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An effective *C. elegans* CRISPR training module for high school and undergraduate summer research experiences in molecular biology

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Abstract

Engaging in research experiences as a high school or undergraduate student interested in science, technology, engineering, and mathematics (STEM) is pivotal for their academic and professional development. A structured teaching framework can help cultivate a student's curiosity and passion for learning and research. In this study, an eight-week training program was created to encompass fundamental molecular biology principles and hands-on laboratory activities. This curriculum focuses on using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene editing in the *Caenorhabditis elegans* model organism. Through pre- and post-program assessments, enhancements in students' molecular biology proficiency and enthusiasm for scientific exploration were observed. Overall, this training module demonstrated its accessibility and ability to engage inexperienced students in molecular biology and gene editing methodologies.

Keywords

CRISPR; gene editing; *C. elegans*; training module

1. INTRODUCTION

Research experiences for students interested in science, technology, engineering, and mathematics (STEM)-related fields help foster a student's academic and professional development. Students who participate in research during the first two years of college are more likely to remain in STEM majors (NAGDA *et al.* 1998) and self-report higher confidence in their science learning abilities, especially for women and historically marginalized

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Conflict of interest statement

All authors declare that they have no conflicts of interest.

Ethics approval statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the institutional review board (IRB) through Indiana University Purdue University Indianapolis (#19152).

minorities (AUCHINCLOSS *et al.* 2014; BANGERA AND BROWNELL 2014). Through research experiences, students develop critical thinking skills, gain confidence in their ability to become successful professionals (ADEBISI 2022) and are more engaged with their coursework after their summer experiences (LOPATTO 2007). STEM students also benefited from targeted one-on-one mentoring (MCSWEENEY *et al.* 2018). Positive research experiences increase participants' desires to earn a doctoral-level degree (LESSARD *et al.* 2021) and contribute to their overall success in graduate school (VINCENT-RUZ, GRABOWSKI AND SCHUNN 2018). Thus, effective research training modules can directly improve STEM learning for all students, regardless of their academic background or career goals.

The establishment of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene editing in science and popular culture opens opportunities to engage students in molecular biology concepts. CRISPR–CRISPR-associated protein (Cas) mediated genome editing is a prokaryotic mechanism for adaptive immunity against viruses and other foreign invaders (JIANG AND DOUDNA 2017). CRISPRs were first discovered in the sequences of DNA from *Escherichia coli* (ISHINO *et al.* 1987) and *cas* genes later shown to encode proteins with endonuclease activity (JINEK *et al.* 2012; JIANG AND DOUDNA 2017). Currently, CRISPR-Cas has become a widespread method used in scientific laboratories and a common topic in biology curricula (DAHLBERG AND GROAT CARMONA 2018). Recombinant Cas proteins, like the *S. pyogenes* Cas9 (e.g. (JINEK *et al.* 2012)), can be combined with chemically synthesized RNAs to form an enzyme complex capable of targeted DNA cleavage (Fig 1A). Cas9-mediated genome editing can be divided into three steps (JIANG AND DOUDNA 2017): 1) DNA site recognition, 2) DNA cleavage, and 3) DNA repair. RNA directs Cas9 to the gene target sequence through complementary base pairing (JIANG AND DOUDNA 2017). Once paired with the specific sequence, Cas9 will cleave the DNA site, creating a double-stranded break (DSB) (Fig 1A) (JIANG AND DOUDNA 2017). The DSB is repaired by the host cellular machinery (JIANG AND DOUDNA 2017), either by error-prone nonhomologous end joining (NHEJ) (LIEBER 2010) or by homology direct repair (SAN FILIPPO, SUNG AND KLEIN 2008). Through this method, genetic regions can be removed, or coding regions inserted to create null mutations, large deletions, point mutants, addition of protein or fluorescent tags, and other modifications to study the biology and pathology of a gene of interest.

Caenorhabditis elegans is a simple model organism that can be modified by CRISPR-Cas9 to train inexperienced students in molecular biology and laboratory skills. The advantages of *C. elegans* include their small size for easy manipulation, transparent body for imaging, simple anatomy, ability to self-fertilize for straightforward genetics, and short life cycle. The adult hermaphrodite worm contains two large germlines with germ cells processing through cell development into oocytes (KIMBLE AND CRITTENDEN 2007) (Fig 1B). Sperm made in the larval stages of development is stored in the spermatheca. Oocytes cross through the spermatheca, are fertilized by sperm, and form embryos in the uterus. Despite its advantages, targeted gene editing in *C. elegans* historically has been challenging. Homologous recombination is inefficient (PLASTERK AND GROENEN 1992; BEREZIKOV 2004), and thus the manipulation of specific gene loci relied on forward genetic screens (KUTSCHER AND SHAHAM 2014). The discovery of CRISPR-Cas9 gene editing capabilities enabled a tractable method in *C. elegans* (DICKINSON *et al.* 2013; FRIEDLAND *et al.* 2013; LO *et al.* 2013) to mutate genes and examine their phenotypes in a relatively short amount of time.

