

A Course-Based Undergraduate Research Experience for High-Throughput Reverse Genetic Studies in *Arabidopsis Thaliana* with CRISPR-Cas9

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Abstract

Gene editing tools such as CRISPR-Cas9 have created unprecedented opportunities for genetic studies in plants and animals. We designed a course-based undergraduate research experience (CURE) to train introductory biology students in the concepts and implementation of gene editing technology as well as develop their soft skills in data management and scientific communication. We present two versions of the course that can be implemented with twice-weekly meetings over a five-week period. In the remote-learning version, students perform homology searches, design guide RNAs and primers, and learn the principles of molecular cloning. This version is appropriate when access to laboratory equipment or in-person instruction is limited, such as closures that have occurred in response to the Covid-19 pandemic. In the in-person version, students design guide RNAs, clone CRISPR-Cas9 constructs, and perform genetic transformation of the model plant *Arabidopsis thaliana*. The highly parallel nature of the CURE makes it possible to target dozens to hundreds of genes, depending on the number of course sections available. Applying this approach in a sensitized mutant background enables focused reverse genetic screens for genetic suppressors or enhancers. The course can be readily adapted to other organisms or projects that employ gene editing.

Keywords: CRISPR-Cas9, course-based undergraduate research experience, CURE, remote learning, plant biology

Introduction

Course-based undergraduate research experiences (CUREs) are credit-based classes in which students investigate an unresolved research question rather than carry out experimental steps with a well-defined outcome (1). CUREs can have powerful positive impacts on students by providing a more accurate representation of the process of science and introducing them to problem-solving required to answer open-ended questions (2, 3). Students that experience CUREs are more likely to remain in science, technology, engineering and mathematics (STEM) during college and enter STEM graduate programs (4). CUREs also increase diversity in STEM fields by promoting higher levels of retention of traditionally underrepresented students (5). Because CUREs require few or no prerequisites, they provide unique opportunities to students from diverse backgrounds to experience research, and serve a larger number of students than traditional undergraduate research experiences (6). Research experiences contribute to students' long-term personal and professional growth, such as identifying as a scientist, and confirmation of their career goals (7).

Despite the benefits, CUREs are not yet widely implemented for several reasons. Often, CUREs require both coordination and flexibility between lab instructors and the individual researcher providing research objectives and materials. During CURE labs the outcome of the experiments are unknown and modifications to the project may need to be made dynamically. CUREs do not predictably follow lecture-based courses due to potential differences between course materials and individual research topics (8). Finally, CUREs represent a significant departure from lab classes with a predetermined and solvable solution: the corresponding complexity introduced by solving unknowns may be unsettling to students (9).

In response to these challenges, several strategies are recommended to promote success of a CURE. First, students should learn the process of science through the direct participation of professors and graduate students who are knowledgeable about, and invested in, the outcomes of the research project (2, 10). Second, monitoring understanding frequently

through quizzes, lab notebook entries, and writing assignments keeps students engaged and highlights when concepts need clarification (11). Third, using simple laboratory techniques that can be quickly learned and implemented encourages student success (12). Finally, building in redundancy within or between sections ensures that research goals are met despite potential novice errors. Striking the correct balance between independent research and structured instruction is therefore essential to an effective CURE. Projects that combine an unknown biological outcome with a well-structured pipeline of reasonably simple techniques can maximize potential gains for the research program while building interest, confidence, and project ownership in first-time researchers.

We envisioned a CURE that could take advantage of the simplicity of gene editing techniques and large numbers of students to enable high-throughput characterization of dozens to hundreds of genes. Students would gain experience with cutting-edge technology that regularly features in the news, while advancing current research objectives.

