CRISPR-Cas Technology In and Out of the Classroom

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Abstract
Student-centered practices, including student-focused research opportunities, enhance biology education and comprehension. One way to support student interest is through research opportunities in faculty laboratories. However, alternatives to traditional research apprenticeships are important for the inclusion of more undergraduate students in CRISPR-Cas–based research. Student interest in CRISPR-Cas technologies serves as a timely focal point for deepening undergraduate student engagement in biology courses. In this article, we describe some of the ongoing efforts to bring CRISPR-Cas technology out of the classroom and into the teaching laboratory.

Introduction
The most effective biology teaching actively engages students in their classrooms and coursework through group-work, activities, and discussion, which all access higher-order thinking and problem-solving skills. While student-centered classroom teaching, including reading primary literature articles, can enhance students’ understanding of complex biological topics, an additional way to bring undergraduate students into the discussion is through research opportunities outside of the traditional lecture or seminar classroom.

The widespread interest in CRISPR* and its subsequent use as a technological tool in concert with Cas nucleases provides a direct link between student interest and biology instruction. In-depth conversations about the biochemical and ethical nuances of CRISPR-Cas–based technologies are topics that undergraduate biology students gravitate toward, in part because of the widespread “user-friendly” descriptions of CRISPR-Cas technology (i.e., precise genome editing and gene expression modulation) that are available through the news and social media. Educators are therefore in a powerful position to help counteract misconceptions or biases that students may bring to the classroom by engaging students in CRISPR-Cas–based research programs. Formal discussions of how the CRISPR-Cas systems were discovered and how they are being used, exploited, and modified ensures that graduates from undergraduate institutions in biology-related departments and programs can enhance the discourse around genetic engineering and genomic technologies.

In this essay, we describe a variety of approaches that are currently being used to address the scientific basis, limitations, and misunderstandings surrounding CRISPR-Cas technologies by bringing students beyond lectures or interactive classroom activities. While our discussion here largely represents published or publicized work, we are aware that many instructors are currently carrying out this work at diverse institutions. We specifically hope that this essay will inspire others to bring discussions of CRISPR-Cas technology to as-yet unserved or underserved undergraduate populations. We highlight novel implementations of CRISPR-Cas technology in laboratory-based courses, including ongoing projects, though we also suggest ideas for potential educational collaborations.

Faculty Research Opportunities
Introducing undergraduate students to research through faculty laboratory experiences increases the students’ sense of scientific identity and constitutes an especially powerful learning tool for students from traditionally underrepresented groups. Students who participate in research programs before graduation often have increased comprehension of biological concepts and self-report higher confidence in their science learning. Moreover,
they often receive focused training in the field of their choice, introductions into the language and culture of science, and increased access to mentorship. Thus, student excitement around CRISPR and CRISPR-based technologies adds another layer of possibility to research experiences: the chance to learn—first-hand—how cutting-edge science is done and how it is evolving.

There are many models that offer research experiences to currently enrolled undergraduate students, including intensive training opportunities funded by the National Science Foundation, National Institutes of Health, and Howard Hughes Medical Institute. These programs place students in active research laboratories providing them opportunities to participate (often over the course of 10 weeks during the summer) in potentially high-profile, cutting-edge research. In particular, a growing number of these programs allow students to interact with technologies, such as CRISPR-Cas, as part of their research projects. Other examples of intensive, non-academic research opportunities that are putting increased focus on having students use CRISPR-Cas technologies include outreach initiatives run by private industries (e.g., Jackson Laboratories), nonprofit organizations (e.g., Innovative Genomics), and competitive university partnerships (e.g., International Genetically Engineered Machine). These programs tend to be selective (requiring individual or team applications), but they provide participants with the chance to build or be a part of a student-driven research program. For individuals with the mentorship and/or other support to participate, intensive training programs such as those funded by federal or other sources are invaluable for understanding the fundamental principles behind as well as the research being done with CRISPR-Cas systems. Moreover, they provide undergraduate students with the opportunities to use the technologies to solve emerging problems.

