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Pipette Olympics: An Engaging Exercise for Undergraduate Laboratory Training

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Pipetting is an important technique used in almost every molecular neuroscience method including but not limited to, PCR, reverse transcription, immunohistochemistry, chromatin immunoprecipitation, and cell culture. The COVID-19 pandemic has robbed the undergraduate population of time to practice in person laboratory techniques. In response, we have devised a standardized, quick, and fun way to instruct students on the fundamentals of pipetting, serial dilutions, and basic statistical analysis. Here, we offer a standardized protocol for instructors to use to teach undergraduates valuable skills while providing friendly competition. We also offer an example of an undergraduate performing the steps of this protocol with example results and the results from three separate undergrads' first two attempts. This exercise provides laboratories with a method to reintroduce undergraduates to lab basics while standardizing the training thereby saving time lost to the pandemic.

Key words: micro pipetting, polymerase chain reaction (PCR), serial dilution, statistical analysis, instructor, undergraduate, neuroscience

Emphasis on laboratory fundamentals in scientific curricula is now especially important due to the impacts of the COVID-19 pandemic on secondary and higher education. The pandemic and resultant lockdown restrictions have reduced both laboratory access and utilization over recent years (Durant et al., 2020; Stenson et al., 2022). Consequently, many educators have had to rely on remote learning modalities for laboratory courses (Seifert et al., 2021; Desa et al., 2022). While the results are far from conclusive, some STEM student populations have demonstrated diminished satisfaction and/or academic performance as a result of the switch to remote learning (Seifert et al., 2021; Wester et al., 2021; Wilhelm et al., 2022). In one study of medical students, many expressed frustrations with the loss of physical and tactile experiences as a result of remote learning (Wilhelm et al., 2022). Students and educators alike can benefit from engaging standardized training exercises when they return to in person laboratory work. One technique commonly used in neuroscientific research that requires hands-on experience is micropipetting.

Proper micropipette technique is imperative for generating accurate and reproducible results as misuse can introduce both systematic and random errors into data (Kumar and Gill, 2018). This makes thorough micropipette training important for any student looking to perform "wet lab" research. While there have been many advancements in the development of robotic liquid-handling technologies, they may be impractical or not cost-effective for many laboratories (Tegally et al., 2020). High-throughput methods involving manual liquid transfer are still prevalent, and often involve the usage of 96-well microplates. PCR is one such method that is of particular importance to the field of neuroscience (Benson, 2020). When PCR is carried out in a 96-well plate, the addition of multiple reagents to each well will require hundreds of liquid transfers. The tedious process of individually pipetting each reagent into each well can result in human error through duplicate additions, skipping wells, incomplete dispensing, etc. Carry-over contamination is also a major concern due to the high sensitivity of PCR (Hu, 2016). Similarly, serial dilutions, often performed in order to create calibration curves for PCR, are also highly sensitive to error due to the logarithmic relationship between number of dilutions and concentration, which amplifies error (Nordgård et al., 2006; Popa-Burke et al., 2009). Proper technique, however, can reduce errors due to pipetting to statistically insignificant levels (Grgicak et al., 2010). Through enough practice, students can master the use of micropipettes for preparing PCR reactions and serial dilutions in 96-well microplates.

In a time where many students have suffered from a lack of hands-on learning experiences in their STEM education, methods for accounting for this deficit are especially in demand. Additionally, novel educational experiences are always beneficial in order to enhance existing curricula. Here we provide a method for training proper micropipetting technique, as well as serial dilution preparation and quantification that simulates common PCR procedures. This process involves using standard food dye as a solvent to mimic the addition of PCR reagents in a 96-well plate. Students also calculate and prepare a serial dilution using the dye. A microplate reader can then be used to quantify absorbance values, and students can apply basic statistical analysis to evaluate their performance. The process is highly reproducible, inexpensive, and provides students with valuable experience in core scientific practices. This standardized method is suitable for undergraduates, or even high school students at all experience levels, whether they are learning laboratory fundamentals, accounting for educational gaps due to remote learning, or simply looking to test their existing skills.

