incubation periods. For a more detailed weekly protocol, please see the Supplemental Material.

Research Methods Course

Several students were enrolled in both genetics and genomics lab and an upper-level research methods course during the same semester. Those students first learned the techniques in the research methods lab and were able to lead their group-mates through the

Table 3. Genetics and genomics module schedule.

Week	Activity	Description & References	Associated Paper
1	Bacterial conjugation	Student-obtained bacteria were conjugated with pSam-Ec for transposon insertion (Wiles et al., 2013); conjugating plates were stored at 4°C after five-hour incubation at 37°C.	Gene recombination in *Escherichia coli* (Lederberg & Tatum, 1946)
2	Conjugant collection and plating	Conjugants were collected from the filter and plated on LB + Kanamycin plates.	In vivo transposition of *mariner*-based elements in enteric bacteria and mycobacteria (Rubin et al., 1999)
3	Replica plating	Colonies were replica plated to chosen LB agar + antibiotic plates to test for lost resistance.	
	Colony selection	Bacterial colonies from the original plates that had lost antibiotic resistance were selected to grow as overnight cultures in LB broth. *Performed outside of class time.*	
4	DNA prep and restriction digest	DNA was prepared using a standard kit and digested using the restriction enzyme *MmeI* (van Opijnen & Camilli, 2013).	
5	Tag ligation and PCR	A tag was ligated to the end of each digest product using T4 DNA ligase to allow for sequencing. Primers for the Kanamycin cassette and the tag were used to amplify the intervening region, and the PCR products were sent for Sanger sequencing.	
6	Sequence analysis	The sequences were analyzed using BLAST (Altschul et al., 1990) to identify genes conferring antibiotic resistance to the bacteria.	

techniques in the genetics and genomics lab. This was a boon for lab instructors, but it also allowed dually enrolled students to act as team leaders in the other course. As the genetics and genomics lab was finishing the project, students in this research methods class were able to carry on with the project and see it progress further. As an example, students enrolled in this course screened the mutagenized bacteria against multiple antibiotics, with a goal of identifying individual mutations that abrogated resistance to more than one type of antibiotic. In this way, the two courses reinforced and expanded the students' experience.

Independent Research

Interested students could easily continue working with the bacteria from the lab sections in an independent research project in a traditional apprenticeship model. Several students continued research on bacteria isolated in the microbiology lab, including projects to screen for multidrug resistance past that of the lab course and assaying the growth of isolates in different concentrations and types of heavy metals. Such projects, after having completed the microbiology lab, can be relatively self-directed by an enterprising and motivated student.

○ Summary

Over the course of a year and a half, we integrated a multicourse CURE across four lab courses alongside independent research. This project began as a single module in a microbiology lab and expanded to encompass techniques in molecular biology, genetics, and genomics. It gave more than 100 students experience in implementing lab-based research and will lead to at least 11 students getting their names on publications, including one first-author paper in preparation. Six students participated across a variety of three lab courses and were able to see the entire scientific process from inception to screening for phenotypes, while another 16 were able to participate in two of the associated lab courses. This iteration allowed students to see how different disciplines would tackle the same question. This CURE shows the potential for an integrative biology curriculum with a research focus.

The integrative, multidisciplinary nature of this CURE should allow any single section to be implemented at another institution in any of the three disciplines: microbiology, molecular biology, and genetics/genomics. For microbiology, the rise of antibiotic-resistant bacteria has become a major public health problem and their persistence in the environment is an open question (Genné-Bacon & Bascom-Slack, 2018). The cost of isolating and characterizing environmental isolates is minimal and represents the most accessible part of this CURE. For molecular biology, in order to understand what is driving the rise in antibiotic resistance, the genome of these bacteria needs to be sequenced. The rapid decrease in the cost of high-throughput sequencing has created a need for scientists who have experience with sequencing and analysis, and it is imperative that we introduce this relatively cheap procedure into the training of biology majors. This was the most technically challenging portion of the CURE and likely would be challenging to scale up to a class larger than 20 students. For genetics, the final class that participated in this CURE, we took a classical genetic screen approach to understanding antibiotic resistance, but with a modern twist; by using Tn mutagenesis, we were able to minimize risk to students while still allowing them to participate in a fundamental genetic technique. Each of these different modules could be used either in combination with each other or separately.

The three courses are either taught by individual instructors (such as the microbiology and molecular biology labs) or team-taught by two instructors, both of whom contributed to this CURE (the genetics and genomics lab). Although this has been implemented in a small department, there is no obvious reason why it could not be expanded for use at larger institutions with multiple instructors and sections per module. Each module can be run independently, and only a background knowledge of the other modules is necessary for both instructors and students.