Course-Based Undergraduate Research Experiences

Because research opportunities for undergraduate students promote retention in STEM fields and solidify STEM identities, many educators are introducing their own students to scientific research at their home institutions. One strategy is through the introduction of Course-Based Undergraduate Research Experiences (CUREs) into undergraduate curricula. CUREs often have students plan and execute a series of experiments that are directed at solving a scientific problem, sometimes in direct connection with the research interests of the faculty teaching the course. Often, these research projects are a quarter, semester, or year in length and guide students through one or more aspects of a larger research project. In some cases, these experiences can be used to generate both laboratory reagents and data as well as data pertaining to student outcomes resulting from the implementation of CUREs. Table 1 lists a selection of recent courses that have been established to take advantage of student and community interest in CRISPR-Cas technology by engaging undergraduate students in CRISPR-Cas–based CUREs.

Given student interest and the increased need to communicate cutting-edge research to undergraduate students, colleges and universities have begun instituting CRISPR-Cas–related CUREs with great success. Part of the reason why CUREs and other forms of course-based research that utilize CRISPR-Cas technologies are successful is due to the current curricula in molecular biology-based laboratory courses at diverse academic institutions, particularly four-year colleges and universities. Undergraduate students with molecular biology interests often take laboratory-based courses that teach them how to perform polymerase chain reactions (PCRs), genomic and plasmid DNA extractions, plasmid design and construction, restriction digests, primer design, and the techniques necessary to create transgenic organisms (i.e., *Escherichia coli*, *Caenorhabditis elegans*, etc.). These molecular techniques are valuable resources for undergraduates entering the biomedical or biotechnology workforce, but they also provide a preexisting framework that easily lends itself to instituting CRISPR-Cas–based CUREs. Such CUREs can be an exciting opportunity for undergraduate students to hone their previous knowledge of molecular biological techniques by engaging in CRISPR-Cas research firsthand. Thus far, no institution has reported a CRISPR-Cas–based CURE that transitions students in CRISPR-Cas technology from start to finish, likely due to time constraints. However, a limited scientific scope can still provide sufficient rationale for student work. There is increasing interest in developing CUREs that incorporate CRISPR-Cas technologies. Readers who are inspired to develop their own CUREs using CRISPR-Cas may be interested in workshops such as the one offered in advance of the 2018 Association for Biology Laboratory Education (ABLE). These workshops help participants hone ideas and plan curricula in hands-on, collaborative forums.

Postsecondary institutions that have implemented a CRISPR-Cas research component into their preexisting coursework report increased student engagement. For example, a collaboration between Rollins College and Stetson University has led to an ongoing CRISPR-based CURE with students reporting better understanding of the possible applications of using plasmid-editing tools to study multifaceted issues in molecular biology (J. Pieczynski, Rollins College, H. L. Kee, Stetson University, personal communication). CUREs can also feed directly into faculty research, where instructors will use their

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3http://www.ableweb.org/conferences/able2018/pre-conference-workshop-on-integrating-crispr-cas9-into-the-undergraduate-classroom/
| Institution(s) | Lead instructor | Course type | Biological system or model organism | Reported student activities and/or prerequisites | Citation |
|---------------|----------------|-------------|-------------------------------------|-----------------------------------------------|----------|
| Davidson College  
*Genetic Editing* | Dr. Rachid El Bejjani | Lecture/lab seminar/ “dry lab” | *Caenorhabditis elegans* | • Critical reading of primary literature  
• Sequence analysis  
• Plasmid design  
• Phenotypic assays of engineered *C. elegans* strains | 28 and personal communication |
| Haverford College, University of Pennsylvania | Dr. R. Jain, Dr. M. Granato | Independent research | *Danio rerio* | • Behavior and development assays | 25 |
| Stetson University, *Genetics*  
Rollins College, *Molecular Biology* | Dr. H. Lynn Kee,  
Dr. Jay Pieczynski | Lecture/lab | *Caenorhabditis elegans* | • Plasmid design  
• Plasmid engineering | Personal communication |
| State University of New York, Geneseo  
*Genome Analysis*  
*Molecular Techniques* | Dr. Kevin Militello | Lab only | *Escherichia coli* | • DNA sequencing and analysis  
• Genomic DNA extractions  
• PCR | 24 |
| University of Colorado  
*Molecular Biology Lab* | Dr. Christopher Phiel | Lecture/lab | Mammalian tissue culture | • A semester of preparatory lectures is required  
• Plasmid design and engineering  
• Tissue culture transfection  
• DNA sequencing and analysis  
• Genomic DNA extractions  
• Bacteriophage lytic infection  
• Students generate valuable bacterial strains with multiple potential uses | 27 |
| Université Laval  
*Laboratoire de physiologie et de génétique microbiennes*  
(*Physiology and Genetics of Microbiology Laboratory*) | Dr. Sylvain Moineau | Lecture/lab | *Streptococcus thermophiles* | | 21,22 |
| University of New Mexico  
*Genome Editing* | Dr. Richard Cripps | Lecture/lab | *Drosophila melanogaster* | • Plasmid design  
• Plasmid engineering | 3 |
| Western Washington University  
*Methods in Molecular Biology* | Various, including  
Dr. Lina Dahlberg,  
Dr. Anna Groat Carmona | Lecture/lab | *Caenorhabditis elegans* | • Plasmid design  
• PCR | Personal communication |

