ARTICLES

Infusing Bioinformatics and Research-Like Experience into a Molecular Biology Laboratory Course

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Abstract: A nine-week laboratory project designed for a sophomore level molecular biology course is described. Small groups of students (3-4 per group) choose a tumor suppressor gene (TSG) or an oncogene for this project. Each group researches the role of their TSG/oncogene from primary literature articles and uses bioinformatics engines to find the gene and promoter sequence of their TSG/oncogene. Based on the promoter sequences, students design appropriate primers for the PCR amplification and cloning of the promoter of the gene of interest and perform a diagnostic digest to confirm the results. Finally, each student writes an individual report about his or her findings and results and each group presents the results to the class. This laboratory sequence teaches students how to read primary literature, use common bioinformatics engines, clone a DNA sequence, and present the results in an oral and written format.

Key words: project-based learning, molecular biology laboratory, bioinformatics, molecular cloning

INTRODUCTION

Traditional molecular biology and biochemistry laboratory courses are composed of individual and very prescriptive exercises. Those exercises teach students techniques but they do not show the scientific process or excite the students to continue doing research (Burnette & Wessler, 2003). Students performing those laboratory exercises do not design experiments or develop the critical thinking skills so crucial in undergraduate education (Glidon and Rosengren, 2012; Knutson et al, 2010; Coil et al, 2010). Evidence suggests that research-focused instruction is more effective for developing those skills (Vision and Change in Undergraduate Biology Education, 2011; Anderson et al, 2005; Treacy et al, 2011). In addition to implementing active learning and problem solving approaches into the biology and biochemistry curriculum, it is also important to integrate modern computational skills and bioinformatics (Badotti et al, 2014; Honts, 2003; Voet, 2003; Voet, 2004). Numerous institutions are introducing bioinformatics into existing biology and biochemistry curricula at multiple levels (Wightam and Hark, 2012; Hydorn et al, 2005; Furge et al, 2009).

In an effort to engage students in an authentic research experience and to bring a sophomore level molecular biology laboratory curriculum into the 21st century, a nine-week laboratory project was developed. Students enrolled in the course meet once a week for three hours and are simultaneously taking a molecular biology lecture (3 hours per week for 16 weeks). Students taking molecular biology – lecture and laboratory - have already completed two semesters of general biology and general chemistry courses. The total number of students taking the laboratory course varied between 30 and 50 people. During the nine-week project students examine primary literature, mine bioinformatics engines for gene and promoter sequences, and clone promoter sequences into vectors. Additionally, students present the results to the class and write individual laboratory reports about the project. The goal of this student-centered adventure is to combine computational skills, bioinformatics, and molecular cloning techniques into one cohesive whole. It also gives students the freedom to choose a gene and its promoter, learn about it from primary literature and then present the findings in an oral and written format.

METHODS

The project begins with a short introduction to bioinformatics and the structure of a typical gene and its promoter. During the first session students complete an activity that familiarizes them with different search engines (Bioinformatics Resource Portal ExPASy, National Center for Biotechnology Information – NCBI, Google Scholar) and tests their understanding of the presented material on bioinformatics and gene structure. Students are placed in groups of 3-4 and given an assignment for the project. Each group chooses a tumor suppressor gene (TSG) or oncogene and conducts research about its role in normal cells and in cancer. Students are asked to research the gene, protein and promoter for the TSG/oncogene. In the second week, students

complete a micropipetting exercise that reinforces proper technique while introducing them to basic computational skills using Excel. In week 3, students are introduced to mammalian tissue culture techniques where each group isolates genomic DNA from HeLa cells and calculates the concentration of that genomic DNA. Weeks 2 and 3 provide time for students to address their concerns about the project and ask questions about the assignment. Learning about gene and promoter sequences that can cause cell transformation is very interesting and exciting to the students but it also creates a level of uncertainty regarding how to complete the assignment using search engines that are new to them. In week 4, students present literature findings about the TSG or oncogene to the rest of the class and design primers to clone the gene promoters. In the following weeks students perform molecular cloning techniques – they design PCR conditions, run PCR reactions and agarose gels, ligate, transform the DNA products, and finally perform diagnostic restriction digests to see the results of the cloning. During the last week of the project, each group presents and interprets its results in front of the class. This session gives the member of each group an opportunity to talk about their results before the individual laboratory report is due.

