Integrating CRISPR-Cas9 Technology into Undergraduate Courses: Perspectives from a National Science Foundation (NSF) Workshop for Undergraduate Faculty, June 2018

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As CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology becomes more mainstream in life science research, it becomes critical for undergraduate instructors to devise engaging ways to bring the technology into their classrooms. To help meet this challenge, the National Science Foundation sponsored a workshop for undergraduate instructors in June 2018 at The Ohio State University in conjunction with the annual Association of Biology Laboratory Educators meeting based on a workflow developed by the workshop's facilitators. Over the course of two and a half days, participants worked through a modular workflow for the use of CRISPR-Cas9 in a course-based (undergraduate) research experience (CURE) setting while discussing the barriers each of their institutions had to implementing such work, and how such barriers could be overcome. The result of the workshop was a team with newfound energy and confidence to implement CRISPR-Cas9 technology in their courses and the development of a community of undergraduate educators dedicated to supporting each other in the implementation of the workflow either in a CURE or modular format. In this article, we review the activities and discussions from the workshop that helped each participant devise their own tailored approaches of how best to bring this exciting new technology into their classes.

INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology is rapidly becoming commonplace in genetics research laboratories to manipulate genes and genomes to investigate important biological questions. Despite its newfound mainstream presence in research, it has not yet become a standard offering in undergraduate biology courses. To date, CRISPR-Cas9 technology has been successfully integrated in some undergraduate lecture and laboratory curricula (1–5), course-based undergraduate research experiences (CUREs) (6), undergraduate research projects (7), and intensive undergraduate-level workshops (8). However, these reports of CRISPR-Cas9 introductions into undergraduate curricula have been mostly published by highly experienced researchers who have well-established laboratories and research programs in molecular genetics, making accessibility of CRISPR-Cas9 in the undergraduate classroom a persistent issue. The challenges of efforts to include CRISPR-Cas9 in undergraduate biology curricula are likely due to instructors’ inexperience with this new technology and the absence of a straightforward workflow for implementing these techniques in undergraduate biology classrooms at different levels.
To help facilitate easy transfer of CRISPR-Cas9 technology into undergraduate classrooms, a two-and-a-half-day workshop sponsored by the National Science Foundation (Award # 1823595) was held immediately preceding the 2018 Association of Biology Educators (ABLE) Annual Meeting at The Ohio State University. Educators from the spectrum of undergraduate institutions participated in the workshop, including those from two-year colleges, liberal arts institutions, and research universities. Although only 25% of participants had prior experience with CRISPR-Cas9, everyone was prepared to incorporate CRISPR-Cas9 into their undergraduate courses by the end of the workshop. This report summarizes the work done at this workshop, including experimentation with a workflow that can be employed at a variety of institutions and adapted for use in a variety of model organisms as well as ways in which participants could overcome hurdles in CRISPR-Cas9 implementation.

BACKGROUND

The ability to easily target specific genes to knockdown their function or integrate specific mutations in a wide variety of model organisms is now possible with CRISPR-Cas9 technology. A PubMed search reveals that of the approximately 9,000 publications referencing CRISPR in the database, 88% have been published since January 2015. This fact illustrates the rapid growth of the field (for reviews on CRISPR-Cas9 basics, see [3] and [9]). The workshop organizers introduced a CRISPR-Cas9 workflow (Fig. 1) to participants that was designed to implement this simple yet powerfully effective genome editing tool in undergraduate classrooms, with an eye to institutions with limited scientific resources. Interspersed with laboratory activities, discussions on pedagogical issues and classroom activities were also conducted.

