Using a Molecular-Genetic Approach to Investigate Bacterial Physiology in a Continuous, Research-Based, Semester-Long Laboratory for Undergraduates †

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Designing investigative laboratory exercises that encourage critical thinking, problem solving, and independent thought for upper-division biology courses is a difficult but worthwhile task. In an effort to do so, we developed a semester-long, continuous, research-based investigative laboratory that integrates numerous genetic and molecular biology methods into the investigation of a bacterial physiological process. In this lab, students use random Tn5 transposon mutagenesis to create prodigiosin pigment mutants in the bacterium, Serratia marcescens. This is followed by phenotypic characterization, cloning, and sequencing the Tn insertion site to identify genes involved in pigment biosynthesis. During this lab, students gain ample experience performing basic lab techniques while learning about — and applying — methods for elucidating gene function. The approach to the laboratory and the outcomes are intimately integrated into the teaching of many fundamental physiological processes underlying prodigiosin production in bacteria. The result is a cohesive course that integrates the theory and application of molecular genetic techniques with the study of bacterial physiology. Assessments of student learning objectives demonstrated that students greatly improved their understanding of both physiological processes and the genetic techniques used to investigate them. In addition, students felt that this semester-long exercise provided the necessary laboratory experience they needed and desired in preparation for careers in molecular biology, microbiology, and biochemistry.

INTRODUCTION

This 15-week laboratory activity was designed to integrate the teaching of fundamental bacterial physiological topics with an investigative, research-based, laboratory experience that provides students with ample hands-on experience with a large variety of basic molecular biology and genetic techniques. The design of this laboratory exercise stems from observations made by many colleagues who have taught upper division undergraduate courses in molecular biology, microbiology, and genetics. The observation is that students are much more engaged and invested in laboratory experiences that are cumulative in nature, that take place over a large portion of the semester, and in which an active investigation with unknown outcomes is carried out. From these teaching experiences, it has also been observed that students take more initiative in the investigation process and routinely demonstrate critical thinking and problem-solving skills to guide them through the experiments. During these labs, students appear to be integrating information from the current course and previous courses, to come up with solutions. Students demonstrate a greater appreciation for the scientific method and the process of discovery, and also seem to better retain the concepts they are learning because they are required to put them into practice to solve the problem at hand. Finally, students become more competent in their basic laboratory manipulations because they have ample opportunity to practice them. As a result, students are more confident in their skill set and knowledge base, which creates a positive feedback loop improving information retention, involvement, and the overall class experience for everyone.

This is in stark contrast to what is typically observed in laboratory courses that consist of a number of prefabricated, “cook book” activities that are unrelated and that do not allow students opportunity to integrate lab experiences with the informational content they are receiving. During these labs, students typically come to class with an agenda to get it over with as quickly as possible and to memorize facts needed to pass an exam, without the sense that they need to understand topics introduced in one lab to understand what is going to happen during the next. This experience is highly unsatisfying for students and professors alike, and does not allow for the numerous greater benefits that a laboratory course can offer.
These experiences related by college professors have been supported by scientific organizations urging the integration of research with undergraduate education. The National Research Council and the National Science Foundation recommend that any undergraduate education involve students in as many aspects of a research project as possible, and that this process include experimental design, literature searches, performing the experiments, interpreting data, and communicating the outcome in a professional manner (7, 8). Engaging more than a few students a semester in this manner is a daunting task for any professor teaching a full load unless the research project can be integrated into the classroom curriculum — thus serving an entire class of students, rather than the few seeking independent research opportunities.

In the laboratory described in this report, students use transposon mutagenesis to identify the genes involved in the production of a red pigment, prodigiosin, in the bacterium Serratia marcescens. Identification of genes involved in prodigiosin production is an active area of research, which allows the students in this lab to be researchers, carrying out a progression of experiments to answer a real life question, which could yield novel results. As in any research, there are pitfalls and unknowns and a great deal of problem solving. This laboratory curriculum is designed to take advantage of these to teach critical-thinking skills and to challenge the students to solve problems based on their previously obtained knowledge, as would be done in any research laboratory.

