“Designer babies?!” A CRISPR-based learning module for undergraduates built around the CCR5 gene

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Abstract
CRISPR-cas technology is being incorporated into undergraduate biology curriculum through lab experiences to immerse students in modern technology that is rapidly changing the landscape of science, medicine and agriculture. We developed and implemented an educational module that introduces students to CRISPR-cas technology in a Genetic course and an Advanced Genetics course. Our primary teaching objective was to immerse students in the design, strategy, conceptual modeling, and application of CRISPR-cas technology using the current research claim of the modification of the CCR5 gene in twin girls. This also allowed us to engage students in an open conversation about the bioethical implications of heritable germline and non-heritable somatic genomic editing. We assessed student-learning outcomes and conclude that this learning module is an effective strategy for teaching undergraduates the fundamentals and application of CRISPR-cas gene editing technology and can be adapted to other genes and diseases that are currently being treated with CRISPR-cas technology.

KEYWORDS
CCR5, CRISPR-cas, gene-editing, germ-line gene editing

1 INTRODUCTION
CRISPR-cas technology is touted as the “game-changing technology” for its ability to modify nature at its most fundamental level through the programmable editing of DNA with precision and speed. CRISPR-cas technology is bound to impact medicine, agriculture, biosphere, and potentially future generations of humans. Broadly speaking, we wondered what the extent of mid to upper-career science student knowledge was on CRISPR-cas-based technology and its uses. Thus, to determine the prior knowledge level of CRISPR-cas-based technologies, we surveyed undergraduate sophomore-junior-level Genetics students at the onset of the semester. Pre-surveys indicated that 76.5% of students (13/17) in a Genetics course were minimally aware of CRISPR-cas technology, while the remainder of the students either had not heard of the technology or were not sure if they had heard of it. Of the students identifying as aware of this technology, our survey results indicated their knowledge concerning CRISPR mostly came from a previous course; however, the news, social media, podcasts/radio and textbooks were also cited as sources for this information. The results of this questionnaire indicated that CRISPR-cas associated technologies are becoming fundamental components of an undergraduate STEM...
CRISPR technology (Table 2). Although Dr. He’s work first known births of genetically modified humans using yoghurt company Danisco,1,2 have been repurposed by tive immune response in bacteria against phages by ing of CRISPR-cas technology and its diverse uses. Thus, although student knew of its existence or were formally introduced through course work in other courses, this informal survey demonstrated that most students did not have or retain a comprehensive understanding of CRISPR-cas technology and its diverse uses.

CRISPR-cas systems, originally identified as an adaptive immune response in bacteria against phages by yoghurt company Danisco,1,2 have been repurposed by scientists for genome-engineering applications in basic science (reviewed in Chen and Doudna.3) Recently, the technology provoked a significant reaction that swept through the scientific community. In November of 2018, Dr. He Jiankui claimed to have produced the first human babies born with CRISPR-cas edited genomes,4 affirming Dr. He Jiankui claimed to have produced the first human babies born with CRISPR-cas edited genomes,4 affirming comments focused on the novelty of these experiments has hastened the debate over the bioethical implications and the guidelines in human heri- tage, necessitating critical dialogues on ways to effectively bringing commonplace in both research and in higher education, let alone provide high impact course-based undergraduate research experiences (CUREs) for students using the technologies. Additionally, not every institution requires genetics or molecular biology courses to have an experien- tial learning opportunity in the lab. Despite these barriers, evidence suggests that students can achieve meaningful learning gains by performing alternative activities such as interactive computer simulations,22–24 kinesthetic modeling activities,25,26 or case-based learning modules.27

CRISPR-cas-based genomic editing tools are becoming commonplace in both research and in higher education, necessitating critical dialogues on ways to effectively bring this technology into the undergraduate classroom. We asked whether we can provide effective instruction on genomic editing technologies in educational settings where wet-lab resources are unavailable, are cost prohibi- via CRISPR-cas-based systems has necessitated further change to curricula. One such example is textbook resources. Many recent editions published just a few years ago lack deep descriptions and applications of CRISPR technologies. Although this is surely to change, the speed of the science will always outpace the rate of textbook publication. From a content perspective, working with recombinant DNA requires students to understand and apply specific fundamental molecular concepts; for example, that DNA is double stranded and antiparallel, the structure and components of plasmids, specificity of restriction enzymes, and the theory of PCR amplification using primers and heat-stable DNA poly- merases. With CRISPR-cas-based approaches, students are required not only understand the basic concepts above, but also adequately understand and apply additional molecular concepts such as hybridization between DNA and functional RNA molecules, cas-nuclease activ- ity, endogenous DNA repair mechanisms, the effect on reading frames/codons, loss-of-function and gain-of-function effects, and genotype–phenotype relationships.

