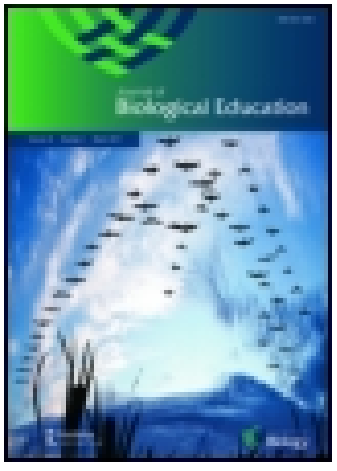

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A Laboratory Exercise for Genotyping Two Human Single Nucleotide Polymorphisms

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The dramatic decrease in the cost of sequencing a human genome is leading to an era in which a wide range of students will benefit from having an understanding of human genetic variation. Since over 90% of sequence variation between humans is in the form of single nucleotide polymorphisms (SNPs), a laboratory exercise has been devised in order to illustrate the importance of SNPs. Two separate SNPs are analysed, one of which has a significant effect on a person's phenotype and one which does not. The genotyping protocol is relatively inexpensive and uses standard molecular biology reagents and equipment.

Keywords: *Single nucleotide polymorphism; DNA sequencing; Bitter taste; Genomics; Genetic variation*

Introduction

Background

Dramatic reductions in the cost of DNA sequencing is leading to an explosion of available human genetic information. Sequencing costs for a human genome have already dropped to the point where whole genome sequencing is a realistic option for routine medical diagnostics (Hayden 2013). This, combined with new applications in other fields, means that an increasing number of professions will need to be fluent in the language of genomics.

Single nucleotide polymorphisms (SNPs, pronounced 'snips') are one of the most important concepts in understanding human genetic variation. SNPs are single base pair differences between individuals of the same species (Pierce 2010). Over 90% of sequence

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variation between humans is in the form of these polymorphisms (Collins, Brooks, and Chakravarti 1998). Some SNPs have no observable influence, some result in intermediate phenotypic differences and a few result in dramatic phenotypic differences (Sulem et al. 2007).

Rationale

A simple and cost-effective method allowing students to genotype two of their own SNPs has been developed. Unlike Merritt et al. (2008), this protocol clearly illustrates how some polymorphisms can completely determine phenotype, while other SNPs have significantly less impact. This laboratory exercise is appropriate for a range of courses from introductory biology to more advanced genetics courses. To assess student learning, we created a short quiz to give after the exercise has been completed (see Appendix 1).

The first SNP chosen illustrates how some SNPs can have a dramatic effect on phenotype. rs713598 is located on chromosome seven within the *TAS2R38* gene (http://www.ncbi.nlm.nih.gov/gene/5726). *TAS2R38* encodes a bitter taste receptor that is expressed on the tongue (for review see Drayna 2005). When phenylthiocarbamide (PTC) binds to this receptor, a signal interpreted as 'bitter' is passed to the brain. Bitter foods and beverages, such as Brussels sprouts or coffee, contain chemicals similar to PTC, which can also be detected by TAS2R38. An individual's sequence at the rs713598 SNP has a potent effect on their ability to taste bitter chemicals (Figure 1(A)). Interestingly, a large proportion of the general population cannot taste PTC or chemicals of similar structure (Drayna 2005). When the alleles of rs713598 are compared, the allele of rs713598 that most commonly detects a bitter taste encodes for an alanine at position 49 of the TAS2R38 protein. In contrast, the most common allele carried when the bitter taste of PTC is not recognised, encodes for a proline in position 49 of the TAS2R38 protein. Approximately 90% of the variance in PTC-tasting phenotypes is explained by the sequence of the rs713598 SNP (Kim et al. 2003). Students can readily determine their phenotype for PTC tasting by placing paper imbued with the chemical onto their tongues. This combination of straightforward genetics and simple laboratory test makes the rs713598 SNP an ideal candidate for inclusion in this exercise.

The second SNP in the exercise was chosen to illustrate how some SNPs have a modest effect on phenotype. rs1815739 is located on chromosome 11 within the *ACTN3* gene (http://www.ncbi.nlm.nih.gov/gene/89). *ACTN3* encodes for α-actinin protein-3 that functions as a structural component in fast twitch muscle fibres. The mutant allele contains a thymine (T) base which codes for a premature stop codon (TGA) instead of the functional arginine codon. This results in an absence of any detectable α-actinin protein-3 (Figure 1(B)). Surprisingly, this nonsense mutation does not lead to a radically altered phenotype (death, major loss of muscle function, etc.). In fact, individuals without any detectable α-actinin-3 protein are still fit enough to compete in Olympic events (Lucia et al. 2007). Although the functional allele is associated with elite-level athletes who compete in 'power events', only 2.3% of the observed variance in muscular power in the general population is explained by the sequence of the rs1815739 SNP (Yang et al. 2003; Clarkson et al. 2005; Moran et al. 2007).