Our specific research goals were to discover plant genes that function in the same pathways as genes involved in division plane orientation, or in karrikin signaling (13–15). Classical forward genetic approaches to this problem would involve mutagenesis of a known mutant of interest and screens for novel mutations that enhance or suppress the original mutant phenotype. However, reverse genetic approaches can also be used to characterize a set of candidate genes that have been associated with the gene of interest, for example through biochemical screens for protein interactors. Vast collections of defined *Arabidopsis thaliana* mutants have been generated through insertional mutagenesis and sequencing (16, 17). These mutant collections are enviable resources for reverse genetic studies in plants, but are not as well-suited to high-throughput tests for genetic modifiers, which would require extensive crossing and isolation of double mutants. In the event of functional redundancy, even higher order combinations of mutations may be required to observe a modifier effect. By contrast, CRISPR-Cas9 can introduce mutations into two or more related genes at once. Biallelic or even

homozygous mutations are common even in the first transformed generation (18). Much effort in CRISPR-Cas9 approaches goes toward identification of useful alleles and deriving homozygous mutant lines. We reasoned that this effort could be substantially reduced by applying a forward genetic strategy: i.e. screen pooled progeny of CRISPR-Cas9 transgenic plants for individuals with suppressor or synthetic enhancer phenotypes of interest, and then focus further studies on those individuals and their targeted candidate gene(s) (Figure 1). This overall approach could also be used to evaluate how mutations in a set of candidate genes affect a transcriptional or translational reporter carried by a transgenic line.

We adopted this approach to evaluate a list of proteins that had been identified as candidate interactors with TANGLED1 (TAN1), which is involved in division plane orientation, or KARRIKIN UPREGULATED F-BOX1 (KUF1) through yeast two-hybrid library screens, affinity purification-mass spectrometry, or predicted function (19, 20). In the remote-learning CURE, we asked students to design guide RNAs to mutate 46 target genes encoding potential TAN1 interactors using CRISPR-Cas9-mediated gene editing. The need to design guide RNAs for so many targets and the easily learned technical skills needed for guide RNA design makes this project well suited for a large undergraduate class. Later, these guide RNAs will be cloned into vectors, and transformed into plants to generate mutants that will be examined for phenotypes of interest. This CURE can be altered to suit similar CRISPR-Cas9 projects. Examples of both in-person and remote learning modifications are provided (Figure 1).

In the in-person CURE, students were asked to use CRISPR-Cas9 to target 64 genes encoding candidate protein interactors of KUF1. Students progressed from guide RNA design to generation of CRISPR-Cas9 constructs and transformation of *Arabidopsis thaliana kuf1* mutants. Students gained experience in molecular biology techniques such as PCR, Golden Gate cloning, bacterial transformation, DNA purification, Sanger sequencing analysis, and *Agrobacterium tumefaciens*-mediated plant transformation. Here we provide detailed protocols to implement either version of the CURE depending upon the availability of laboratory facilities.

Intended audience:

This course is intended for instructors of lab courses for first-year biology students. It was implemented in-person in Spring 2019 for six sections and online in Spring 2020 for eight sections with 18-24 students enrolled in each section. This course, listed as BIOL20, is a long-running CURE offered to first-year life science students at UCR as an alternative to a traditional first-year biology lab course (21). The course is designed for UCR faculty to offer 5-6 week long CUREs while course administration, grading, and most technical support is handled by instructors, graduate teaching assistants, and staff. Thus, faculty can focus on the research project to deliver the best possible experience to the students. The remote learning option was a direct result of the cancellation of in-person instruction due to the Covid-19 pandemic. However, instructions presented here can be applied to online courses or used in settings where laboratory space for students is unavailable.

Prerequisite student knowledge:

This course includes a 5-week period prior to the start of the research project during which students learn and practice several common lab techniques. Structured lab activities with predetermined outcomes introduce students to pipetting technique and dilutions (22), PCR, gene expression and structure, DNA polymorphisms (21), and bacterial transformation. During in-class instruction, students master the techniques through repetition so that they are able to produce quality results during the research project. During remote instruction, students watched videos demonstrating the techniques and analyzed past student data. In this way students learned how to analyze “real” data as opposed to hypothetical or perfect data provided by the instructors. Since this class is intended for first-year students, the introductory activities and labs provide required basic information needed to perform the experiments and understand the key concepts of genetic information transfer and genome organization. Students will become

familiar with PCR and genetics, especially if the project will be done in a lab. All students have taken high-school biology or an equivalent science course.

