Laboratory Exercise

An Alternative Laboratory Designed to Address Ethical Concerns Associated with Traditional *TAS2R38* Student Genotyping[S]

Michelle L. LaBonte†*
Melissa A. Beers‡

From the †Department of the History of Science, Harvard University, Cambridge, Massachusetts 02138, ‡Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts 02481

Abstract

The *TAS2R38* alleles that code for the PAV/AVI T2R38 proteins have long been viewed as benign taste receptor variants. However, recent studies have demonstrated an expanding and medically relevant role for *TAS2R38*. The AVI variant of T2R38 is associated with an increased risk of both colorectal cancer and *Pseudomonas aeruginosa*-associated sinus infection and T2R38 variants have been implicated in off-target drug responses. To address ethical concerns associated with continued student *TAS2R38* gene testing, we developed an alternative to the traditional laboratory genotyping exercise. Instead of determining their own genotype, introductory level students isolated plasmid DNA containing a section of the human *TAS2R38* gene from *Escherichia coli*. Following PCR-mediated amplification of a section of the *TAS2R38* gene spanning the SNP at position 785, students determined their assigned genotype by restriction enzyme digestion and agarose gel electrophoresis. Using the course wide genotype and phenotype data, students found that there was an association between *TAS2R38* genotype and the age of persistent *P. aeruginosa* acquisition in cystic fibrosis "patients." Assessment data demonstrated that students taking part in this new *TAS2R38* laboratory activity made clear learning gains.

Keywords: TAS2R38*; SNP; genotyping; ethics*

Introduction

The *TAS2R38* gene is one of a large family of taste receptor genes and the corresponding T2R38 protein is a G-protein coupled receptor [1, 2]. *TAS2R38* genotype correlates with the ability to taste the chemical phenylthiocarbamide (PTC), and thus originally *TAS2R38* was thought to be a Mendelian inherited trait. However, the broad spectrum of PTC sensitivity across different worldwide populations led to a closer investigation of the gene. The ability to taste PTC is actually much more complex, with 21 different single nucleotide polymorphisms (SNPs) identified in the coding region of the gene and the majority of these occurring solely in African populations [3]. In most other populations there are five common SNPs encoding seven different haplotypes [4]. These seven haplotypes include the two most common haplotypes, PAV, corresponding to the ability to taste PTC and AVI, corresponding to the inability to taste PTC [3, 4].

In recent years a number of new potential associations have been found between *TAS2R38* genotype and disease risk. Carrai *et al.* demonstrated an association between AVI/AVI individuals and an increased risk of colorectal cancer in Caucasian populations [5]. This association was not seen in a more recent study, but the study did not have sufficient statistical power to detect a possible association in the Caucasian population [6]. A 2012 study claimed that *TAS2R38* genotype is associated with an increased risk of schizophrenia, but scientific evidence regarding this association has been controversial [7, 8]. A new study proposes a role for the T2R family of receptors, including T2R38, in off target effects of pharmaceuticals [9]. In addition, evidence suggests that the PAV version of T2R38 functions in innate immunity by binding to acyl-homoserine lactones (AHLs) secreted from *Pseudomonas aeruginosa* [10]. Thus, individuals with the AVI version of T2R38 are more susceptible to *P. aeruginosa* sinus infection because their cells are unable

[S] *Additional Supporting Information may be found in the online version of this article.*

**Address for correspondence to: Department of the History of Science, Harvard University, Cambridge, MA 02138.*
E-mail: mlabonte@post.harvard.edu.

Received 4 June 2014; Revised 27 October 2014; Accepted 17 November 2014
DOI 10.1002/bmb.20846
Published online 20 February 2015 in Wiley Online Library (wileyonlinelibrary.com)

to bind to the AHLs produced by *P. aeruginosa*. As a result, downstream events that would lead to pathogen clearance attempts are not as effectively triggered in AVI/AVI or AVI/PAV individuals [10]. These findings have important implications for the possible role of T2R38 variants in chronic rhinosinusitis and respiratory disease susceptibility [11–13]. Taken together, recent studies demonstrate that T2R38 functions as more than simply a taste receptor.

Before the nontaste functions of T2R38 were known, Merritt *et al*. described a laboratory exercise in which students determine their PTC-tasting phenotype and their *TAS2R38* genotype [14]. This laboratory exercise makes use of a SNP at position 785 of the *TAS2R38* gene to determine genotype. Presence of a C at position 785 in the DNA is most commonly associated with the PAV (proline-alanine-valine) protein variant that is able to bind to the PTC chemical. In contrast, a T at position 785 in the DNA is typically associated with the AVI (alanine-valine-isoleucine) protein variant that is unable to bind to PTC [14]. Educational laboratory exercises designed to analyze student *TAS2R38* variants have become quite widespread, and Carolina Biological sells a *TAS2R38* genotyping kit [15, 16]. Students in the BISC110/112 Introductory Cellular and Molecular Biology with Laboratory course at Wellesley College participated in a version of the Merritt *TAS2R38* laboratory for several years until reports of potential disease risks associated with *TAS2R38* genotype were published. In deciding whether to continue student *TAS2R38* genotyping in light of the expanding knowledge of the biology of taste receptors, faculty at Wellesley explored how others have dealt with similar concerns [17, 18].

Many university instructors have tried to address the ethical concerns associated with student DNA testing by implementing an informed consent process in which students are given the ability to opt out of such exercises or in which instructors ask for volunteers for the testing [19, 20]. However, some worry that students may feel pressure to participate in the testing out of concern that their grade will be affected if they opt out [17, 21]. Stanford Medical School addressed this issue of perceived coercion by having students send testing out to a third party and mandating that students do not tell their professors whether they got tested [19]. The University of California at Berkeley provided incoming students with aggregated data on three metabolically relevant genes after the California Department of Public Health determined that students could not receive their individual test results upon arrival to campus in the fall of 2010 [22]. Others have opted to anonymize the samples during the DNA testing process and then provide students with aggregated data [23].

After considering how best to respond to the expanding understanding of taste receptors, we developed a new laboratory exercise in which students are assigned cloned versions of *TAS2R38* alleles and experimentally determine their "genotype." In addition, students are assigned a phenotype so that they are still able to analyze course-wide data to determine whether there is an association between genotype and phenotype. Given the concern that the removal of the student genotyping might affect student engagement with the laboratory series as well as the ability to generate and analyze authentic data, we took the opportunity to add a complex simulation to model the process of scientific investigation [17, 24–27]. Thus, our modified *TAS2R38* laboratory unit allows instructors to avoid the ethical issues associated with student DNA testing while still providing students with a laboratory experience focused on the increasingly relevant issue of human DNA testing.

Laboratory Series Background and Design

In this laboratory unit, students experimentally determine their assigned *TAS2R38* genotype by isolation of a section of human *TAS2R38* DNA. Instead of providing students with human genomic DNA, they are given cloned *TAS2R38* DNA in the context of a bacterial plasmid. After amplifying a region of the *TAS2R38* gene by PCR, students are able to determine their assigned genotype based on restriction enzyme mediated analysis of the SNP at position 785. Using this approach, students gain experience with the same experimental techniques that are used in traditional student *TAS2R38* DNA testing laboratory exercises.