A bevy of evidence suggests a common set of student misconceptions concerning molecular genetics.10–14 Traditionally, instruction has relied upon these core molecular concepts being reinforced in a laboratory setting, where these misconceptions may be addressed through hands-on application-based lab work. CRISPR-cas-based methodologies have been used successfully to train stu- dents in fundamental molecular biology skills15 and in a variety of model organisms, including yeast,16–18 Escherichia coli,19 Drosophila melanogaster,20 and Ara- bidopsis thaliana.21 However, not all undergraduate teaching laboratories are equipped to sufficiently perform gene editing experiments in live organisms and conduct the subsequent molecular and phenotypic analysis, let alone provide high impact course-based undergraduate research experiences (CUREs) for students using the technol- ogies. Additionally, not every institution requires genetics or molecular biology courses to have an experien- tial learning opportunity in the lab. Despite these barriers, evidence suggests that students can achieve meaningful learning gains by performing alternative activities such as interactive computer simulations,22–24 kinesthetic modeling activities,25,26 or case-based learning modules.27

Advancements in the ability to edit genomes of organ- isms have permanently changed the landscape of science education particularly in areas of molecular genetics. In the 1980s, the identification and uses of restriction enzymes and Polymerase Chain Reaction (PCR) ushered in the revolutionary recombinant DNA technology, leading to instructional design and curricula changes in biol- ogy education. Thirty plus years later, genomic editing curriculum, even at the introductory level. Interestingly, although many students had been previously introduced to CRISPR technology, when asked to elaborate on the depth and extent of their knowledge, 66.6% of written responses were coded as “I don’t know” or “I can’t remember.” Of students remaining, 33.4% of students with a cursory knowledge of CRISPR, comments focused on the novelty and basic components of this technology, however their descriptions lacked accuracy or in-depth discussion of its applications. Thus, although student knew of its existence or were formally introduced through course work in other courses, students remaining, 33.4% of students with a cursory understanding of CRISPR, comments focused on the novelty of these experiments has hastened the debate over the bioethical implications and the guidelines in human heritable germline editing, but also highlighted the immediate need for both current instruction on genome editing technologies and bioethical discussions in the classroom.

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students still understand and apply the principles of CRISPR without physically performing gene-editing experiments on organisms or cells in lab? Researchers in lab spend a considerable amount of time devoted to the technical aspects of CRISPR-cas-based studies, including designing the tools to generate the type of modification desired, target cells/tissues, germline vs. somatic modification, and mode of delivery of CRISPR-cas tools. Thus, we sought to build a case-based learning module that centers around the technical design, implementation, and consequence of CRISPR-cas gene editing.

Using the manipulation of the gene CCR5 in human embryos as a model example, we describe the design and organization of a gene editing experiential lesson where students apply key concepts in molecular genetics to learn the principles of a specific CRISPR-cas editing design strategy and analysis of the molecular and phenotypic outcomes, with an intentional focus on the bioethics associated with this technology (Figure 1). We specifically are focusing on the use of the cas9 endonuclease due to its popularity, ease, and ubiquitous use in many CRISPR-based manipulations. We combined computer-based work utilizing basic bioinformatics and sequencing software with modeling to educate students on the essential background, planning and design of CRISPR-cas9-based edits. We then challenged students to demonstrate their transference of knowledge from the CCR5 case-based module by having them investigate and propose their own research projects utilizing CRISPR-cas-technologies. Our learning outcomes and student work are described in Table 1. When assessed, we determined that our CCR5 case-based learning module was an effective method for introducing the fundamental technical aspects of molecular genetics and importantly CRIPSR-cas system. We found that students are eager to learn about CRISPR-cas technology and its applications in today's world, and students gain a deeper understanding of the mechanisms behind the technology that has significant power to change human biology.

### 2 | MATERIAL & METHODS

#### 2.1 | Intended audience and learning time

This work is intended for sophomore- and junior-level undergraduate Biology students enrolled in a Genetics or Biotechnology course, but can be integrated and modified for Introductory Biology course or upper-level Molecular Biology course. The main activity was implemented during a 2-hour 45-minute laboratory period. The activity can be adapted to fit two traditional 90-minute class/lecture periods. Subsequent post-activity student work where students created a research proposal was carried out over the next two laboratory periods and during students’ own time.

#### 2.1.1 | Prerequisite student knowledge

Student should have prior exposure to basic molecular genetics principles that include DNA/RNA structure, codons, reading frames, mutation. However, this lesson

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**FIGURE 1** Overview of the CRISPR-cas case-based learning module workflow. The center green box shows the flow of the central dogma of molecular biology. The purple boxes on the left show how comparison of unmodified CCR5 allele and modified alleles are compared to enable analysis of the genotypic and phenotypic effects of CRISPR-cas modifications. The blue boxes on the right show three sequential student activities centered on the technical and application of CRISPR-cas editing strategy [Color figure can be viewed at wileyonlinelibrary.com]
2.2 | Materials

Each student will require a computer/laptop with access to the internet and the software program, SnapGene Viewer (full version, https://www.snapgene.com). A free 30-day trial to SnapGene Viewer can be downloaded by each student with their email address. Alternative molecular biology software could be used with modifications to the below resources.