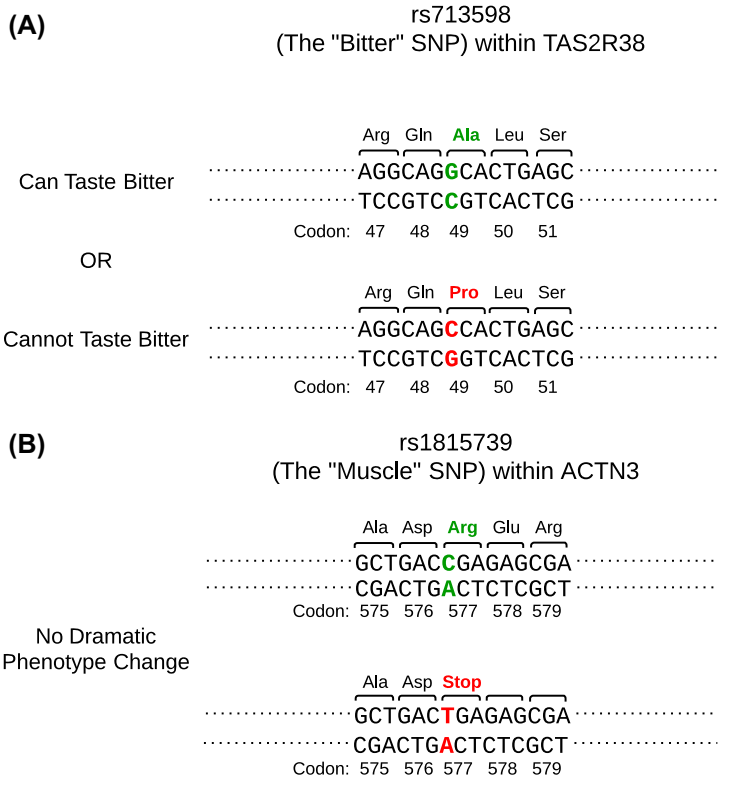

Figure 1. Diagram of the sequence surrounding the TAS2R38 SNP and ACTN3 SNP. Each illustrates the two possible allele sequences. (A) rs713598 affects the *TAS2R38* gene by changing Ala49Pro. The change in DNA sequence from the SNP results in an alteration in the ability to taste PTC. (B) rs1815739 affects the *ACTN3* gene with a premature stop codon. As this gene is responsible for only 2.3% of variance in muscular power, the change in DNA sequence from this SNP results in no dramatic phenotypic change (full colour figure available online)

Compared side by side, these two SNPs illustrate a key principle of genetic variation. Some sequences and polymorphisms are crucial to creating our phenotypes, while others hardly make a noticeable change. As stated previously, the 'bitter' SNP is on the higher end of the scale with regard to its phenotypic impact, with over 90% of the variance in PTC-tasting phenotypes explained by the 'bitter' SNP (Kim et al. 2003). In contrast, the 'muscle' SNP has a more modest impact on phenotype, accounting for only 2.3% of the observed variance in muscular power amongst the general population (Clarkson et al. 2005; Moran et al. 2007). Both SNPs have a high population diversity, so instructors can expect to find examples of both heterozygous and homozygous genotypes within any population of students.

Educational Implications

This practical is primarily designed to illustrate how SNPs can affect phenotype to a varying degree. However, the exercise can be tailored to illustrate a variety of topics,

including: Mendelian genetics, haplotypes, the Hardy–Weinberg equilibrium, translation, coding mutations, protein function and redundancy, and cell signalling, as well as to facilitate discussion on the effects of inheritable factors vs. environmental factors on phenotype.

Materials and Methods

Ethical Approval

Prior to development of this novel activity, ethical approval was obtained from Union College's institutional review board. Informed consent was also obtained from each participant. Since this exercise deals with students' personal health information, it is recommended that institutional review board/ethics committee approval and participant informed consent should be obtained before carrying out the lab exercise.

