ARTICLE
DRD4 Allele Frequency as a Lab Exercise in Neuroscience and Genetics Courses

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DNA segments with variable number tandem repeats (VNTR) serve as a model for students to learn DNA extraction and polymerase chain reaction (PCR) techniques in biology laboratory courses from the high school to the graduate level. Because of a growing interest in the neurosciences among undergraduates, we have developed a PCR exercise with a focus on the nervous system and behavior, with the aim of inspiring students from all aspects of the neurosciences to appreciate the central dogma and neurogenetics. DRD4 was a good candidate to provide a lab exercise that would be more engaging than VNTR analysis of a non-coding segment. DRD4 encodes for the dopamine D4 receptor and has been controversially associated with 'novelty seeking' or 'wanderlust' behavior. DRD4 has three common variants of the 48 bp sequence on exon 3, easily differentiated through gel electrophoresis. The 2 repeat (2R), 4 repeat (4R) and 7 repeat (7R) of the 48 bp sequence are the most common alleles. The 7R sequences result in the expressed dopamine D4 receptor having less affinity for dopamine binding, which was proposed to be the reason individuals engage in 'novelty seeking' behavior, to increase dopamine release to facilitate more binding to the receptor. Here we demonstrate an enjoyable and simple two lab sequence to analyze DRD4 genotypes that is appropriate for neuroscience and genetics courses.

Key Words: PCR, DRD4, VNTR, Novelty Seeking, Wanderlust, Dopamine

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Polymerase chain reaction (PCR) techniques are now routine in biology laboratory courses at all educational levels. Genetic polymorphisms such as variable number tandem repeat (VNTR) or Alu insertions provide useful learning tools to illustrate population genetics, human demographics, DNA fingerprinting, and other molecular concepts. Common examples of PCR labs include PV92 alu insertion on chromosome 16 (Novick et al., 1993) and D1S80 DNA fingerprinting lab on chromosome 1 (Budowle et al., 1991). DNA can easily be extracted by students in the class, with analysis completed within the class population. Due to allele variation in the population and the size difference between alleles or fragments after restriction enzyme digestion, DNA extraction and PCR of a gene can be analyzed through gel electrophoresis (Saiki et al., 1988; Mullis, 1990).

With the advent of undergraduate neuroscience programs combining backgrounds among disciplines, notably in psychology and biology, a PCR laboratory exercise that utilizes a genetic polymorphism that has a behavioral link would be a useful teaching tool. One example that includes behavior is TAS2R38 for tasting bitterness, where tasters have three single nucleotide polymorphisms (SNPs) (Merritt et al., 2008), one of which is in a sequence recognized by restriction enzyme Fnu4H1 that can be employed after PCR and before gel electrophoresis (PCR-RFLP). The resulting fragments can distinguish homozygote from heterozygote tasters and homozygote non-tasters for phenylthiocarbamide (Reinking et al., 2013). Olfaction could be paired in a similar manner, such as TAAR5 for detecting fish odor containing trimethylamine or OR6C70 for licorice (trans-anethole) (Gisladottir et al., 2020), also both due to SNPs that could plausibly be determined via sequencing or with a restriction enzyme lookup tool to recognize sequences after PCR and before gel electrophoresis (Grisham et al., 2015).

However, we chose DRD4, which encodes for the dopamine 4 receptor, has been linked to novelty seeking, and has the advantage of polymorphisms as the result of a VNTR (Van Tol et al., 1992). Therefore, the different sized alleles can readily be distinguished via gel electrophoresis after PCR without a restriction enzyme step or sequencing.

The dopamine D4 receptor is coded by the *DRD4* gene on chromosome 11 (Petronis et al., 1993) with a VNTR in exon 3 that creates alleles that can have 2 to 11 repeats, with 2R, 4R and 7R alleles being the most common in the human population (Van Tol et al., 1992). The 4R allele has been observed in 64.3% of the global population, the 7R allele observed in 20.6%, and the 2R allele in 8.2% of the population, with other repeats occurring at lesser frequencies, and none greater than 2.5% (Chang et al., 1996; Kidd et al., 2014). All alleles in this polymorphism likely occurred before early human migration. The 7R allele is 5-10 fold younger than the common 4R allele and appears to have been selected for 40,000 years ago (Ding et al., 2002). Populations who have migrated further in the past 30,000 to 1000 years had a higher frequency of 7R or greater alleles (Chen et al., 1999). Likewise, nomadic populations had higher frequencies of 7R or greater alleles than sedentary ones. In indigenous Americans the frequency of the 7R allele is 48.3% of the population, or 27.7% higher than the global population (Chang et al., 1996;

Kidd et al., 2014).

The binding affinity of dopamine to the dopamine 4 receptor with 7R or greater allele is less than the binding affinity for dopamine to receptors of the 4R or 2R allele (O'Dowd, 1992; Van Tol et al., 1992). Also, because of differential binding of the antipsychotic clozapine, it was quickly speculated that different alleles of DRD4 might increase the risk for schizophrenia and bipolar disorder (Nanko et al., 1993). However, soon it became clear there was no link for schizophrenia (Barr et al., 1993; Shaikh et al., 1994) or bipolar affective disorder (Lim et al., 1994).