During the course of the year, as we first implemented the CURE, we encountered several issues that leave room for improvement in future cycles. First, this CURE came about organically, as the instructors in charge of the courses found common research interests that would provide students a feel for real research. Since this was initially developed as an interesting project for students, rather than established as a pedagogical research process, we did not seek to measure student outcomes across the CURE or in individual modules. In the future, we plan to demonstrate measurable learning outcomes, especially for those students who had participated in multiple parts of the CURE. Secondly, we observed that as part of the longer genetics and genomics lab, there were striking gaps in students' memory and understanding of the goals of the lab module. By the final lab in this module, many students had forgotten earlier components of the module, noticeably the most important part: the underlying importance of understanding antibiotic resistance. We plan to rectify this gap by having more consistent lab quizzes throughout the module and emphasizing the connection of each single week to the overall goals. Additionally, a single, coherent lab manual for the CURE portion of each teaching lab, separate from the rest of the lab manual, may help to reinforce the scientific reason for each CURE module. Finally, there were some failures and setbacks during various modules as part of the CURE. This is ultimately an accurate representation of how lab-based inquiry proceeds, but students who are used to packaged lab experiments experienced frustration when their work did not yield results or got contaminated. However, we know that failure is an integral part of science and has a number of benefits even in the classroom (Gin et al., 2018), and we plan to better prepare students for failure and to help them understand that science is not a linear path. Ultimately, we believe that each of these issues represents opportunity; the learning outcomes can be measured, the gaps can be used to create a better lab experience, and the failures can be used to show students what science really looks like.

○ Acknowledgments

We thank Drs. Jay Campisi, Ashley Fricks-Gleason, and Conner Sandefur for guidance in writing the manuscript; all the students who participated in our labs; Dr. Tim Loveless for his design work on the header image of this article; and Regis University's URSC committee for partially funding this research.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215,* 403–410.

Bangera, G. & Brownell, S.E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE–Life Sciences Education, 13,* 602–606.

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*(4), 36–45.

Cooper, K.M., Gin, L.E., Akeeh, B., Clark, C.E., Hunter, J.S., Roderick, T.B., et al. (2019). Factors that predict life sciences student persistence in undergraduate research experiences. *PLoS ONE, 14*(8).

Corwin, L.A., Runyon, C.R., Ghanem, E., Sandy, M., Clark, G., Palmer, G.C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE–Life Sciences Education, 17*(2).

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2017). Antibiotic discovery throughout the Small World Initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *MicrobiologyOpen, 6*(3).

Esparza, D., Wagler, A.E. & Olimpo, J.T. (2020). Characterization of instructor and student behaviors in CURE and non-CURE learning environments: impacts on student motivation, science identity development, and perceptions of the laboratory experience. *CBE Life Sciences Education, 19*(1).

Genné-Bacon, E.A. & Bascom-Slack, C.A. (2018). The PARE Project: a short course-based research project for national surveillance of antibiotic-resistant microbes in environmental samples. *Journal of Microbiology & Biology Education, 19*(3).

Genné-Bacon, E.A., Wilks, J. & Bascom-Slack, C. (2020). Uncovering factors influencing instructors' decision process when considering implementation of a course-based research experience. *CBE–Life Sciences Education, 19*(2).

Gin, L.E., Rowland, A.A., Steinwand, B., Bruno, J. & Corwin, L.A. (2018). Students who fail to achieve predefined research goals may still experience many positive outcomes as a result of CURE participation. *CBE–Life Sciences Education, 17*(4).

Graham, M.J., Frederick, J., Byars-Winston, A., Hunter, A.B. & Handelsman, J. (2013). Increasing persistence of college students in STEM. *Science, 341,* 1455–1456.

Hanauer, D.I. & Dolan, E.L. (2014). The project ownership survey: measuring differences in scientific inquiry experiences. *CBE–Life Sciences Education, 13,* 149–158.

Hanauer, D.I., Graham, M.J., Betancur, L., Bobrownicki, A., Cresawn, S.G., Garlena, R.A., et al. (2017). An inclusive Research Education Community (iREC): impact of the SEA-PHAGES program on research outcomes and student learning. *Proceedings of the National Academy of Sciences USA, 114,* 13531–13536.

Harrison, M., Dunbar, D., Ratmansky, L., Boyd, K. & Lopatto, D. (2011). Classroom-based science research at the introductory level: changes in career choices and attitude. *CBE–Life Sciences Education, 10,* 279–286.

Hunter, A.B., Laursen, S.L. & Seymour, E. (2007). Becoming a scientist: the role of undergraduate research in students' cognitive, personal, and professional development. *Science Education, 91*(1), 36–74.