The student handouts and accompanying instructor guide/key are provided in Supporting Information.

2.3 | Instructions for faculty and students

2.3.1 | Comparative analysis of CCR5 gene and modified allele variants on paper

The activity utilizes the gene encoding human CCR5; the intended target gene used in Dr. He’s study. CCR5 gene encodes for a chemokine GPCR in T cells, and was targeted because of the known role of the CCR5 as a co-receptor in HIV infection of humans.28 CCR5 delta 32 (Δ32) is a naturally occurring allele lacking 32 nucleotides that correspond to a sequence that normally codes for part of the co-receptors second extracellular loop.28 Removing these 32 nucleotides results in a premature stop codon due to frameshift, and the resultant truncated protein product can no longer be exocytosed to the cell membrane.29 Individuals homozygous for the CCR5 Δ32 variant are resistant to HIV infection, as CCR5 is required for membrane fusion during HIV infection. The HIV life cycle and the key molecular components of a HIV infection cycle in immune T cell can be introduced to students using this animation by Janet Iwasa.30

Dr. He’s goal of editing human embryos was to create a similar non-functional HIV-resistant CCR5 variants using CRISPR-cas technology.31 However, the results of these experiments have purportedly generated three new CCR5 allelic variants named after the twin girls, Lulu and Nana, with Lulu being heterozygotic for a novel CCR5 variant (the Lulu CCR5 allele) and wild-type allele, and Nana being heterozygotic at the CCR5 locus (Nana CCR5 alleles 1 and 2 respectively).4,31 He also claimed that a third child has been genetically modified in a similar manner.

To begin their comparative analysis of the different alleles, students were given a worksheet (Supporting Information) that contains the partial coding DNA sequence of an unmodified CCR5 allele, the HIV resistant allele Δ32 CCR5, and three novel alleles generated via CRISPR-cas9-based modification. They were tasked to determine the nucleotide differences between them and the effect on the reading frame, codon and amino acid (Figure 2a, example of student work; Table 2, summary of CRISPR alleles). Students worked on their own initially and then in a group to compare answers.

2.3.2 | Comparative analysis of CCR5 gene and modified allele variants on computer

Students were guided to obtain the full reference coding sequence of CCR5 from NCBI (Accession number...
NM_000579). They created a “new sequence” in the DNA analysis software SnapGene. Students were tasked to create “new features” that represent the three exons to show where intron/exon boundaries would exist in genomic DNA. Using the open reading frame feature, the students determine the translated protein sequence and normal length of protein. They were given the three other new allele variants (Δ32, Lulu, Nana allele 1 and Nana allele 2) that had been created by the instructor based on reported CRISPR modified sequences. Students were guided to align these sequences together and determine the nucleotide, reading frame, amino acid sequence and protein length changes that were created. The use of the full version SnapGene is key in visualizing the alignment. Although NCBI and free DNA softwares ApE and Benchling enables alignment of sequences together, the alignment in SnapGene is presented in an effective scheme that enables students to still visualize the double-stranded DNA molecule with polarity of each DNA strain, the resulting translation, and the features labeled in one window (Figure 2b,c, Figure S1). Students compared their analysis on SnapGene to their worksheet. Using SnapGene allows students to determine whether premature stop codons are formed further downstream from the nucleotide change.

2.3.3 Predicting the effect of the mutations on protein structure

The nucleotide sequence alignment allows students to visualize the effect on the linear primary sequence of the

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**FIGURE 2** Student work aligning unmodified CCR5 allele to HIV resistant Δ32 and CRISPR-cas modified alleles (a) on paper, and (b) using computer software program SnapGene. B) shows all alleles aligned to the unmodified allele. Panel (c) shows an expanded alignment view with the change in reading frame and early stop codon (red asterisks). Expanded alignment views for other modified alleles are shown in Figure S1.
protein, but it is important that students move beyond the linear protein sequence and establish the effects of changes in the primary sequence to the tertiary structure. Students were shown published tertiary structures of the CCR5 protein\textsuperscript{33,34} where the unmodified protein consists of seven transmembrane helices, characteristic of all G protein-coupled receptors. Additionally, the web-based 3D structure viewer iCn3D can be used by students to visualize the tertiary structure of the protein. The students were tasked to discuss and predict how the new alleles affect protein structure and draw the various predicted protein structures out on paper. Students of the Advanced Genetics class utilized the software BioRender (https://biorender.com), a free biology drawing software to create their schematics. Examples of student work are shown in Figure 3a,b.

