Using Student-Generated UV-Induced *Escherichia coli* Mutants

in a Directed Inquiry Undergraduate Genetics Laboratory

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ABSTRACT

We report a thematic sequence of directed inquiry-based labs taking students from bacterial mutagenesis and phenotypic identification of their own self-created mutant, through identification of mutated genes by biochemical testing, to verification of mutant alleles by complementation, and lastly to mutant allele characterization by DNA sequence analysis. The lab utilizes UV mutagenesis with wild type *Escherichia coli* and a UV-sensitive isogenic derivative optimized for undergraduate use. The labs take advantage of the simplicity of *E. coli* in a realistic genetic investigation using safe UV irradiation methods for creation and characterization of novel mutants. Assessment data collected over three offerings of the course suggest that the labs, which combine original investigation in a scientifically realistic intellectual environment with learned techniques and concepts, were instrumental in improving students' learning in a number of areas. These include the development of critical thinking skills and understanding of concepts and methods. Student responses also suggest the labs were helpful in improving students' understanding of the scientific process as a rational series of experimental investigations, each building upon interpretation of previously acquired results. Survey data also suggest the labs are helpful in improving student awareness of the interdisciplinary nature of scientific inquiry.
INTRODUCTION

As scientist-educators we strive to involve students in the process of scientific inquiry in classrooms and research labs. Students can benefit in a number of ways from involvement in projects where they are “doing real science” as opposed to performing scripted “cookbook” activities. These include (1) gaining an understanding of the process of how scientific studies are conducted; (2) confirming that such studies produce credible results; and (3) seeing how engaging the process of scientific discovery can be. The report of the Committee on Undergraduate Biology Education to Prepare Scientists for the 21st Century underscored the significance of involving and inspiring undergraduate students through active learning as a means to better prepare them as future scientists and to “give them an enduring sense of the power and beauty of creative inquiry” (NATIONAL RESEARCH COUNCIL 2003). Committee findings also emphasized the importance of interdisciplinary research experiences and the utilization of “examples of research showing that science consists of unanswered questions” in an effort to “intrigue and inspire students to probe problems in depth”.

The integration of discovery-based exercises into undergraduate genetics laboratory curricula poses many challenges. These include budget limitations, performing activities within the constraints of prescribed laboratory periods over a term or semester, and student mastery of laboratory techniques as a prerequisite to performing actual experiments. However, the rewards of implementing active and discovery-based teaching approaches have been shown to be substantial (HANDELSMAN et al. 2004). As compared to traditional approaches, teaching methods utilizing active-learning exercises can result in increases in knowledge retention, student confidence, enthusiasm, and
satisfaction (SMITH et al. 2005; WYCKOFF 2001). Marcus and Hughes reported the development of inquiry-based genetics lab exercises using *Drosophila melanogaster* strains harboring *P*-element transposon insertions in novel recombination mapping activities (MARCUS and HUGHES 2009). While student responses to the lab exercises were variable, many students were reported to be excited by the prospect of doing real experimental science.

With the intent of improving the undergraduate biology curriculum at Trinity University through the introduction of new discovery-based and active-learning exercises, we developed a lab sequence in which students create and analyze novel mutations affecting lactose metabolism in *Escherichia coli*. The lab sequence intellectually and experimentally links techniques learned at the outset to an original, unscripted scientific investigation taking place over an 8-week period. Instead of being provided organisms with known genotypes, students create and maintain their own strains, thereby gaining a sense of project ownership. Students use safe UV mutagenesis to generate mutants and perform facile screens for mutant identification. This is followed by a logical sequence of directed inquiry activities: biochemical characterization of mutants using enzymatic and substrate transport assays, complementation analysis using simple plasmid transformations, and PCR amplification and sequencing of mutant alleles. The activity culminates in comparisons of mutant and wild type sequences to facilitate an understanding of how mutations manifest themselves in altered protein structures.