Metazoan germ cells contain discrete cytoplasmic assemblies of RNAs and proteins collectively referred to as germ granules (Fig 1C). In *C. elegans*, P granules are a type of germ granule that contain specific RNAs and proteins essential for germ cell development and RNA metabolism (PHILLIPS AND UPDIKE 2022). Proper P granule assembly is dependent on the PGL-1 scaffold protein. Structurally, PGL-1 contains an N-terminal domain (Nterm), dimerization domain (DD), and a C-terminal region with RGG repeats (Cterm) (Fig 1D) (KAWASAKI *et al.* 1998; AOKI *et al.* 2016; AOKI *et al.* 2021). PGL-1 and its homologs can self-assemble into liquid condensates (Fig 1C) through liquid-liquid phase separation (HYMAN, WEBER AND JULICHER 2014). Little is known regarding the role of PGL-1 Cterm protein region in regards to self-assembly, P granule assembly, and function in germ cell development.

This summer research module was designed to teach students the fundamentals of molecular biology through experimentation with *C. elegans* and CRISPR-Cas9 (Fig 1E). The module introduced an innovating and dynamic approach that combined hands-on laboratory exposure and measurable learning assessment. In this study, students used CRISPR-Cas9 to map the protein regions in PGL-1 necessary for protein expression and P granule assembly, but the laboratory project could have been adapted to any *C. elegans* and CRISPR gene editing target. Thus, this model was a practical template to teach students basic science concepts, engage students in independent laboratory research, and generate reagents for future studies.

2. MATERIALS AND METHODS

2.1 Recruitment and Assessment.

Students were recruited to the lab via high school and undergraduate summer research programs at the Indiana University School of Medicine (IUSM). These programs included the Indiana University – Purdue University Indianapolis (IUPUI) Life Health Science Internship (LHSI), Indiana University Simon Comprehensive Cancer Center (IUSCCC), Indiana Medical Scientist/Engineer Training Program’s Undergraduate Summer Research Program (MSTP-USRP), and Indiana Clinical and Translational Sciences Institute (CTSI) summer research program. The applicants were chosen by their specific programs, and in most cases had the opportunity to indicate their scientific interests. Final matches were made dependent on these interests and lab availability. The Institutional Review Board (IRB) required study participants to be over 18 years of age (IRB #19152). All guidelines were followed according to their training “Legacy Human Subjects Research - Biomedical Researchers, IRB Administrators, and IRB Members (ID 201217).” All participants gave their informed consent for inclusion before joining the study.

A Qualtrics (Qualtrics XM; www.qualtrics.com) pre- and post-test and survey were offered to summer students participating in the program. IRB exemption was given through Indiana University Purdue University Indianapolis (#19152). The test probed the student’s knowledge base in basic molecular biology and genetics, CRISPR, and model organisms. The survey measured an individual’s current interest in science. The pre- and post- test and survey were administered on the first and last day of the student’s summer research experience. They were performed unanimously without identifiers. Six participants took the pre-test and survey, but only four students completed the post-test and survey. The

discrepancy arose because two students were unable to conclude their internships for personal reasons. The individual pre- and post-responses could not be discerned, and thus all test and survey results were included in this report. The pre- and post-tests were scored, and the results were graphed using GraphPad Prism and Microsoft Excel software.

2.2 Data Analyses.

GraphPad Prism software was used to present the graphical data. For the multiple-choice survey responses, the accuracy was calculated as a percentage. This was done by summing the number of correct responses for each question and dividing by the total number of participants who answered that question. The resulting value was then multiplied by 100 to convert it to a percentage. Science interest was assessed using a questionnaire, and the results were reported as the percentage based on the responses from participants. This percentage was calculated by dividing the number of participants who chose a particular response by the total number of respondents to that question, and then multiplying by 100. The pre-survey responses included data from 6 participants (n=6), while the post-survey responses included data from 4 participants (n=4). Given the small sample sizes, statistics were not performed to compare the groups. Instead, the results were summarized and presented as descriptions of the data collected.