Learning time:

The BIOL20 course is 10 weeks, with a 5-week CURE consisting of online class meetings twice a week for 1-1.5 hours. For classes that meet in lab, lab periods occur twice a week for 3 hours each. All protocols are designed to fit in the 3-hour sessions because students do not have free access to the laboratory.

Learning outcomes:

The goal of this course is to introduce the concepts of problem solving during research to students using an entirely online platform, with an option for in lab learning. In addition to participating in research, students will learn about PCR, primer design, and gene editing. Students also practice communicating scientific concepts and results with a written report and short presentation about the research project. Three of six core competencies and three of five core concepts for biological literacy outlined by the *Vision and Change* framework are addressed (Table 1; (2)).

Procedure:

Student and faculty instructions:

This course is designed for students to work in pairs on a single gene. This gives students an opportunity to discuss guide RNA design with a peer. Redundancy also ensures that if one student is not successful in their cloning or if the guide RNA is ineffective, the research goal will be met. Students are paired within a section rather than across sections to prevent selection of the same guide RNAs. All other lab work and assignments are completed

individually by students. Below we present an overview of the workflow first for the remote-learning version and then the in-person version.

Remote Learning CURE

Week 1. The research project is introduced. A video created by someone familiar with the project provides background information and rationale for the project (example in Appendix 1). Specific guidelines and writing tips are provided for the written project report due at the end (Appendix 2). Examples of published papers relating to the project are discussed in class to help students conceptualize how to format and write a scientific report. Students are expected to complete a draft of their report introduction over the following week. This draft is then graded and individual feedback on how to improve scientific writing is given.

Primer design concepts are introduced, with an emphasis on using primers to detect insertions and deletions (indels) within a gene. A step-by-step guide for primer design is provided using the maize *ACTIN-1* gene (Appendix 3). Students then choose or are assigned their genes and asked to create primers to amplify their gene's coding sequence. Additional instructions are supplied on how to look up *A. thaliana* genes and their sequences on The Arabidopsis Information Resource (TAIR) (<https://www.arabidopsis.org/>, Appendix 4). Students record their primer design results in an electronic lab notebook which is graded for completeness. Creating an online blog using Wordpress or a similar service, is a convenient way to keep all lab notebook entries in a single, accessible place (<https://wordpress.com/>), as described here (23) . An example notebook entry is supplied for students (Appendix 5). Student understanding of how to use BLAST to examine gene structure and general knowledge of the project is assessed with Quiz 1 (Appendix 6).

Week 2. In week 2, the concepts of gene evolution and homologs are explored with a video lecture (Appendix 1). Students create a phylogenetic tree for their assigned gene using Plaza

4.0 (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v4_dicots/, Appendix 7). Students analyze their phylogenetic tree by identifying paralogs and orthologs for their assigned gene. Students determine whether their assigned gene has any paralogs that might be off-target sites for guide RNAs and record their findings in an electronic lab notebook entry (example in Appendix 8).

Week 3. In week 3, CRISPR-Cas9 is explained in greater detail and students design guide RNAs for their assigned genes. The online E-CRISP (<http://www.e-crisp.org/E-CRISP/>) tool is used to design guide RNAs (Appendix 9). Other guide RNA design websites may suit other projects better, depending on the goals of the project. The instructor demonstrates how to use E-CRISP with students and then students design their own guide RNAs using the provided protocol. The instructor explains features of acceptable guide RNAs including location of the guide RNAs within the gene, the presence of a PAM sequence, and lack of off-target sequences. This project uses dual guide RNAs, rather than a single guide RNA, to delete a region of the gene. To gain more experience designing guide RNAs, students create two pairs targeting a small region of their gene and two pairs targeting the entire gene for deletion. Students record their results in an electronic laboratory notebook and explain which pairs they designed are better suited for rendering their assigned gene nonfunctional (example in Appendix 10). Students then add their best guide RNA pairs into a class spreadsheet where they record their name, gene, region targeted by their guides, and guide sequence (example in Appendix 11). The project report guidelines are revisited and students are asked to complete a draft for the methods and data sections within the following week (Appendix 2). This draft is then graded and given back to the students with feedback for improvement. Student understanding of how to create a phylogenetic tree and design guide RNAs for *A. thaliana* genes is assessed with Quiz 2 (Appendix 12).