Experimental workflow: an authentic CRISPR-Cas9-based research experience for undergraduates

Although the workshop was developed with the zebrafish (Danio rerio) system in mind, the entire workflow shown in Figure 2 can be implemented in vitro and extended to be used in a variety of model organisms. The workflow is designed to enable students to select a gene of interest, design an appropriate single guide RNA (sgRNA) to cut a specific gene target via Cas9, and use in vitro techniques to investigate whether the design worked (see Supplemental Materials for specific protocols and reagent lists). The techniques outlined in Figure 2 done during the course of the workshop include in vitro transcription and nuclease assays to illustrate a simple way in which students could test whether CRISPR-induced double strand breaks (DSBs) in the DNA they targeted were successful (Fig. 3).
implementation plan devised by participants is shown in Fig. 4). An instructor may elect to use select portions of the workflow in order to leave time for other activities/projects that are important for a particular group of students. Also, an instructor can choose to emphasize bioinformatics, molecular biology, principles of cell and developmental biology, or genetic applications, depending on the needs of a particular class (see Table 1 for a breakdown of modules).

**CRISPR-Cas9 COURSE INTEGRATION USING BACKWARD DESIGN**

We support a backward design approach in the development of a CRISPR-Cas9 laboratory experience (10) that follows a previously described three-step framework (11). Like most CUREs/discovery-based lab activities, the overarching pedagogical goal of the CRISPR-Cas9 laboratory experience could be to engage students in authentic scientific research. Through such research experiences, students will likely gain valuable technical expertise, science process skills, improved scientific identity, increased project ownership, and more scientifically sound thinking (12).

Both the research and pedagogical goals can be further subdivided into manageable scientific discovery milestones for research and student-centered learning objectives (13) (Table 2). At this level of backward design and planning, research milestones are often best assessed by students acquiring and making claims from their own data that advance the overall research question. It is recommended that these goals be established in terms of the tangible data students will collect, and that formative and summative learning assessments be designed around these data milestones. Table 2 illustrates how the backward design process could be applied to the CRISPR-Cas9 curriculum.

**Knowledge and skills**

A laboratory experience centered around the CRISPR-Cas9 system has the potential to introduce students to a variety of crucial life science topics, including bioinformatics, genetics, and molecular/cellular biology. For example, bioinformatics modules can introduce students to gene annotations, navigating gene browsers, and using sequence analysis tools. Through the *in vitro* techniques sampled in this workshop, students learn about DNA/RNA synthesis, quantification, and analysis by agarose/polyacrylamide gel electrophoresis in a rich application-oriented context. Extensions could include genotyping, Western blotting,
pulse field electrophoresis, and immunohistochemistry. Because CRISPR-Cas9 can be applied to any number of organisms (e.g., bacteria, yeast, plant, zebrafish, and mammalian systems), students could explore differences in genome organization, processing of genetic information, and gene conservation. Utilizing CRISPR-Cas9 also allows authentic research opportunities. Students can research genes of unknown function and those that have not yet been fully annotated.

**Integration of ethics considerations into the curriculum**

By taking an *in vitro* approach to CRISPR-Cas9 work, students make and test the tools for genome editing without actually injecting them into organisms or cells. In this manner, they learn the underlying concepts and methodology while circumventing the ethical issues of working with whole animals. In addition, the work provides an opportunity to discuss real and potential ethical, legal, and social impacts (ELSI), and related considerations involving the use of CRISPR-Cas9 technology in crops/animals/humans, and the implications on biodiversity and the natural environment. (For more information on these considerations, consult [13–16].)

**Evidence and assessment of the CRISPR-Cas9 lab experience**

After the overarching scientific goals and learning outcomes for the lab experience have been identified, the next step is to consider the types of evidence that would signify attainment of these goals. The workflow in Figure 2 produces at least five pieces of qualitative data: 1) *in silico* sgRNA design, 2) preparation of dsDNA template, 3) sgRNA synthesis, 4) PCR amplification of the target gene/genomic fragment, and 5) cleavage of the PCR product by the sgRNA-Cas9 ribonucleoprotein complex via *in vitro* nuclease assay. It is therefore recommended that the backward design process be structured around these research products. The ability of students to collect and interpret the data can indicate successful achievement of the research goals.