2.3 Nematode Strains and Maintenance.

Nematodes were grown on Nematode Growth Medium (NGM) plates with HB101 bacteria as food source, as described previously (BRENNER 1974). All strains were propagated at 20°C. Worms were outcrossed with a wildtype N2 strain.

2.4 CRISPR-Cas9.

Trained lab members performed all CRISPR microinjections into the gonads of young adult worms. The CRISPR mix included recombinant *S. pyogenes* Cas9 (Integrated DNA Technologies, IDT), tracrRNA and *pgl-1* targeting crRNAs (IDT), and repair DNA oligo (IDT). The co-conversion approach was implemented, which involves co-injection of CRISPR-Cas9 ribonucleoproteins (RNPs) targeting the *unc-58* or *dpy-10* gene, producing uncoordinated worms that have impaired locomotion (*unc* or *rol* phenotype, respectively), to select and screen worm progeny modified by the CRISPR microinjection (ARRIBERE *et al.* 2014; KIM *et al.* 2014; PAIX *et al.* 2015; GHANTA, ISHIDATE AND MELLO 2021). These reagents were used to modify a worm strain expressing Halo-V5 tagged PGL-1 protein. Halo is a protein tag that permits straightforward imaging with commercially available fluorescent ligands (LOS *et al.* 2008; DANIELS *et al.* 2014; ENGLAND, LUO AND CAI 2015). V5 is a 13 amino acid epitope tag that can be detected by immunoblot with commercially available antibodies. Thus, all CRISPR worms expressed mutant PGL-1 protein that could be detected by imaging and immunoblot.

2.5 Polymerase Chain Reaction (PCR) and DNA sequencing.

F1 *unc* L4 larvae were singled onto NGM plates with HB101 bacteria and allowed to lay eggs for approximately one day. These F1 animals were then transferred into 2x worm lysis buffer (50 mM KCl, 10 mM Tris pH 8.3, 2.5 mM MgCl₂, 0.01% gelatin, 0.45%

NP-40, 0.45% Tween 20 detergent, 8 units/ml Proteinase K (New England Biolabs)), lysed at 60°C for 1 hour, and PCR screened to detect the desired *pgl-1* deletions. The PCR screen used Taq polymerase (NEB), dNTPs (NEB), targeted *pgl-1* primers (IDT), and the acquired worm lysis buffer as DNA template. All primers were designed on SNAPGene software (GSL Biotech LLC; snapgene.com). F1 worms that generated the expected PCR product deletion were selected and their F2 progeny singled. These singled worms were lysed and analyzed again by PCR to identify homozygous animals. Homozygous animal samples were PCR amplified with Q5 (NEB) or KOD (Sigma) polymerase with the same primers. This PCR product was PCR purified (NEB) and Sanger sequencing performed using SupraDye v3.1 (Calibre Scientific). Unincorporated dNTPs were removed from the samples with AxyDye cleanseq magnetic beads (ThermoFisher). Samples were sequenced by ACGT (www.acgtinc.com). Sequences were analyzed by SNAPgene to confirm proper editing.

2.6 Immunoblot.

Worms were collected in 2x sodium dodecyl-sulfate polyacrylamide (SDS) sample buffer (Bio-Rad), denatured for 10 minutes at 95°C, loaded onto 12% SDS-page gels, and transferred onto PVDF membrane (Bio Rad) using a Trans-Blot Turbo Transfer System (Bio Rad). After transfer, membrane was blocked with 5% non-fat milk in PBS-T (127 mM NaCl, 2.7 mM KCL, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 0.1% (w/v) Tween 20 detergent) for one hour followed by primary V5 antibody (1:500; R&D Systems Bio-technie) overnight to probe for PGL-1. Next, membranes were washed with PBS-T, and incubated in secondary Goat anti-mouse HRP antibody (1:4000; R&D Systems Bio-technie) for at least one hour. Membrane was then washed with PBS-T and developed using SuperSignal West Pico Stable Peroxide Solution (ThermoFisher) and SuperSignal West Pico Luminol Enhancer Solution (ThermoFisher). Developed blots were imaged on a ChemiDoc MP Imaging System (Bio Rad) and analyzed on its Image Lab software (Bio Rad).