The topic of investigation of prodigiosin biosynthesis was chosen because it is a process that integrates many fundamental principles of physiology such as primary and secondary metabolism, biosynthetic pathways, gene organization and regulation of expression, quorum sensing, and effects of nutritional status on cellular physiology (1-4, 10, 11). In addition, students are likely to find this topic of research particularly interesting due to its potential medical application, as prodigiosines are currently being researched for their anti-tumor properties and pharmaceutical potential (9). A genetic approach using random transposon mutagenesis was chosen as a method to investigate the genes for prodigiosin production because this method incorporates, and allows students to master, a great number of molecular biology and genetic techniques. These include conjugation, transformation, chromosomal and plasmid DNA isolations, agarose gel electrophoresis, selections and screens, cloning and DNA sequencing and analysis, and are all integral to the process of disrupting, cloning, and sequencing genes with a particular physiological function. Thus, the student’s experience with these techniques is grounded in a real research question with the potential to add to the body of science rather than being merely theoretical. It is our hypothesis that applying these techniques to a real research question, while having the students be actively engaged in the research process, would result in both a greater understanding of the physiological processes being addressed and the application of genetic and molecular techniques to answer physiological questions.

Intended audience and prerequisite student knowledge

This laboratory was designed for upper-division Bacterial Physiology and Microbial Genetics courses attended primarily by Microbiology and Biology majors in their junior or senior years. Students should have an introductory course in genetics with some introduction to molecular biology, and an introductory course in microbiology and chemistry. Although there is opportunity to review basic microbiology skills such as micropipetting, aseptic technique, and bacterial culture manipulations in the first recommended laboratory exercises, these exercises are designed to be only a review, and do not provide enough practice for a student to become proficient. Thus, we highly recommend that students have some previous experience with these topics, as well as with the concepts of pure culture, basic bacterial growth traits, and culturing techniques.

The described laboratory is for a class size of approximately 20 students, broken into groups of two to three students. However, this exercise could be scaled up or down for any class size. The smaller group size is recommended to allow each student maximum involvement in both the hands-on aspects of the lab and in the intellectual challenges of planning and predicting experimental outcomes.

Learning time

The full-length version of the laboratory is designed to be run the entire length of a 15-week semester course that meets for three hours, twice weekly (approximately 27 lab days total). However, several modifications are possible that could allow it to be run over 6, 11, or 27 lab sessions to fit other course needs and formats. Examples of these modifications are included in the detailed Instructor’s version in the Supplemental Materials, Appendix 2A.

For each three-hour lab session, approximately 20 to 40 minutes of introduction time is recommended. This time is used in recapping what was done in the previous lab session and describing what will be done next. Periodically, up to an hour of “lecture” might be required to deliver adequate material for the students to put that day’s lab activity into perspective. Details of the information provided to the students for each lab day are provided in the Instructor’s version of the laboratory manual in Appendix 2A.

Learning objectives

By the completion of this course students should be able to:

1. Describe the methodology and rationale for two general approaches to identify genes with a particular function, random transposon mutagenesis, and construction of DNA libraries.
2. Demonstrate how to determine how many mutants should be examined in a Tn mutagenesis experiment (or in analyzing a chromosomal library) to ensure complete coverage of the genome using the Poisson distribution.

3. Define the difference between a selection and a screen and describe how, and for what purpose, each one is carried out.

4. Indicate under what circumstances polar effects could be observed in bacterial mutants and how to interpret phenotypes in these cases.

5. Outline and carry out the steps in DNA manipulation and cloning protocols including DNA isolations, precipitations, visualization via agarose gel electrophoresis, and the properties and roles of restriction enzymes and DNA ligase.

6. Compare and contrast the methods for introducing exogenous DNA into a bacterial cell, transformation, conjugation, and transposition.

7. Assemble DNA sequences, navigate sequence databases NCBI and JCVI, and use bioinformatics programs such as BLAST to make predictions about the function of genes.

