Laboratory Exercise

An Investigative Graduate Laboratory Course for Teaching Modern DNA Techniques[S]

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Abstract

This graduate-level DNA methods laboratory course is designed to model a discovery-based research project and engages students in both traditional DNA analysis methods and modern recombinant DNA cloning techniques. In the first part of the course, students clone the *Drosophila* ortholog of a human disease gene of their choosing using Gateway® cloning. In the second part of the course, students examine the expression of their gene of interest in human cell lines by reverse transcription PCR and learn how to analyze data from quantitative reverse transcription PCR (qRT-PCR) experiments. The adaptability of the Gateway® cloning system is ideally suited for students to design and create different types of expression constructs to achieve a particular experimental goal (e.g., protein purification, expression in cell culture, and/or subcellular localization), and the genes chosen can be aligned to the research interests of the instructor and/or ongoing research in a department. Student evaluations indicate that the course fostered a genuine excitement for research and in depth knowledge of both the techniques performed and the theory behind them. Our long-term goal is to incorporate this DNA methods laboratory as the foundation for an integrated laboratory sequence for the Master of Science degree program in Molecular and Cellular Biology at Quinnipiac University, where students use the reagents and concepts they developed in this course in subsequent laboratory courses, including a protein methods and cell culture laboratory.

Keywords: *Integration of research into undergraduate teaching; laboratory exercises; new course development; molecular biology; Gateway® system; DNA techniques; cloning*

Introduction

The benefits of replacing standard "cookbook" laboratories with project-based laboratory courses that reflect the nature and excitement of the scientific process are well established [1, 2]. Ideally, all undergraduate and graduate students would have the opportunity to contribute to an ongoing research project with a faculty mentor. However, practical considerations, including funding and time constraints often make it difficult to achieve that goal. Accordingly, many examples of laboratory courses that engage groups of students in discovery-based research related to ongoing faculty research have been reported [3–9].

At Quinnipiac University, one of the goals of the Master of Science in Molecular and Cellular Biology degree program is to ensure that all students acquire the scientific habits of mind and practical skills developed from participating in research, even if they do not engage in an independent thesis project with a faculty member. Accordingly, we developed a one semester DNA methods laboratory course in which students clone an ortholog of a human disease gene from either *Drosophila melanogaster* or *Caenorhabditis elegans* using the Gateway® cloning system (Thermo Fisher Scientific) and characterize the expression of that gene in human cell lines using reverse transcription PCR.

We chose to use the Gateway® cloning system over traditional, ligase-mediated molecular cloning for several reasons: it is ideally suited for students to create different

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types of expression constructs depending on their experimental objectives (e.g., for protein purification or subcellular localization), it is based on site-specific recombination, which enables us to explore that topic in greater depth with students, and the Gateway® Open Architecture Policy has led to it being widely used in both industry and academic labs (Thermo Fisher Scientific).

The Gateway® cloning system enables researchers to easily transfer DNA fragments between cloning vectors using the site-specific recombination system from phage lambda. Very briefly, gene specific PCR primers containing *att*B sites are used to generate *att*B-flanked PCR products from cDNA clones. The BP clonase enzyme mix is then used to catalyze the recombination of the *att*B-flanked PCR product into a donor vector (which contains *att*P sites) to create an entry vector. Once the PCR product is cloned into an entry vector, it can be transferred into any Gateway® destination vector to create an expression vector. Different destination vectors containing different promoters, fluorescent protein genes, and purification tags are available, and the power of the Gateway® cloning stems from its adaptability: once the desired DNA fragment is cloned into an entry vector, it can be transferred into any of several destination vectors to create an expression vector suitable for the experimental purpose. This system is ideally suited for teaching laboratories because it enables students to design and create expression vectors that are suited to their experimental goals. Furthermore, it is very efficient: the manufacturer reports 95% cloning efficiency, and over three years of using this approach in our course, students obtained their clone of interest 100% of the time.

Importantly, the model organisms and genes chosen are closely related to ongoing faculty research in the department, and the adaptability of the Gateway® cloning system enables students to design and create different types of expression constructs to achieve their experimental goals (e.g., protein purification, expression in cell culture, and subcellular localization). As an example of how the course described here can be modified based on the research interests of the instructor, for the first two years the course was run, students cloned human disease gene orthologs from *Drosophila* because the instructor (L. Keller) works on neurodegeneration in this system. When a different instructor (A. de Lencastre, who studies microRNAs in *C. elegans)* taught the course, he added the option for students to clone either genes or untranslated regions from *C. elegans* to the course using the *C. elegans* MultiSite Gateway Cloning vectors [10].

