An integrated biochemistry and genetics outreach program designed for elementary school students

Eric D. Ross, Sarah K. Lee¹, Catherine A. Radebaugh, Laurie A. Stargell

Department of Biochemistry and Molecular Biology, Colorado State University,
Fort Collins, CO 80523-1870

¹Present address: Chemistry and Biochemistry, Abilene Christian University, Abilene
TX 79699
Running Title: Biochemistry and genetics outreach

Key words: Outreach, elementary school students, yeast, genetics, enzymes

Corresponding author:

Laurie A. Stargell

Mailing address: Department of Biochemistry and Molecular Biology
Colorado State University
Fort Collins, CO 80523-1870.

Phone: (970) 491-5068
Fax: (970) 491-0494
E-mail: Laurie.Stargell@Colostate.edu
ABSTRACT
Exposure to genetic and biochemical experiments typically occurs late in one’s academic career. By the time a student has the opportunity to select specialized courses in these areas, many have already developed negative attitudes towards the sciences. Given little or no direct experience with the fields of genetics and biochemistry, it is likely that many young people rule these out as potential areas of study or career path. To address this problem, we developed a seven-week (~1 hour per week) hands-on course to introduce fifth grade students to basic concepts in genetics and biochemistry. These young students performed a series of investigations (ranging from examining phenotypic variation, in vitro enzymatic assays and yeast genetic experiments) to explore scientific reasoning through direct experimentation. Despite the challenging material, the vast majority of students successfully completed each experiment, and most students reported that the experience increased their interest in science. Additionally, the experiments within the 7 week program are easily performed by instructors with basic skills in biological sciences. As such, this program can be implemented by others motivated to achieve a broader impact by increasing the accessibility of their university, and communicating to a young audience a positive impression of the sciences and the potential for science as a career.
INTRODUCTION

As students progress from elementary school through high school, their attitudes towards science tend to become progressively more negative (Ayers and Price 1975; Jarvis and Pell 2002; Morrell and Lederman 1998; Simpson and Oliver 1985). Attitudes towards science may affect both classroom performance (Koballa and Crawley 1985; Rennie and Punch 1991; Tuan et al. 2005) and the courses in which students choose to enroll (Farenga and Joyce 1998). This presents a significant challenge for recruiting students into fields such as biochemistry and genetics, where the difficult subject matter means that students are traditionally not exposed to even the basic concepts of these fields until late in their academic careers. By this point, negative attitudes towards the sciences have often solidified; consequently, many students rule out these career choices without ever having been exposed to the fields.

To address this problem, we developed a curriculum that introduces very young students to basic biochemical and genetic concepts. We chose to target fifth graders, because they tend to still have a relatively positive view of the sciences (Morrell and Lederman 1998), and are old enough to perform basic biochemical experiments.

In teaching basic genetic and biochemical concepts, we focused on the following major areas: genetic variation, the function of enzymes in biological systems, the role of DNA mutation in conferring phenotypic changes, and the use of model systems. The popular press frequently discusses each of these topics, and therefore a basic understanding of these areas promotes scientific literacy. Numerous products, ranging from detergents to shampoos, advertise that they contain enzymes, yet almost none of the fifth grade students that we worked with had even a basic understanding of the function
of enzymes. Understanding the effects of DNA mutation is essential for understanding evolution. Finally, while essential to biological research, the value of model systems is not always intuitively obvious to those outside of the field (PETSKO 2011), where basic research takes a backseat to translation studies that appear to more directly impact the human condition.

Our program development focused on four goals. First, we aimed to provide a positive environment for elementary school students to interact with professional scientists. We reasoned that many students do not have direct contact with scientists, and in the absence of direct interaction, student views of scientists are shaped by outside factors. These factors likely include the overwhelmingly negative and/or misleading portrayals of scientists in the media and entertainment (HAYNES 1994; JONES 2001; JONES 2005; MAUGH II 1978). Second, we wanted to develop experiments that realistically reflect the scientific method, yet are simple enough that elementary school students in a large classroom setting could reproducibly perform them. Third, we sought to utilize experiments that are transferrable and transportable, using reagents that are accessible to most biological scientists. Fourth, we wanted to introduce students to basic concepts in biochemistry and genetics, including the types of questions that are posed and the approaches used to address them.

To accomplish these goals, we developed a seven-week course, entitled Biochemistry is Elementary, in which students worked through five basic biochemical and genetic experiments in a ~1 hour session per week. The vast majority of the students successfully completed each experiment. Although longitudinal studies would be needed
to determine whether this course had a lasting impact, student surveys indicated that in
the short term, we were successful in accomplishing each of our goals.

MATERIALS AND METHODS

Week One, Introduction to genotype and phenotype:

*Required Materials:* Additional materials beyond the work book were not required.

*Student Activity:* Students record the number of individuals in the class and a description of their experimental group (e.g., fifth grade students at Skyview Elementary School in Windsor, Colorado). In pairs, students record whether they have: a widow’s peak, dimples, freckles, a cleft chin, and unattached ear lobes. Students also record whether they can roll their tongue and whether, when they clasp their hands together, their left or right thumb is overtop of the other and held closest to their body. Data from the entire class is assembled to determine the fraction of students with the dominant trait.

Week Two, Enzymes as builders or breakers:

*Required Materials:* The following materials are required per group of 2-3 students: two 1.7 ml microcentrifuge tubes; three 15 ml screw cap conical tubes, four solution droppers, five glucose test strips (Bayer Diastix #2803), two lactase tablets (Fast Act, Vanilla twist flavor 9,000 FCC units per tablet), 6 mls water, 1 ml 2% (w/v) glucose in water, 10 mls milk; labeling markers, tube racks and paper towels as needed.
**Student Activity:** Students first test positive and negative control samples. Working in groups of 2-3, students label two microcentrifuge tubes 1 and 2. Each group then transfers approximately 1 ml of water and glucose solution to tubes 1 and 2, respectively. Each student records the tube number and its contents in a results table. Students then dip a glucose test strip into tube 1, and quickly remove it after the test square becomes wet. The test strip is placed on a paper towel for one minute. Then the color of the test square is recorded in the results table. This process is repeated for tube 2.