**PROCEDURE**

**Materials**

Both students and instructor will need the corresponding laboratory manuals provided in Appendix 2 of the Supplemental Materials. A detailed list of required materials is provided in Appendix IA in the Supplementary Materials and is organized as “Group materials,” “Classroom materials,” and “Daily Materials.” Due to the ongoing and semester-long nature of the laboratory, it is recommended that each group of students maintain their own stocks of routinely used reagents and own sterile supplies that they can keep in the classroom and are responsible for obtaining, maintaining sterility, and storing as needed (“Group materials”). It is also recommended that basic molecular biology instruments such as microcentrifuges, micropipettors, etc., be available in the classroom daily, if possible, to facilitate carrying out impromptu experiments (“Classroom materials”). “Daily Materials” listed are those required for a specific day’s lab which also require the “Room materials” and the “Group materials.” With the exception of a few bacterial strains indicated in Appendix IA, the required materials are readily available and commonly used in molecular and microbiology laboratories.

In addition to laboratory materials, several journal articles were used in class as a preparatory assignment to introduce the laboratory topic and which formed the basis of a class discussion. Thomson et al. (10), and Harris et al. (2) were used to introduce the topic of prodigiosin production, methods for gene identification via construction of chromosomal libraries and Tn mutagenesis, and to establish what is known about this process. Larsen et al. (5) describes the transposon delivery vector used in this laboratory. The journal article student assignments associated with these articles are listed in Appendix 4 of the Supplementary Materials.

**Student instructions**

Detailed student instructions are provided in the Student Laboratory Manual in Appendix 2B of the supplementary Materials. In addition to the information provided for the students in the lab manual, it is recommended that each of the daily activities be introduced and further detailed in class with drawings on the black board or with similar visual aids (see instructor’s version for additional information on introducing individual laboratories). Students are introduced to the overall goals and approach of the laboratory on the first day of class, and are instructed to obtain a laboratory manual and a notebook for recording the daily laboratory activities. The daily lab activities are outlined in the laboratory manual, and students are required to read each lab prior to coming to class.

**Instructor version**

During phase 1 of this project (approximately the first six laboratory sessions), Tn5 random transposon mutagenesis will be used to generate a pool of kanamycin resistant S. marcescens mutants, some of which show altered pigment production as compared to wild type. Following mutagenesis, students will isolate mutants showing either increased or decreased pigment production, and perform phenotypic analysis on various minimal and rich media. During phase 2 (approximately lab session seven through lab session 11), students will attempt to clone the transposon insertion site. During phase 3, (lab session 12 to 27) students will attempt to obtain sequence flanking the Tn insertion site and to identify the gene(s) disrupted by the transposon (see Fig. 1 and Appendix 8 in the Supplementary Materials for flow charts with time lines detailing these steps).

**Fig. 1. Flow chart of daily laboratory activities.** The semester long laboratory experiment can be broken down into three phases, each of which builds upon the previous phase. Each day indicated refers to a three-hour laboratory period. This schedule is designed to complete all three phases in a typical 15-week semester with two, three hour laboratory sessions per week, totalling approximately 27 lab sessions.
Each laboratory session is introduced by the instructor to both provide adequate information for students to carry out the lab exercise, as well as to assess how well students understand the process. For example, on lab day 2 of the mutagenesis experiment, the task at hand is to determine the frequency of transposition. This requires a discussion about selection and requires the instructor to discuss the genotypes and phenotypes of the donor and recipient strains used in the conjugation. We recommend starting this discussion by first asking the students what is growing on their nonselective plates after filter mating. This requires them to remember what they did in the previous lab and asks that they connect this with what they are actually observing growing on their plates. Based on their responses, we recommend drawing the cell types they indicate on the board. They will likely immediately identify that E. coli donor cells and recipient cells with plasmid are present, and they might be able to tell you that some recipient cells that did not receive plasmid are also there. We recommend using this time to introduce the terms transconjugant or exconjugant and to reinforce the proper use of genetic terminology such as donor, recipient, and phenotype and genotype nomenclature. After drawing each of these cell types, the instructor could ask students the phenotypes of each, suggesting that they might not know the whole story yet, but should be able to come up with kanamycin-resistant for the donor and kanamycin sensitive for the recipient based on discussions from the previous lab session. We recommend writing these phenotypes under each of the corresponding cell types, and then asking the students to explain why the E. coli donor is kanamycin-resistant to make sure they understand the connection with this phenotype and the Tn delivery vector. We then recommend asking the students how they would isolate the desired transconjugants from that mixture given the phenotypes they come up with. Students will quickly realize that selection for kanamycin resistance is important for isolating the transconjugants from the wild type S. marcescens, but then they quickly get stuck with how to select against the E. coli donor. We recommend then introducing the leucine auxotrophy of the E. coli BW20767 (6) donor and explaining what this means. Based on the information provided, we recommend asking them to devise a selection medium that will accomplish the desired task. Once students seem to have an answer, we recommend having them share this with the class and evaluating each of the choices on the board while indicating which of the cell types present would be selected for or against, and why, on the media students recommend.

