Laboratory Exercise

Integration of a Zebrafish Research Project into a Molecular Biology Course to Support Critical Thinking and Course Content Goals[S]

Lisa K. Felzien*

Biology Department, Rockhurst University, Kansas City, Missouri 64110

Abstract

Engaging undergraduates in research is essential for teaching them to think like scientists, and it has become a desired component of classroom and laboratory instruction. Research projects that span an entire semester expose students to a variety of concepts and techniques and allow students to use experiments to learn scientific principles, understand why specific techniques are applicable, critically analyze varied data, and examine how experimentation leads to acquiring knowledge. To provide an experience with these features, a semester long research project was integrated into a combined lecture and laboratory course, Molecular Biology. The project utilized the zebrafish model to examine gene expression during embryonic development and required students to develop and test hypotheses about the timing of expression of previously uncharacterized genes. The main goals for the project were to provide opportunities for students to develop critical thinking skills required for conducting research and to support the content goals of the course. To determine whether these goals were met, student performance on the steps of the project and related pre-test and post-test questions was examined. © 2016 by The International Union of Biochemistry and Molecular Biology, 00:000–000, 2016.

Keywords: *integration of research into undergraduate teaching; gene expression; molecular biology*

Introduction

The value of and need for involving undergraduate students in research projects has been well documented [1, 2]. Research projects rely on student creativity and higher order thinking skills and have been shown to increase the retention of students underrepresented in the sciences, prepare students for careers in science, and create essential interactions between students and faculty [3, 4]. While the benefits are immense, providing mentoring for large numbers of students is a challenge. This issue has resulted in increased incorporation of research approaches within courses, such as inquiry-based laboratories [5–7], which have demonstrated success in increasing student knowledge, writing skills, and critical thinking.

In addition to inquiry-based approaches that span one to several weeks of a course, larger projects that more extensively mimic the research process have also been developed [8, 9]. While progress has been made in this area, there exists an ongoing need to provide tested and adaptable models for research projects within courses and new ways of integrating research across the curriculum [10]. The approach described here is the development of a semester-long, integrated research experience in a combined lecture and laboratory molecular biology course. This project required students to learn multiple techniques, use analytical skills, and connect technical and analytical approaches with concepts from lectures; thus, providing an opportunity to explore the relationship between research, molecular biology principles, and the acquisition of knowledge.

Two major topics in the molecular biology course were the genetics of development and gene expression. Therefore, a project that emphasized gene expression during zebrafish embryogenesis was developed. Using zebrafish as a model with undergraduates has many advantages, including ease of use and adaptability to primarily undergraduate institutions with limited research funding. Students see the relevance and significance of this model as they read the literature and use online resources. Students interested in health-related careers, a majority population

Volume 00, Number 00, Month/Month 2016, Pages 00–00
[S] *Additional Supporting Information may be found in the online version of this article.*
**Address for correspondence to: Biology Department, Rockhurst University, Kansas City, Missouri 64110. E-mail: lisa.felzien@rockhurst.edu*
Received 22 October 2015; Revised 27 January 2016; Accepted 3 April 2016
DOI 10.1002/bmb.20983
Published online 00 Month 2016 in Wiley Online Library (wileyonlinelibrary.com)

in many biology and biochemistry programs, appreciate the zebrafish model because of its relevance to human health and disease. Zebrafish homologs have been found for approximately 70% of human genes, and genes have been identified in zebrafish that are homologous to those involved in human disorders related to the blood, heart, kidneys, muscles, and central nervous system [11]. Cancer, a disease of interest to many students, shares similarities with development in the areas of gene expression, cell division, and cell migration [12, 13]. Finally, zebrafish are commonly used to screen drugs for their effects on genetic defects as well as for toxicity, both issues with which students can relate.

Zebrafish development is very well characterized, and is divided into the specific stages of cleavage, blastula, gastrula, segmentation, pharyngula, and hatching, all of which are completed within ~72 h post fertilization (hpf) [14]. The formation of most organs has begun by 24 h [15], and developmental stages are easily observed and distinguished due to the transparency of the chorion and developing embryo. Tissue specific expression of a number of zebrafish genes has been examined during development, and the effects of mutations of many genes critical for development have been determined. The expression of additional genes during zebrafish development has been detected through the generation of mRNA sequences from pools of RNA purified at various developmental time points. The specific timing of expression, however, remains unknown for some genes with characterized homologs in other species as well as gene families in which only some members have been studied. This gap in our current understanding provides opportunities for students to make novel contributions in this area.

