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A modular laboratory course using planarians to study genes involved in tissue regeneration

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Abstract

Undergraduate research experiences are excellent opportunities to engage students in science alongside experienced scientists, but at large institutions, it is challenging to accommodate all students. To address and engage a larger number of students, we developed a modular laboratory course based on the Course-based Undergraduate Research Experiences (CURE) model. This new course was integrated with the scientific aims of a research laboratory studying the cellular and molecular mechanisms underlying tissue regeneration in planarians. In this course, students were asked to identify genes with roles in planarian biology. Students analyzed and cloned an assigned gene, determined its expression pattern *in situ* and examined its function in regeneration. Additionally, we developed critical thinking and scientific communication skills by incorporating activities focused on critical concepts. Students obtained high quality primary data and were successful in completing and mastering the course learning outcomes. They benefitted by developing basic research skills, learning to perform, trouble-shooting experiments, reading and critically analyzing primary literature, and using the information to defend and explain their experimental results. Through this course, students also increased their confidence and ability to perform independent scientific research. The course was designed to make it accessible to the community to implement and adapt as appropriate in diverse institutions.

Keywords

undergraduate; planarians; stem cells; course-based undergraduate research experience (CURE)

Introduction

Undergraduate biology courses have been traditionally lecture-based with labs that attempt to teach introductory techniques and skills. Such courses are not universally effective in increasing students' subject comprehension, which may be due in part to a lack of active learning during lecture, and an absence of opportunities to develop independent thinking skills that are beneficial for diverse learning styles. Student assessments demonstrate that the use of active learning improves the performance of underrepresented groups in biology courses [1,2], demonstrating that the incorporation of hands-on training experiences in science classes improves classroom diversity and inclusion. Accordingly, undergraduate

research experiences are excellent opportunities for engaging students in scientific practices while being mentored by experienced scientists. Experiences in research allow students to practice how to address hypotheses, perform experiments, and analyze data while incorporating fundamental concepts learned in introductory courses.

San Diego State University (SDSU) is a large and diverse **Hispanic Serving Institution** [3] that plays a pivotal role in training the local workforce. SDSU also provides an affordable education to students from diverse backgrounds, and an outstanding opportunity to recruit and train underrepresented students interested in pursuing scientific careers. However, like many large state universities, it is challenging for students to find researcher labs on their home campus to engage in an authentic research experience. As an alternative, laboratory courses can provide an authentic research experience through the development of a curriculum that incorporates critical independent thinking skills and hypothesis-driven exercises. However, current traditional curricular lab design and implementation lacks these critical features. In fact, many students fail to integrate what they learn from lecture with concepts presented in lab because of the focus on acquiring skills/techniques-based on following protocols and not developing critical scientific thinking skills. Students often complain how introductory labs are generally tedious and focused on obtaining a result by strictly following a protocol and do not provide them an opportunity to learn from mistakes. Fortunately, the creation and implementation of the course-based undergraduate research experience (CURE) has promoted changes to the way we can help students to learn, retain and develop critical thinking skills in a laboratory course setting [4–6]. Reports such as *Vision and Change in Undergraduate Biology Education* have made a call for integration of CURE type courses into biology curricula and less emphasis on “cookbook” laboratories [7]. A CURE lab is centered around a compelling question that can be addressed experimentally, where students are required to use content knowledge previously learned from lectures or previous courses as a foundation to investigate critical scientific questions using tools accessible to institutions at all levels and sizes.

In this study, we have designed a laboratory course based on models of previously published CURE-type courses [4,6,8,9]. This new course is integrated with the research focus of the Zayas laboratory, which studies the cellular and molecular mechanisms of tissue regeneration using freshwater planarians. These animals can completely regenerate entire worms from small body fragments and are ideally suited for screening gene function in regeneration. Their amazing capacity for tissue replacement is sustained by adult pluripotent stem cells (called neoblasts) that replace cells lost during normal cell turnover and after wounding [10–13]. Planarians are an excellent model system to use in a classroom laboratory setting due to their low cost and ease to maintain and propagate [14]. The planarian genome has been sequenced and databases like PlanMine [15] are critical resources for designing *in vivo* studies that connect concepts in genomics and gene expression with key biological mechanisms like organism development, patterning and cellular signaling.

In this course, students were tasked with identifying genes with roles in planarian regeneration. We assigned each student a specific gene based on current Zayas laboratory experimental findings. Throughout the semester, students analyzed their assigned gene

sequence, cloned it into a plasmid vector, determined its expression pattern in whole animals and examined the function of the gene in tissue regeneration. We also aimed to develop students' critical thinking and scientific communication skills (written and oral) by utilizing non-experimental down time and incorporating activities focused on reinforcing and understanding critical concepts needed to complete, troubleshoot experiments and present data in class. Finally, a workshop was developed and aimed at exposing them to the variety of professional opportunities that they could take advantage of with a science degree. We hypothesized that this course would be beneficial to students because students that engage in authentic research are more likely to have a continued interest in science [16–18], and pursue scientific careers [5]. Based on students' responses and surveys after taking this course, they more likely identify themselves as scientists, and have become more confident in their scientific skills. In this paper, we present our course design and all of its components so that it may be shared and utilized at a variety of institutions.

Materials and Methods

Planarian Care

A clonal, asexual strain of *Schmidtea mediterranea* (CIW4) was maintained in food grade plastic containers [19]. Each student was provided with a population of ten planarians, which they were responsible for expanding by performing 1–3 rounds of cutting [14]. Each group maintained their population in a single container throughout the semester. All students had sufficient animals to perform their experiments. Animals ranging in length from 3–6 mm were starved for at least one week prior to all experiments.

Informatics

For assigned gene sequences, students performed Blastx searches to identify homology, and analyzed the predicted protein sequences using the NCBI Conserved Domain Database Search tool (nucleotide sequences), SMART (longest ORF translation), and InterPro Scan (longest ORF translation).