After each student has determined her assigned genotype, she then conducts a course-wide analysis of data to see if there is an association between assigned phenotype and *TAS2R38* genotype. While this laboratory exercise historically has used PTC-tasting ability as the associated phenotype, we took the opportunity to choose a more medically relevant phenotype since students would not be working with their own DNA. Since evidence from the scientific literature suggested an interesting potential (and untested) association between *TAS2R38* genotype and the ability of cystic fibrosis (CF) patients to respond to *P. aeruginosa* exposure, we chose to assign students a phenotype based on the age at which persistent *P. aeruginosa* is acquired in CF patients [5].

CF is an autosomal recessive disease caused by a faulty chloride channel, termed the cystic fibrosis transmembrane conductance regulator (CFTR) [28, 29]. While the mutant chloride channel affects many organs, the resulting thick, sticky mucus in the lungs is responsible for significant morbidity and mortality in CF patients. As a result of the thick mucus and possibly the altered ion content in the CF lung, individuals with CF are prone to serious bacterial infections [30]. One such bacterium, *P. aeruginosa*, is present in many CF patients and increased morbidity and mortality is associated with early *P. aeruginosa* acquisition [31].

Many factors contribute to the age of persistent *P. aeruginosa* acquisition, including both genetic and

environmental influences [32]. As mentioned above, a study of sinonasal surgery patients (excluding patients with CF) demonstrated that infection with *P. aeruginosa* is influenced by the *TAS2R38* genotype [10]. In the individuals studied, the PAV variant of the T2R38 protein is able to bind to AHLs produced by *P. aeruginosa*, resulting in calcium flux, increased ciliary beat frequency, and clearance of *P. aeruginosa*. In contrast, the AVI variant of the T2R38 protein is not able to recognize the AHLs produced by *P. aeruginosa*. As a result, homozygous AVI/AVI and heterozygous PAV/AVI individuals have an increased risk of *P. aeruginosa*-associated sinus infection [10].

Given the role of *TAS2R38* genotype in *P. aeruginosa* infection of individuals without CF and the association of *P. aeruginosa* with morbidity in CF patients, we had students explore the simulated association between age of persistent *P. aeruginosa* acquisition in CF patients and *TAS2R38* genotype.

Methods

Laboratory Schedule

This laboratory unit is placed in the schedule such that the lecture portion of the course has covered the basic information regarding DNA, mutation, and heritability. The lab series is designed to expand upon this knowledge and introduce new concepts like SNPs and the "taster" gene *TAS2R38* as well as lab techniques including PCR, restriction enzyme digestion, and agarose gel electrophoresis. We also use this lab as an opportunity to introduce students to the ethical issues associated with personal genetic testing.

During week 1 of the module, we begin with a lecture providing the scientific background associated with the experiment. During this time, we also discuss gene testing in the context of direct to consumer genetic testing and informed consent. Students watch the NOVA scienceNow Personal DNA Testing video as a starting point for future discussions regarding the ethical issues associated with DNA testing [33]. To begin the experiment, students are given *Escherichia coli* containing either a plasmid with the T at position 785 of the *TAS2R38* gene, a C at position 785, or a combination of both types of bacteria. Students are also assigned an age of acquisition of *P. aeruginosa* as the phenotype of the cystic fibrosis "patient."

After isolating the plasmid DNA from their assigned *E. coli* sample, students practice dilutions by serially diluting their DNA for use in a PCR reaction. Part of the class is reserved for working as a group to discuss the process of PCR and the design of the primers used to amplify a 303 base pair region surrounding the SNP at position 785 of the *TAS2R38* gene. The students set up a PCR reaction using their diluted DNA, the reaction is run in the thermocycler overnight, and the instructor freezes the reactions until the next lab period. In addition, each pair of students amplifies a "control" heterozygous DNA sample to facilitate analysis in the second week.

In week 2, the students digest their amplified *TAS2R38* gene fragment with the restriction enzyme *Fnu4HI*. This enzyme recognizes the restriction site 5'-GCNGC-3'. The "taster" C785 variant of the *TAS2R38* gene creates an *Fnu4HI* cut site (5'-GCTGC-3'), while the "nontaster" T785 variant does not (5'-GTTGC-3'). The ability of *Fnu4HI* to differentiate the *TAS2R38* variants allows the students to visually determine the genotype of their "patient" via agarose gel electrophoresis. At the beginning of lab the students set up digests of "patient" and heterozygous control DNA. While the DNA is being digested by the restriction enzyme, pairs of students work together to pour 1.5% agarose gels. Students then separate both undigested and digested DNA samples by agarose gel electrophoresis. While waiting, students discuss possible outcomes to prepare for the subsequent interpretation of their gels.

Next, genotypes are recorded on a course data sheet (Supporting Information A) next to the corresponding "patient" number and age of acquisition. The students then analyze the data (101 samples during the fall 2013 semester) to determine if there is an association between age of acquisition of *P. aeruginosa* and genotype at position 785 in the *TAS2R38* gene. The analysis results in students writing a partial lab report in the style of a scientific article. This article includes Title, Authors, Introduction, and Results text with at least three figures, References, and a short student self-assessment about the lab and writing process. The lab work is completed in pairs, yet the final written work is completed independently. A writing workshop a week before the article is due allows the students to present their figures to their lab section for peer review and analysis before incorporation into their final article. Throughout the unit, the simulated nature of the experiment is stressed.

Generation of T785 and C785 *TAS2R38* Plasmids

The instructors generated the *TAS2R38* plasmids before the laboratory unit commenced. To this end, purified genomic DNA from a T785/C785 individual was used to amplify a 303 bp region of the *TAS2R38* gene by PCR with illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare catalog #27–9559-01). Primers were designed such that the 303 bp section of *TAS2R38* DNA contained the SNP at position 785 (Forward primer: 5′ AACTGGCAGATTAAAGATCT CAATTTAT 3′; Reverse primer: 5′ AACACAAACCATCACC CCTATTTT 3′). The PCR product was visualized by 1.5 % agarose gel electrophoresis with SYBR Safe DNA Gel Stain (Life Technologies catalog #S33102) and then purified using the QIAQuick Gel Extraction kit (Qiagen catalog #28704). To ultimately clone both the T785 and C785 alleles, the PCR product was ligated to the pGEM-T Easy vector (Promega catalog #A1360). DH5 alpha *E. coli* were transformed with the pGEM-T Easy ligation reaction and colonies

containing *TAS2R38* inserts were selected by blue/white screening using X-gal (Promega catalog #V3941). Plasmids containing *TAS2R38* inserts were purified using the QIAprep Spin Miniprep Kit (Qiagen catalog #27104) and the SNP at position 785 was determined by restriction enzyme digestion with *Fnu4HI* (New England Biolabs catalog #R0178S).