Materials and Methods

Participants and Course Characteristics

Student participants were juniors and seniors enrolled in the course, Molecular Biology, which has been offered one time per year for several years with an enrollment of ~24 students. The prerequisite course was an upper level genetics course, and many students had completed additional coursework in biology and chemistry. Students majoring in biochemistry and a molecular track in a biology degree program were required to take the course, which met for 85 min two times per week. Approximately 1/3 of the class meetings were spent completing the experimental steps for the project. Students worked in groups of three to four and were required to participate equally. Equal participation was encouraged during class by the instructor and evaluated through a peer evaluation process at the end of the class.

Zebrafish Maintenance and Embryo Cultures

Wild type AB zebrafish were obtained from the Zebrafish International Resource Center. Basic care and breeding procedures are well described [14] and included maintaining adult fish at 28°C in a 14-h light 10-h dark cycle and housing adults overnight in a 2 gallon aquarium containing a layer of marbles at the bottom to isolate fertilized eggs from adults. Students were not directly involved in the maintenance and breeding of fish, but they observed demonstrations of embryo collection and were provided with cultures of embryos fertilized ~1, 24, 48, and 72 h prior to class and maintained in embryo water [16] at 28°C. Embryos were examined with a stereoscope at 20–40× magnification, and images were captured with a UHCCD Series C-Mount USB2.0 CCD Camera (ToupTek). Use of zebrafish for this project was approved by the Rockhurst University Institutional Animal Care and Use Committee.

RNA Extraction

RNA was extracted from zebrafish embryos using the Trizol reagent (Life Technologies). Manufacturer's instructions were followed, with minor modifications. Specifically, embryos were washed with sterile dH_2O, and 100 μL Trizol was added for 15–50 embryos. Embryos were homogenized in 1.5 mL tubes with Teflon pestles, until evidence of physical disruption of the chorion and/or the embryo was present (~2 min of gentle agitation). Final RNA pellets were resuspended in 25 μL nuclease free water and quantified using UV spectrophotometry. Students were required to use gloves and wear protective clothing throughout the RNA isolation steps, in part, because Trizol contains phenol.

Gene Selection and Hypothesis Generation

Hypotheses were student-generated through a systematic framework. Groups were initially encouraged to identify areas of interest from the molecular biology course, consider prior knowledge from other classes, generate ideas through popular press articles and websites, and consider the career interests of group members. Students generated a preliminary list of candidate genes for study by examining peer-reviewed literature. Additional literature and nucleotide searches through the National Center for Biotechnology Information (NCBI) were performed with instructor guidance, to ensure that groups selected a gene in which the timing of expression had not yet been determined during zebrafish embryogenesis. Some students chose genes encoding transcription factors or proteins involved in epigenetic regulation that were already characterized in zebrafish. In these cases, students were led through the process of determining possible uncharacterized downstream targets identified in other species by examining results from published microarray analyses or regulatory pathway diagrams. The NCBI basic local alignment sequence tool (BLAST) was also frequently used to identify unstudied genes related to genes that were already characterized. Following gene selection, students explored knowledge about similar genes in other species or cells in culture to formulate their hypotheses about the expected timing of expression during zebrafish embryogenesis.

Gene Sequence Identification, BLAST Searches, and Primer Design

mRNA sequences were retrieved through nucleotide searches using the NCBI website. Coding sequences were used in BLAST searches to identify similar genes in other species. Students chose various databases in the BLAST program, including the human, mouse, and other (including a variety of species) options, to determine levels of similarity between zebrafish genes and those in other species.

To design primers that amplified a 400–600 base pair region of the gene of interest, students used the primer BLAST option through NCBI. All default settings were utilized, except the requirement that one primer spanned an exon–exon junction, so that primers would selectively bind to mature mRNA sequences. Students were required to identify the regions in their sequences corresponding to the primers and demonstrate that the forward primer is the exact sequence of the mRNA, while the reverse primer is complementary to the mRNA.

Reverse Transcriptase PCR

Primers were purchased (Life Technologies) and given to students in lyophilized form for resuspension and use in RT-PCR. GAPDH was used as a control in some semesters, using the forward primer, 5′ GGGTGATGCAGGTGCTACTT 3′, and the reverse primer, 5′ GGCAGGTTTCTCAAGACGGA 3′, encompassing a 499 bp region. More recently, β-actin was used as a control, using the forward primer, 5′ CAGCC TTCCTTCCTGGGTATG 3′, and the reverse primer, 5′ AGGT TGGTCGTTCGTTTGAA 3′, encompassing a 400 bp region. Data shown from students on ddx46 were generated with a forward primer: 5′ ACGCGCTGAAGAAACACAAC 3′ and a reverse primer: 5′ TCCACGATACGCATCACCTG 3′, encompassing a 490 bp region. Data shown from students on cflara were generated with a forward primer: 5′ AGCAGCG ACGAGTCTAAGAT 3′ and a reverse primer 5′ CATGGCA TGTTCTGACCTCT 3′, encompassing a 517 bp region.

