HOW CRISPR-MEDIATED GENOME EDITING IS AFFECTING UNDERGRADUATE BIOLOGY EDUCATION

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ABSTRACT

In 2010, the CRISPR/Cas system of *Streptococcus thermophilus* was found necessary and sufficient to cleave bacteriophage DNA. Since this time, CRISPR went from a niche scientific field to the laboratories of major research institutions, undergraduate classrooms, and popular culture. In the future, CRISPR may stand along with PCR, DNA sequencing, and transformation as paradigm shifting discoveries in molecular biology. CRISPR genome editing is technically uncomplicated and relatively inexpensive. Thus, CRISPR-mediated genome editing has been adopted by and applied to undergraduate curricula in a wide variety of ways. In this review, we provide an overview of CRISPR-mediated genome editing and examine some of the ways this technology is being leveraged to train students in the classroom and laboratory.

CRISPR PROTECTS BACTERIA FROM VIRUSES

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are short DNA repeats found in prokaryotic genomes (1). These repeats are interspersed with non-repetitive short sequences homologous to bacteriophage DNA (Figure 1) (2). The discovery that the interspersed elements possess homology to bacteriophage genomes lead investigators to propose that these short viral elements provide bacteria with immunity against invading viruses. Indeed, CRISPR sequences were found to mediate bacteriophage resistance and investigators identified CRISPR-associated genes (Cas genes) that were also important for this viral immunity (3, 4). Bacteria can encode a wide variety of Cas proteins which play diverse functions in bacteriophage DNA cleavage reviewed in (5). Briefly, 20 nucleotide CRISPR-RNA (crRNA) guides transcribed from the spacer regions inhibit bacteriophage infection by targeting a Cas nuclease to the viral DNA using complementary base pairing. Some CRISPR-mediated viral cleavage systems also require a trans-activating CRISPR-RNA (tracrRNA) for maturation of crRNA (6). In addition, cleavage of viral genome also requires a short, 2-6 nucleotide, Protospacer Adjacent Motif (PAM) in the viral sequence (7-9). The dual requirement of the 20 base guide and adjacent PAM sequence provides a high degree of specificity limiting off-target or bacterial genome cleavage.