Our long-term goal is to incorporate the DNA methods laboratory course reported here as the foundation for an integrated laboratory sequence for the Master of Science degree program in Molecular and Cellular Biology, where the reagents and concepts developed in the DNA methods course are used in subsequent courses, including a protein methods course [8] and a cell culture laboratory course.

Course Synopsis

The course described here has been offered since 2013 and exists as an updated version of a long-running DNA laboratory techniques course, which is one of five core courses required for all students in the Master of Science degree program in Molecular and Cellular Biology at Quinnipiac University. Enrollment is typically between 30 and 45 students each spring, which are split into two or three sections of 12-15 students per section. A prerequisite is graduate level molecular genetics. This course is often the student's first exposure to a graduate laboratory course, and is usually followed by a second required laboratory course in protein techniques (previously described in [8]). This laboratory course is taught in an interactive, hands-on learning format as a semester-long, 3-h/week introductory course. It could easily be adapted, however, to be delivered as an accompanying laboratory course within upper-level undergraduate courses in biochemistry, molecular biology, or cell biology. Teams of two to four students are created on the first day of the course and work as collaborative student research teams throughout the semester. Each research team works in a pod-style lab station with a computer providing continual access to the internet, on-line lecture material, laboratory protocols, and all necessary programs and databases such as Excel, FlyBase (http://flybase.org/), wormbase (http://wormbase.org) the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), and the plasmid editing freeware, Ape: A plasmid Editor (http://biologylabs.utah.edu/jorgensen/wayned/ape/). The hands-on laboratory exercises (Table I) encourage collaborative problem solving and sharing of knowledge. Lecture material is provided primarily through on-line lectures, and students are expected to complete assigned reading from the required text and watch the on-line course material before class to facilitate full participation in problem-solving and hands-on experiments. The required text, "From Genes to Genomes: Concepts and Applications of DNA Technology" [11] provides a comprehensive introduction to the concepts and applications of DNA technology. This reference text is supplemented with current materials such as primary literature, manufacturer-supplied protocols, and in-depth explanations of contemporary topics such as the Gateway® system. All prelaboratory preparation is done by the instructor, with the help of a graduate assistant who previously took the course.

Each student is assessed on their participation, laboratory notebook, weekly in-class learning assessments, and two laboratory exams. The in-class learning assessments and laboratory exams are based solely on the theory and practical applications of the material. The course culminates in a class poster presentation during which each student group presents a poster based on their semester-long research project.

TABLE I *Week-by-week summary of laboratory experiments*

1	Bacterial transformation
2	Calculation of transformation efficiency and PCR
3	DNA gel electrophoresis and extraction
4	Introduction to the Gateway® system and construction of entry clones
5	Miniprep of plasmid DNA and diagnostic restriction digests; sequencing reaction setup
6	Analysis of DNA sequencing results and *in silico* creation of expression clones
7	BLAST analysis and RT PCR primer design
8	Human RNA extraction and quantification
9	Human cDNA synthesis
10	PCR from cDNA, gel electrophoresis and DNA analysis
11	Data analysis of quantitative real-time PCR results
12	Student research team poster presentations

Expenses

All reagents were purchased using funds specifically set aside for the Master of Science degree program in Molecular and Cellular Biology in the Department of Biological Sciences at Quinnipiac University. All standard laboratory equipment including power supplies, gel boxes, incubators, shakers, vortexes, centrifuges, pipetmen, and a thermocycler were already in place within the functional teaching laboratory. Initial costs associated with this course could run anywhere between $500 and 3000 depending on institutional equipment and available reagents. Subsequent offerings of this course are substantially less expensive because most reagents can be used for several semesters. The main costs associated with this course include the Gateway® enzymes ($207-231), the Reverse Transcriptase enzyme ($324), Qiagen kits ($112-332), and *Drosophila* cDNA clones ($25 each).