RT-PCR was performed with the Access RT-PCR system (Promega) using manufacturer's directions and 2 mM $MgCl_2$, an estimated amount that provides maximal amplification of several target genes previously tested. RT reactions were carried out for 45 min at 45°C, followed by a denaturation step at 94°C for 2 min. 50 cycles of PCR were performed, with denaturation at 94°C for 30 sec, annealing at 50°C for 1 min, and elongation at 57°C for 2 min. A final extension step at 57°C for 7 min was also completed. Half of each reaction (25 μL) was separated on a 2% agarose gel in 1X TAE, containing 0.2 μg mL^{-1} ethidium bromide. Gels were exposed to UV light and images were documented with a Sony 5.0 megapixel, 3.0× zoom digital camera. Students were required to wear gloves, protective clothing, and goggles when working with ethidium bromide gels and the UV light box. Ethidium bromide was added to the gel for containment and was used at the lowest possible concentration needed to visualize bands. Recently, SYBR safe (Life Technologies) has been used as a safe replacement for ethidium bromide.

Assessment

A multiple choice pre-test was taken on the first day of class to determine incoming knowledge about the concepts and skills related to the research project. The pre-tests were not returned to students, and the same questions were used on the final exam. Results from 66 students over the course of 3 semesters were included in this analysis. Use of pooled assessment results was approved by the Rockhurst Institutional Review Board.

Results

Project Overview and Goals

The project was designed to support course content while providing an authentic research experience. The overall, content, and skills goals for the project are shown in Table I. Developmental genetics, genomes and evolution, and gene expression were major course content areas, and the research project supported each of these topics. Two additional goals relating to the process of research were: (1) exposure of students to a variety of experimental techniques and (2) extensive student involvement in project development, data analysis, and dissemination. This combination of goals provided a framework for creating a meaningful student experience.

Stages of the Project and Sample Student Data

Background and theoretical information essential for the project was provided through lectures, activities, or assignments prior to the completion of each experimental stage. The project stages are represented in Table II. Students worked in groups and used a laboratory guide (Supporting Information) that included protocols, images of zebrafish developmental stages, and diagrams demonstrating experimental approaches. Students completed several assignments over the course of the semester from the laboratory guide related to data documentation and analysis, acquisition of background information, hypothesis generation, and gene sequence analysis. The individual assignments were used as the basis for formal scientific presentations and papers completed at the end of the semester.

In preparation for the examination of zebrafish embryos and RNA extraction from embryos, students were first introduced to concepts related to *Drosophila* development, a topic supported by information in the course textbook. Students also reviewed images and descriptions of the stages of zebrafish development found in standard embryo staging guidelines [14]. Zebrafish embryos at various developmental stages were examined during two class periods, and representative images were recorded (Fig. 1). Two additional class periods were dedicated to purifying RNA from embryos. The quality of RNA samples was evaluated by comparing the total amount of isolated RNA to the number of embryos, documenting the 260/280 ratio, and determining the RNA concentration (see Table III for sample student data). Because each

TABLE I *Goals of the research project*

OVERALL GOALS

1. Make connections between the research project and related course concepts.
2. Experience research in an authentic way.
3. Gain an appreciation for animal models and molecular biology research.
4. Gain experience that supports further engagement in research.

GOALS RELATED TO COURSE CONTENT

1. Developmental genetics—Describe the significance and examples of homology among organisms with respect to developmental genes; Compare and contrast development in *Drosophila* and zebrafish, including physical and genetic aspects.
2. Genomics and evolution—Compare zebrafish genes from our research project with those in other organisms; Explain how genomes change through mutation, including how the zebrafish genome has been mutated for the study of specific genes; compare the features of the zebrafish genome with other eukaryotic and bacterial genomes.
3. Gene expression—Explain how transcription is regulated in eukaryotes; Compare gene expression in zebrafish and other eukaryotes with gene expression models in prokaryotes; Use standard approaches to examine gene expression.

EXPERIMENTAL TECHNIQUES

1. Stage zebrafish embryos and document the status of embryonic cultures.
2. Complete BLAST searches.
3. Purify RNA from zebrafish embryos at specific developmental stages.
4. Design PCR primers using primer BLAST.
5. Complete calculations required for nucleic acid quantification, dilution, and concentration.
6. Perform RT-PCR to examine gene expression.