Cell Lysis and DNA Extraction

Cell lysis and extraction of DNA was conducted using a modified version of the Hotshot method of Truett et al. (2000). Students begin by swishing 7.5 mL of filter-sterilised phosphate-buffered saline in their mouths for 30 s to extract cheek epithelial cells. One millilitre of this solution is then transferred into a clean 1.5 mL or 1.7 mL microcentrifuge tube, and labelled. To protect student privacy, it is recommended that each student choose a secret random number, which is then used to identify their samples during the test. Teachers should also avoid compiling any list that could be used to correlate students with their genetic material. Each tube is centrifuged at $5000 \times g$ for five minutes to collect the cheek cells in a pellet. The supernatant is decanted and discarded, and 75 μL of alkaline lysis solution (25 mM NaOH, 0.2 mM disodium EDTA, pH 12) is added to the pellet, which is suspended by vigorous vortexing. The cells are lysed at 95 °C for 30 min; then 75 μL of neutralising buffer (40 mM Tris-HCl, pH 5) is added.

Genotyping

Each SNP is genotyped using an adapted version of the Amplification Refractory Mutation System or ARMS method (Newton et al. 1989), in which four primers are supplied concurrently. Figure 2 shows the arrangement of the primers around a hypothetical SNP. Each SNP study uses a different set of four primers (Table 1), but both studies can be run simultaneously in the thermal cycler. The outer primers are exactly complementary to the target sequence and their product serves as an allele-independent positive control.

The internal primers are allele-specific. Each internal primer's 3′ base pair complements only one form of the SNP. The specificity is derived from the inhibition of *Taq* DNA Polymerase due to one of the internal primers having a mismatched nucleotide. This polymerase inhibition, caused by the base pair mismatch, is the foundation of the ARMS procedure experimentally proven by Newton et al. (1989). As seen in Figure 2, the mismatch keeps the 3′ end from binding, which stops amplification. The specificity of each inner primer is further enhanced with an additional mismatch three base pairs back from

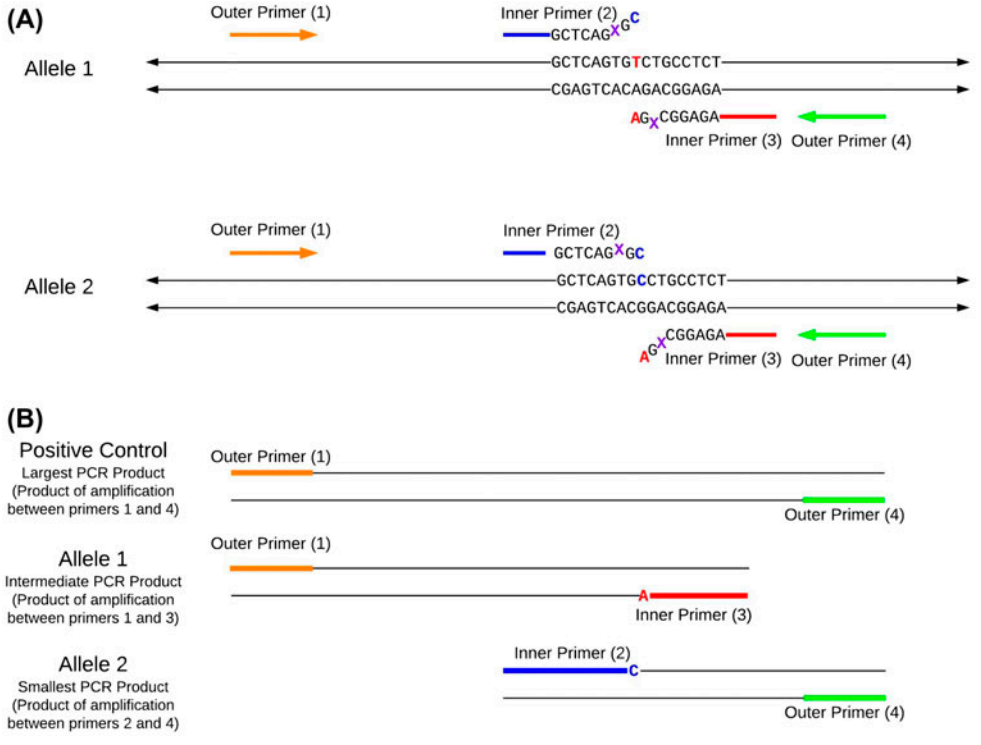

Figure 2. (A) Four ARMS primers are shown interacting with the variable region containing two alleles of a SNP (allele 1 and allele 2). The outer primers (primers 1 and 4) complement conserved regions of DNA, and therefore bind to allele 1 and allele 2. The inner primers (primer 2 and primer 3) bind in a variable region, and thus exhibit allele-specific binding. For example, primer 2 binds if a G is present in the bottom strand; primer 3 binds if a T is present in the top strand. The X's within primers 2 and 3 represent a deliberate base pair mismatch that causes a 'bump' and enhances primer-specific binding. (B) Three different lengths of PCR products are produced from the four primers. Amplification between 1 and 4 results in the largest DNA band and is produced allele independent. Primers 1 and 3 produce a DNA band of intermediate length and indicate that allele 1 is present. Amplification between primer 2 and primer 4 results in the smallest DNA band and indicates that allele 2 is present (full colour figure available online)

the 3′ end. This mismatch causes a 'bump' that mildly inhibits the polymerase, increasing specificity as seen in Figure 3.