Weeks 4 and 5. If needed, more time can be taken to design guide RNAs. Students prepare a five-minute presentation about the project, their assigned gene, and their results that is presented to the class in week 5 (Appendix 13 and 17). Because the scope of the project is limited by time and access to a lab, future project directions are discussed in class along with additional background reading (Appendix 14). Students are then expected to complete their project report using the feedback on their drafts. Students complete conclusions and future directions sections in their report (Appendix 15). Finally, students take a final exam during which they are assigned a rice gene and are asked to locate the gene sequence and design guide RNAs (Appendix 18).

In-lab CURE

Week 1. The in-lab timeline for the research project differs from the online version in its progression (Table 3). In addition to performing “wet lab” techniques, guide RNA selection is done the first week only. Students write a report, record their results in a lab notebook, complete quizzes, and present their results as described in the remote learning version.

First, students are introduced to the project goals with a slide presentation (Appendix 19). To increase student buy-in, we recommend starting with a big-picture overview that explains why the project is interesting and important. This is followed by an introduction to *Arabidopsis thaliana* as a genetic model system, and a broad explanation of forward and reverse genetic strategies to understand gene function. We find the metaphor of “how a biologist would fix a radio” makes these concepts accessible to introductory biology students (24). Then, we explain how candidate genes were obtained. Finally, we introduce CRISPR-Cas9 as a gene-editing tool, starting with newsworthy developments in its application for gene editing in humans before discussing how the system works, features of synthetic guide RNAs, and the consequences of double-stranded DNA breaks. Each student is assigned “their” target gene. Students use

BLAST and phylogenetic comparisons to identify close homologs of their target that may be functionally redundant (Appendix 20). When genetic redundancy is likely, the effort required to generate triple and higher order mutants becomes prohibitive for evaluating more than a few candidate genes. We reasoned that CRISPR-Cas9 could be used to efficiently generate knockout mutations in a candidate gene and its homologs simultaneously in a sensitized mutant background. In the second lab period, students use CRISPR-P 2.0 to select two guide sequences that target their assigned gene and its homolog(s), if any (Appendix 21) (25). Students submit their selected 23-nucleotide guide plus protospacer adjacent motif (PAM) sequence to an online form with notes on their guide selection rationale. Instructors order four oligonucleotide primers for each student that will be used to incorporate the two guide sequences into a CRISPR-Cas9 construct in the following week (Appendix 22).

Week 2. Students set up PCR reactions using their four primers to incorporate both the two RNA-encoding sequences into the ends of a cassette containing a U6-26 terminator and a U6-29 promoter (Appendix 23). A brief lecture introduces students to the individual components of the CRISPR-Cas9 construct, how PCR with non-matching 5' primer extensions can be used to incorporate new sequences into the ends of a PCR product, and what PCR product they will be generating at an annotated nucleotide sequence level (26, 27). After checking for successful cassette amplification, the PCR products are purified and quantified. This is followed by a Golden Gate cloning reaction, which uses a Type IIS restriction enzyme to incorporate the guide RNA cassette into the CRISPR-Cas9 vector backbone such as pHEE401E or pYUU (27–29). We prefer pYUU because it enables visual selection of transgenic seed by fluorescence, which is appealing to undergraduate students and allows a pool of seed from a transformed plant to be easily screened (29). Furthermore, the ubiquitin promoter that drives Cas9 expression in pYUU is useful if transgenic plants will be subjected to heat stress to increase Cas9 cleavage efficiency (30). A brief lecture or handout that compares Golden Gate cloning to more the

traditional Type II restriction enzyme subcloning is advisable. If time permits, students may transform *E. coli* with the Golden Gate reaction (Appendix 24). After incubating on LB plates with appropriate antibiotic selection at 37°C overnight, instructors store the plates with colonies at 4°C for the following week. A presentation detailing the experiments to be completed in the following weeks is given in class (Appendix 25).