Students then label three 15 ml tubes 3, 4 and 5, and mark the 5 ml line on each tube. Students transfer 5 mls of milk to tubes 3 and 4, and 5 mls of water to tube 5. Students record the tube number and contents in their results table. Students add half of an enzyme tablet each to tubes 4 and 5, cap the tubes tightly and mix all three tubes by inverting for five minutes. Students then dip a glucose test strip into tube 3, quickly remove it after the test square becomes wet, and place it on a paper towel for one minute. Then, they record the color of the test square in the results table. This process is repeated for tubes 4 and 5.

**Week Three, Salivary amylase:**

**Required Materials:** Each student requires ~4 mls of starch solution, ~1 ml of iodine solution, ~4 mls of distilled water, six 1.7 ml microcentrifuge tubes, 1 solution dropper and 1 Petri dish top or bottom. Extra solution droppers are needed to dispense the starch solution, iodine solution and distilled water. Labeling markers, paper towels and tube racks are also needed.

Starch solution is prepared by adding 10 grams of soluble starch (JT Baker, Cat. # JT4006-4) to 1 L water (tap or distilled). The mixture is heated with constant stirring until
boiling. The mixture is then removed from heat, placed on a cool stir plate, and stirred until the solution has cooled to room temperature. The solution is stored in a 1 L bottle.

To prepare 500 mls of iodine solution, 10 grams of potassium iodide (JT Baker, Cat. # JT3168-4) are added to 480 mls distilled water and stirred until completely dissolved. Two grams of iodine crystals (JT Baker, Cat. # JT2211-0) and enough distilled water to bring the total volume to 500 mls are then added. The solution is stirred until the iodine crystals are completely dissolved. The iodine solution is stored in a brown bottle in the dark, as the solution breaks down in the light. (Iodine solution stored in the dark can be used for at least two years.) The iodine solution is diluted 1:4 prior to use.

*Student Activity:* Students work in groups of four to five to share reagents, but each student completes the experiment individually. Students then compare their results to those obtained by the other students in their group.

Students label six microcentrifuge tubes 1, 2, 3, 4, 5, and 6. Using a solution dropper, students transfer 1 ml of distilled water to tube 1 and 1 ml of starch solution to tube 2. Students record the tube number and its contents in a results table. Students add three drops of iodine solution, cap the tubes and mix by inverting for 1 minute. Students record the color of the liquid in each tube in their results tables. They then add 10 drops of starch in tubes 3, 4, 5, and 6. Students spit into their Petri dish until they have at least 1 ml of saliva. Students add 10 drops of distilled water to tube 3, and 10 drops of saliva to tube 4 using a clean dropper. Students record the tube number and its contents in their results table. Students then add two drops of iodine solution to tubes 3 and 4, close the tubes, mix both tubes by gently inverting the tubes for 1 minute, and record the final
color of the liquid in each tube. To ensure accuracy and introduce the importance of reproducibility, students repeat the assay using tubes 5 and 6.

**Week Four, Using yeast to study enzyme defects (part 1):**

*Required Materials:* The following materials are needed per student: 3 yeast agar plates, 3 sterile cotton tipped applicator packs, 1 paper towel, 1 white tube containing sterile distilled water, 1 blue tube containing wild type yeast, one yellow tube containing mutant yeast and one pair of gloves. The following materials are required for each group of 4-5 students: 2 labeling markers, 2 tube racks, 1 roll of tape and 1 zip-lock waste bag (one gallon size). One plastic container to serve as an incubator is also required per class.

To prepare dilute yeast (wild type and mutant) solutions, BY4741 (wild type yeast) and BY4741 ade2Δ (mutant yeast) cells are streaked from glycerol stocks onto YPD plates. Plates are incubated at 30°C for two days, and then stored at 4°C. Two days prior to use, one colony each from the wild type and mutant yeast streaks is used to inoculate 5 ml cultures of sterile YPD medium (10 g yeast extract, 20 g bacto peptone and 20 g glucose in 1 liter of distilled water). Cultures are incubated overnight at 30°C with agitation. The next day, the O.D.₆₀₀ of each culture is determined. Cultures are diluted to an O.D.₆₀₀ of 1 and then 4 ten-fold serial dilutions are prepared in distilled water so that the final concentration is equivalent to an O.D.₆₀₀ of 10⁻⁴. One ml aliquots of the dilute (10⁻⁴) wild type or mutant cells are transferred to sterile blue or yellow microcentrifuge tubes, respectively. The dilute cells can be used immediately or stored overnight at 4°C prior to use.
Yeast agar plates are prepared as follows: In a two liter flask, 20 g glucose, 5 g yeast extract, and 20 g bacto peptone are dissolved in 1 liter of distilled water. (To ensure an obvious red phenotype for the mutant cells, the amount of yeast extract is reduced in these plates from the standard YPD recipe.) 20 g agar is added. The mixture is autoclaved for 40 minutes and swirled to mix. The solution is allowed to cool to approximately 50°C and then 30-35 mls per plate is poured into 100 mm x 15 mm Petri dishes. The plates are allowed to sit overnight at room temperature and then stored at 4°C. These plates are marked with a black stripe on one side of the lid.

**Student Activity:** Students work in groups of four to five to share reagents, but each student completes the experiment individually. Students then compare their results to those obtained by the other students in their group.