2.7 Confocal Microscopy.

Worms were fixed and permeabilized in 3% paraformaldehyde (PFA) followed by DAPI (0.5 ug/ml) and Halo-Oregon Green ligand (300 nM, Promega) staining in PBS-T for one hour. Worms were washed with PBS-T after fixing and staining before being placed on slides with VECTASHIELD Mounting Medium (Vector Laboratories) for imaging. Fluorescent confocal microscopy was performed using a Zeiss AxioObserverZ1 by 3i (www.intelligent-imaging.com). Adult germline images were taken using Slidebook software (Intelligent Imaging Innovations) and a 63x objective. All images were analyzed using Fiji image-processing package (<http://fiji.sc/Fiji>) (SCHINDELIN *et al.* 2012).

3. RESULTS AND DISCUSSION

Research for high school and undergraduate students promotes their retention in STEM-related fields (NAGDA *et al.* 1998) and enhances students' learning experiences (PENDER *et al.* 2010). Summer break is a common time to fully immerse themselves in a research experience. Therefore, a full-time, 8-week summer teaching module was created that used CRISPR gene editing and the *C. elegans* model organism as an entrée into molecular

biology. CRISPR technology, widely used in scientific laboratories and in *C. elegans* research, is currently being developed as a cancer therapy (Baylis and McLeod, 2017). Therefore, the students' education in current gene editing methods has direct relevance to human health, meeting key criteria for many biomedical summer research programs (see Methods).

This training module included:

1. An independent research project centered around CRISPR and *C. elegans* (Fig 2)
2. One-on-one personalized mentorship with a training mentor (e.g., graduate student)
3. Weekly wet lab assignments to provide hands-on training and step-by-step instruction toward a research project goal(s) (Fig 2, Supplemental Table 1)
4. Weekly dry lab assignments to provide step-by-step learning on the fundamentals of molecular biology (Fig 2, Supplemental Table 1)
5. Weekly, hour-long molecular biology teaching and review sessions with the Lab mentor (i.e., Lab supervisor) (Supplemental Table 1)
6. A pre- and post-test and survey to measure students' scientific knowledge and self-assurance (Supplemental Table 2)
7. End-of-the-term summer research presentation for their individual programs

The goals of the training module were to enhance students' scientific knowledge, develop laboratory and logic skills, and explore their enthusiasm for STEM. The one-on-one mentoring enhanced communication among lab members and students and fostered meaningful interpersonal bonds between mentor and mentee. Thus, this hands-on summer research experience was tailored for inexperienced high school and undergraduate students to provide them with invaluable research exposure, nurture their academic self-assurance, and instill fundamental concepts in molecular biology.

Training ran for 8 weeks, with an expected commitment of 35–40 hours per week. At the beginning of each week, students were given specific terms or questions outlined in the “dry lab” section of the summer strategic plan (Supplemental Table 1). These questions focused on basic concepts in molecular biology, model organisms, CRISPR and gene editing, standard methods in DNA and protein detection, and basic laboratory techniques. Students met with their training mentors, typically a graduate student mentor, throughout the week to discuss dry lab prompts in an informal setting. This established a baseline understanding of scientific terminologies and techniques critical to the activities scheduled. At the end of each week, a comprehensive review session was facilitated by the Lab supervisor, wherein both the dry and wet lab concepts were revisited with the students. At least one training mentor was present in the weekly reviews to ensure clarity and to know what was discussed with the students. These weekly reviews enabled the mentors to gauge students' level of comprehension and decide what to emphasize in the following weeks to fill knowledge gaps.

The student research projects used CRISPR-Cas9 gene editing to modify a gene of interest in *C. elegans*. The CRISPR injections were conducted by laboratory personnel, while all follow up screening, experiments, and animal maintenance were performed by students with close supervision. The targeted gene and its desired mutants were central to other research projects being concurrently pursued in the lab, with plans of using the mutant animals generated for future research. The students in this cohort aimed to delete regions of *pgl-1*, a *C. elegans* gene expressed in its germline and required for proper germ cell development (KAWASAKI *et al.* 1998). Prior work had determined that PGL-1 protein could conceptually be divided into Nterm, DD, and Cterm protein regions (Fig 1D) (KAWASAKI *et al.* 1998; AOKI *et al.* 2016; AOKI *et al.* 2021). Students were tasked to use CRISPR-Cas9 to delete genomic portions of *pgl-1* associated with these protein regions and test their necessity for protein expression and cell localization. All students worked with a worm strain expressing PGL-1 tagged with Halo, a modified enzyme that enabled easy labeling and protein detection (LOS *et al.* 2008; DANIELS *et al.* 2014; ENGLAND, LUO AND CAI 2015), and a V5 epitope tag for antibody binding.