These labs reflect a methodical investigative approach using concepts and techniques to address questions lacking scripted answers. It was our hope that this active-learning investigative approach would captivate students and aid in their understanding of
technical concepts associated with the study of genetics. Additionally, students might develop an improved understanding of the process of scientific inquiry and the linkage between the larger picture of a scientific investigation and the stepwise accumulation of findings leading toward scientific discovery. Assessment data were collected following three consecutive years of teaching this sequence. Our findings suggest that this lab sequence is effective in developing student understanding of genetics concepts, the process of scientific inquiry, and the interdisciplinary crosstalk between scientific disciplines (e.g., biochemistry, genetics, and chemistry) in the process of scientific investigation.

MATERIALS AND METHODS

*Escherichia coli* was chosen for a number of reasons: i) handling and maintenance of the organisms requires little training and is inexpensive; ii) *E. coli* grows rapidly, with doubling times of ~ 30 minutes in rich media, allowing completion of exercises within a day or two; iii) many individuals can be analyzed (at appropriate densities, mutant phenotypes can be readily distinguished from among hundreds of surrounding colonies in a single dish); iv) phenotypically-interesting mutants are readily recovered following mutagenesis of the haploid genome; v) plasmid complementation can be done in a day; and vi) mutant allele PCR amplification and nucleotide sequencing allow comparative analysis with wild type sequences.

We focused on the *lac* operon, because it is usually covered in introductory genetics courses as a model of gene regulation, and can serve as a “Rosetta Stone” connecting textbook to lab. A wealth of information is also available regarding *lac* operon
component structure-function relationships, making it a logical place to expose students to gene structure, function, regulation, and protein structure.

We use these exercises as part of a required laboratory component of a 12-week sophomore-level undergraduate genetics course. The lab activities cover eight weeks, leaving time to repeat experiments if necessary. At the end of the sequence, students’ findings are reported in a research paper. The course is taught to three or four sections of approximately 16 students each (depending on enrollments); each section meets once a week for approximately three hours. Students work in pairs and are required to complete a 2-semester introductory biology course before enrolling in the course. Students learn pipet use in the introductory biology course, and are taught bacteriology skills in the genetics lab. Protocols and notes are available as a supplementary file (File S1), and all strains and plasmids are available upon request.

**Bacterial strains and plasmids:** *Escherichia coli* K12 strains were cultured at 37° C in Luria Bertani (LB) broth, or on LB or MacConkey agar medium with added kanamycin (Km, 50 μg/mL), tetracycline (15 μg/mL), or ampicillin (100 μg/mL) where appropriate. UV mutagenesis was done using strains W3110 (F-, λ-, IN(rrnD-rrnE)I) and an isogenic uvrA-phr mutant, constructed using standard P1 transduction methods with strains JW0698 (Δ(araD-araB)567 ΔlacZ4787(::rrnB-3) Δphr-758::Km λ- rph-1 Δ(rhaD-rhaB)568 hsdR514) and N3055 (λ- IN(rrnD-rrnE)I uvrA277::Tn10). lacY and lacZ complementing plasmids were prepared using pLC20-30 from the Clarke and Carbon collection (CLARKE and CARBON 1976), and standard cloning and PCR methods; details are given in Supplementary File S1.

**Week 1. Handling of bacterial cultures and dilution plating.**
Students are introduced to the philosophy and scope of the investigative mutagenesis experiment, and are instructed that they will generate their own mutants and characterize them in detail, from phenotypic analysis to nucleotide sequence analysis. We emphasize they will be doing a real genetics experiment in the sense that neither they nor the instructor have any \textit{a priori} knowledge of the properties of mutants they will create. We also emphasize that students need to think about how a geneticist would methodically approach a problem using a variety of techniques in a logical sequence, each bringing the researcher closer to the answer.

Students learn microbiological techniques for use throughout the semester. They perform serial dilutions in preparation for mutagenesis the following week. Aseptic techniques are demonstrated and each student inoculates and streaks out cultures.

