An Undergraduate Laboratory Exploring Mutational Mechanisms in *Escherichia coli* Based on the Luria-Delbrück Experiment

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Understanding the mechanism for DNA mutations is a key concept in most genetics and microbiology courses. In addition, understanding that most mutations occur prior to exposure to selection is an important yet often difficult concept for students to grasp. We developed an undergraduate laboratory activity on mutation mechanisms based on the classic experiment from Luria and Delbrück. The activity uses *Escherichia coli* as the model organism and the antibiotic streptomycin for selection. Students gain hands-on experience with an important experiment in genetics, and the laboratory contains an investigative component in having students calculate mutation rate for streptomycin resistance and in having the students design a follow-up experiment. *E. coli* has a knockout collection available, and we used a wild-type strain and a ΔmutS strain in the laboratory exercise. The ΔmutS strain is missing an enzyme in the mismatch repair pathway, and students calculate and compare the mutation rate and frequency for both the wild type and the knockout strain. Assessment of student learning showed that students had a significant gain in understanding of mutational mechanisms. An optional, additional experiment involving PCR and DNA sequencing of streptomycin-resistant mutants is also presented.

**KEYWORDS** mutation, Luria-Delbrück, *Escherichia coli*, genetics, antibiotic resistance, mismatch repair

**INTRODUCTION**

**Background**

Understanding mutations, including when they occur relative to selection and how they are repaired, is a key concept covered in most introductory genetics courses. Students frequently have misconceptions about topics related to mutation (1), and additional learning opportunities are necessary to address these misconceptions. Laboratory activities have the potential to increase student learning (2, 3), and this activity provides a laboratory-based exploration of the topic of mutation using the classic genetics experiment by Luria and Delbrück (4).

Luria and Delbrück’s 1943 experiment used *Escherichia coli* as the model organism and showed that phage resistance was preexisting in populations (“spontaneous” model for mutation) rather than occurring after exposure to phage (“acquired” model of mutation). Briefly, the experiment involved growing individual cultures in the absence of selection and then plating each on selective media (media with phage). If the acquired model of mutation was correct, all plates from the individual cultures would contain similar numbers of mutants, since they were all exposed to the same selective pressure at the same time. However, if the spontaneous model of mutation was correct, there would be fluctuation in the number of resistant cells between individual culture tubes. This is because mutations could occur late in growth (producing few resistant cells) or early in growth (producing many resistant cells, so-called “jackpot” plates; Fig. 1). A batch culture was used as a control to show that variance was low if all mutants were present in a single population rather than individual tubes.

Previous undergraduate laboratories have incorporated the Luria-Delbrück design using the bacterium *Serratia marcescens* (5, 6) and yeast (7) as model organisms, with both using an antibiotic as the selective agent. We designed our laboratory activity to use *E. coli* as the model organism and an antibiotic (streptomycin) as the selective agent. We chose *E. coli* as our model organism primarily because there is a knockout collection available (Keio Collection [8]). This provides an opportunity for instructors and/or students to choose test strains from the knockout collection and compare how the Luria-Delbrück data from these strains compare to those for the wild-type strain. In addition, BSL-1 wild-type *E. coli* strains are readily available. We designed the laboratory to use streptomycin for selection instead of phage. This allows students to conduct an inquiry-based experiment with respect to mutation rate and mechanism, separate from that of the original Luria-Delbrück paper. Antibiotic resistance is also an...
important topic in genetics and microbiology and is often of interest to students since most are familiar with this issue. In addition, using antibiotics instead of phage allows for a simpler experimental setup for instructors and a more accessible lab for students, especially if students are new to microbiology lab skills.

The topic of DNA repair is typically covered with mutation in most introductory genetics courses, and we include this concept in our laboratory, as well. Students run the Luria-Delbrück experiment on a DNA repair mutant (ΔmutS) in addition to the wild-type strain. The MutS protein is involved in recognizing mismatched DNA bases after replication and initiating the mismatch repair pathway, and thus ΔmutS strains have a mutation rate higher than that of wild-type strains (9). The student protocol contains questions that prompt students to compare wild-type and ΔmutS strain data and to explain the observed differences between these strains. The addition of the ΔmutS strain broadens the scope of this laboratory and encourages students to think critically about factors that may affect mutation rate.

Intended audience and prerequisite student knowledge

The intended audience for this laboratory is an introductory genetics or microbiology course, and this is typically a sophomore-level lab for students. Students should be familiar with concepts of mutation and selection, have basic knowledge of what microbes are and how they grow, and be able to use aseptic techniques when working with microbes. Most students who completed this lab at SUNY Geneseo were biology or biochemistry majors and had basic microbiology skills (plating, dilution series, aseptic technique). Basic background knowledge of microbes, mutations, and evolution (from an introductory biology course) is required for this laboratory exercise.