Isolation of Plasmid DNA Containing the TAS2R38 Gene

Each student was given a microfuge tube containing a frozen pellet of *E. coli* that contained the plasmid with the 303 bp *TAS2R38* gene segment (see Generation of T785 and C785 TAS2R38 Plasmids for cloning procedure). Each student then isolated the plasmid DNA using the manufacturer instructions included with the Qiagen QIAprep Spin Miniprep Kit (Catalog #27104).

PCR of the Region Surrounding SNP 785 in *TAS2R38*

Students diluted their isolated plasmid DNA (see Isolation of plasmid DNA containing the TAS2R38 gene) 1:10,000 in water. The diluted plasmid DNA was mixed with illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare catalog #27–9559-01). The same primers from Generation of T785 and C785 TAS2R38 Plasmids were used to amplify a 303 bp region around the SNP at position 785 in the *TAS2R38* gene. Amplification occurred using the following conditions: 95°C for 10 min; 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; followed by a final 10 min incubation at 72°C. Each pair of students also amplified a prediluted heterozygous sample of *TAS2R38* DNA to use as a control in their analysis.

Restriction Enzyme Digest of the SNP at Position 785 in *TAS2R38*

Following PCR amplification of the 303 bp region surrounding SNP 785 in *TAS2R38*, each sample was digested with the enzyme *Fnu4HI* (New England Biolabs catalog #R0178S) following manufacturer instructions. Digests were incubated at 37°C for 1 h to allow for complete digestion of the DNA.

Agarose Gel Determination of Genotype at SNP 785

Each pair of students was tasked with working through all calculations and procedures necessary to make and pour a 1.5% agarose gel dissolved in 1× TBE (89 mM Tris Base (TRIZMA), 89 mM boric acid, 2 mM EDTA (pH 8.0)). Students mixed their cut and uncut DNA samples with 6× loading dye (0.4% Ficoll 400, 1.8 mM EDTA, 0.55 mM Tris-HCl, 0.001% SDS, 0.025% Bromophenol Blue pH 8.0 at 25°C) and loaded the samples into individual wells. Gels were stained with SYBR Safe DNA Gel Stain (Life Technologies catalog #S33102) and photographed under UV light. Banding patterns of the "patient" DNA were compared with the known heterozygous control and the PCR Marker ladder (NEB catalog #N3234S).

Data Analysis

Genotypes determined by each student from their analysis of the agarose gel were recorded on a composite data sheet (Supporting Information A). The students then sorted the data using Microsoft Excel or any other spreadsheet program in order to create figures that best answered their experimental question.

Assessment Methods

Formal evaluation of this laboratory unit was conducted during fall 2013, the second semester this unit was offered, during which 108 students were enrolled in eight laboratory sections. The two identical surveys, pre- and postunit, are included as Supporting Information B. The survey is divided into four multiple choice sections. Part 1 (one question) addresses prior student exposure to biology in high school. Part 2 (five questions) identifies prior student experience with the techniques in this laboratory unit, with responses ranging from "I can perform this procedure on my own" to "I have never heard of this procedure." Part 3 (four questions) is designed to highlight general knowledge about genetics concepts and Part 4 (five questions) highlights knowledge surrounding concepts specific to the laboratory unit. Part 4 has "I do not know" as one of the answers for each of the five questions to prevent students from guessing. Participation in the survey was anonymous and did not involve any personal identifying information, so the study was exempt from review by the Wellesley College Institutional Review Board.

Results

Determination of *TAS2R38* Genotype

The two most common T2R38 receptor variants, PAV and AVI, can be determined by analysis of a single SNP at position 785. To determine their assigned genotypes, students in the BISC 110/112 laboratory course at Wellesley College isolated human *TAS2R38*-containing plasmid DNA, amplified a 303 bp region of the gene by PCR, digested the resulting PCR product with the enzyme *Fnu4HI*, and separated the DNA fragments by 1.5% agarose gel electrophoresis. As seen in Fig. 1, the three possible *TAS2R38* genotypes can easily be distinguished using this technique. Although the 65 bp band is often difficult to see, the two larger bands can be used to determine genotype. Students with the assigned T785/T785 genotype will have a single band at 303 bp and students with the assigned C785/C785 genotype with have bands at 238 bp and 65 bp. Students with the heterozygous C785/T785 genotype will have three bands at 303 bp, 238 bp, and 65 bp. Once each student completed the genotyping analysis, results were entered into a course wide spreadsheet. The resulting data sheet, containing course wide assigned genotype and assigned phenotype data, was used by students to determine if there was an association between *TAS2R38* genotype and age of

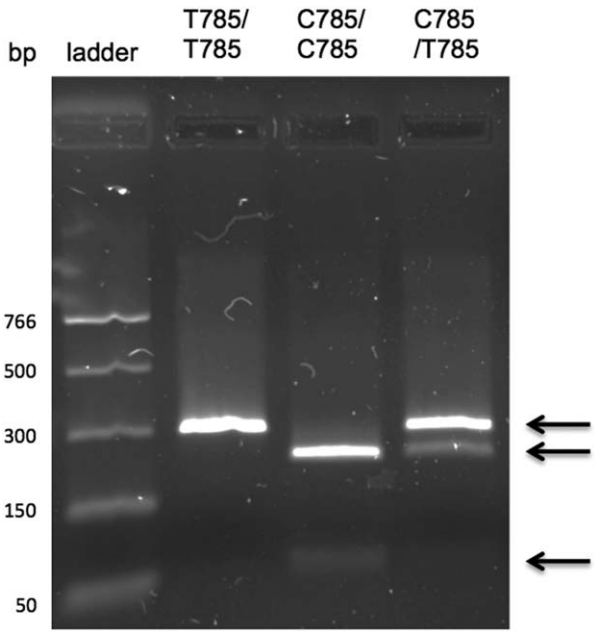

FIG 1

Determination of TAS2R38 *genotype. Plasmid DNA containing a section of the* TAS2R38 *gene was isolated from* E. coli *and a 303 bp region of the* TAS2R38 *gene was amplified using PCR. The PCR products were digested with* Fnu4HI *and the resulting DNA fragments were separated by 1.5% agarose gel electrophoresis and visualized with SyberSafe DNA stain. A single 303 bp band corresponds to the T785/T785 genotype and the C785/C785 genotype is represented by two bands of 238 bp and 65 bp. The heterozygous C785/T785 genotype is represented by three bands of 303 bp, 238 bp, and 65 bp. The three possible fragment sizes are indicated by arrows to the right of the gel.*

persistent *P. aeruginosa* acquisition. The fall 2013 course data sheet can be seen in Supporting Information A.