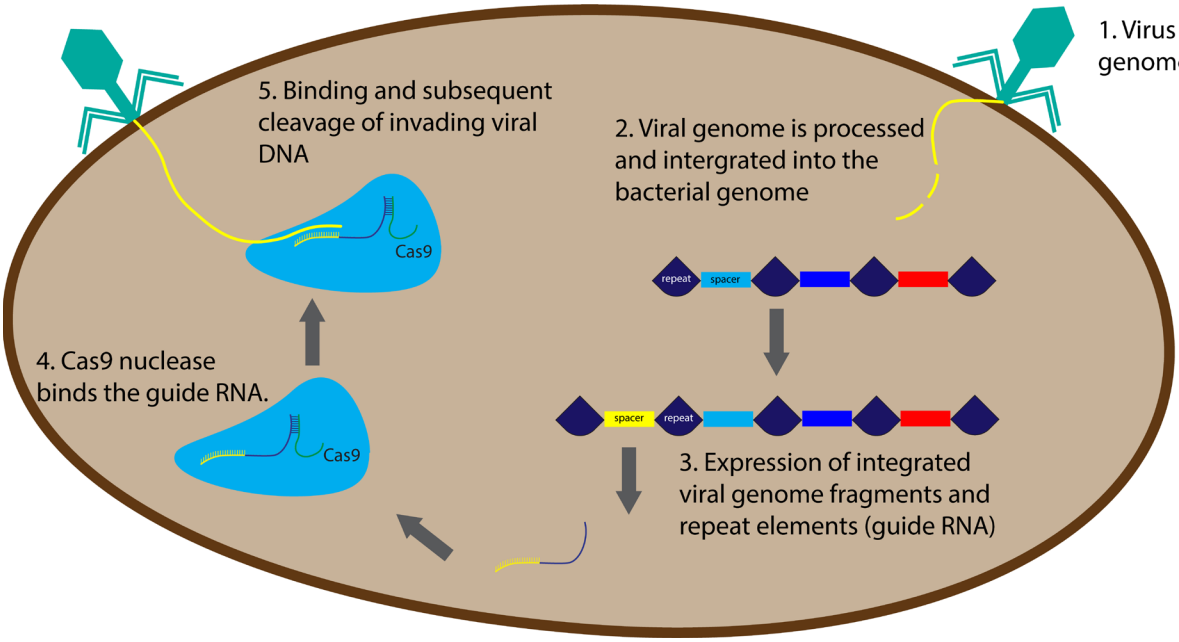


Figure 1. CRISPR is used by bacteria to cleave viral DNA. crRNA is yellow and navy blue. tracrRNA is in green.

CRISPR-MEDIATED GENOME EDITING

The discovery that crRNA guides, in coordination with Cas nuclease mediate viral genome cleavage in bacteria provided an elegant method by which bacteria defend themselves from viral invasion. This discovery however begged the question, could this system cleave DNA *in vitro* or in other organisms? Investigators determined Cas9, crRNA, and tracrRNA were sufficient to cleave plasmid DNA *in vitro* in a sequence specific manner (8). Building upon these results, multiple investigators tested and found Cas9, crRNA, and tracrRNA could cleave DNA *in vivo* in organisms other than bacteria including mammalian cells (10-12). This technique is now commonly referred to as CRISPR-mediated genome cleavage or merely CRISPR.

For CRISPR-mediated genome cleavage to occur, a guide RNA must target a Cas9 nuclease to a DNA sequence of interest. However, once cleavage occurs it is critical that the host cell's DNA repair machinery efficiently repairs the DNA break or the cell will die. DNA breaks are repaired by two distinct mechanisms. Nonhomologous End Joining (NHEJ), reviewed in (13), requires that the cleaved ends of the DNA are trimmed (Figure 2). The ends are then joined, resulting in deletion of the trimmed sequence. Such deletions can shift the reading frame causing premature translation termination. Homologous Recombination (HR), reviewed in (14), is another mechanism used to repair Cas9-mediated cleavage. During HR cells use a homologous DNA sequence to repair the cut site. DNA encoding a desired genome edit, the repair template, is cotransformed with the Cas9 nuclease and guide RNA. When the cell uses the repair template to fix the cleaved DNA, changes encoded by repair template are introduced at the site of cleavage (Figure 2). Thus by designing the sequence of the repair template, the investigator can precisely edit the genome, introducing desired point mutations, insertions, or deletions. Such precise changes are commonly referred to as CRISPR- mediated genome editing.

A number of nucleases can be used to perform genome cleavage, and these nucleases have distinct requirements. For instance, Cas9 nuclease requires a tracrRNA as well as guide RNA to mediate cleavage (6, 9), while Cpf1, another Cas nuclease, does not require tracrRNA (15). Cas9 and Cpf1 also use distinct PAM sequences and cut at different lengths away from their PAM sequence. In addition to genome editing, Cas9 can be used to alter gene expression by creating a gene fusion a transcriptional activator or repressor to catalytically active Cas9 and targeting these complexes to the gene of interest (16). Furthermore, inactive Cas9 nuclease has been used to fluorescently label the genomic loci enabling investigation of the 3D architecture of the nucleus (17). These discoveries have fundamentally changed the field of biology allowing researchers unprecedented flexibility to manipulate an organism's genome and transcriptome.