Week 3. Students transform *E. coli* with the Golden Gate reaction if it wasn't completed during week 2. This is an appropriate time to give a short lecture on transformation approaches (e.g. electroporation, chemical transformation, particle bombardment, and infection with *Agrobacterium tumefaciens*) for bacteria and plants, as well as the importance of selectable markers. On the next lab day, students use colony PCR to test individual colonies for insertion of the guide RNA cassette into the plant transformation vector (Appendix 26).

Week 4. Overnight cultures of two or three PCR-positive colonies are initiated the evening before the first lab day, to be performed by the instructor or teaching assistants. The next day, students prepare glycerol stocks and plasmid miniprep isolations from the cultures (Appendix 27). After determining the concentration of the plasmids, the constructs are sent for sequencing. Ideally, sequencing results will be obtained by the next class period. Regardless, transformation of *A. tumefaciens* (e.g. strain GV3101 (31)) must occur by the end of the week, as it requires two days to grow usable colonies (Appendix 28). It may be appropriate to perform transformations with all constructs, and filter them later based upon sequence results. Sequences are compared to a template sequence provided in Appendix 29 to ensure that both guide sequences are incorporated without errors. Benchling (www.benchling.com) offers a free tool to compare sequencing results to the template and visualize sequencing chromatograms. We typically observe a high rate of success from PCR-positive colonies. We recommend a brief lecture on the Sanger sequencing method with fluorescent dideoxynTPs.

Week 5. Cultures of an *A. tumefaciens* colony for each construct are initiated the evening before the first lab day. The floral dip transformation method for *A. thaliana* is simple, effective, and tolerant of experimental variation (32). However, the large number of transformations that will be performed across the sections necessitates smaller-than-normal culture volumes. This will make it easier to find enough shaker and centrifuge capacity, and to dispose of biohazardous waste. We use 20 mL cultures of *A. tumefaciens* that are resuspended in 10 mL volumes before manual-drip application to flowering plants. Students transform one pot of five healthy, flowering *A. thaliana* plants. Plants are kept in dark, humid conditions overnight to promote infection and then grown normally. Seed can be collected 3–4 weeks later. We obtained as many as 74 transformants from a single pot, but more typically about 16. Because the students' transformations won't be ready for several weeks, a demonstration sample must be prepared.

Safety concerns:

No additional safety training is required when the course is conducted remotely. When in-lab activities are performed, the instructors review lab safety protocols the first day of class. Students are instructed how to appropriately dispose of hazardous and biohazardous waste, and the locations of biohazard waste bins and fire extinguishers are reviewed. In the lab, students wear proper personal protective equipment at all times including lab coat, eye protection, gloves, closed toe shoes, and long pants or skirt. Ethidium bromide, a mutagen, is incorporated into the agarose gels, but alternatives such as Sybr Safe (Thermo Fisher) can be substituted. All ethidium bromide-contaminated gels, gloves and pipette tips are picked up by environmental health and safety personnel for proper disposal. *E. coli* and *A. tumefaciens* contaminated materials are placed in appropriate biohazard bins and later autoclaved. Bleach is added to bacteria contaminated solutions. Students disinfect bench surfaces with antibacterial wipes after each laboratory session.

Discussion:

This course is designed to provide first-year undergraduate students with research experience while building core understanding of molecular biology and laboratory techniques. Although one version of the course was held remotely, students also performed these activities in the laboratory, indicating its flexibility. In addition, specific goals and organisms used in the project can be modified, making this type of CURE adaptable and generalizable.

Evidence of student learning:

Students record their work in online notebook posts which are graded by the teaching assistants. This allows the teaching assistants to assess student understanding of the project and monitor the quality of the results obtained by the students. Students also work on a scientific report about the research project. Drafts for the report introduction, materials and methods, and results are completed by the student and teaching assistants provide feedback for the student. This provides opportunities for students to improve their reports and writing over time. A final report is then submitted for grading at the end of the project. The report helps students solidify their understanding of the experiments and form their own hypothesis about the potential future outcome of the project. Additionally, scientific writing improves students' overall academic performance and critical thinking skills (33). Project presentations are presented individually by students to the class and are scored by the instructor according to the rubric (Appendix 16). Presentations both introduce concepts of sharing scientific ideas and results with peers, and allow the instructor to assess students' understanding of the project.