Course schedule and experimental details

The overall agenda for the nine-week project is presented in Table 1. Each session is accompanied by a 30 minute lecture from the instructor and a 10 minute quiz to gauge student understanding of the material from the previous week. All course materials – power point lectures and detailed protocols - can be obtained by contacting the author.

Table 1. Weekly agenda for the nine-week project.

Week	Title
Week 1	Introduction to bioinformatics and explanation of the project
Week 2	Micropipetting and serial dilution exercise
Week 3	Genomic DNA isolation
Week 4	Presentations and primer design
Week 5	PCR and restriction digest exercise
Week 6	Agarose gel electrophoresis and gel purification
Week 7	Ligation and transformation
Week 8	Mini-prep and diagnostic restriction digest
Week 9	Interpretation of results in preparation for a formal laboratory report

Week 1: Introduction to bioinformatics and explanation of the project.

During this 3 hour session students learn: (1) what bioinformatics is; (2) how to read a DNA sequence; (3) the differences between prokaryotic and eukaryotic sequences; (4) how to search for prokaryotic and eukaryotic gene and promoter sequences using search engines such as ExPASy and NCBI; (5) how to compare multiple sequences to each other. After a short presentation from the instructor, students are placed in groups (3-4 people per group) and complete an activity. The goals of the activity are to give students hands-on experience with the search engines and to check their understanding of the previous material. Each group is required to: (1) find the gene and protein sequence of human Rb; (2) indicate the start, stop, and length of the Rb coding sequence and protein sequence; (3) using BLAST, compare the Rb protein to other proteins in the database.

At the end of the session, students are given the following assignment:

1. Define a tumor suppressor gene and an oncogene.
2. Give an example of each.
3. Define a gene promoter.
4. A. If you are in a Monday laboratory, choose one tumor suppressor gene (one per group)

B. If you are in a Tuesday laboratory, choose one oncogene (one per group)

5. Find one research article describing the promoter of your chosen tumor suppressor/oncogene.

a. find the promoter sequence (using any resource you learned about during the first week)

b. where is the promoter located?

c. how long is it?

d. what is the minimum length of the promoter for the gene to be transcribed?

6. Find one review article describing the tumor suppressor gene/oncogene that you chose.

a. why is this gene important?

b. what happens when the gene is not expressed/overexpressed/mutated?

7. Find a gene sequence of your tumor suppressor/oncogene (using any resource you learned about during the first week).

a. can you see the start and the stop of the gene?

b. how long is the sequence?

Please provide a list of references at the end.

8. Prepare a 10 minute presentation showing your results to the rest of the class.

Equipment list week 1: access to computers.

Week 2: Micropipetting and serial dilution exercise.

During this session students practice micropipetting, make serial dilutions using colored solutions, and practice using a spectrophotometer. They also use Excel for calculating averages, calculating standard deviations, and graphing. Most importantly, this session also gives students an opportunity for students to ask questions about the assignment.

Students use small (P-20 and P-200) and large (P-1000) pipettes to measure and combine colored solutions. Then, using an appropriate micropipette, they check their accuracy in pipetting. All students are asked to practice this portion of the exercise until they become proficient and accurate.

In the second part of the meeting students dilute a yellow stock solution by combining 150 µL of stock solution and 850 µL of water (dilution #1). Then, they take 150 µL of dilution #1 and mix it with 850 µL of water (dilution #2). They are asked to perform this task in triplicate, to measure the absorbance of each solution in a spectrophotometer using a 570 nm wavelength and to calculate the dilution factor. Students input the serial dilution data into an Excel spreadsheet, calculate averages and standard deviations when appropriate and plot the data using a bar graph.

Finally, this is the time for students to ask for help with the assignment and for instructors to provide support finding sequences and appropriate articles.

Equipment list week 2: food coloring, micropipettes, spectrophotometer, access to computers.

Week 3: Genomic DNA isolation.

Each group is given a confluent plate of HeLa cells. Students scrape the cells off of the plate and isolate genomic DNA using Wizard Genomic DNA purification Kit (Promega). At the end of the protocol, DNA is rehydrated with 40 µl of rehydration solution provided in the kit. To verify successful DNA isolation students calculate the concentration and purity of the sample based on the absorbance values at A260 and A280 using a spectrophotometer.