PROJECT DEVELOPMENT, DATA ANALYSIS AND DISSEMINATION

1. Review the literature to identify interests and candidate genes whose expression has not been previously characterized in zebrafish development.
2. Create a hypothesis about the timing of expression of the chosen gene based on information from other species.
3. Access and use online zebrafish resources.
4. Analyze results that determine RNA quality and expression of experimental and control genes.
5. Complete further literature and BLAST searches to explain unexpected results.
6. Evaluate the hypothesis.
7. Present background and results to peers.
8. Write a scientific paper documenting results and analysis.

class had six groups, failures at this stage were overcome by the ability to use others' RNA samples. Groups shared the results of their RNA purification with the class, allowing students to request the use of RNA from a different group if their purity levels (a 260/280 ratio lower than 1.1) or concentrations ($<0.2\ \mu g\ \mu L^{-1}$) were too low.

Following RNA purification, students were engaged in assignments and exposed to lecture material about prokaryotic and eukaryotic models of gene expression. They then spent four class days and time outside of class performing background literature searches and BLAST searches through NCBI that led to gene selection and hypothesis formation on the expected timing of the expression of their gene. BLAST searches taught students how to work with databases and helped with hypothesis development, because students were able to determine the level of similarity between their gene and similar genes in other species and examine any known functions for the related genes in another species. Once a gene was selected and a hypothesis formed, students used the primer BLAST tool at NCBI to generate primer sequences for the next stage of the project, RT-PCR.

RT-PCR on zebrafish RNA was performed following a lecture on the process, including a discussion about positive controls and possible false positive and negative results. Students spent four class periods on RT-PCR, and activities included planning, set-up, electrophoresis, and data analysis. Most groups (an average of five out of six total groups per semester) obtained PCR products. A range of experimental results was observed, including a single product of the expected size with or without additional products and one or more products of the wrong size. The absence of a product in the experimental samples was uncommon, but

TABLE II *Experimental steps of the research project*

Identification of embryonic stages, events, and characteristics
↓
Extraction and quantification of RNA
↓
Background research on development and candidate genes
↓
Hypothesis about when gene is expressed
↓
RT-PCR to detect mRNA from the gene
↓
Data interpretation and evaluation of hypothesis
↓
Formal presentation and scientific paper documenting the project

when this occurred, students had to consider sources of error. If control PCR reactions were successful, students could discuss the possibility that their gene was not expressed during zebrafish development, even though they had to consider the absence of a positive control for their gene. Figure 2 shows two student examples of RT-PCR results, demonstrating some of the possible scenarios faced by students as they analyzed their data. In Fig. 2a, genomic DNA contamination was likely in the 48-h sample for the gene, ddx46. Figure 2b shows a product at the expected size as well as smaller products in the experimental samples for the gene, cflara, and one β-actin control sample with no product.

Groups frequently obtained unexpected results in RT-PCR, which forced them to analyze whether their gene was expressed, whether nonspecific amplification had occurred, or both. Several websites provide information about issues related to RT-PCR [17, 18], and students used this type of information to discuss what may have resulted in the absence of a product or products at the wrong size. Students identified ideas such as poor primer design, suboptimal $MgCl_2$ concentration, low annealing temperatures, and multiple target sequences as reasons for their unexpected results.