### TABLE 2  The effect of CCR5 mutant alleles on reading frame and protein structure

| Allele            | Nucleotide change in coding sequence | Effect on reading frame (RF) | Effect on amino acids                                                                 | Protein length | Protein structure                                      |
|-------------------|--------------------------------------|-----------------------------|--------------------------------------------------------------------------------------|----------------|---------------------------------------------------------|
| Unmodified CCR5   | Normal (RF +1)                        | New amino acids on C terminus (31 different amino acids from AA185)      | 352                                                                                   | 7 TM domains with extracellular N' and intracellular C' |
| Delta 32 (Δ32) CCR5 | Deletion of 32 nucleotides (911..942) | Frameshift (RF +3) leading to early stop codon | Lacks 3 TM domains, altered C' protein sequence after TM 3                             | 215            |                                                          |
| Lulu allele (Δelta 15) | Deletion of 15 nucleotides (900..914) | In-frame deletion | Deletion of 5 amino acids from AA181 in extracellular loop 2                          | 347            | Extracellular loop 2 missing 5 AA                      |
| Nana allele 1 (Delta 4) | Deletion of 4 nucleotides (913..916) | Frameshift leading to early stop codon (RF +2) | 9 different amino acids on C terminus from AA186                                       | 194            | Lacks 3 TM domains, altered C' protein sequence after TM 3 |
| Nana allele 2 (Plus 1) | Addition of 1 nucleotide at bp 911    | Frameshift (RF +3) leading to early stop codon | 42 different amino acids on C terminus from AA185(31 of the amino acids are the same as Δ32) | 226            | Lacks 3 TM domains, altered C’ protein sequence after TM 3 |

**FIGURE 3**  Student work depicting protein structure of CCR5 and predicted structures of its various modified versions (a) on paper and (b) using Biorender. Nucleotide sequences that were inserted or deleted are indicated, and nucleotides shared between Δ32 and Nana+1 alleles are highlighted [Color figure can be viewed at wileyonlinelibrary.com]
appropriate region in order to create a loss-of-function CCR5 protein. Specifically, students identified and designed 3 key components: (a) the cas9 nuclear target sequence in CCR5, (b) the protospacer adjacent motif (PAM) sequence in CCR5 gene utilized by cas9, and (c) the complementary guide RNA that is guided to the target sequence by cas9 (Figure 4).

Using the reference unmodified CCR5 gene, students utilized the “features” tool on SnapGene to identify and annotate the cas9 specific PAM sequence (5’NGG3’) chosen by Dr. He that was presented in his oral presentation at the Second International Summit on Human Genome Editing meeting (Start at: 1:17:57). The guanine dinucleotides of the PAM sequence that is on the non-target strand of the gRNA interacts with arginine amino acids of cas9 to assist the unwinding of double-stranded DNA for subsequence nuclease activity (as reviewed in Chen and Doudna3). Next, students were guided to label the target sequence of the cas9 nuclease, which are the 20 nucleotides upstream of the 5’TGG3’ PAM sequence (Figure 5a). The cas9 nuclease is brought to the target sequence by the single guide RNA, which is composed of an RNA sequence complementary to the target strand and an 80-mer universal scaffold region that aids in cas9 binding. To model formation of gRNA-cas9-target DNA complexes, students were tasked to hand draw how all these components interact, labeling important components and sites including their PAM sequence, the location of the cas9 cut site, the annealing of the gRNA to its specific target nucleotide sequence. We intentionally had students draw this out based on our prior teaching experiences where assessment revealed students found it challenging to conceptualize the action and polarity of gRNA and cas9 complex with two complementary DNA strands (target and non-target strands) of the target gene.19

Students were prompted to discuss why this specific PAM associated with the target sequence was chosen of all possible PAM sequences of (5’NGG3’) as a quick “Find” of “GG” highlights in yellow all the GGs present in the sequence (yellow boxes, Figure 5a), and students observe that there are many potential PAM sequences present throughout the gene. The discussion led the class to conclude that this specific 5’NGG3’ was chosen of many potential 5’NGG3’ because of the necessity for cas9 cut the CCR5 genomic DNA in close proximity to the physical location of the desired nucleotide change that could potentially give rise to Δ32 allele variant.