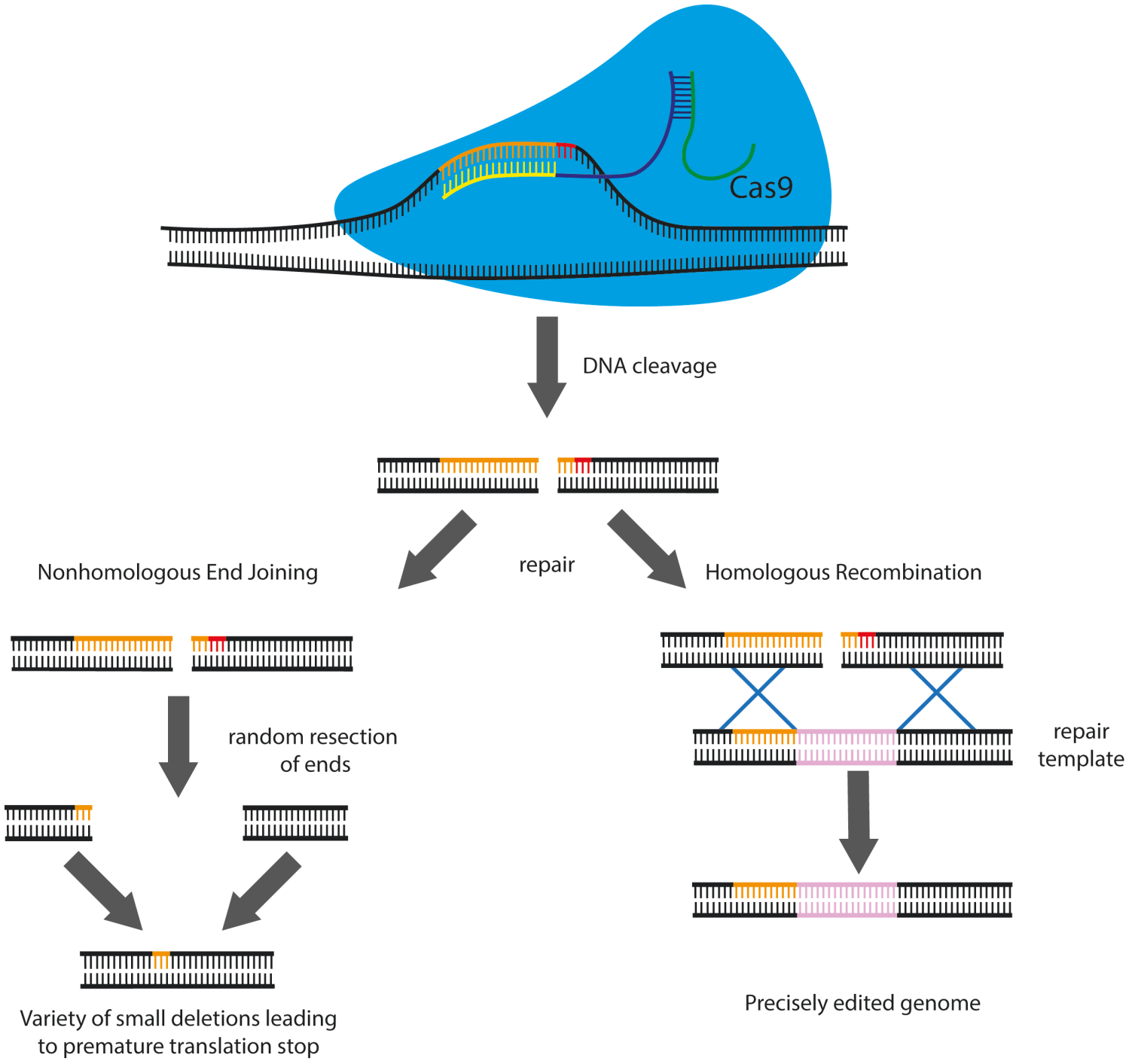


Figure 2. DNA cleaved by Cas9 is repaired by either nonhomologous end joining or homologous recombination. PAM site is red.

CRISPR MEDIATED GENOME EDITING BECOMES WIDESPREAD

The groundbreaking realization that CRISPR genome editing was broadly applicable has since lead to the development of CRISPR systems in numerous organisms. For instance, in the fungal kingdom, CRISPR-mediated genome editing systems have been established for traditional yeast model systems such as *Schizosaccharomyces pombe* (18) and *Saccharomyces cerevisiae* (19), as well as non-conventional yeast *Candida glabrata* (20), *Candida albicans* (21), Yarrowia lipolytica (22), *Kluyveromyces lactis* (23), *Pichia pastoris* (24), *Naumovozyma castellii* (25), *Ogataea thermomethanolica* (26), *Candida lusitaniae* (27), Candida parapsilosis (28), *Cordyceps militaris* (29), *Cryptococcus neoformans* (30), *Shefferomyces stipites* (31), and *Aspergillus fumigatus* (32) as well as others. In addition, CRISPR-mediated editing systems have been developed for many mammalian (33) and plant model systems (34), as well as other organisms whose genomes have historically been challenging to manipulate (21, 35). This expansion of the use of CRISPR is similarly demonstrated by online mentions. In 2004 a keyword search for CRISPR of the PubMed database would have retrieved under ten papers. In 2017 alone, over 3000 papers with the key word CRISPR were deposited and indexed on PubMed (Figure 3). Google searches for CRISPR have had a similar meteoric rise in popularity over the same time period (Figure 3) and this rise in popularity has led to articles and features in popular media (36, 37).

