

# UC San Diego

## UC San Diego Previously Published Works

### Title

CRISPR in Your Kitchen: an At-Home CRISPR Kit to Edit Genes in *Saccharomyces cerevisiae* Used during a Remote Lab Course.

### Permalink

<https://escholarship.org/uc/item/2nf7k3m2>

### Journal

Journal of microbiology & biology education, 23(1)

### ISSN

1935-7877

### Authors

McDonnell, Lisa  
Moore, Andrew  
Micou, Melissa  
[et al.](#)

### Publication Date

2022-04-01

### DOI

10.1128/jmbe.00321-21

Peer reviewed



## CRISPR in Your Kitchen: an At-Home CRISPR Kit to Edit Genes in *Saccharomyces cerevisiae* Used during a Remote Lab Course

Lisa McDonnell,<sup>a</sup> Andrew Moore,<sup>a</sup> Melissa Micou,<sup>a</sup> Christopher Day,<sup>a</sup>  
Emily Grossman,<sup>a</sup> and Clara Meaders<sup>a</sup>

<sup>a</sup>Division of Biological Sciences, University of California, San Diego, La Jolla, California, USA

**KEYWORDS** CRISPR, remote laboratory course, COVID-19, pandemic, home kit, online course, hands-on experience, Cas9, *Saccharomyces cerevisiae*

### INTRODUCTION

Before the coronavirus disease 2019 (COVID-19) pandemic, our in-person, upper-division molecular biology lab course was focused on students using CRISPR-Cas9 editing to generate loss-of-function mutations in the *ADE2* gene of *Saccharomyces cerevisiae* (1). Student-generated phenotype and genotype data allowed the class to measure the frequency of *ADE2* loss of function as a result of nonhomologous end joining (NHEJ) and homology-directed repair (HDR). When the lab course moved online in March 2020, we lost the hands-on component of our CRISPR-editing focused lab course and, instead, focused on analyzing previously generated results (2). To add hands-on experience to the remote version of the course and allow students to generate the phenotype and genotype data once again for analysis, we developed an at-home CRISPR editing kit that we shipped to over 600 enrolled students during the remote offerings of the course from Fall 2020 through Spring 2021. The experimental work for the kit spread over 2 to 3 weeks. Students performed the experimental steps during synchronous video lab sessions, or they had access to video recordings of the instructor working through each step to complete the work asynchronously. The kit's experimental work generated robust phenotype results, and students were provided with the opportunity to ship a sample back to campus for sequencing to generate genotype results.

Although other yeast-based CRISPR lab curriculums have been published (1–3), our kit protocols varied in a few key ways that made it quite easy for students to do CRISPR at home. Most notably, our protocol for yeast transformation did not require competent cells and all transformation incubation steps could be done at room temperature. The kits costed \$70–\$80

each to produce, including return shipping (mostly within the state) and sequencing of samples. The cost of the kit was covered by the \$80 lab fee that each enrolled student paid. The addition of the at-home CRISPR kit to our remote lab course offered valuable hands-on experience, ownership over the results generated, and helped students connect the course content to a tangible research experience.

### HOME-KIT EXPERIMENTAL PROCEDURE OVERVIEW

The home-kit was used in an upper-division molecular biology lab course. Students in this course were largely biology majors that had taken introductory biology and genetics before the course, so most had some incoming knowledge about the central dogma. About 50% of students had taken molecular biology. Most students were aware of CRISPR but were not familiar with how it worked, nor had they used it experimentally (except for one or two students). The goal of the CRISPR experiment was for students to set up multiple yeast transformations to test for the frequency of loss of function in *ADE2* as a result of NHEJ repair (no donor template added) and HDR (donor template added) after Cas9 cutting. An overview of the flow of the experimental work can be found in Fig. 1. Before starting the experimental work in the kit, instructors will have spent several class sessions discussing how CRISPR works, important aspects of experimental design, and how one makes recombinant plasmids for use in a CRISPR experiment.