*Course titles are italicized.*  
*Housed in the Integrative Biology department.*
laboratory courses to bolster discussions of the CRISPR-Cas mechanism and/or the process of science. Davidson College (Davidson, NC) recently introduced a course that explores the technical literature on genome editing and its current uses. There are two variations of the course, one in which primary literature instruction accompanies a series of experiments that allow students to engineer plasmids for genome editing in C. elegans using CRISPR-Cas technology, the other focuses on sequence analysis, experimental design, and plasmid design in silico. The plasmids and experiments that result from these courses have been used to engineer transgenic worms for research purposes in Dr. Rachid El Bejjani’s laboratory (Davidson College, Davidson, NC). The University of Colorado, Denver offers a similar course model. After taking a semester of preparatory lectures, students can participate in a laboratory-based course to introduce CRISPR-Cas into mammalian tissue culture cells as part of Dr. Christopher Phiel’s research into the roles of glycogen synthase kinase 3 in mammalian stem cells and epigenetics. At the University of New Mexico, Albuquerque, students design the CRISPR RNA guides that will eventually be used to knock out an assigned gene of interest in Drosophila. Importantly, although Dr. Richard Cripps only just introduced this course in 2015, he has since published his laboratory-based course model, including the data generated by his students during the course.

Some CUREs allow faculty researchers to generate valuable tools and/or reagents that can be used in independent research projects for students within the faculty member’s laboratory. For instance, Dr. Kevin Militello at the State University of New York, Geneseo recently published a paper illustrating the power of his laboratory-based course model wherein students examined the CRISPR loci of uncharacterized E. coli strains. Students were asked to isolate genomic DNA from various E. coli strains, amplify the CRISPR locus, and then run DNA sequence analyses in order to identify previously uncharacterized CRISPR loci that can be studied in greater detail later in the future. The Université Laval (Quebec City, Canada) has also used CRISPR-Cas–based CUREs to further examine the complexities of the CRISPR-Cas system. Dr. Sylvain Moineau’s CRISPR-Cas–based CURE investigates natural CRISPR-Cas immunization in Streptococcus thermophilus and introduces students to how bacteria can expand their CRISPR array in response to challenge with virulent bacteriophage. Students can utilize lytic phage infection models and screen surviving S. thermophilus cultures for changes in their CRISPR locus using PCR and DNA sequencing. The benefit of this multifaceted CURE is that it offers many variations with many possible avenues for future exploration since students are generating valuable bacterial strains with multiple potential uses, and indeed this sort of discovery by research laboratories has been particularly important in for the dairy industry. A variety of student products from CUREs can therefore contribute to ongoing faculty research programs.

Qualitative and quantitative assessments show that undergraduate students leave CUREs that focus on CRISPR with a better understanding of the principles, research concepts, and applications of CRISPR-Cas technology. Because CUREs that utilize CRISPR-Cas technology are relatively new, there is limited information about how these academic gains translate into student career choices or persistence in scientific (and particularly those involving genetic engineering) fields. However, as the availability of CRISPR-Cas–based CUREs increases, more information regarding the benefits to students will likely become available. Beyond undergraduate engagement, Simon Levien recently published an article with the New Jersey Science Teachers Association that explored some of the benefits of exposing high school students to CRISPR-Cas systems using CUREs, even suggesting low-budget means of introducing the subject matter in advanced placement courses. Mr. Levien’s post regarding the impact that CRISPR-based CUREs have on high school students reflects some of the preliminary data regarding student outcomes resulting from implementing CRISPR-Cas–based CUREs. In light of the prevalence of CRISPR-Cas technology in the scientific community, offering high school students and undergraduates an opportunity to take part in CRISPR-Cas–based CUREs can also have a lasting benefit once students graduate from college and enter the workforce.