Student Analysis of Genotype and Phenotype Data

Students in the BISC 110/112 laboratory course are asked to write a partial article based on the genetics data. Using the simulated data, students determine whether there is an association between *TAS2R38* genotype and the age of persistent *P. aeruginosa* acquisition in CF patients. At least three distinct figures addressing this question are required. Many students begin their analysis by presenting a figure of their gel. Their second figure often represents the mean age of persistent *P. aeruginosa* acquisition for each genotype. As shown in Fig. 2*a*, the mean age of persistent *P. aeruginosa* acquisition in cystic fibrosis patients with the *TAS2R38* T785/T785 genotype is 5.5 (±2.7) years. In contrast, the mean age of *P. aeruginosa* acquisition is 11.6 (±3.2) and 12.1 (±3.1) years in C785/T785 and C785/C785 individuals, respectively. Students generating this type of figure conclude that the mean age of *P. aeruginosa* acquisition is lower for cystic fibrosis patients with the T785/T785 *TAS2R38* genotype as compared with those with the C785/T785 or C785/C785 genotypes.

For their third figure, students will often look at the *TAS2R38* genotype distribution either in different age groups or at each age individually. As seen in Fig. 2*b*, one student analyzed, for each *TAS2R38* genotype, the percentage of cystic fibrosis patients who acquired *P. aeruginosa* in the 2- to 8-year and 9- to 18-year age ranges, deciding to cut the range of acquisition from 2 to 18 years in half. She found that the majority of cystic fibrosis patients with the T785/T785 *TAS2R38* genotype acquired *P. aeruginosa* during the younger age range of 2 to 8 years. In contrast, the majority of cystic fibrosis patients with the C785/T785 and C785/C785 *TAS2R38* genotypes acquired *P. aeruginosa* at the older age range of 9 to 18 years. Another student looked in even more detail at the relationship between *TAS2R38* genotype and age (Fig. 2*c*). Based on this analysis of simulated data, it is clear that cystic fibrosis patients who acquire *P. aeruginosa* at younger ages are more likely to have the T785/T785 *TAS2R38* genotype. As the age of *P. aeruginosa* acquisition increases, the *TAS2R38* genotype is more likely to be C785/T785 or C785/C785. Thus, students are able to see a clear relationship between *TAS2R38* genotype and age of *P. aeruginosa* acquisition through analysis of the simulated course wide data. Note: student figures and legends are presented as they were designed and written. No modifications were made for publication.

Evaluation of Student Outcomes

To evaluate the efficacy of the *TAS2R38* laboratory unit, we asked students to complete anonymous pre- and postunit surveys (see Materials and Methods and Supporting Information B). Wellesley College has a diverse student background, with students from all walks of life and from many different countries throughout the world. Since Wellesley College does not give course credit for high scores on AP/IB biology exams, all students interested in a major in several biologically relevant fields must complete the BISC 110/112 course with lab. To assess their variable backgrounds, we asked students to provide us with information regarding their high school biology coursework (Fig. 3). We found that 99% of our students had a prior biology course. More specifically, 68% of students had AP/IB biology with or without lab, while 31% had regular or non-AP/IB biology with or without lab. Interestingly, 26% of our students had no prior laboratory experience.

In addition to the high school biology background of our students, we sought to assess their general genetics knowledge by asking multiple-choice questions covering concepts such as molecular biology, genotype, and phenotype. Responses demonstrated very little if any increase in student knowledge comparing pre- and post survey data (Fig. 4, questions 2–4). This outcome was expected, as we felt that most students with a high school biology background should be able to correctly answer these questions.