The length of the primers (29–45 bp) not only further increases specificity, but also allows the annealing and extension temperatures to be identical. As a result, the duration of each PCR is reduced and, as a consequence, the overall time that students will have to wait in the lab is limited to 80 min. At this point, the PCR products can either be stored at −20 °C or run immediately on an agarose gel.

The students' PCR products are resolved on a 1.5% agarose gel. The three possible bands for each SNP study reveal the individual's genotype. The largest band is a positive control that exists independent of the SNP and confirms the presence of DNA. The intermediate band indicates the non-functional alleles and the smallest band indicates a functional allele. If a student has all three bands, they are heterozygous for the trait.

Table 1. Sequences of primers designed using the ARMS method, for both the rs713598 (bitter) and rs1815739 (muscle) SNPs and the exact PCR product lengths for the positive control and two alleles studied

'Bitter' SNP: rs713598	
Primer sequences	
Flank Forward	5′ GGTGTGAGAGAAACGGATGAGCTTGGAGCAGTAAA 3′
Flank Reverse	5′ ACCATCAGTCTTCCACCCTATGATAAGCTCTTACGTGT 3′
Inner Forward	5′ ACACAGCAGCACACAATCACTGTTGCTCAGaGc 3′
Inner Reverse	5′ CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCcGc 3′
PCR band length	**Genotype interpretation**
640 Base Pairs	Positive Control
436 Base Pairs	Non-Taster
281 Base Pairs	Taster
'Muscle' SNP: rs1815739	
Primer sequences	
Flank Forward	5′ GCTTCTGACCCACTACGCCTCCCACCTCTA 3′
Flank Reverse	5′ CCCGAGATTTCAGGGTGGTCACAGTATGCAGGAG 3′
Inner Forward	5′ AGTTCAAGGCAACACTGCCCGAGGCTGtCc 3′
Inner Reverse	5′ TGGATGCCCATGATGGCACCTCGCTCgCa 3′
PCR band length	**Genotype interpretation**
682 Base Pairs	Positive Control
513 Base Pairs	Mutant Allele
227 Base Pairs	Functional Allele

Note: Lower case nucleotides indicate potential base pair mismatches.

Presentation of only two bands indicates a homozygous individual. The exact size of the bands and their interpretations are listed in Table 1. Examples of all three genotypes are in Figure 3.

PCR

Each group requires both a 'bitter' and a 'muscle' master mix (Table 2). Students receive four PCR tubes. Two tubes contain 1.5 µL of their extracted DNA for genotyping, and two tubes contain 1.5 µL of water as a negative control. 23.5 µL of muscle master mix (Table 2) is added to one DNA tube and one water tube. 23.5 µL of bitter master mix (Table 2) is then added to the other two tubes. Reactions are run simultaneously on a Bio-Rad MyCycler Thermal Cycler at initial denaturation 95.0 °C 2:00 (1x); denaturing step 95.0 °C 0:30, annealing and extension 72.0 °C 1:40 (30x); final extension 72.0 °C 10:00 (1x); hold 4.0 °C infinity. Table 1 lists all primer sequences.

Agarose Gel Electrophoresis

PCR products are separated with a 1.5% agarose gel (1.5 g agarose per 100 mL 1X TAE) using 1X TAE (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA). In order to visualise