CRISPR's appeal is in part due to the relative ease by which it can be applied to a wide variety of biological systems. Such promiscuity gives CRISPR-mediated genome editing the potential to effect human well-being in a multitude of ways (38). Genome engineering in agriculture has the potential to increase yields and develop more nutritional food products (39). Gene drive technologies that use CRISPR Cas9 to spread genes in wild arthropod populations could fundamentally alter crop management strategies and reduce pesticide use (40). Similar gene drive strategies could be employed to limit the spread of diseases such as malaria (41). However the release of genetically modified organisms to the wild is controversial and has the potential to cause unforeseen consequences. Beyond regulating the spread of disease, increased genetic engineering efficiency via CRISPR enables investigators to better model diseases *in vivo* (42-44). Unlike transplant or *in vitro* experiments, CRISPR-Cas9 can be used to introduce a specific genotype. This is ideal for studying disease progression and variability, as such studies more closely maintain tissue microenvironments and can be performed in fully immunocompetent animals (45). Genome editing systems in human pathogens such as Trypanosoma brucei (46, 47) or C. albicans (21) may enable researchers to identify new therapeutic targets. Collectively, these models have the power to open doors to new therapies and a better understanding of human health. Finally, CRISPR-mediated genome editing could be used to alter the human genome to improve health and change phenotype (48). While CRISPR-mediated genome editing has the potential to improve human health in a variety of ways many such applications are provocative and controversial. The scientific community and public must wrestle with not only the technical but ethical challenges presented by such avenues of investigation (49).

HOW CRISPR IS BEING APPLIED TO UNDERGRADUATE EDUCATION

These potential applications can bring about significant technical challenges and require students balance complex ethical considerations as well. As such, it is imperative students are exposed to genome editing technology during their undergraduate education. This exposure will provide students the best opportunity to efficiently and responsibly apply CRISPR technology to important research problems. These experiences can come in a variety of forms (50).

Figure 3. The increase in CRISPR related academic and social materials over time. **A.** CRISPR publications indexed on PubMed. Number of publications submitted to PubMed indexed with the key word CRISPR by year. **B.** CRISPR Google search trends. Google search frequency from 2004 to 2017. Each data point is divided by the total searches of the geography and time range it represents to compare relative popularity. Time period with the highest search frequency will score 100, while the time period with the lowest frequency of searches will score 1.