Students label the bottom of three agar plates with the numbers 1, 2, 3 and their initials. Wearing gloves, students pick up the white tube (containing sterile water) and invert it ten times. Students open a sterile applicator package approximately one inch and set it down on their paper towel. Students open the white tube, remove the sterile applicator from its package, and dip the applicator into the tube as far as it will go. They then remove the applicator from the tube and touch it to the center of the plate labeled “1”. Students use the applicator to spread the liquid around the plate and then discard the applicator into a waste bag. Plate #1 is then set aside to allow the liquid to soak into the agar. The same technique is then used to spread liquid from the blue (wild type) and yellow (mutant) tubes onto plates 2 and 3, respectively, using a new applicator each time. In a results table, students record the plate number and the liquid that was spread on it.
When all of the plates are dry, students tape the lids to the bottoms on two sides and put them into the plastic incubator. After 3-4 days, students record the growth phenotypes in a results table and draw a picture of each plate. This process is then repeated after another 3-4 days.

**Week Five, Using yeast to study enzyme defects (part 2):**

Week 5 is used to examine the results of the Week 4 experiment. Instructors discuss the results of the experiments and encourage the students to hypothesize about the basis for the observed phenotypes. Instructors photograph the plates with a digital camera and print a picture of each student’s plates. To maximize the visibility of the colonies, plates should be photographed bottom-side up on a dark surface. To identify the plates, a strip of white paper with the student’s name should be included in each photograph. All yeast plates are removed from the classroom for autoclaving and proper disposal.

**Week Six, Using yeast to study enzyme defects (part 3):**

*Required Materials:* The following materials are required per student: 1 yeast agar plate (black stripe), 1 yeast agar plate plus extra glucose (green stripe), 1 yeast agar plate plus extra protein (blue stripe), 1 yeast agar plate plus extra DNA components (purple stripe), 1 paper towel, 4 sterile cotton tipped applicator packs, 1 pair of gloves, 1 labeling template (Supplemental Figure S1), 1 experimental template (Supplemental Figure S2) and 1 master template (Supplemental Figure S3). Required per 2 students: 1 labeling marker and 1 master plate with wild type and mutant yeast cells. Required per 4-5
students: 1 waste bag and 1 roll of tape. One plastic container to serve as an incubator is also required per class.

A yeast master plate contains ~100 single colonies of wild type and mutant cells on 2 halves of a single plate. To prepare these plates, cultures are grown overnight and diluted to an O.D. $\text{$_{600}$}$, $10^{-4}$ as described above. Using an applicator saturated with this suspension (similar to the technique that the students used in Week 4), the instructor spreads dilute ($10^{-4}$) wild type and mutant cells onto a single yeast agar plate (divided in half). When the plates are dry, they are placed in an incubator at 30°C for two days. The plates may be used immediately or stored at 4°C.

Yeast agar plates are prepared as described above. Yeast agar plates plus extra glucose contain 40 grams of glucose per liter. These plates are marked with a green stripe on one side of the lid. Yeast agar plates plus extra protein contain 30 grams peptone per liter. These plates are marked with a blue stripe on one side of the lid. Yeast agar plates plus extra DNA components contain 50 milligrams of adenine hemisulfate per liter. These plates are marked with a purple stripe on one side of the lid.

To further distinguish the yeast agar plates with extra components, food coloring (yellow, blue or green) may be added to the plates prior to autoclaving. Red food coloring should be avoided, as it will decrease the contrast between the white and red yeast colonies.

*Student Activity:* Students work in groups of four to five to share reagents, but each student completes the experiment individually.
Students label the bottom of each plate by (a) placing the plate on the labeling template bottom side up (b) tracing the line down the middle of the plate (c) numbering the plate in the upper left part (#1 for yeast agar, #2 for the plate with extra sugar, #3 for the plate with extra protein and #4 for the plate with extra DNA components) (d) labeling the left side of the plate “mut” for mutant (e) labeling the right side of the plate “wt” for wild type (f) writing their initials on the bottom of the plate and (g) drawing a big black dot on the upper right part of the plate.

After putting on a pair of gloves, students place plates 1 and 2 onto their experimental template with the lids facing up, and line up the black dots on the plates with the dots on the template. Students place the master plate with wild type and mutant yeast cells on the master template with the lid facing up. Using a sterile applicator, students pick up one wild type (white) colony from the master plate and spread the yeast cells onto the wild type side of the plate by zigzagging across the plate. The same applicator is then used to pick another wild type (white) colony and spread it onto the wild type side of plate 2. The applicator is then discarded into a waste bag. Using a sterile applicator, students pick up one mutant (red) colony from the master plate and spread the yeast cells onto the mutant side of plate 1. The same applicator is then used to pick another mutant (red) colony and spread it onto the mutant side of plate 2. The applicator is then discarded into a waste bag. Students then set plates 1 and 2 aside and repeat this process for plates 3 and 4. For all four plates, students tape the lid to the bottom on two sides and then place them in the plastic incubator. After 2-3 days, students record their results in a results table and draw a picture of each plate. This process is then repeated after another 2-3 days.
**Week Seven, Using yeast to study enzyme defects (part 4):**

Week 7 is used to examine the results of the Week 6 experiment and discuss the significance of these results. All yeast plates are removed from the classroom for autoclaving and proper disposal.

**Vocabulary Analysis:** Student surveys were used to assess improvements in vocabulary (see Supplementary Materials for complete course surveys). In the surveys, students were asked to indicate for a given word whether they "owned it," meaning that they felt comfortable defining and using the word; had "heard of it," meaning that were familiar with the word, but could not precisely define it; or "never heard of it." The meaning of each category was re-iterated before the first survey. Statistical significance of changes in the fraction of students who owned a given word was evaluated using Fisher’s Exact Test. When analyzing differences in distribution among all three categories, the Freeman-Halton extension of Fisher’s Exact Test for 2x3 tables was used (FREEMAN and HALTON 1951).

**RESULTS**

**Course structure and metrics:** The course was designed as a series of seven one-hour classes (Table 1). During each of the first three weeks, students performed a single stand-alone experiment. During the final four weeks, students performed two separate two-week experiments. Students were provided a workbook outlining a basic question (see Supplemental Document 1 for the work book for Weeks 4 and 5; the complete work
book for all 7 weeks is available upon request). We discussed the concepts that underlie the question; the students collectively developed a hypothesis, which they entered into their book. Basic biochemical and genetic experiments were then conducted to address the hypothesis. The workbooks included the detailed protocol for the experiment, as well as cues for where to note experimental results.