**FIGURE 4** Necessary components for CRISPR-cas9-based genetic modifications. (a) cas9 and gRNA form a complex, then gRNA base pairs with complementary sequence in CCR5 gene with PAM aiding in bringing cas9 nuclease in, resulting in cas9 double-stranded cleavage 3 nucleotides from PAM sequence. (b) A comparison of endogenous DNA repair mechanisms, non-homologous end joining (NHEJ) and homology directed repair (HDR), which utilizes a repair template containing the desired nucleotide change. Nana alleles Δ4 and +1 are shown as outcomes of NHEJ, and HIV resistant Δ32 is shown as an outcome of HDR with the use of an appropriate repair template.
2.3.5 | Interpreting DNA repair mechanisms that created Nana and Lulu alleles; designing a homology directed repair (HDR) repair template

Identification of the likely CCR5 PAM sequence and cas9 cut site was followed by discussion of the outcomes for the CCR5 gene after cas9 cleavage, and how different endogenous repair mechanisms are used to achieve different research goals. Animation are shown to illustrate the two processes.\textsuperscript{35,36} The process of non-homologous end joining (NHEJ) is used after cas9 makes the double stranded cut to produce indels and potential loss-of-function alleles due to frameshift mutations. Homology Directed Repair (HDR) is typically utilized when a specific nucleotide change is desired. HDR requires the addition of a repair template with homology arms that enables the cell to utilize HDR to incorporate a desired nucleotide sequence after the cas9-mediated dsDNA cut. As a class, we discussed the features that the HDR must contain: (a) the desired nucleotide change, (b) regions of DNA that were similar to the original allele (homology arms), and (c) a PAM sequence that was altered such that the repair template would not be cut by cas9. Students were guided to create an HDR template in SnapGene (Figure 5b). Students were then asked to work in groups to discuss which DNA repair strategy was likely utilized by Dr. He to produce the three new variant alleles found in Nana and Lulu. Most students report that NHEJ occurred in the embryos to create the new variants and that a repair template was likely not used as the HIV resistant Δ32 allele was not found in Lulu and Nana.

2.3.6 | Putting knowledge into practice: CRISPR-cas9 gene editing strategy with butterfly wing patterning gene optix and human oncogenic Ras gene

Students worked on two follow-up hypothetical scenarios to practice the CRISPR design and strategy of sgRNA and
target sequence. The first scenario was that students were investigating the function of the gene optix in Lepidoptera butterflies, and were tasked to design a CRISPR-cas9 strategy to create a loss-of-function optix gene. The students were shown published results of a CRISPR-cas9 strategy, where optix CRISPR-ed butterflies displayed color and patterning defects. An example of student work is shown in Figure 5c. The second scenario was that they had cultured cancer cells growing in lab with Ras gene that has the oncogenic mutation that causes abnormal cell proliferation. The students’ goal was to design a strategy to take this mutant Ras and genetically modify it to wild-type Ras. They were given the coding sequence for the Ras gene in SnapGene with the oncogenic mutation causing 12th amino acid Glycine to Valine change (G12V). Students were asked to discuss in pairs what they would predict the phenotypic change would be after successful gene edit. The work was submitted to be checked by the instructor.

2.3.7 | Bioethics discussion on implications of CRISPR-cas9 technology and CCR5 editing in human society

Following the exercise, we engaged the students in a robust bioethical discussion on the bioethical implications of CRISPR-cas9 technology and the modification of the CCR5 gene. Questions that were posed to students for discussion are included in the Tables S2 and S3. One topic was the discussion of the novel and Δ32 mutations on CCR5’s normal function in human health and disease. Whether Dr. He’s novel CCR5 mutations in the twins actually confer HIV protection or has an effect on normal immune function has not been published yet. We asked students whether Lulu and Nana should be monitored by a specialized group of doctors and researchers for unintended consequences of their genetic edits since they have novel modified alleles that have not been studied before. Since Dr. He’s made his claim, studies show that humans who are carriers for the Δ32 CCR5 allele have a faster recovery from strokes. Furthermore, CCR5 plays a role in brain cognition, as mice that lack CCR5 have improved memory.

3 | RESULTS AND DISCUSSION

3.1 | Student learning of CRISPR-based concepts

To assess if students could effectively learn the basics of CRISPR-based gene editing, without traditional “wet laboratory” bench-based manipulations, we deployed a “dry lab” computer-based module using human germline editing of CCR5 as a case-based learning study. This was implemented in two separate courses at a primarily undergraduate institution (PUI) in Spring 2018 semester. Courses were designed for mid to upper level science majors (300-level Genetics course with 17 students, and 400-level Advanced Genetics course with 11 students) with a vested interest in advanced topics in genetics and genetic engineering. In general, students were enthusiastic about studying CRISPR-cas technology, especially using an example that was a very recent and controversial development in the field. Our learning objectives (Table 1) were framed around core components of CRISPR-cas editing, focusing on the technical design and outcomes of gene editing concepts. To assess student achievement of these learning objectives, we chose not to formally assess student work on the CCR5 gene, as this work was instructor guided. Instead, we assessed students on learning objectives in a post-activity assignment where students could effectively showcase their knowledge (Table 3, Table S3/Rubric).

Pre and post-surveys asked students to “explain their current understanding of how the process of CRISPR-cas gene editing works, and describe the molecular components, and how they utilized by the system.” In pre-surveys, most answers were unacceptable (93.33%) or developing (0.07%), and in post-surveys there is an increase in students’ understanding of CRISPR-cas9 technology with 50% achieving acceptable or higher (Figure 6). These results indicate that the use of a case-based module is an effective strategy for disseminating the fundamentals of CRISPR-cas9-based technology.