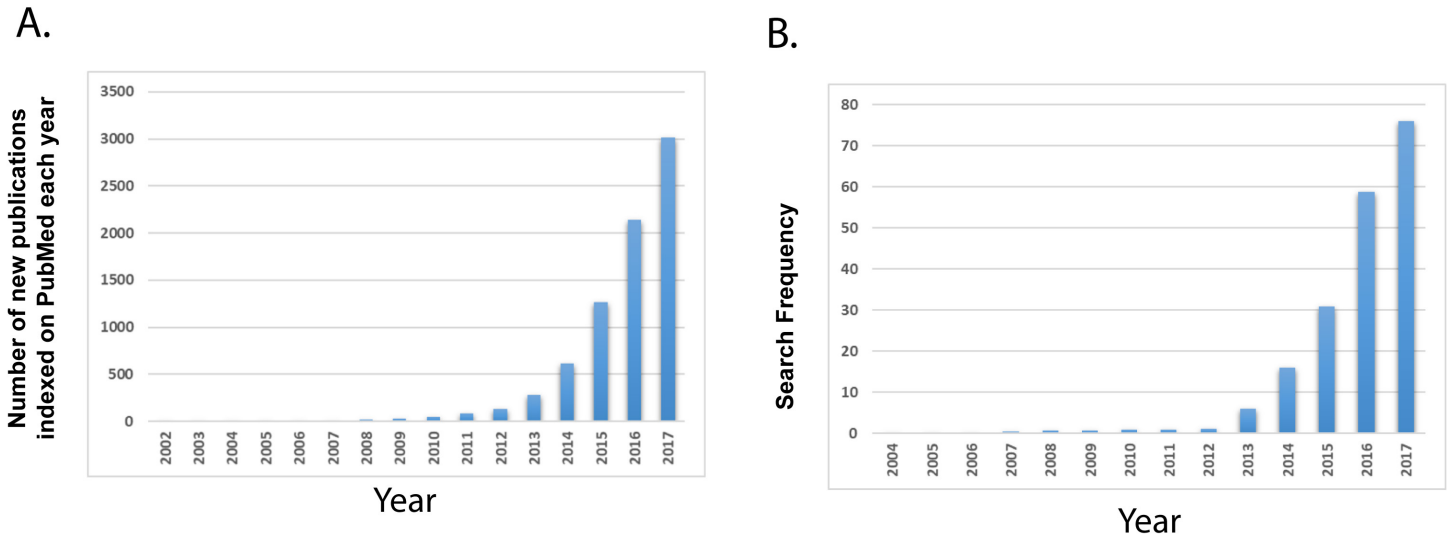
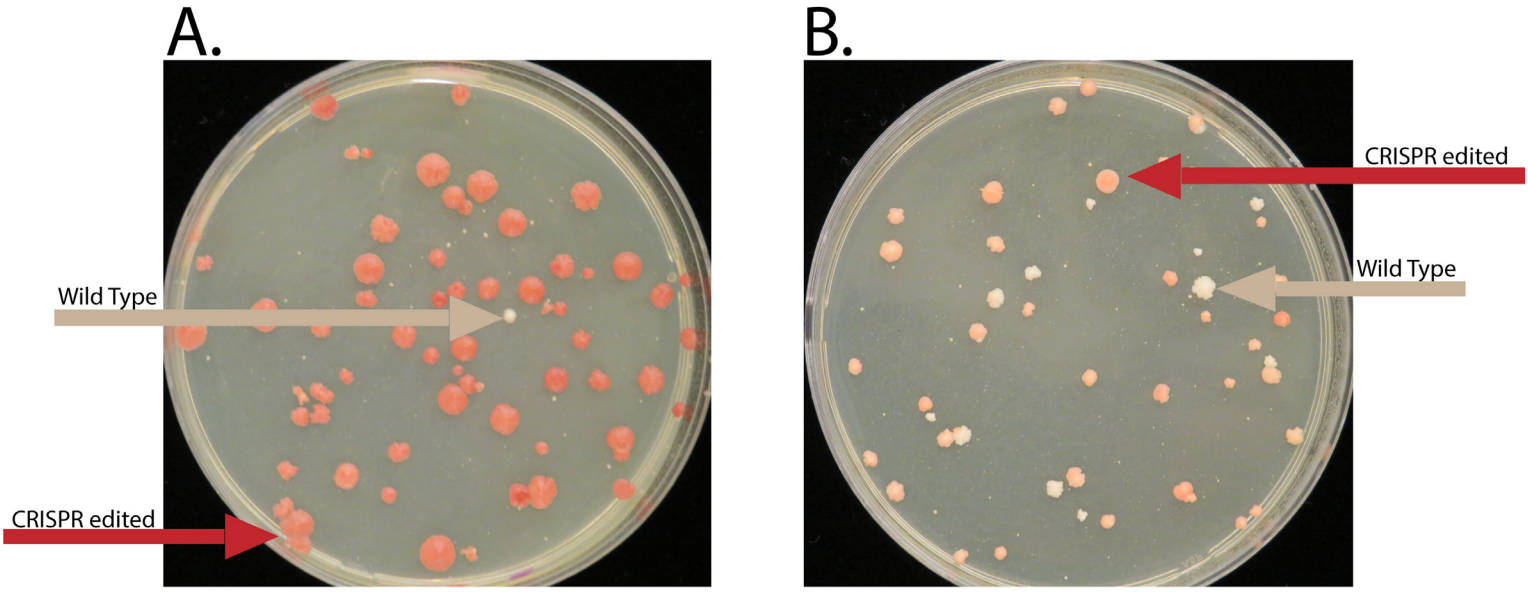