Course Essential Questions

The main goal of this course is to introduce students to modern techniques in DNA manipulation. Course essential questions have been defined previously [12] and are intended to set the stage for further questioning. The course syllabus outlines several essential questions that provide a sense of purpose and relevance as they delve into the field of recombinant DNA technology: 1) How is genetic material extracted from a cell? 2) What is the anatomy of a gene? 3) How can a genetic test for a mutation in a disease gene be developed? 4) What are the steps necessary to determine the *in vitro* localization of a protein involved in a disease? 5) How have advances in recombinant DNA technology impacted individuals and society?

Laboratory Experiments

Laboratory Experiment 1: Bacterial Transformation

The overall goal of this initial laboratory experiment is to get students comfortable pipetting and to introduce the concepts and experimental methods of bacterial transformation by transforming chemically competent cells with a test plasmid. Any test plasmid with an antibiotic resistance gene can be used to transform either commercially purchased or in-house prepared chemically competent bacterial cells. We have successfully used test plasmids containing either the kanamycin resistance gene (*KanR*) or the ampicillin resistance gene (*AmpR*). Care should be taken to ensure that proper institutional recombinant DNA protocols are followed and that bacterial cultures are disposed of properly.

After completing this laboratory, students should be able to describe bacterial competence and transformation, explain the role of calcium ions in making cells chemically competent and what happens to the cell wall during electroporation, explain why we incubate cells in non-selective medium prior to plating, and propose how to select for transformants given the selectable marker present in plasmid being used. Students should also be able to describe the role of each of the common features of a plasmid cloning vector, including the origin of replication, multiple cloning site (polylinker), and selectable marker.

In this first lab, students perform a bacterial transformation and plate their transformants. In the subsequent laboratory, students will record the experimental results from the first laboratory and calculate transformation efficiencies. Practical and instructional details for this and all laboratory experiments are included within Supporting Information.

Laboratory Experiment 2: Calculation of Transformation Efficiency and Polymerase Chain Reaction (PCR)

In this laboratory, students will 1) calculate the bacterial transformation efficiency from the plates they prepared in the previous laboratory and 2) use PCR to amplify a gene of interest using oligonucleotides containing appropriate flaking Gateway® cloning sequences (*attB* sites). For the PCR experiment, each group chooses a gene from a list of *Drosophila* orthologs of human disease genes compiled by the instructor. That gene becomes the student group's focus of study for the remainder of the semester. Figure 1 illustrates student-generated PCR amplification for a gene of

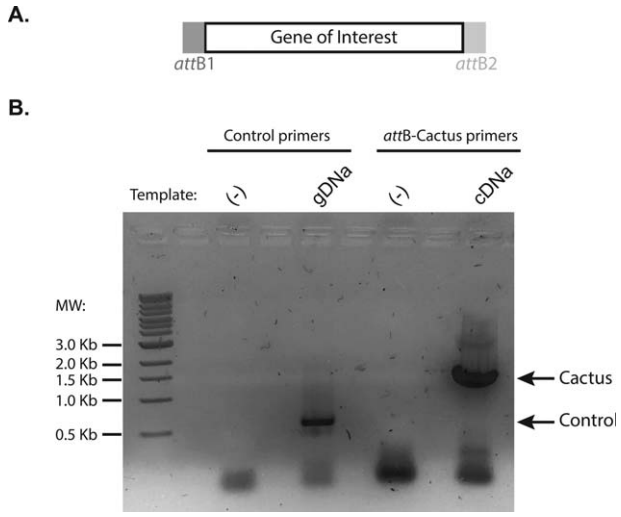

FIG 1

Student PCR amplification of* attB*-flanked Cactus ORF. *A: Schematic diagram of* att*B-flanked PCR product. B: Student PCR amplification of a control amplicon (lanes 3 and 5) and an* att*B-flanked Cactus ORF amplification (lanes 7 and 9). The control primer pair amplifies a 610 bp fragment from* C. elegans *genomic DNA (lane 5), and the* att*B*-Cactus *primer pair amplifies a 1,566 bp fragment from the* Drosophila Cactus *cDNA clone (*Drosophila *Genome Resource Center clone ID # LD10168, Lane 9). PCR products were separated by 1.0% agarose gel electrophoresis and visualized with ethidium bromide. Every other lane was loaded to facilitate gel extraction of* att*B*-Cactus *amplicon.*

interest (the *Drosophila* Cactus gene). In practice, we have students set up their PCR reactions and then calculate their transformation efficiency during PCR thermocycling. After completing these experiments, students should be able to explain why PCR is a useful laboratory technique, describe the function of each key component of a PCR, and illustrate what happens during each of the thermocycling steps. In addition, students will learn how to calculate the volume of reagents necessary to set up a typical PCR reaction and understand how to set up master mixes for any PCR reaction. Finally, since this is the first step of the Gateway® cloning process, students should be able to summarize the overall approach of the Gateway® cloning system, explain what advantages it has over "traditional" ligase-mediated cloning, and why the PCR primers include Gateway® adapter sequences.