MATERIALS AND METHODS

Materials

Transparent 96-well flat bottom plate (Fisher Scientific), 100–1000 µL and 20–200 µL micropipettes and micropipette tips, 8 1.5 mL microtubes, food coloring (yellow #5,

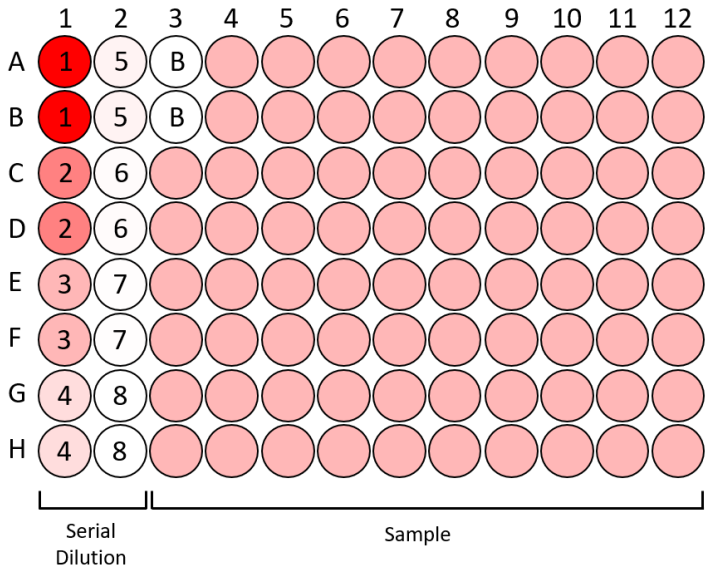

Figure 1. A sample layout guide for the setup of the 96-well plate.

Standard	Relative Concentration	Absorbance
1	1	3.44
2	0.2	0.821
3	0.04	0.1695
4	0.008	0.0325
5	0.0016	0.009
6	0.00032	0.004
7	0.000064	0.003
8	0.0000128	0.0025

Table 1. The background adjusted and averaged absorbance values from each duplicate pair correlating to the proportion of sample concentration in the serial dilution.

Tartrazine), microplate reader (BioTek), and a PCR workstation (optional) are suggested for this training.

Protocol

1. To prepare the "sample," add 50µL of food coloring (~2 drops) into 10 mL of water and shake.
2. Label the 8 1.5 mL microtubes as numbers 1–8 and pipette 440 µL of water into tubes 2–8 by pushing the plunger to the first stop, pulling up the liquid, and dispensing into a tube by plunging to the first stop.
3. Transfer 550 µL of the sample into tube 1.
4. Transfer 110 µL of the diluted sample from tube 1 into tube 2 and mix by pipetting. This can be accomplished by pulling the liquid mixture up and down a few times. Repeat step 4 (transferring 110 µL from tube 2 into tube 3 and mixing by pipetting up and down, etc.) until tubes 1–**7** contain 440 µL of solution and tube 8 contains 550 µL. Remove 110 µL of solution from tube 8 and throw it into waste.
5. Transfer 200 µL of the solution in tube 1 into both wells A1 and B1 in the 96-well microliter plate in order to set up the serial dilution in duplicate.
6. Repeat step 6 (transferring 200 µL from tube 2 into both wells C1 and D1, etc.) for the remaining vertical pairs of wells in columns 1 and 2. See Figure 1 for a visual guide.
7. Pipette 200 µL of water into wells A3 and B3 to serve as blanks.
8. In the remaining wells in columns (3–12), pipette 190 µL of water into each well.
9. Add 10 µL of the sample to each well in columns 3-12. Plunge to first stop, take up, and plunge to first stop again.
10. Using a microplate reader, measure the absorbance of each well. The wavelength varies depending on color of dye, yellow 5 absorbance is 400-460nm range. Set the microplate reader to measure at 450nm. Save and export the to a Microsoft Excel spreadsheet (see Table 2 for an example output.
11. In Microsoft Excel, measure the average absorbance of the blank wells and subtract this value from all other wells to eliminate background absorbance.
12. Take the average absorbance of each duplicate pair in columns 1 and 2 and generate a table (see Table 1 for an example).
13. Generate a scatterplot with these averages on the y axis and the proportion of sample concentration in each solution on the x axis (see Figure 2 for an example).
14. Add a linear trendline to the plot along with an r^2 value.
15. This is the final metric for measuring performance in creating the serial dilution.
16. Obtain the mean and standard deviation of the absorbance of the sample solutions. Divide the standard deviation by the mean to obtain the coefficient of variance (CV). Subtract the CV from 1 to obtain the final

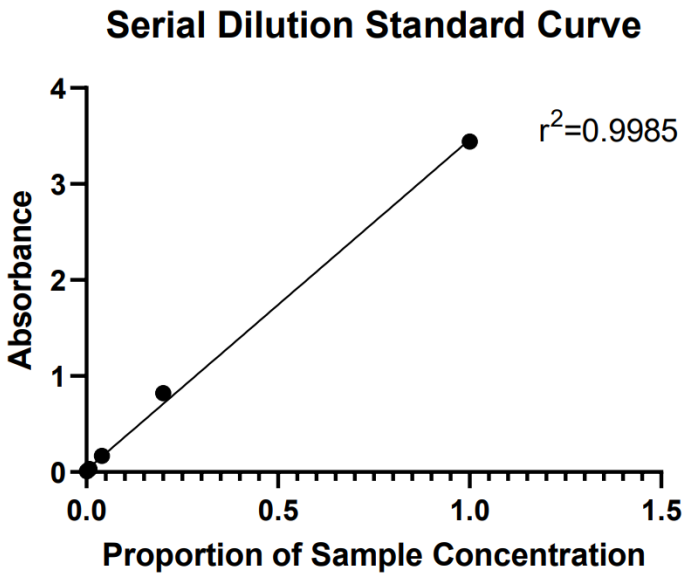

Figure 2. The serial dilution standard curve with a linear regression line and r^2 value.

metric of pipetting precision.