A similar approach to delivering introductory material each day provides an opportunity for the instructor and students to catch any misunderstanding of the experimental procedure before proceeding to the next step. This approach also encourages active participation of the students because they are asked to describe to the instructor what is happening, how to proceed and for what purpose, and what controls are needed. Additional detailed instructions for the instructor are provided in the instructor's version of the lab manual found in the Supplementary Materials, Appendix 2A. This version of the lab manual is identical to the student's version, but has additional notes for the instructor on how each daily activity might be introduced, on any difficulties students might have in comprehension or in the manipulations, and it also has descriptions of possible outcomes to guide them.

Suggestions for determining student learning

Both informal and formal assessments were used to determine student learning over the course of this laboratory. Informal assessments were carried out at the beginning of each lab session during class discussions of the progress of the lab, as described above. Each discussion would be led with questions directed to the class such as, “Where did we leave off at the end of the last class?” Based on student’s answers, we recommend drawing a diagram representing the current phase of the project. After establishing the current status of the lab, questions such as “What should our next step be?“ would be posed to the class. Because this teaching style and interaction with the students had been established the first day of class and used throughout, we have observed that students are actively engaged in these discussions and we highly recommend waiting for students to answer, picking students that are typically silent to answer, and asking students to elaborate on any ideas they may provide. Based on the student's answers, it is easy to assess their overall understanding of the lab and to remediate any misconceptions prior to proceeding with the current lab activities. During these informal assessments, we have observed that approximately 50% of the students are able to provide sound suggestions for what should be done next in the lab, although they might not be able to include the details and may not have the appropriate terminology to describe it.

Formal assessments to determine student learning include exam questions that ask students to provide a rationale for certain steps in a particular suite of lab experiments, or to describe the objective of an experiment and what controls should be used. A few examples of these are provided in the Supplemental Materials in Appendix 7; however, many more could be designed specifically to address learning of topics critical to a particular course.

Another form of formal assessment used in this class was a laboratory report assignment. Students were asked to submit a “journal article” style paper summarizing their lab activities and results after phase 1 of the project, and then again after phases 2 and 3 of the project, which was at the end of the course. These lab reports were to be written in a professional and polished manner following the format of any ASM journal, and were to include figures and tables, whenever appropriate. The general assignment description, examples of student’s laboratory reports, and a detailed grading rubric used for this assessment are provided in the Supplementary Materials, Appendix 6. From the descriptions of the “Materials and Methods,” the “Results” sections, and
the “Discussion” sections of these lab reports, it is easy to determine the level of comprehension the students had of the lab exercises. For example, based on a student’s description of their sequence analysis and the figure they are instructed to make, diagramming the transposon insertion site, it is easy to determine if the student is able to correctly assemble their sequences, carry out a BLAST search, and understands the information received from these activities — an exercise that addresses student learning objective 7. From this lab report assignment, it was evident that the understanding of the methods and the significance of the results improved dramatically from the first laboratory report to the last.