Cloning

Genes of interest were amplified from cDNA with gene specific primers containing overhangs homologous to the pPR-T4P vector [20] and cloned using ligation-independent cloning (Table S3) [21,22].

Riboprobe synthesis and whole-mount in situ hybridization

Antisense RNA probes were produced [23]. Briefly, DNA templates were PCR amplified from cDNA clones in pPR-T4P plasmid vectors. Antisense riboprobes labeled with digoxigenin (DIG) were synthesized at 37°C. Probes were purified via ethanol precipitation and whole-mount in situ hybridization was performed manually [24] (see step by step protocol) with some modifications to fit the classroom settings. Planarians were sacrificed using a 5% N-Acetyl Cysteine solution prior to fixation in 4% formaldehyde. Fixed worms were bleached in formamide solution under bright light for 2 hours before lab time. During class, bleached worms were treated with Proteinase K solution, post-fixed with 4% formaldehyde and transferred to screw-capped tubes for subsequent steps. Samples were

then incubated with PBSTx:Pre-Hyb solution (ratio 1:1) for 10 min. at room temperature before exchanging with Pre-Hybridization solution preheated to 58°C and incubating at 58°C on a heat block until the end of class (at least 1.5 hr.). Before class ended, the pre-hybridization solution was exchanged with the riboprobe solution. Samples were then incubated at 58°C for >16 hr., after which they were transferred to 24-well plates for equilibration and stringent washing as described [24] with the following adaptations: volume of sodium citrate washing buffer were increased to 500 µl, washing temperature was 58°C, and duration of each wash was 15 min. Afterwards, samples were returned to room temperature, washed in MABTw and stored at 4°C until next class for adding anti-digoxigenin AP antibody (Roche, 1:2000) and incubating overnight. The day after antibody incubation, samples were washed for 5 min., 10 min. and then 3 times for 20 min. each in MABTw at room temperature with generous amount of solution to compensate for the reduced number of washes. Samples were stored at 4°C in MABTw until next class for development.

RNA interference

In vitro transcription of double stranded RNA (dsRNA) was performed using either commercial transcription buffer (10X Lucigen Transcription Buffer) or 10X homemade transcription buffer [25]. The homemade buffer, however, was found to yield comparable RNA products as commercial buffer with less amount of DNA template, and was used since for this course. After 4 hours of transcription at 37°C, the reactions were treated with RNAse-free DNAse I, resulting in a crude *in vitro* transcribed dsRNA (mixture of ssRNA and dsRNA species). The crude transcription reactions were mixed with food dye solution (1:10 dilution in RNAse-free water) to achieve volume of 30 μL equivalent to 6 × 5 μL aliquots. Each aliquot was mixed with 20 μL of approximate 3:1 ratio of liver: water paste. Five to six RNAi feedings were performed over a period of four weeks and animals were cut pre-pharyngeally 48–96 hr. after the last feed (ideally, 72 hr. would be desired, but the amputation time can be adjusted within the range mentioned here for classroom settings). Planarians were monitored regularly following 10 days of regeneration and then fed with normal mixture of liver paste and food dye to observe any additional behavioral defects. All experiments were performed in duplicate at a minimum.

Image Acquisition and Processing

Live images of RNAi-treated planarians or images of fixed in situ-labeled planarians were acquired by students on a Leica M205 microscope fitted with either a Leica DFC290 or DFC450 camera. All images are of the dorsal worm with the anterior at the top. Images were processed for brightness and the figures organized using Creative Suite (Adobe).

Results

Functional Genomics Course Organization

The overall experimental goal of the *Functional Genomics Laboratory* course was to guide each student through the process of cloning a gene, determining the pattern of expression in the animal and examining the function the gene in planarian regeneration. The class had an enrollment of 18–22 students, a teaching ratio of 9:1, and was organized in groups of 3–4

students. The course met twice a week for 5.5 hours total. In order to complete the assigned experiments, students occasionally stayed late or came in to the laboratory outside of class times. However, flexible time frames were arranged at the beginning of the semester and to minimize outside time commitments group members took turns completing experiments. The course prerequisites were introductory cell and molecular biology and intermediate genetics. Students enrolled in the course were mostly graduating seniors (4^{th} or 5^{th} year undergraduates), with a small number of juniors (3^{rd} year students). The data included in this study is from 58 undergraduates enrolled from each of three spring semesters during 2015–2018.

The laboratory was organized into three sections (Figure 1). The first section introduced undergraduates to planarians (*Schmidtea mediterranea*) as a model system for investigating regeneration and included training on how to maintain, propagate, and feed their population of animals. Each student was assigned a gene from a list of candidates identified experimentally in the Zayas laboratory. Although students worked in small groups, each individual was tasked with acquiring, recording, and analyzing data pertaining to their assigned gene. Group work was ideally suited for combining some tasks (e.g., running an agarose gel) or covering steps of longer protocols outside of class. To accomplish this goal, they first learned how to use bioinformatic software to determine gene homology and find predicted protein domains (Supp. Figure 1). Students then used their sequences to design primers to clone their genes using ligation independent cloning [22]. The first unit concluded with each student successfully cloning the assigned gene into the pPR-T4P vector: a plasmid that can be used for generating riboprobes and dsRNA for *in situ* hybridization and RNA interference experiments, respectively (Figure 1, green). Students who were unable to amplify and clone their gene from *S. mediterranea* cDNA had to troubleshoot their cloning protocol and were given an additional gene to clone in case their original gene cloning proved challenging.

Sections 2 and 3 guided students through inquiry-based experimentation utilizing their cDNA clones. To investigate the function, students performed RNA interference (RNAi) by feeding planarians *in vitro* transcribed dsRNA (see Methods). Planarians are fed dsRNA over multiple weeks, and then are subjected to amputation. Students then observe the extent and rate of regeneration. In section 3, students synthesized riboprobes to perform whole mount *in situ* hybridization (Figure 1, green). To provide a detailed depiction of the course we created a weekly outline, which includes the experimental procedures performed, instruments needed, and the concepts discussed (Table 1). Moreover, we developed an alignment table that we utilized each semester that explicitly states daily learning objectives, experimental plans, activities, assessments, and bench materials needed for each day (Supp. Table 1).

