Dilution and Pipetting Lesson Using Food Dyes

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Abstract

In this lesson students learn how to use micropipettors to accurately measure small volumes of liquids and perform dilutions using food dyes. Students dilute four food dyes at 10X concentration to 1X working stocks. Using the formula \(C_1V_1 = C_2V_2\), each student completes a table requiring four dilution calculations for different final volumes. Using the values they generated, students make the solutions of 1X food dye. Reference solutions furnished by the instructors provide students with immediate feedback and self-assessment. To practice adjusting the micropipette volumes, students combine their 1X solutions to produce four new colors. Comparison of these to reference solutions provides students with immediate feedback. This lesson works for students in grades 6-14 and adult learners in continuing education workshops. It requires about an hour and after they complete the lesson, students have confidence to move on to more challenging solution preparations with colorless reagents.

INTRODUCTION

The first day in a molecular biology laboratory can be an intimidating experience for students. In addition to discussions of biological concepts and research methodology, students must learn how to manipulate unfamiliar equipment with precision. Prior to implementing the lesson described in this report, students in our class had persistent problems calculating simple dilutions and pipetting small volumes. Their inability to perform these basic tasks from the very beginning of the course led to a high frequency of failed experiments which in turn led to losses in confidence that seriously interfered with their enjoyment of laboratory activities. In response, we developed this lesson to provide a thorough yet enjoyable introduction to the essential skills of dilution, pipetting, and the clear labeling of test tubes. Published pipetting guides were too complex for our purposes because they introduced several types of pipettors (1), were discipline specific (2), or did not include dilution exercises (3). None of the pipetting lessons we found available on the web (4) addressed the skills in context of a molecular biology laboratory or did not develop the lesson within the framework of Scientific Teaching (5). This lesson combines active laboratory work with immediate feedback. Furthermore we provide examples of common pipetting errors.

Intended Audience

The lesson was developed for use in the Dynamic Genome class at UCR by first-year undergraduates (6) and the Evolutionary Genetics course at Athens Academy by second-year high school students. Both courses are authentic research experiences in which students learn molecular biology skills including PCR,
gel electrophoresis, and DNA sequence analysis. We have also used the lesson with middle and high school students during outreach programs and with high school teachers in continuing education workshops. The lesson requires about sixty minutes and can be completed in one laboratory period or it can be spread over multiple days. The lesson assumes no prior knowledge of dilutions or pipetting.

**SCIENTIFIC TEACHING THEMES**

The active exercise requires students to work both individually and in small groups. Instruction is divided between short discussions and completion of a written exercise or laboratory work with built-in self-checks so that errors can be corrected quickly. The lesson is inclusive of all learning abilities, as it assumes no prior knowledge of pipetting or dilution, and requires the use of math reasoning, motor skills, and self-assessment. Colorblind individuals have completed the lesson without difficulty.

**LESSON PLAN**

**Pre-class Preparation**

**Reagents**

We purchase generic liquid food dyes (red, blue, green and yellow) from a local grocery store and dilute the stocks 1:20 to generate “10X” solutions (Figure 1A, page 4). A pair of students is given 500 ul of each 10X dye solution in a separate 1.5 ml tube (Figure 1A) along with a 15 ml tube of water. The instructor prepares one set of reference solutions for the class in 1.5 ml tubes, using Tables 2 and 3 (page 3), and places these reference on white paper labeled according to Tables 2 and 3 or in a tube rack (see Figure 1, page 4). All students complete the exercise individually. Instructors may want to include a list of common errors (see Figure 2 and 3 legends, page 4) beside the reference solutions.

**Equipment**

Each student is provided with a set of pipettors (P1000, P200, and P20) with a supply of tips and empty 1.5 ml tubes. We use Gilson Classic Pipettmen (Middleton, WI), but any brand of air displacement pipettors will work.

**In-class Script**

**Performing dilution calculations**

The lesson begins with a discussion of the need to dilute concentrated stock solutions to final (working) concentrations (for an overview of activity and time requirement, please see Table 1, page 3). A useful example of an everyday concentrated stock is Campbell’s Condensed Soups. Most of us have prepared soup and are aware that one can of water is added to one can of soup base. Thus the condensed soup is a 2X stock. Another example is frozen orange juice where one can of concentrated juice is diluted with three cans of water and thus the stock is 4X. After using these examples, the instructor discusses a relevant concentrated laboratory solution such as 50X TAE (used for electrophoresis) or 2X Taq mix (used for PCR). The instructor shows how the formula \( c_{v_i} = \frac{c_{v_j}}{m_{v_i}} \) is used to calculate dilutions (some students may recognize \( m_{v_i} = m_{v_j} \)). Students are then given four tubes of 10X food dyes (i.e. a concentrated stock solution) and instructed to dilute each to 1X final concentration. Students use Table 2 to calculate the volumes of 10X dye and water required to make 1X solutions of differing final volume. The volumes were chosen to require the students to use the P1000, P200 and P20 pipettes at various volume settings. After the discussion of dilutions, students are instructed about the importance of clearly labeling test tubes and are asked to label four with their initials, the specific dye color, and the final concentration.

**Using a pipettor to make solutions**

Instructors first demonstrate the proper use of the different pipettors and then each student prepares the four dilutions. For a detailed description of pipettor usage see Miller et al (3). Students can also view a video that demonstrates the proper use of a pipettor (7). Students receive immediate feedback when they compare their results to the reference solutions provided by the instructor (Figure 1B and C). Examples of errors are shown in Figure 1C. Once the 1X dyes are made, students can photograph their results with a cell phone or tablet computer for inclusion in the lab notebook. An electronic laboratory notebook, e-LN, is used in the Dynamic Genome course (8, http://dgenotebook.ucr.edu/notebooks/public/2015/03/25/example-dilution--...). If students make errors they can photograph the tubes and comment on what they did wrong. They then repeat the dilution to correct any mistakes until each student has one correct dilution for each of the four colors.

