HOW-TO-DO-IT

Trekking Through THE HUMAN GENOME: An Individualized Laboratory Project

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On February 15, 2001, the International Human Genome Sequencing Consortium published a draft sequence and initial analysis of the 3.2 gigabase human genome (Sachidanandam et al., 2001). Eric Lander, Director of the Whitehead Institute Center for Genomic Research stated, "We are standing at an extraordinary moment in scientific history. It is as though we have climbed to the top of the Himalayas. We can for the first time see the breathtaking vista of the human genome." (U.S. Department of Energy, 2001).

While experts like Eric Lander readily appreciate these sights, students studying genetics from traditional textbooks and labs have little frame of reference from which to evaluate the utility and complexities of the Human Genome Project. Many students have the misconception that by sequencing the entire human genome, virtually all genes are identified and their function known. They often believe that a single gene is responsible for a single trait, and any disease is the result of one "bad gene." Despite this simplistic viewpoint, students are usually unaware of the vast amount of personally relevant genetic information that is available in the human genome databases by a few clicks on the Internet. No password required.

The project presented here allows students to develop their own incentive for researching the human genome databases, and along the way, to resolve for themselves some genetic misconceptions. Several good computer-based projects have been described previously that introduce students to the fields of genomics and bioinformatics (Puterbaugh & Burleigh, 2001; Smith & Emmeluth, 2002; Morvillo et al., 2000; Wefer, 2003). Our goal was to develop a hands-on exploratory project that combines such computer database research with laboratory experimentation, and is individualized in order to maximize student ownership and interest. We developed a multi-week project where students research and design a PCR assay to identify a gene of personal interest in their own genome. This project is driven by the student's interest in the topic and the need to design his/her own experiment, allowing students a firsthand experience with the challenges and rewards of deriving information from the Human Genome Project. We incorporated this project into an upper-level Molecular Genetics course, but it could be adapted readily for courses at the introductory undergraduate or advanced high school level.

The project is spread over an eight-week period (see Table 2). In the first two weeks, students explore human traits or diseases of interest to them, search the literature and molecular databases of the Human Genome Project to pinpoint genes potentially involved in the characteristic, and locate the DNA sequence corresponding to such a gene. In the third week, students design their own PCR primers to amplify a portion of the gene, and over the subsequent three weeks, they conduct a PCR assay using their own cheek cell DNA as a template to identify the gene in their own genome. Students communicate their findings with each other twice during the course of the project. At the completion, our students were fully equipped to view the human genome from the top of Mt. Everest.

The Base Camp

The National Center for Biotechnology Information (NCBI) Web site (www.ncbi.nlm.nih.gov) is the base camp for our project, as the work of thousands of scientists is collected here in the public genomic databases. We begin by introducing students to the various tools on the NCBI Web site, explaining how anyone can access human genomic DNA sequences, search an annotated version of the human genome, find relevant literature, and actually see the chromosomal position of a gene and find its corresponding DNA sequence. For a guide to using the NCBI Web site, see http://www.ncbi.nlm.nih.gov/Education/index.html.

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Acclimatizing: Living Within the Databases

Once students have been introduced to the NCBI Web site, they are ready to dig in and start using it. We tell students they are responsible for identifying a usable target gene sequence (one of roughly 30,000 in the human genome) that has been shown to play some role in a trait or disease of interest to them. To achieve this, they must search the literature to find genes involved in a characteristic of interest, and evaluate the DNA sequence of the gene to determine whether it will be a useful target for genomic PCR.

Students begin by thinking of virtually any trait or genetically based disease that has piqued their curiosity. Students may have personal experiences with particular diseases or physical or behavioral characteristics (a relative with sickle cell anemia, red-green color blindness, or sleep disorder), or perhaps they recently heard something interesting on the news (a new gene involved in breast cancer).