Equipment list week 3: HeLa cells, tissue culture incubator, tissue culture hood, cell scrapers, Wizard Genomic DNA Purification Kit (Promega), heat block, quartz cuvettes, spectrophotometer, computers

Week 4: Presentations and primer design.

Each group presents the background information on the chosen tumor suppressor gene or oncogene; shows the sequences; and addresses assignment questions 5-7 (listed in the description of week 1 above) in detail. The presentations allow students to learn about the various genes investigated by other groups. The presentations also allow the instructor to verify the quality and accuracy of the findings before the students begin the molecular cloning portion of the project.

Based on the presented findings, each group designs primers necessary for cloning a tumor suppressor gene promoter or an oncogene promoter. Primers are submitted to the instructor at the end of the session and sent to Eurofins MWG Operon for synthesis. The primers are typically received within 48 hours.

Equipment list week 4: computers

Week 5: PCR and restriction digest exercise.

Students dilute the PCR primers to a final concentration of 0.1 µg/µl in water and calculate the volume of genomic DNA needed for the PCR reaction. Thermal cycler conditions appropriate for amplifying the promoter sequence are used. The PCR reaction components and the thermal cycler conditions are listed in Table 2 and Table 3. After the PCR is complete, the reactions are stored at -20^{o}C until the next week.

Table 2. Ingredients for the PCR Reaction.

Ingredient	Volume Added
Deionized Sterile H_2O	to 50 µL
Genomic DNA	(50 ng) x µL
10x buffer solution	5 µL
0.1 µg/µl forward primer	2 µL
0.1 µg/µl reverse primer	2 µL
10 mM dNTP	2 µL
Taq polymerase	1 µL

While the PCR is under way, students complete the virtual restriction digest activity provided below:

1. Find the sequence for a circular plasmid called pET24c. Please download the sequence.
   a) How many places on this vector do EcoRI and NdeI cut? (use NEB Cutter)
   b) When a double digest of the vector is performed with EcoRI and NdeI, two linear fragments are released. What are the sizes of the two fragments? (Digest #1)
   c) When the vector is digested with EcoRI alone, what DNA fragments would you expect on the gel? (Digest #2)
   d) When the vector is double digested with EcoRI and EcoRV, what fragments are released? What are the fragment sizes? (Digest #3)
   e) Draw a picture of a gel and position a ladder next to it (you may use 1kb ladder from NEB); draw the DNA bands for each of the digests on the gel.
2. EcoRI and NdeI restriction enzymes produce "sticky ends" when they cut DNA.
   a) What sequences do EcoRI and NdeI recognize? (use NEB website)
   b) What do the "sticky ends" look like after being cut by EcoRI and NdeI?
   c) Why might restriction enzymes that produce "sticky ends" be better when ligating DNA han enzymes like BsaBI which produce "blunt ends" after digestion?
3. Open a file with the promoter sequence you are investigating.
   a) Is Bam HI, XhoI, and EcoRI cutting the sequence? If yes, how many times?
   b) Choose 1 restriction enzyme that cuts the promoter sequence only once; what fragments do you see on an agarose gel after cutting the sequence? (draw bands on the gel; digest #4)
   c) Choose 1 restriction enzyme that cuts the promoter sequence twice; what fragments are on an agarose gel after cutting the sequence? (draw bands on the gel; digest #5)

Table 3. Thermal cycler conditions. "x" needs to be determined based on the designed primer's melting temperatures.

Steps	Temperature	Time
1	95°C	2 min
2	95°C	30 seconds
3	x°C	30 seconds
4	65°C	50 seconds/kb
5	Go To Step 2. Repeat 34 times	
6	65°C	10 min
7	4°C	1 hr

Equipment list week 5: thermal cyclers, PCR reagents (New England Biolabs or Promega), primers (Eurofins MWG Operon), computers.

Week 6: Agarose gel electrophoresis and gel purification.

