Grocery Store Genetics: A PCR-Based Genetics Lab that Links Genotype to Phenotype

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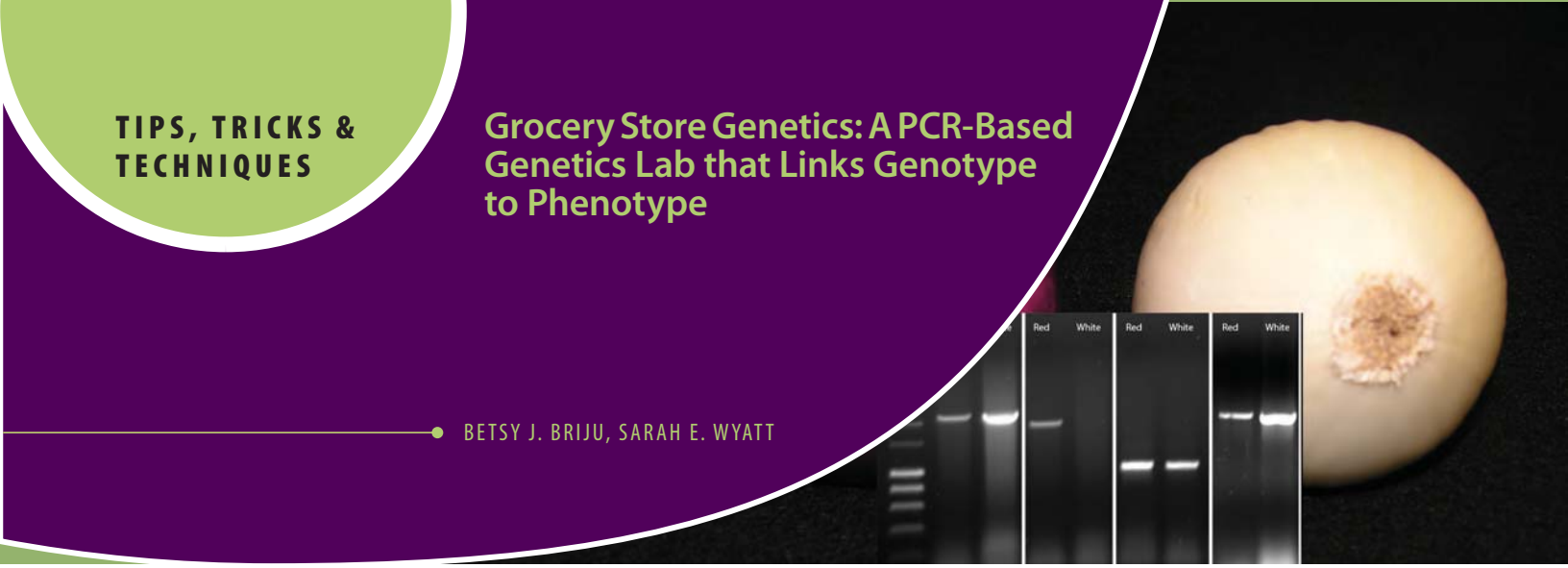
TIPS, TRICKS & TECHNIQUES

Grocery Store Genetics: A PCR-Based Genetics Lab that Links Genotype to Phenotype



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Abstract

Instructors often present Mendelian genetics and molecular biology separately. As a result, students often fail to connect the two topics in a tangible manner. We have adopted a simple experiment to help link these two important topics in a basic biology course, using red and white onions bought from a local grocery store. A lack of red coloration in white onions is a result of one or more mutations in the color production pathway. This mutation can be seen by the use of polymerase chain reaction (PCR) followed by gel electrophoresis. An absence of an amplified PCR product for one of the genes necessary for color production is associated with a lack of color production – an obvious trait in white onion. The students are able to "see" the difference at the DNA level between the red and white onion.