Mentors provided attentive supervision throughout experimental preparation and execution until the students were proficient and confident in the methods used. Ample practice before initiating any of the project experiments ensured a progressive transition toward independence at the bench. The students that participated had varying degrees of innate skill and dedication. Students that struggled with the techniques were watched closely throughout the summer, while students that picked up skills quickly were given independence early in the research experience. While CRISPR-Cas9 is a relatively established method in *C. elegans*, the difficulty obtaining specific mutations in target genes can be variable. In the cases where mutation was difficult, students joined the effort to create another mutation, often by pursuing a different allele. Animal phenotype analyses were chosen based on whether they could be performed quickly in a short time span. For example, target protein expression by immunoblot only takes two days and thus could be easily performed in the final week of the summer research experience. The short generational time and self-fertilization of *C. elegans* allowed for straightforward collection of animal samples for these analyses.

The *C. elegans* CRISPR-Cas9 protocol for the training module was as follows:

1. CRISPR mix designed and made. CRISPR guide RNAs were designed prior to the start of the module and based on the desired cleavage site and standard RNA requirements by Cas9 (ARRIBERE *et al.* 2014; KIM *et al.* 2014; PAIX *et al.* 2015; GHANTA, ISHIDATE AND MELLO 2021). Recombinant Cas9 protein was incubated with commercially synthesized RNAs that targeted the DNA site of interest and another gene used for phenotypic screening. DNA repair oligos were included in the mix for the proper repair of *pgl-1* and for the addition of a dominant mutation in the co-injection target to cause a phenotype that could be used for animal screening. The *dpy-10* and *unc-58* genes were used as co-CRISPR targets, both of which are commonly used in *C. elegans* CRISPR gene editing (ARRIBERE *et al.* 2014). All CRISPR reagents were designed and ordered by the mentors prior to the students' arrival. The mentor also assembled the mix itself prior to use.

2. CRISPR mix injected into worms. The science mentors performed all injections. The gonads of adult hermaphrodite worms were microinjected with the CRISPR mix by the mentors (Fig 2A). These P₀ parental worms were placed on single plates and incubated with food for 3–4 days until their F₁ offspring were older larvae or adults. Before and during this incubation period, students were learning basic molecular biology and *C. elegans* methods in preparation for the subsequent steps.
3. Worms with the co-CRISPR phenotype were identified and screened. Successful gene editing of the *dpy-10* or *unc-58* co-CRISPR targets results in worms with impaired locomotion phenotype, thereby providing a distinctive phenotypic marker for the identification of CRISPR-modified worms (ARRIBERE *et al.* 2014). Under the guidance of their mentors, students were expected to independently identify CRISPR-mutated *C. elegans* based on their unique phenotype (Fig 2B), lyse worms to extract their DNA, and screen the worms by PCR analysis and gel electrophoresis (Fig 2C). Modified worm samples were expected to generate smaller DNA PCR bands compared to wildtype, indicating a genomic deletion at the desired site. Progeny of these worms were singled onto new plates, incubated for 1–3 days, lysed to isolate their DNA, and PCR screened again to isolate worms homozygous for the CRISPR modification.
4. Worms that were edited in the region of interest were sequenced to confirm proper repair. Once homozygous mutants were identified via PCR analysis, students independently sequenced their worms to determine whether the editing was correct. Homozygous worm DNA was PCR amplified again with a high-fidelity Q5 or KOD DNA polymerase (see Methods), and samples were sequenced by Sanger sequencing (Fig 2C). Under the mentor's guidance, gene sequence files were aligned to the expected reference *pgl-1* genomic region to confirm proper CRISPR deletion and repair. Worms with the desired alleles were outcrossed with wildtype (N2) worms twice to lower the chances of off-target CRISPR modifications. The genetic deletions were tracked by the student using PCR, as described previously.
5. Properly edited worms were analyzed by immunoblot and imaging to detect protein expression and localization (Fig 2D). After the *pgl-1* CRISPR deletions were successfully completed, students were given the opportunity to analyze the worm strains for protein expression and cell localization by immunoblot or imaging. N2 and Halo-tagged PGL-1 worms were used as negative and positive controls, respectively. In immunoblots, students collected adult worms in protein sample buffer, ran an SDS-PAGE gel electrophoresis, transferred the gel to a membrane, and probed the membrane for antibodies that detected the V5 epitope on Halo-tagged PGL-1 (see Methods). In imaging experiments, students collected and fixed adult worms, stained the worms with Halo ligands and DNA-binding stain, and imaged them by confocal microscopy with help from their science mentors. Thus, this experience provided students with the opportunity to learn new lab techniques, method concepts, and different perspectives on how to analyze for proteins in animals.