Although students will not uncover their own predisposition for the trait or disease of their choice from this project, it is helpful to integrate discussions of relevant ethical issues surrounding the knowledge of genetic information. We asked students whether they would want certain genetic information even if it is, as Ridley (2000) points out, "the bleakest kind of self-knowledge: the knowledge of our destiny, not the kind of knowledge that you can do something about." There are many excellent resources for discussing the social, economic, and ethical issues that result from advances in biotechnology and the genome project, including Nancy Wexler's efforts to understand Huntington's disease (Wexler, 1995), Videodiscovery's BioEthics laser disc materials, and the Department of Energy Web site (http://www.ornl.gov/hgmis/elsi/elsi.html).

In order to investigate what is known about the molecular genetics of their chosen trait, students search the Online Mendelian Inheritance in Man (OMIM) database using the pulldown menu at the NCBI Web site. OMIM contains records about human genes, traits, and disorders that are inherited in a Mendelian manner. This is a particularly useful starting point because OMIM will search using simple, lay terms for traits or disorders (e.g., freckles, sweating, diabetes) and therefore students can begin the process without knowing gene names or sophisticated medical terminology. Nonetheless, they may need to sift through a number of links in order to find the most relevant for their purposes. Students might need to limit search terms if there are too many results, or consider a different topic if too little is available.

Since students need to identify a single gene for this project, they are often frustrated when they learn that their chosen disease or characteristic might be attributed to multiple genetic loci and that environmental factors are involved. From a pedagogical perspective, we are pleased with this frustration since they are experiencing firsthand the complexities of genetics. In some cases, students may need to make arbitrary decisions in order to choose a single genetic locus. Students may also find conflicting information on OMIM, as this database provides unbiased research information from a wide range of sources. We remind students that ongoing scientific discovery yields many discrepancies that await resolution by further research such as theirs.

Table 1. Equipment and reagents

General
Set of micropipettors with tips
Refrigerator
1.5 ml microcentrifuge tubes, racks
Ice buckets
Lab markers
Genomic DNA isolation from cheek cells
BuccalAmp Kit (Epicentre)
Microcentrifuge for 1.5 ml tubes
Hot plate and beaker for boiling water bath
Water bath at 65˚C
Vortexer
Paper cups
Saline solution
PCR
DNA Thermalcycler and appropriate size PCR tubes
JumpStart RedTaq DNA Polymerase reagent (Sigma)
Human pMCT118 locus, a VNTR (Carolina)
Custom ordered PCR primers (Qiagen)
Nanopure water
Gel electrophoresis materials
Weighing station—balance, weigh boats, spatulas
Agarose powder
125 ml Erlenmeyer flasks
TBE (1x) buffer
Microwave oven
Gel electrophoresis boxes, combs, and power supplies
DNA ladder
Gel imaging camera
Ultraviolet light box viewer
Ethidium Bromide or Gel Staining solution
Saran Wrap™

By the end of their OMIM session(s), students will have obtained the official gene symbol of at least one gene that is potentially (perhaps tenuously) involved in a trait or disease of interest. Examples of genes chosen by students in our class include alcohol dehydrogenase (ADH1B); melanocortin receptor (MC1R); dopamine receptor (DRD4); and Von Hippel-Lindau (VHL), potentially involved in alcoholism; red hair and fair skin production; novelty-seeking behavior; and renal cancer, respectively.

Planning the Route: Evaluating the Exons

Once a potential gene of interest has been identified, students determine whether enough basic information on

the gene and gene product is available to make the project feasible. A relatively well-characterized gene will have one or more RefSeqs, which are Reference Sequences compiled by NCBI, based on all available public sequences and relevant literature. The RefSeq serves as the standard sequence to which students ultimately will design primers for their PCR project.

Since genomic DNA is easy to isolate and manipulate, we developed a genomic PCR approach to this project. However, many published sequences, including RefSeqs, correspond to cDNA that is derived from cellular messenger RNA lacking introns. To use genomic DNA as a template for the PCR reactions, students must take into account the possibility of introns in their gene before they can design PCR primers. If primers were designed to the cDNA sequence, and the regions corresponding to the two primers happened to be separated by a large intron in the genomic DNA, the PCR product obtained from the genomic DNA template would be much greater than predicted from the cDNA sequence. Moreover, amplification of a region spanning a large human intron (e.g., 100 kb) is highly inefficient and not feasible for this project. If students identify a single exon sequence, however, they can design primers within this region and avoid any potential complications by introns.