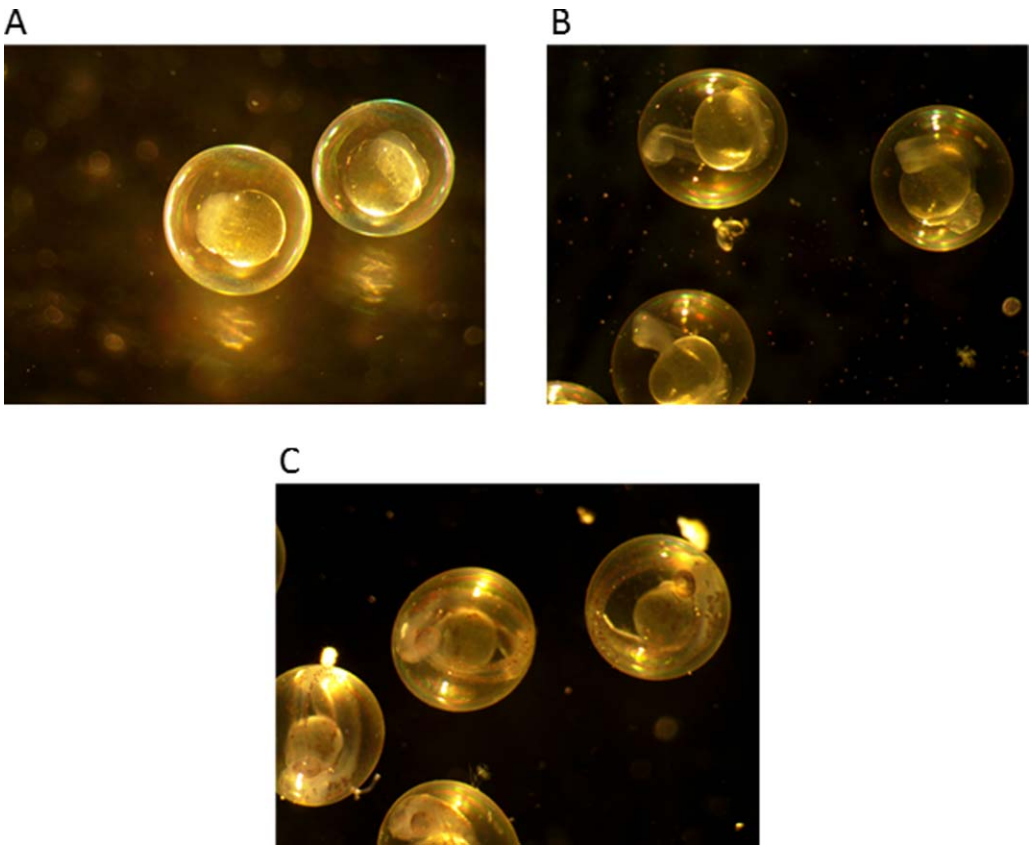

FIG 1 *Student images of zebrafish embryos at 1, 24, and 48 hpf. Stereomicroscopy and digital imaging were used to create images of embryos at 1 (a), 24 (b), and 48 (c) hpf. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

TABLE III *Example of student data collected on embryo staging and RNA purification*

Hours post-fertilization (hpf)	*Embryo stages, numbers, and % of total culture*	*Final RNA concentration ($\mu g\ \mu L^{-1}$)*	*260/280*	*Total RNA (μg)*
2	Cleavage (64 cell stage): 11 embryos (42%) Blastula (256 cell stage): 10 embryos (38%) 20% dead, removed	0.6	1.21	14.9
24	Segmentation (25 somite stage): 22 embryos (100%)	0.31	1.61	7.8
48	Pharyngula: 25 embryos (100%)	0.63	1.69	15.8
72	Hatching: 22 embryos (100%)	0.46	1.5	11.5

Pitfalls in RT-PCR included students failing to set up a successful experiment due to pipetting errors or leaving out a necessary component for the reactions. The latter issue was partially overcome by having students create a master mix for their PCR reactions. Pipetting errors were difficult to overcome, but Rockhurst's biology department is attempting to build stronger pipetting skills in lower level classes by using practical exams to test for skill and accuracy. Another issue was the lack of time to repeat the PCR experiment with modifications, such as different RNA samples, different annealing temperatures, and different primers. To overcome this issue, recent approval to increase the weekly contact time for the class from 2 h and 50 min to 5 h has been obtained. Additional class time will also allow more support for students as they choose their gene and perform BLAST searches, areas of the project with which students feel frustration.

Assessment of Student Learning

Possible gains in student learning were examined by a set of nine multiple choice questions related to experimental and conceptual aspects of the project and administered as a pretest on the first day of class. The same questions were used as a post-test on the final exam, and the average was calculated and compared from pre to post-test. Table IV shows the topics covered by each question, the Blooms Taxonomy level, the post-test to pre-test ratio, and the delivery method (lecture or steps of the research project) for material relating to each question. Figure 3 demonstrates the averages for each question on the pre-test as compared to the post-test. Substantial increases were seen for most questions, with the greatest increase observed in the area of analyzing the hypothesis on gene expression (question 9), an idea that was also supported in opinion surveys in which students were asked to identify the most important aspect of the project that contributed to their learning. The areas showing the least improvement were comprehension level questions over material presented through lecture (questions 2 and 4). This finding supports the idea that active learning improves student outcomes.

An area of concern was the post-test performance levels on questions 4 and 7, with averages of <60 and 50%, respectively. Both of these topics were related to the process of RT-PCR and were covered in a lecture prior to the completion of the RT-PCR step of the project. This finding has led to a modification of the laboratory guide to improve learning in these areas by including an assignment on the role of polymerases in RT-PCR and a section containing student examples of RT-PCR results, such as Fig. 2a above, that address DNA contamination in RT-PCR.

Follow-up Research Projects

Two of the overall goals of the research project (Table I) were related to student work beyond the course. These goals were: (1) for students to gain an appreciation for molecular biology research and working with biological models and (2) for students to gain the experience needed to engage in independent research projects. The first goal was developed to elicit interest in follow-up independent research projects. The second goal was intended to make independent projects more feasible, requiring less student training by the faculty mentor. While students were poised to continue their research, continuation of course projects was somewhat limited due to the fact that most students took the class as seniors and had little time left to do research. A new curricular approach in the Biology department will likely make it possible for more students to take the course earlier, thus overcoming this issue.