All the necessary materials to grow and transform yeast are provided to students in the kit (see a detailed list of supplies and procedures in Appendix 1–3). The kit contains a haploid strain of nonpathogenic yeast (*Saccharomyces cerevisiae*), and aliquots of Cas9-expressing plasmid with or without a gRNA that targets *ADE2*. We used the pML104 plasmid available from Addgene (<https://www.addgene.org/67638/>) and had previously added a guide RNA (gRNA) to the plasmid that targets the *ADE2* gene 38 base pairs downstream of the start codon, (gRNA sequence originally from Seghal et al. [3]). Students were also given an “empty” pML104 plasmid (no gRNA) as a

---

Address correspondence to Division of Biological Sciences, University of California, San Diego, La Jolla, California, USA. E-mail: [lmcdonnell@ucsd.edu](mailto:lmcdonnell@ucsd.edu)

The authors declare no conflict of interest.

Received: 23 November 2021, Accepted: 30 November 2021,

Published: 28 March 2022



control. For instructors that do not want to construct a Cas9-gRNA plasmid, there are a variety of plasmid-gRNA samples available from Addgene that could be used (<https://www.addgene.org/browse/article/28215495/> [4]). The kit also contained a sample of double-stranded HDR templates that encoded six-point mutations (Fig. 1B). Four of the mutations resulted in three different premature stop codons and two were silent mutations. With subsequent sequencing of the yeast transformants, we could track how often all six mutations were incorporated as a result of HDR repair after Cas9 cutting. A list of the transformations conducted using the experimental kit was listed in Step 2 in Fig. 1. We chose overnight shipping to ensure most would arrive in 1 to 2 days after shipping, although some took up to 5 days to arrive. The materials in the kit were quite stable at room temperature and, given that we shipped many kits within California, we expected some would have been exposed to elevated temperatures. We were not aware of fluctuations in temperature during shipping posing a problem for using the kit, although we did not explore the effects of kits being stored below 4°C or above 30°C for multiple days.

We developed a yeast transformation protocol that did not require making competent yeast cultures, and all incubations could be done at room temperature. By incubating freshly grown yeast in a polyethylene glycol (PEG)-based transformation mixture, average transformation efficiencies were approximately 300 colonies/ $\mu$ g plasmid (control reactions with pML104), and between around 12 and 70 colonies/ $\mu$ g plasmid for the pML104-gRNA and pML104-gRNA + HDR template transformations, respectively. Yeast that successfully took up the pML104 plasmid could grow on uracil-minus selective media. Students counted colonies to document the number of red (*ADE2* loss-of-function) and total colonies from each transformation, and they entered their results into a shared class spreadsheet (e.g., Google Sheets). Pooling the data in this manner created a larger data set for phenotype analysis. The frequency of red colonies could be used to infer the frequency of loss-of-function mutation introduced by HDR when the template was added to a transformation, or by NHEJ when no template was added (Fig. 1C). In addition, the relative abundance of total colonies could be used to infer the frequency of Cas9 cutting and resulting in cell death (5) (Fig. 1C).

The kit also provided a return shipping label so that students could mail a plate of yeast colonies back to campus lab

for sequencing of *ADE2*. Students drew a circle on the plate to identify the colony they wanted to be sequenced, labeled the plate/colony with a unique identifier (provided by their instructor), sealed the plate with tape or parafilm, and mailed it back to campus with the prepaid return label provided in the kit. Once received in the lab, our lab research associate amplified the targeted region of *ADE2* using PCR and sent the resulting products for sequencing to a local sequencing company. Students were given access to their sequences and those generated from their classmates' samples. Students performed multiple sequence alignments (comparing to wild-type *ADE2*) to determine the types of mutations introduced by NHEJ, as well as the variations in the incorporation of all six-point mutations from the HDR template (Fig. 1D). In past course iterations, we sequenced *ADE2* from both red (loss-of-function) and white colonies. By examining both red and white colonies, we discussed how often non-loss-of-function mutations occurred (e.g., silent or in-frame insertions/deletions, or repair back to wild-type).