The course was offered at Skyview Elementary, a Title I public school in Windsor, Colorado. Here we discuss the results from the three fifth grade classes (74 students, 37 females, 37 males) that participated in the complete course. Each class had 24-25 students, and one professor and one graduate student were assigned to each class. Because the same professor and graduate student stayed with each class for the duration of the course, the three classrooms serve as three independent replicates for evaluating the effectiveness of the course.

Since the course included challenging vocabulary, it was important to provide students a forum to express whether the concepts were being clearly explained. Therefore, before and after most classes, students were given assessments. Since a major goal of this project was to make the science enjoyable for the students, we did not want to introduce onerous testing. Indeed, the teachers specifically requested that any assessments have the feel of surveys rather than tests, and that assessments be limited in length. Thus, surveys were given that consisted largely of self-reporting, focusing predominantly on vocabulary (see Supplementary Materials for complete course surveys). The teachers suggested using a template that was already frequently used in these classrooms, in which students were asked to classify words into one of three categories: “never heard it,” “heard it” or “own it” (indicating that the student is fully
comfortable defining and using the word). Because of the obvious limits of self-reporting, a few questions were included in the surveys to objectively test knowledge of key concepts.

**Week 1: Introduction to genotype and phenotype:** The first class introduced students to the basic concepts that served as the foundation for the course. We discussed what the job of a research scientist entails – essentially, identifying a question with an unknown answer, and working to find the answer. Next, we discussed the four basic steps in the scientific method: i) identify a question, ii) develop a hypothesis, iii) perform experiments to test the hypothesis, and iv) if necessary, revise the hypothesis and try again.

We designed the activity for week 1 to reinforce these ideas, while introducing the concept that differences in many observable characteristics among the students can be explained by differences in genes. Our question for the week was, “Why are some traits more common than others?” To facilitate the development of a hypothesis, we explained the concept of dominant and recessive traits, using eye color as an example. Predictably, the students hypothesized that dominant traits will be more common than recessive traits. In pairs, the students then determined their phenotype for a range of characteristics (Table 2). Upon assembling the data for the class, in many cases the recessive trait was more common, disproving our hypothesis.

We finished the class by discussing theories about why some traits are more common than other traits. With some guidance, the students pointed out that certain physical traits are more common in people from some parts of the world, and suggested that perhaps
these traits might be beneficial in those specific climates. In fact, it is unclear for the specific traits discussed whether their regional distribution is a result of genetic drift or due to a specific selective advantage. Nevertheless, this dialogue allowed students to reason their way towards a process that underlies evolution, even though evolution was not explicitly discussed.

**Week 2: Enzymes as builders and breakers:** In week two, we conducted our first biochemical experiment, in which we examined the basis for lactose intolerance. We started by discussing the expression, “you are what you eat,” and asked students why they do not resemble the foods that they eat. We then introduced enzymes as the “builders and breakers” in our body, and explained that different enzymes are responsible for breaking down or assembling chemical complexes. We introduced the generic mechanism for an enzymatic reaction as:

\[
\text{Substrate} + \text{Enzyme} \rightarrow \text{Product} + \text{Enzyme}
\]

The digestion of lactose was used as a paradigm to demonstrate the mechanism of enzymes. Lactose intolerance results from a lack of the lactase enzyme (JÄRVELÄ *et al.* 2009). Lactase catalyzes the hydrolysis of lactose in the following reaction:

\[
\text{Lactose} + \text{Lactase} \rightarrow \text{Glucose} + \text{Galactose} + \text{Lactase}
\]

Therefore, the question for the class was, “Will adding the lactase enzyme to milk cause the breakdown of lactose?” Our hypothesis was, “If we add the lactase enzyme to milk, then we will detect glucose.” Thus, we proposed to treat milk with lactase, and use glucose test strips to monitor the formation of glucose. Before conducting the experiments, we asked two questions to introduce the concept of negative and positive
controls. First, how do we know that there is not already glucose present in milk, or that some other chemical in milk could not result in a false positive? Second, how do we know what a positive result for the presence of glucose will look like? To address these questions, milk without enzyme and a glucose solution were tested as controls. For the actual experiment, students added lactase to milk and mixed the tube by inversion for two minutes. As a control to ensure that the handling of the milk did not result in glucose formation, an identical sample was prepared without enzyme. Both samples were then tested using glucose test strips. All students got the same result – the addition of lactase resulted in glucose formation.

We then discussed the implications of these experiments. We explained to the students that most mammals have reduced expression of lactase in adulthood because they no longer need the enzyme. Thus, the ability to digest lactose after weaning is actually the result of a mutation (JÄRVELÄ et al. 2009), demonstrating the important point that mutations can confer a benefit (it was not hard to convince students that the ability to eat ice cream and pizza is a benefit). We discussed how understanding the basis for lactose intolerance allows scientists to develop treatments for people with this condition. When asked how they would treat lactose intolerance, students suggested some of the currently used methods, including removing lactose from milk and ingesting lactase enzyme when eating dairy products.

**Week 3: Salivary amylase:** To reinforce the concept of enzymes, in the third week we examined another enzyme whose activity varies among people, the human salivary amylase enzyme. Salivary amylase hydrolyzes α-1,4-glucan bonds in starch. This enzyme
is found in saliva, and starts the process of digestion of starchy foods. Within the human population, there are variations in the number of amylase gene copies present. Indeed, in terms of copy number variation, the amylase locus is one of the most variable in the human genome (IAFRATE et al. 2004). The result of copy number variation is very different amylase protein levels between individuals (PERRY et al. 2007). Furthermore, certain mutations in the human salivary amylase enzyme result in decreased starch hydrolysis (RAGUNATH et al. 2008; RAMASUBBU et al. 2004).