Indorf, J.L., Weremijewicz, J., Janos, D.P. & Gaines, M.S. (2019). Adding authenticity to inquiry in a first-year, research-based, biology laboratory course. *CBE–Life Sciences Education, 18*(3).

Jones, M.T., Barlow, A.E.L. & Villarejo, M. (2010). Importance of undergraduate research for minority persistence and achievement in biology. *Journal of Higher Education, 81,* 82–115.

Jordan, T.C., Burnett, S.H., Carson, S., Caruso, S.M., Clase, K., DeJong, R.J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *MBio, 5*(1).

Lederberg, J. & Tatum, E.L. (1946). Gene recombination in *Escherichia coli*. *Nature, 158,* 558.

Light, C.J., Fegley, M. & Stamp, N. (2019). Emphasizing iterative practices for a sequential course-based undergraduate research experience in microbial biofilms. *FEMS Microbiology Letters, 366*(23).

Lopatto, D. (2004). Survey of Undergraduate Research Experiences (SURE): first findings. In *Cell Biology Education, 3,* 270–277.

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

Mitchell, A.L., Attwood, T.K., Babbitt, P.C., Blum, M., Bridge, A., Brown, S.D., et al. (2018). InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Research, 47,* D351–D360.

Olimpo, J.T., Fisher, G.R. & Dechenne-Peters, S.E. (2016). Development and evaluation of the tigriopus course-based undergraduate research experience: impacts on students' content knowledge, attitudes, and motivation in a majors introductory biology course. *CBE–Life Sciences Education, 15*(4).

Parks, S., Joyner, J.L. & Nusnbaum, M. (2020). Reaching a large urban undergraduate population through microbial ecology course-based research experiences. *Journal of Microbiology & Biology Education, 21*(1).

Rodenbusch, S.E., Hernandez, P.R., Simmons, S.L. & Dolan, E.L. (2016). Early engagement in course-based research increases graduation rates and completion of science, engineering, and mathematics degrees. *CBE–Life Sciences Education, 15*(2).

Rubin, E.J., Akerley, B.J., Novik, V.N., Lampe, D.J., Husson, R.N. & Mekalanos, J.J. (1999). In vivo transposition of mariner-based elements in enteric bacteria and mycobacteria. *Proceedings of the National Academy of Sciences USA, 96,* 1645–1650.

Russell, S.H., Hancock, M.P. & McCullough, J. (2007). Benefits of undergraduate research experiences. *Science, 316,* 548–549.

Seymour, E., Hunter, A.B., Laursen, S.L. & Deantoni, T. (2004). Establishing the benefits of research experiences for undergraduates in the sciences: first findings from a three-year study. *Science Education, 88,* 493–534.

Shortlidge, E.E., Bangera, G. & Brownell, S.E. (2017). Each to their own CURE: faculty who teach course-based undergraduate research experiences report why you too should teach a CURE. *Journal of Microbiology & Biology Education, 18*(2).

Stanford, J.S., Rocheleau, S.E., Smith, K.P.W. & Mohan, J. (2017). Early undergraduate research experiences lead to similar learning gains for STEM and non-STEM undergraduates. *Studies in Higher Education, 42,* 115–129.

van Opijnen, T., Bodi, K.L. & Camilli, A. (2009). Tn-seq: high-throughput parallel sequencing for fitness and genetic interaction studies in microorganisms. *Nature Methods, 6,* 767–772.

van Opijnen, T. & Camilli, A. (2013). Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. *Nature Reviews Microbiology, 11,* 435–442.

Wei, C.A. & Woodin, T. (2011). Undergraduate research experiences in biology: alternatives to the apprenticeship model. *CBE–Life Sciences Education, 10,* 123–131.

White, A.K., Renfro, B.M. & Ault, J.F. (2011). Using a molecular-genetic approach to investigate bacterial physiology in a continuous, research-based, semester-long laboratory for undergraduates. *Journal of Microbiology & Biology Education, 12,* 185–193.

Whitley, K.V., Tueller, J.A. & Weber, K.S. (2020). Genomics education in the era of personal genomics: academic, professional, and public considerations. *International Journal of Molecular Sciences, 21*(3).

Wiles, T.J., Norton, J.P., Russell, C.W., Dalley, B.K., Fischer, K.F. & Mulvey, M.A. (2013). Combining quantitative genetic footprinting and trait enrichment analysis to identify fitness determinants of a bacterial pathogen. *PLoS Genetics, 9*(8).

BETHANY LUCAS, GENA NICHOLS, and MAX BOECK are Assistant Professors in the Department of Biology, Regis University, Denver, CO 80226; e-mail: blucas001@regis.edu, gnichols@regis.edu, mboeck@regis.edu.