Students prepare a 1% agarose gel containing 126 nM ethidium bromide. The genomic DNA and the PCR reactions are resolved on a gel and visualized on a transilluminator. A 1 kb ladder from NEB is used for band size comparison. PCR bands of appropriate size are excised from the gel and purified using Wizard SV gel and PCR clean-up system (Promega). PCR bands are eluted with 20 µl of water and the concentration of purified fragment is verified using NanoDrop (Thermo Scientific).

Equipment list week 6: low melting agarose, TAE buffer, ethidium bromide solution, 6x DNA loading dye (NEB), 1 kb ladder (NEB), Wizard SV gel and PCR clean-up system (Promega), NanoDrop spectrophotometer (Thermo Scientific).

Week 7: Ligation and transformation.

Students use a Quick ligation kit (NEB) to ligate the PCR fragment (150 ng) into the pGEM-T Easy vector (50 ng) (Promega). After a 5 minute incubation at room temperature, students transform the recombinant DNA into sub-cloning efficiency DH5α chemically competent cells (Invitrogen). The transformants are spread on LB plates containing ampicillin and grow over night at 37°C. Students are asked to view the transformation plates sometime during the week in order to determine the number of colonies.

Equipment list week 7: Quick ligation kit (NEB), pGEM-T Easy vector (Promega), DH5α competent cells (Invitrogen), LB Amp plates, 37°C incubator/shaker.

Week 8: Mini-prep and diagnostic restriction digest.

The day before each lab session this week, the instructor picks 3 colonies per group from the transformation plates and starts over-night liquid cultures. Students isolate the promoter-pGEM-T Easy plasmid from each culture using the QIAprep spin miniprep kit (Qiagen). DNA is eluted with 50 µl of water. Using the purified plasmid DNA, students perform a diagnostic restriction digest according to Table 4. The reactions are incubated for 30 minutes at 37°C, loading dye is added after the incubation, and all samples are separated on an agarose gel.

Table 4. Diagnostic Restriction Digest.

Ingredient	Volume
Plasmid DNA	17 µl
10x CutSmart buffer	2 µl
EcoRI endonuclease	1 µl

Equipment list week 8: LB media, ampicillin, QIAprep spin miniprep kit (Qiagen), EcoRI restriction endonuclease, agarose gel

Week 9: Interpretation of results.

Students collect the data and share results with the rest of the class. They explain each gel, band sizes and other results. In addition to modeling a talk that might be given at a scientific meeting, the presentations help the students prepare for writing the final lab report that is due the following week.

Equipment list week 9: computers

RESULTS AND DISCUSSION

Project-based laboratory activities should be considered as an addition to every biology and biochemistry curricula. The long-term projects involving numerous scientific assays, that may or may not successfully produce usable data, gives students an idea of what research is really like. The laboratory sequence described here began, just like any "real" research project, with an exploration of the primary literature. In the project described here, the literature search is primarily a fact-finding mission about the characteristics of a chosen TSG or oncogene and its promoter. During this literature search, students learn about a real gene that has a real impact on cells. The freedom to choose a TSG or oncogene puts students in control and gives them an opportunity to research something that interests them. Finding a gene sequence of interest tends to be the least challenging portion of the project. Finding the promoter sequence and evidence from primary literature that this is the minimum sequence necessary for transcription is typically much more challenging. Students must find not only the promoter sequence but also an article that supports their claim. That is why students were given three weeks before they presented their findings and continued with the project. Each of our groups had successfully found the gene and promoter sequences. All of our students also designed the forward primer with ease but some of them had trouble with the design of the reverse primer (4 out of 12 groups). For this reason, every group had to show both primers to the instructor before submitting the final primer sequences for ordering. The most exciting part of the project was running PCR products on an agarose gel. Students were eager to see the results. As expected, there were four possible outcomes: 1) no PCR band; 2) a PCR band that did not correspond to the expected promoter size; 3) multiple PCR

(a)