During the concluding week, students showcased their immersive summer experience and research endeavors through a short slideshow or poster session, required by their funding summer program (Fig 2D). This allowed students to convey their scientific findings, improve their communication skills, apply critical thinking, and showcase their intellectual efforts.

The core objectives of this training module were to teach fundamental concepts in molecular biology and inspire students to think as scientists in a research laboratory setting. To evaluate the success of these goals, a pre- and post- test and survey were administered to students participating in the training module and over 18 years of age. The pre- and post-test and survey were identical to evaluate learning and growth. The test evaluated students' knowledge of molecular biology, gene editing and CRISPR, and model organisms and *C. elegans* (Supplemental Table 2). The survey measured students' interest in science, STEM confidence, and independent learning (Supplemental Table 2). The test and survey were administered at the beginning and end of the training module. Participation was optional and all results were blinded. A total of 6 students participated in the test and survey, two whom did not complete the program and thus did not take the post-evaluation.

Student testing results supported the training module as a valuable strategy to teach molecular biology and instill enthusiasm for STEM research. The multiple-choice questions tested fundamental concepts in molecular biology, gene editing and CRISPR, and model organisms and *C. elegans* to quantitatively gauge whether students learned these undergraduate level scientific principles within a condensed period. In all three areas of study, students demonstrated improved percentages at the end of the summer (Fig 3A). The survey portion of the evaluation indicated that student enthusiasm for science increased after the training module (Fig 3B). Interest in the science section was evaluated across levels ranging from "Definitely false" to "Definitely true." Students reported an increase in their confidence to perform scientific tasks. 100% of students reported enjoyment for scientific learning at the end of the training module (Fig 3B). Students left the training module interested in pursuing further experiences and careers in STEM. In summary, the participating students learned basic molecular biology concepts in tandem with their summer research experience and left the program interested in pursuing further STEM experiences, meeting the objectives of the program.

The pre- and post-surveys helped evaluate the scientific knowledge and interest gained over the experience and helped remove biases. Results from this summer training module indicated enhancements in students' molecular biology proficiency and enthusiasm for scientific exploration. Other studies have performed pre- and post-surveys with CRISPR study but noted variable gains in improvement. For example, an undergraduate laboratory course in CRISPR noted some RNA design concepts learned but others needing improvement (MILITELLO AND LAZATIN 2017). This may be due to the differences in examination, using multiple choice in this module (Supplemental Table 2) versus short answer in the other study. Due to the timing and design, this study also allowed a full immersion in lab bench research. The students had a full work week to study the concepts and lab methods and had one-on-one mentoring. Mentors handled the advanced technical aspects of the projects, such as oligonucleotide design and CRISPR injections, while mentees were responsible for basic molecular biology tasks like PCRs, immunoblots, and

DNA sequencing. This may have promoted a sense of teamwork and shared responsibility among participants in both the lab research and concept study. The other study was an undergraduate laboratory course and most likely could not afford the same dedication of work hours.

The opportunity to perform hands-on wet lab experiments connects molecular biology concepts with real world experience. Other studies have improved student comprehension of CRISPR-Cas9 technology solely through dry lab exposure (PIECZYNSKI AND KEE 2021). But both wet and dry lab experience has distinct benefits. Another CRISPR laboratory training study noted gains in experimental understanding but losses in data interpretation (ADAME *et al.* 2016). While this is a negative, it may also reflect students realizing that scientific research requires more knowledge and training than can be achieved in the classroom. Students remained enthusiastic about STEM careers. Thus, combining research with learning concepts can maximize a student's overall experience.

Overall, this training module incorporated assessment methods, hands-on experience, and a collaborative learning environment to enhance science education. While the study focused on molecular biology and CRISPR gene editing, the approach can be implemented in any science topic being investigated by research laboratories accepting summer high school and undergraduate students.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

The data that support the findings in this study is available on request from the corresponding author, S.T.A.

