Tips & Tools
DOI: https://doi.org/10.1128/jmbe.v21i3.2173
JMBE Journal of Microbiology & Biology Education

A Student-Focused Lab Module To Investigate Single-Nucleotide Polymorphisms of Common Heritable Traits†

Erin K. Shanle* and Denis Trubitsyn
Department of Biological and Environmental Sciences, Longwood University, Farmville, VA 23909

INTRODUCTION

Introductory biology and genetics courses typically use monogenic biallelic Mendelian traits to teach the fundamental relationship between genotype and phenotype. Although this is an invaluable starting point for understanding the inheritance of traits, students often carry misconceptions about a single gene being the sole determinant for a given phenotype (1). With the wide availability of personalized genomics through direct-to-consumer services such as 23andMe, it is critical that biology students and the general public understand that this deterministic view is not accurate in most cases. Environmental factors, variable penetrance, and interplay among an array of genetic factors ultimately lead to a given phenotype in most human traits. Given these layers of complexity, it can be difficult for students to gain a deep understanding of the true relationship between phenotype and genotype for many human traits.

Here, we describe a student-centered lab module that builds on the Mendelian principles of trait determination and culminates in students discovering the limitations of the assumption that a single genotype determines a given phenotype. Single-nucleotide polymorphisms (SNPs) for eye color, phenylthyocarbamide (PTC, bitter) taste, hair curl, cilantro aversion, and photic sneeze reflex were chosen for this investigation because they had limited associations with human disease and were easily characterized by students. This lab module was designed to be completed over four lab periods and was assessed by an individual lab report and group poster presentation (Fig. 1). Students were also surveyed before and after the module for self-reported confidence performing research, engagement in inquiry-based labs, perspective on personalized genomics, and interest in pursuing a research career. The lab module was implemented in an introductory cell biology and genetics course that biology major undergraduates take after one introductory biology course. This lab module provides a foundation for upper-level genetics courses and may be adapted for introductory biology courses as well. After completing this lab module, students should be able to:

1. Analyze and interpret SNP sequences to compare the relationship between genotypes and phenotypes for common human traits.
2. Describe factors that limit the use of a SNP as a sole predictor of a human trait.
3. Design primers and perform PCRs.

PROCEDURE

In the first lab of this module, students navigated two publicly available databases (ClinVar and dbSNP) to learn about SNPs in the context of a case study centered around Wilson's disease. Teacher notes and student handouts for the labs are provided in Appendix 1, and the case study is provided in Appendix 2. Within the case, students also participated in a class discussion centered around personalized genomics and the concept of genetic self-awareness. Finally, students were introduced to five common traits with known SNP associations: eye color, PTC taste, hair curl, cilantro aversion, and photic sneeze reflex (Table 1). Students learned about primer design and the overview of the lab module. Students recorded their phenotypes and predicted their genotypes using the associations provided in Student Handout 1 (Appendix 1). At the end of the first lab, students chose one SNP to investigate in the module. This student-centered approach allowed students to focus on the traits they found most interesting.

In the second lab, students used tissue-based PCR to amplify the region surrounding the SNP. Primer sequences and reaction conditions are provided in Appendix 1. Gel electrophoresis results for a subset of student samples are shown in Fig. 2 (top). In the third lab, students purified the PCR mixture to prepare for sequencing. At the end of the third lab, student samples were deidentified within each SNP group. In the final lab, students analyzed the sequences

*Corresponding author. Mailing address: Department of Biological and Environmental Sciences, Longwood University, 201 High Street, Farmville, VA 23909. Phone: 434-395-2584. E-mail: shanleek@longwood.edu.
Received: 24 May 2020, Accepted: 9 August 2020, Published: 12 November 2020
†Supplemental materials available at http://asmscience.org/jmbe

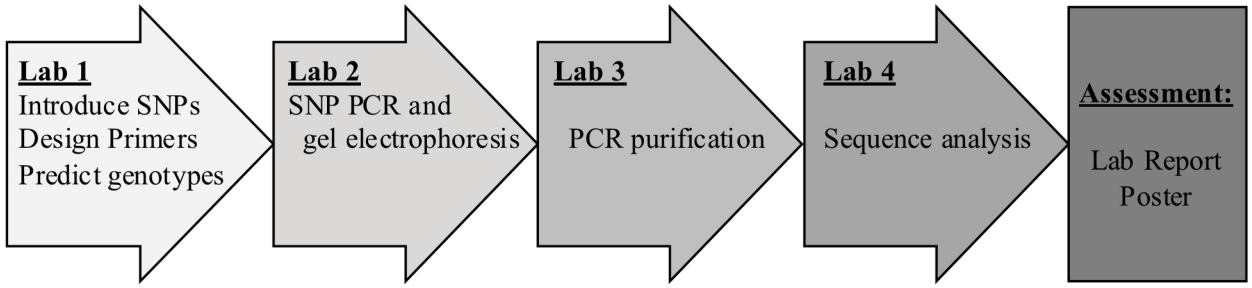

FIGURE 1. Sequence of labs in the SNP module. Each lab activity can be separated for shorter lab periods or performed by the instructor outside of class to reduce the number of labs required for the module.