In 1996 two studies indicated that people with the DRD4 7R allele engage in more 'novelty seeking' (impulsivity, thrill seeking) behavior presumably to stimulate more dopamine release in reward pathways because of the low binding affinity for dopamine in their D4 receptors (Benjamin et al., 1996; Ebstein et al., 1996). Since these publications, an explosion of studies indicating having 7 repeats or more on at least one DRD4 allele may be linked to: attention deficit hyperactivity disorder in children (Rowe et al., 1998), alcohol and drug abuse (Kotler et al., 1997), spirituality (Comings et al., 2000), empathy in women (Uzefovsky et al., 2014), delinquency, anger and thrill seeking in adolescent boys (Dmitrieva et al., 2011), infidelity and sexual promiscuity (Garcia et al., 2010) and increased creativity (Mayseless et al., 2013) among others.

Controversially, other than human migration studies, metadata analysis has determined the link may not be solid between personality traits and the 7R allele, which is not surprising considering the polygenic nature of human behaviors such as 'wanderlust' or 'novelty seeking (Munafò et al., 2008; Pappa et al., 2015).'

Because the DRD4 7R allele has been implicated in these behaviors, we hypothesize that students will be more curious about the lab and therefore learn concepts more effectively. It is well known that curiosity in students can enhance learning (Berlyne, 1960; Renninger et al., 1992), and even DRD4 7R carriers will show heightened attention to high priority items (Gorlick et al., 2015). It has also previously been shown that learning and motivation improves when concepts in the classroom relate to everyday life (Borges and Mello-Carpes, 2014). Therefore, this lab exercise that incorporates aspects of familiar behavior, that is exciting to stimulate curiosity, could facilitate increased learning retention and student performance.

LEARNING AND SKILLS OBJECTIVES

Learning Objectives

- Comprehend genetic principles underlying PCR, gel electrophoresis, and population genetics
- Analyze, interpret, and critique experimental data
- Perform calculations involving the Hardy-Weinberg equation
- Appreciate the complexity of the genetics underlying personality-related traits

Skills Objectives

- DNA extraction
- PCR
- Gel Electrophoresis

MATERIALS AND METHODS

Human Subjects

Seventy-nine students in seven lab sections with different instructors in a 200 level genetics course participated in this study. The experiment was divided into two parts: a two week PCR experiment to test the class DRD4 allele frequencies early in the semester, and a one week population genetics experiment later in the semester to compare with the DRD4 labs for learning retention. Before the study, students were given a consent form to indicate their participation was voluntary, and that they could opt out at any time without any impact on their grade. If they chose to participate, the form indicated that their answers to a pre- and post-test questionnaire and DNA sample would remain anonymous, and that their DNA sample would be discarded and disposed of after the lab exercise. Five students withdrew over the course of the semester and 74 of the original student population participated in the post-test. All procedures were approved by the University of Hartford Human Subjects Committee (ID# PRO16090003).

Lab Design and Equipment

The experiment was divided into three separate weekly lab sections, each 2.5 hours (see timeline, Figure 1). The first two weeks comprised the core DRD4 lab: DNA Extraction in week 1, followed by PCR between labs and gel electrophoresis in week 2. Therefore, this exercise could be completed in two weeks. In the design presented here, the first two weeks were completed towards the beginning of the semester. The gel analysis was conducted in a third lab period towards the end of the semester when students were learning about population genetics. This coincided with the timeline of the genetics lecture in this course, which covers molecular genetics during the first half of the semester and classical genetics during the second half. All needed supplies were purchased with lab fees paid by the students.

In week 1, the students were taught the relevant background information about PCR and the DRD4 alleles for about 0.5 hr-0.75 hr: including the different alleles and their frequency in the population, how dopamine binds to the dopamine 4 receptor and the theory of novelty seeking, and the history and technical aspects of DNA amplification through PCR. They then performed DNA extraction, which takes about an hour. For the purposes of this course, students also analyzed the results of a transformation experiment they completed the previous week during this lab period, which was not associated with the DRD4 labs.

At the end of lab, the instructor collected the DNA samples and ran the PCR reaction overnight at some point between the first and second lab. The lab setup for DNA extraction in week 1 is as follows: each pair of students was given the following materials: 2- 1.5 ml tubes containing 1mL of a 0.9% sterile saline or sterile phosphate buffered saline (PBS), a 1.5 ml tube with 0.5 mL of InstaGene Matrix (Bio-rad, 7326030), p20, p200 and p1000 micropipettes with sterile tips, a waste container, an individually wrapped sterile cotton swab, and two heat blocks: one set to 56°C, and one set to 100°C. At the end of this lab, students added their DNA to a PCR tube with necessary reaction mix (see below for details). For these labs, the PCR mixture was made

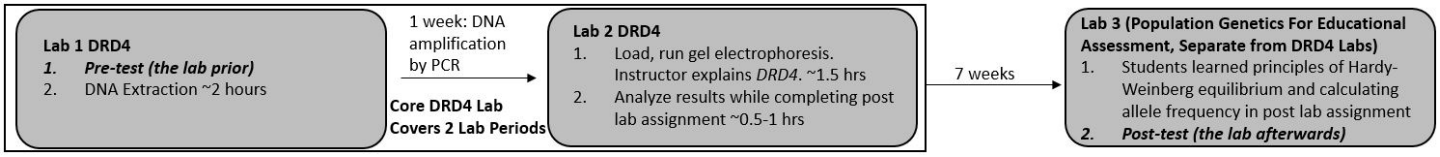

Figure 1. Timeline of the Lab Exercises. The lab exercise requires two 2.5 hour lab classes, one for DNA extraction and one for gel electrophoresis and analysis, while DNA amplification through PCR occurs at some point between the labs. An extra lab on population genetics was conducted later in the semester to compare retention of information from the DRD4 lab with information from the recently learned concepts.

ahead of time by the instructor to cover the number of students in the section, but this could also be completed by the students themselves.