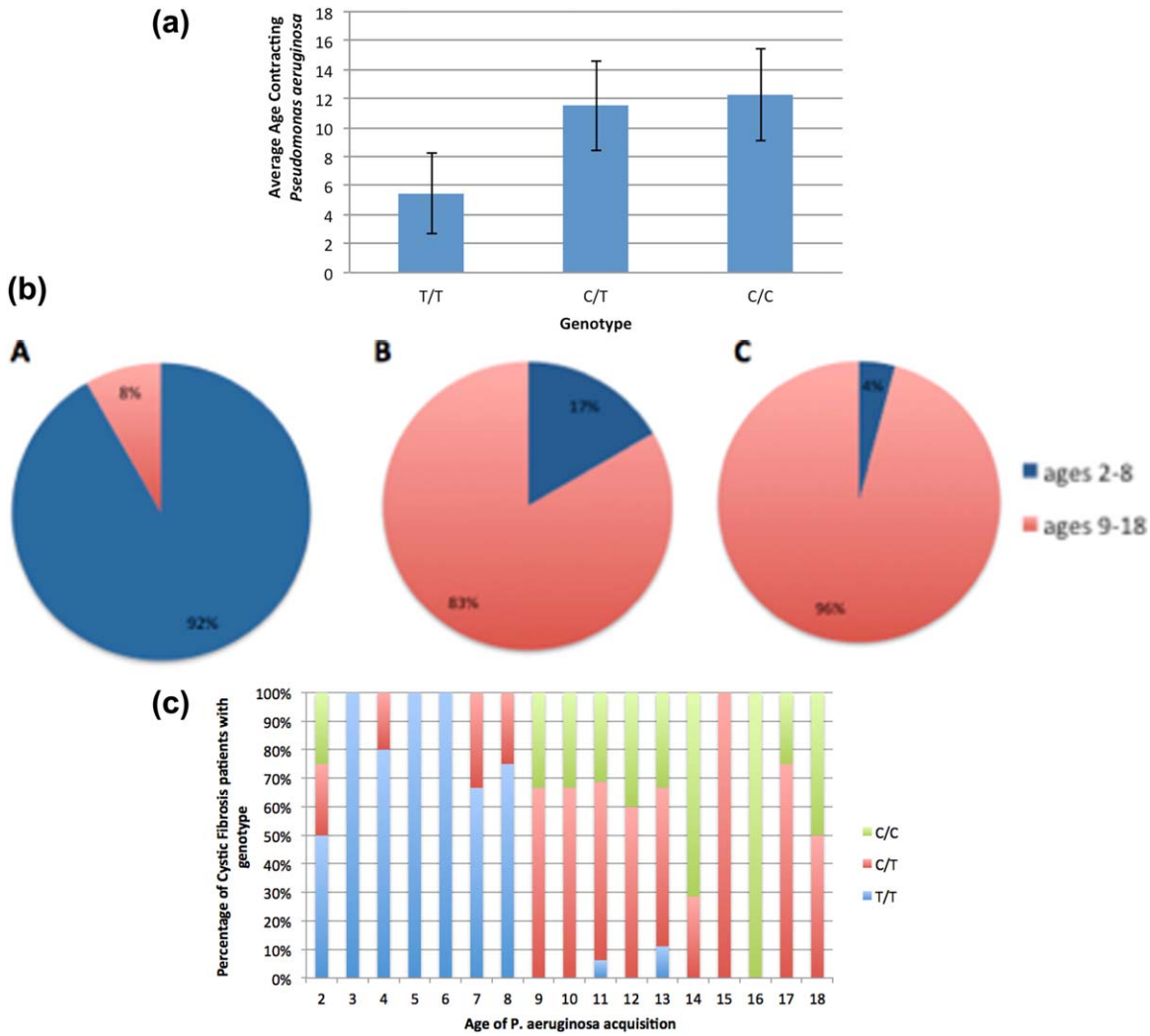

FIG 2

(a) Correlation between average age of acquiring P. aeruginosa *and SNP position 785 on the TAS2R38 gene. Average age at which cystic fibrosis patients were infected with* P. aeruginosa *was determined for each genotype group, where C represents C785 and T represents T785. C/C and T/T are homozygous alleles for cytosine and thymine bases, respectively, while C/T is a heterozygous allele for both cytosine and thymine bases. Standard deviation is depicted as error bars on the graph. Data was collected from 101 individuals. (Figure and legend prepared by Christina Lepore, a Fall 2013 BISC110/112 Laboratory student at Wellesley College.) (*b*) Percentage of cystic fibrosis (CF) patients who acquired* P. aeruginosa *in certain age groups sorted by genotype of TAS2R38 gene. One hundred one CF patients were sorted according to their genotype through PCR and restriction enzyme analysis. (A) Shows CF patients with genotype T785/T785; (B) shows CF patients with genotype C785/T785; (C) shows CF patients with genotype C785/C785. CF patients were also separated into two groups based on age: 2 to 8 yrs and 9 to 18 yrs. These divisions were made based on data from Fig. 2. In A, 91.7% of CF patients with T785/T785 genotype acquired* P. aeruginosa *in the 2 to 8 yrs group. In B, however, 83.5% of CF patients with C785/T785 genotype acquired* P. aeruginosa *between 9 and 18 yrs. In C, the split is even clearer, with 95.8% of CF patients with C785/C785 genotype who acquired* P. aeruginosa *between 9 and 18 yrs. These results suggest that CF patients with a C allele at position 785 are more likely to acquire* P. aeurginosa *at a later age than those without a C allele at position 785. (Figure and legend prepared by Crysti Wang, a Fall 2013 BISC110/112 Laboratory student at Wellesley College.) (*c*) Early acquisition of* P. aeruginosa *in cystic fibrosis patients associated with T785 SNP in the TAS2R38 gene. Genotypes of 96 individuals with cystic fibrosis were determined by using PCR, restriction digestion with enzyme* Fnu4H1*, and gel electrophoresis analysis. Phenotypes, age of* P. aeruginosa *acquisition, were given by each individual. Phenotypes of early acquisition of* P. aeruginosa *had the highest percentage of individuals with the genotype T785/T785. Phenotypes of late acquisition of* P. aeruginosa *had the highest percentage of individuals with the genotype C785/T785 and C785/C785. Early acquisition of* P. aeruginosa *in cystic fibrosis patients is associated with the T785 SNP within the TAS2R38 gene. Late acquisition of* P. aeruginosa *in Cystic Fibrosis patients is associated with C785 SNP within the TAS2R38 gene. (Figure and legend prepared by Anna Ha, a Fall 2013 BISC110/112 Laboratory student at Wellesley College.) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

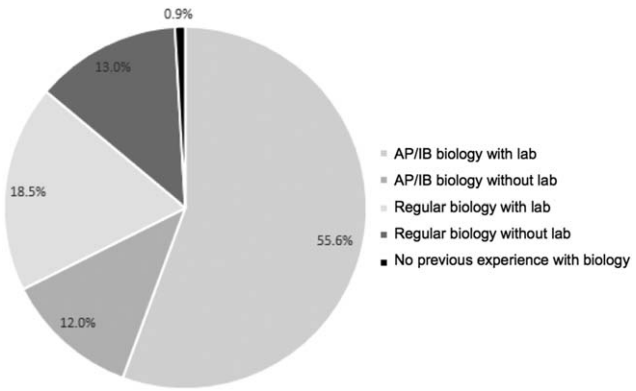

FIG 3 *Variation in student preparedness before enrolling in BISC110/112 at Wellesley College. 108 students completed the genetics survey.*

However, we were surprised by the moderate student understanding of DNA (Fig. 4, question 1). We had expected that the vast majority of students would be aware of the three important features of DNA included in the question, yet those who answered incorrectly chose only the unabbreviated name of DNA rather than all of the above. While the most students had at least a very basic understanding of DNA, a more detailed discussion of the structure of DNA in class may be important.

Students were also asked multiple choice questions covering unit specific concepts such as the definition of a SNP, the symptoms involved in cystic fibrosis, and the basic methodology behind the relevant laboratory tools. Students exhibited varying levels of understanding on the preassessment surveys, yet approximately 89 to 99% of the students responded with the correct answer on the postassessment survey (Fig. 4, questions 5–9). Before the lab unit, only 18% of the students knew that a SNP was a specific location in the genome where greater than 1% of the population has been shown to have a different nucleotide present. After the lab unit, 92% of the students answered this question correctly (Fig. 4, question 5). Only 40% of the students understood before the unit that cystic fibrosis is characterized by abnormal transport of chloride and sodium ions across an epithelium, leading to thick, viscous secretions. This increased drastically to 90% after the unit (Fig. 4, question 6). When asked to define what a restriction enzyme is, only 44% of students answered correctly before the unit. In contrast, 92% of students correctly defined a restriction enzyme at the end of the unit. Similar results were obtained for understanding the concept of PCR. Before the lab unit, only 35% of students knew that PCR allows scientists to amplify a few copies of DNA to generate thousands to millions of copies of a particular sequence. In contrast, 94% of the students could properly define PCR at the end of the unit. When asked about agarose gel electrophoresis before the unit, 64% of students knew that smaller pieces of DNA move faster through the gel matrix. At then end of the lab unit, 99% of the students answered the question correctly. These data demonstrate that students taking part in the genetics laboratory unit made important conceptual knowledge gains about advanced topics and methodologies.

In addition to evaluating student understanding of relevant scientific concepts and laboratory techniques, we also assessed levels of self-determined competency with laboratory methods (Fig. 5). We were interested in analyzing student perceptions of their laboratory skills, as many Wellesley students go on to work in research labs on campus and the methods we emphasize in this laboratory unit are all basic techniques in molecular biology. Before the lab unit, 46% of students felt they could perform a plasmid mini-prep on their own or with guidance, while 21% had never heard of the procedure. At the end of the unit, 99% felt confident they could perform a mini-prep on their own or with guidance. Performing a PCR reaction showed similar pre- and postcompetency levels (43% pre and 97% post). Interestingly, restriction enzyme digestion showed the highest gains from pre- to postlaboratory in that 32% of students before the unit and 98% at the conclusion of the unit felt they could perform a restriction enzyme digest on their own or with guidance. Students were initially most comfortable with agarose gel electrophoresis (66% could perform on their own or with guidance before and 99% after the lab unit) and using Microsoft Excel to sort and analyze a large data set (89% could perform on their own or with guidance before and 99% after the lab unit). Taken together these data suggest that a majority of students feel an increased competency with methods commonly used in a molecular biology lab.