Learning time

This lab is designed to be conducted primarily over two laboratory sessions, with a short amount of time during the third session for data collection. The first session takes approximately 1.5 h, depending on how much introductory material is provided for the students. This would allow additional laboratory work to be conducted during this time. The second laboratory session takes approximately 2 to 3 h, depending on whether students design and set up a follow-up experiment. Approximately half an hour is needed during the third session for data collection. Optionally, students can collect these data outside the designated laboratory time, and in this case no third session is needed.

Learning objectives

Upon completing this laboratory exercise, students will be able to (i) explain mutation mechanisms for the mismatch repair pathway and argue that mutations occur prior to selection exposure, (ii) differentiate between mutation rate and mutation frequency, (iii) explain the setup of the Luria-Delbrück experiment, (iv) calculate and interpret data from this experiment, including mutation rate and frequency, and (v) compare and contrast DNA repair mutant strain data to those of a wild-type strain.
PROCEDURE

Materials

A detailed description of the materials required for this laboratory activity is provided in Appendix 1.

Student instructions

Depending on the prerequisite knowledge of the students, a brief review of the Luria-Delbrück experiment and its significance may be necessary. In addition, before the second laboratory session, we typically review concepts related to mutation and DNA repair, and students should read the article by Meneely (10). A student protocol is included in Appendix 2.

Laboratory week number 1

The goals of the laboratory this week are for students to (i) determine the culture concentration (cells/mL) of batch and individual cultures and (ii) plate these cultures onto selective medium (medium plus streptomycin). The student protocol includes detailed information on steps that were conducted by the instructor prior to the laboratory session. Students will be provided with batch and individual cultures that were grown overnight. There is one batch flask per lab section and at least one individual culture tube per student. Each student measures the concentration of the batch culture and of individual culture samples. We used the conversion factor $8 \times 10^8$ cells/mL per optical density at 600 nm (OD$_{600}$) to convert our absorbance values to cells/mL. Students then centrifuge 1 mL of individual or batch cultures and plate the cells onto Luria broth agar plates containing 25 $\mu$g/mL streptomycin (LB+Strep). Students use sterile spreaders to disperse the sample on the plate and incubate the plate upside down at 37°C for 24 to 48 h.

Students also plate cells from the parent culture to confirm that streptomycin resistance mutations were not already present in the parent culture before the batch and individual cultures were inoculated. Students should be reminded that the parent culture was kept at 4°C after inoculating the individual and batch cultures and should refer to Fig. 1 of the student protocol. Students spin down and plate the parent culture sample onto both LB and LB+Strep plates. This control confirms that the parent strain was viable but not streptomycin resistant. The parent culture plates are also incubated upside down at 37°C for 24 to 48 h.

Laboratory week number 2

The goals of the laboratory this week include the following: (i) students run and analyze a simulation of the experiment, (ii) students count colonies on their plates and use class data for analysis, and (iii) students design and set up a follow-up experiment. Before students collect data, we have students run simulations using the tools developed in reference 11. The simulation is an excel-based tool that allows students to visualize hypothetical individual and batch culture data and is valuable for helping students understand what to expect for their individual versus batch cultures. Students count colonies on their batch and individual culture plates and record their group data. Data are also input into a shared spreadsheet.

After data are collected, the instructor reviews the topic of mutation rate versus frequency and discusses the calculations for mean, variance, and mutation rate. Students use the data set from the class as a whole to calculate mean and variance and discuss whether the data are consistent with the spontaneous or acquired model of mutation. Students then compute the average mutation frequency and compare these values between strains. Finally, students calculate the mutation rate for the wild-type strains. Students discuss whether the mutation rate is similar to known values for E. coli.

If time permits, students design and set up a follow-up experiment. Students discuss their experiment as a group and obtain feedback from the instructor on their proposed experiment and their overall experimental design. Student experiments from prior semesters have included (i) testing the streptomycin-resistant colonies for resistance to other antibiotics, (ii) testing the MIC for streptomycin-resistant colonies, and (iii) testing for contamination, particularly for any colonies that do not have a typical E. coli colony morphology. Instructors typically provide a list of available materials for students to use. If materials are not available, instructors have also given students the option to meet at an additional time and prepare materials in order to set up their experiment, at the discretion of the instructor. Laboratories that incorporate opportunities for students to ask and answer their own questions are typically more successful in terms of learning outcomes (12), and we encourage instructors to incorporate this section of the laboratory.