Laboratory Experiment 3: DNA Gel Electrophoresis and Extraction

In this laboratory, students conduct agarose gel electrophoresis on their Gateway® PCR reactions from the previous lab and use a gel extraction kit to purify the DNA for subsequent cloning. It is important that students learn how to appropriately and safely handle agarose gels, in particular if they contain the nucleic acid intercalating stain ethidium bromide. Several alternatives to ethidium bromide, such as SYBR® safe DNA gel stain (Invitrogen #S33102) are also commercially available.

Upon completing this laboratory exercise, students should be able to describe the basic principles underlying gel electrophoresis, illustrate the relationship between DNA charge and size/shape on electrophoretic mobility, and explain how nucleic acids are visualized after electrophoresis. Additionally, students should be able to diagram the steps involved in gel extraction of nucleic acids, describe the role that the active ingredients in each of the buffers plays in the purification procedure, track the location of the DNA (e.g., in the supernatant versus column) throughout the purification process, and explain the effect of salt and pH on the DNA binding characteristics of the membrane in the column.

Laboratory Experiment 4: Introduction to the Gateway® System and Construction of Entry Clones

In this laboratory exercise, students use Gateway® cloning technology to clone their PCR product (generated in Laboratory Exercise 2 and purified in Laboratory Exercise 3) into a pDONR vector, generating the entry vector that is the "doorway" to the Gateway® system. Upon completing this laboratory, students should be able to outline the basic experimental steps involved in cloning with either restriction enzymes or the Gateway® cloning system and describe the advantages and disadvantages of each method. Of particular importance, students should be able to describe the key features of donor, entry, and destination vectors and diagram the reactants and products of Gateway® cloning reactions.

We use this laboratory exercise as an opportunity to compare and contrast the history, theory, and practical considerations of ligase-mediated cloning (which many students have previous experience with) and modern recombination based cloning approaches, which students are unlikely to have used before. Topics we emphasize include the biological basis of the Gateway® system, the similarities, and differences between ligase-mediated cloning and cloning by site-specific recombination, and how to design a Gateway® cloning experimental strategy. We suggest that instructors familiarize themselves with the Gateway® Recombination Cloning Technology, which is explained in detail on http://www.lifetechnologies.com/us/en/home/life-science/cloning/gateway-cloning.html.

At the end of this laboratory exercise, students will have performed a Gateway® BP reaction to generate an entry clone containing the PCR product of interest in a vector that is suitable for a variety of downstream applications. Once the entry clone is generated, the DNA fragment of interest can be moved into any Gateway® expression construct with a one-step, 1 h recombination reaction (LR reaction).

Briefly, our overall Gateway® cloning strategy is as follows. The PCR products from Laboratory Exercise 2 contain flanking Gateway® cloning sequences termed *att*B sites that recombine, in a directional manner, with *att*P sites in a donor vector (Fig. 1*A* and Supporting Information Table S1). The donor vector also contains a positive selection cassette (*KanR*, which confers resistance to Kanamycin) and a negative selection cassette (the *ccdB* gene, which prevents growth of bacteria containing donor vector that did not undergo site-specific recombination). The BP Clonase II enzyme mix (Invitrogen #11789-020) catalyzes recombination between the *att*B sites on the PCR fragment and the *att*P sites on the donor vector to form an entry vector containing the gene of interest inserted with the recombined sites (now referred to as *att*L1 and *att*L2), as well as the positive selection cassette (*KanR*). The products of the BP reaction are then transformed into bacteria and plasmid DNA is isolated from independent colonies before being mini-prepped and confirmed by restriction analysis and sequencing in subsequent laboratory exercises.