RESULTS AND DISCUSSION

The goal of this protocol is to create a standardized method for undergrads to practice micropipetting, serial dilutions, and basic statistical analysis. Perfecting these ubiquitous skills before performing them in a live study will reduce error and increase confidence in the student.

A majority of the undergraduates in our lab have successfully completed the procedures above. We suggest that students practice their pipetting until the 1-CV hovers around 0.99. This indicates high precision in their pipetting skills. In our experience, undergraduates may range from 1-5 tries to perfect this technique. To help instructors with these procedures, we have provided a sample of one undergraduate attempt at this protocol. The student began by creating a standard dilution by a factor of 5 into 8 microtubes. After loading these into the proper wells the student then loaded water into the rest of the wells mimicking the addition of master mix in a PCR plate. After this the student added the yellow dye marked "sample" into every well with water mimicking the addition of the cDNA in a PCR plate. After running the plate through the microplate reader, an excel file was generated, similar to Table 2. Taking the duplicate values of the blank wells and subtracting this value from every other well in Microsoft excel, the student generated a table similar to Table 1. This table shows the background adjusted absorbance values of each dilution. A standard curve can be plotted from these values and an r^2 calculated. This r^2 value represents the final performance of the standard dilution. This serves as a great practice without wasting valuable, expensive laboratory reagents.

Three students from our laboratory were asked to follow the Pipette Olympics procedure twice and record their resulting R^2 and 1-CV values. The change of these values between the students' first and second attempts was measured, then averaged together to analyze the development of pipetting precision through this procedure (Table 3). Two out of three students improved their pipetting with the second attempt.

Due to the COVID-19 pandemic, it is understandable that neuroscience majors have spent less time in the laboratory, therefore have missed out on basic lab training. It is important, therefore, to have these students trained in laboratory techniques in an expedited way to reduce gaps in learning. Neuroscience educators lacked a gamified standard quantitative method to teach and practice pipetting techniques, hence the focus of this paper. Through friendly competition fostered by the principal investigator or instructor, students compete to achieve the best r^2 and 1-CV value in the least amount of tries. In our laboratory, the winner is announced during lab meeting and congratulated with a paper Olympic gold medal. This way, students enjoy the experience while also fostering friendly competition.

In summary, this protocol provides undergraduate students hands-on experience with basic laboratory techniques they will utilize in every neuroscience wet lab.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	3.44	0.008	0.000	0.199	0.199	0.202	0.201	0.201	0.201	0.200	0.201	0.200	**$\bar{x}$**
B	3.44	0.01	0.000	0.200	0.202	0.201	0.201	0.199	0.201	0.200	0.200	0.201	0.200
C	0.839	0.003	0.199	0.202	0.201	0.201	0.201	0.202	0.202	0.200	0.199	0.198	**σ**
D	0.803	0.005	0.199	0.201	0.200	0.199	0.200	0.200	0.199	0.200	0.201	0.199	0.001
E	0.173	0.002	0.199	0.200	0.200	0.198	0.201	0.202	0.199	0.200	0.201	0.202	**CV**
F	0.166	0.004	0.201	0.199	0.198	0.198	0.199	0.199	0.199	0.201	0.202	0.200	0.006
G	0.034	0.004	0.201	0.200	0.199	0.200	0.201	0.200	0.202	0.199	0.200	0.200	**1-CV**
H	0.031	0.001	0.202	0.199	0.200	0.201	0.202	0.200	0.202	0.199	0.200	0.201	0.994

Table 2. A student's example of the background adjusted absorbance values for the entire 96 well plate. The mean ($\bar{x}$), standard deviation (σ), coefficient of variance (CV), and 1-CV value are shown.

	r^2			1-CV		
Student	**Attempt 1**	**Attempt 2**	**Change**	**Attempt 1**	**Attempt 2**	**Change**
1	0.9549	0.9810	0.0261	0.8760	0.9060	0.0300
2	0.9787	0.9761	-0.0026	0.8797	0.8779	-0.0018
3	0.9797	0.9804	0.0007	0.7323	0.7757	0.0434
Average			0.0081			0.0239

Table 3. A sample of student data that displays the R^2 and 1-CV values of 3 students' first and second attempts, as well as the change between the attempts.

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