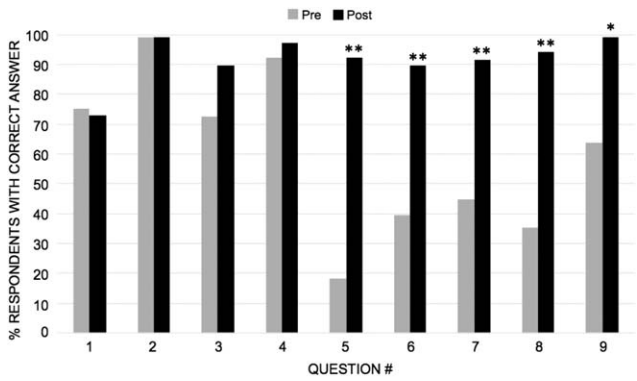

FIG 4 *Percent of students who answered concept questions correctly one week before the beginning of the laboratory unit (pre) and after the unit was completed (post). Questions 1 to 4 highlight general knowledge about genetics concepts and questions 5 to 9 highlight more advanced knowledge surrounding concepts specific to this laboratory unit. See Supporting Information B for specific questions). Using a two-tailed* t*-test, significant differences are indicated by* $*p < 0.01$ *or* $**p < 0.0001$.

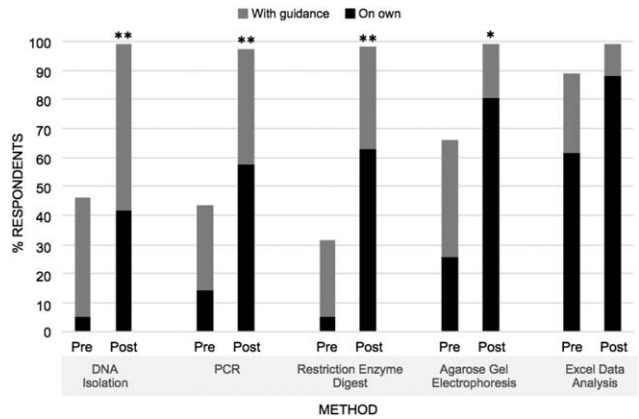

FIG 5

Student self-determined competency with laboratory unit methods. One hundred eight students completed the genetics survey. Using a two-tailed t*-test, significant differences are indicated by* $*p < 0.01$ *or* $**p < 0.0001$.

Given the concerns regarding lower student interest associated with removing student DNA testing from this lab, we also compared end of semester student responses about personal relevance from before and after the simulation was introduced. While this comparison asks students about the entire semester of laboratory concepts, the only substantial change that was made in that time period was to the genetics series. As shown in Fig. 6, student perception of personal relevance was not negatively impacted by the switch from student DNA testing to analysis of plasmid DNA. Overall, these data demonstrate that the simulated laboratory exercise results in student learning gains and does not affect student perceptions of personal relevance.

Discussion

It is important that instructors have alternatives to traditional student DNA testing laboratories, so that they can decide how best to address ethical concerns raised by such educational experiences. In fact, the FDA has expressed concerns with personal genetic testing that provides consumers with medically relevant information. In November of 2013, the FDA sent a warning letter to the personal genotyping company 23andMe asking them to stop marketing a genotyping kit until they could provide evidence of clinical and analytical validity [34]. Due to continued FDA involvement as of October 2014, 23andMe is no longer marketing their health product or providing customers with health reports [35, 36]. The role of the FDA in regulating personal genomics, although contested by some, further highlights the need for careful consideration of student DNA testing. In this study, we present a viable alternative to the student *TAS2R38* genotyping laboratory that eliminates the ethical concerns associated with student DNA testing. Our approach could be applied to virtually any current laboratory exercise in which students genotype their own DNA, provided instructors are able to generate or obtain cloned versions of the possible alleles of the gene of interest.

Our assessment data demonstrate that this new alternative to student DNA testing resulted in clear student learning gains. Not only did students learn important information about SNPs and cystic fibrosis, they also learned about a number of molecular biology laboratory techniques (Fig. 4). We saw an increase in student-perceived competency with the tools used in lab, such as DNA mini-prep, PCR, restriction enzyme digest and agarose gel electrophoresis (Fig. 5). Furthermore, students taking part in the simulated laboratory reported that the semester lab experience was at least as personally relevant to them as it was to students who had done their own genotyping in the original iteration of the *TAS2R38* laboratory at Wellesley.

While we found our approach to be quite effective, a very different approach to dealing with the ethical concerns of student DNA testing has been taken by Taylor and Rogers [18]. Instead of carrying out genotyping with cloned DNA, students at Wabash College did only the first part of a traditional genotyping experiment. In this case, students isolated their own DNA and amplified a region of the p53 gene, variants of which are associated with increased cancer risk. Instead of completing the genotyping component of the laboratory, a genetic counselor was brought in to discuss the ethical implications of such a laboratory experience [18]. Using this approach, they avoided some of the ethical concerns associated with traditional student p53 analysis that has been

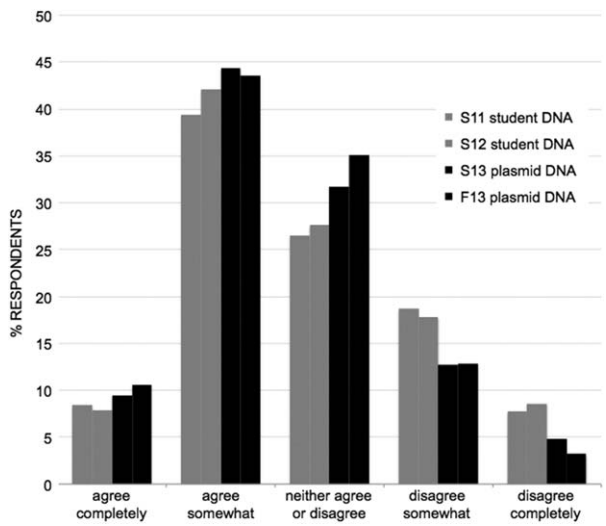

FIG 6

Student perceptions of personal relevance of the semester long laboratory series. This exit survey is distinct from the genetics survey and was taken at the end of the laboratory session each semester indicated. Answers are in response to the question "The concepts that we covered in lab were relevant to my personal life." 155 students took the S11 survey, 152 students took the S12 survey, 63 students took the S13 survey, and 94 students took the F14 survey.

described in the classroom setting [37, 38]. Thus, several viable alternatives are available to those who wish to address the ethical issues associated with student DNA testing by avoiding the student testing altogether, as opposed to other approaches such as anonymizing results or allowing students to opt out of the experience. Furthermore, these alternatives to student DNA testing still allow for rich classroom discussion about the socioscientific topic of genetic testing in general [39, 40]. By covering the topic of genetic testing in the context of the hands-on laboratory simulation, students are introduced to some of the ethical issues associated with personal genetic testing before possibly being faced with the decision to obtain individual genetic test results in the future [41].