Journal article assignments were also used to determine student learning and to help students put the concepts and techniques they were using into a broader context. For the first journal article assignment, students were instructed to read two assigned journal articles about prodigiosin biosynthesis and one about the transposon mutagenesis method we would be employing (Supplementary Materials, Appendix 4A). After reading the articles, students were to answer a number of set questions designed to determine their basic understanding of the content and methods of the paper, and to introduce appropriate background for their lab research project. For example, questions about the methodology used in each paper to identify genes with a particular function provided an opportunity to discuss the concepts of Tn insertion libraries and chromosomal libraries, DNA manipulations and cloning, and selections and screens (these address student learning objectives 1, and 3–6). The answers to these questions were discussed in class and were also turned in for a grade. At the end of the semester, timed with the laboratory when students would be getting back sequence data indicating into what genes the Tn had been inserted, students were assigned a second journal article. For this assignment however, students were to find their own primary article with the direction that it had to describe the investigation of genes involved in prodigiosin production and it had to be more recent than 2005. The purpose for this was to lead them to do a literature search that would aid them in forming predictions about what genes were disrupted in their own pigment mutants. Students were to summarize the pertinent methods and results of the paper (see assignment description in Supplementary Materials, Appendix 4C). From these summaries, it was observed that most students understood much more of the content in the journal articles they chose than they did in the first journal article assignment. These assignments also permitted an assessment of student learning objectives 1, and 3–6 again at the end of the semester (see Supplementary Materials, Appendix 4B for student answers to journal article assignment 1).

Finally, protocol development assignments served the purposes of preparing students for upcoming lab exercises in DNA isolation, having students carry out literature searches to find references on molecular protocols, and assessing students’ understanding of DNA manipulation and isolation methods (lesson objective 5). The first protocol development asks students to look up and outline the steps in isolating chromosomal DNA from bacterial cells. The focus of the assignment is to understand how each step works and why it is performed. This assignment is collected and the steps are discussed in class. The next assignment is similar in purpose and format but asks students to devise a protocol for plasmid purification from bacterial cells. By completing this assignment, students are given the opportunity to apply what they have learned about DNA isolations to the isolation of plasmid DNA. Instructors are able to assess how well students understood these principles based on their descriptions of plasmid DNA isolations in this second assignment, which occurs later in the semester.

**Sample data**

Examples of results obtained during this laboratory exercise are summarized in the student laboratory reports in the Supplementary Materials, Appendix 6B. The laboratory reports contain the results obtained by students during this lab and include: images of agarose electrophoresis results, pictures of *S. marcescens* pigment mutants, figures indicating the Tn insertion site of the student’s mutants, and student descriptions of the lab exercises and outcomes. Class results from this laboratory are also summarized in Table 1 and can be found in the Supplementary Materials, Appendix 9. Transposon mutagenesis of *S. marcescens* was very successful in these courses. From the 33 pigment mutants obtained, 17 were selected for further study by pigment variation from the wild type and 11 different transposon insertion sequences were obtained. Examples of student responses to exam questions and examples of student’s assignments are also provided in Supplementary Materials, Appendices 4–7.

**Safety issues**

Common-sense caution is urged during this laboratory in the manipulation of bacterial cultures and in using standard molecular biology reagents such as sodium acetate, NaOH, and UV light. We recommend that each student be required to wear a laboratory coat and safety goggles during liquid or culture transfers and when using UV light. While the bacterial cultures used in this lab are not pathogenic, they should be treated as such to teach students proper aseptic technique.

**DISCUSSION**

**Field testing**

This lab has been administered in a Microbial Genetics course, and in two sections of a Bacterial Physiology course. Each course consisted of approximately 20 students, and each was a 15-week course with either two 2-hour labs
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Table 1. Class summary results of *Serratia marcescens* transposon mutagenesis, isolation of prodigiosin pigment mutants, and cloning the Tn insertions site.