Through each experimental section, students were provided with the background knowledge and scaffolding through mini-lectures (Supp. Table 1), primary literature discussions or peer learning activities to help them master the course concepts and objectives (Figure 1, purple). These activities and discussions also ensured that the students learned key skills like following scientific protocols and maintaining lab notebooks, but also practiced learning how to think and propose experiments like a scientist. Students were expected to read

primary literature to provide them with sufficient background knowledge to develop a hypothesis for the function of their genes; thus, we incorporated key papers throughout the semester to model how to read and analyze results. Each section culminated with the students submitting a written report in a primary article format with literature references to provide background to support their hypotheses (Figure 1, grey). As the semester progressed, the students were expected to expand the report with new experimental methodologies and results. The instructors graded each written report using a rubric that provided students feedback for subsequent submissions (Supp. Table 2). Feedback was provided for all these components so students could identify gaps in their knowledge and improve their communication skills throughout the semester. The final assessment included both the final written report and an oral group presentation.

Each time this course was taught the experimental procedures were more streamlined and successful, which was evident by the quality of data, presentations and written reports that were produced by the students. We selected a few representative genes that students investigated to highlight the quality and type of results the students can obtain in this type of course.

Gene expression screen exhibited patterns in variety of tissues and organs of planarians

After the students designed primers and cloned their gene (Supp. Table 2), they synthesized riboprobes to perform *in situ* hybridization experiments (see Methods). The students were able to observe diverse expression patterns in a variety of tissues and organs as illustrated in the schematic of the planarian anatomy (Figure 2A). Selected genes were chosen to represent the quality and variety of results that the students obtained. Many genes were expressed in the central nervous system (*vglut3, soc7, lrsam1, march6*, and *lim3*) (Figure 2B) and displayed additional distinct expression in other tissues. For example, *vglut3* was specifically expressed in neuronal cells in the cephalic ganglia, brain branches, ventral nerve cords, and distal tip of the pharynx (known to be enriched for neural factors [26], as well as a punctate pattern around the epidermis. *socs7* was expressed in the cephalic ganglia and pharynx in addition to diffuse expression in the mesenchyme. In contrast, *lim3* had strong expression in the midline and posterior boundaries of the cephalic ganglia and a punctate expression pattern throughout the rest of the brain area, in the ventral nerve cords and along the lateral periphery of the animal. We also observed expression of genes like *bre1* that in addition to a diffuse parenchymal pattern, were expressed in a pattern that outlined parts of the nervous system including the cephalic ganglia and ventral nerve cords (Figure 2B). Other genes like *klf2*, *rnf44* and *tvag* were predominantly expressed in the intestine, whereas, *hox4a, traf2*, and *nompC* showed epithelial expression. Interestingly, *hox4a* had epithelial puncta throughout the worm with stronger expression in the posterior tip whereas *nompC* had stronger expression in the anterior tip (Figure 2B). The students used their *in situ* hybridization results together with the proposed expression of their gene from the single cell planarian database analysis [27], to construct a rationale for the hypothesis and proposed function(s) for their assigned gene.

Regeneration defects uncovered by RNAi screen

The process of treating planarians with dsRNA to knockdown mRNA levels for genes of interest is straightforward and does not require specialized equipment or reagents. We followed a step-by-step protocol [25] to generate *in vitro* transcribed dsRNA (from the same cDNA clone used for riboprobe synthesis) and fed the reactions to the planarians over six weeks (see methods). Amputation of planarian tissues provides a simple way to assess the role of these genes in stem cell-based tissue renewal. After perturbing individual genes using RNAi, the worms were amputated pre-pharyngeally and allowed to regenerate for up to 10 days, a period over which *S. mediterranea* regenerate and repattern their tissues appropriately.

Positive and negative controls were used to validate the RNAi experiment. *gfp(RNAi)* worms (negative control) exhibited a normal regeneration as expected, whereas *cul4(RNAi)* worms (positive control) showed defects in blastema formation and head regression (arrowhead) with subsequent lysing of the worms after amputation (100% n = 10 and n = 25, respectively) (Figure 3) [28]. Additional positive controls were used with varying degrees of success (data not shown), which was indicative of the technical variation that can be expected in a course where students perform a technique for the first time. Over the course of three semesters, 67 genes were screened and 5 genes displayed defects in tissue regeneration and homeostatic maintenance. All of the genes resulting in RNAi phenotypes were subsequently validated in the summer months. For example, *vglut3(RNAi)* worms developed an uneven or “ruffled” edges (arrow) and also exhibited a locomotory defect wherein planarians were sticking to the plate and were unable to glide properly (red asterisk; n = 7 of 10 animals). Consistent with a previous report [38], *hox4a(RNAi)* worms did not display overt regeneration or homeostasis defects after RNAi feedings. However, we were surprised that *hox4a(RNAi)* worms continued to exhibit eating behaviors, but developed epidermal lesions anterior to the pharynx (white asterisk) and the ingested food were observed escaping through the lesions (n = 15 of 30). Finally, *bre1(RNAi)* worms exhibited head regression (arrowhead; n = 19 of 36), ventral curling (n = 2 of 36), or developed lesions (n = 10 of 18) (white asterisk) (Figure 3). After amputation, *bre1(RNAi)* planarians failed to regenerate and eventually lysed (Figure 3). These results demonstrate the ability of students to identify and characterize novel genes involved in planarian regeneration.

In summary, RNAi experiments identified roles in planarian regeneration and homeostasis for a subset of the genes screened. The quality of these results demonstrates that it is possible to use an undergraduate research-based course to identify candidate genes for further investigations. These results validated that our students were able to follow experimental protocols to perform molecular cloning and screen genes for function in planarian regeneration.