Laboratory week number 3

The goals for this week are to collect and analyze data from the follow-up experiment. Equipment and materials needed will depend on the follow-up experiments chosen by students.

Faculty instructions

A detailed description of the instructor protocol is found in Appendix 3, and a spreadsheet to aid in experimental setup is found in Appendix 4. Briefly, the instructor will streak out E. coli strains onto LB agar plates, test those colonies for existing streptomycin resistance mutations, inoculate a streptomycin-sensitive colony as the parental culture, and inoculate batch and individual cultures for students to use in week 1 of the lab. All E. coli strains should be streaked out fresh from a glycerol freezer stock, and a freshly made stock of streptomycin should be used to make plates. For
the most consistent results, use plates within 1 week, though streptomycin should be stable in plates for up to 1 month (13).

Students should read the paper by Meneely (10) before week 2 of the lab, and this paper will also be useful for instructors who would like a review of the Poisson distribution and how it is used to calculate mutation rate. Instructors may also want to read the original Luria-Delbrück paper (4) and assign and discuss this paper with students if time permits. The authors typically include a discussion of the original Luria-Delbrück data in the lecture portion of the genetics course. Depending on the size of the lab section, the instructor can choose how many individual culture tubes to inoculate. Across each section, we suggest 10 or more total individual and batch cultures be plated in order to obtain reliable estimates of variance. Depending on the section size and resources available, this could require one of each type of culture per student or per lab group. This will increase the likelihood that at least one produces a plate with zero colonies (for the Poisson distribution mutation rate calculation) and that at least one is a “jackpot” plate with many colonies.

Due to its high mutation rate, the ΔmutS strain may have streptomycin-resistant colonies on the parent culture plate, and it does not typically produce individual culture plates with zero colonies. In this case, students can compare values on their averaged, normalized mutant frequency counts between the wild type and the ΔmutS strain instead of comparing mutation rates. This strain provides an interesting point of conversation for students about why we check for streptomycin-resistant colonies in the parental strain and about how the mutation rate calculation works.

This experiment has been conducted over five separate semesters at SUNY Geneseo, and while the actual number of streptomycin-resistant mutants can vary somewhat between semester to semester, the data consistently illustrate the principles from the Luria-Delbrück experiment and allow students to calculate mutation rate (Appendix 6, exceptions being one section in Fall 2019 and one section in Spring 2021). It is important that incubation times of the parent culture, individual, and batch cultures are kept consistent, such that the number of cell divisions (and opportunities for mutation) is the same between sections; variations in growth time likely contribute to the variation we observed between semesters. However, the lab is robust and has worked well for different sections and different instructors.

Overall, this laboratory is accessible for introductory genetics or microbiology students, particularly if they have already covered basic plating and aseptic techniques. We have gotten repeatable and usable results from this lab over several semesters and with 3 to 4 different faculty using the protocol. This laboratory works best if multiple sections can pool their data and/or collect data from different strains (one section uses wild type, the other uses ΔmutS, for example).

Suggestions for determining student learning

In order to assess learning, pre- and posttest questions were administered to students, and these questions can be found in Appendix 5. In addition, students in the genetics laboratory could choose one of their semester experiments, including this one, to write up as a laboratory report and for which to prepare an oral presentation.

Sample data

Sample data for the streptomycin-resistant colony counts for both wild-type and ΔmutS strains are shown in Table 1. Sample data for corresponding mean, variance, average mutation frequency, and mutation rate data from this experiment are shown in Table 2. Due to its high mutation rate, the ΔmutS strain data, the Poisson distribution mutation rate equation cannot be used since none of the plates had zero colonies. Students should note the difference in mutation frequency between individual and batch cultures and between wild-type and ΔmutS strains. They should interpret the differences in variance between individual and batch cultures for both strains. The data set from Table 1 is provided in Appendix 6, along with data sets from additional semesters in which we ran this experiment. This may be useful for instructors in case the experiment fails and example data are needed, or in case the lab is being conducted in an online format and students cannot collect data themselves.

Safety issues

All E. coli strains used in this laboratory are BSL-1. Students are required to wear the appropriate personal protective equipment and instructed on appropriate handling of biohazardous waste. In the genetics lab, students have already conducted prior experiments involving aseptic technique and plating, so this information will need to be addressed if it was not covered previously. All contaminated materials are either autoclaved or treated with a 10% bleach solution before disposal. Students may need additional safety instructions based on their follow-up experiments, and any necessary safety discussion should be included when the instructor approves their experimental design and setup.

