11-14-2017

An Undergraduate Laboratory Manual for Analyzing a CRISPR Mutant with a Predicted Role in Regeneration

Susan Walsh
Rollins College, sjwalsh@rollins.edu

Ashley Becker
Rollins College, abecker@rollins.edu

Paxton S. Sickler
Rollins College, paxton.sickler@gmail.com

Damian G. Clarke
Rollins College, DCLARKE@Rollins.edu

Erin Jimenez
NHGRC, NIH, erin.jimenez@nih.gov

Journal of Human Biology & Health Education

BIO ACCENT

Susan Walsh, et al. J Hum Bio & Health Edu 2017, 1: 2

1: 008

Research

An Undergraduate Laboratory Manual for Analyzing a CRISPR Mutant with a Predicted Role in Regeneration

Susan Walsh[1*], Ashley Becker[1], Paxton S Sickler[1], Damian G Clarke[1], and Erin Jimenez[2]

[1]Department of Biology, Rollins College, Winter Park, FL, 32789, USA

[2] Translational and Functional Genomics Branch, National Human Genome Research Center, National Institutes of Health, Bethesda, MD, 20892, USA

Abstract

Exposing students to undergraduate research has reportedly improved students' development of knowledge and skills in the laboratory, self-efficacy, satisfaction with their research, retention, and perseverance when faced with obstacles. Furthermore, utilizing authentic course-based undergraduate research experiences (CUREs) includes all students enrolled in the class, giving those who may not otherwise have access to an independent undergraduate research project an opportunity to engage in the scientific process in context of an original, unanswered question. In the fall of 2016, second semester introductory biology students conducted a semester-long research project on the transcription factor Lin28a to determine the effect of Lin28a on regeneration in a CRISPR mutant. During ten laboratory periods, students completed four experiments: 1) genotyping mutants by PCR and RFLP, 2) neuromast regeneration after copper sulfate treatment, 3) measuring changes in gene expression by RT-PCR after fin clipping, and 4) swimming behavior. In the context of this class, students were challenged to design their own experiments, interpret their own data, and make connections among the experiments to draft a final paper presenting their results and conclusions. Here, we present a student laboratory manual that can be adapted to other relevant CRISPR mutants. Overall, this coursework aligns with Vision and Change, and these experiments gave students a taste of the questions, techniques, and experimental design currently used in the field of regenerative biology.

Keywords: Zebrafish; Neuromast Regeneration; CRISPR; Undergraduate Education; Lin28a; Course-Based Undergraduate Research; Genetics

Introduction

Students learn best by practicing science [1]. Student research in the undergraduate environment is mainly done through apprenticeships in faculty research labs; however, there are rarely enough resources or positions to provide every student with this opportunity [2]. Thus, integrating authentic research experiences into the curriculum through a course-based undergraduate research experience (CURE) enables all students to engage in the scientific process [3-5]. As a high impact practice, CUREs provide many advantages. Exposing students to research early in their undergraduate curriculum can potentially influence their retention, academic success, and career goals. Furthermore, utilizing authentic CUREs includes all students enrolled in the class, giving those who may not otherwise have access to research an opportunity. Finally, CUREs may increase laboratory skills, self-efficacy, personal satisfaction, and resilience[4].

This paper provides a CURE laboratory manual for a semester-long zebrafish research project used with biology majors enrolled in the second semester of an introductory biology course. Zebrafish are an accessible vertebrate model system for undergraduates, as development occurs rapidly and many embryos are produced from a single breeding pair. Unlike mammals, zebrafish possess the remarkable ability to regenerate a number of tissues, from tail fins to hair cells to optic nerves [6]. The gene of interest here, *lin28a*, has been suspected to play a role in tissue regeneration, based on studies of development, pluripotency and metabolism [7-9]. Therefore, to understand if knockout of *lin28a* would be sufficient to cause defects in regeneration, a CRISPR mutant fish was

***Corresponding Author**: Susan Walsh, Department of Biology, Rollins College, Winter Park, FL, 32789, USA, Tel: +407-646-2534; E-mail: sjwalsh@rollins.edu

Sub Date: October 30th 2017, **Acc Date**: November 13th 2017, **Pub Date**: November 14th 2017.