**Week 2. UV mutagenesis of wild type \textit{E. coli} strain W3110 and UV-sensitive strain W3110-\textit{uvr-phrA6}.*

This lab introduces genetic selections versus screens. Mutant generation as a powerful genetics tool is discussed with the connection between phenotypic variation and nucleotide sequence level genotypic differences. DNA damage, mutation and repair provide the rationale for using isogenic strains. We contrast the use of a genetic \textit{screen} for defects in lactose fermentation on MacConkey agar with a \textit{selection} for antibiotic-resistant derivatives from an antibiotic-sensitive bacterial population, which they see in complementation assays.

UV mutagenesis was selected over more efficient chemical mutagens for safety reasons; it was selected over transposition-based methods because it allows for the
recovery of broader mutant classes with unique and subtle properties, for example temperature-sensitivity or alterations in catalysis due to amino acid substitutions.

The class is divided into two teams for mutagenesis. This lab requires organization and attention to detail; for this reason, one student from the lab section is chosen to organize and oversee the mutagenesis treatments. She or he is responsible for assigning students to timing UV exposures of cultures, assigning teams to plate out time points, etc. One team performs mutagenesis of the wild type strain, while the second team carries out mutagenesis of the UV repair mutant. Bacterial cell suspensions are exposed to UV irradiation for four different times at each of four different distances, giving a total of sixteen different treatments.

1.5 mL aliquots of cells in cold 0.1 M MgSO₄ are dispensed into 35 mm petri dishes for mutagenesis. We use a 15 W Sylvania G15T8 germicidal lamp as a source of UV-C radiation with overall irradiance and fluence rates of 600 mW/m² and 1.3 μmol/m²/sec, respectively (SHINKLE et al. 2005). To protect students, the lamp is hung behind a black curtain. Dishes are placed on a tray and the lids are removed immediately before being placed behind the curtain. It is imperative that the lids are removed to permit UV exposure; it is also critical that the dishes (not the lids) are labeled before irradiation to keep track of strains and treatments. Dishes are then removed from the UV source at appropriate time points.

Following UV exposure, students plate serial dilutions of each strain × distance × time on MacConkey agar to determine survival frequencies in irradiated cultures. Non-irradiated cells of both strains are plated out to provide initial culture densities. These data are used to determine survival frequencies between the strains, illustrating the
deleterious effects of UV exposure and the importance of photoreactivation and nuclease excision in UV-induced lesion repair.

Teams plate out aliquots of mutagenized cells of W3110-uvrA-pher to identify mutants. This activity need only be done with the UV repair mutant, as little mutagenesis will occur with strain W3110. Optimal exposure conditions for our lamp setup are described in File S1; for UV irradiation experiments, survival frequencies in the range of 0.1% - 1% result in the highest mutant recovery frequencies (MILLER 1972).

Following outgrowth, plating, and overnight growth of mutants, students inspect plates for rare Lac- colonies among many red Lac+ colonies. In the optimal irradiation range, 2–10 lactose-nonfermenting mutants can be found on plates containing ~500 colonies. Having identified putative mutants based on colony color, cultures are streaked onto MacConkey-Km plates to obtain single colonies. We encourage students to find as many mutants as possible in a reasonable time, since students may find false positives and/or strains with mutations lying outside of lacY or lacZ (e.g., lacI or crp; see Results and Discussion). Persistent students will find mutants, and these are maintained on plates for the duration of the lab by weekly streaking. Depending on colony density, mutant genotype stability, and colony picking skills, students may streak more than once to ensure they have stable single colony Lac- isolates before proceeding to subsequent experiments. We have found it valuable to build extra time into the schedule to allow students to establish stable Lac- strains before proceeding to the next exercise.

Alternatively, irradiated cultures can be screened for other mutants, e.g., auxotrophs or temperature-sensitive mutants; and/or mutagenesis can be modified to a more open-
ended approach where students are given license to come up with their own screens/selections.

**Week 3. The β-galactosidase assay for biochemical characterization.**

Here a simple biochemical assay is used to characterize Lac⁻ mutants. *lac* operon components are presented, including *lacY*, encoding the proton symport membrane protein required for lactose uptake; *lacZ*, encoding β-galactosidase, required for hydrolytic cleavage of lactose into glucose and galactose; and *lacI*, encoding the lactose repressor, which prevents *lac* expression in the absence of lactose. The *lac* operon is described in many if not all undergraduate genetics textbooks.