Our ultimate goal of this exercise is for students to then be able to apply their acquired knowledge, requiring them to demonstrate knowledge beyond simple comprehension and reiteration of facts. We therefore asked students to complete exercises demonstrating their ability to apply CRISPR-related concepts to novel scenarios. Students in our 300-level Genetics cohort were tasked to create a research proposal that asks a scientific question utilizing CRISPR-technology in their organism of choice (Table 3). Specifically, students determined a gene of their choice that they would like to modify and proposed how they would phenotypically assay the success of their edit. Some students were initially overwhelmed by the open-ended nature of the research proposal, but once they started investigated their gene of interest, they became more engaged. Student and faculty hesitation with novel research in courses is not uncommon; however, there is overwhelming evidence to suggest that once adopted, novel research is an effective teaching and learning practice.
TABLE 3  Student learning objectives for application of CRISPR-cas in research proposal

| Student learning objective                                                                 | Means of assessment       | Percent of students achieving LO |
|--------------------------------------------------------------------------------------------|---------------------------|--------------------------------|
| 1  Explain gene chosen for modification and the justification for gene and desired change in cell/organism of choice | Introduction in research proposal | 77.78%a                      |
| 2  Describe the CRISPR-cas9 strategy                                                       |                           |                                |
| A. Determine appropriate target sequence and PAM Sequence in target gene                   | Methodology in research proposal | 100%a                        |
| B. Determine the gRNA sequence utilized                                                    |                           |                                |
| C. Create repair template if HDR is utilized                                               |                           |                                |
| 3  Describe how the effect of the gene modification will be measured if gene modification is successful, and expected results and challenges | Methodology in research proposal | 66.67%                       |
| 4  Describe the bioethical implications of using CRISPR-cas9 system in your system         | Discussion in research proposal | 55.56%                       |

aNine groups of two students.
bEight groups of two students, one group did not choose to utilize HDR mechanism.

FIGURE 6  Student pre- and post-survey responses to the question “Explain your current understanding is of how the process of CRISPR-cas9 gene editing works. What are the molecular components, and how are they utilized by the system?”  [Color figure can be viewed at wileyonlinelibrary.com]

Students proposal topics and results of assessment are summarized in Table 4. Students were assessed on their justification of their gene and desired change, and 77.78% of students achieved acceptable or higher in their justification. Assessment showed that all students could describe their CRISPR-cas strategy in their proposal through accurately determining appropriate target sequence and PAM sequence in desired gene, and designing the gRNA and repair template utilized to make their desired nucleotide change. This shows transference of knowledge from the CCR5 exercise, and that students were able to master the technical aspects of CRISPR-cas9 technology design. However, 66.67% of students achieved the learning objective of effectively describing how the effect of the gene modification would be measured and their expected results and challenges. Achieving this learning objectives requires higher order thinking in terms of the relationship between genotypes and phenotypes by determining and describing a valid assay to measure the success of their gene modification. All teams were also required to rationalize the bioethical implications of using the CRISPR-cas system in their system of choice. 55.56% of the students could effectively describe the bioethical implications of using the technology in their system. Notably, only one team proposed a strategy to modify a gene in human embryos, while all other teams chose either human somatic cells or other organisms. Considering the required bioethical component of this proposal, this likely signifies that students may be unwilling to engage in such controversial dialog. In anything, student resistance highlights a need for bioethical training, even at the undergraduate level.

In the 400-level advanced genetics course, students worked in groups to create a podcast for the general topic focused on a theme of CRISPR-cas technology. We chose to create podcasts over research proposals for two main reasons. Firstly, one of the course goals was to develop student communication skills, and secondly, this exercise was used intentionally in the first weeks of Advanced Genetics as an introduction into the rest of course material focusing on current findings and applications of CRISPR-cas technology through primary literature. The three topics chosen for each group’s podcast included: (a) CRISPR-cas history, guidelines, and treatment of disease; (b) CRISPR-cas gene editing in human embryos and live humans; and (c) CRISPR-cas agriculture and food. (Rubric for the podcast, Table S4). When assessed for content and depth of knowledge, student work again demonstrated that fundamental aspects of CRISPR-mediated gene edits were being understood by students (data not shown). Again, these data signify that learning modules such as the one herein is an effective and engaging means to teach these concepts to undergraduates.
3.2 Case-based learning modules as gateway into authentic research practices

Bringing CRISPR-cas9 technology to undergraduate curriculum can prove challenging especially in circumstances where resources or instructor familiarity might be limiting factors. Although hands-on experimentation with CRISPR systems is not feasible for every institute, we tested whether a case-based learning module is sufficient to teach students about the effective use of CRISPR-cas technology in authentic research. We asked students to report the effectiveness of using CRISPR-cas technology in (a) learning how authentic biology research is conducted, and (b) their abilities to apply tools learnt in experimental research and design (Figure S2a,b). The majority of students who did not see the importance of these examples before engaging in the exercise could clearly see the relevance upon completion of the module. These data suggest that students found they could apply this case-based module to authentic practices in the research process.