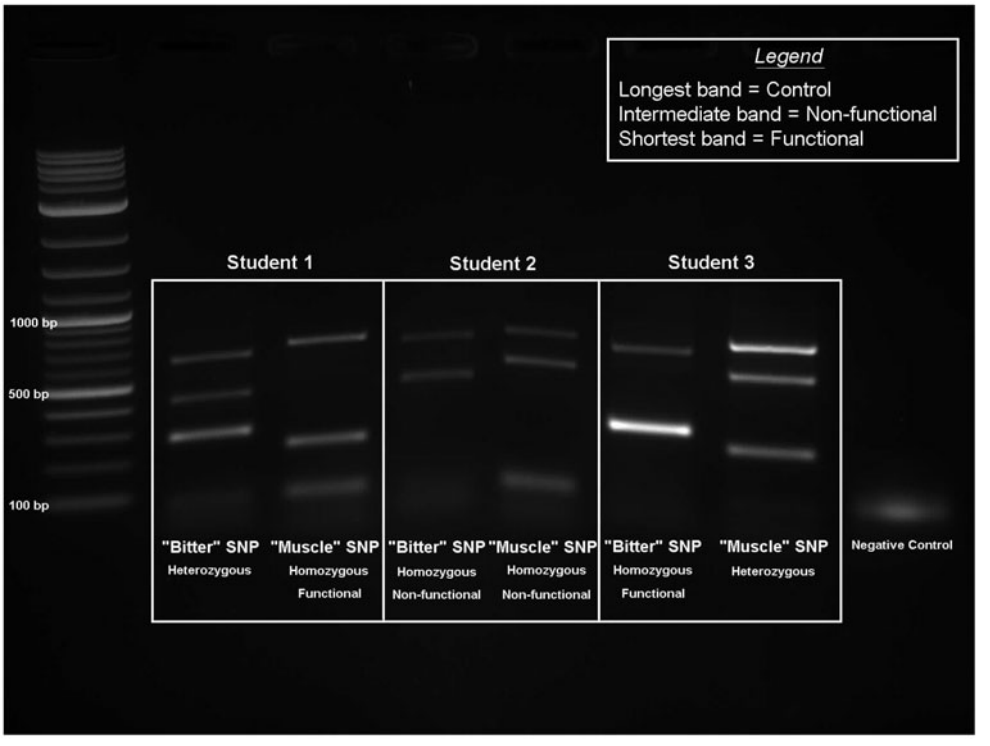

Figure 3. Gel electrophoresis of PCR products from three different individuals, illustrating all six possible genotypes. Refer to Table 1 for exact bp sizes for each band. Negative control was produced by a PCR with all the reagents except DNA. The ~100 bp band is a known artefact created by primer dimer amplification (Altshuler 2006). The standard on the left is 2-log DNA Ladder from New England Biolabs

the DNA, a stain is required. Traditionally, and in the development of this assay, ethidium bromide is used at a concentration of 0.5 μg/mL, that is added before the gel is poured. In many settings, such as schools, using a safer, non-toxic DNA stain, such as RedSafe Nucleic Acid Staining Solution(iNtRON Bio), is recommended. Once the gel is cast, 15 μL of PCR product is mixed with 3μL of 6x loading dye (New England Biolabs) and then loaded into each well; the gel is then run at 80 V for ~55 min.

Gel Analysis

Using UV light, students visualise the stained DNA and can deduce the genotype of each individual. Each lane is identified only by the student's random number. Each SNP has three possible bands. As long as the target DNA is present, the largest band appears as an allele-independent positive control. The smallest band indicates a functioning allele (either bitter taste perception or synthesis of ACTN3), and the intermediate band is the non-functional allele. Refer to Table 1 for band size and interpretations, and Figure 3 for a gel with all three genotypes.

Table 2. Reagents utilised in one PCR reaction for either *ACTN3* ('Muscle' SNP) or *TAS2R38* ('Bitter' SNP). Instructors should create a master mix by multiplying each of the ingredients by the number of reactions needed to be run and then adding all reagents together except the cheek DNA. Students will then pipette 23.5 μL of the master mix onto their 1.5 μL of cheek DNA

Master mix reagents	Amount per reaction (μL)	Final concentration
'Bitter' SNP rs713598		
Water	16.5	
10x Thermopol Buffer	4	1.6X concentration
10 mM dNTP Mix	0.625	0.25 mM each nucleotide
Outer Fwd (20 μM)	0.5	0.4 μM
Outer Rev (20 μM)	0.5	0.4 μM
Inner Fwd (20 μM)	0.5	0.4 μM
Inner Rev (20 μM)	0.5	0.4 μM
Cheek DNA	1.5	
Taq (homemade)	0.375	
Total reaction volume	25 μL	
'Muscle' SNP rs1815739		
Water	18.25	
10x Thermopol Buffer	2.5	1X concentration
10 mM dNTP Mix	0.625	0.25 mM each nucleotide
Outer Fwd (20 μM)	0.5	0.4 μM
Outer Rev (20 μM)	0.5	0.4 μM
Inner Fwd (20 μM)	0.25	0.2 μM
Inner Rev (20 μM)	0.5	0.4 μM
Cheek DNA	1.5	
Taq (homemade)	0.375	
Total reaction volume	25 μL	

Bitter Taste Phenotype

Bitter taste was assessed by having students place one strip of PTC-imbibed paper (NASCO) on their tongues for a minimum of 30 s. Students who can taste PTC will report tasting a strong bitter flavor. Those who cannot taste PTC will report tasting only the paper itself. Each strip contains about 0.007 mg of PTC, which is approximately 100 times less toxic than the salt contained in one small bag of potato chips (Merritt et al. 2008).