Participants in the workshop noted increased ownership for the research questions of colleagues after investing time designing their own individual chosen sgRNAs as part of the workflow. In addition to improved technical proficiency and knowledge of the CRISPR-Cas9 system, the workshop participants forged new collaborations and connections. It is hypothesized that students may

| Module | Outcome/output | Suggested time |
|--------|----------------|----------------|
| Bioinformatics | • Analyze gene sequences | 3–4 hours |
| | • Identify regions that can be effectively targeted based on available high-scoring guides and biological relevance | |
| | • Primer design for PCR genotyping (on-target and off-target) | |
| sgRNA synthesis | • ssDNA → dsDNA template → ssRNA lab workflow | 6–8 hours |
| | • Compare how ssDNA, dsDNA, and ssRNA behave during electrophoresis | |
| | • Basic qualitative (gel) and quantitative (spec) analysis of sgRNA | |
| Genotyping assay | • PCR optimization (gradient PCR) | 2–3 hours |
| In vitro nuclease activity | • *In vitro* validation of sgRNA | 2–3 hours |
| | • Functional QC of sgRNA | |
| | • Off-target efficiency | |
| Embryo injections | • Observe or perform microinjection into 1–2 cell stage embryos | 4–5 hours |
| Phenotypic analysis (of injected embryos) | • Scoring embryos injected with sgRNA-Cas9 RNP complex | 2–3 hours |
| | • Identification of potential phenotypes | |
| Basic genotyping (of injected embryos) | • Analyze presence of potential indel mutations (on-target and off-target) | 2–3 hours |
| Advanced genotyping (to detect mutations present in injected embryos) | • Cloning of PCR products | 6–8 hours |
| | • Sequencing cloned PCR products | |
| | • Analyze mutations | |

CRISPR = clustered regularly interspaced short palindromic repeats; ssDNA = single-stranded DNA; dsDNA = double-stranded DNA; sgRNA = single guide RNA; QC = quality control.
Sample implementations of CRISPR-Cas9 in lab and non-lab courses

Support for implementation of the workflow was provided beyond the workshop by online (e-mail and videoconference) discussions and through a shared common technical document with frequently asked questions.

Upon completion of the CRISPR-Cas9 workshop, one workshop participant was able to successfully implement the in vitro steps from the workshop (Fig. 2) in a molecular biology summer course. The class met for about three hours Monday through Thursday for five weeks. The overarching goal of this pilot was for students to apply the theory of the CRISPR-Cas9 system using molecular biology methodology. Students worked in pairs using a single sgRNA per group, replicating the experiment that the instructor had conducted during the workshop. Students designed their own guides for the same region of this gene in order to mirror closely those sgRNAs that they were working with. Although extensive assessments have not yet been carried out, qualitative data indicate that the experience proved to be enriching. Students learned key molecular biology techniques, designed experiments with appropriate controls, troubleshooting and working through perceived failure/frustration. Importantly, students reported having learned the most when their experiment did not work the first time and parameters/conditions had to be adjusted. Figure 3 illustrates students’ in vitro cleavage of template DNA by sgRNA-Cas9 ribonucleoprotein complex, revealing that their sgRNA synthesis and in vitro nuclease assays were successful.

Another workshop participant implemented CRISPR-Cas9 theory and applications in her five-week, non-lab course (Scientific Process and Biological Discovery) in a pre-college institute for incoming freshmen. Using the CREATE pedagogical method (26), the instructor introduced CRISPR-Cas9 to her students through newspaper articles and public web-resources. Next, students found and shared newspaper articles involving CRISPR-Cas9. Then, the class chose one topic (eliminating endogenous retroviruses in pigs using CRISPR) and read and discussed content from a primary research article about the topic.

CRISPR = clustered regularly interspaced short palindromic repeats; sgRNA = single guide RNA.
Revision and iteration

As with scientific discovery, curriculum design is an iterative process. After pilot testing, we highly recommended that revision and iteration take place. For example, assessments and lab materials can be customized and more scaffolding added to enhance the learning experience of a specific cohort of students. Participants in the workshop hope to share their experiences of pilot implementations during a symposium in 2019, as well as on an online resources page.