### Safety issues

Before using the kits, students completed safety training as part of their course. Our university had online lab safety training that all lab-enrolled students took. Safety recommendations are included in Table 1, based on recommendations from the American Society of Microbiology (ASM) Biosafety Guidelines (6) and included in the student instructions (Appendix 3).

Students were provided with nitrile gloves to wear during all steps of the experiment and were encouraged to use sterile and safe practices, including wearing safety glasses if they had them, and to take caution when handling the liquidized bleach (used to kill yeast samples at the end of the experiment).

### Preparing and shipping the kits

Preparing 250 kits took approximately 3 to 4 weeks of work. See Appendix 1 and 2 for more specific details on kit material preparation and more explanation of the preparation timeline in Appendix 2. Before preparing the kits to ship to students, our Environmental Health and Safety (EHS) officers reviewed a detailed list of the kit materials and protocols and examined the kit contents. We worked with our local FedEx

### FIG 1 Legend (Continued)

need to repeat the experimental work. The kit protocol takes approximately 20 to 25 days to complete, including receiving sequencing results after returning a sample to campus. (B) schematic of *ADE2* gene region targeted and point-mutations introduced by HDR. The gRNA was designed to target 38 bp downstream of the start codon (gRNA sequence highlighted in green and underlined). The HDR template was about 130 bp long, flanking the predicted Cas9 cut site (black arrow). The template encoded six-point mutations (labeled 1 to 6). Mutations 1 and 6 are silent (1, A-G; 6, A-T); mutations 2 and 3 are nonsense mutations (2, C-T and 3, G-T), and mutations 4 and 5 combined also cause a nonsense mutation as well as modify the PAM sequence (4 and 5, GC-TA). (C) Example colony abundance and phenotype results; Cas9 cutting typically results in lowered colony abundance, and significantly more loss of function of *ADE2* phenotypes are observed when the HDR template is included. (D) Example sequencing results of a portion of the *ADE2* gene near the Cas9 cut site, from the CRISPR experiment. The top sequence (WT) is the wild-type, and the subsequent seven rows below WT are each from a different yeast colony exhibiting the red loss-of-function phenotype, that had been transformed with Cas9 plasmid with gRNA, as well as HDR template. Numbered arrows indicate the point mutations encoded in the HDR template, as in panel C. Results show various degrees of incorporation of all six of the point mutations encoded in the HDR template. (E) Example sequencing results showing NHEJ-induced mutations after Cas9 cutting. The top sequence is the wild-type with the gRNA target site boxed and the predicted Cas9 cut site indicated with a black triangle. The three sequences below wild-type are from three colonies that showed the red loss-of-function phenotype after transformation with pML104+gRNA and no HDR template (NHEJ mutations).

TABLE I  
Safety recommendations for use of the at-home kit

Experimental step	Recommendation
Before use	General lab safety training (online training offered by your institution, if possible, others are available such as <a href="https://www.youtube.com/watch?v=ge8I4fSdbPM">https://www.youtube.com/watch?v=ge8I4fSdbPM</a> . Instructors may also wish for students to sign a safety statement confirming they understand the safety procedures and if their university requires a liability waiver.
During use	Disinfecting workspace before and after experimental steps using provided disinfectant wipes.
	Wearing a lab coat and goggles if available; wearing nitrile gloves when using the kit contents.
	Disinfecting materials such as tips and spreaders that were in contact with yeast culture using bleach provided in the kit.
When the experiment was completed	Shipping yeast plates back to campus for autoclaving and disposal.

representative to develop a plan for shipping, including providing them with a list of student mailing addresses (collected by surveying registered students) so they could batch print the labels and provide them to us, as well as return shipping labels included in the kits. At a predetermined date, FedEx picked up all the labeled kits. Students typically received their kits within 1 to 5 days after shipping. We did not ship to on-campus students. They came to a predetermined location to pick up a kit. Although we received approval for international shipping and completed all the necessary documentation, very few of our international students received the kits during the Fall 2020 term. As such, we stopped shipping internationally during Winter and Spring 2021. Kits could be stored at room temperature for multiple days (or a few weeks) before shipping and can be stored at room temperature once received by students.