of all of the samples within the SNP group to determine whether the predicted genotypes matched the sequencing results. Example chromatograms showing homozygous and heterozygous genotypes are shown in Fig. 2 (bottom). In most cases, the heterozygous condition showed two distinct overlapping peaks (Fig. 2A, bottom). However, two peaks for different bases showed an exact overlap in some cases (Fig. 2C, bottom). It is helpful for students to compare their final analysis with those of other students in the same SNP group so they can discuss why and how they determined whether each SNP was homozygous or heterozygous. Safety considerations and required materials are provided in Appendix 1.

It is important to have groups of several students who choose the same SNP for several reasons. By combining two sections of students (total of 34 students), SNP groups contained 3 to 11 student samples. First, some sequencing results were not interpretable, likely due to poor PCR amplification or purification. However, several samples in each group were interpretable, so students were able to complete the lab module for every chosen SNP. Second, there will be a greater likelihood for variable phenotypes and genotypes for a given SNP. Within the smallest group of three students who chose cilantro aversion, two students were recorded as having the trait and one student did not. Sequencing results were successful for every sample in this group, so students could compare the predicted and observed genotypes. Finally, combining students in different sections and having larger groups ensure the samples are not identifiable and maintain genetic privacy. This is an important consideration, particularly if a chosen SNP is associated with a health condition. The SNPs chosen for this lab module had limited, if any, published association with health conditions. However, the eye color SNP is associated with iris pigmentation, which is a marker for increased risk of melanoma (7). Because the final assessment was a lab report, some students made health risk connections in their report.

RESULTS AND CONCLUSIONS

In order to assess the lab module, students completed an individual lab report, a poster presentation, and pre- and post-assessment surveys. Students reported a significant

TABLE 1
Possible genotypes and associated phenotypes for the SNPs used in the lab module.

Phenotype (trait)	SNP ID	Upstream Sequence (5' to 3')	SNP Alleles	Genotypes	Association	Reference
Photic sneeze reflex	rs10427255	GGATTTAGCCCA	C>T	CC/CT/TT	CC more likely to sneeze in response to sunlight	(2)
Curly hair	rs17646946	AGGAACTGGAGT	A>G	AA/AG/GG	GG more likely to have curly or wavy hair	(3)
PTC (bitter) taste	rs713598	TGTTGCTCAGTG	C>G	CC/CG/GG	GG less likely to taste bitter	(4)
Cilantro aversion	rs2741762	ATTCCATATTCA	G>A	GG/GA/AA	AA less likely to like cilantro	(5)
Blue eyes	rs12913832	TTTGAGCATTAA	A>G	AA/AG/GG	GG more likely to have blue eyes	(6)

One SNP allele (a letter of DNA sequence) is inherited from each parent, resulting in a homo- or heterozygous genotype. Short sequences directly upstream of the SNP location are provided to aid in student-generated sequence analysis.