The variability in amylase activity can be monitored using an assay that detects the presence of starch, the substrate for amylase. When iodine is mixed with starch, the resulting solution is bright blue. When active amylase from human saliva is present in the mixture, the starch is broken down, resulting in a brown color. If the saliva contains low levels of amylase, or if mutations are present in the enzyme that result in a less effective amylase, the starch is not broken down, resulting in a blue color. This colorimetric assay allows students to easily test for the activity of amylase in their own saliva.

To encourage the concept of reproduction of results, each student performed the amylase assay two times. We found the students were able to reproduce their own results in 95% of the cases. The failures were most likely due to not adding enough saliva to the tube. This provides the opportunity for discussing the importance of repetition in biological experiments. The power of this experiment is that it reinforces the concept that enzymes are present in our own bodies, which was introduced in Week 2 (the lactase experiment) and that people have variation (Week 1).
**Weeks 4-7: Using yeast to study enzyme defects:** The fourth week of class started with the question, “if you identify an enzyme mutation that causes disease in humans, how would you determine the function of the enzyme and basis for disease?” In discussing this question, students very quickly realized the challenges of researching on human subjects. In all classes, students suggested using other organisms to study the disease (usually they suggested mice). At this point, we discussed the significant genetic similarity among eukaryotes, and the advantages of utilizing simple model organisms to study diseases.

In weeks 4-7, we conducted two experiments to highlight the value of model systems for studying human disease. The experiments centered on the yeast ADE2 gene. Ade2 has previously been used as the focus for other project-oriented lab courses, albeit for much more senior students (ARONSON and SILVEIRA 2009). The Ade2 protein provides a convenient model, both because of its well-studied nature, and because simple selective and non-selective assays exist to monitor its activity. Ade2 is a phosphoribosylaminoimidazole carboxylase involved in the highly conserved de novo purine biosynthesis pathway (SILVER and EATON 1969; SMIRNOV et al. 1967). Yeast cells lacking the Ade2 enzyme are unable to grow in the absence of adenine; in the presence of limiting adenine, cells lacking Ade2 activity turn red due to accumulation of a product derived from the substrate of Ade2 (SILVER and EATON 1969; SMIRNOV et al. 1967).

In humans, the bifunctional enzyme PAICS performs the function of the yeast Ade2 enzyme, as well as that of Ade1, the enzyme responsible for the subsequent step in adenine biosynthesis (MINET and LACROUTE 1990; SCHILD et al. 1990). PAICS is highly
conserved in higher eukaryotes, and mutations in PAICS can result in developmental defects and in some cases embryonic lethality (Ng et al. 2009; Tiong et al. 1989). Additionally, because cancer cells rely heavily on the de novo purine biosynthesis pathway while normal cells preferentially utilize salvage pathways (Li et al. 2007), PAICS has emerged as a potential target for anticancer drugs.

Therefore, we told the students that an enzyme had been identified that is important in both development and cancer. We explained that a similar gene had been identified in yeast, and that we were going to try to use yeast to determine the function of the enzyme. The first step, and the goal for weeks four and five, was to determine whether mutations in ADE2 confer a phenotype in yeast. The students hypothesized that deletion of ADE2 would make the cells sick or dead, resulting in a detectable phenotype.

To practice sterile technique, and as a negative control, each student first streaked water onto a YPD plate using a sterile applicator. The students then streaked the wild type and ade2Δ deletion strains onto YPD plates. The plates were incubated in each classroom at room temperature in a large plastic container for one week. Because YPD contains limiting amounts of adenine, the ade2Δ mutant cells appear red, while wild type cells appear white.

In week five, we compiled the data from the students’ plates. Although 5-10% of the plates had some level of contamination, in all cases a clear color difference was detected between the wild type and ade2Δ strains (Figure 1; Supplemental Figure S4). Interestingly, although color differences were observed in all classrooms, the extent of these differences varied among the classrooms. This likely resulted from subtle
temperature differences among the rooms, which can result in disparate yeast growth rates and consequently, differences in colony color.

Students then brainstormed possible causes of the red color phenotype observed in the mutant strain. In each class, the students proposed that the red phenotype in the ade2Δ strain was due to a failure to make some essential nutrient. This theory is not exactly correct, as the color is actually due to build-up of the substrate of ade2Δ, not absence of the product. Nevertheless, the theory provided a simple testable hypothesis. The students hypothesized that if the medium was supplemented with the product that Ade2 generates, the ade2Δ strain would be “cured” of its red phenotype. Therefore, students proposed different supplements that could be added to the medium. Student suggestions included extra sugar, protein, fats, DNA components, agar and vitamins.

In week six, we tested some of these supplements. YPD plates were prepared with extra sugar (4% glucose as opposed to 2%), protein (3% peptone as opposed to 2%) or DNA components (50 mg adenine hemisulfate per liter). Each student streaked wild type and ade2Δ strains side-by-side on each medium. As a control to ensure that the color difference was reproducible, both strains were also streaked onto standard YPD. Plates were again incubated for one week at room temperature.

In week seven, we analyzed the results. Although a small fraction of the plates were again contaminated, all students observed that the ade2Δ mutant strain grew white on medium supplemented with excess DNA components. However, the mutant strain grew red on the other media (Figure 2; Supplemental Figure S3). Therefore, the students correctly theorized that the ade2Δ strain could be “cured” of its red phenotype by supplementing the normal growth medium, and discovered that only DNA components
could cure the phenotype. This suggests that the Ade2 enzyme is responsible for synthesizing DNA components. Based on their results, the students then proposed possible treatments for defects in the \( ade2^\Delta \) gene. Students suggested dietary supplements, introducing a good copy of the gene back into cells, and enzyme replacement therapy. We then discussed how each of these methods has been used successfully for various diseases.

**Assessing effectiveness of the course:** Pre- and post-course surveys were used to give students a forum to provide feedback, and to evaluate our effectiveness in each of the four major goals of the course. The first goal was to provide students the opportunity to interact with professional scientists. In pre-course surveys, only 21% of students said that they knew any scientists; 45% said that they did not know any scientists, while an additional 34% said that they were not sure. These numbers highlight the importance of giving students a forum to interact with professional scientists.