Key Words: *PCR; onion; genetics; trait; genotype; phenotype; biochemistry.*

A typical genetics module in an introductory biology class might begin with a lesson on Mendel's laws of inheritance. For a laboratory, some teachers have adopted the use of mutants, ordering plant or animal/insect mutants from stock centers or commercial vendors, to demonstrate variations in phenotype. These modules may end with lessons on DNA, transcription, and translation. Many high schools and introductory college/nonmajors biology laboratories now use polymerase chain reaction (PCR) to amplify DNA – often using commercial kits for DNA fingerprinting to illustrate DNA polymorphisms. Although these laboratories enhance student learning, students still don't always grasp the link between a gene that encodes a protein (or molecular marker, in the case of DNA fingerprinting) and the phenotype of an organism (Bahar et al., 1999; Marbach-Ad & Stavy, 2000; Lewis & Kattmann, 2004; Mills Shaw et al., 2008). Here, we present a PCR-based experiment to help bridge this gap using red and white onions bought from a local grocery store. Not only are students presented direct DNA evidence for a phenotype, the laboratory exercise also helps demonstrate the genetic variation (i.e., polymorphisms) that has resulted in the phenotypic variation seen in the world around us.

Many high schools and introductory college/ nonmajors biology laboratories now use polymerase chain reaction (PCR) to amplify DNA.

Color production in onion bulbs can serve to illustrate the relationship between the genetic condition (genotype) of a plant and the external expression of the trait (phenotype). Red coloration in onion bulbs is caused by the presence of pigments (anthocyanins) dissolved within the vacuole of the epidermal cells of the onion bulb. Conversely, the absence of red color in the white or yellow onion is associated with a defective gene in the anthocyanin biosynthetic pathway (Figure 1; Kim et al., 2004a). Among the many enzymes that act in tandem to produce the red pigment, four enzymes have been selected for this lab: phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), dihydro flavanol 4-reductase A (DFR-A), and anthocyanidin synthase (ANS). Presence and action of these enzymes to convert one metabolite to another in the anthocyanin biosynthesis pathway result in the production of the red pigment in onion cells. Each of the corresponding genes must be both present and functional in the genome of the onion plant to make an onion bulb red. Inability of the onion cell to produce one or more of these enzymes leads to formation of a white or yellow bulb. Because of this, students are able to correlate what they see as phenotype (onion color) to the presence of the corresponding genes as indicated by amplification of a marker for the gene through PCR.

For this exercise, students work in groups of four, with each student selecting one of the four genes to investigate. Onions can be bought at a local grocery store. We have obtained onions from a variety of sources (grocery store, garden supply store, local farmer's market), and all consistently displayed differences in at least one of these four genes in the anthocyanin biosynthetic pathway. Here, we have focused on the red and white onions as an initial experiment for students to perform. The students' task is

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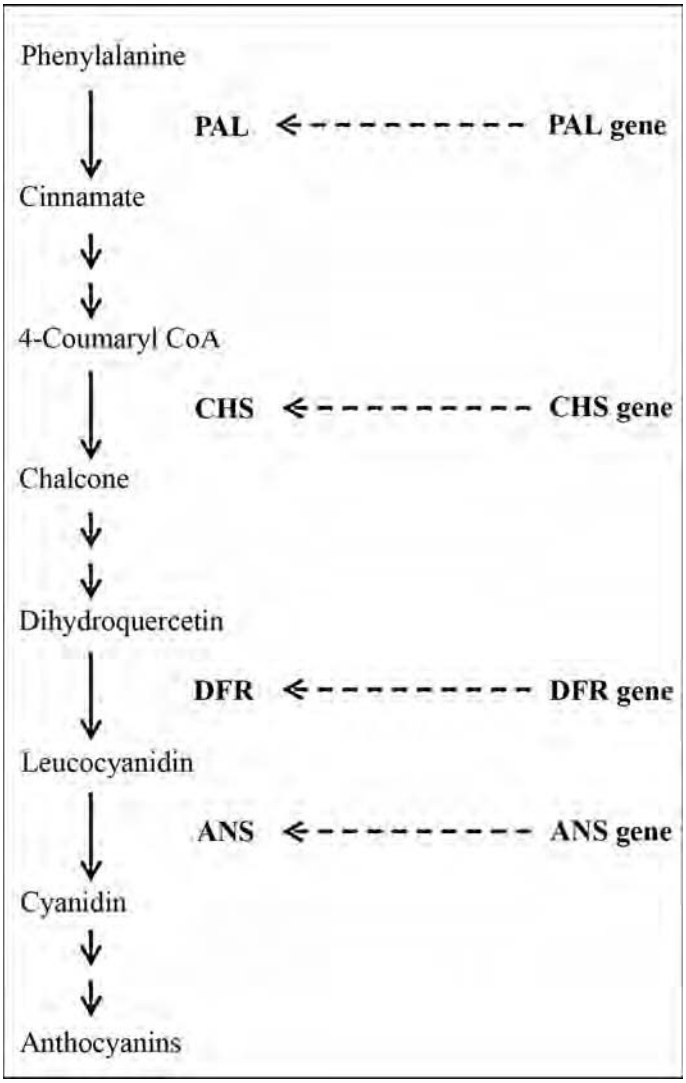