Citation: Susan Walsh, Ashley Becker, Paxton S Sickler[1], Damian G Clarke, and Erin Jimenez et al. (2017) An Undergraduate Laboratory Manual for Analyzing a CRISPR Mutant with a Predicted Role in Regeneration J Hum Bio & Health Edu 1: 008

created [10] and tested against wild-type fish for larval regeneration in neuromasts, altered gene expression of pluripotency factors, and rheotaxis behavior. The experiments dovetailed with lecture material focused on genetics, physiology, and neuroscience. In the context of understanding *lin28a*, students were encouraged to read primary literature, consider experimental design and controls, make testable hypotheses, interpret their data, and connect experiments to generate a cohesive semester-long project that could be assembled into a singular paper about one gene. Although our data suggest that *lin28a* may not affect the variables measured, students had the opportunity to engage in a novel research project through their enrollment in a required introductory course.

Methods

Animal Care

This research was approved by the Rollins College Institutional Animal Care and Use Committee. Because students were working with live vertebrate animals, all students were required to pass an electronically graded animal care quiz with a score of 85% or higher before the first laboratory period (Appendix 1). Full animal protocols can be requested from the author.

The *lin28a* fish (Z001470) was made according to standard procedures [10,11]. To breed embryos, adult zebrafish were segregated by gender and used as a breeding population. In brief, 2-3 females and 2-3 males were placed in a breeding tank .This tank was set up after the fish ate their evening meal, and the fish were left in the breeding tanks overnight. In the morning, the fish mated shortly after the lights turned on. After mating, embryos were collected and grown at room temperature in Petri dishes and 1X E3 embryo media. These embryos and the adults were brought into the classroom laboratory for students to examine.

For the adult zebrafish fin clipping experiment, adults were anesthetized in 1X tricaine (16 mg/mL) until they were motionless. The caudal fin was clipped with a pair of clean, dissection scissors and used to isolate mRNA or genomic DNA for analysis. After clipping the adult fish was returned to system water to recover.

Laboratory Manual and Classes

Detailed, student-friendly procedures with hyperlinks to videos and other resources are described in the complete laboratory manual (Appendix 2). The lab manual includes questions and activities that are italicized and red. The answer key includes answers in blue (Appendix 3). An instructional prep sheet with specific catalog numbers where appropriate is also included (Appendix 4). Students were scheduled with a weekly three-hour laboratory period that complemented three 50-minute a week lectures covering typical introductory biology material (metabolism, genetics, cell division, and neuroscience).

Project Ownership Survey

On the first day of lab, students consented to use of their survey data through an informed consent form approved by the Institutional Review Board. The participants in this study were 56 sophomore and junior undergraduate students enrolled in three different laboratory sections of General Biology II at Rollins College in Fall 2016. Each section was taught by a different instructor. The traditional group comprised of 37 students split between two sections. The experimental group included one section of 19 students who participated in the CURE. The survey was administered via Qualtrics using validated questions from the Project Ownership Survey [12] and a 5 part likert with strongly disagree (1) to strongly agree (5) during the last laboratory period. Student's scores were averaged for each traditional and experimental group, and a t-test ($p<0.1$) was used to compare any statistical mean difference.

Results and Discussion

To understand the role of *lin28a* in regeneration, students genotyped progeny of CRISPR heterozygotes, observed regeneration in neuromasts, examined gene expression, and finally tested rheotaxis. A full description of the procedures, the background information, and concept questions can be found in Appendix 2 with an answer key and student data in Appendix 3. In the context of this ten-week series of experiments, there was not a significant difference in neuromast regeneration, gene expression, or rheotaxis between the mutant and wild-type zebrafish. Although these data exhibited a high amount of variability, most likely due to the novice skill set of the undergraduate researchers, these data are congruent with observations made by a postdoctoral researcher at the NIH [10]. Indeed, after the students had completed the first two experiments (genotyping and regeneration), the postdoc visited the class to discuss her own research data on regeneration that matched that of the students. Informally, students remarked that they felt validated through this experience.