DISCUSSION

Field testing

This activity was implemented in the introductory genetics laboratory course at SUNY Geneseo during the Spring 2019 semester and has been a part of the curriculum for most subsequent semesters. Laboratory sections are typically 10 to 16 students in size. The laboratory was implemented successfully across several semesters and with several different instructors, indicating that the student and instructor protocols are clear and effective. In addition, the lab was successfully conducted in a hybrid setting during one semester, in which the experimental portion was done during one lab session and all of the data analysis was conducted virtually.
Evidence of student learning

Student learning was assessed with pre- and posttest questions during the Spring 2021 semester (collection of student data was approved for exempt status by SUNY Geneseo’s Institutional Review Board, IRB no. 202021037). We asked the following four assessment questions. (i) True or False: A mutation for antibiotic resistance will occur in response to (after) exposure to the antibiotic. (ii) You grow two independent cultures of *E. coli* overnight in LB medium. The next day, you plate the cells on LB+streptomycin. Would you expect the plates from the independent cultures to have the same number of streptomycin-resistant colonies (yes or no)? (iii) In the Luria-Delbrück fluctuation test, which of the following values “fluctuates” (varies): mutation rate, mutation frequency, both, neither? (iv) You conduct a Luria-Delbrück experiment and get the following data (see appendix for table). Would you predict that these data are from the individual cultures or the batch culture? Assessment questions can be found in Appendix 5. The first assessment question addresses our learning objective i (LO i) and tests whether students grasp the key concept from the experiment, that mutations occur prior to selection. The second assessment question addresses LO iii and indicates whether students understand the basic setup of the experiment. The third assessment question addresses LO ii and tests whether students understand the difference between mutation frequency and mutation rate. Finally, question four addresses LO iv and requires that students interpret example data from an experiment. Our assessment questions do not specifically address the mutant (ΔmutS) strain and LO v, primarily because this leaves the option open for instructors to choose a different mutant strain if they prefer.

### TABLE 1

| Tube no. | No. of StrR colonies of wild-type strains in: | No. of StrR colonies of ΔmutS strains in: |
|----------|---------------------------------------------|------------------------------------------|
|          | Individual cultures | Batch culture | Individual cultures | Batch culture |
| 1        | 264             | 27           | 327             | 261           |
| 2        | 171             | 25           | 193             | 239           |
| 3        | 136             | 12           | 191             | 219           |
| 4        | 64              | 4            | 180             | 201           |
| 5        | 57              | 3            | 166             | 194           |
| 6        | 42              | 3            | 154             | 188           |
| 7        | 32              | 2            | 139             | 188           |
| 8        | 32              | 2            | 137             | 178           |
| 9        | 19              | 2            | 117             | 171           |
| 10       | 12              | 2            | 112             | 149           |
| 11       | 11              | 1            | 107             | 110           |
| 12       | 3               | 1            | 90              | 107           |
| 13       | 3               | 1            | 75              | 104           |
| 14       | 2               | 1            | 72              |               |
| 15       | 1               | 1            | 72              |               |
| 16       | 1               | 1            | 57              |               |
| 17       | 1               | 0            |                 |               |
| 18       | 0               | 0            |                 |               |
| 19       | 0               | 0            |                 |               |
| 20       | 0               | 0            |                 |               |
| 21       | 0               | 0            |                 |               |
| 22       | 0               | 0            |                 |               |
| 23       | 0               | 0            |                 |               |
| 24       | 0               | 0            |                 |               |
| 25       | 0               | 0            |                 |               |
| 26       | 0               | 0            |                 |               |
| 27       | 0               | 0            |                 |               |

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Students in all semesters improved on each of the four questions, despite some variation between semesters, though statistically significant \((P<0.05, \text{t test, unpaired})\) gains were observed for only question 3 in semester 2 (Fig. 2A). No improvement was observed for question 4 in semester 3, but students scored highly on this question to begin with. Learning gains (calculated from data for all three semesters combined) for questions 1 to 4 were 8.9%, 10.4%, 9.6%, and 14.5%, respectively. The overall averages on student posttests improved for all semesters individually (Fig. 2B), though the improvement was statistically significant \((P<0.05, \text{t test, unpaired})\) for only semesters 2 and 3. Since our assessment questions cover most of our learning objectives, this suggests that students are meeting the learning objectives for the lab.

Overall, questions three and four were the more difficult questions for students. Assessment question three dealt with differences in mutation rate and frequency, which is one of the more difficult concepts in this laboratory for students to grasp. We have added an additional question to the student protocol to address this concept specifically so that students get experience in the lab tying this back to their data directly. Question four has students interpret example data from a Luria-Delbrück experiment, and it makes sense that most students would not perform as well on this question before the lab. This question showed the most student improvement on the posttest in the combined data set, indicating gains in student learning with respect to data analysis for the experiment.