The second goal of the course was to develop realistic biochemical and genetic experiments that could be easily reproduced by elementary students. Therefore, we designed each experiment to follow the key steps of the scientific method, including the use of controls. The high success rate of each experiment (Table 1) clearly demonstrates that these experiments were easily reproducible by elementary school students, even in a large classroom setting.

The third goal was to utilize reagents that would be easily accessible to most biological scientists. The reagents for these experiments are relatively inexpensive and the only equipment required for these experiments is an autoclave for sterilizing yeast
growth medium, tubes, and pipette tips, basic lab glassware, and a plastic container for incubating the yeast plates. Therefore, almost any biological scientist would be able to replicate these experiments at modest cost.

The final goal was to teach basic biochemical and genetic concepts, focusing on the function of enzymes, the role of mutation in creating phenotypic variation, and the use of model systems. Therefore, the surveys included questions assessing each of these areas. As previously discussed, self-reporting was used throughout the course to allow students the opportunity to provide feedback about their level of understanding of vocabulary. This also provided us information about how student confidence with vocabulary changed during the course. Student confidence with key terminology significantly increased over the course of the program (Figure 3A; Table S1).

An obvious concern is whether students accurately self-report. Three results from the surveys give us confidence that student self-reporting is reasonably accurate. First, we included “genotype” and “phenotype” on the survey even during some weeks where these words were not mentioned in class. While self-reporting scores increased significantly during weeks where these words were discussed (Weeks 1 and 4-5), no significant improvement was seen during weeks where these terms were not discussed (Weeks 2, 3, 6 and 7; Figure 3B). This indicates that the students did not simply reflexively claim greater understanding over time. By contrast, relative understanding of the terms “enzyme” and “substrate,” which were mentioned in every class after Week 1, showed continual improvement (Figure 3C), although the individual week-to-week changes were not statistically significant.
Second, when asked to provide written definitions, most of the students who claimed to “own” a vocabulary word were able to successfully define it. For example, in the survey following Week 2, 50% of the students claimed to “own” the word “enzyme” (Figure 3C). When those students who claimed to own enzyme were then asked to define the word, more than half (53%) provided a definition consistent with what we had taught them – i.e. that enzymes are responsible for building up and breaking down molecules. Another 22% gave a partially accurate definition; most of these gave a definition that was specific for lactase (for example, “an enzyme turns lactose into glucose”). Thus, 75% of the students who claimed to “own” the word “enzyme” were able to give a fully or partially accurate definition.

Third, we were concerned that, rather than separately considering each word, students might simply place every word into the same category. To address this, we analyzed the correlations between answers for week 2; this week was chosen because the pre-class survey showed the most equal mix of “never heard of it,” “heard of it” and “own it.” For each word in the pre- and post-class surveys, we compared the each student’s response to their response for “enzyme” (the primary topic of week 2). In the pre-class survey, there was no significant correlation (P>0.1 using Fisher Exact test with the Freeman-Halton extension for 2x3 tables; FREEMAN and HALTON 1951) between how a student categorized “enzyme” and how they categorized any of the other seven words (Figure S6A). This indicates that students really do independently consider each vocabulary word. Intriguingly, we expected that some students would be predisposed to score their knowledge higher due to differences in confidence; however, these results suggest that any such differences are modest. In the post-class surveys, there were statistically
significant correlations between how students self-scored their knowledge of “enzyme” and how they scored each of the other words except “milk” (Figure S6B). This is to be expected, as there are likely differences in the rate at which students learn new vocabulary. Furthermore, even among students who self-scored identically for “enzyme,” there still were substantial differences in how they self-scored for each of the other vocabulary word (Figure S6B), suggesting that students consider each word independently. For example, among students who “owned” the word enzyme, a minority “owned” substrate, while more than 90% owned product, milk and lactose intolerance. Collectively, these results support the utility of self-reporting.

Nevertheless, because of the limits of self-reporting, a few additional objective questions were included to measure progress in key areas. Although less than 10% of the students reported “owning” the term “model organism” (i.e., felt that they could define and use the term) before the class (Table S1), after the final class a significant fraction of the students (55%) were able to write a definition for the term. Students provided answers such as, “Something other than a human that can be tested [sic] to learn something about a human,” “Yeast is a model organism because [sic] it is easier to work with than humans,” and “An object that takes the place of a human to experiment on.” Likewise, after week five, 66% of the class successfully filled in the blanks in the following equation describing a generic enzymatic reaction:

\[ \text{_____} + \text{_____} \rightarrow \text{_____} + \text{_____} \]

By week seven, 91% answered this question correctly. This improvement is not a result of direct instructor feedback on incorrect answers; because we did not want the students to feel like they were being tested, the corrected surveys were not returned to the students.
and the results of surveys were not discussed with either the teachers or the students. Therefore, this ongoing improvement in the objective ability to write the equation for an enzymatic reaction, along with the improvement in self-reported understanding of the definition of enzyme (Figure 3C) highlights the value of having multiple activities reinforce a given concept.

DISCUSSION

One objective driving the development of this program was to provide students with the opportunity to interact with professional scientists. This was accomplished simply by our presence in the classroom each week. Therefore, our biggest challenge was designing legitimate biochemical experiments that are doable and enjoyable for such young students. The high success rates for each of the experiments (Table 1) and the enthusiastic reviews of the class clearly demonstrate that we accomplished this goal. When students were asked “Do you have any suggestions for us?” at the completion of the course, the majority of responses were simply “no”. More elaborate comments were generally positive and enthusiastic. Such comments included, “No I think all is perfect,” “No it was really fun,” “No. You did everything right,” “No, well just keep being great!” and “No I think that you should do all the things you did this year.” Indeed, none of the students offered any substantial criticism (see Supplementary Table 1 for a complete list of student comments); and a common “complaint” was that students wished we had more time with them (some suggestions from the final evaluations included, “come earlier so have you [sic] longer” and “come with us to middle school”). When asked in the final course survey whether they had “fun doing experiments with us,” 65 out of 66 students
answered “yes,” none of the students answered “no,” and 1 student answered “not sure.” Additionally, in the final survey, students were asked whether the experiments made them “more interested in science,” “less interested in science” or “not sure.” 62% of students said that the experience made them more interested in science, while only 1% said that the experiments made them less interested.