Undergraduate classroom laboratories can be categorized according to their scientific process, where a higher ranking indicates a more authentic research experience (Table 1). Even though some laboratories may require more technical ability, research involves the generation of novel data through well-controlled experiments. This particular CURE incorporated an experiment characterizing unknowns where some of the genotypes were known to the instructor (wild-type controls), but all other laboratory sessions were guided design on original research with no known answer (Table 1). For some experiments, students were encouraged to develop their own experimental design, but through class discussion, the experiments were limited to predetermined controls and samples. Emphasizing primary literature enhanced the rigor of the design, as students had to interpret peer-reviewed, published figures and methods sections before initiating an experiment. Furthermore, in the context of this project, students kept a laboratory notebook, created figures and figure legends, and wrote a full-length paper, reinforcing the communication details necessary for the scientific process.

Table 1: Some categories of typical undergraduate laboratories where a higher ranking suggests a more authentic research experience, irrelevant of the techniques and skills required to complete the lab.

Type of Lab Experience	Ranking	Associated Experiment
"Cookbook Lab": Known Procedure and Answer	0	Heart Beat Rate
Characterize "Unknown" using Set Procedures: Instructor Knows Answer	1	Zebrafish Genotyping
Known Answer, Student-Led Experimental Design	2	N/A
Guided Design on Original Research Question	2	Neuromast Regeneration, RT-PCR, Rheotaxis
Student-Led Experimental Design on Original Research Question	3	N/A

At the end of the semester, students were asked to take the Project Ownership Survey [12], and responses from the CURE students were compared to those students enrolled in the more traditional laboratory sections. These traditional sections engaged in some experimental design and unknown characterization (ranked 1 or 2 in Table 1). However, the experiments were proof of concepts where the answers were known, and the scientific questions were typically only addressed over one or two weeks. Among questions from the POS, the only answer with a large difference (t-test, $p<0.1$) by the experimental group was, "my findings were important to the scientific community" (Table 2). This is likely because the experimental group was aware that their research was a collaborative project with the NIH with an unknown answer. The two groups reported almost equal scores on their research being interesting and exciting (Table 2). We hypothesize that one reason the differences in the other responses of the POS were not significant was because the survey was given during a time when the traditional section was doing an experimental design (ranked 2 in Table 1). Thus, these students may have answered the POS questions based on the lab currently underway rather than considering the semester as a whole.

This study had several confounding variables. The study was limited to a small sample size of less than 20 students in the experimental group. The average science GPAs among the two groups differed, with 3.01 for traditional and 2.78 for experimental, which may have correlated to the students' interest and engagement in the course as a whole. Another limitation is that the study was not randomized in that the students were not able to choose their lab section, and the sections additionally varied by both professor and timing. Providing the choice of traditional lab sections versus a CURE section before course registration may have allowed some students who were academically weaker or simply less interested in authentic research to opt for the traditional laboratory curriculum instead of the CURE, thereby increasing interest and excitement about the project. In contrast, a random assignment of students would make a better study. Having different professors for each section may have skewed results due to different teaching styles and experience teaching these labs. For example, the traditional series of laboratories has been taught and refined for three years at Rollins, but the experimental curriculum was novel so troubleshooting and refinement of the instructions was happening as the

Table 2: Some POS questions and responses comparing the traditional and the CURE courses. *indicates $p<0.1$

Question	Traditional	CURE
My research will help to solve a problem in the world.	2.85	3.31
My findings were important to the scientific community.	2.82*	3.50*
My research project was interesting.	4.09	3.94
My research project was exciting.	3.71	3.69

students piloted these laboratories for the first time. Also, the traditional General Biology labs already included some experimental design and multiple week labs, such as a C. *elegans* chemotaxis taste lab. Finally, the fact that *lin28a* did not have an effect in the experiments utilized and therefore contradicted the initial hypothesis may had led to lower morale since this was likely a first encounter refuting a hypothesis for these introductory students. In the context of this perceived failure, students were encouraged to not only report their results, but also discuss future directions and alternative hypotheses in their final paper. Indeed, among the final submitted papers, 17of 19 students recognized the disagreement with the initial hypothesis and in response, two of these students proposed additional trials of the experiments, and six of these students suggested an analysis of other genes, i.e. *lin28a* is not solely responsible for regeneration. Although the first suggestion is weak, the second is viable based on the primary literature [8, 9].