After preparation of the 1X solutions, students label four new tubes and mix the 1X solution according to Table 3. If they have successfully pipetted the correct solutions in the correct amounts, the students will generate orange, purple, cyan, and olive colors (Figure 2A). To evaluate pipetting skills, they again compare their results with the reference set. Small errors in pipetting will result in color variations (Figure 2B) and small volume differences are easy to spot due to the dye. Again the results are photographed for inclusion in a notebook. Students can comment in the notebook entry about any mistakes they made before trying again. Students are given time to repeat any step until they get it right.

**TEACHING DISCUSSION**

Inclusion of this lesson in our Dynamic Genome course (Burnette and Wessler 2013) and the Evolutionary Genetics course at Athens Academy resulted in an increase in the success rate of the first PCR experiment. The visual feedback from the food dyes grabs the student attention and provides immediate feedback, so there is time to correct errors if necessary. Students especially enjoyed using their personal electronic devices to photograph the tubes and many even shared them on social media. In addition, many students ask if they can take the dyes home to show family and friends. The lesson is inexpensive and safe because it uses only food dyes and water. The four dye set cost less than $5 and will serve at least 500 students.

In addition to the college course, we have successfully used this lesson in outreach workshops for middle and high school students. Hundreds of students have used this lesson before moving on to molecular biology experiments that require precision pipetting skills. Middle and high school teachers who participated appreciated the combination of math and biology skills and several have included the module in their curriculum.
Table 1. Dilution Pipetting—Approximate duration of each Lesson activity

<table>
<thead>
<tr>
<th>Activity</th>
<th>Estimated Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background Discussion</td>
<td>10 min</td>
</tr>
<tr>
<td>Demonstration of dilution calculations</td>
<td>10 min</td>
</tr>
<tr>
<td>Students complete Table 2</td>
<td>10 min</td>
</tr>
<tr>
<td>Students prepare 1X dye solutions</td>
<td>30 min</td>
</tr>
<tr>
<td>Students prepare new colors based on Table 3</td>
<td>30 min</td>
</tr>
</tbody>
</table>

Table 2. Volume calculations for the 1X solutions

<table>
<thead>
<tr>
<th>Tube Label</th>
<th>Amount of 10X dye (μL)*</th>
<th>Amount of H₂O (μL)*</th>
<th>Total Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>50</td>
<td>450</td>
<td>500</td>
</tr>
<tr>
<td>Blue</td>
<td>100</td>
<td>900</td>
<td>1000</td>
</tr>
<tr>
<td>Green</td>
<td>75</td>
<td>675</td>
<td>750</td>
</tr>
<tr>
<td>Yellow</td>
<td>87</td>
<td>783</td>
<td>870</td>
</tr>
</tbody>
</table>

* Students would fill in these columns.

Table 3. Volumes for pipetting practice

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Green (μL)</th>
<th>Yellow (μL)</th>
<th>Red (μL)</th>
<th>Blue (μL)</th>
<th>Total Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>300</td>
<td>60</td>
<td>0</td>
<td>360</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>16</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td>147</td>
</tr>
<tr>
<td>4</td>
<td>27.5</td>
<td>50</td>
<td>57</td>
<td>12.5</td>
<td>147</td>
</tr>
</tbody>
</table>
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Figure 1. Dilution exercise outcomes. 1A. 10X dyes supplied to the student. 1B. Reference (R) and student (S) 1X solutions based on values calculated from Table 2. 1C. Common errors made by students while preparing the 1X solutions. The most common error is seen in the red solution. The volume is too high due to the student pushing the plunger to the second stop. For the blue solution the student used the P200 set to 10µl instead of the P1000 set to 100 µl. The volume of the student’s green solution is slightly off because the student pushed the plunger to the second stop before pipetting the 10X green dye. While preparing the yellow solution the student used the P20 instead of the P200 for both water and dye. Note that all solutions were made from the same stocks. Color variation is due to lighting differences in the photograph.

Figure 2. Pipetting exercise. 1A. Reference (R) and student (S) results based on the volumes found in Table 3. 2B. Examples of errors made by students. The yellow solution is too dark because the student used the 10X stock solution. For solution 2 the student pushed the plunger to the second stop when pipetting the water. The volume for solution 3 is too high because the student used the P1000 instead of the P200 and pipetted 270 µl of green dye. When preparing the final solution the student used the P200 pipette resulting in pipetting 120µl of the blue dye instead of 12 µl.

Figure 3. Pipetting “art” exercise.
**Extension and Adaptations**

If time permits a fun extension of the lesson is shown in Figure 3 and gives student more practice with the pipettors to further hone their skills before moving on to mixing enzyme reactions. Groups of students are provided with a 96-well plate and they generate a picture of their own design by filling the wells using all of the available dyes (10X, 1X, and new colors) or use a prescribed template (4). Completed plates can be photographed with cell phones and shared on social media and plates can be washed and re-used. Other extensions could include a discussion of pipetting precision and accuracy using the procedure included in the Gilson Guide to Pipetting (9).

**SUPPORTING MATERIALS**

- S1. Dilution Pipetting-Student Handout

**ACKNOWLEDGMENTS**

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**REFERENCES**