Anecdotally, students have difficulty visualizing the design, implementation and expected results of manipulating sequences. Here we took a backwards design approach, working from affected protein produced from CRISPR-cas-based edits to the unmodified CCR5 gene for students to visualize how their desired outcome necessitate thinking critically about each process of the central dogma. This technical design can be done without the use of sequencing computer software; however, we find that the use of SnapGene (or similar software) is helpful in allowing students to conceptualize how changes at the genomic level affect the gene product. When asked students for written anonymous feedback about the use of SnapGene to support our lab learning outcomes, students commented on the effectiveness of visualizing nucleotides, amino acids and features easily. Additionally, the ability to make desired nucleotide changes to simulate gene edits/modification was beneficial. A few students did comment on the challenges of learning how to use the program initially, but that with practice they were able to use the program more effectively. We should also note that student understanding seemed to be drastically improved when they could visualize both strands of the DNA and its polarity, not just coding strand of the desired target.

### TABLE 4 Student proposed CRISPR-cas target genes and strategies in research proposal

| Target gene | Target organism | Strategy | Phenotype |
|-------------|----------------|----------|-----------|
| Pax 6       | *C. elegans*   | Create loss-of-function Pax 6 | Nervous system development |
| TRAK1       | Human T-cell   | Create wild-type version from a mutant version | Trak1 stability and GABA signaling |
| Dystrophin  | Human muscle cells | Create wild-type version from a mutant version | Gain in muscle mass and strength |
| CH1 gene encoding the Fel d 1 protein | Cat | Create loss-of-function CH1 | Reduction in human allergies to cat |
| Hairless (HR) | Naked mole rat | Create specific nucleotide change to cause tryptophan to cysteine change | Hair growth |
| Opsin       | Mouse          | Create loss-of-function opsin | Reduce blue color vision |
| tga1        | Maize          | Create specific nucleotide change (to cause asparagine to lysine change) | Increase in encased corn kernels (teosinte-like phenotype) |
| KIAA0319    | Human embryos | Create specific nucleotide change of T to C; changing a SNP associated with dyslexia | Change in dyslexia symptoms |
| CFTR        | Human lung cells | Inserting 508th amino acid that is deleted in CFTR patients | Reduction in CFTR symptoms |

3.3 Beyond case-based learning modules—Expanding into other high impact practices

In the future, we can imagine instructors expanding this case-based learning module focused on CCR5 into other
areas of biology and medicine. For example, bringing into class a published example where students can analyze data from an individual with HIV and leukemia was given a stem-cell transplant from a donor homozygous for CCR5 Δ32.48 As a result, genotyping and phenotypic analysis revealed the individual had switched to homozygous Δ32 genotype and was cured of HIV and leukemia and HIV. This allows for deeper discussions into experimental data that probes the relationship between genotypes and phenotypes.

In our iteration of this module in Advanced Genetics, complementing this exercise with primary literature led us to analyze and discuss methodology and data of two scientific papers. The first paper was used as a historical perspective of what had been done previously in the field using Zinc Finger Nucleases to genetically modify T cells of individuals to Δ32 version and then transplant back into an individual (autologous transplantation).49 As a class we identified the Zinc Finger Nuclease target sites within the CCR5 gene. The second paper was used to discuss how scientists have used CRISPR-cas9 technology to create indels in CCR5 of T cells through NHEJ and test its effect on HIV infection.50 Following the focus on CCR5, we went on to study the recent work on gene editing of dystrophin gene in muscle dystrophy in dogs, and continued to use SnapGene to analyze CRISPR genes.

This module can also be used as a framework to create additional case-based learning studies for different diseases that are currently being targeted with somatic, non-heritable/germline CRISPR-cas technology. For example, currently the first human clinical trial utilizing CRISPR technology for immunotherapy cancer treatment is underway at University of Pennsylvania by removing individual T cells, modifying genes of T cells in lab, and then putting back the modified T cells into individuals to attack cancer cells.51,52 Other current examples include, companies working to modify the BCL11A gene and beta-globin gene to treat inherited blood disorders beta thalassemia and, sickle-cell disease53–55 (Vertex, Editas, Sanford University). These “ex vivo” methodologies involve a different set of considerations, both from the scientific and ethical perspectives. A notable case is the first human in vivo CRISPR editing clinical trial called EDIT-101 at the genome editing company Editas. Editas has received FDA approval for utilizing CRISPR technology to modify the CEP290 gene in photoreceptor cells to treat retinal degeneration condition called Leber Congenital Amaurosis 10.56–58 The methodology differs here because scientists will use CRISPR technology and adeno-associated viral (AAV) delivery system to deliver CRISPR components to edit genes inside the human body in vivo rather than ex vivo. Collectively, these different studies and clinical trials can be used to immerse students in analyzing biomedically relevant genes and associated diseases in the classroom, and bring forward the discussion on how both non-heritable and heritable CRISPR gene editing is being designed and applied in basic science and medicine.