The qualitative β-galactosidase assay is used to determine whether cells produce functional *lacZ* gene products. This is done by assaying cell suspensions for the appearance of o-nitrophenol, the yellow hydrolysis product resulting from cleavage of the chromogenic LacZ substrate o-nitrophenyl-β-D-galactopyranoside (ONPG). The assay also demonstrates that *lac* expression is inducible using cultures grown in the presence and absence of the inducer isopropyl-β-D-thiogalactopyranoside (IPTG). Instructors can discuss reaction chemistry involved in glycosidic bond cleavage, particularly regarding reactive amino acids in the protein, as this can later be tied into *lacZ* mutant analysis at the nucleotide sequencing stage.

It is apparent to students that a Lac⁻ phenotype could be attributed to *lacZ* mutations resulting in nonfunctional enzymes, or mutations that affect *lacZ* expression. However, it is usually less obvious that Lac⁻ mutants may produce functional β-galactosidase, but possess defects in LacY: such mutants fail to produce colored products from ONPG because ONPG cannot enter the cell. *lacY* mutants can be distinguished from *lacZ*
mutants using whole intact cells and cells whose membranes have been permeabilized with a mild chloroform treatment. *lacZ* mutants will yield a negative reaction using either whole or permeabilized cells, whereas mutations in *lacY* will give negative results in whole cells but will yield positive results with permeabilized cells. A wealth of knowledge is also available regarding LacY: it is arguably the best understood proton symport protein (Guan and Kaback 2009, Liu et al. 2009, Smirnova et al. 2008, Zhou et al. 2009), and structural relationships can be drawn between wild type and mutant forms of LacY following sequencing of mutant *lacY* alleles.

In our experience, the majority of mutants identified by students carry mutations in *lacZ* or *lacY*, but alternative outcomes are possible (see Results and Discussion).

Following completion of this lab, students have solid biochemical evidence to support predictions of what type of mutant they have recovered and are ready to test these predictions by complementation analysis using plasmids carrying wild type *lac* alleles.

**Week 4. Plasmid extraction.**

Students are introduced to complementation and are shown how mutant phenotypes could be attributed to specific gene defects by introducing wild type alleles into mutants to see if the wild type phenotype is restored. Students are given strains carrying *lacZ* and *lacY* plasmids, and control vector pBR322, and they perform plasmid extractions. We use alkaline lysis for this but kits are also available.

**Week 5. Agarose gel electrophoresis, spectroscopy, and complementation of Lac- mutants.**

Agarose gel electrophoresis and UV spectroscopy are introduced for nucleic acid visualization and analysis using a Nanodrop instrument or UV spectrophotometer to
determine plasmid concentrations. Students prepare competent cells of their mutants and control \( lacZ \) and \( lacY \) mutants for plasmid transformation using a previously published procedure (CHUNG et al. 1989). Students inoculate media with strains the day before the lab, and subculture them the following morning for competent cell preparation and transformation that afternoon. Complementation with \( lacZ \)- and \( lacY \)-containing plasmids and the interpretation of results is typically straightforward. If a Lac\(^+\) phenotype is not restored to mutants carrying either \( lacZ^+ \) or \( lacY^+ \) plasmids, students can perform transformation using plasmids carrying \( lacI \) and \( crp \) (see Results and Discussion).

**Week 6. PCR amplification of mutant alleles from Lac\(^-\) mutants.**

Based on the previous weeks’ findings, students use PCR to amplify alleles from DNA of their mutants and control sequences from wild type parent DNA. Primer sequences and PCR protocols are given in the supplementary file File S1.

**Week 7. Agarose gel electrophoresis and DNA sequencing of PCR products.**

Students perform agarose gel electrophoresis to assess the success and specificity of PCR amplification of mutant and wild type control alleles. We have found it useful to build extra time into the schedule to troubleshoot and/or repeat failed PCR assays. Over the course of the week, PCR products are sent to the University of Texas in Austin, TX for sequencing.