Students take a survey at the beginning and end of the course on their self-perceptions of learning. Students that completed either the in-lab or remote learning CURE showed an increased interest in participating in research on campus. In the pre-survey, ~24% of students responded that they were interested in pursuing research with a specific instructor on campus. In the post-survey, research interest increased to ~90% for both in-lab and remote learning

classes. This indicates that students that are exposed to primary research express increased interest.

Eight sections containing 142 students participated in this remote-learning CURE by designing guide RNAs for 46 *A. thaliana* genes. Each instructor was responsible for two sections and 12 genes. Both sections targeted the same gene sets to ensure redundancy. Typically 4 students were asked to create 2 pairs of guide RNAs for one gene. Students generated a total of 285 guide RNA pairs. 245 of the pairs correctly and specifically targeted the assigned genes, representing an 86% success rate for guide RNA design. All assigned genes had at least one correctly designed pair of guide RNAs. The most common error made by students was selecting overlapping guide RNA pairs within the gene (3% error 9 /285 guide RNA pairs overlapped). In the future, instructors will mention a minimum expected deletion size. In two instances, one of the guide RNAs was predicted to target both the gene of interest and a different gene. Altogether this demonstrates that first-year undergraduates learned how to use free and online software to identify homologs, and design guide RNAs, and explain their research using the approach outlined in this remote-learning CURE.

Possible modifications:

Selecting a Guide RNA Design Program:

There are a number of guide RNA design programs online. The program chosen for the remote learning course was E-CRISP (<http://www.e-crisp.org/E-CRISP/index.html>). The interface, which includes simple bar graphs that show the specificity, annotation score, and efficiency of each guide RNA, provides intuitively simple parameters for judging guide RNAs. E-CRISP works for very large genes (over 10kb in length) and provides options to adjust guide RNA specificity and to filter guide RNAs based on a variety of parameters such as 5' proceeding base preference, guide RNA length, and GC-content. The program chosen for the in-lab course was CRISPR-P 2.0 (<http://crispr.hzau.edu.cn/CRISPR2/>). The CRISPR-P 2.0 output shows all

potential guide RNAs, which is useful if the goal of the project includes mutating more than one gene with per guide RNA.

Challenges Specific to Remote Learning Classes:

Remote learning has a number of unique challenges. Based on conversations with students, it is important to provide step-by-step instructions with appropriate time allowed to demonstrate the protocols and go through an example. Online polls are used to determine whether students need more time to complete steps. The professional version of Zoom, an online platform for remote teaching, includes a polling feature. Online quiz tools such as Kahoot can be used to assess conceptual understanding (<https://kahoot.com/>). Students may experience technical issues including slow internet connection that may limit class participation. Therefore, it is vital to offer additional one-on-one help from teaching assistants or instructors during office hours. Similarly, not all students have devices that support certain platforms or online tools. The projects presented here can be completed on tablets or Chromebooks and do not require a desktop or laptop computer. Identifying and selecting online tools that are supported across multiple device types promotes more inclusion.

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Competing Interests:

The authors declare no conflict of interest.

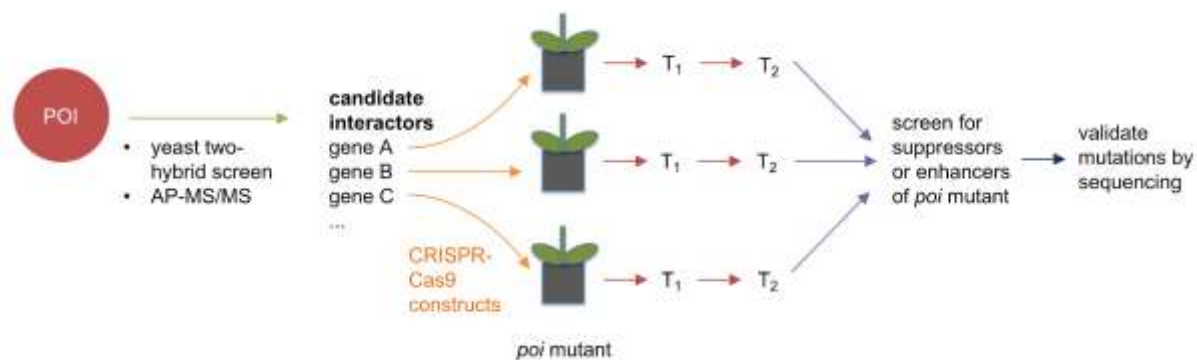


Figure 1. Strategy to identify genetic modifiers through CRISPR-Cas9.