When students who took the course as juniors continued their projects, the results were outstanding. With preliminary data generated through the course experience, students were able to engage in projects in which they examined coexpression of related genes or treated zebrafish embryos with compounds to test the effects on the

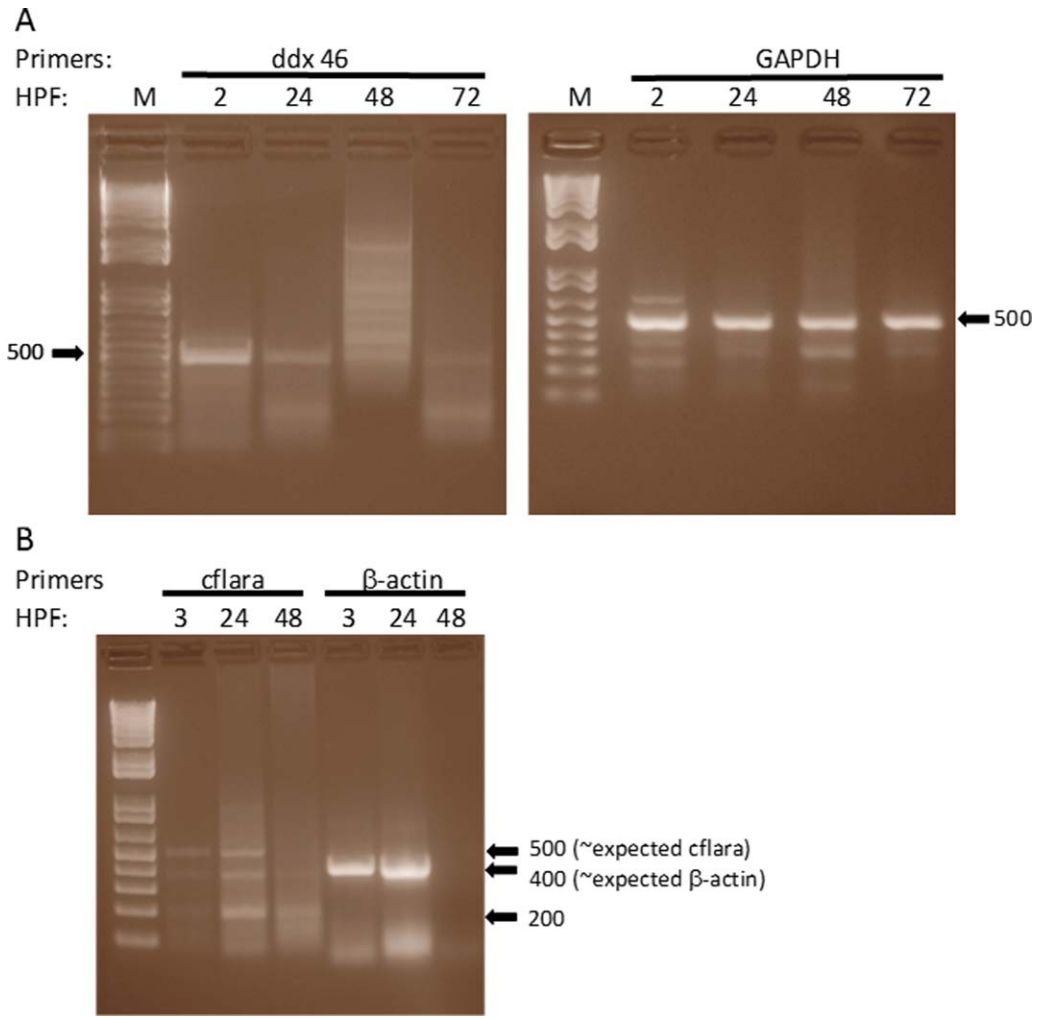

FIG 2

*Sample RT-PCR results generated by students. RT-PCR results from RNA samples isolated at 2, 24, 48, and 72 hpf for ddx46 and GAPDH from one student group were analyzed by electrophoresis on two separate gels (*a*). The expected size of the ddx46 product is 490 bp, and the size of the GAPDH product is 499 bp. RT-PCR results from RNA samples isolated at 3, 24, and 48 hpf for cflara and β-actin from a second student group were analyzed on a single gel (*b*). The expected size of the cflara product is 517, and the β-actin product is 400 bp. The 1 Kb plus marker (M) (Invitrogen) was used in all gels to estimate PCR product size. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

expression of their gene. Some students accumulated enough data to present their work at local and national conferences. In one case, a longer-term project that several additional students continue to pursue was generated. The progress in this area will hopefully result in a publication. In addition, a group of students is currently examining genes that have shown promising results in the course in recent years to see which ones have not been characterized by other researchers in the intervening time and by repeating RT-PCR experiments to see whether results are reproducible. This project may also lead to a publication.