Laboratory Experiment 5: Miniprep and Diagnostic Restriction Digest of Plasmid DNA; Sequencing Reaction Setup

This laboratory introduces several fundamental aspects of molecular biology (plasmid DNA isolation and restriction enzyme digestion) while reinforcing skills such as agarose gel electrophoresis and DNA gel analysis. More specifically, students conduct a miniprep to isolate entry clone plasmid DNA, perform one or more diagnostic restriction enzyme digestions to confirm the identity of their plasmid (Fig. 2), and set up a DNA sequencing reaction. (Note: although students set up sequencing reactions during this laboratory, we do not discuss the details of DNA sequencing until the next week.) After completing these laboratory activities, students should be able to describe the role that the active ingredients in the lysis, neutralization, wash, and elution buffers plays in the purification procedure, track the location of the plasmid DNA (e.g., in supernatant versus column) throughout the purification process, explain the effect of salt and pH on the DNA binding characteristics of the membrane in the column, and design a strategy that uses diagnostic restriction digests to confirm the identity of a plasmid.

Procedurally, overnight cultures of single bacterial colonies from the BP transformation plates are grown, plasmid DNA is isolated from those cultures using a commercially available mini-prep kit, and the plasmid DNA is verified by diagnostic restriction enzyme digestion and gel electrophoresis. We used the Qiagen mini-prep kit and the New England Biolabs Educational Course Support Program to obtain a panel of commonly used restriction enzymes. After restriction digest verification of plasmid DNA (Fig. 2), students prepare forward and reverse sequencing reactions

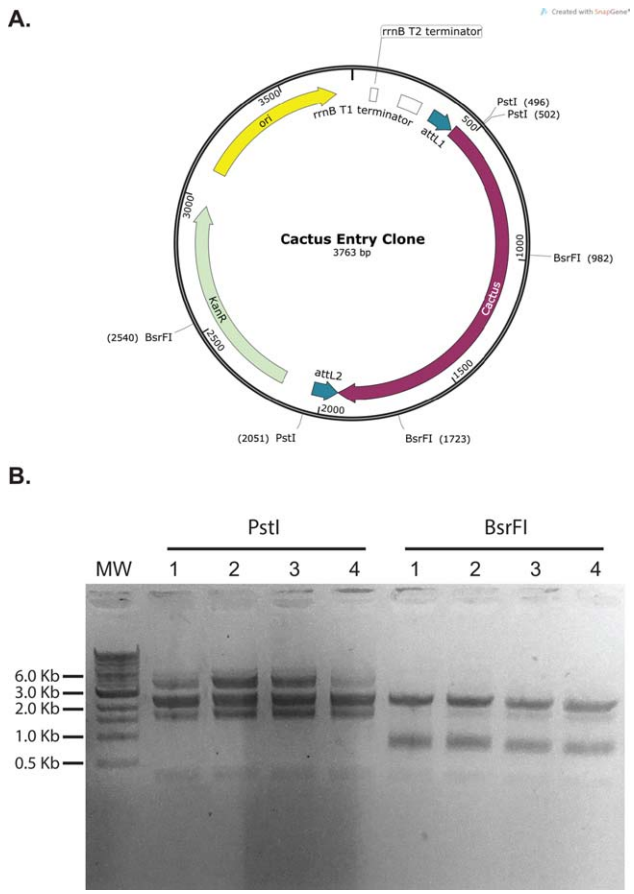

FIG 2

Restriction Map and Student Restriction Digest of Cactus Gateway Clone. *A: Plasmid map of Cactus Entry Clone. B: Plasmid DNA was isolated from liquid cultures grown from four independent kanamycin resistant clones, digested with either PstI or BsrFI, separated by 1.0% agarose gel electrophoresis, and visualized with ethidium bromide. Expected fragment sizes: PstI: 2208, 1549, and 6 bp; BsrFI: 2205, 817, and 741 bp. The higher molecular weight bands in the PstI lanes likely result from incomplete digestion of the 3763 bp plasmid. [Color figure can be viewed at wileyonlinelibrary.com]*

by combining plasmid DNA and primer into tubes, which are then submitted to a commercial sequencing facility.