We chose to have students analyze the association between *TAS2R38* genotype and the age of persistent *P. aeruginosa* acquisition in cystic fibrosis patients, but many other approaches could be taken using the genotyping portion of the laboratory series that we developed in conjunction with different phenotypic assignments. When we first implemented this new lab using cloned *TAS2R38* DNA in place of student DNA, we assigned students a phenotype based on PTC tasting ability instead of using the cystic fibrosis disease phenotype. Students were assigned the phenotype of taster, partial taster, or non-taster. The *TAS2R38* genotype and PTC-tasting phenotype combinations were assigned based on the distributions seen in earlier iterations of the Wellesley College BISC 110/112 laboratory course (when our students determined their *TAS2R38* genotype after isolating their own DNA). However, the phenotype and genotype distributions could also be determined based on published literature on this topic [3, 42]. This approach would presumably be the easiest for instructors to implement in their courses if they are currently having students genotype their own *TAS2R38* DNA, because all that would need to be changed is the initial laboratory component in which students isolate their own DNA.

If instructors wish to move away from the PTC phenotype but time constraints don't allow for sufficient background regarding cystic fibrosis, another option is to have the phenotype be simply the presence or absence of *P. aeruginosa* associated sinus infection in patients without cystic fibrosis. In this case, one could make genotype/phenotype assignments based on the findings of Lee and colleagues, such that students assigned T785-containing genotypes would predominantly be assigned the phenotype of *P. aeruginosa* associated sinus infection [10]. Alternatively, labs could be designed using other published phenotypes associated with *TAS2R38* variants, such as schizophrenia or colorectal cancer [5, 7, 8]. Finally, if instructors prefer that students meet the learning objective of working with authentic, student-produced data and they are comfortable dispensing with the human DNA testing learning objective, a variety of alternative labs could be implemented [43–45].

As discussed above, our revised *TAS2R38* laboratory in which we use plasmid DNA in place of student DNA can be modified in a variety of ways to best suit instructor and student needs. The use of plasmid DNA allows instructors to eliminate ethical concerns associated with student DNA testing, yet students are still exposed to all of the relevant laboratory techniques used in the original version of this lab. We have shown through this modified laboratory series that students gain important knowledge about general and unit specific concepts, as well as an increase in self-perceived competency with several basic molecular biological methods. Using this approach, we were able to engage students personally and scientifically while eliminating the complex ethical issues surrounding the analysis of student DNA in the classroom.

Acknowledgements

The authors thank Sherly Veeraragavan and Mbaira Maorongarti for providing the skilled technical expertise needed for implementation of this laboratory exercise. The authors also thank the Wellesley College BISC110/112 laboratory students who took part in this course and the additional laboratory instructors who taught the course, including Louise Darling, Jocelyne Dolce, John Goss, Martina Köniğer, Adam Matthews, and Kaye Peterman. In addition to the instructors in the course, Tucker Crum, Gary Harris, Yui Suzuki, John Cameron, Andrea Sequeira, and Drew Webb provided valuable input as the authors made initial plans to redesign this laboratory series. The authors also thank Kaye Peterman for the implementation of the previous iteration of this laboratory exercise at Wellesley College, modeled after the work of Merritt *et al.* [14]. The authors thank Wellesley College students Christina Lepore, Crysti Wang, and Anna Ha for allowing them to include their figures in the article and also thank Jocelyne Dolce for careful review of an earlier draft the article. The Department of Biological Sciences at Wellesley College and an HHMI Curriculum Development Grant provided funding this work.

References

[1] Adler, E., Hoon, M. A., Mueller, K. L., Chandrashekar, J., Ryba, N. J., and Zuker, C. S. (2000) A novel family of mammalian taste receptors. Cell 100, 693–702.

[2] Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., Zuker, C. S., and Ryba, N. J. (2000) T2Rs function as bitter taste receptors. Cell 100, 703–711.

[3] Campbell, M. C., Ranciaro, A., Froment, A., Hirbo, J., Omar, S., Bodo, J. M., Nyambo, T., Lema, G., Zinshteyn, D., Drayna, D., Breslin, P. A., and Tishkoff, S. A. (2012) Evolution of functionally diverse alleles associated with PTC bitter taste sensitivity in Africa. Mol. Biol. Evol. 29, 1141–1153.

[4] Drayna, D. (2005) Human taste genetics. Ann. Rev. Genom. Hum. Genet. 6, 217–235.

[5] Carrai, M., Steinke, V., Vodicka, P., Pardini, B., Rahner, N., Holinski-Feder, E., Morak, M., Schackert, H. K., Gorgens, H., Stemmler, S., Betz, B., Kloor, M., Engel, C., Buttner, R., Naccarati, A., Vodickova, L., Novotny, J., Stein, A., Hemminki, K., Propping, P., Forsti, A., Canzian, F., Barale, R., and Campa, D. (2011) Association between TAS2R38 gene polymorphisms and colorectal cancer risk: A case-control study in two independent populations of Caucasian origin. PLoS One 6, e20464.

[6] Schembre, S. M., Cheng, I., Wilkens, L. R., Albright, C. L., and Marchand le , L. (2013) Variations in bitter-taste receptor genes, dietary intake, and colorectal adenoma risk. Nutr. Cancer 65, 982–990.

[7] Brewer, W. J., Lin, A., Moberg, P. J., Smutzer, G., Nelson, B., Yung, A. R., Pantelis, C., McGorry, P. D., Turetsky, B. I., and Wood, S. J. (2012) Phenylthiocarbamide (PTC) perception in ultra-high risk for psychosis participants who develop schizophrenia: Testing the evidence for an endophenotypic marker. Psychiatry Res. 199, 8–11.

[8] Moberg, P. J., Li, M., Kanes, S. J., Gur, R. E., Kamath, V., and Turetsky, B. I. (2012) Association of schizophrenia with the phenylthiocarbamide taste receptor haplotype on chromosome 7q. Psychiatr. Genet. 22, 286–289.

[9] Clark, A. A., Liggett, S. B., and Munger, S. D. (2012) Extraoral bitter taste receptors as mediators of off-target drug effects. FASEB J. 26, 4827–4831.

[10] Lee, R. J., Xiong, G., Kofonow, J. M., Chen, B., Lysenko, A., Jiang, P., Abraham, V., Doghramji, L., Adappa, N. D., Palmer, J. N., Kennedy, D. W., Beauchamp, G. K., Doulias, P. T., Ischiropoulos, H., Kreindler, J. L., Reed, D. R., and Cohen, N. A. (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. J. Clin. Invest. 122, 4145–4159.

[11] Adappa, N. D., Zhang, Z., Palmer, J. N., Kennedy, D. W., Doghramji, L., Lysenko, A., Reed, D. R., Scott, T., Zhao, N. W., Owens, D., Lee, R. J., and Cohen, N. A. (2014) The bitter taste receptor T2R38 is an independent risk factor for chronic rhinosinusitis requiring sinus surgery. Int. Forum Allergy Rhinol. 4, 3–7.

[12] Lee, R. J. and Cohen, N. A. (2013) The emerging role of the bitter taste receptor T2R38 in upper respiratory infection and chronic rhinosinusitis. Am. J. Rhinol. Allergy 27, 283–286.