Figure 1. Abbreviated anthocyanin biosynthetic pathway in onions. Solid arrows indicate biochemical reactions; dashed arrows indicate transcription and translation. Phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), dihydro flavanol 4-reductase A (DFR-A), and anthocyanidin synthase (ANS) are necessary to convert the amino acid phenylalanine into anthocyanins. Adapted from Kim et al. (2004a).

to analyze the genes to see which one is defective in the white onion, resulting in the lack of color. The majority of non-red onions contain the DFR-A^{PS} allele, having a premature stop codon in the coding region that results in inactivation of the DFR-A protein and lack of anthocyanin production (Kim et al., 2009). To identify the DFR-A^{PS} allele for onion-breeding programs, Kim et al. (2009) developed a PCR-based assay that takes advantage of a deletion of a simple sequence repeat in the promoter region of the gene. Although the repeat does not result in the inactivation of the gene, it does serve as a reliable marker for the defective DFR-A^{PS} allele, and so we used it to develop this lab exercise.

Students will need to extract genomic DNA (gDNA) from both red and white onions using any available method, such as that presented by Averill (1993); the CTAB method (Ausubel, 1987); or any commercially available kit, following the manufacturer's directions (e.g., Promega Wizard Genomic DNA Purification Kit, catalog no. A1120; Qiagen QIAmp DNA Mini Kit, catalog no. 51304; or Biobasic One-4-ALL Genomics DNA Miniprep Kit, catalog no. BS88504). We have found that the method described by Averill (1993) was the least expensive and most visual protocol to isolate DNA for use as a crude template for PCR. Students will then try to amplify their gene-of-interest using the corresponding primers from Table 1 and the PCR reaction mixture as indicated in Table 2. The primers were chosen on the basis of the primary literature (CHS: Kim et al., 2004a; DFR-A and ANS: Kim et al., 2009) and personal communication (PAL: S. Kim). A standard PCR program is used – an initial denature step at 94°C for 3 minutes; then 35 cycles: 94°C for 30 seconds (denature), 60°C for 30 seconds (anneal), and 68°C for 3 minutes (extension). This is followed by a final extension at 68°C for 10 minutes and a 4°C hold until the students are ready to analyze the results. A specific positive control to ensure that the PCR components – buffer, deoxynucleotides (dNTPs), and Taq DNA polymerase – are functional is not necessary. The other genes being amplified serve that function. Negative controls (see Figure 2) can be useful to determine whether cross-contamination has occurred.

To analyze the PCR results, students can use standard gel electrophoresis (e.g., a 1% agarose gel in 1X TBE run at 115 V). Although ethidium bromide was used here to detect the DNA (1 mg/mL), GreenGlo (Denville Scientific, CA), a potentially safer, but more expensive, detection method may also be used successfully. Gel electrophoresis of the PCR products will allow students to see the presence or absence of the genes in the white onion sample as compared with the red onion (Figure 2).