Laboratory Experiment 6: Analysis of DNA Sequencing Results and In Silico Creation of Expression Clones

In this laboratory, students analyze the results of their DNA sequencing reactions from the previous laboratory. After completing this laboratory, students should be able to list the components of a dideoxy sequencing reaction, illustrate the chemical difference between deoxynucleotides (dNTPs) and dideoxynucleotides (ddNTPs), explain how ddNTPs terminate chain growth, and predict the effect that changing the ratio of dNTPs to ddNTPs would have on a sequencing

reaction. Furthermore, students should be able to describe how polyacrylamide gel electrophoresis and capillary electrophoresis can be used to separate sequencing products, and how the results of a sequencing experiment are "read" and interpreted.

Instructors should familiarize students with Sanger Sequencing. In our experience, the following topics are essential to emphasize the role of dideoxy terminators, how the location of primer binding sites influences the orientation of the resulting sequence, and that the sequences at the very beginning and end of a read are often inaccurate. For a good overview on the topic, instructors can refer to Jonathan Weissman's iBioSeminar on DNA sequencing (http://www.ibiology.org/ibioseminars/techniques/jonathan-weissman-part-1.html).

We encourage instructors to guide students in proposing an experiment to explore the function of their gene of interest using the expression clone they generated. For example, one student created an expression clone to GFP-tag the *Drosophila* homolog of superoxide dismutase (SOD), which when mutated can cause familial amyotrophic lateral sclerosis in humans. The student then proposed an experiment to visualize the movement of this GFP-tagged protein in motor neurons of living *Drosophila* to determine if SOD resides within axons or is primarily at neuromuscular junctions where degeneration initiates.

Laboratory Experiment 7: BLAST Analysis and Reverse Transcription PCR Primer Design

This laboratory experiment marks the beginning of the second half of the course, during which students will characterize the human homologue of their gene of interest. By the end of this laboratory, students should be able to describe why sequence alignment is a powerful tool for biologists, explain how a BLAST search "works," execute nucleotide and protein BLAST searches, and be able to interpret the results of a BLAST search, including what information is given by the BLAST score (*S*) and Expect value (*E*). Additionally, students should be able to obtain information about a gene of interest using MedGen/OMIM and species-specific databases and use online PCR primer design tools to design PCR primers, which can be used to determine whether their gene of interest is present in cDNA from human cell lines.

Students first perform a BLAST search to identify the human homolog of their genes of interest. In doing so, students learn common uses of BLAST, including how to identify the species of origin for an unknown DNA sequence, how to examine the genomic context of a DNA sequence, and how to determine the degree of sequence similarity between various species.

After the human homolog of their gene is identified, students design PCR primers to amplify their gene from human cDNA using an online primer design tool such as Primer3 (http://bioinfo.ut.ee/primer3/). To confirm that their primers only amplify cDNA and not genomic DNA, students perform *in silico* PCR (https://genome.ucsc.edu/cgi-bin/hgPcr) using their primers against either a genomic database or a cDNA database. Since these activities only require access to a computer and the internet, instructors can choose to make this exercise an off-site take-home exercise.

Laboratory Experiment 8: Human RNA Extraction and Quantification

In this experiment, students extract RNA from human cell lines to test the expression of their human gene of interest by reverse transcription PCR. By the end of this lab, students should be able to compare and contrast the common methods of RNA isolation (e.g., Trizol vs. column-based RNA extraction methods), describe the advantages and disadvantages of each, and explain the importance of using RNAse free reagents in maintaining the integrity of the RNA throughout the experiment. Additionally, students will be able to describe how the concentration and purity of an RNA sample can be assessed by UV spectroscopy and compare and contrast various methods for gene expression analysis (e.g., northern blot, microarray, quantitative Real-Time PCR, and *in-situ* RNA hybridization).

In practice, students use a column-based purification procedure to extract RNA from various cell lines. We provide cell pellets from several human cell lines, and students choose, which they would like to work with. Using multiple cell lines encourages students to generate hypotheses about the expression levels of their genes of interest in different medically relevant biological settings, and provides an opportunity to engage students in a discussion about factors to consider when using cell lines (transformed vs. non-transformed, immortal vs. primary, cancerous vs. noncancerous, etc.) The cell lines we use include HeLa, RPE-1, and 501-mel, MCF-7, HEK, and HEK-T, which can be obtained from commercial sources (such as the American Type Culture Collection, ATCC®: https://www.atcc.org) or from in-house resources, such as another laboratory at the university.