In our experience, the biggest challenge in performing experiments with elementary school students is that they are eager to jump in to the experiments, so sometimes they struggled to listen through detailed instructions. We used a number of specific techniques to combat this problem. First, we had students read written instructions out loud, which was much more effective than having each student silently read through the instructions. We generally waited to hand out reagents until we had read through the instructions. For the more complicated experiments, we handed out reagents incrementally, which prevented the students from moving ahead with the experiments. Finally, for complicated procedures, students took turns reading each step out loud, then the teachers demonstrated each step. We found the students were more attentive when the teacher was performing the experiment; therefore, this provided a captive audience for us to explain the challenges of each step.

One strength of the course is that, with the exception of the last few class periods, each class can function as an independent module. Therefore, contraction of the class to fit a shorter timeline would be a simple task. For example, Weeks 2 and 3 are conceptually redundant, in that both demonstrate the role of enzymes. Thus, if time were limiting, sessions could be eliminated. Additionally, there are easy ways that the class could be expanded. For example, it would be simple to add an additional yeast genetics
section. At the end of the final class, students hypothesized that Ade2 must be involved in synthesis of DNA components. One additional experiment that would have supported this hypothesis would be to test the effects of excluding DNA components from the growth medium. When ade2Δ mutant strains are cultured in the absence of adenine, they do not grow, supporting the hypothesis that Ade2 is involved in synthesis of DNA components (specifically adenine).

Interestingly, the yeast experiments were the most popular among the students, with 57% of the students rating one of the two yeast experiments as their favorite (Table 1). By contrast, the observation of phenotypes was rated as the favorite of only 8.7% of students. This was somewhat surprising, as the yeast experiments were the most technically challenging, while the phenotype observation was the simplest. This may in part be because the yeast experiments were freshest in students’ minds when they filled out the final survey. Nevertheless, it highlights the fact that students at this age are not afraid of a challenge.

One question in setting up the course was the best way to evaluate student progress. As previously discussed, because a major goal of the class was to provide students with an enjoyable scientific experience, we did not want to introduce onerous testing. Ideally, students would be objectively tested on every vocabulary word every week; however, this was not possible, as we were limited in the number of questions that could be asked each week due to time constraints, and it was specifically requested that the assessments not have the feel of tests. Thus, we worked extensively with the teachers to develop assessments that balanced the need for useful feedback with our desire to provide an enjoyable experience. Although there are well-established limits to the extent to which
self-reporting can be used to objectively evaluate students (COOK and CAMPBELL 1979), there were two factors that made us comfortable in our decision to utilize self-reporting. First, the teachers had used this self-reporting system previously, and felt that the students tended to accurately self-report. The few objective questions that we inserted into the surveys supported this observation. Second, conveying didactic information represented a very small goal of the course. Realistically, it is unlikely that a few years from now the students will remember the scientific vocabulary that they learned during the course; however, we hope that their positive experiences with science and with scientists will have a lasting impact. As confidence with science may affect selection of scientific classes (FARENGA and JOYCE 1998), future attitudes towards science (GEORGE 2000; TALTON and SIMPSON 1986), and scientific achievement (HILL et al. 2010; OLIVER and SIMPSON 1988; OSBORNE et al. 2003; SHRIGLEY 1990), the students’ perceived understanding of the vocabulary may be as important as their true level of understanding.

As we did not want to create controversy that might compromise our core mission, we chose not to directly address the topic of evolution. Instead, our experiments demonstrated many of the key concepts that underlie evolution, including: i) the idea that genetics can explain many of the visible differences among people, ii) the fact that many genetic characteristics are regionally specific, iii) the idea that mutations can be beneficial, and iv) the fact that even very simple organisms like yeast are highly genetically similar to humans. Our hope is that these key concepts will lay the groundwork for future understanding of evolution presented in upper-level science classes.
In sum, teaching the “Biochemistry is Elementary” course provided elementary students with a positive and challenging environment to experience biochemistry and genetics firsthand. We believe the value of this course lies in its ability to engage students in science at a young age, which may help cultivate a life-long interest in science.

Acknowledgments

This work was supported by a National Science Foundation grant (MCB-0843073) to L.A.S and a National Science Foundation grant (MCB-1023771) to E.D.R., as well as by a generous gift to Colorado State University from the Tsao family. We would like to thank Skyview Elementary for hosting this program and the fifth grade teachers – Teri Romshek, Amy Nicholl, Amy Sassano and Nona Winder– for their help in establishing this program. We would also like to thank the graduate student and postdocs who assisted in development and implementation of this program: Keely Sudhoff, Kristopher Hite, Troy Sorensen, Courtney Croke, Lynsie Sundin, Sarah Hobdey, Kyle MacLea, Whitney Luebben, Lindsey Long, Marie Yearling, Sheena D’Arcy, Aaron Nelson and Kristi Barker. Finally, we thank Professor Alan Wheals for the scanning electron micrograph of yeast cells shown in Workbook 4.
### TABLE 1. Program statistical analyses by experimental weeks

<table>
<thead>
<tr>
<th>WEEK(S)</th>
<th>ACTIVITY</th>
<th>KEY CONCEPTS</th>
<th>SUCCESS RATE (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>FAVORITE EXPERIMENT (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Examining differences in phenotypes among the students</td>
<td>Phenotype versus genotype, dominant and recessive, hypotheses</td>
<td>100</td>
<td>8.7</td>
</tr>
<tr>
<td>2</td>
<td>Studying lactose hydrolysis</td>
<td>Enzymes, positive control, negative control, mutations</td>
<td>100</td>
<td>15.9</td>
</tr>
<tr>
<td>3</td>
<td>Testing for salivary amylase activity</td>
<td>Enzymes, mutations</td>
<td>95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.3</td>
</tr>
<tr>
<td>4-5</td>
<td>Using yeast to study defects in the ADE2 gene</td>
<td>Model organisms, enzymes, mutations</td>
<td>100</td>
<td>24.6</td>
</tr>
<tr>
<td>6-7</td>
<td>“Curing” cells of the ade2Δ phenotype</td>
<td>Model organisms, enzymes, mutations</td>
<td>95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of students who successfully completed the experiment, and got the expected result.