**Week 8. Analysis of DNA sequencing results.**

The full scope and value of the lab is realized in the eighth week when students receive electropherogram and DNA sequence text files for comparison with wild type sequences. Here a fundamental connection is established between phenotypic defects of
mutants with genotypic alterations reflected in altered allele sequences. Students construct alignments of mutant and wild type sequences to identify sequence changes.

RESULTS AND DISCUSSION

Experimental results

The mutagenesis protocol is standard but utilizes a DNA repair mutant we constructed for the labs along with the isogenic Lac\(^+\) parent. Students directly compare the effects of irradiation at different distances and times within and between the two strains (Figure 1).

The MacConkey screen permits easy visualization of white Lac\(^-\) colonies amidst red Lac\(^+\) colonies (Figure 2). Student success in picking mutants varies and as mentioned previously, false positives will arise on these plates. In particular, we see Lac\(^+\) "fish eye" morphologies that initially appear white but which, following isolation, are shown to be Lac\(^+\). False positives may be accompanied by the production of elevated levels of secreted polysaccharides, which can initially be interpreted as Lac\(^-\) due to abundant whitish extracellular material. Students are encouraged to cast wide nets and spend time outside of normal lab times to obtain bona fide Lac\(^-\) derivatives.

The $\beta$-galactosidase assay provides a good indication of the genetic basis underlying mutant phenotypes (Figure 3). $\text{lacZ}$ mutant extracts will not produce a yellow reaction product, whereas yellow reactions indicate functional LacZ. Non-permeabilized $\text{lacY}$ mutant extracts will not produce yellow color over short time courses, whereas chloroform permeabilization of $\text{lacY}$ extracts will produce a yellow color within minutes. Wild type extracts produce a yellow product although product appearance is more rapid with permeabilized cells than with non-permeabilized cells, since permeabilization allows
more rapid interaction between substrate and enzyme. Positive and negative controls are included to highlight the importance of controls.

Plasmid extraction and transformation are straightforward and serve to connect complementation and cell biochemistry data. After this experiment, students can usually conclude whether they have isolated \textit{lacZ} or \textit{lacY} mutants.

As mentioned previously, students may isolate strains with mutations in genes other than \textit{lacY} or \textit{lacZ}. Among these alternative Lac\textsuperscript{−} isolates, one might find: i) mutations in \textit{lacI}, ii) mutations in \textit{lac} promoter or sequences bound by LacI, or iii) mutations involved in CRP-dependent catabolite control. On the one hand, the MacConkey screen is not suitable for identification of \textit{lacI} null alleles that do not efficiently bind \textit{lac} operator DNA: mutants of this type are typically Lac\textsuperscript{+} on MacConkey medium and would evade detection. However, it is possible to generate \textit{lacI} alleles that bind \textit{lac} operator DNA but which do not bind lactose or IPTG (ROMANUKA \textit{et al.} 2009). These mutants could appear as Lac\textsuperscript{−} or weakly Lac\textsuperscript{+}. Such mutants could be found using the MacConkey screen, and we suspect they would have variable $\beta$-galactosidase activities depending on their DNA binding kinetics. We would predict that $\beta$-galactosidase activities with these mutants would be identical regardless of whether they were grown in the presence or absence of IPTG. A \textit{lacI}\textsuperscript{+} plasmid is available for use although results may vary depending on a number of factors, such as the DNA binding properties of the mutant gene product and dimerization interactions with itself and plasmid-encoded wild type LacI.

Students could recover strains with mutations in \textit{crp}, encoding the cyclic-AMP receptor protein (CRP or CAP), as efficient \textit{lac} transcription following lactose- or IPTG-dependent induction requires RNA polymerase-CRP interactions (SHARMA \textit{et al.} 2009).
crp mutations adversely affecting RNA polymerase-promoter interactions could yield Lac’ mutants. We have not needed to use crp+ plasmids but these are available. If students pick multiple mutants, the odds of recovering lacZ and/or lacY mutants are very high; alternatively, the less straightforward characterization of a mutant yielding atypical β-galactosidase results may be an appropriate project for a particularly gifted, invested or enthusiastic student.