<table>
<thead>
<tr>
<th>Group</th>
<th>Transposition Frequency</th>
<th># of Mutants Screened</th>
<th># of Mutants Used for Phenotypic Analysis</th>
<th># of Mutants to Clone Tn Insertion Site</th>
<th>Mutant Name</th>
<th>Phenotypec</th>
<th>Name of Plasmopson Sequenced</th>
<th>Size of Plasmopson BamHI Fragments (Kb)</th>
<th>Insertion Site Sequence Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 × 10⁻⁵</td>
<td>2000</td>
<td>4</td>
<td>2</td>
<td>VEK1</td>
<td>Pig-, white</td>
<td>pVEK1</td>
<td>7</td>
<td>none</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VEK2</td>
<td>Pig++, magenta</td>
<td>pVEK2</td>
<td>3/5</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>2.3 × 10⁻⁵</td>
<td>4200</td>
<td>5</td>
<td>2</td>
<td>JATD</td>
<td>Pig+/-, peach</td>
<td>pJATD</td>
<td>10/7</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>JATH</td>
<td>Pig+/-, white outer edge</td>
<td>pJATH</td>
<td>10/7</td>
<td>Sensor histidine kinase⁸</td>
</tr>
<tr>
<td>3</td>
<td>3.4 × 10⁻⁶</td>
<td>750</td>
<td>2</td>
<td>2</td>
<td>TLC1</td>
<td>Pig-, white</td>
<td>pTLC1</td>
<td>8</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TLC2</td>
<td>Pig+/-, sectoring white to red</td>
<td>pTLC2</td>
<td>10</td>
<td>Comp transcriptional regulator⁷</td>
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<tr>
<td>4</td>
<td>2.7 × 10⁻⁴</td>
<td>1780</td>
<td>3</td>
<td>2</td>
<td>GAG1</td>
<td>Pig-, white</td>
<td>pGAGM1</td>
<td>5.5</td>
<td>phosphopantethenyl transferase⁸</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GAG2</td>
<td>Pig-, white</td>
<td>pGAGM2</td>
<td>4.5</td>
<td>SAM dependent methyltransferase⁷</td>
</tr>
<tr>
<td>5</td>
<td>7.9 × 10⁻⁶</td>
<td>2436</td>
<td>8</td>
<td>2</td>
<td>DJZ8</td>
<td>Pig+/-, Salmon</td>
<td>pDJZ8</td>
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<td>DNA topoisomerase</td>
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<td></td>
<td></td>
<td></td>
<td>DJZ9</td>
<td>Pig+/-, orange</td>
<td>pDJZ9</td>
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<td>Polyribonucleotide nucleotidytransferase</td>
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<tr>
<td>6</td>
<td>4.4 × 10⁻⁵</td>
<td>4706</td>
<td>6</td>
<td>2</td>
<td>GG8a</td>
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<td>pGG8a</td>
<td>11</td>
<td>Conserved hypothetical protein⁷</td>
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<td></td>
<td></td>
<td>GG9b</td>
<td>Pig+, dark red with gold sheen</td>
<td>pGG9b</td>
<td>10</td>
<td>Phosphate transporter⁷</td>
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<td>7</td>
<td>3.7 × 10⁻⁵</td>
<td>4700</td>
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<td>3</td>
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<td>Pig+/-, orange</td>
<td>pMJB4</td>
<td>9</td>
<td>pigM</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>MJB5</td>
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<td>none</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>JAT1</td>
<td>Pig-, white</td>
<td>pJAT1</td>
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<td>pigJ</td>
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<tr>
<td>8</td>
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<td>4880</td>
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<td>2</td>
<td>RBC2</td>
<td>Pig+/-, white w/red streaks</td>
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<td>N/A</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RBC4</td>
<td>Pig-, white/yellow</td>
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<td>N/A</td>
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<tr>
<td>Total</td>
<td>5.9 × 10⁻⁵</td>
<td>25452</td>
<td>33</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

a Obtained sequence is from *Serratia proteamaculans*.
b Obtained sequence is from *Hahella chejuensis*.
c Pigment phenotypes are as follows: pig++, hyperpigmented; pig+, same as wild type; pig+/-, intermediate pigmentation; pig +/-, minimal pigment; pig-, no pigment.
per week, or two 3-hour labs per week. The lab activities described in the lab manual worked well for both of these formats. Both courses are upper-division biology courses comprised of junior and senior Biology, Microbiology and Biochemistry majors in addition to a few Master’s level students. While conducting this lab in the Microbial Genetics course, much more emphasis was placed on instruction of the molecular and genetic methods used. During the implementation of this lab in the Bacterial Physiology course, while students were required to understand the theory of the method used, much more emphasis was placed on integrating the physiological aspects of prodigiosin production into the lab.