<sup>b</sup> Percentage of students who indicated on the final survey that this was their favorite experiment.

<sup>c</sup> Percentage of students who got the same result for their two independent replicates.

<sup>d</sup> Unexpected results were due to either incorrect labeling or failure to apply the yeast strain.

### TABLE 2. Phenotypes examined in Week 1

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>DOMINANT OR RECESSIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widow’s peak</td>
<td>Dominant</td>
</tr>
<tr>
<td>Dimples</td>
<td>Dominant</td>
</tr>
<tr>
<td>Freckles</td>
<td>Dominant</td>
</tr>
<tr>
<td>Cleft chin</td>
<td>Recessive</td>
</tr>
<tr>
<td>Hand folding: left over right&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dominant</td>
</tr>
<tr>
<td>Tongue rolling&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dominant</td>
</tr>
<tr>
<td>Attached ear lobes</td>
<td>Recessive</td>
</tr>
</tbody>
</table>

<sup>a</sup> When a person clasps their two hands together (without interlocking fingers), most people will always clasp their hands with the same thumb nearest to their body. Folding one’s hands with the left thumb towards the body is dominant.

<sup>b</sup> Although being born with the ability to roll one’s tongue seems to be genetic, this skill can be learned.
Figure Legends

FIGURE 1: - The ade2Δ strain produces red colonies when grown on YPD. (A) Wild type and (B) ade2Δ cells were diluted to 10⁻⁴ and spread on YPD plates. The plates were incubated at room temperature for seven days and then photographed.

FIGURE 2: - The red phenotype of the ade2Δ strain can be “cured” by the addition of DNA components (adenine) to YPD plates. Wild type and ade2Δ cells were streaked side-by-side on YPD plates containing: (A) extra sugar, (B) extra protein or (C) extra DNA components. The cells were also streaked onto a YPD plate (D) as a control. The plates were incubated at room temperature for seven days and then photographed.

FIGURE 3. - Improvement in self-reported vocabulary knowledge. (A) In student surveys, students were asked to indicate for a given word whether they "owned it," meaning that they felt comfortable defining and using the word; had "heard of it," meaning that were familiar with the word, but could not precisely define it; or "never heard of it." Statistical significance of changes in the percent that “owned” a given word, evaluated using Fisher’s Exact Test, is indicated (* is P<0.05; ** is P<0.01; *** is P<0.001; NS is not significant) (B) Self-reported vocabulary knowledge only showed significant improvement when vocabulary words were explicitly discussed. "Phenotype" and "genotype" were only discussed in Weeks 1 and 4-5; students were surveyed about these words at various points throughout the course. (C) Continual improvement was
seen for "enzyme" and "substrate." Both of these words were used continually throughout the course.

**Supplementary Figure Legends**

FIGURE S1. - Labeling template. This template is designed to help students properly label their yeast plates in Week 6. Students place their plate with the lid facing down onto the template, and then use the template to label the plate.

FIGURE S2. - Experimental template. This template is designed to help the students remember which side of their plates they should streak the mutant and wild type yeast in Week 6. Prior to streaking each plate, students place the plate onto the template with the lid facing up, matching the black dot on the plate with the black dot on the template.

FIGURE S3. - Master template. This template is designed to help the students in Week 6 remember which side of the master plate contains the wild type strain, and which side contains the mutant. Students place their master plate on the template, with the lid facing up.

FIGURE S4. - Sample of actual student results from Weeks 4 and 5. The \( ade2\Delta \) strain produces red colonies when grown on YPD. (A) Water control (no yeast), (B) Wild type and (C) \( ade2\Delta \) cells were diluted to \( 10^{-4} \) and spread on YPD plates. The plates were incubated at room temperature in the classroom for seven days and then photographed next to the student’s name.
FIGURE S5. - Sample of actual student results from Weeks 6-7. The red phenotype of the ade2Δ strain can be “cured” by the addition of DNA components (adenine) to YPD plates. Wild type and ade2Δ cells were streaked side-by-side on YPD plates containing: (A) extra sugar, (B) extra protein or (C) extra DNA components. The cells were also streaked onto a YPD plate (D) as a control. The plates were incubated at room temperature in the classroom for seven days and then photographed next to the student’s name.

FIGURE S6. - Correlation between student survey answers for “enzyme” and for each of the other terms surveyed in week 2. Before (A) and after (B) week 2, students were surveyed on 8 terms: enzyme, substrate, product, milk, positive control, negative control, and lactose intolerance. Shown is the relationship between the students’ answers for “enzyme” and their answers for each of the other terms. The distribution of survey answers for each term is shown among students who “never heard of” (n=38 before week 2, n=1 after week 2), “heard of” (n=18 before week 2, n=35 after week 2) or “owned” enzyme (n=3 before week 2, n=36 after week 2). Statistically significant differences in the fraction of students who “owned” a given word between each pair of adjacent columns, as evaluated by two-tailed Fisher’s Exact Test, are indicated (* is P<0.05; ** is P<0.01; *** is P<0.001).
Supplementary Materials:

Document 1: Workbook weeks 4 and 5

Complete Student Survey Set

Supplementary Table S1: Complete vocabulary survey data
References:


HAYNES, R. D., 1994 From Faust to Strangelove: Representations of the Scientist in Western Literature. The Johns Hopkins University Press, Baltimore, MD, USA.


Figure 1
Figure 2
Figure 3