In the second week, the students loaded samples onto agarose gels; this week's work took about 1.5-2.5 hours including the time spent for gel electrophoresis. During gel electrophoresis, the students performed a pharmacogenetics case study on an unrelated topic. Aheadof the second lab, the instructor made 1.5% agarose gels in 1x TAE buffer, with GelRed (Biotium #41002), a non-mutagenic, non-toxic, nucleic acid stain for DNA fluorescence. 5 μL of GelRed was added to every 200-250 ml of 1.5% agarose in 1x TAE buffer. About 50 ml is poured into each gel box with 10 wells, with two power supplies running to cover the 12 students. At the beginning of the second lab, students were given a p20 for gel loading, as well as a 100 bp ladder and the anonymous PCR samples. Please note that students can also be instructed to make the gels themselves at the beginning of the lab, which is something done successfully in other labs.

For the third week, towards the end of the semester, students used the results from the gels to calculate allele frequencies. This was part of a larger population genetics activity that involved analysis of easily observable traits such as tongue rolling and widow's peak as well as blood types. For week 3 there is minimal preparation or materials required for this lab apart from the blood typing kits (Carolina Biologicals) unrelated to the DRD4 labs.

Another instructor using this experiment could easily modify to fit their own class design and goals. For example, the population genetics lab could be left out and only the two-week DNA extraction, PCR and analysis of gel electrophoresis in the second week could be performed.

Lab Procedure: Buccal DNA Extraction

To obtain their buccal sample, students were instructed to swipe with good pressure up and down on the inside of their cheek for 15 seconds with the sterile cotton swab, and swish the swab around inside of their microcentrifuge tube with saline for 15 seconds. After disposing of the cotton swab, students placed their microcentrifuge tubes with their buccal swab sample into a microcentrifuge, making sure that their tube was balanced against another tube in the opposite position in the rotor. The microcentrifuge was run for 1 minute at 6,000 rpm to pellet the cells to the bottom of the tube. Students were then told to pour off the supernatant into the waste container, being careful not to shake their tube prior, and disturb or lose the pellet. In this step a small amount of liquid will remain in the tube with the pellet even after they pour off the supernatant. Students were then instructed to vigorously shake the Instagene Matrix to suspend and quickly transfer 200 μl of InstaGene Matrix to the cell pellet in their microcentrifuge tube. Students then broke up their pellet to mix their cells with InstaGene Matrix by vortexing the tube for 10 sec. All vortexing in this procedure was done at high speed setting. Their tubes were then placed in 56°C in a heat block for 30 minutes, vortexing the tube for 10 second at the 15 and 30 minute marks. Afterwards, their tubes were placed in another heat block set at 100°C for 8 minute, vortexing again on high speed afterwards for 10 second. They then placed the tubes back in the microcentrifuge at 6,000 rpm for 1 minute. After this last centrifugation step, the supernatant contained the students' DNA.

Lab Procedure: Polymerase Chain Reaction and Gel Electrophoresis

The DRD4 gene is rich in G/C content >65%, so One*Taq*® Hot Start Quick-Load® 2X Master Mix with GC Buffer was used (New England Biolabs, #M0489). Note: during troubleshooting, it is very important to take into account the high G/C content of the DRD4 sequence for quality results. PCR tubes were prepared by the instructor a couple of days before the lab and stored at -20, then given to the students at the end of the first lab after the DNA extraction procedure above. The PCR tubes were prepared with the following mixture: 12.5 μL of 2x Master Mix, 2.5 μL of GC enhancers, and 2.5 μL each of 3 μM forward 5'-GCGACTACGTGGTCTACTCG - 3' and reverse 5' - AGGACCCTCATGGCCTTG - 3' primers (Sigma, Easy Oligos) for 20 μL total. For the purposes of this lab, students weren't involved in primer design, and the standard effective primers for DRD4 studies were used, which include 282 bp outside the VNTR area of interest (Lichter et al., 1993). At the end of the first lab, students added their DNA to the unlabeled tube using the p20 micropipete. It was found that if students added 5 μL from the supernatant with their DNA to total 25 μL in the tube, it was generally effective. In some courses, students measured their DNA using the Nanodrop spectrophotometer, and added the amount equivalent to 100 ng DNA, with nanopure water to bring the total volume to 30 μl in the PCR tube. The PCR tubes were unlabeled and mixed in an opaque bag to ensure anonymity, and the students' remaining DNA samples were discarded.