[13] Lee, R. J., Kofonow, J. M., Rosen, P. L., Siebert, A. P., Chen, B., Doghramji, L., Xiong, G., Adappa, N. D., Palmer, J. N., Kennedy, D. W., Kreindler, J. L., Margolskee, R. F., and Cohen, N. A. (2014) Bitter and sweet taste receptors regulate human upper respiratory innate immunity. J. Clin. Invest. 124, 1393–1405.

[14] Merritt, R. B., Bierwert, L. A., Slatko, B., Weiner, M. P., Ingram, J., Sciarra, K., and Weiner, E. (2008) Tasting phenylthiocarbamide (PTC): A new integrative genetics lab with an old flavor. Am. Biol. Teach. May, 23–28.

[15] PTC Extraction and Amplification Kit. Available at: http://www.carolina.com/pcr-kits/ptc-extraction-and-amplification-kit-with-02-ml-tubes-with-perishables/211377P.pr?catId=&mCat=&sCat=&ssCat=&question=ptc. Accessed on 14 January, 2015.

[16] Reinking, J. L., Waldo, J. T., Dinsmore, J. (2013) A trio of human molecular genetics PCR assays. Biochem. Mol. Biol. Educ. 41, 173–179.

[17] Callier, S. L. (2012) Swabbing students: Should universities be allowed to facilitate educational DNA testing? Am. J. Bioeth. 12, 32–40.

[18] Taylor, A. T. and Rogers, J. C. (2011) The ethical implications of genetic testing in the classroom. Biochem. Mol. Biol. Educ. 39, 253–260.

[19] Salari, K., Pizzo, P. A., and Prober, C. G. (2011) Commentary: to genotype or not to genotype? Addressing the debate through the development of a genomics and personalized medicine curriculum. Acad. Med. 86, 925–927.

[20] Knoell, D. L., Johnston, J. S., Bao, S., and Kelley, K. A. (2009) A genotyping exercise for pharmacogenetics in pharmacy practice. Am. J. Pharm. Educ. 73, 43.

[21] Kirkwood, K. W. (2012) The professor really wants me to do my homework: conflicts of interest in educational research. Am. J. Bioeth. 12, 47–48.

[22] Corbyn, Z.(2010) California's genetic education. Nature. Available at: http://www.nature.com/news/2010/100921/full/news.2010.484.html. Accessed on 14 January, 2015. doi:10.1038/news.2010.484

[23] Weinlander, K. M. and Hall, D. J. (2010) Designing laboratory exercises for the undergraduate molecular biology/biochemistry student: Techniques and ethical implications involved in personalized medicine. Biochem. Mol. Biol. Educ. 38, 180–187.

[24] Bryce, T. A. G. D. (2004) Tough acts to follow: The challenge to science teachers presented by biotechnological progress. Int. J. Sci. Educ. 26, 717–733.

[25] Daley, L. A., Wagner, J. K., Himmel, T. L., McPartland, K. A., Katsanis, S. H., Shriver, M. D., and Royal, C. D. (2013) Personal DNA testing in college classrooms: Perspectives of students and professors. Genet. Test. Mol. Biomarkers 17, 446–452.

[26] Salari, K., Karczewski, K. J., Hudgins, L., and Ormond, K. E. (2013) Evidence that personal genome testing enhances student learning in a course on genomics and personalized medicine. PLoS One 8, e68853.

[27] Silveira, L. A. (2008) Experimenting with spirituality: analyzing The God Gene in a nonmajors laboratory course. CBE Life Sci. Educ. 7, 132–145.

[28] Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J. L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., and Tsui, L. (1989) Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. Science 245, 1066–1073.

[29] Rich, D. P., Anderson, M. P., Gregory, R. J., Cheng, S. H., Paul, S., Jefferson, D. M., McCann, J. D., Klinger, K. W., Smith, A. E., and Welsh, M. J. (1990) Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature 347, 358–363.

[30] Ciofu, O., Hansen, C. R., and Hoiby, N. (2013) Respiratory bacterial infections in cystic fibrosis. Curr. Opin. Pulm. Med. 19, 251–258.

[31] Emerson, J., Rosenfeld, M., McNamara, S., Ramsey, B., and Gibson, R. L. (2002) Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr. Pulmonol. 34, 91–100.

[32] Green, D. M., Collaco, J. M., McDougal, K. E., Naughton, K. M., Blackman, S. M., and Cutting, G. R. (2012) Heritability of respiratory infection with *Pseudomonas aeruginosa* in cystic fibrosis. J. Pediatr. 161, 290–295 e291.

[33] Personal DNA Testing. Available at: http://video.pbs.org/video/2281242004/. Accessed on 14 January, 2015.

[34] 23andMe, Inc. US Food and Drug Administration Warning Letter. Available at: http://www.fda.gov/iceci/enforcementactions/warningletters/2013/ucm376296.htm. Last accessed 22 November, 2013.

[35] 23andMe, Inc. US Food and Drug Administration—Close Out Letter. Available at: http://www.fda.gov/iceci/enforcementactions/warningletters/ucm391016.htm. Last accessed 14 January, 2015.

[36] An FDA Update For 23andMe Customers. Available at: http://blog.23andme.com/news/an-fda-update-for-23andme-customers/. Accessed on 14 January, 2015.

[37] Soto-Cruz, I. and Legorreta-Herrera, M. (2009) Analysis of a p53 mutation associated with cancer susceptibility for biochemistry and genetic laboratory courses. Biochem. Mol. Biol. Educ. 37, 236–242.

[38] Ouyang, L., Ge, C., Wu, H., Li, S., and Zhang, H. (2009) PCR-RFLP to detect codon 248 mutation in exon 7 of p53 tumor suppressor gene. Biochem. Mol. Biol. Educ. 37, 106–109.

[39] Van der Zande, P. A., Brekelmans, S. F., Waarlo, M., and Vermunt, J. D. (2012) Expertise for teaching biology situated in the context of genetic testing. Int. J. Sci. Educ. 34, 1741–1767.

[40] Sadler, T. D., Amirshokoohi, A., Kazempour, M., and Allspaw, K. M. (2006) Socioscience and ethics in science classrooms: teacher perspectives and strategies. J. Res. Sci. Teach. 43, 353–376.

[41] Zeidler, D. L. and Sadler, T. D. (2008) Social and ethical issues in science education: A prelude to action. Sci. Educ. 17, 799–803.

[42] Wooding, S. (2006) Phenylthiocarbamide: A 75-year adventure in genetics and natural selection. Genetics 172, 2015–2023.

[43] Burnette, J. M., III and Wessler, S. R. (2013) Transposing from the laboratory to the classroom to generate authentic research experiences for undergraduates. Genetics 193, 367–375.

[44] Gasper, B. J. and Gardner, S. M. (2013) Engaging students in authentic microbiology research in an introductory biology laboratory course is correlated with gains in student understanding of the nature of authentic research and critical thinking. J. Microbiol. Biol. Educ. 14, 25–34.

[45] Graham, M. J., Frederick, J., Byars-Winston, A., Hunter, A. B., and Handelsman, J. (2013) Science education. Increasing persistence of college students in STEM. Science 341, 1455–1456.