Following PCR, amplification products are sometimes larger than expected due to the occurrence of Tn10 insertions (Figure 4). The uvrA allele used for construction of the mutagenesis strain carries a Tn10 insertion, and while we didn’t initially intend for students to find transposon insertion mutants, the unexpected discovery of insertions serves our initial aims well: that is, to have students discover something in an unscripted investigation and to feel engaged in and excited about the science.

Sequence analysis of alleles reveals a variety of alterations. Close examination of fifteen lacY mutant sequences revealed the following: eight sequences carried Tn10 insertions in three different sites; one sequence carried an indel of >1.5 kb that extended beyond the lacY coding sequence; one sequence carried both an indel and a missense substitution; one sequence carried a frameshift mutation resulting from a single nucleotide indel; and four sequences were incomplete, likely resulting from low quality sequence templates following PCR amplification and cleanup. Representative mutant allele sequences are shown in Figure 5. We have found that sequence quality is directly affected by the quality of the sequence template; therefore care must be taken to ensure that PCR products are adequately cleaned up prior to sequencing.
We have thus far concluded the lab activities with the sequence analysis. It is possible to broaden the lab by including protein analysis. For example, available high-resolution structures of LacY, LacZ, LacI could be used as templates to generate predictive structures using homology modeling applications (e.g. SWISS-MODEL or 3Djigsaw) to explore how mutant alleles yield defective proteins.

**Student evaluation of experience**

In broad terms, our goal was to use a guided inquiry-based sequence to facilitate understanding of the scientific approach to problem solving and development of critical thinking skills. In addition to learning genetics concepts, we wanted students to appreciate the importance of bringing different disciplines (e.g., genetics, biochemistry, etc.) together to solve problems, as increasing interdisciplinary content is beneficial to student learning and development (NATIONAL RESEARCH COUNCIL 2003). To assess these outcomes, student surveys were administered over three years based on an instrument reported by Sleister (SLEISTER 2007). Survey questions used a 5-point scale intended to assess perceptions of what students gained from taking the labs (Q1-Q4), as well as asking them to compare the value of the directed inquiry approach to that of traditional labs (Q5-Q9) (Table 1). In this context, traditional labs previously taken by students would likely include, e.g., an introductory chemistry laboratory course utilizing scripted activities with *a priori* known outcomes.

Compared to before their participation, after taking the genetics lab students agreed that they had noticed improvements in all areas queried. These areas included understanding of scientific concepts (Q1, $\mu \geq 4$ over all three years) and the process of scientific inquiry itself (Q2, $\mu \geq 3.8$ over all three years). Students also perceived
improvements in their abilities to think critically (Q3, $\mu \geq 3.7$ over all three years) and see “big picture” connections between individual experiments (Q4, $\mu \geq 4.1$ over all three years). Additionally, mean values for Q1-Q3 increased over the three year assessment period, while the mean value for Q4 over survey year three was also higher relative to survey year one.

Questions Q5-Q7 queried students regarding course value relative to previously taken traditional lab courses in terms of understanding technical concepts and methods, and understanding the interdisciplinary linkage of scientific inquiry. Students generally agreed that the approach taken by the labs was helpful in their understanding of technical concepts and methods (Q5, $\mu \geq 4.1$ over all three years). Survey data also suggest that the thematic nature of the labs facilitated student understanding of the project as a whole and their abilities to make connections between experiments (Q6, $\mu \geq 4$ over all three years). The results also suggest the labs helped students to see the multidisciplinary nature of scientific inquiry (Q7, $\mu \geq 4.1$ over all three years). Questions Q8 and Q9 were used to assess the perceived value of the labs vis-à-vis students’ futures at the institution and beyond. Most students entering the course follow a pre-professional track toward medical school or other health professions. Students felt these labs were helpful in achieving academic (Q8, $\mu \geq 3.7$ over all three years) as well as professional goals (Q9, $\mu \geq 3.6$ over all three years).