Additionally, students were assessed via lab reports and presentations (completed in groups). Typically, we allow students to choose an experiment they have done over the course of the semester to write up and/or present on, so not all students in the lab will necessarily choose this experiment. Though all of the learning objectives can be assessed in a lab report or presentation format, they are an especially ideal place for students to compare and contrast mutant and wild-type strain data and to go into detail on how their calculations of mutation rate and frequency support Luria and Delbrück’s original conclusions about mutations. In addition, this type of assessment allows students to analyze data from their follow-up experiments and to place them within the larger context of the Luria-Delbrück experiment. In general, students were successful on these assignments; for example, in the spring 2021 semester, the average lab report grade was a 93.4% \((n=15 \text{ groups})\). Some students still struggled to interpret their mutation rate and frequency data in terms of how they can be used to support the fact that mutations occur prior to selection. We recommend, when possible, that instructors have students write a lab report and/or presentation on this experiment in order to reinforce the learning objectives.

**Possible modifications**

It is possible to extend this lab to include follow-up experiments incorporating PCR and DNA sequencing, two topics

| Strain and culture | Mean | Variance | Avg cells plated | Avg mutation frequency | Mutation rate (Poisson equation) |
|-------------------|------|----------|------------------|------------------------|----------------------------------|
| Wild-type E. coli B |      |          |                  |                        |                                  |
| Individual        | 31.5 | 3,793.3  | 3.23E+09         | 9.76E–09               | 3.08E–10                         |
| Batch             | 4.6  | 60.3     | 2.85E+09         | 1.62E–09               | 6.47E–10                         |
| ΔmutS strain      |      |          |                  |                        |                                  |
| Individual        | 136.8| 4,247.7  | 3.73E+09         | 3.66E–08               |                                  |
| Batch             | 177.6| 2,254.2  | 3.92E+09         | 4.53E–08               |                                  |

**FIG 2.** Assessment data from three semesters, each taught by a different faculty member. (A) Students improved on each of the four assessment questions, except for question 4 in semester 3. Significant differences are indicated by an asterisk \((P<0.05, \text{t test, unpaired})\). (B) The average score on the posttest was higher than that on the pretest for all semesters and was statistically significant for semesters 2 and 3 \((P<0.05, \text{t test, unpaired})\). Median values for sections 1, 2, and 3 for the pretest were 50%, 75%, and 50%, respectively. Median values for the posttest were 75% for all semesters.
which are often covered in an introductory genetics laboratory. We have not yet incorporated these follow-up experiments into our laboratory activity, but we include an instructor protocol and preliminary data (Appendix 3 and 7) and have piloted the sequencing of the \( rpsL \) gene during one semester. In the pilot, 11/12 student groups obtained usable \( rpsL \) sequencing data. A suggested timeline and an approximate required laboratory time for this addition are included in the instructor protocol, as well. The additional experiments involve investigating specific DNA mutations that confer streptomycin resistance in \( E. coli \). Most streptomycin resistance mutations in \( E. coli \) occur within the \( rpsL \) gene (14), and students will PCR amplify and sequence the \( rpsL \) gene in streptomycin resistance colonies from their Luria-Delbrück plates. First, students would use colony PCR to amplify the \( rpsL \) gene from one of the streptomycin-resistant colonies that occurred in their Luria-Delbrück experiment. Students run the PCR products on a gel to confirm the PCR was successful. Next, students would conduct a PCR purification protocol and then measure the concentration (ng/\( \mu \)L) of their purified PCR product. If time and/or resources are limited, the purification step can be done by the sequencing center instead. PCR products are sent for sequencing, and students compare the \( rpsL \) sequence from their streptomycin-resistant mutant to the wild-type \( rpsL \) sequence in order to identify the mutation. Students can classify the mutation based on the specific base change, as well as how the protein was affected (nonsense, missense, etc.). Example data of streptomycin-resistant mutants obtained from this experiment are provided in Appendix 7. From 16 different streptomycin-resistant colonies sequences, we observed seven different mutations; even if the laboratory section is small, students are likely to observe a variety of mutations.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

- **SUPPLEMENTAL FILE 1**, XLSX file, 0.1 MB.
- **SUPPLEMENTAL FILE 2**, XLSX file, 0.1 MB.
- **SUPPLEMENTAL FILE 3**, PDF file, 0.4 MB.

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