**Suggestions for using the kit**

The students found it helpful to become oriented to the kit when we spent time during a real-time video session going over the kit contents, opening our kits together, and reviewing each component. It was also helpful to do the experimental steps together in real-time (via video conferencing) with the instructor first demonstrating a technique (e.g., creating a

clean workspace and streaking the yeast) and then allowing for students to do the same procedure using their kits, and ask any questions about the technique they may have.

We highly recommend having students pool their resulting yeast colony counts (loss-of-function red versus non-loss-of-function white) and use the pooled data for analysis. By pooling the results students were able to see the variation and trends in colony counts and frequency of loss of function (Fig. 1C). We also recommend giving students access to multiple sequences so they can again assess the variation in mutations that occur as a result of NHEJ and by HDR (Fig. 1D and E).

**RESULTS AND CONCLUSION**

Students surveyed (IRB approval was granted by our institutional review board for project no. 170886SX entitled “Investigating the Impact of Pedagogical Choices on University Student Learning and Engagement”) at the end of the course largely agreed that the at-home experiment provided opportunities to help them learn about experiments, CRISPR, and concepts learned in class, made molecular biology feel more “real,” and that reading about the experiment would not have been as effective as the hands-on experience (Fig. 2).

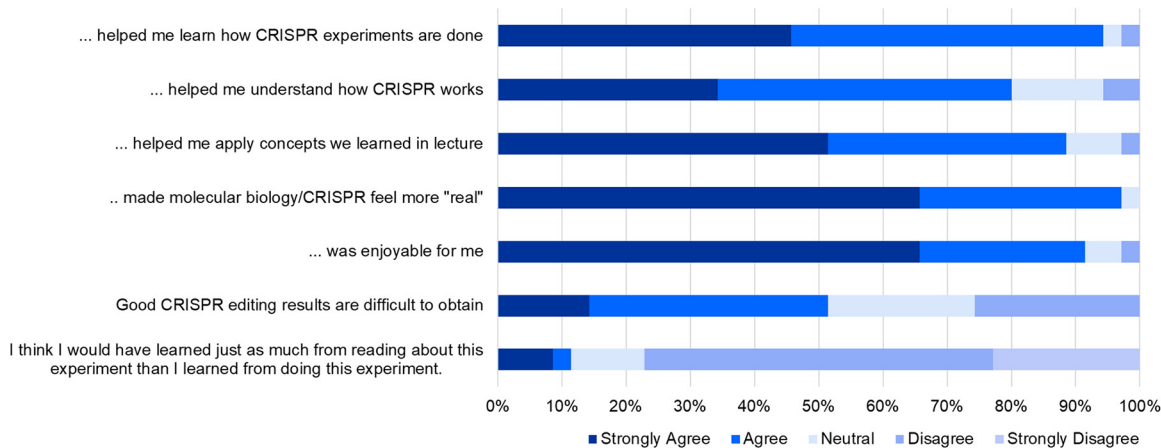


FIG 2. Students reported a positive experience with the at-home CRISPR kits. Students were surveyed from the Spring 2021 iteration of the course. Results presented are from 35 students from a one-course section (48 students).

Many students reported that “good CRISPR editing results are difficult to obtain,” suggesting that the home kit, and likely the subsequent data analysis, provides an experience that conveys the realistic challenges of doing research. Over 79% of surveyed students reported talking about the at-home work with someone other than a classmate such as friends, family, and roommates (data not shown). We postulate that having the experimental supplies at home might make it more likely that students talk to others about what they are learning and doing in the course. One student commented “It was so fun to be able to actually do the experiment. Opening the box and seeing all of the tools and samples brought so much joy to my day and then when I actually had colonies grow red I was super excited.” Regarding challenges with the kit, a few students commented that it was hard to find appropriate space in their shared accommodations and that having to wait several days for colonies to grow was frustrating. Less than 10% of students reported challenges with contamination. No other common challenges were reported by students.