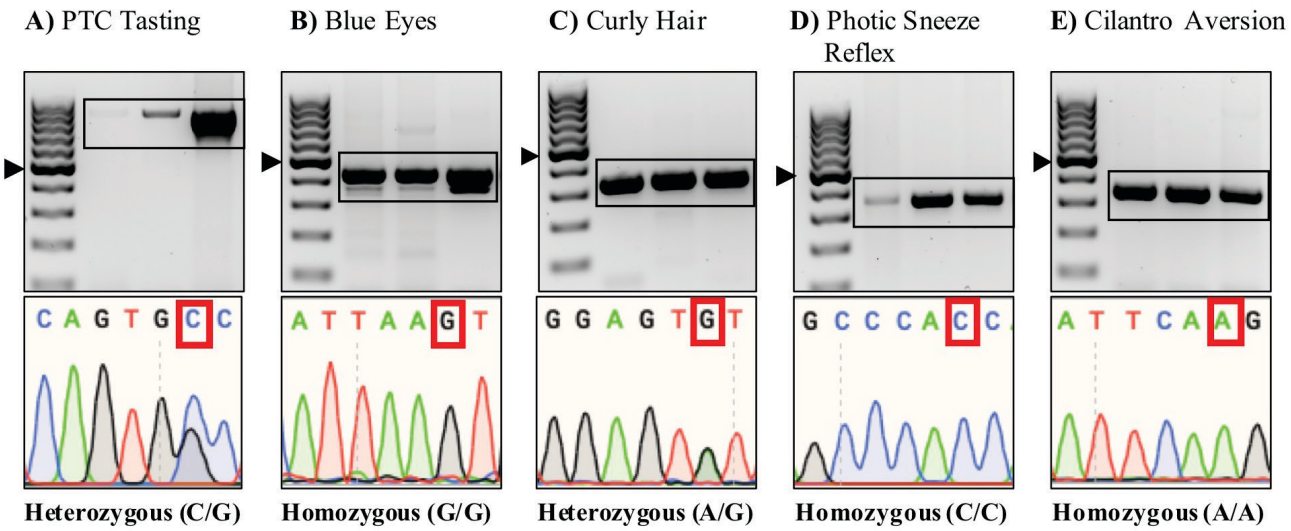

FIGURE 2. Results for SNP PCR and Sanger sequencing. A subset of gel electrophoresis results (top) for the chosen SNPs (A–E). A 100-bp DNA ladder was used in the first lane to confirm PCR product sizes (500 bp, indicated by ▶). Frames highlight the products of expected sizes for each SNP. Example DNA sequencing spectra are shown below each gel with the genotype interpretation for the given spectrum. Spectra were visualized with SnapGene software (Insightful Science).

increase in their ability to find novel results, but there were no significant changes in perceptions of engagement, personalized genomics, or interest in pursuing a research career (Fig. 3, Appendix 3). Because Longwood University biology students are required to complete research projects in the first semester of the curriculum prior to taking this course, it may not be surprising that this lab module did not change student perceptions on engaging in inquiry. Final lab reports and poster presentations demonstrated that students initially predicted genotypes based on the association information provided (see Appendix 4 for lab report and poster guidelines). Students found that SNP genotypes did not always align with associated phenotypes, particularly for SNPs associated with hair curl, eye color, and photic sneeze reflex. In final lab reports, most students identified the following factors to explain the discrepancies between genotype and phenotype: (i) variability in self-reporting phenotypes, (ii) contribution of other genes that determine the trait, and (iii) environmental factors. This suggests that the lab module described herein helps students gain a deeper understanding of the complex relationship between genotype and phenotype.

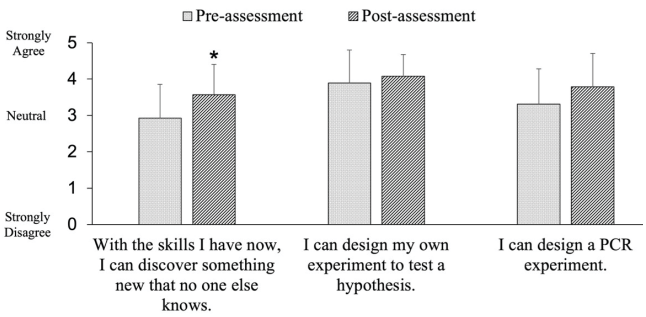

FIGURE 3. Sample results of pre- and post-assessment surveys. *, $p < 0.05$ (two-tailed Student's *t*-test). Additional suggested assessment questions can be found in Appendix 3.

SUPPLEMENTAL MATERIALS

Appendix 1: Teacher notes and student handouts
Appendix 2: Wilson's disease case study
Appendix 3: Pre- and post-assessment surveys
Appendix 4: Lab report and poster guidelines

ACKNOWLEDGMENTS

The authors declare that they have no conflicts of interest. This work was approved by the Longwood University Institutional Review Board. A Longwood University Faculty Research and Development Grant helped fund the project.

REFERENCES

1. Shaw KR M, Van Horne K, Zhang H, Boughman J. 2008. Essay contest reveals misconceptions of high school students in genetics content. Genetics 178:1157–1168.
2. Eriksson N, Macpherson JM, Tung JY, Hon LS, Naughton B, Saxonov S, Avey L, Wojcicki A, Pe'er I, Mountain J. 2010. Web-based, participant-driven studies yield novel genetic associations for common traits. PLOS Genet 6(6):e1000993.
3. Medland SE, Nyholt DR, Painter JN, McEvoy BP, McRae AF, Zhu G, Gordon SD, Ferreira MAR, Wright MJ, Henders AK. 2009. Common variants in the trichohyalin gene are associated with straight hair in Europeans. Am J Hum Genet 85:750–755.

4. Kim U, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. 2003. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science 299:1221–1225.
5. Eriksson N, Wu S, Do CB, Kiefer AK, Tung JY, Mountain JL, Hinds DA, Francke U. 2012. A genetic variant near olfactory receptor genes influences cilantro preference. Flavour 1:22.
6. Sturm RA, Duffy DL, Zhao ZZ, Leite FPN, Stark MS, Hayward NK, Martin NG, Montgomery GW. 2008. A single SNP in an evolutionary conserved region within intron 86 of the HERC2 gene determines human blue-brown eye color. Am J Hum Genet 82:424–431.
7. Laino AM, Berry EG, Jagirdar K, Lee KJ, Duffy DL, Soyer HP, Sturm RA. 2018. Iris pigmented lesions as a marker of cutaneous melanoma risk: an Australian case-control study. Br J Dermatol 178:1119–1127.