Laboratory Experiment 9: Human cDNA Synthesis

In this laboratory, students reverse transcribe the previously extracted total RNA into cDNA, which is the first step in the reverse transcription PCR method of determining gene expression. After completing this laboratory, students will be able to list the key components of a reverse transcription (RT) reaction and illustrate what is happening during each of the RT incubation steps. We used oligo dT as our primer for the reverse transcriptase reactions, which enriches for mRNA transcripts. However, instructors may choose to discuss the pros and cons of using alternative methods such as random hexamers and gene specific primers and discuss downstream applications of gene expression analysis using cDNA. By the end of the exercise, the students will also be familiar with possible downstream applications of cDNAs generated by this type of experiment,

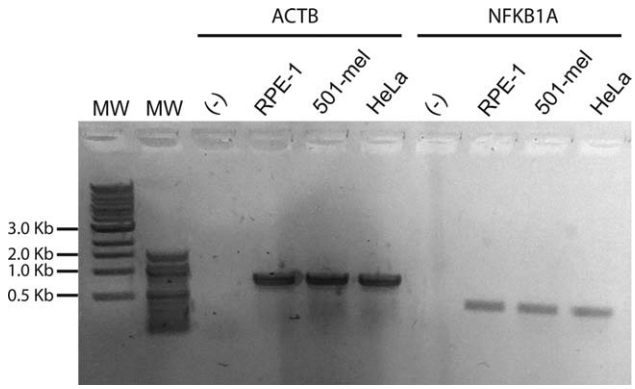

FIG 3

Student PCR of human cDNA. *cDNAs were prepared from three human cell lines (RPE-1, 501-mel, and HeLa) and PCR amplified using primers specific to either human* ACTB *(beta-actin) or* NFK1A *(the human homologue of the* Drosophila cactus *gene). Expected product sizes: ACTB = 733 bp, NFK1A = 340 bp.*

including methods of measuring gene expression by quantitative PCR, microarray analysis or modern next-generation sequencing technologies.

Laboratory Experiment 10: PCR from cDNA, Gel Electrophoresis, and DNA Analysis

This laboratory experiment provides the opportunity for students to hone their PCR and gel electrophoresis skills to determine if their gene of interest is expressed in any of the human cDNAs prepared in the previous laboratory. Each student research group sets up multiple PCR reactions containing either control (human beta actin) primers or the primers they previously designed to amplify their gene of interest. This is also an excellent opportunity to discuss the importance of including "no-template" negative controls in PCR experiments. See Fig. 3 for an example of a student-generated experimental data illustrating amplification of Actin and the human homologue of the *Drosophila* Cactus gene (*NFK1A)*. By the end of this activity, students will be able to use their experimental results to determine whether their gene of interest was expressed in the human cells; they tested and propose a plausible explanation as to why their gene is or is not expressed those cells their laboratory notebook.

Laboratory Experiment 11: Data Analysis of Quantitative Real-Time PCR Results

In this laboratory experiment, students analyze quantitative Real-Time PCR (qRT-PCR) data to determine the relative expression levels of their genes of interest in different human cell lines. By the end of this laboratory, students should be able to explain why electrophoresis of PCR products followed by visualization using fluorescent stains (such as ethidium bromide) is not quantitative, understand that methods that do not involve amplification (such as northern blotting) are the "gold standards" for measuring changes in gene expression, and describe the conceptual basis for two commonly used quantitative Real-Time PCR approaches (TaqMan and SYBR®). Furthermore, students should be able to explain the importance of using an appropriate reference gene, describe the relationship between the melting curve and PCR specificity, define Ct, illustrate how one can create a standard curve from a series qRT-PCR reactions using 10-fold dilutions of template, and the advantages of using the $\Delta\Delta C_t$ method to measure changes in gene expression.

These qRT-PCR data can be generated in class using the primers that the students designed and tested in Laboratory experiments 7–10. However, setting up a fully controlled qPCR experiment in a group/class setting can be challenging and is unlikely to generate useful results, as precise repetitive pipetting is critical for accurate qPCR results. Therefore, we chose to generate representative qPCR data for the students to interpret (Supporting Information Figure S1) which allows students to focus on the generation of hypotheses about what to expect regarding expression of their genes of interest in different cell lines and the interpretation of data in the context of these hypotheses.