At the end of the semester, a survey was conducted asking the students to rate each of the lab-related assignments for their usefulness in achieving the student learning objectives appropriate to that particular assignment. Students indicated that the protocol development assignments (provided in Supplementary Materials, Appendix 5) were extremely useful to their understanding of the molecular methods used. The laboratory reports were also rated very high, with students indicating that these assignments helped “bring an understanding to the big picture of what we were doing,” “helped me to write more professionally and to be able to put my raw data and analysis into a form that can be understood.” The journal article assignments were the least useful based on the student’s responses, with over half of the students indicating that they already felt comfortable getting information from journal articles. However, these assignments were still considered to be very useful in providing important background information for this particular lab, and for explaining various genetic methods and techniques.

When asked about the lab exercise in general and its usefulness with respect to the learning objectives, students gave overwhelmingly positive comments, such as “I loved this lab!”, and “This was the best class I’ve ever taken because the lab made me feel like a researcher.” Students indicated that they really liked that it was a semester-long project that built on itself and allowed them to continually build on their knowledge base and to think on their own. Students indicated that they much preferred this lab format to those in which students simply follow directions and move on to a new and unrelated lab activity each day (i.e., “cook book labs”). Although this is anecdotal evidence, these sentiments were consistent among all the students and across all three semesters that the lab was taught.

From an instructor’s perspective, I found this lab to be much more rewarding to teach than a traditional lab that has been run and repeated semester after semester, with little continuity. Although there is always some risk involved — certain steps not working as they should or students having a lower skill level than others — the fact that the outcomes vary from semester to semester and from student group to group means that both the students and the instructor have to be prepared for anything. This requires troubleshooting and critical thinking, and most importantly, that students really understand what they are doing so that they can think through a problem. This last point is what students and instructor seemed to value most about this lab.

Evidence of student learning

An assessment was devised to determine the level of understanding students had of basic approaches to understanding gene function and of basic methods that would be used during the lab. This assessment was given to the students on the first day of class, and again on the last day of class. The assessment, as well as examples of incorrect and correct student answers, can be found in the Supplementary Materials, Appendix 3. The results of these assessments are summarized in Fig. 2. For each question, the percentage of correct answers increased 10%–70% in the postproject assessment, indicating that students did improve their understanding of the processes explored in this laboratory exercise. Most significantly, students greatly improved their understanding of approaches to determine gene function and were able to apply what they learned in this lab to investigating other physiological processes, as seen by the 60% and 40% increase in correct responses to questions 3 and 4, respectively.

One aspect of student learning that greatly increased based on instructor observation, but which was not formally assessed, was a dramatic increase in student confidence and independence in guiding a research project and designing experiments, and in performing routine laboratory manipulations such as pipetting, making serial dilutions, making reagents, and culturing bacteria. One of the major concerns as an instructor is that students may graduate with a Bachelor’s degree in a field of biological sciences with only a theoretical knowledge of laboratory work, having only experienced these types of manipulations as a demonstration, or perhaps only being able to do them once. This typical, and minimal, exposure does not adequately train students in the skills desired from biotechnology companies, graduate schools or diagnostic labs, and it does not allow them the
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opportunities to develop confidence in their skill set. The laboratory exercise described here results in significantly increased confidence in, and mastery of, these important and basic skills.

Possible modifications

The first phase of this lab, consisting of the mutagenesis experiment and phenotypic analysis of obtained mutants, could be a stand-alone lab appropriate for the classes described above or for a lower-division Biology course, as well. During this phase, basic microbiology and genetic techniques are used, and the time and cost of the lab is minimal. To expand this lab to suit a more advanced audience, or one more focused on molecular biology and physiology, we recommend carrying out phases 2 and 3, as well. These phases involve cloning of the Tn insertion site, and sequence analysis of the disrupted genes. Additional optional labs are also suggested in the laboratory manual (Supplemental Materials, Appendix 2). These could be stand-alone labs, or could be combined with phase 1 and/or phases 1, 2 and 3.