Students improved their scientific thinking and communication skills through course objectives

In addition to the experimental outcomes, we also set out to develop students’ critical thinking and communication skills. The development of students’ scientific thinking skills were assessed through graded assessments (quizzes) as well as through the quality of lab

notebook entries and written reports. The assessment questions were aligned with the cognitive level of the learning outcomes and represent the students' knowledge of the course topics at the beginning, middle, and end of the semester. Each learning outcome was assessed both experimentally and conceptually as shown in Table 2. Assessment questions were consistent between course offerings with the exception of those referring to primary articles, which were selected to fit the course focus for a particular semester. A comparison of grades from each semester indicated no statistical difference from the first course offering (Supp. Figure 2). When data from all three course offerings were combined, the average score of Assessment 2, 85.8%, was similar to the average score of Assessment 1, 84.8% (Figure 4A). While the distribution of scores on Assessment 3 was similar to that of Assessment 2, students' overall scores improved by an average of 6.8% by Assessment 3 (Figure 4A). These results indicate that student performance on summative assessments improved over the duration of the course.

Student scientific thinking skills were also assessed qualitatively using graded writing scientific reports. Each report focused on a different aspect of the planarian project (Figure 1), requiring students to perform independent literature searches pertinent to their gene of interest and to use course materials to construct an introduction, methods, data analysis, and a discussion section. Students were graded on the quality of their writing using a rubric designed for this course (Supp. Table 2), with a focus on how students interpreted their collected data and how they used existing scientific literature to inform their reports. Data from three semesters of the course indicate that students' grades improved from Written Report 1 (75.3%) to Written Report 2 (79.7%) (Figure 4B). In addition, student performance on scientific writing assignments showed a 12.4% improvement by Written Report 3 (Figure 4B). Undergraduates in each of the three semesters demonstrated progressive improvement in scientific writing throughout the course (Supp. Figure 2). Students were required to combine and use feedback on their writing and data-interpretation skills to improve subsequent reports. These results suggest that students in each of the course offerings were processing scientific information better and meeting the course objectives by the end of the semester.

In addition to quantifying students' assessments, individual representative examples of student responses were chosen from the qualitative assessments (lab notebooks and written reports) and organized by learning outcome (Table 2 and Supp. Table 4). Individual student responses demonstrate that the students were able to communicate more scientifically (i.e., using correct scientific terminology), were more focused on the project hypothesis versus what the instructor was asking them to do, were able to propose alternative steps to troubleshoot experimental obstacles and be more descriptive with results that tied back to their central hypothesis (Supp. Table 4). These results suggest that these students benefited in their scientific thinking and lab skills by taking this undergraduate research course.

Students reported higher perceived gains than other course-based undergraduate research experiences

Students participated in the CURE survey where their attitudes of the course was assessed and compared with students who have participated in the CURE course survey nationally

[6,29]. Our students reported many perceived gains on the course elements listed on the CURE survey and specifically to many of the areas that were associated with the learning outcomes for our course including reading primary literature, collecting and analyzing data and communicating their science. The greatest gains were in areas where students had ownership of their project (unknown outcome of course, student having input into topic) and areas that were focused on and practiced during the course (reading primary scientific literature, present results written and orally, and maintaining a lab notebook) (Figure 5A). Overall, when compared to other CURE courses our students perceived higher gains, especially in scientific communication (reading and discussing primary literature, presenting their research and maintaining lab notebooks (Figure 5A). Students were also surveyed on learning gains in skills such as “clarification of a career path,” “understanding the research process” and “becoming part of a learning community.” The overall trend for our course in the post-survey was that our students reported higher gains when compared to the other students that participated in the CURE survey (Figure 5B).

Finally, students were surveyed at the beginning and end of the course on their expectations of the course. As shown in (Table 3), most students at the beginning of the semester were focused on the technical aspects and lab skills that they expected to learn and acquire. When surveyed again at the end of the semester, we noticed a trend that had shifted to be more focused on the knowledge and critical thinking skills they had learned and how they had benefited from the experience. This indicated to us that the students were engaged in the research process and invested on the outcome of the project. They were becoming “deep learners” [30,31] and not just focused on getting a good grade for the course.

Conclusions and Implications

We created a new laboratory course to provide undergraduate students in biology with an authentic stem cell biology and regeneration science research experience at SDSU using planarians as a model system. One of our main objectives was to create a modular course structure that could be easily shared to promote the community to co-opt and modify the lab course to fit within the goals and needs of other institutions. An advantage of our design is its modularity. Depending on the course level and background, each section (Figure 1) can be used independently, can be omitted or swapped to fit within the labs teaching and research goals. Additionally, the topic question, experimental technique and/or model system can also be changed. For example, the first section of bioinformatics and cloning could be integrated into any introductory biology course where the students learn about biotechnology and each student investigates a gene and clones it into a vector. To be authentic, we would recommend it be connected with a lab or labs that will use these cloned genes so students can develop an applicable hypothesis. We can see this course design also working with a variety of model systems including *C. elegans*, drosophila, zebrafish, yeast, cell lines or commercially available planarians [32]. Although these model systems have been used in other developed CURE courses the detailed course formats have not been made readily available [8,9,14,33–36]. This course was designed with the goal that other researchers on our campus could easily adapt and incorporate it in the curriculum to provide additional research opportunities for our undergraduate population.