Proteins that potentially interact with a protein-of-interest (POI) are identified through yeast two-hybrid library screens or affinity-purification tandem mass spectrometry. The list of candidate interactors is evaluated through a CURE-based approach. Each candidate interactor gene is assigned to two students. Students identify homologs that may be functionally redundant with a candidate interactor, and select two guide RNA (gRNA) sequences to target the candidate and its close homologs, if any. Students use PCR and GoldenGate cloning to insert both gRNAs into a CRISPR-Cas9 construct. Correct constructs are identified after *E. coli* transformation with colony PCR, plasmid preparation, and Sanger sequencing. Constructs are then transformed into the *Arabidopsis thaliana* *poi* mutant background. Transformed seed (T1) are selected, heat-shocked to improve editing efficiency, and selfed to produce T2 seed. Pooled T2 seed from different T1 lines carrying a single construct are phenotyped for either suppression or synthetic enhancement of the *poi* mutant phenotype. CRISPR-induced mutations are then validated by sequencing of the target gene(s).

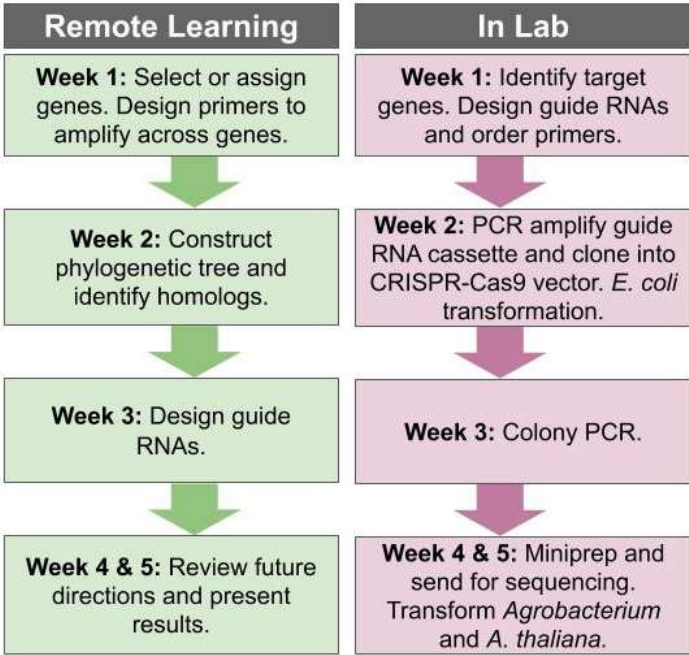


Figure 2. Timeline of the research project. Steps shown on the right column require laboratory facilities, and may be incorporated if lab space is available.

Table 1. Learning outcomes for each week of the remote learning CURE.

Week	Learning Outcomes
1	Students will be able to: • Explain the overall goal of the project and identify their specific role. • Compare and contrast regulated cell division and consequences of misplaced cell division on plant growth and development. • Use online resources to research <i>A. thaliana</i> gene information. • Design PCR primers with the aid of online tools to amplify a part of an <i>A. thaliana</i> gene. • Create a detailed lab notebook entry.
2	Students will be able to: • Define homology and identify gene homologs through DNA database searches. • Discuss the evolutionary relationships between paralogs and orthologs. • Create a phylogenetic tree for a gene family using online tools and identify paralogs and orthologs for that gene.
3	Students will be able to: • Discuss the mechanism of CRISPR-Cas9 mutagenesis. • Form a hypothesis about the potential consequences of mutating a gene and what that suggests about the gene's function. • Design guideRNAs to target different regions of a gene. • Evaluate and select guide RNAs based on a given set of criteria.
4 and 5	Students will be able to: • Analyze DNA sequence for PCR induced mutations (in lab only). • Effectively communicate the project and results in a scientific report. • Create and present slides with a summary and interpretation of their results to their peers.