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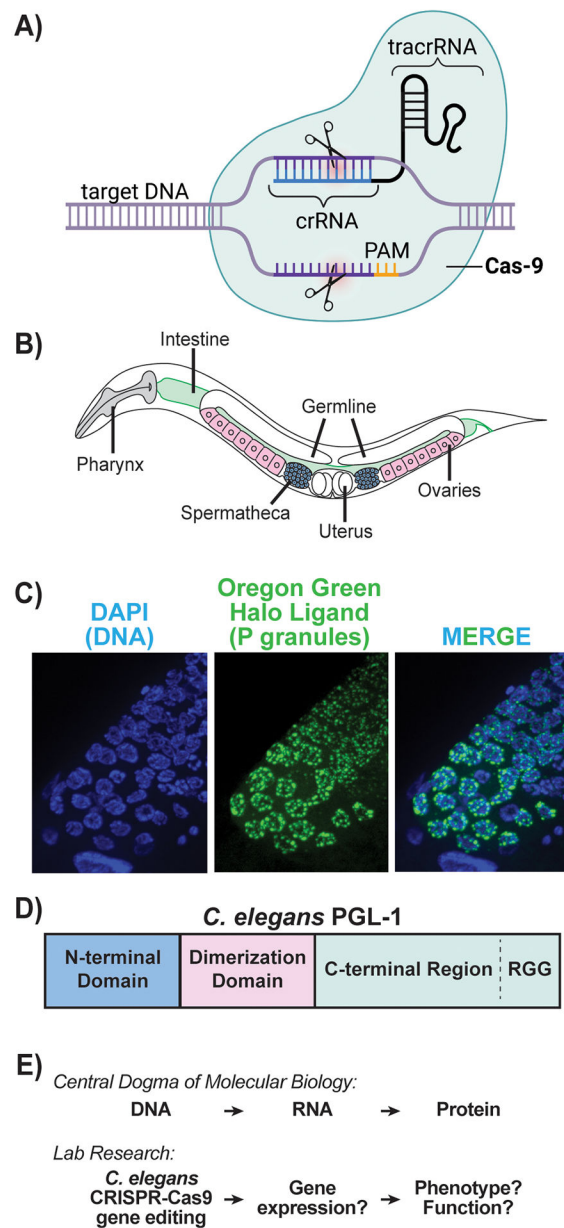


Figure 1. Essential concepts for summer students' research experience in CRISPR and *C. elegans*.

(A) Diagram of CRISPR-Cas9 and RNA-mediated cleavage. Trans-activating CRISPR RNA (tracrRNA) base pairs with CRISPR RNA (crRNA) to form guide RNA. tracrRNA and crRNA interaction is crucial for target recognition and cleavage. The protospacer adjacent motif (PAM) sequence is required for Cas9 nuclease activity, causing double-stranded DNA breaks 3–4 nucleotides downstream from the PAM site. The canonical PAM sequence is 5'-NGG-3', where "N" is any RNA base followed by two guanine (G) RNA bases. Figure created with Biorender (www.biorender.com). (B) *C. elegans* anatomy of the adult hermaphrodite. The CRISPR-Cas9 mix is injected into the germline directly. PGL-1 is also expressed in the germline. Image adapted from (KIMBLE AND CRITTENDEN 2007). (C) Confocal microscopy of P granules in the *C. elegans* adult germline. These germ granules are found at

the nuclear periphery of developing germ cells. Halo-tagged PGL-1 stained with an Oregon Green Halo ligand (green) and DNA with DAPI stain (blue). Images made in FIJI/ImageJ (SCHINDELIN *et al.* 2012). **(D)** Linear diagram of *C. elegans* PGL-1. Not to scale. **(E)** Central dogma of molecular biology paired with an outline of the summer training module.