Between the first and second lab, samples were spun

briefly in a microcentrifuge and then placed in the thermocycler at the following specifications overnight by instructor before second lab: Initial denaturation 94ºC for 4 minute, then Cycle 35x the following: 94ºC for 30 sec, 55 ºC for 1 minute, 68ºC for 1.5 min; then final elongation at 68ºC for 7 minute, and held at 4ºC. PCR tubes were then stored at -20ºC until the second lab.

Ahead of the second lab, the instructor made 1.5% agarose gels in 1x TAE buffer, with GelRed and allowed it to solidify. Students were instructed at the beginning of the lab to remove the comb from the gel to expose the wells for sample loading, fitting their gel into the electrophoresis chamber with the wells opposite the positive electrode, and filling the chamber with 1x TAE buffer. Each student was given experience loading a sample into a well. And then the 100 bp ladder was loaded by a student at the end. Students then ran the 1.5% agarose gel at 80-100 mV for approximately 45 minute or until the yellow light weight dye in the samples neared the bottom of the gel. Afterwards, the gel was placed under UV light for analysis.

Assessment

Quantitative Pre- and Post-test

After signing the consent form, students were given a pre-test to quantify prior knowledge to the lab exercise. After the completion of the two lab DRD4 sequence, and the population genetics lab later in the semester, the students were given the same test as a post-test 11 weeks later to assess how well their knowledge improved and was retained over time on relevant concepts. For both the pre- and post-test the instructor stepped out of the room and another member of the department administered the tests to ensure anonymity. When considering questions for assessment, they were designed to determine learning outcomes in PCR, which was learned earlier in the semester with the DRD4 two-lab sequence, as well as to assess allele frequency analysis and Hardy-Weinberg equilibrium, which was learned in the population genetics lab later in the semester. Both topics are appropriate for Biology majors in a 200 level genetics course. This lab has also been used effectively at the freshman level for incoming neuroscience majors at the University of Wisconsin-River Falls, as well as at the graduate level for students in a neurophysiology course at the University of Hartford. As the learning outcomes are different in courses like these, the DRD4 lab could be used to assess different concepts, such as more emphasis on the understanding of the link between the molecular level and the behavioral level.

In the genetics course at the University of Hartford, the following six multiple choice questions were asked the week before the DNA extraction lab, the first lab in the DRD4 two-lab sequence, and also 11 weeks later after the population genetics lab. The first three questions (1-3) focus on the material learned in the DRD4 lab at the beginning of the semester, and the second three questions (4-6) focus on the material learned immediately before the post-test, eight weeks after the DRD4 lab exercises:

Question 1: Which of the following best describes the complete sequence of steps occurring during every cycle of PCR?

1. The primers hybridize to the target DNA.
2. The mixture is heated to a high temperature to denature the double-stranded target DNA.
3. Fresh DNA polymerase is added.
4. DNA polymerase extends the primers to make a copy of the target DNA.

A) 2, 1, 4 (correct) B) 1, 3, 2, 4
C) 3, 4, 1, 2 D) 3, 4, 2
E) 2, 3, 4

Question 2: The sequences below are different DNA primers that could be used for PCR. Which of these would have the highest annealing temperature?

A) 5'AAGAAAAGTTAGATTTAAG3'
B) 5'GCGTTAGCCAGACGTACGG3' (correct)
C) 5'GAACAGAAATTGCAGACGG3'
D) 5'GAGAAGTTTAGCAAGGAAA3'

Question 3 includes figure 3B: The image shows the results of an agarose gel following PCR amplification of a VNTR locus in a couple and their 6 children. Which lanes represent the parents?

A) 1 and 2 B) 2 and 3 C) 3 and 6
D) 1 and 5 (correct) E) 5 and 8

Question 4: In a Hardy-Weinberg population with two alleles, A and a, that are in equilibrium, the frequency of allele a is 0.1. What is the frequency of individuals with AA genotype?

A) 0.20 B) 0.32
C) 0.42 D) 0.81 (correct)

Question 5: In peas, a gene controls flower color such that R = purple and r = white. In an isolated pea patch, there are 36 purple-flowering plants and 64 white-flowering plants. Assuming Hardy-Weinberg equilibrium, what proportion of the population is heterozygous?

A) 0.16 B) 0.80
C) 0.32 (correct) D) 0.64

Question 6 includes figure 3C: DNA typing is used to compare evidence DNA (E) left at a crime scene to two suspects (S1 and S2). Suspect 1 is excluded by the evidence, but suspect 2 remains included. What is the frequency of suspect 2's genotype if the allele frequencies in the population are $f(A_1) = 0.1$, $f(A_2) = 0.2$, and $f(A_3) = 0.7$ and the population is at Hardy–Weinberg equilibrium?

A) 0.01 B) 0.02
C) 0.04 (correct) D) 0.28

Qualitative Assessments

After the second lab in the DRD4 sequence, students answered a series of qualitative questions as a post-lab assignment. The questions included the following:

1) In PCR, the annealing temperature is critical. Altering the temperature can greatly impact the stringency (specificity) of primer binding. Why do you think this is the case?

2) The annealing temperature normally varies from 50-70°C. The specific temperature is chosen, in part, based on the sequence of the primers. When deciding on the annealing temperature to use in PCR, you need to take into consideration the G/C versus A/T content of your primers. Why do you think this is the case?