Although the biosynthesis of prodigiosin pigment production in Serratia marcescens was examined in this laboratory exercise, this curriculum could be adapted to investigating any number of physiological processes for which a screen for altered phenotypes is readily available, such as motility, auxotrophies, chemical resistance, or secondary metabolite production. This transposon delivery system has been shown to work in a wide variety of bacteria, allowing the use of this lab experiment to investigate processes in numerous bacterial species (5).

Potential problems encountered

The following is a list of problems that have arisen in at least one group per class, followed by a possible solution.

Potential problem 1: Contamination of media and cultures.

Possible solution: Carefully review aseptic technique during the first lab and carry out recommended labs A and B to allow students to observe their own aseptic technique, or lack thereof. In rooms that are particularly dusty or moldy, sterilized media can be dispensed and inoculated in a culture hood to help minimize contamination.

Potential problem 2: None or too few Tn-induced pigment mutants isolated by a group.

Possible solution: Typically, students in each group are able to find at least one pigment mutant on the very first round of selection in the process of determining the Tn insertion frequency on day 3 of the mutagenesis lab. Whether a group immediately finds a pigment mutant or not, we recommend that each group carry out the large scale selection of Tn-induced pigment mutants on day 3, with a goal of screening at least 2,000 mutants per group. If each group plates about 10 petri plates with approximately 200 colonies per plate to screen, they should be able to find at least one pigment mutant per person in that group to work with.

Potential problem 3: A particular group is unable to clone the Tn insertion site of their mutants.

Possible solution: This event is highly likely to happen for at least one mutant each group has chosen to work with, as there are many places for error in this process. To remedy this, we recommend that each group start with at least one mutant per person in the group to work with, so that the chance of the group getting at least one Tn insertion site cloned is higher. In the event that none of the Tn insertion sites of the mutants are successfully cloned for a group, we recommend having a group that has had more than one successfully cloned share one of their clones with the others. There is also time built in at the end of the lab to repeat cloning steps, if necessary.

Potential problem 4: No sequence data is acquired from the cloned Tn insertions sites.

Possible solution: This is also likely to occur for at least one of the mutants whose Tn insertion site has been successfully cloned. The primary reason for this is probably insufficient plasmid DNA used as a template in the sequencing reactions. To remedy this, if time and resources permit, multiple plasmid preps of the same plasmid template could be prepared to ensure there will be enough for the sequencing reaction. Alternately, as done above, groups that have been able to successfully get sequence data for more than one mutant might share one of their sequences with another group. As a class, this exercise works best if each group has at least one sequence to process.

Supplemental Materials

Appendix 1A: Materials & Instruments
Appendix 1B: Recipes
Appendix 2A: Instructor’s Lab Manual
Appendix 2B: Student’s Lab manual
Appendix 3A: Pre/Post Project Assessment
Appendix 3B: Pre/Post Project Assessment Answer Key
Appendix 4A: Journal Article Assignment 1
Appendix 4B: Journal Article Assignment 1 Answer Key
Appendix 4C: Journal Article Assignment 2
Appendix 5A: Chromosomal DNA Prep Protocol Development Assignment
Appendix 5B: Chromosomal DNA Prep Protocol Development Assignment Student Example Answer Key
Appendix 5C: Plasmid DNA Prep Protocol Development Assignment
Appendix 5D: Plasmid Prep Protocol Development Assignment Student Example/Answer Key
Appendix 6A: Laboratory Report Assignments 1 & 2
Appendix 6B: Laboratory Report 1 and 2 student examples
Appendix 7: Example Exam questions and student’s answers addressing lab theory
Appendix 8: Flow chart diagram of the steps in the Tn mutagenesis and cloning procedure in S. marcescens
Appendix 9: Agarose gel electrophoresis results of BamHI digested Plasposon DNA isolated from E. coli transformants

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