Figure 4. CRISPR mediated genome editing of *S. cerevisiae* ADE2. Repair of the cleavage sites in the two strains of yeast were shown was done by homologous recombination. Stop codons were inserted into the 5' end of the ADE2 gene generating a functional deletion. Pink and red colonies indicate successful genome editing while white colonies are unedited *S. cerevisiae*. **A.** Commercially available baking strain, Red Star Quick Rise Instant Yeast. **B.** Commercially available brewing strain, Lallemand Nottingham Ale Brewing Yeast.



UNDERGRADUATE RESEARCH PROJECTS

One way students can be exposed to CRISPR-mediated genome editing is by applying it to an undergraduate research project. Well-designed undergraduate research projects both train students and move a larger research program forward. Undergraduates typically have limited time they can devote to a research project and their tenure in a lab is likely to be shorter than that of a graduate student or more senior scientist. Such limitations make it crucial that students generate strains and reagents required to test a hypothesis in a timely manner. In some fields, CRISPR-mediated genome editing has increased the speed by which investigators can generate such materials. For instance, the human fungal pathogen C. albicans is diploid (51), does not undergo meiosis, and cannot maintain plasmids. Historically, genetic manipulation of C. albicans required multiple rounds of homologous recombination as each allele must be mutated independently and screened in succession (52). As ploidy increases genetic manipulation generally becomes more laborious. These characteristics made it considerably more challenging to work with than other fungal model systems such *S. cerevisiae*. The development of CRISPR-mediated genome editing in C. albicans has greatly increased the speed at which genetic engineering can be performed and allows undergraduate researchers to generate strains quickly (25, 53). Such efficiency is important so students make progress on a project and are afforded the opportunity to witness the payoff of performing hypothesis-driven experiments.

CRISPR IN THE CLASSROOM

Applying CRISPR to a mentored research project provides a tremendous opportunity for undergraduates to develop a deep understanding of how genome editing can be applied to a research problem. However, it is unrealistic to expect that all undergraduates could gain experience in this way. Laboratory courses that employ CRISPR as a technique are an alternative method of teaching genome editing to larger groups of students. How CRISPR can be applied in an undergraduate curriculum depends upon the experience level of the students. Introductory courses pose unique challenges due to students' relative unfamiliarity with bench science and the financial and equipment constraints inherent to many such courses. Thus, it is important that exercises yield easily interpretable results while staying within the course's technical and financial boundaries. One example of a model system well suited for introductory biology courses is CRISPR-mediated genome editing of yeast ADE2. Mutation of *S. cerevisiae* ADE2 leads to a buildup of an adenine precursor pigmenting the yeast red (Figure 4) (25). The intensity of the color and efficiency of gene editing will depend upon the length of the incubation as well as S. cerevisiae strain. This easily observable phenotype, and the practicality of yeast, make them an exceptional system to expose undergraduate students to CRISPR-mediated genome editing in introductory courses (54).

As students gain more experience, the experiments they can perform and data they can interpret during laboratory classes broadens. This provides the instructor with significantly more latitude when designing exercises. There has been a shift recently in higher education away from laboratories that merely teach a technique towards Course-Based Undergraduate Research Experiences (CUREs) (55). CUREs allow students to develop and address a scientific question using a variety of methodologies and techniques. The speed and relatively simple nature of CRISPR-mediated genome editing makes it particularly well suited for upper level biology CUREs where faculty address a research question of interest. CRISPR based CUREs have been developed using a variety of different model systems including zebrafish (56), *Drosophila melanogaster* (57), and mammalian tissue culture (58). The framework for many of these courses is similar; students design and clone guide RNAs into appropriate expression vectors and then use these vectors to edit the genome of their model system. These courses allow faculty to address an important research question and introduce cutting edge genome editing technology when appropriate to students concurrently. Students then assess how edits they introduce affect phenotype. In the case that guide generation or editing takes longer than predicted, phenotypic assessment can be performed in other classes or in the research lab of the principal investigator leading the exercise.