Course-Based Research and Institutional Collaborations
Research opportunities, including CUREs, require intensive planning and can be difficult to implement or change at large research institutions, or at institutions with short instruction periods (i.e., academic quarters). However, peripheral interactions with research (in the form of collaborations or contributions) can still provide undergraduate students with a meaningful and flexible way to work as researchers without requiring the planning and implementation of an entire CURE. Scientific collaborations and undergraduate participation in science communication projects can provide another mechanism for students to contribute to both the scientific and lay communities.

A collaboration between researchers at the University of British Columbia (UBC; Vancouver) and at Western Washington University (WWU; Bellingham, WA) provides an example of how collaboration-based research...
projects can introduce students to CRISPR-Cas technology without requiring a new or fully redesigned course model (Fig. 1A). Students in WWU’s molecular biology laboratory course use a variety of molecular techniques in an 11-week introduction to plasmid design and genetic engineering. In addition to being introduced to concepts relating to genome editing, students in this course become accustomed to isolating genomic DNA from various samples (both plants and bacteria) in order to genotype genetically modified organisms using PCR. This course was easily adapted so students use PCR to genotype *Caenorhabditis elegans* strains that are generated as part of a large-scale CRISPR-Cas initiative undertaken at UBC.34 While the WWU students do not design the plasmids, or inject the animals, they design and run genotyping PCRs to determine whether the CRISPR-Cas mediated gene disruption conducted at UBC was successful. This collaborative project gives the students an opportunity to learn about the components that are required for CRISPR-Cas mediated genome editing as well as providing an avenue to ongoing research. As a final project, the students return their annotated genotyping data to the UBC laboratory, which serves as a quality control measure for the *C. elegans* research community at UBC (Fig. 1B). Thus, research opportunities involving CRISPR-Cas technology can be successfully implemented in preexisting laboratory courses, and these efforts can be used to foster collaborations between institutions.

**Measuring Success**

Genetic engineering is not trivial, and the molecular techniques required to engineer a transgenic organism depend on knowledge of complex biological systems along with practice to ensure reproducible success. Students engaged in CRISPR-Cas–based CUREs are able to engage in small research projects (Table 1); however, these projects may be left unfinished at the completion of the
course. While incomplete projects may not provide the closure that traditional laboratory courses might, they give realistic insight into the scientific experience, which can prompt students to seek additional research opportunities outside of the classroom. Although data collection on the outcomes of CRISPR-Cas–based CUREs is ongoing, instructors report increased student learning and engagement after implementing CRISPR-Cas–based CUREs using various metrics.\textsuperscript{21–26} Academic institutions have only recently started using CRISPR-Cas–based CUREs in their coursework; with increased interest in CRISPR-Cas–based CUREs, more rigorous studies on student achievement will undoubtedly help inform future activities at more institutions.

### Additional Mechanisms for Student Engagement in CRISPR-Cas Technology

Scientific and other publications are also an accessible entry point for student participation in CRISPR-Cas technology without requiring a redesigned course or ongoing faculty research. We highlight three different examples to illustrate potential avenues (blogs, websites, and peer-reviewed publications) for student publication and research dissemination. Haverford College (Philadelphia) students who engaged in summer independent research (Table 1) had the opportunity to write about their experiences conducting independent research via the university’s “Speaking of Science” blog.\textsuperscript{26} Similarly, students from Tufts University (Medford, MA) created and published a publicly accessible website on the mechanisms of CRISPR-Cas modification and DNA repair.\textsuperscript{35} While this website is not currently maintained, it is a clear example of students synthesizing their knowledge into a reader-friendly, factually accurate publication. One feature of this website is the comments section, which serves to provide accolades as well as critical feedback to the authors. Finally, many institutions have student-managed newspapers or other campus publications that can be accessed by potential matriculating students. Utilizing a model similar to those used at Haverford College and Tufts University, students engaged in scientific inquiry could have the opportunity to report their findings or experiences in undergraduate journalism projects to help promote student engagement and course enrollment and provide perspective to the challenges of engaging in student research.