Discussion

The main goals of the integrated research project were to provide a real research experience and to make connections between molecular biology concepts and the research project. Support of these goals was demonstrated through the experimental results obtained and analysis accomplished by students. Pre and post testing also showed several gains in critical thinking related to data analysis (application and synthesis questions) and knowledge about experiments and concepts related to the project.

The most important benefits of the research project were seen when students developed their own hypotheses and analyzed unexpected or unclear data. These two steps were crucial for eliciting critical thinking. The extensive student involvement in the planning and set-up for experiments was also valuable and was possible, in part, because each group worked on a different gene. This involvement increased student ownership. In addition, having students purify their own material for analysis, select their own

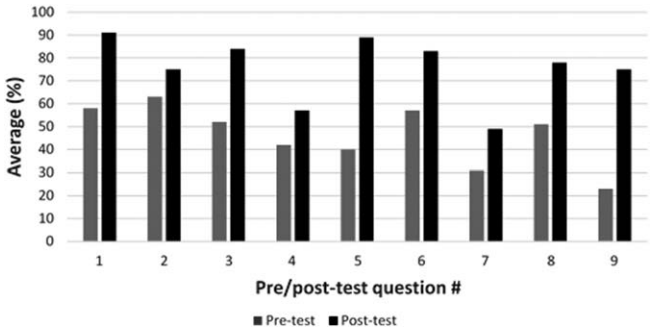

FIG 3

Comparison of student performance on pre-test and post-test questions. Average performance by 66 students over three semesters was calculated for pre-tests taken at the beginning of the class and post-tests, taken with the final exam. Pre and post-test scores were compared for each question, with the content of the questions listed in Table IV.

primers, and quantify and dilute items that were different for each group allowed them to experience critical components involved in research but rarely achieved in a classroom setting. A final benefit was the ability to reach some students who were less strong academically but felt inspired by a research project. These students, who may not normally have stood out in a classroom setting, sometimes became group leaders for the project. Because students had a clear set of instructions for the presentation and paper and because the presentation was public, most students took pride in their work and had a high level of motivation to produce quality products.

Challenges associated with the project included increasing difficulty in finding relevant zebrafish genes not previously studied due to the pace of current research on zebrafish. In most semesters, gene choice was completely open for students. Occasionally, a theme was created for the semester, however, in which students identified genes related to a specific process, such as innate immunity or apoptosis. Apoptosis is an area that has not been well characterized during zebrafish development, so it was a topic that was successful in helping guide students to more easily identify genes not previously characterized.

Difficulties associated with student failures were an additional challenge. Some students experienced frustration with failure and expected to be given a project that would work. Because the typical lab experience is one in which students obtain anticipated results, it was difficult for them to understand that failures are expected as a part of research and that the process is as important as the end result. Because the project was designed to allow the collection of data at each stage, groups that were not successful with RT-PCR (the main area where failure was experienced) were still able to present and write about background and hypothesis development, embryo images, information about the quantity and quality of their purified RNA, BLAST data, and control RT-PCR results. The absence of results in the controls for RT-PCR was rare, but when that occurred, students had to identify their possible errors and share that information in the presentation and paper. Even with some significant challenges, the project was valuable and rewarding in an environment with limited funding, variable student interests and skills, and a great need to expose students to research.

While the project as described was accomplished in a single, small, integrated class, it could be successfully

TABLE IV

Pre and post-test question performance, Blooms taxonomy level, and teaching method

Question #	*Content covered*	*Post:pre-test ratio*	*Blooms taxonomy level*	*Primary teaching method*
1	Major events in embryonic development	1.57	Knowledge	Lecture
2	Common features of development in varied organisms	1.19	Comprehension	Lecture
3	RNA preparation	1.62	Application	Experiment
4	Directionality/function of reverse transcriptase	1.36	Comprehension	Lecture
5	Definition of PCR	2.23	Knowledge	Lecture
6	Identification of relative band size on agarose gel	1.46	Application	Experiment
7	RT-PCR control for presence of DNA	1.58	Application	Lecture
8	Hypothesis on gene function and expression	1.53	Synthesis	Literature searches
9	Hypothesis testing for gene expression	3.26	Application	Experimental analysis

adapted to a variety of settings. First, it could be completed in a separate 3-h laboratory course, with introductions to the experiments and connections to lecture material addressed at the beginning of the laboratory. This model would allow more time for greater student guidance and repetition of the experiments. Second, the project could be expanded in a setting where multiple laboratory sections are offered, as only approximately six female and three male fish must be maintained per laboratory section for sufficient embryo production. Finally, the scope of the project could be modified, including the use of real time PCR to determine levels of gene expression, the use of mutant strains of zebrafish to look for the effects of a mutation on the expression of related genes, and the treatment of embryos with compounds expected or known to affect a process of interest. These additions would help sustain the project over time as research in this area continues to evolve.