○ Conclusions

The genetics that underlie the color trait in red onion can be identified using PCR amplification. Therefore, a simple experiment with onions from the grocery store, coupled with the technique of PCR, may prove valuable to help students associate genotype to phenotype and achieve the intended pedagogical goal. This experiment can also serve as a launching point for additional exercises and more in-depth analyses of these genes through bioinformatics analysis of available databases. For example, students could search the available genome databases (National Center for Biotechnology Information [NCBI], http://www.ncbi.nlm.nih.gov/; Arabidopsis Information Resource, http://www.arabidopsis.org) to find orthologs of their genes of interest. Or, if time and cost permit, students could amply the full-length onion gene, clone it into a plasmid vector and transform it into bacteria, and extract the plasmid DNA and have it sequenced. Once they have obtained a DNA sequence from whichever method is selected, students can use this sequence for bioinformatics analysis using the tools available from NCBI. They can identify exons, introns, and promoter regions and search for the primer-binding sites. Instructors could also use inquiry-based laboratories and allow students to evaluate the genetic cause of other phenotypes (i.e., accumulation of metabolites [and not the end-products] within the pathway may impart minor coloration, evident by the color gradient in onions – yellow, golden, pink, etc.; Kim et al., 2004a, 2004b, 2005). Using the literature presented here, students could set up hypotheses and test color-related genes in unknown varieties of yellow, golden, and pink onions from the grocery to determine which genes might be defective. For more advanced inquiry, students could use RNA extraction and reverse transcriptase (RT) with PCR to evaluate gene