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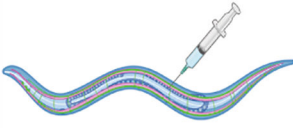
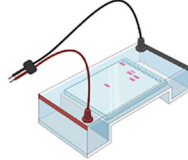
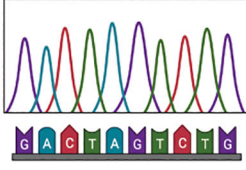
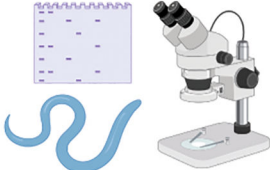
	Lab Research	Learning Objectives
A) Week 1 	<ul style="list-style-type: none"> • Practice worm picking • Perform worm lysis • Practice PCR and gel electrophoresis 	<ul style="list-style-type: none"> • Find science information • Identify reliable scientific resources • Gene editing overview • Applications of gene editing in medicine
B) Week 2 	<ul style="list-style-type: none"> • Perform PCR and gel electrophoresis independently • Perform primer design • Begin CRISPR injections 	<ul style="list-style-type: none"> • Basic molecular biology definitions • Learn about model organisms • Learn about <i>C. elegans</i> • Step by step PCR mechanism
C) Week 3-6 	<ul style="list-style-type: none"> • Identified CRISPR modified worms and isolate DNA • Identify gene modifications via PCR • Perform DNA sanger sequencing 	<ul style="list-style-type: none"> • CRISPR overview • CRISPR mechanism • CRISPR in <i>C. elegans</i> • DNA Sanger sequencing
D) Week 7-8 	<ul style="list-style-type: none"> • Perform immunoblots and confocal imaging • Create poster of summer project • Formally present project 	<ul style="list-style-type: none"> • Learn about immunoblot • Learn about confocal imaging • Freeze worms

Figure 2. *C. elegans* CRISPR mutant experimental outline and paired student learning objectives.

Overview of research expectations with accompanying learning objectives. **(A)** Week 1. Students are introduced to *C. elegans* and are taught basic molecular biology techniques such as PCR. Concurrently, they learn about gene editing technology and how to use search engines to find science information. **(B)** Week 2. Students initiate their independent work and learn to select CRISPR-modified worms based on the *unc* phenotype. Students learn about molecular biology terminology and how to use *C. elegans* as a model organism in research. Mentors inject pre-designed CRISPR-Cas9 mix into worms. **(C)** Weeks 3–6. Students further develop their skills by collecting DNA from CRISPR-modified worms and screening for gene modifications via PCR and Sanger sequencing. **(D)** Weeks 7–8. Once desired mutations are identified, students can perform immunoblots and fluorescent confocal microscopy. They will gather data and prepare a final presentation of their summer experience.

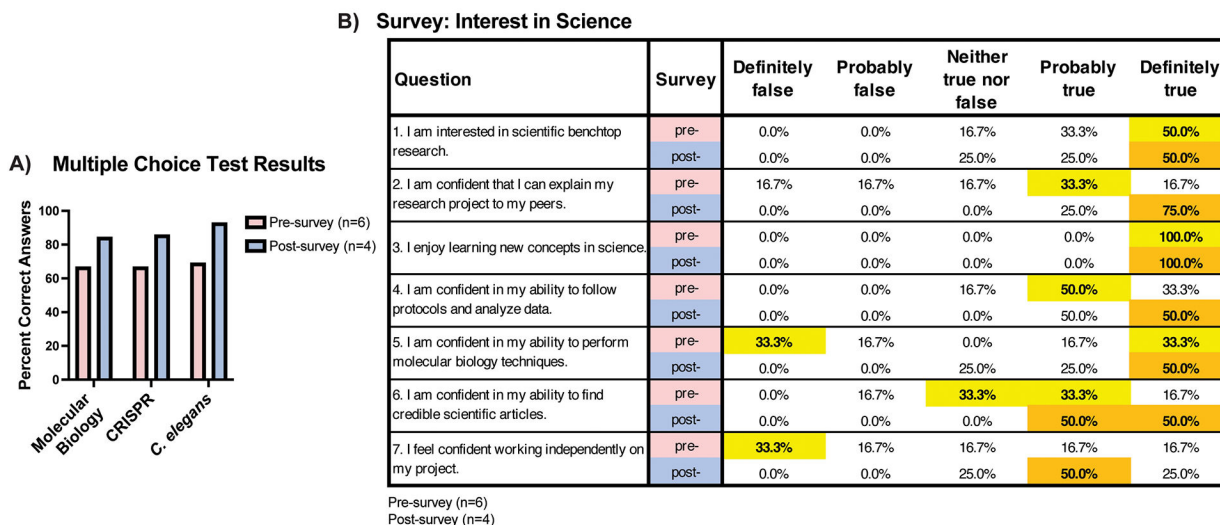


Figure 3. Results from student pre- and post-tests and surveys.

(A) Percent correct answers for the molecular biology, CRISPR, and *C. elegans* pre- and post-survey sections. Students improved their scores in all study sections. (B) Table depicting the participant's self-assurance in the training module. Between pre- and post-surveys, students reported increased confidence in all questions. Highlighted areas (yellow or orange) indicate the highest score per question. If a tie, both boxes were highlighted.