The other main objective of this work was to develop and test the effectiveness of an inquiry-based course at our institution. Here, we show that students were successful in completing and mastering our learning outcomes and contributed to a functional genomics screen (Table 2). They were able to independently complete experiments and produce new data that could be investigated further (Figure 2 and 3). For example elements of the *socs7 in situ* pattern were similar to previously described patterns for *hedgehog* and *wnt11–6* in the medial boundary and posterior end of the cephalic ganglia, respectively [37]. Follow-up double *in situ* hybridization experiments would be informative to determine if *socs7* gene expression overlaps with these two important signaling genes and could give insight into the function of this particular gene. In addition, a phenotype was not previously detected for *hox4a* [38]. Driven by curiosity, students tested the ability of worms to eat following RNAi to examine behavior (e.g., chemotaxis) and discovered that feeding in of *hox4a(RNAi)* animals allowed to visualize the subtle epidermal lesions (Figure 3). This exciting prospect that a *hox* gene does affect planarian tissues represents a potential future project. An example of a follow up project initiated by undergraduates was the discovery of light-induced depigmentation in planarians that helped establish these animals as an experimentally tractable model for research into the pathophysiology of acute porphyrias [39].

Our students benefitted by developing basic research skills, performing, analyzing and troubleshooting experiments, and practicing reading and critically analyze primary literature to defend and explain their experimental results. We were able to assess that the level of students' critical and scientific skills improved, most clearly evident by their performance on their written reports and oral presentations (Figure 4). The students were able to clearly discuss how they came to their hypothesis, used correct scientific terminology and referenced primary studies to defend their results. By the end of the course, students were fully engaged in the research and not so focused on their performance or grades.

We showed that our students reported higher learning gains in science communication, science careers and being part of the learning community when compared to other CURE courses (Figure 5A and 5B). This is noteworthy because developing a self-efficacy and scientific identity is critical for students to remain in science, especially at a Hispanic serving institution like SDSU [40]. In the future, to determine what aspects of our course are most effective for our students we will need to utilize more quantitative and validated approaches [41]. As we teach future iterations of this course, we foresee updating and providing additional opportunities for students to follow up with experiments that they propose or that further look into the function of their individual genes (e.g., qPCR to assess the effectiveness of RNAi knockdowns or immunostaining to examine underlying causes of phenotypes) or to have the course be two semesters long to allow students even more independence to design and complete proposed experiments. Any changes made to the curriculum come with the caveat that it should be focused on the student having a feeling of ownership of their data for them to fully engage.

Finally, this course also successfully exposed more students to the possibilities of careers in STEM. In our three semesters of teaching this course, we have mentored and written many letters of support for our students to continue in higher education and STEM careers. This

course naturally fosters more interaction with instructors and we purposefully made a point to provide a place to speak about careers, and thus one can expect an increased number of requests. As of now we have three students who have continued in the SDSU Bridges to Stem Cell Internship Program sponsored by the California Institute for Regenerative Medicine, two who have continued in the SDSU Masters degree program, four to Ph.D. programs, one to become a science teacher, and several in biotech. Many of our students did not know what options other than medical school were there for them. Preparing students and exposing them to careers in science has been well received in our course and our experience coincides with studies that show students lack this information even at the time of graduation and are not prepared to go into the workforce [42]. These types of courses and discussions are important for our field and should be incorporated to provide mentorship and guidance to our undergraduates and to increase the level of our students' learning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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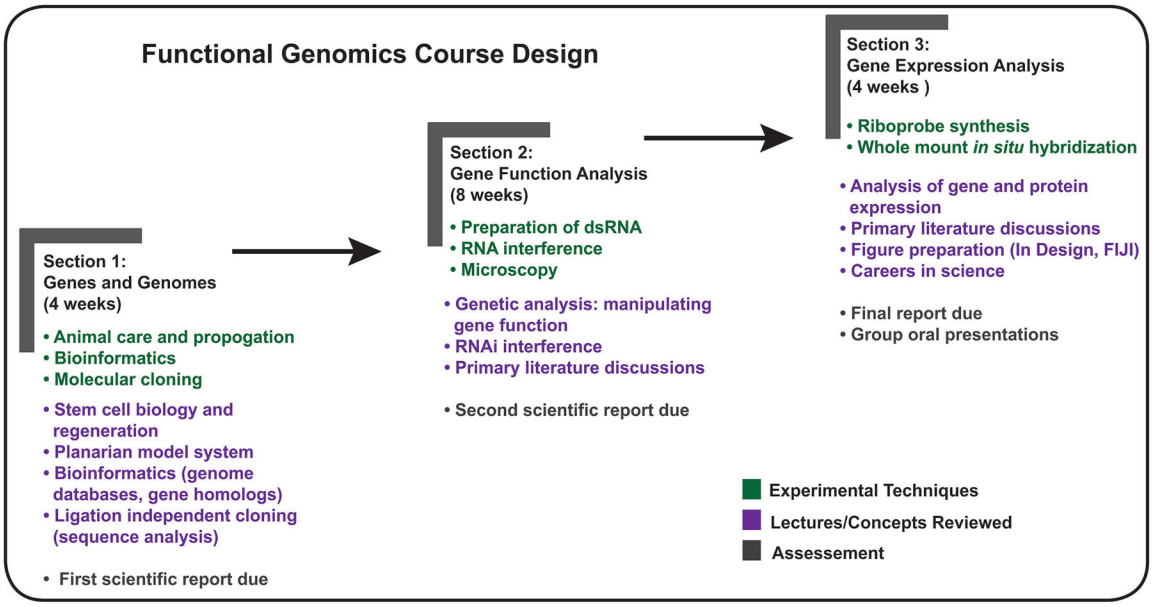

Figure 1: Flowchart of course and experimental design.

The course required three major sections: genes and genomes (section 1), analysis of gene function (section 2) and analysis of gene expression (section 3). The first section included using bioinformatic software and tools to identify and confirm homologs, design primers and clone their assigned gene with ligation independent cloning. The second and third sections students utilized their cloned gene to prepare dsRNA and riboprobe respectively to knockdown and examine expression of their gene of interest (green). Each section included brief lectures, paper discussion and/or activities to provide necessary background and provide guidance on critical concepts needed to master learning objectives (purple). Each section concluded with the submission of a written scientific report to assess their experimental and scientific process and provide an opportunity for written feedback (grey).