3) Considering that there are three alleles of the DRD4 locus, and each individual would have two alleles, what are the possible genotypes that exist in the population?

4) The picture (Figure 2) shows the results of PCR analysis of the DRD4 locus in several individuals. Which lanes have the 7R, 4R, and 2R alleles? Lanes 5 and 10 contain the DNA molecular weight standard

5) Observe the pictures of the gels posted on blackboard. Compare lanes containing different results. Why do you think the results are different or similar? Do you see examples of each allele (2R, 4R, 7R)? Which lanes are heterozygous individuals and which are homozygous? Explain your answers.

6) If any lanes did not have any bands, what would be some possible sources of error? If everyone did have bands, you should still answer this question as a hypothetical.

The following questions appeared on the post-lab assignment for a population genetics lab that focused on Hardy-Weinberg equilibrium and calculating allele frequencies. PCR was not discussed, but students used the DRD4 results to calculate allele frequencies:

7) Determine the frequencies of the 2R, 4R, and 7R alleles in the class using data obtained from the PCR experiment

8) In a 1996 study of the DRD4 allele frequencies, the following data was obtained (from Chang et al., 1996, *Human Genetics* 98(1):91-101). Compare and contrast the data obtained from the class data with the data presented in that study.

"We have determined the repeat number genotype of the DRD4 repeat polymorphism in 1,327 individuals from 36 different populations. The allele frequencies differ considerably among the different populations. The 4-repeat allele was the most prevalent (global mean allele frequency = 64.3%) and appeared in every population with a frequency ranging from 0.16 to 0.96. The 7-repeat allele was the second most common (global mean = 20.6%), appearing quite frequently in the Americas (mean frequency = 48.3%) but only occasionally in East and South Asia (mean frequency = 1.9%). The 2-repeat allele was the third most common (global mean frequency = 8.2%) and was quite frequent in East and South Asia (mean frequency = 18.1%) while uncommon in the Americas (mean frequency = 2.9%) and Africa (mean frequency = 1.7%). The universality of the polymorphism with only three common repeat-number alleles (4, 7, and 2) indicates that the polymorphism is ancient and arose before the global dispersion of modern humans. The diversity of actual allele frequencies for this expressed polymorphism among different populations emphasizes the importance of population considerations in the design and interpretation of any association studies carried out with this polymorphism."

Student Pitfalls

In some cases, the experiment did not work well, and some students had no bands. If the yield for an entire section was low, students were provided with sample gels from other sections to interpret. Since the samples were anonymous, it wasn't essential the analysis was from another section. In the post-lab assignment no. 6 (see above), students were required to consider what technical aspect explained why bands were not visible in a lane.

Data Analysis

Statistical significance within this study was determined by using an unpaired 1-tailed t-test for analysis between pre and post-tests, and paired 2 tailed t-test for analysis within the pre-test and post-test. The results from methodologies were considered to be statistically significance if the p-value was less than 0.05.

RESULTS

DRD4 Allele Analysis

The timeline of the two labs for this exercise is represented in Figure 1. Students were given the pre-test the week before they began the experiment. The first week of the lab, students listened to the instructor explain *DRD4* allele differences and the implications of 'novelty seeking' in the literature. The instructor also explained the PCR technique. Student DNA was PCR amplified between the first and second labs, and in the second lab students performed gel electrophoresis of their sample. Students then analyzed their results while answering the post-lab qualitative questions. When analyzing their results in the post-lab qualitative questions, students were tasked with determining the different alleles in their class. Figure 2 shows a gel from 8 different DNA samples amplified for the *DRD4* VNTR, in addition to the 282 bp sequence within the primer area. Each VNTR is 48 bp, therefore, 2 copies is 48+48+282 = 378 bp. 4 copies would then be 474, and 7 copies 618. Comparing with the 100 bp ladder, students would determine that for this example, with 16 alleles in the 8 subjects in Figure 1, that lane 1 contained 4R/4R, lane 2: 4R/4R, lane 3: 4R/4R, lane 4: 2R/7R, lane 6 2R/4R, lane 7 4R/4R, lane 8 4R/4R, lane 9 4R/7R. Therefore, if this was the result of the class gel, students would ascertain that lanes 1, 2, 3, 7 and 8 are homozygous, while lanes 4, 6 and 9 are heterozygous. This would effectively answer questions 4 and 5 in the post-lab questions. They would also discuss which lanes have the novelty seeking allele. In this case, it would be lanes 4 and 9.