Acknowledgements

The author thanks Dr. Elizabeth Evans for support with zebrafish husbandry and IACUC approval, Mr. Aaron Bossert for assistance with zebrafish maintenance, and Dr. Richard Thompson for his thorough review of the manuscript. The author acknowledges the following students: Melanie Sowadski and Chelsey Garbs for the use of their RNA and RT-PCR data for the ddx46 project, and Leah Grant, Jackie Piszsczek, and Linda Dang for the use of their RT-PCR data for the cflara project.

References

[1] National Research Council (2003) BIO 2010: Transforming Undergraduate Education for Future Research Biologists, The National Academies Press, Washington, DC. Available at: http://www.nap.edu/openbook.php?record_id=10497

[2] Project Kaleidoscope (2002) Recommendations for Action in Support of Undergraduate Science, Technology, Engineering and Mathematics: Report on Reports. Available at: www.pkal.org/documents/REportOnReports.cfm

[3] Lopatto, D. (2004) Survey of undergraduate research experiences (SURE): [Database]. First Findings. Cell Biol. Educ. 3, 270–277.

[4] Brownell, J. E. and Swaner, L. E. (2010) Five High-Impact Practices: Research on Learning Outcomes, Completion, and Quality, Association of American Colleges and Universities Press, Washington, DC.

[5] Windschitl, M. and Buttemer, H. (2000) What should the inquiry experience be for the learner? Am. Biol. Teach 62, 346–350.

[6] Eberhardt, E. S., Hansen, J., Riservato, L., Cole, M., Smaglo, B., and Szaniawski, P. (2003) Preparing undergraduates to participate in the post-genome era. Biochem. Mol. Biol. Educ. 31, 402–409.

[7] Billingsley, J. and Carlson, K. A. (2010) Epigenetic effects of diet on fruit fly lifespan: An investigation to teach epigenetics to biology students. Am. Biol. Teach 72, 231–234.

[8] Carson, S. (2007) A new paradigm for mentored undergraduate research in molecular microbiology. CBE Life Sci. Educ. 6, 343–349.

[9] Brame, C. J. Pruitt, W. M., and Robinson, L. C. (2008) A molecular genetics laboratory course applying bioinformatics and cell biology in the context of original research. CBE Life Sci. Educ. 7, 410–421.

[10] The Coalition for Reform of Undergraduate STEM Education, C.L. Fry, Ed. (2014) Achieving Systemic Change: A Sourcebook for Advancing and Funding Undergraduate STEM Education, Association of American Colleges and Universities Press, Washington, DC.

[11] Santoriello, C. and Zon, L. I. (2012) Hooked! Modeling human disease in zebrafish. J. Clin. Invest. 122, 2337–2343.

[12] Kikuchi, A. (2003) Tumor formation by genetic mutations in the components of the Wnt signaling pathway. Cancer Sci. 94, 225–229.

[13] Montell, D. J. (2003) Border-cell migration: The race is on. Nat. Rev. 4, 13–24.

[14] Bradford, Y., Conlin, T., Dunn, N., Fashena, D., Frazer, K., Howe, D. G., Knight, J., Mani, P., Martin, R., Moxon, S. A., Paddock, H., Pich, C., Ramachandran, S., Ruef, B. J., Ruzicka, L., Bauer Schaper, H., Schaper, K., Shao, X., Singer, A., Sprague, J., Sprunger, B., Van Slyke, C., and Westerfield, M. (2011) ZFIN: Enhancements and updates to the zebrafish model organism database. Nucleic Acids Res. 39, D822–D829.

[15] Chu, J. and Sadler, K. C. (2009) A new school in liver development: Lessons from zebrafish. Hepatology 50, 1656–1663.

[16] Westerfield, M. (2000) The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio), 4th ed., Univ. of Oregon Press, Eugene, OR.

[17] Life Technologies protocols: PCR and RT-PCR overview. Available at: https://www.lifetechnologies.com/us/en/home/references/protocols/nucleic-acid-amplification-and-expression-profiling/pcr-protocol/pcr-and-rt-pcr.html

[18] Promega corporation PCR amplification. Available at: https://www.promega.com/resources/product-guides-and-selectors/protocols-and-applications-guide/ pcr-amplification/