Students can also publish their data from CRISPR-Cas projects in peer-reviewed journals that are geared toward emerging scientists. For example, the Journal of Young Investigators\textsuperscript{3} and Frontiers for Young Minds** publish perspective pieces as well as student research projects. These venues cater to undergraduate (and younger) writers while introducing them to the collaborative and rigorous nature of the peer-review process.

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\textsuperscript{3}www.jyi.org  
\textsuperscript{*https://kids.frontiersin.org
Student interaction with CRISPR-Cas technology before entering college is also possible, and we offer two examples of inquiry- rather than research-based activities for high school students. In one example, students used their high-school biology course meetings (often only 35 minutes per day, several times a week) to plan in vivo and in vitro approaches to replicating Jennifer Doudna’s seminal experiment (highlighted in her iBiology video talk) (Fig. 2A). Students designed strategies to make guide CRISPR RNAs, transform cells, and in the end, specifically cleave the pGLO plasmid (BioRad) containing the sequence for green fluorescent protein (Fig. 2B). In the second example, students used a commercially available kit (Odin) to induce cleavage of an antibiotic resistance gene. While neither of these examples falls under the category of original research, student responses were overwhelmingly positive. Notably, students reported arriving at college with a firm understanding of the mechanisms behind CRISPR-based technologies and of contextualize the social media–based description of “CRISPR babies” in terms of biological and ethical realities (J. Punt, University of Pennsylvania, Philadelphia, PA, personal communication).

In light of this last comment, it is also worth highlighting the need to address ethical concerns in student-centered research endeavors at any level. The ethics of genome editing (including complex ideas such as gene drives and gene therapy) and scientific research (for example, data collection and analysis, collaboration, and dissemination) are most fruitful when students can grasp the complexities, power, and limitations of molecular techniques and scientific practices. By bringing a discussion of ethics into the classroom or laboratory, instructors can help students make connections between their academic studies, scientific progress, and society. As students mature, they can become “ambassadors” for science, perhaps initially among their friends and family. Their expertise can begin to inform discussions based on reporting in the lay media in their own social spheres, and then outward to impact society more generally. Direct student engagement with scientific research and writing can become a bridge to opening evidence-based, fruitful discussions on the ethics regarding CRISPR-Cas technologies specifically, and in scientific research, generally.

“Kitchen Counter” CRISPR

The level of interest in the general public regarding the power of CRISPR-Cas technology is reflected in the flood of news reports, podcasts, magazines, and fictional stories that focus on directed gene editing and its ethics. So-called “kitchen counter” kits for CRISPR-Cas editing, which require no expensive laboratory equipment, are advertised as simple, do-it-yourself opportunities for anyone to work with CRISPR-Cas as gene editing tools (for example, The ODIN). However, while these kits are available to the general public, it is important to note that they are not foolproof. Online reviews from products reflect the fact that even a “failsafe” kit can require troubleshooting. Anecdotally, the previously mentioned high school students were able to recreate a previously published experiment with moderate success (Fig. 2B), but the following year, students found the “kitchen counter” activity more challenging, and did not generate the expected, and advertised, results. As more CRISPR-Cas–based CUREs are developed at academic institutions, the nuances of using this system should be communicated to the general public.

Conclusions

Public and scientific interest in CRISPR-Cas technology and CRISPR-based tools make these fertile areas for student exploration and learning. Research experiences that are dedicated to investigating biological problems using CRISPR-based solutions are already being introduced to undergraduate students through a wide variety of venues. Even more peripheral opportunities, which introduce students to collaborating and communicating, provide valuable information to students and the wider scientific community without requiring entirely new laboratory courses or intensive redesign of existing course. User-friendly explorations of CRISPR-Cas technology through media and kits also suggest that students can begin to engage in meaningful ways with CRISPR-Cas technologies even before they begin their postsecondary careers. Scientific literacy is one of the most important skills undergraduate students gain as they earn their degrees. By working to provide them with research skills that foster conceptual understanding of cutting-edge research techniques, such as CRISPR-Cas, we will encourage undergraduate students to bring critical reasoning to the public discourse of complex scientific and ethical topics.

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