Later in the semester, eight weeks after performing the DNA extraction, students again revisited the results of the DRD4 gels while learning about population genetics and the

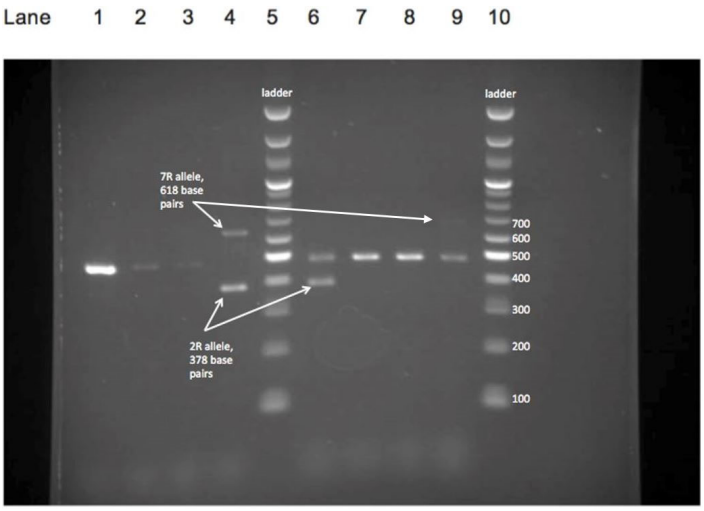

Figure 2. Representative Gel for Analysis. The PCR products after gel electrophoresis of 8 faculty members in a Biology Department in a mid-sized university in the United States. Each band refers to the number of the 48 bp variable number tandem repeats (VNTRs) plus a 282 bp flanking sequence within the amplified area. Therefore, 2 copies = 378 bp. The most common is 4 repeats at 474 bp, while repeats greater than 7 that have been associated with 'novelty seeking' are at 618 bp. There are also fragmented repeats and other variations, but 48 bp is most common. A 100 bp ladder is included in lanes 5 and 10 which will have a band every 100 bp, and a strong band at 500 bp, which closely aligns with the most common human allele, the 4 VNTR at 474 bp.

Hardy Weinberg Equation. Students were tasked with calculating the frequencies of the 2R, 4R, and 7R alleles in the class and comparing with the population at large. This would answer questions 7 and 8 from the qualitative post lab questions.

Pre- and Post- Assessment

In order to determine if students retained information related to the desired concepts in the DRD4 lab, they were given three multiple choice questions assessing their understanding of PCR. They were also given three questions assessing population genetics and Hardy - Weinberg equilibrium which was learned later in the course. The pre-test was given the week before the first DRD4 lab and the post-test was given the week after the population genetics lab (8 weeks after the second DRD4 lab, a timeframe of 11 weeks between pre- and post-tests). In Figure 3A, Students performed better on all quantitative questions in the post-test compared to the pre-test with one exception. The most improvement occurred in the understanding of how to analyze PCR products after they are separated by gel electrophoresis as shown in question 3 and in Figure 3B. Likewise, students performed much better on question 4, which involved determining the frequency of a homozygous genotype when given allele frequencies in a population and assuming Hardy-Weinberg equilibrium. Additionally, likely due to the high G/C content in the area amplified on the DRD4 gene, students improved on a question about which primer would require a high annealing temperature in question 2, knowing that this would correlate with the G/C content of the DNA fragment. However, students had a much greater prior knowledge on this question compared to the others before the lab. Students did not perform as well on questions determining the frequency of heterozygote genotypes, assuming Hardy-Weinberg equilibrium, and when given allele frequencies. They also improved slightly when they were required to combine their knowledge of Hardy-Weinberg equilibrium with DNA data from a crime scene. The figure of the crime scene data shown for question 6 is represented in Figure 3C.

The only question where students had a lower performance on the post-test compared to the pre-test was question 1, which assessed the sequence that occurs during the PCR technique. However, students performed much better overall when comparing the total answers correct on the pre-test n=79, which was 1.96 with total answers correct on the post-test n=74, 2.67, $p < 0.001$.

Assessment of Learning Retention

The responses to the three questions that focused on the PCR aspect of the experiment during the two lab DRD4 sequence, which occurred eight weeks prior to the post-test, was compared to the responses of the three questions that focused on the population genetics lab the week before the post-test (Figure 4). Surprisingly, there was no difference observed between the post-test performance on the PCR questions and the Hardy-Weinberg questions, even though the Hardy-Weinberg questions were directly related to what the students had just learned. Additionally, there was actually a slightly greater positive difference on the post-test compared to the pre-test on questions related to PCR material learned during the DRD4 lab eight weeks prior compared to the questions related to Hardy-Weinberg equilibrium learned just before the post-test (Figure 4A), indicating memory retention of material from the DRD4 lab. On both PCR and Hardy-Weinberg questions, improvement was significant from pre-test n=79 to post-test n=74, $p < 0.01$, which was expected based on the total correct overall. No significance was observed on the pre-test PCR questions vs. the pre-test Hardy-Weinberg questions indicating that students had the same level of understanding of both types of material before learning, or the post-test PCR questions vs. the post-test Hardy-Weinberg questions (Figure 4B).

DISCUSSION

DRD4 VNTR allele analysis is an effective simple lab, which is easy to establish and run over two lab sections. It provides a link between the molecular and behavioral levels that would be an effective learning tool in the neurosciences. Also, since the polymorphism is the result of a VNTR, allele differences are readily detectable via gel electrophoresis after PCR without time consuming and technically challenging restriction enzyme digestion steps required to analyze polymorphisms due to SNPs.