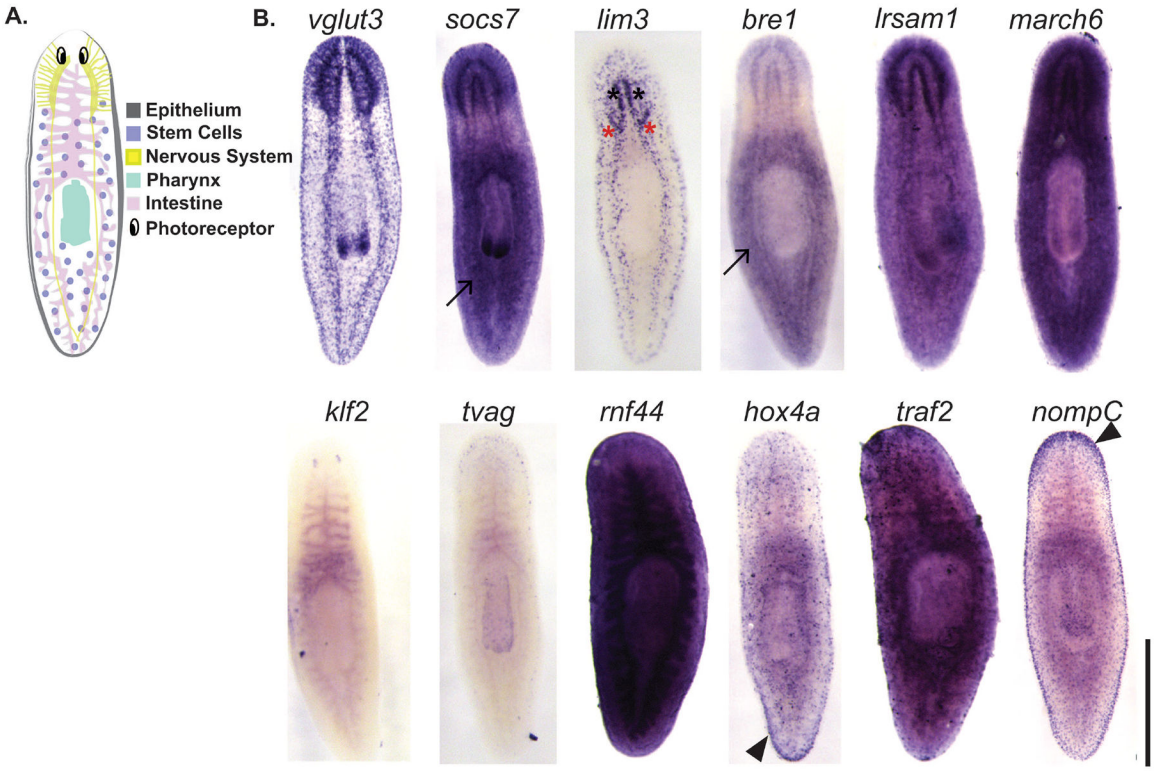

Figure 2: Expression Analysis of genes screened in S. mediterranea during course.

A) Planarian anatomy illustration of organs and cell types. B) Whole mount *in situ* hybridization (colorimetric) expression patterns of selected genes that show expression in a variety of cells and tissues. *vglut3*, nervous system punctate epithelium and pharynx; *socs7*, nervous system, pharynx, and parenchymal tissue (black arrow); *lrsam1* and *march6*, nervous system with no pharynx expression; *lim3*, cephalic ganglia in areas of possible *wnt11–6* (red asterisk) and *hedgehog expression* (black asterisk) [37] and ventral nerve cord; *bre1*, parenchyma (black arrow); *klf2*, photoreceptors; *tvag*, pharynx; *rnf44*, ubiquitous with strong intestine expression; *hox4a, traf2* and *nompc*, epithelium. *hox4a* has strong expression in the posterior epithelial tip whereas *nompc* has stronger expression in the anterior tip (black arrowheads). Gene names as indicated are the top blastx hit from Planmine. Scale bar, 500 μm.

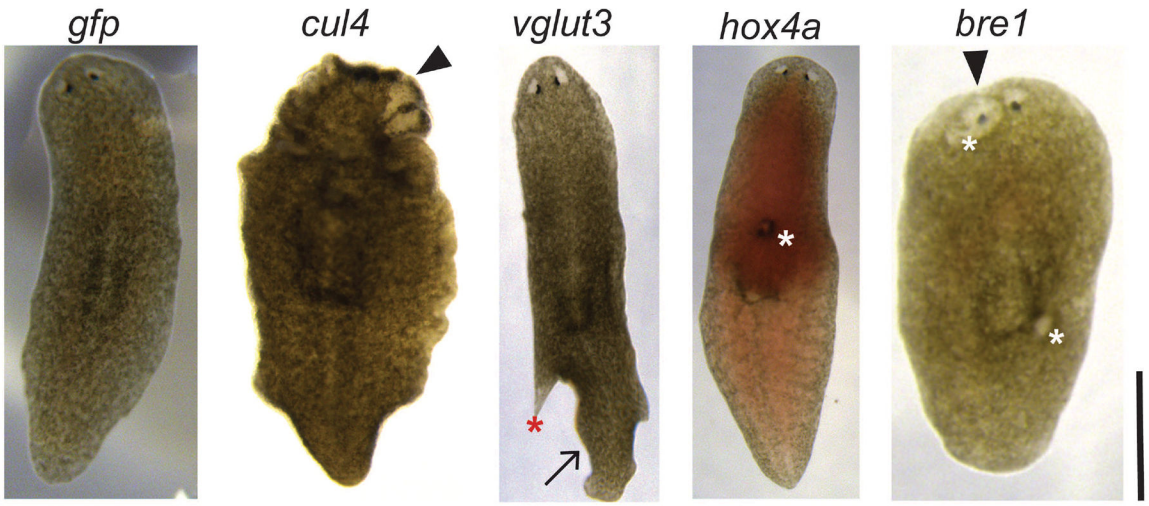

Figure 3: Phenotypes from the RNAi screen in S. mediterranea.