A year-to-year variation in survey results was also observed. This variation likely stems from improved delivery of course material and participation by multiple faculty. However the labs were taught by a single instructor during year three, thereby creating greater course continuity (all year three questions had $\mu \geq 4$).
In summary, we believe this laboratory experience may be valuable in reaching goals that active, engaging teaching methods hope to achieve. We observe students to be engaged in the labs as they work through the scientific process in a real experimental context and are simultaneously increasing their understanding of basic genetics techniques and concepts. Also, students see this lab as a valuable experience, and significantly, we find many of them having fun in the process.

LITERATURE CITED


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Table 1. Assessment questions and responses.

Think about your abilities at the beginning versus end of the semester. Using the scale 1–5, please indicate the level to which you agree with the following statements:

5=Strongly Agree, 4=Agree, 3=Neutral, 2=Disagree, 1=Strongly Disagree:

As compared to before participating in genetics lab, after taking genetics lab I noticed an improvement in:

<table>
<thead>
<tr>
<th>Survey Question</th>
<th>F ’07 (N=36)</th>
<th>F ’08 (N=29)</th>
<th>F ’09 (N=37)</th>
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<tbody>
<tr>
<td>1. My understanding of technical/scientific concepts (e.g., PCR, molecular cloning).</td>
<td>4.03 ±0.91</td>
<td>4.21 ±0.56</td>
<td>4.32 ±0.75</td>
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<td>2. My understanding of how science is done (i.e., the range of activities from asking a biological question to conducting an experiment and interpreting data).</td>
<td>3.81 ±0.86</td>
<td>4.00 ±0.76</td>
<td>4.05 ±0.70</td>
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<td>3. My ability to think critically.</td>
<td>3.75 ±0.91</td>
<td>4.17 ±0.60</td>
<td>4.24 ±0.72</td>
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<td>4. My ability to make connections between individual experiments (e.g., the “big picture” of the project).</td>
<td>4.14 ±0.93</td>
<td>4.07 ±0.70</td>
<td>4.46 ±0.73</td>
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Have you taken a traditional science laboratory course? ______ If you answered “yes” to the previous question, please use the scale 1–5 to indicate the level to which you agree with the following statements.

5=Strongly Agree, 4=Agree, 3=Neutral, 2=Disagree, 1=Strongly Disagree

Relative to other more traditional science laboratory courses, I believe…

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<th>Survey Question</th>
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<th>F ’08 (N=25)</th>
<th>F ’09 (N=30)</th>
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<td>5. The inquiry-based approach used in lab helped me better understand genetics concepts and methods (e.g., mutation, gel electrophoresis).</td>
<td>4.27 ±0.67</td>
<td>4.12 ±0.78</td>
<td>4.47 ±0.57</td>
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<td>6. The inquiry-based approach helped me to make connections between different concepts/experiments.</td>
<td>4.00 ±0.94</td>
<td>4.00 ±0.82</td>
<td>4.33 ±0.84</td>
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<td>7. The inquiry-based approach helped me realize that research is interdisciplinary (e.g., a mutation at the level of DNA affects the</td>
<td>4.27 ±0.80</td>
<td>4.16 ±0.69</td>
<td>4.37 ±0.67</td>
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cellular/biochemical level; methods are required from more than one subject area—e.g., genetics and chemistry).

8. The concepts and technical skills I gained while participating in lab helped me/will help me in my further studies at Trinity and beyond.

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<td>3.73 ±1.18</td>
<td>4.24 ±0.72</td>
<td>4.10 ±0.80</td>
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9. The concepts and skills I gained while participating in lab will help me in my future career.

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<td>3.58 ±1.09</td>
<td>3.84 ±1.11</td>
<td>4.07 ±1.11</td>
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FIGURE LEGENDS

Figure 1. Student-generated survival curves of *Escherichia coli* W3110 wild type (solid symbols) and W3110-uvrA-phr (open symbols) following UV irradiation. Exponential phase cultures were pelleted by centrifugation and resuspended in cold 0.1 M MgSO₄ and cell suspensions were exposed to UV source for the indicated times at distances of 45 inches (wild type, ■; mutant, □) and 25 inches (wild type, ●; mutant, ○). Following irradiation, cells were plated onto MacConkey agar medium and enumerated following overnight growth at 37°C.