Cost

As stated previously, this exercise is quite cost-effective. An initial outlay of approximately US$620 provides materials for around 210 students. However, since some of the resources are reusable, the long-term cost per session would be much lower, possibly as little as US$1.15 per student.

Contact Information

For more detailed information, please contact frrose@ucollege.edu or visit http://goo.gl/HFP4tP.

Procedure

Step 1. Introduction

Begin with a brief lecture describing the topics and SNPs to be studied, the methods and appropriate laboratory safety procedures. Instruct the students to predict their bitter and muscle genotypes before they have tasted the PTC paper or genotyped themselves. Then divide the students into groups appropriate for the number of lanes the agarose gel contains. Each student will need two lanes, one for each SNP. In addition, it is recommended to run at least one negative control lane for each master mix using water instead of student DNA to ensure the PCR reactions have not been contaminated.

Step 2. Experiments

To genotype these two SNPs, follow the steps below.

(1) Extract DNA from cheek cells: it takes approximately 10 min to prepare the reaction and 30 min for the heating step.
(2) PCR: each student receives two PCR tubes. Add 23.5 μL of master Mix (Table 2) to 1.5 μL of the student's DNA for the two separate SNP studies. Have a teaching assistant prepare a PCR tube with water instead of DNA to act as a negative control for each SNP master mix (Table 2). The thermal cycler runs for about 80 min depending on its ramp speed.
(3) (*)Create Agarose Gels: have each group make a 1.5% agarose gel.
(4) (*)Check 'bitter' SNP phenotype: have each student taste the PTC paper to check their phenotype and compare it with their prediction.
(5) Run Gels: have each student load both of their PCR products into adjacent lanes and run the gel for 55 min at 80 V.
(6) Analyse: examine the gels under UV light and have the students compare their predicted genotypes with the PCR results.

(*)Steps with an asterisk can be performed during the wait times of previous steps.

Step 3. Conclusion

Briefly discuss with the students the importance of SNPs and how various genes have differing impact on phenotype. Encourage students to share their results and correlate their genotype with daily activities. Administer the supplied exit quiz.

Results

Assay Accuracy Validation

To verify the accuracy of our method, 37 anonymous students were genotyped. All of the resulting genotypes were interpreted and correlated with their phenotype for bitter taste. To further validate the method, we sequenced select bands and confirmed that results matched sequences published by NCBI (*TAS2R38* Gene: NG_016141.1, *ACTN3* Gene:

NG_013304.1, accessed 16 April 2013). One example of each possible sequence was cloned into Promega's PGem-T Easy plasmids for use as positive controls.

Evidence of Impact

In order to measure educational impact, the lab exercise was conducted with a cohort of 24 students. A nine-question quiz was designed to measure student learning (see Appendix 1). Students took the quiz the day before the lab exercise, and then again one week after the lab exercise. Questions were scored either correct or incorrect with each question being worth one point. The mean pre-lab quiz score was 2.2 points (standard deviation of 1.1 points), while the mean post-lab quiz score was 6.7 points (standard deviation of 1.3 points). In addition to the quiz, the same students completed a short survey. An overwhelming majority of students agreed with the statement 'The time spent on this lab was worthwhile' (95% agreed or strongly agreed on a 5-point Likert scale).

Discussion

Creation of this lab exercise provides a cost-effective and robust PCR assay that illustrates the importance of SNPs. Students are able to view their own DNA sequence and then correlate the results with their phenotype for one of the SNPs. Students have expressed excitement about the experience of taking a small peek at their genome, which is believed to facilitate a deeper learning about SNPs.

The advent of rapid, low-cost DNA sequencing is leading to an era in which DNA testing is used in a broad range of applications. Medicine will be a field impacted significantly by this innovation. Whole genome sequencing is anticipated to replace a variety of existing medical tests, and it will allow medical practice to be tailored to the patient based on their DNA sequence (Bick and Dimmock 2011). For example, sequence variation between individual patients can dramatically affect drug responses. Adverse drug reactions can be predicted based on certain SNPs for certain cholesterol-lowering drugs and for the anticoagulant warfarin (Ware, Roberts, and Cook 2012). Since 90% of intraspecies sequence variation is due to SNPs, it is reasonable to assume that much of the clinically important DNA sequence will be in the form of SNPs.

As DNA testing becomes a more routine part of clinical medicine, an understanding of the implications of SNPs is important to the general public, particularly biology students. By focusing on the 'bitter' SNP and the 'muscle' SNP, this exercise demonstrates how some variability can have significant effects, whilst others are almost inconsequential.