Animals were fed dsRNA 6 times over 4–5 weeks against controls *gfp* (negative n = 10) and *cul4* (positive n = 25). All treated animals were observed during RNAi feeding for defects in homeostasis phenotypes and then amputated pre-pharyngeally 5 days after last RNAi feeding and allowed to regenerate. Planarians were observed for defects in regeneration. Selected genes showed defects during the regeneration process. All *gfp* treated worms exhibited no defects as expected n = 10. All c*ul4* worms exhibited head regression depicted by black arrowhead and eventual lysis (n = 25). *vglut3* worms had ruffled edges (red asterisk) and defects in gliding where they would stick to the plate (black thin arrow) n = 7/10. *hox4a* developed a lesion (white asterisk) anterior to the junction between the pharynx and the intestine n = 15/30. *bre1* treated worms developed head regression n = 19/36 (black arrowhead), ventral curling n = 2/36 and lesions n = 10/36 (white asterisk) before amputation. After amputations all were severely impaired and lysed. Gene names as indicated are the top blastx hit from planmine. Scale bar, 1mm.

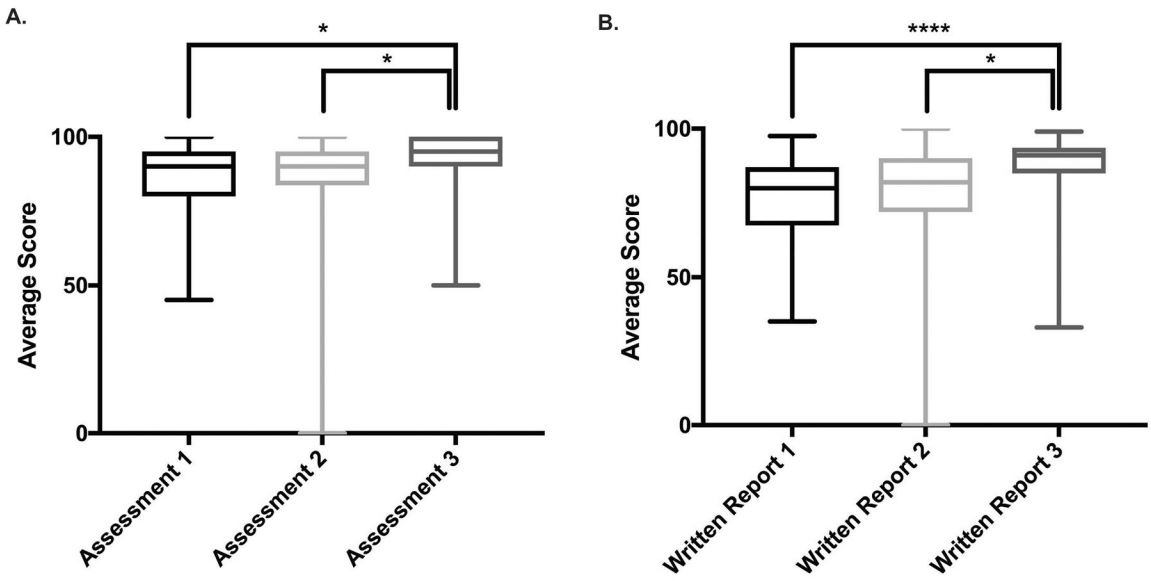

Figure 4: Student performance on summative assessments improved during duration of course.
A) Graph of students' average scores on summative assessments (quizzes) throughout the duration of the course. B) Graph of students' average scores of written reports that were graded based off of written report rubric. Each average score is the calculated average total of the three years that the course was taught. The average score is a percentage of 100 and statistical analysis used was a one-way ANOVA with Bonferroni post-test (n = 58).
*, $p<0.05$, ****, $p<0.0001$

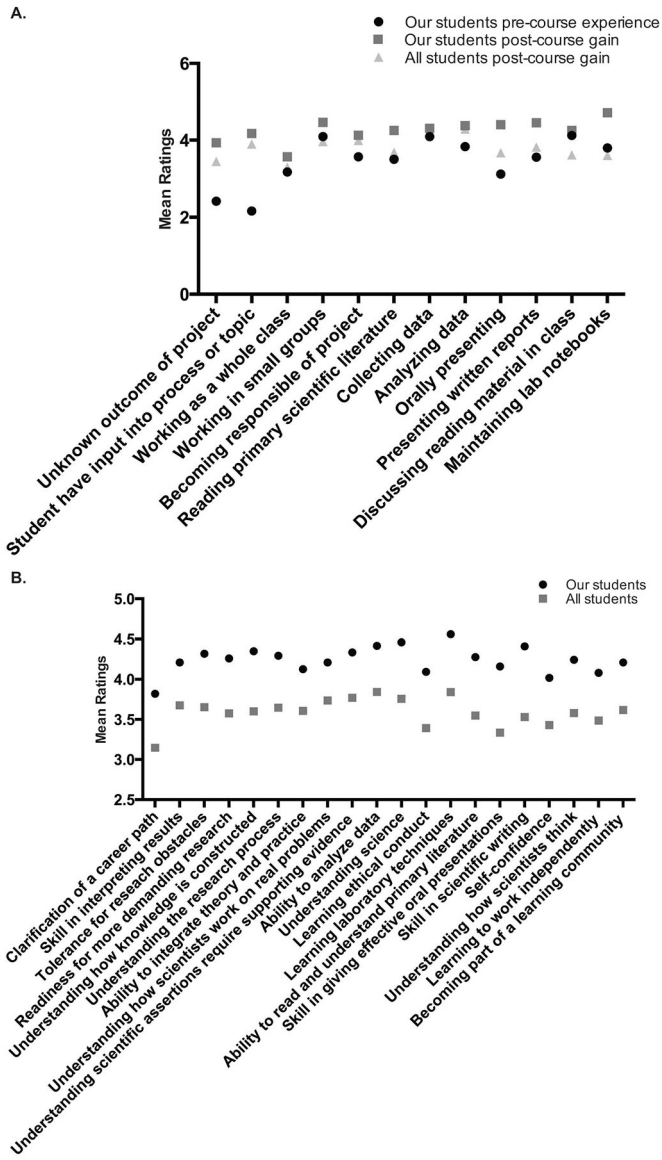

Figure 5: Course and learning gains higher in our course when compared to other research courses based on CURE (course undergraduate research experience) survey.