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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

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REFERENCES
1. Mojica FJM, Diez-Villasenor C, Garcia-Martinez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol. 2005;60:174–182.
2. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709–1712.
3. Chen JS, Doudna JA. The chemistry of Cas9 and its CRISPR colleagues. Nat Rev Chem. 2017;1:1–15.
4. Regalado A. EXCLUSIVE: Chinese scientists are creating CRISPR babies. MIT Technology Review; 2018. Available at: https://www.technologyreview.com/s/612458/exclusive-chinese-scientists-are-creating-crispr-babies/. Accessed on January 6, 2020.
5. Lannphier E, Urnov F, Haecker SE, Werner M, Smolenski J. Don’t edit the human germ line. Nature. 2015;519:410–411.
6. Oppermann M. Chemokine receptor CCR5: Insights into structure, function, and regulation. Cell Signal. 2004;16:1201–1210.
7. He J. About Lulu and Nana: Twin Girls Born Healthy After Gene Surgery As Single-Cell Embryos. YouTube; 2018. Available at: https://www.youtube.com/watch?v=th0vnOMFltc. Accessed on January 6, 2020.
8. Second International Summit on Human Genome Editing: Continuing the Global Discussion; 2019. Available at: http://www.nationalacademies.org/gene-editing/2nd_summit/. Accessed on January 6, 2020.
45. Rodenbusch SE, Hernandez PR, Simmons SL, Dolan EL. Early engagement in course-based research increases graduation rates and completion of science, Engineering, and Mathematics Degrees. CBE—Life Sci Educ. 2016;15:ar20.
46. Auchincloss LC, Laursen SL, Branchaw JL, et al. Assessment of course-based undergraduate research experiences: A meeting report. CBE—Life Sci Educ. 2014;13:29–40.
47. Kirkpatrick C, Schuchardt A, Cotner S. Computer-based and bench-based undergraduate research experiences produce similar attitudinal outcomes. CBE—Life Sci Educ. 2019;18:ar10.
48. Hütter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med. 2009;360:692–698.
49. Tebas P, Stein D, Tang WW, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med. 2014;370:901–910.
50. Liu Z, Chen S, Jin X, et al. Genome editing of the HIV coreceptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4+ T cells from HIV-1 infection. Cell Biosci. 2017;7(7):47.
51. Early Results from First-In-U.S. Trial of CRISPR-Edited Immune Cells for Cancer Patients Suggest Safety of Approach—PR News; n.d. Available at: https://www.pennmedicine.org/news/news-releases/2019/november/results-first-us-trial-crispr-edited-immune-cells-cancer-patients-safety-of-approach. Accessed on January 6, 2020.
52. NY-ESO-1-redirected CRISPR (TCRendo and PD1) Edited T Cells (NYCE T Cells)—Full Text View—ClinicalTrials.gov; n.d. Available at: https://clinicaltrials.gov/ct2/show/NCT03399448?term=Crispr. Accessed on January 6, 2020.
53. CRISPR Therapeutics and Vertex Announce Progress in Clinical Development Programs for the Investigational CRISPR/Cas9 Gene-Editing Therapy CTX001 | CRISPR Therapeutics; n.d. Available at: http://ir.crisprtx.com/news-releases/news-release-details/crispr-therapeutics-and-vertex-announce-progress-clinical. Accessed on January 6, 2020.
54. A Safety and Efficacy Study Evaluating CTX001 in Subjects With Severe Sickle Cell Disease; n.d. Available at: https://clinicaltrials.gov/ct2/show/NCT03745287. Accessed on January 14, 2020.
55. Bak RO, Dever DP, Porteus MH. CRISPR/Cas9 genome editing in human hematopoietic stem cells. Nat Protoc. 2018;13:358–376.
56. Editas Wins FDA Approval for IND of CRISPR Treatment for LCA10; n.d. Available at: https://www.genengnews.com/news/editas-wins-fda-approval-for-ind-of-crispr-treatment-for-lca10/. Accessed on January 6, 2020.
57. Single Ascending Dose Study in Participants With LCA10; n.d. Available at: https://clinicaltrials.gov/ct2/show/NCT03872479?term=Crispr&draw=3. Accessed on January 6, 2020.
58. Mullard A. First in vivo CRISPR candidate enters the clinic. Nat Rev Drug Discov. 2019;18:656.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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