Because of the constraints of mentors and resources, research opportunities for undergraduates are often limited. The main goal of a CURE is to provide students with a taste of authentic research and a reflection of how scientific research is done[3]. Although this CURE did not result in publishable findings regarding *lin28a* and regeneration, it provided students the opportunity to work on a novel project where the answers were unknown. With CRISPR mutant zebrafish becoming more readily available and easy to generate [11, 13, 14], the hope is that other zebrafish teacher-scholars will be able to extrapolate and modify this lab manual to fit their needs and their mutant fish of interest. Indeed, some individual components could be combined into a single laboratory period or expanded to generate additional periods. Certainly, this project could be used in a course focused entirely on genetics, particularly with additional discussion of the current technique of CRISPR/Cas9 genetic engineering. Ultimately, the hope is that integrating authentic research projects into the classroom laboratory curriculum will enrich students' understanding of the scientific process and better prepare them for the critical thinking and problem solving necessary to their success in STEM.

Acknowledgements

The authors acknowledge that this research would not have been possible without the active engagement and participation of the students enrolled in the fall 2016 General Biology II courses. Karen Jackson and Jay Piecyznski were the instructors of the other two courses. We thank Shawn Burgess' lab at NHGRI/NIH for validating the CRISPR *lin28a* target in zebrafish and for sharing protocols. The authors would like to thank Zeynep Teymuroglu for her critical evaluation of the survey data.

References

1. (2011) American Association for the Advancement of Science. Vision and Change in Undergraduate Biology Education: A Call to Action.
2. Russell SH, Hancock MP, McCullough J (2007) Benefits of Undergraduate Research Experiences. Science 316(5824): 548-549.
3. Shortlidge EE, Brownell SE (2016) How to Assess Your CURE: A Practical Guide for Instructors of Course-Based Undergraduate Research Experiences . J Microbiol Biol Educ 17(3): 399-408.
4. Corwin LA, Graham MJ, Dolan EL (2015) Modeling Course-Based Undergraduate Research Experiences: An Agenda for Future Research and Evaluation. Cell Biol Educ 14(1): 1-13.
5. Corwin LA, Runyon C, Robinson A, Dolan EL (2015) The Laboratory Course Assessment Survey: A Tool to Measure Three Dimensions of Research Course Design. Cell Biol Educ 14(4): 1-11.
6. Stoick-Cooper CL, Moon RT, Weidinger G (2007) Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. Genes Dev 21(11): 1292-1315.
7. Shyh-Chang N, Zhu H, Yvanka De Soysa T, Shinoda G, Seligson MT, et al. (2013) Lin28 Enhances Tissue Repair by Reprogramming Cellular Metabolism. Cell 155(4): 778-792.
8. Ouchi Y, Yamamoto J, Iwamoto T (2014) The Heterochronic Genes lin-28a and lin-28b Play an Essential and Evolutionarily Conserved Role in Early Zebrafish Development. PLoS One 9(2): e88086.
9. Ramachandran R, Fausett B V, Goldman D (2010) Ascl1a regulates Müller glia dedifferentiation and retinal regeneration through a Lin-28-dependent, let-7 microRNA signalling pathway. Nat Cell Biol 12(11): 1101-1107.
10. Pei W, Xu L, Huang SC, Pettie K, Idol J, et al. (2017) Large-scale, guided genetic screen to identify genes essential in the regeneration of hair cells and other tissues. npj Regen Med submitted.
11. Varshney GK, Zhang S, Pei W, Adomako-Ankomah A, Fohtung J, et al. (2016) CRISPRz: a database of zebrafish validated sgRNAs. Nucleic Acids Res 44(D1): D822-826.
12. Hanauer DI, Dolan EL. (2014) The Project Ownership Survey: Measuring Differences in Scientific Inquiry Experiences. Cell Biol Educ 13(1): 149-158.
13. Varshney GK, Carrington B, Pei W, Bishop K, Chen Z, et al. (2016) A high-throughput functional genomics workflow based on CRISPR/Cas9-mediated targeted mutagenesis in zebrafish. Nat Protoc 11(12): 2357-2375.
14. Varshney GK, Pei W, LaFave MC, Idol J, Xu L, et al. (2015) High-throughput gene targeting and phenotyping in zebrafish using CRISPR/Cas9. Genome Res 25: 1030-1042.