A) Course elements. Graph shows mean ratings by students of gains in 12 areas corresponding to the course elements above. Pre-course survey asked students to rate their experience with the course elements. B) Learning gains. Graph shows mean ratings by students in 20 corresponding areas. Mean ratings in both graphs were measured by post-course survey where they were asked to “rate the gains they made due to the course” on a 1–5 scale (1 = no or very small gain to 5 = very large gain). Means are an average of the three years students participated in the CURE survey (n = 35).

Table 1:

Outline of 16-week undergraduate research course

Week No.	Procedures Performed	Apparatus Utilized	Techniques/Concepts Discussed
1		N/A	Course Expectations, Lab Safety
2	Informatics, Planarian Care and Propagation (performed every week following)	Computers, Stereoscopes (be used with any planarians procedures)	Experimental Background and Design, Identifying and Analyzing Gene Homologs, Animal Husbandry
3	Cloning: Primer Design, PCR	Computers, Thermocycler	Primer Design, DNA In vitro amplification (PCR)
4	Cloning- Gel Electrophoresis, Ethidium Bromide Staining, Gel Analysis and Extraction, DNA Quantification, T4P Treatment	DNA boxes, Gel Scanner, UV/Vis Spectrometer, Microcentrifuge, Heat Block	Ligation Independent Cloning, Electrophoretic Mobility, UV Absorbance of DNA
5	Annealing, Transformation, Colony PCR	Incubator, Thermocycler, Heat Block	Recombinant bacterial systems, Plasmid structure and Antibiotic resistance, Data Analysis for Verification of Cloning
6	DNA Extraction, (mini-prep), Sequence Analysis	Microcentrifuge, UV/Vis Spectrometer, Computers	Mini-prep, Data and Software Analysis for DNA Sequencing
7	PCR for dsRNA, DNA Purification, Quantification and In vitro Transcription	Thermocycler, UV/Vis Spectrometer, Microcentrifuge	Transcription and In vitro transcription technique
8	RNAi Preparation and feeding, gel electrophoresis	DNA boxes, Gel Scanner, UV/Vis Spectrometer	RNAi
9	RNAi feeding (performed weekly until end of semester), PCR for Riboprobe Synthesis	Thermocycler	Riboprobes Synthesis, RNAi Methods (journal discussion)
10	DNA Purification, Quantification and In vitro Transcription, Planarian Propagation	Thermocycler, UV/Vis Spectrometer, Microcentrifuge, Stereoscope	In situ hybridization, RNAi Data Analysis (journal discussion)
11	RNAi observations and feeding		
12	RNA Purification and Quantification, Planarian Fixation and Bleaching	Microcentrifuge, UV/Vis Spectrometer, Light Box,	Riboprobe Synthesis, In situ hybridization
13	In situ hybridization: Riboprobe hybridization and antibody incubation and Planarian Amputation (RNAi treated)	Nutator, Stereoscopes, Incubator	Conclusion of Gene Expression from In situs
14	In situ hybridization Color Development and Journal Discussion	Nutator, Stereoscopes	Insitu hybridization Analysis (journal discussion), Data Collection of Regeneration of RNAi treated planarian
15	Figure Preparation and Formatting, Acquiring Images for Report.	Computer, Stereoscope with image capturing	Adobe, In design, Careers in science
16	Research Presentation, Data Analysis	Computers	

Table 2:

Learning outcomes and assessments of the course

Learning Outcome	**Assessment**
Identify human gene homologs in the genomes of research model organisms	Lab notebooks, quizzes, BLAST validation of sequence and homolog in a related species,
Design experiments and clone genes	Lab notebooks, quizzes, primer design, PCR validation of product size, colony PCR validation of cloned product in vector, DNA sequence analysis
Analyze gene expression and function in animal models	Lab notebooks, quizzes, PCR Validation of product size and quantification of transcribed product of dsRNA and riboprobe synthesis, Observation of RNAi fed animals and colorimetric reaction of *in situ* hybridization of animals
Evaluate primary research data and defend finding in scientific reports	Quizzes, journal discussion and scientific research reports (3 submissions)
Prepare laboratory presentations to defend and discuss project findings	Oral Presentations and scientific research report (3 submissions)

Table 3:

Students Responses on Course:

Beginning of Semester Survey	**End of Semester Survey:**
"To learn and experience following protocols for lab experiments… to learn and not be intimidated by what I don't know."	"I did not like that it only last one semester and I did not have the opportunity to take it earlier in my undergrad career"
"I expect to learn about planarians, stem cells and more detailed gene cloning."	"I love that this class was a real ongoing study. It gave me more of a motivation to do it all."
"I expect to learn more about genes and how to work with them in a lab setting."	"This is probably the most in depth information I have learned in the shortest amount of time… the vibe of the class is very open and welcoming, as well as highly enjoyable."
"I expect to learn how to apply molecular biology techniques."	"I feel a lot more confident with lab techniques…"
"I expect to get familiar with doing experiments, following protocols methodologically, trouble shooting experience, lab note taking. Etc. so that I can be confident working in any experiments."	"I enjoyed the reasoning behind these techniques and how they can be applied…"
"Learn a little about functional genomics and lab techniques to prepare for lab work."	"What I like the most about the course is the useful lab skills I developed throughout the course, how I learned to think critically and being skeptical in positive manner about experimental results and other research work.