Some theoretical limitations to this approach exist. Since the assay is based on the inhibition of *Taq* DNA polymerase, there is the possibility of some unwanted amplification. Due to the design of the assay, the chance of this occurring is very low. Should it occur, however, compare the suspected band to the band created by the flanking primers. The unwanted band will usually be dimmer. Given the authors have genotyped over 100 samples without evidence of unwanted amplification, this is not likely a significant problem. If this is a recurring problem, it is possible to decrease the number of amplification cycles to diminish the brightness of the false positive bands. Another potential limitation is that,

in rare cases, a student's bitter taste phenotype may not match their genotype. This is likely due to a rare combination of SNPs within the gene (Drayna 2005).

Many educational institutions are under pressure to reduce expenditure on teaching. This method is a cost-effective laboratory exercise that requires minimal laboratory resources. Initial purchase of all the reagents necessary to run the practical was approximately US$620. This was sufficient for about 210 students. In reality, the outlay is likely to be less than this, since most labs will already have acids, bases and buffers available. The ARMS method eliminates the need to purify the PCR products, which also saves both time and money. We further reduced the costs by making the *Taq* DNA Polymerase ourselves (for which the method of Ferralli, Egan, and Erickson 2007 is recommended).

By studying SNPs associated with daily activities, such as exercise or food choices, students will remember their genotype and correlate it with their phenotype. This connection between the theoretical principles of genetics and the practical application of genetic literacy engages students and promotes retention of the material. It also prepares them for a future in which DNA sequencing plays a much larger role in everyday life.

Conclusion

In light of the rapid advances in sequencing technology and its broad applications, all bioscience students will need some basic genetic literacy. Therefore, a simple, cost-effective laboratory exercise was created to allow students to genotype two of their own SNPs. The use of the 'bitter' SNP, affecting the TAS2R38 protein, is designed to illustrate that one minor genetic change can have drastic effects on bitter taste perception. In contrast, the 'muscle' SNP, demonstrates that some genetic variations can have modest effects on an individual's phenotype. Together, these two SNPs show that genetic changes can have a wide range of effects, from drastic to inconsequential. Understanding the variety of genetic ramifications will be crucial for bioscience students in their future careers and life decisions.

If you are interested in control plasmids or have questions, comments or suggestions, please contact the corresponding author.

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We would like to thank Union College (Lincoln, Nebraska) for providing the supplies and equipment to create this method. Thanks also to the professor and students of the Union College general biology class for donating DNA and working with the plasmids and also to the physician assistant class of 2016 for their time and donated DNA. We also thank Dr Salvador Moguel, Brianna Payne, Scott Cushman, Victoria Leddy and Ellen Rose for their helpful comments and suggestions. This work was made possible through a grant from Union College.

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References

Altshuler, M. L. 2006. *PCR Troubleshooting: The Essential Guide*. Wymondham: Caister Academic Press.

Bick, D., and D. Dimmock. 2011. "Whole Exome and Whole Genome Sequencing." *Current Opinion in Pediatrics* 23 (6): 594–600. doi:10.1097/MOP.0b013e32834b20ec.

Clarkson, P. M., J. M. Devaney, H. Gordish-Dressman, P. D. Thompson, M. J. Hubal, M. Urso, T. B. Price, et al. 2005. "ACTN3 Genotype is Associated with Increases in Muscle Strength in Response to Resistance Training in Women." *Journal of Applied Physiology* 99 (1): 154–163. doi:10.1152/japplphysiol.01139.2004.

Collins, F. S., L. D. Brooks, and A. Chakravarti. 1998. "A DNA Polymorphism Discovery Resource for Research on Human Genetic Variation." *Genome Research* 8 (12): 1229–1231. doi:10.1101/gr.8.12.1229.

Drayna, D. 2005. "Human Taste Genetics." *Annual Review of Genomics & Human Genetics* 6 (1): 217–235. doi:10.1146/annurev.genom.6.080604.162340.

Ferralli, P., J. D. Egan, and F. L. Erickson. 2007. "Making *Taq* DNA Polymerase in the Undergraduate Biology Laboratory." *BIOS* 78 (2): 69–74. doi:10.1893/0005-3155(2007)78[69:MTDPIT]2.0.CO;2.

Hayden, E. 2013. "Gene Sequencing Leaves the Laboratory: Maturing Technology Speeds Medical Diagnoses." *Nature* 494 (7437): 290–291. doi:10.1038/494290a.

Kim, U., E. Jorgenson, H. Coon, M. Leppert, N. Risch, and D. Drayna. 2003. "Positional Cloning of the Human Quantitative Trait Locus Underlying Taste Sensitivity to Phenylthiocarbamide." *Science* 299 (5610): 1221–1225. doi:10.1126/science.1080190.