Students are given representative qRT-PCR data and use the $\Delta\Delta C_t$ method [13] to determine the expression changes of their gene of interest between two different cell lines. Students calculate primer efficiency by generating standard curves for different primers from the qRT-PCR data. Students are given Ct values for Real-Time qPCR experiments using primers that amplify their genes of interest and cDNA generated in the previous laboratory by reverse-transcription. They then use the $\Delta\Delta C_t$ method [13] to calculate the relative expression of their gene of interest in two cell lines after normalizing to an endogenous housekeeping gene (human beta actin). Since the $\Delta\Delta C_t$ method requires that both primers have similar efficiencies, the students generate standard curves for Ct values at various dilutions of template cDNA and use the slopes of these lines to calculate primer efficiencies for their genes of interest.

Finally, the students prepare a bar graph in which they plot the relative expression of their gene of interest in a cancer cell versus a noncancerous cell line (Supporting Information Figure S1). These results may then be compared to the hypotheses that students proposed at the beginning of the exercise—namely, if their genes would be expected to be upregulated or downregulated in cancer cell lines such as HeLa or MCF-7 compared with a noncancerous cell line.

Week 12: Student Research Team Poster Presentations

To stress the importance of scientific dissemination and effective communication, students create a scientific research poster based on their results from the entire semester. Student then present their posters to the class on

TABLE II *Course evaluations demonstrate a high degree of student satisfaction with the course*

Course evaluation	*5 (strongly agree)*	*4*	*3*	*2*	*1 (strongly disagree)*	n
Course overall: Overall, I would rate this course as a valuable learning experience.	69.6%	30.4%	0.0%	0.0%	0.0%	69
Knowledge: I understand the central concepts and ideas in this course.	72.9	27.1	0.0	0.0	0.0	70
Commitment: I did my part to learn as much as possible in this course.	74.3	25.7	0.0	0.0	0.0	70
Learning: I can apply information/skills learned in this course.	72.9	27.1	0.0	0.0	0.0	70
Texts: Class resources (i.e., text, other materials effectively contributed to learning.	41.2	50.0	5.9	2.9	0.0	68
Assignments and exams: The assignments and/or examinations were relevant and meaningful for the content of the course.	60.9	33.3	5.8	0.0	0.0	69

*Course evaluations were compiled by Quinnipiac University and presented as average numbers based on a 5-1Likert scale: 5 = strongly agree, 4 = agree, 3 = neutral, 2 = disagree, and 1 = strongly disagree. The data represent the combined results for eight individual sections of Bio605 laboratory between 2013 and 2016 (*N = *70). The total number of students between 2013 and 2016 = 82 and the overall average student response rate was 85.4%.*

the last meeting day of the semester. Each student research group includes only the experiments that they think are most important and are encouraged to include data that was unexpected, which allows for active discussion. To help students learn how to use Powerpoint to create professional posters, we have refer students to various freely available on-line poster guides such as, http://guides.nyu.edu/posters and http://undergraduateresearch.as.ua.edu/presenting-your-work/making-posters/. Additionally, to cut costs, we now use large format posters (48″ × 36″), which can be printed as engineering prints, in color, for ∼$7 at Staples (http://www.staples.com).

Student Evaluation and Feedback

At the conclusion of each semester that this course has been offered (2013–2016) all students were encouraged to complete an anonymous on-line course evaluation. As shown in Table II, the student evaluations demonstrate a high degree of student satisfaction with the course. All students that participated in the survey either "strongly agreed" or "agreed" that the course was a valuable learning experience, that they understood the central concepts and ideas in this course, and that they could apply information/skills learned in this course (Table II). We could improve the response rate of 85.4% by requiring students to complete the evaluation during the last class session.

Conclusions

Described here is an innovative semester-long laboratory course focused on DNA cloning that allows students hands-on experience in a wide-range of techniques. The use of the Gateway® Cloning System allows the instructor maximum flexibility to create plasmids that they can be utilized not only in other advanced molecular biology laboratory courses but also within their research programs [14]. The material presented in these laboratory exercises provides students with the opportunity to practice their critical and quantitative reasoning skills, develop experience with traditional and emerging DNA technologies, and contribute reagents to other courses and faculty research programs. Experience with the techniques in this course, which are widely used in academia and industry, will help prepare students for graduate programs or careers in biotechnology. Importantly, this course can be taught alone or as part of an integrated curriculum in molecular biology, which uses reagents designed and created in this laboratory in multiple other courses such as cell culture, protein purification, and/or cell biology.

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