FUTURE PERSPECTIVES: MOVING KNOWLEDGE GAINED FROM THE WORKSHOP INTO THE CLASSROOM

Workshops such as the one described here provide valuable experiences for undergraduate faculty and staff. In addition to deepening their scientific knowledge, it allows participants to gain similar hands-on experience to their students. Participants are therefore better prepared to anticipate and guide their students through troubleshooting. Communities of support formed during such workshops provide a safety net for trial and error as the workshop content is implemented in the classrooms. Together, the experiences help build the confidence of participants and increase the likelihood that they will incorporate CRISPR-Cas9 into their undergraduate classrooms. However, there are hurdles to overcome.

A concern that most participants had before the workshop was their apprehension about introducing an advanced topic like CRISPR-Cas9 as a genome editing tool effectively in undergraduate classrooms. Providing a hands-on experience of the laboratory workflow in a collaborative environment, along with protocols and some reagents (like CRISPR oligos and PCR primers), was the first step to help the participants take this technology into their classrooms immediately after the workshop. Active discussions of real and potential challenges that may arise in classrooms were deliberately incorporated in the workshop, as was modeling classroom situations. Consequently, one point that was reiterated many times immediately after the completion of the workshop was the clarity that participants gained regarding the underlying concepts and methods involved in CRISPR-Cas9 genome editing technology and the ease with which they can be introduced into classrooms. Open conversations following the workshop enabled the creation of a practical FAQ document that addressed simple challenges faced by the instructors.

Keys to successful completion of the CRISPR-Cas9 workflow include clearly defining learning outcomes, determining the molecular techniques required and how those specific technical skills will be obtained by faculty, staff, and students, incorporating sufficient scaffolding, and selecting the resources required to complete the workflow. Upper-level students conducting independent research projects could pilot CRISPR-Cas9, help develop curriculum, and troubleshoot. They can also serve as valuable teacher assistants in the undergraduate courses that implement the curriculum. As for materials and reagents needed to conduct the workflow, many are becoming available from an increasing number of companies, similar to other molecular biology products like restriction endonucleases, PCR, and cloning reagents.

Implementing the workflow and extending it to in vivo systems in certain model organisms (e.g., Arabidopsis) in a 14-week semester might be challenging or not feasible. Composite courses (parallel courses) are a great way to address this. For example, the first class might perform an in vitro assay and clone the most effective sgRNAs into a plant expression vector, checking plasmids by PCR and then electroporating them into Agrobacterium tumefaciens. Arabidopsis plants could be transformed by floral dip, and the three- to four-week waiting period would allow time to introduce the students to that particular model organism and the phenotypes to look for. The resulting plant seed would be selected on antibiotic plates and used by the next student cohort or as an independent study by a few students. Such a strategy could also increase project ownership and enhance student learning. Another strategy involves collaborations between institutions. For example, students from one undergraduate school could conduct in vitro experiments to identify the most effective sgRNAs, and students from another undergraduate school could test those sgRNAs in an in vivo system that the first school does not have.

A major reason why CRISPR-Cas9 technology has not yet become a standard technology in undergraduate biology courses is that instructors lack experience with the CRISPR-Cas9 system. To help address this issue, we supply the protocols and reagent lists necessary to run the experiments described here in the Supplemental Materials and welcome correspondence on ways that they can be tailored for the needs of a particular class. Workshop participants forged partnerships and teams interested in ongoing collaboration for instructional design and planning. We advocate the free exchange of teaching resources, assessments, and student feedback involving CRISPR-Cas9 and welcome you to join our community. Through such collaborations and workshops, we hope CRISPR-Cas9 becomes a standard technology in undergraduate classrooms, with a resulting increase in undergraduate STEM student retention and research publications.

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