We highly recommend using this at-home kit to bring CRISPR to students in a remote environment. The experiment itself is easy to perform, and the data are interesting to analyze. Variations could include having multiple different gRNA and HDR combinations being tested by a class. With a large enough student population, varying the gRNA/HDR combinations could allow for additional questions to be addressed. Does cutting efficiency vary depending on gRNA composition (e.g., length and/or GC content)? What is the effect of various mutations on regulatory regions, to identify critical components of the promoter? Providing remote students an opportunity to do experimentation with this kit offers a chance for students to have ownership over the data, a connection to the course (which can be challenging in an online environment), and some hands-on skills that are otherwise challenging to accomplish remotely. We imagine our kit could be combined with a do-it-yourself (DIY) gel electrophoresis component, as described by Ens et al. (7) and additional simulated labs (e.g., via <https://www.labster.com/>). With such additions, students in a fully remote lab course could check DNA samples (e.g., the plasmid and amplified HDR samples) before doing the yeast transformation. The addition of more hands-on components, in combination with the extensive data analysis possible with the data generated by the CRISPR experiment, could make such a fully remote lab course suitable as equivalent to some existing fully in-person lab courses. Being able to provide fully remote lab courses that offer interesting and relevant hands-on experience would create opportunities for many more students to engage in biology experimentation.

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.4 MB.

## ACKNOWLEDGMENTS

We sincerely thank Lorraine Pillus and her lab for providing the haploid yeast strain and tips on working with yeast in the lab; Scott Rifkin for a sample of *dam-/dam-E. coli* needed for growing the pML104 plasmid; William McGinnis for helping to conceptualize and pilot the CRISPR lab before the course going remote. We are extremely thankful to the lab staff who helped prepare materials and organize the kits: Ana Gomez, Dennis Hickey, and Bobby Waddell. Thank you to the students for providing their feedback on their experiences using the kits.

We declare no conflicts of interest.

## REFERENCES

1. Jones EW, Fink GR. 1982. Regulation of amino acid and nucleotide biosynthesis in yeast, p 181–299. *In* The molecular biology of the yeast *saccharomyces*: metabolism and gene expression, Strathern JN, Jones EW, Broach JR, (ed.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. McDonnell L, Reuther K, Cooper A, Day C, Gustafson-Brown C. 2021. Converting a face-to-face lab to online: an example of process and outcomes for a CRISPR-based molecular biology lab. CourseSource. <https://doi.org/10.24918/cs.2021.22>.
3. Sehgal N, Sylves ME, Sahoo A, Chow J, Walker SE, Cullen PJ, Berry JO. 2018. Gene Editing in yeast: an experimental protocol for an upper-division undergraduate laboratory course. *Biochem Mol Biol Educ* 46:592–601. <https://doi.org/10.1002/bmb.21175>.
4. Sankaran SM, Smith JD, and, Roy KR. 2021. CRISPR-Cas9 gene editing in yeast: a molecular biology and bioinformatics laboratory module for undergraduate and high school students. *J Microbiol Biol Educ* 22:e00106-21.
5. Dicarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM. 2013. Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res* 41:4336–4343. <https://doi.org/10.1093/nar/gkt135>.
6. ASM Task Committee on Laboratory Biosafety. 2019. Guidelines for Biosafety in Teaching Laboratories Baltimore County, p 7.
7. Ens S, Olson AB, Dudley C, Ross ND, Siddiqi AA, Umoh KM, Schneegurt MA. 2012. Inexpensive and safe DNA gel electrophoresis using household materials. *Biochem Mol Biol Educ* 40:198–203. <https://doi.org/10.1002/bmb.20596>.