In this experiment, we utilized population genetics and PCR as our assessment topics as students were taking a genetics course. However, topics more relatable to the neurosciences could be assessed in an introductory or upper division undergraduate neuroscience course. For

A

Question	Pre-Test	Post-Test	Difference
1 *PCR step sequence*	20.25%	14.87%	-5.39%
2 *PCR primer annealing temp*	55.70%	70.27%	+14.57%
3 *PCR product analysis, reading the gel (see B below)*	21.52%	54.05%	+32.54%
4 *Hardy-Weinberg frequency of homozygotes*	16.46%	44.60%	+28.14%
5 *Hardy-Weinberg frequency of heterozygotes*	43.04%	44.60%	+1.56%
6 *Hardy-Weinberg frequency of crime scene suspect's DNA profile (see C below)*	27.85%	35.14%	+7.29%
Overall	***30.80%***	***43.92%***	***+13.12%***

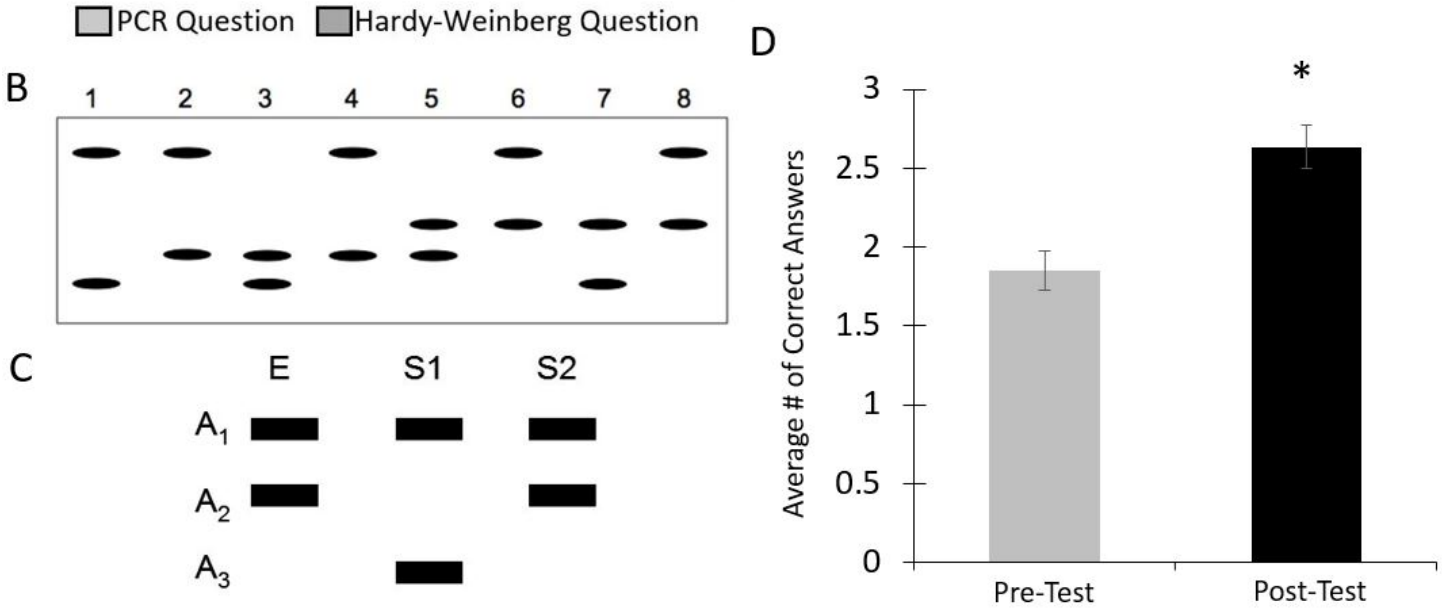

Figure 3. Assessment of Learning Outcomes. *A* shows the percentage correct for each question in the pre-test and the post-test genetics assessment. The students were aware that participation on the questions did not count towards the class grade and were anonymous. 79 students participated in the pre-test questions, and 74 in the post-test over 7 lab sections in a 200-level Genetics course. The difference indicates whether an increase or decrease occurred in the post-test compared with the pre-test after the DRD4 lab exercise on each question. In *B*, the figure refers to question 3 and students were told it is the PCR products on a gel from 8 people, 6 children and their 2 parents and asked to determine which two lanes are the parents. In *C*, the figure refers to question 6 and the students were told the allele frequencies were $f(A_1) = 0.1$, $f(A_2) = 0.2$, and $f(A_3) = 0.7$ and what the frequency is of suspect 2's genotype in the population if it is at Hardy-Weinberg equilibrium. *D* is an analysis of the average of number of questions each student answered correctly on the pre-test assessment n=79 and post-test assessment n=74. Students did significantly better on the post-test than the pre-test on an assessment that had low incentive for high achievement. Unpaired 1-tailed t-test: * = $p < 0.001$.

example, in a graduate level neurophysiology course in our department, this lab was routinely part of the sequence, and students were given questions on the polygenic nature of human behavior. As part of the analysis, they were required to read five papers on DRD4 in opioid dependence, spirituality, promiscuity, creativity and attention (Kotler et al., 1997; Comings et al., 2000; Garcia et al., 2010; Mayseless et al., 2013; Gorlick et al., 2015). Similarly, although it wasn't employed in this study, a coded anonymous questionaire asking about students' novelty seeking behavior could be paired with results to the gel electrophoresis to determine if 7R carriers correlated with novelty seeking behaviors in the class population.