Students search Gene at the NCBI Web site to ultimately obtain an exon sequence from their gene. To do this, they type in their official gene symbol that they obtained from OMIM (e.g., BRCA1). There may be more than one result when they search Gene if the sequence has been identified in multiple organisms, so students should locate the result for *Homo sapiens* (Hs). Click on the official gene symbol to be connected to the Entrez Gene page. The search results will provide a wealth of information on the gene and its protein product. In addition, there are numerous links on the right; look for Evidence Viewer. Click on this link, and the students can locate the exon sequences (or an intron, if desired) for their gene. In many cases, known polymorphisms will be displayed below the sequence, giving students a direct molecular view into human genetic variation.

Students ideally should plan to work from an exon that is at least 400 bp to facilitate analysis of their PCR product in later steps, but shorter exons can also work. Once students have identified such an exon sequence, they should print it or copy it into a text document for later use in PCR primer design.

Having chosen a gene for further study, students may wish to obtain additional information on their gene or gene product, depending on the level of the student. In our upper division course, students investigated such aspects as the molecular and cellular function of the gene, the cytogenetic map position, any potential sequence variants and known contributions to the trait or disease, size of the gene and/or coding region, and homologous genes identified in animal genetic model systems. In addition to the background literature on the trait or disease obtained from OMIM, our students used PubMed on the NCBI Web site to obtain primary research articles. This background research was used for a later oral presentation about their project to their peers.

Table 2. The Project at a Glance

Weeks 1-2	Search OMIM at NCBI and identify a gene of interest to you.
Week 3	Using NCBI, find an exon within your favorite gene. Design outer and semi-nested PCR primers within this exon and order them online.
Week 4	Isolate genomic DNA from your cheek cells. Set up PCR using the human VNTR locus as control.
Week 5	Analyze PCR using the human VNTR locus by electrophoresis. Set up PCR using outer primers to gene of interest, ordered online.
Week 6	Analyze PCR using outer primers by electrophoresis. Set up PCR using semi-nested primers, ordered online.
Weeks 7-8	Analyze PCR using semi-nested primers by electrophoresis. Prepare and communicate your findings.

Gathering the Gear: Primers

With exon sequence in hand, students are ready to design their PCR primers. Although PCR reactions using specific primers against total human genomic DNA as template usually yield sufficient product to be visualized by conventional agarose electrophoresis, a second, semi-nested PCR reaction substantially enhances the amplification if input template DNA is limited or of poor quality (Honda et al., 1995), which might be the case with some student samples. Moreover, semi-nested analysis provides verification that the original PCR product was indeed specific for the gene of interest, and gives students a further opportunity to demonstrate their understanding and proficiency of PCR mechanics.

The semi-nested PCR approach requires two rounds of PCR amplification and three primers: two forward and one reverse (Figure 1). In the first PCR reaction, the outer primer pair (FP1 and RP3) is used against the student's genomic DNA as a template for amplification. The product that is obtained from this first reaction is then used as the template in the second, semi-nested PCR reaction. In this second reaction, the semi-nested primer pair (FP2 and RP3) is used. The product obtained from the second PCR reaction is predictably shorter than the first.

Although online primer design algorithms are available (e.g., Whitehead Institute for Biomedical Research: http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi), we opted for students to design their primers "by hand," facilitating their understanding of the process and enhancing their ownership. All student-designed primers resulted in specific product of the predicted size. To design

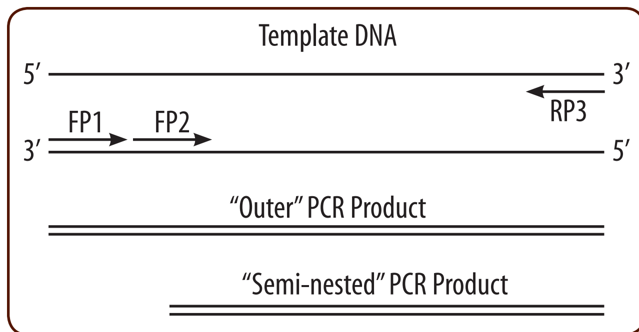

Figure 1. The design of semi-nested primer pairs.
Primer pairs are used in two sequential PCR reactions. The reverse primer (RP_3) is first matched with the outer forward primer (FP_1) and later with the inner, nested forward primer (FP_2) for the second reaction. The semi-nested PCR product is predictably shorter than the first product made from the outer primers.