Lucia, A., J. Olivan, F. Gomez-Gallego, C. Santiago, M. Montil, and C. Foster. 2007. "Citius and Longius (Faster and Longer) with No alpha-Actinin-3 in Skeletal Muscles?" *British Journal of Sports Medicine* 41 (9): 616–617. doi:10.1136/bjsm.2006.034199.

Merritt, R. B., L. A. Bierwert, B. Slatko, M. P. Weiner, J. Ingram, K. Sciarra, and E. Weiner. 2008. "Tasting Phenylthiocarbamide (PTC): A New Integrative Genetics Lab with an Old Flavor." *The American Biology Teacher* 70 (5): e23–e28. doi:10.1662/0002-7685(2008)70[23:TPPANI]2.0.CO;2.

Moran, C. N., N. Yang, M. E. S. Bailey, A. Tsiokanos, A. Jamurtas, D. G. MacArthur, K. North, Y. P. Pitsiladis, and R. H. Wilson. 2007. "Association Analysis of the ACTN3 R577X Polymorphism and Complex Quantitative Body Composition and Performance Phenotypes in Adolescent Greeks." *European Journal of Human Genetics* 15 (1): 88–93. doi:10.1038/sj.ejhg.5201724.

Newton, C. R., A. Graham, L. E. Heptinstall, S. J. Powell, C. Summers, N. Kalsheker, J. C. Smith, and A. F. Markham. 1989. "Analysis of Any Point Mutation in DNA. the Amplification Refractory Mutation System (ARMS)." *Nucleic Acids Research* 17 (7): 2503–2516. doi:10.1093/nar/17.7.2503.

Pierce, B. A. 2010. *Genetics: A Conceptual Approach*. 4th ed. New York: W. H. Freeman.

Sulem, P., D. F. Gudbjartsson, S. N. Stacey, A. Helgason, T. Rafnar, K. P. Magnusson, A. Manolescu, et al. 2007. "Genetic Determinants of Hair, Eye and Skin Pigmentation in Europeans." *Nature Genetics* 39 (12): 1443–1452. doi:10.1038/ng.2007.13.

Truett, G. E., P. Heeger, R. L. Mynatt, A. A. Truett, J. A. Walker, and M. L. Warman. 2000. "Preparation of PCR-quality Mouse Genomic DNA with Hot Sodium Hydroxide and Tris (HotSHOT)." *BioTechniques* 29 (1): 52–54. http://www.biotechniques.com/multimedia/archive/00011/00291bm09_11375a.pdf.

Ware, J. S., A. M. Roberts, and S. A. Cook. 2012. "Next Generation Sequencing for Clinical Diagnostics and Personalised Medicine: Implications for the Next Generation Cardiologist." *Heart* 98 (4): 276–281. doi:10.1136/heartjnl-2011-300742.

Yang, N., D. G. Macarthur, J. P. Gulbin, A. G. Hahn, A. H. Beggs, S. Easteal, and K. North. 2003. "ACTN3 Genotype is Associated with Human Elite Athletic Performance." *The American Journal of Human Genetics* 73 (3): 627–631. doi:10.1086/377590.

Appendix 1

The following quiz can be used as a post-quiz to assess student learning. We recommend modifying the questions to suit the concepts you are seeking to reinforce.

Quiz Questions about *Concepts*

(1) Define SNP.
(2) An individual that has a family history of breast cancer is tested for mutations in the *BRCA1* and *BRCA2* genes. The sequence results reveal they have 5 SNPs from the known normal sequence. Not knowing any more information, discuss the potential implications of these results. [The goal of this question is to test the students ability to *apply* the principle that SNPs have varying influence over phenotype. Meaning the SNPs referenced in the question could significantly increase the chance of cancer or might not have any effect at all upon the phenotype. Because students will likely one day confront the sequence of their own genome (with undoubtedly thousands of SNPs), we wanted them to recognise that just because there is variation in our genomes does not mean each difference is of significance.]
(3) Approximately what percentage of sequence variation between human individuals is due to SNPs? [We felt this question reinforced the importance of SNPs, we emphasize in the pre-lab that 90% of all sequence variation is in the form of SNPs.]
(4) What is the current approximate cost of sequencing an entire human genome? [As of writing this article it has dropped to ~US$1000/genome, but will likely drop further over time. The drop in cost of sequencing is the economic driving force behind the wider use of DNA sequencing].
(5) Please give an example of a SNP that leads to a clear phenotypic change.

Quiz Questions about *Method*

(1) List the three phases of a PCR cycle and describe the role of each phase.
(2) Briefly describe the importance and function of the inner primers in the ARMs genotype PCR.
(3) What is the function of *Taq* DNA polymerase enzyme in the Polymerase Chain Reaction?
(4) Gel electrophoresis separates DNA based on _____.