Students engaging in a hands-on lab with a case-based activity, where they try to determine the DRD4 7R frequency in relation to 'novelty seeking,' could help improve student learning and retention (McFee et al., 2018). In this study a significant increase in performance was observed overall in the responses by students in the post-test indicating learning of the material. Interestingly, students performed slightly better on questions related to PCR, which was learned during the DRD4 lab sequence about two months prior to the post-test, when compared with material learned directly before the post-test.

A

Question Type	Pre-Test	Post-Test	Difference
PCR Questions	32.50%	46.40%	+13.90%
Hardy-Weinberg Questions	29.11%	41.44%	+12.33%

B

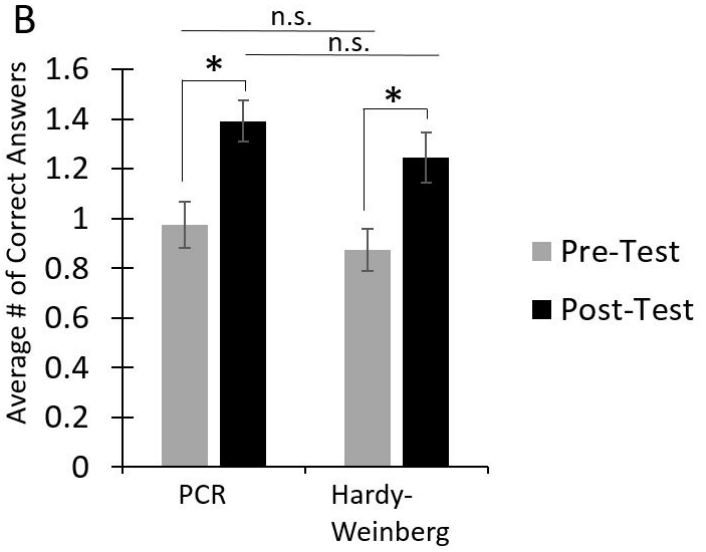

Figure 4. Assessment of Learning Retention. In *A,* the percentage correct on the pre-test and post-test for the three questions that focused on the DRD4 Lab at the beginning of the semester (PCR concepts), with population genetics lab nine weeks later and immediately before the post-test (Hardy-Weinberg concepts). In *B,* the average number of questions each student answered correctly for the three PCR and three Hardy-Weinberg questions on the pre and post-tests. Comparison of pre- and post-tests, unpaired 1-tailed t-test * = $p < 0.01$, comparison of PCR pre-test or post-test with Hardy-Weinberg pre-test or post-test, paired 2-tailed t-test, not significant (n.s.).

Transience is a well-known aspect of memory loss, in which material is forgotten over time, and can be quantified as a negative logarithmic 'Ebbinghaus' curve (Ebbinghaus, 1885). In the time frame, students should have performed at least 5% worse on the PCR questions. In this experiment, no information related to the experiment was reassessed or revisited, which would counterbalance transience of the learned material (Murre and Dros, 2015). Accordingly, most learned material is rapidly forgotten unless it's reinforced repeatedly, or if the learned material was significant, or meaningful (Custers and Ten Cate, 2011). Since DRD4 is dirigible to relatable and meaningful concepts about human behavior, such as novelty seeking or wanderlust behavior, it is likely the reason it was retained over time, and students performed just as well as they did on new material learned.

Although students performed better on nearly all questions that were assessed to determine learning outcomes, one exception was the first question which assessed the sequence of the PCR technique. Students had been exposed to the PCR technique in the pre-lab lecture. It's possible the performance on question 1 about PCR sequence was confusing to some students as 4 options were given to put in the correct order, and one was unused. In educational studies, if prompts do not indicate what's expected it may be an added stressor and impede performance through confusion (Rashid et al., 2021). Some answers on the multiple choice gave the sequence of 3 options, and some 4, but it wasn't stated that students didn't need to use all four, so this may have caused some students to pick an answer that incorporated all 4 options thinking this was one of the requirements.

Overall, the performance was lower than we expected on both the pre- and post-test in general, which was likely due to the low stakes nature of the assessment. Students were completely anonymous in their responses to the pre-test and the post-test and their answers did not contribute to their overall grade in the course, which may have resulted in students not being as motivated to ensure the correct answer for each question due to no value associated with the task (Liem and Martin, 2012). Students were also not told in advance that they would be taking the assessment that day, so there was no chance that they would have invested time in studying beforehand.

Despite this, the improvement was significant after the lab on the topics assessed, with memory on PCR concepts learned on the DRD4 lab retained over time. Likewise, students in the labs were biology students in a genetics course, and we would expect student engagement on the DRD4 lab to be further increased in neurosciences courses due to the relatable behavioral component of 'wanderlust' or 'novelty seeking (Borges and Mello-Carpes, 2014).'

Because of the relative simplicity of performing DNA extraction and PCR, with readily accessible equipment at most institutions, students in genetics and neuroscience courses can learn important hands-on skills, while determining allele frequency differences related to behavioral phenotypes. The DRD4 gene has three prevalent alleles in the human population that are easily distinguishable via gel electrophoresis directly after PCR, with a behavioral phenotype (novelty seeking, wanderlust) implicated for the 7R allele in the literature. Therefore, we find this to be an enjoyable and effective lab exercise that can be easily incorporated into appropriate courses as a useful teaching tool.

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