5′CGAATCCCAGTGGAGGGGACCCTTTTACTTGCCCTGAACATACACAT**GCTG**
GGCCATTGTGATTGAAGTCTTCTAACTCTGTCTCAGTTTTCACTGTC
GACATTTTCCTTTTTCTAATAAAAATGTACCAAATCC**CTGGGGTAAAAG**
CTAGGGTAAGGTAAAGGATAGACTCACATTTACAAGTAGTGAAGGTCCA
AGAGTTCTAAATACAGGAAATTTCTTAGGAACTCAAATAAAATGCCCCACATTTT
ACTACAGTAAATGGCAGTGTTTTTATGACTTTTATACTATTTCTTTATGGTCGATAT
ACAATTGATTTTTTAAAATAATAGCAGATTTCTTGCTTCATATGACAAAGCCTCAA
TTACTAATTGTAAAAACTGAACTATTCCCAGAATCATGTTCAAAAAATCTGTAATT
TTTGCTGATGAAAGTGCTTCATTGACTAAACAGTATTAGTTTGTGGCTATAAATGA
TTATTTAGATGATGACTGAAAATGTGTATAAAGTAATTAAAAGTAATATGGTGGC
TTTAAGTGTAGAGATGGGATGGCAAATGCTGTGAATGCAGAATGTAAAATTGGT
AACTAAGAAATGGCACAAACACCTTAAGCAATATATTTTCCTAGTAGATATATATA
TACACATACATATATACACATATACAAATGTATATTTTTGCAAAATTGTTTTCAATC
TAGAACTTTTCTATTAACTACCATGTCTTAAAATCAAGTCTATAATCCTAGCATTAGT
TTAATATTTTGAATATGTAAAGACCTGTGTTAATGCTTTGTTAATGCTTTTCCCACTC
TCATTTGTTAATGCTTTCCCA***CTCTCAGGGGAAGGATTTGCA***TTTT
GAGCTTTATCTCTAAAT-**3′**

Figure 2. An example of an exon with primer regions chosen by a student.
This is the DNA sequence corresponding to the ninth exon of the ADH1B gene. The orientation of published sequences is rarely indicated, but here we have indicated the 5′ left and 3′ right convention. The sequences used for primer design are underlined. Note that the reverse primer is the complement in the 5′-3′ orientation (italicized).
This is the DNA sequence corresponding to a portion of the ADH1B gene from NCBI.

Actual Student-Designed Primer Sequences:

ADH1B-FP1	5′- GCTGGGCCATTGTGATTGAAG –3′
ADH1B-FP2	5′- CTGGGGTAAAAGCTAGGGTAAG –3′
ADH1B-RP3	5′- TGCAAATCCTTCCCCTGAGAG –3′

effective PCR primers by hand, students must follow specific guidelines. Among the most critical are: distance between the primers in a pair, individual primer length, G/C content, melting temperature, and lack of self-complementarity leading to unwanted hairpin formation (White, 1997).

The outer primer pair should be separated by a minimum of 300 bp so that product can readily be distinguished from low molecular weight primers and artifacts on agarose gel electrophoresis. Since product yield will decrease with increasing distance, more than 700 bp or so should not separate primers. The inner primer can be made virtually anywhere, but we suggest a minimum of 100 bp from one outer primer to readily detect the size difference on the gel.