GGGAGAAAACGTTAGGGTGTGGATATTACGGAAAGCCTTCCTAAAAAATGACATTTAACTGATGAGAAGA
AAGGATCCAGCTGAGAGCAAACGCAAAAGCTTTCTTCCTTCCACCCTTCATATTTGACACAATGCAGGAT
TCCTCCAAAATGATTTCCACCAATTCTGCCCTCACAGCTCTGGCTTGCAGAATTTTCCACCCCAAAATGT
TAGTATCTACGGCACCAGGTCGGCGAGAATCCTGACTCTGCACCCTCCTCCCCAACTCCATTTCCTTTGC
TTCCTCCGGCAGGCGGATTACTTGCCCTTACTTGTCATGGCGACTGTCCAGCTTTGTGCCAGGAGCCTCG
CAGGGGTTGATGGGATTGGGGTTTTCCCCTCCCATGTGCTCAAGACTGGCGCTAAAAGTTTGAGCTTCT
CAAAAGTCTAGAGCCACCGTCCAGGGAGCAGGTAGCTGCTGGGCTCCGGGGACACTTTGCGTTCGGGCTG
GGAGCGTGCTTTCCACGACGGTGACACGCTTCCCTGGATTGG

(b)

	FORWARD PRIMER	REVERSE PRIMER
Sequence	5'GAGAAAACGTTAGGGTGTGGATATT ACGAAG 3'	5'CAATCCAGGGAAGCGTGTCACCG TC 3'
Length	31 nucleotides	25 nucleotides
GC content	42%	60%
Salt adjusted melting temperature	69.7 °C	70.7 °C
Potential for hairpin formation	None	None
Complementarity	None	None
Self-annealing	None	None

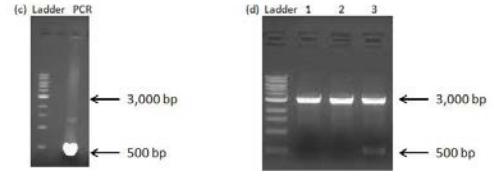

Fig.1. Representative data showing the results of the nine-week project. (a) p53 promoter sequence (GenBank accession number J0438); (b) p53 promoter-specific forward and reverse primers and their characteristics; (c) agarose gel showing a PCR amplified fragment of DNA about 500 nucleotides in length; (d) pGEM-T Easy vector without an insert (lanes 1,2) and pGEM-T Easy vector with a DNA fragment about 500 nucleotides in length inserted (lane 3).

bands; and 4) a PCR band that corresponds to the expected promoter size. Six out of twelve groups had a DNA band that corresponded to the size of their gene promoter. One group had multiple bands on the gel and five groups did not have a band at all. All groups that did not have a PCR product were given a choice of re-doing their PCR with a different polymerase (Expand High-Fidelity PCR system from Roche) or using another group's PCR products. All groups chose to redo their PCRs and four groups had a PCR band on the second try. Every group had colonies on the transformation plates and nine groups had an insert in at least one of their colonies.

Learning assessment

The assessments for the project-based based portion of the laboratory (9 out of 16 weeks) consisted of weekly quizzes, a group power point presentation, and an individual formal laboratory report. Each quiz was designed to assess understanding of the background material presented by the instructor and the experimental techniques performed the previous week. Each student was also responsible for reading and comprehending the material associated with each laboratory exercise. These quizzes represented to 25% of the students' grades for the nine-week project.

On week 4, the members of each group of students gave a power point presentation about their literature findings, gene and promoter sequences. Each presentation submitted to the instructor contained a contribution table describing each student's role in this part of the project. The presentation represented 22% of the total grade for the nine-week project. Upon completion of the project, each student wrote an independent laboratory report that mimicked a manuscript intended for peer review and publication. Each report consisted of an introduction containing a clear objective, materials and methods section, results, discussion, conclusion, and references. This portion of the project amounted to 43% of the total grade for the project. Representative data obtained in the project are shown in Figure 1.

At the end of week 16 of the semester, students filled out an anonymous survey. On the scale of 1-7 (one being lowest and seven being highest) students considered this course challenging (average response 6.7) but found the assessments relevant (average response 6.75) and course objectives well-chosen and appropriate (average response 6.55). When asked about the overall quality of the laboratory course, students showed enthusiasm and appreciation for the project.

Hazards

HeLa cells must be handled according to the rules of biosafety level 2. Protective gloves and clothing must be worn while handling the cells. All waste (plates, scrapers, tubes, tips) must be collected separately and properly disposed. *E. coli* DH5α is a nonpathogenic strain of bacteria. Ethidium bromide is a mutagen and might be harmful if inhaled, ingested, or absorbed through the skin.

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