Table 2. Suggested pacing for the remote learning course organized into 5 weeks of instruction.

Week	Activities	Materials
1	Introduction to Research Project and Primer Design -Watch the project introduction video in class. -Design primers for maize <i>actin</i> as a class. -Assign genes to students or have them choose genes. -Students design primers to amplify across their assigned gene on their own. -Students record their primers in a notebook entry based on the provided example. -Students complete Quiz 1	-Project introduction video (Appendix 1) -Project report guidelines (Appendix 2) -Primer design - maize <i>actin</i> (Appendix 3) -Primer design - <i>A. thaliana</i> (Appendix 4) - Example Notebook Post - Primer Design (Appendix 5) -Quiz 1 (Appendix 6)
2	Phylogeny and Homologs -Watch the “Gene evolution and homology” video in class. -Discuss the “Phylogeny and Using Plaza” reading in class. -Students create a phylogenetic tree for their assigned gene. -Students record and analyze their phylogenetic tree in a notebook entry.	-Gene evolution and homology video (Appendix 1) -Phylogeny and Using Plaza reading (Appendix 7) -Example Notebook Post - phylogenetic tree (Appendix 8)
3	Guide RNA Design -Review CRISPR-Cas9 in class using the E-CRISP protocol. -Discuss an example of how to use E-CRISP to design guide RNAs in class. -Students design guide RNAs for their assigned gene as described in the E-CRISP protocol. -Students record their guide RNAs in a notebook entry based on the provided example. -Students record guide RNAs in a class spreadsheet. -Students complete Quiz 2	-E-CRISP protocol (Appendix 9) -Example Notebook Post - guide RNAs (Appendix 10) -Example class guide RNA spreadsheet (Appendix 11) -Quiz 2 (Appendix 12)
4	Project Presentation and Future Directions -Discuss the Project Presentation guidelines in class. -Students create a short project presentation to be presented the following week. -Discuss future directions for the project using the “Future Directions” reading in class.	-Presentation guidelines (Appendix 13) -Future Directions (Appendix 14) -Example student report (Appendix 15)
5	Student Presentations -Students present their results to the class. -Students take a final exam for formal assessment.	-Example presentation score sheet (Appendix 16) -Example Student Presentation (Appendix 17) -Final exam (Appendix 18)

Table 3. Modified research project course showing additional in lab activities.

Week	Activities	Materials
1	-Introduce project to class. -Assign candidate genes to students. -Students use BLAST and phylogenetic comparison to identify close homologs for their gene. -Design guide RNAs targeting assigned gene and close homolog(s). -Instructor orders primers to PCR amplify guide RNAs the following week.	-Project Introduction Slides (Appendix 19) -Selection of Target Gene(s) (Appendix 20) -Guide RNA Design (Appendix 21) -Designing and Ordering primers (Appendix 22)
2	-PCR amplify guide RNA cassette. -Go over presentation detailing upcoming experiments -Golden Gate clone guide RNAs into CRISPR-Cas9 vector and transform <i>E. coli</i> with Golden Gate reaction mix.	-PCR amplification of guide RNA cassette (Appendix 23) -Golden Gate Cloning Protocol and <i>E. coli</i> transformation (Appendix 24) -Upcoming experiment overview slides (Appendix 25)
3	-Introduction to transformation in bacteria and plants -Colony PCR	-Colony PCR of <i>E. coli</i> transformants (Appendix 26)
4	-Grow positive colonies overnight for minipreps. -Students perform DNA minipreps and make glycerol stocks for their overnight cultures. -Send colonies for sequencing. -Students check sequences for errors. -Transform vector into <i>A. tumefaciens</i>.	-DNA minipreps of CRISPR/Cas9 constructs (Appendix 27) -Transformation of <i>A. tumefaciens</i> (Appendix 28) -Sequence analysis (Appendix 29)
5	-Transform plants using floral dip. -(optional) Demonstrate selection of transgenic seeds.	Transformation of <i>A. thaliana</i> (Appendix 30)

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