Another way CRISPR is being applied in the classroom is by examining how CRISPR helps bacteria defend against viruses. For example, labs have been developed where undergraduates infect *Streptococcus thermophilus* with bacteriophage and sequence the bacterial genomic DNA to see if viral DNA has been incorporated to the CRISPR array (59). Modified bacteria gain resistance to later phage infections and subsequent experiments can be performed that further examine the CRISPR-mediated resistance. Furthermore, CUREs have been developed to identify CRISPR repeats in uncharacterized strains of *Escherichia coli* (60). The CRISPR loci found among different strains and species of bacteria should be distinct and would depend upon the viruses they have encountered. By examining the sequences of CRISPR arrays, students can identify phages the bacteria have encountered. While students that perform these exercises are not editing genomes, they learn about CRISPR biochemical mechanisms and gain an appreciation for how fundamental discoveries can have broad applications.

The exercises described above provide examples of how CRISPR is being applied to undergraduate curriculum. However, CRISPR-based CUREs go beyond just genome editing, exposing students to a variety of other molecular biology techniques. For instance, to clone a guide RNA into an expression vector students need to perform a variety of additional techniques including restriction digestion, plasmid purification, and DNA ligation. CRISPR CUREs therefore represent comprehensive approaches that integrate and expose students to genome editing and foundational molecular biology techniques. CRISPR's broad utility and practicality make CRISPR CUREs attractive pieces from which to shape undergraduate molecular biology curricula. As CRISPR is now part of the molecular biology tool box, instructors should consider adoption of it alongside classic molecular biology tools when developing classroom activities.

CRISPR AND SCIENCE POLICY

In undergraduate curricula, the most immediate impact of cutting edge genome editing technologies like CRISPR is being felt in biology classrooms as the development of genome editing technologies directly affects course material being taught. However, the remarkable rise and accessible nature of CRISPR, combined with the powerful prospect that it will enable us to manipulate genomes at will, brings with it a variety of moral and ethical questions that society must examine. For all its promise, CRISPR raises apprehension regarding human germline manipulation, unforeseen risks, and the potential for these technologies to further social inequities (61). These are by no means novel concerns; since generation of the first transgenic organism, the scientific community has debated the lines that separate the unethical from ethical and the reckless from the enthusiastic. While these debates continue in laboratories and scientific conferences across the world, the discussion should extend beyond the broader science community to the general public and society at large. Evidence for this shift can already be seen by the inclusion of CRISPR debates in academic courses on public policy and science law (62, 63). Diverse enrollments in these types of classes will provide opportunities to engage in discussions and hear various viewpoints relating to genome editing technologies. It is imperative that the scientific community continue to inform the public of the remarkable promise of genome editing, as well as the limitations and hazards it presents. The inclusion of these discussions in classrooms outside of biology departments will provide the best chance for us to make informed decisions on how we choose to apply genome editing technology as a society.

SUMMARY

When the students involved in writing and researching this article started high school, CRISPR-mediated genome engineering had not been invented and now it is being taught in undergraduate curricula across the world. In just one decade, a little known bacterial immune response has profoundly changed

research and molecular biology undergraduate education in ways few envisioned. The potential for CRISPR- mediated genome editing to profoundly affect research, medicine, and society over the next decades is significant. The initial version of any technology invariably requires refinement. Advancements to CRISPR- mediated genome editing will continue as more bacterial CRISPR systems are characterized and the molecular biology of systems in use is further refined. Improvements to genome editing technologies along with the likely continued drop in the price of gene synthesis will open the door to further scientific advances. In addition, as CRISPR-mediated genome editing systems are developed for more organisms, the limitations traditional model systems impose on research may begin to dissipate. Such progress is especially exciting in the context of undergraduate education. A recent paradigm shift has placed more focus on laboratory exercises that not only teach techniques, but also explore important biological problems through guided semi-independent research in the classroom. The confluence of this shift with advances in genome editing technology have the potential to allow students to make significant progress on research questions previously impractical for undergraduate curricula.

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