Figure 2. Screen and single colony isolation of W3110-uvrA-phr Lac⁻ mutants on MacConkey agar medium following UV irradiation. Suspensions of UV repair deficient *E. coli* W3110-uvrA-phr in 0.1 M MgSO₄ were exposed to UV source for varying times and at varying distances as described (File S1). Following outgrowth and segregation of mutagenized populations in Luria Bertani broth, serial dilutions were plated on MacConkey agar medium and incubated overnight at 37°C. (A) Serial dilution plating of mutagenized cells on MacConkey agar. Lac⁻ mutants are indicated with black arrows. (B) Comparison of single colony isolations on MacConkey medium of Lac⁻ mutant (left) and wild type Lac⁺ parent (right).

Figure 3. Differentiation of *E. coli* lacZ and lacY mutants using a qualitative β-galactosidase assay. Bacterial cultures are grown in Luria Bertani medium containing IPTG for 2–3 hr assaying cell extracts (File S1). β-galactosidase-dependent production of o-nitrophenol in chloroform-permeabilized cells (right, + CHCl₃) allows the
discrimination of \textit{lacY} mutants from \textit{lacZ} mutants. \textit{wt}, wild type \textit{Lac}⁺ \textit{E. coli}; \textit{lacY}, \textit{lacY} lactose transport mutant; \textit{lacZ}, \textit{lacZ} \textit{β}-galactosidase mutant. Over the short reaction time course shown (2-4 minutes), no activity is observed in intact cells that have not been permeabilized (left, \text{-CHCl₃}).

Figure 4. Gel electrophoresis of \textit{lac} allele PCR amplification products from genomic DNA of \textit{lacZ} Tn10 insertion mutant (lanes 2-6) and \textit{Lac}+ parent W3110-\textit{uvrA-phr} (lanes 7-11). Lane 1, kb ladder; lanes 2 and 7, \textit{lacI} amplification products; lanes 3 and 8, \textit{lacZ} region 1 products; lanes 4 and 9 \textit{lacZ} region 2 products; lanes 5 and 10, \textit{lacZ} region 3 products; lanes 6 and 11, \textit{lacY} products. Due to its larger size, \textit{lacZ} is amplified and sequenced as three separate \textasciitilde 1 kb fragments; extraction and amplification methods are described in File S1.

Figure 5. Partial alignment of representative \textit{lacY} mutant types with wild type \textit{E. coli} W3110 \textit{lacY} sequences. Wild type \textit{lacY} sequence (\textit{lacY}) is given at the top of each panel. (A) T → A transversion mutation (shaded and underlined) in mutant H9 (MUT H9) sequence found at nt position 59 with respect to wild type \textit{lacY} translation start, resulting in F59Y substitution in LacY permease. Altered phenylalanine residue (shaded and underlined in wild type LacY sequence) occurs in LacY transmembrane domain 1 (\textit{MIRZA \textit{et al.} 2006}). (B) Frameshift mutation resulting from G insertion (shaded and underlined) in mutant A12 (MUT A12) at nt position 492 relative to \textit{lacY} translation start. Mutation gives rise to LacY derivative (LacY⁺) with altered amino acid sequence beginning at residue 172, in transmembrane helix 6 (\textit{MIRZA \textit{et al.} 2006}). (C) Tn10 insertion in mutant
A9 (MUT A9) at nt position 109 relative to wild type lacY translation start. First nucleotide of Tn10 insertion is shaded and underlined. Tn10 sequence is given as reverse complement of GenBank accession AY319289. First four residues of altered peptide sequence (LacY’) are underlined and occur in N-terminal periplasmic domain (MIRZA et al. 2006).
Figure 1.
Figure 2.
Figure 3.
Figure 4.

\[ l_{ac}Z::Tn\,10 \quad \text{Lac}^+ \]

\[ \begin{array}{cccc}
I & Z_1 & Z_2 & Z_3 & Y \\
I & Z_1 & Z_2 & Z_3 & Y \\
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Figure 5.

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