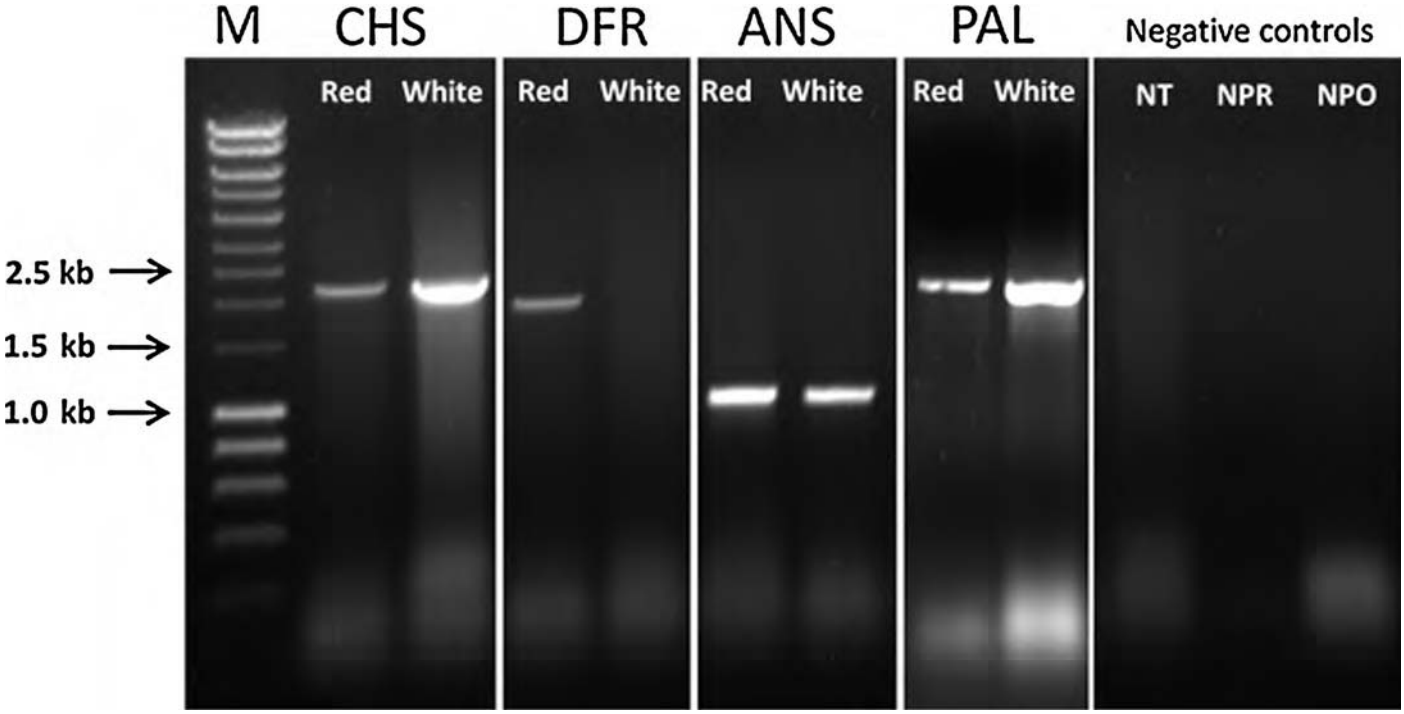

Figure 2. Image of experimental results. PCR products were amplified using gDNA from red (R) and white (W) onions, stained with ethidium bromide, and electrophoresed on a 1% agarose gel. M = marker lane, a 10-kb DNA marker for size comparison (Denville Scientific, http://www.denvillescientific.com). Chalcone synthase (CHS), dihydro flavanol 4-reductase (DFR-A), anthocyanidin synthase (ANS), and phenylalanine ammonia lyase (PAL) have been amplified. Negative controls include no template (NT), no primers (NPR), and no polymerase (NPO).

Table 1. Primer sequences for the four genes – PAL, CHS, DFR, and ANS – and the expected amplified product using gDNA from white or red onions.

Gene	Forward Primer	Reverse Primer	Size Amplified (kb)	
			White	Red
PAL	TGCTGTGAATGGGGATTTTAGCGTCAA	AAGGGGTGAGGAGTGTGTTGTCGGAAG	1.7	1.7
CHS	GAGGGTCCAGCCACGGTGTTAGCTG	ATCAATGGCCACACTCCTAAGCACC	2.3	2.3
DFR	ATGCCAGTGGAGTGCATGTTGAATGGT	TGGGTAGCGATTGGTTCATTCTCTTCA	None	1.9
ANS	TTTGCTCGACGTTTAGCAGAAGAAGA	TGAGGATGATGACAAAGTTAGCGGAGCA	1.0	1.0

Table 2. PCR components, suggested vendors, corresponding concentrations, and final volumes.

PCR Component	Stock Concentration	Suggested Vendors [a]	Diluted Stock	Amount (µL) in 20-µL Reaction Volume
Distilled water	NA	NA	NA	14.5
OneTaq PCR buffer	5X	NEB	NA	2.0
Deoxynucleotides	100 mM	NEB	2 mM	0.25
OneTaq DNA polymerase	5000 U/mL	NEB	NA	0.25
Forward primer	100 µM	IDT	20 µM	1.0
Reverse primer	100 µM	IDT	20 µM	1.0
Onion DNA	Varied	Extracted	50 ng/µL	1.0

[a] NEB = New England Biological (http://www.NEB.com); IDT = Integrated DNA Technology (http://www.idtdna.com); NA = not applicable.

expression (mRNA) in various organs of the plant (i.e., leaves, roots, and bulbs). In instances where the red color is lost because of gene mutation, students could clone the functional gene from a red onion and insert it into the bulb cells of white or yellow onion using a gene gun as described in Scott et al. (1999). If the inserted functional gene restores the red color, it confirms that the defective gene is indeed the cause of the lack of color.

In summary, most genes encode a protein, either an enzyme or a structural protein. An enzyme may be part of a pathway, converting one metabolite to another. Transcription and translation of the gene are crucial for the pathway to be functional and, therefore, for the end-product (pigment in this case) to be made. The end-product, in turn, determines the phenotype of the organism. Study of the gene(s) in a pathway provides insight into the cause of the phenotype displayed by the organism. The exercise presented here provides technical experience with DNA extraction and PCR but also provides a backdrop for discussions of genotype–phenotype interactions, gene–enzyme relationships, the resulting biochemistry and physiology, and how polymorphisms lead to genetic diversity.

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