A good PCR primer for this project is 20-22 nucleotides, has a minimum G/C content of 50%, and has a G or C residue at the 3' end, proposed to stabilize the duplex DNA at the point of DNA synthesis. The melting temperature, T_m, in part dictates the appropriate annealing temperature in the PCR reaction, and can be approximated by the simple equation $T_m = 2\,(A+T) + 4\,(G+C)$. Thus, a 22 nucleotide primer having 50% G/C content will have approximate $T_m = 66^{\circ}C$. Both primers in a pair (e.g., FP1 and RP3) need to have T_ms that are closely matched (within 2-3°C) and in the range of 60-70°C. We found that an annealing temperature several degrees lower than the T_m resulted in a greater product yield for most student primer pairs. Finally, we had students visually inspect primer sequences for self-complementarity that could lead to problematic hairpin formation. We arbitrarily chose four nucleotides as the acceptable limit for self-complementarity. Although all these rules seem daunting at the onset, students have a great deal of flexibility within their exon sequence and primer sequences can be designed with relative ease (Figure 2).

Custom primers, or oligos, can be ordered online from a variety of vendors (we used Qiagen) and cost roughly $10 each plus shipping (Table 3). Primer sequences must be written in the 5'-3' orientation for ordering, including the reverse primer (RP3), which of course must be the complement of the given strand from the NCBI sequence. We suggest that the students read the primer sequences back to each other several times prior to clicking the “submit” button. A mistake here could jeopardize the downstream work. Primers from Qiagen usually arrive within three to four days by overnight courier.

Setting the Pitons: Genomic DNA & Primer Controls

Isolation of PCR-quality human genomic DNA is a simple procedure. By working with their own genomic DNA, students continue to deepen their personal connection to the project. We used the BuccalAmp™ DNA Extraction Kit (EpiCentre #BQ0901S), which takes about one hour and produces a highly reliable template for PCR reactions. Students use a swab to collect their own cheek cells and then liberate the genomic DNA at high temperatures in a PCR-compatible reagent. The genomic DNA samples can be stored at 4°C and used throughout the project until it is disposed of at the project’s completion. Students are informed that their DNA will not be used by anyone other than themselves, and that they will be responsible for its disposal.

Table 3. Vendors

Online custom ordered primers Qiagen http://www.qiagen.com/ 27220 Turnberry Lane, Suite 200 Valencia, CA 9135 1-800-426-8157
BuccalAmp™ DNA Extraction Kit (EpiCentre #BQ0901S) Epicentre www.epicentre.com/main.asp 726 Post Road Madison, WI 53713 1-800-284-8474
The human pMCT118 locus, a VNTR (Carolina Biological Supply #21-1506). Carolina Biological Supply Company www.carolina.com/general/company/Srv.asp 2700 York Road Burlington, NC 27215 1-800-334-5551
JumpStart RedTaq DNA Polymerase reagent (Sigma #D8187) Sigma-Aldrich Corporation www.sigma-aldrich.com/ PO BOX 14508 St Louis, MO 63178 1-800-325-3010

Outer Pair Experimental Reaction	
Student Genomic DNA	5 µl
Primer FP1	1 µl (50 µM)
Primer RP3	1 µl (50 µM)
PCR Reagent	20 µl
Nanopure Water	13 µl
Total =	**40** µl
Positive Control	
Student Genomic DNA	5 µl
pMCT118 primers	15 µl (sold at 0.25 µM)
PCR Reagent	20 µl
Total =	**40** µl
Negative Control	
Student Genomic DNA	0 µl
Primer FP1	1 µl
Primer RP3	1 µl
PCR Reagent	20 µl
Nanopure Water	18 µl
Total =	**40** µl

Figure 3. Setup of outer pair experimental and control PCR reactions.

In order to test (and possibly fine-tune) their PCR skills and verify the integrity of their genomic template DNA, students perform a preliminary PCR reaction using control primers to a human VNTR locus (Carolina Biological Supply #21-1506). The human pMCT118 locus varies between individuals in the number of tandem repeated sequences and therefore has been used extensively in forensic analysis. When the PCR products from many students are analyzed on a single gel, students immediately observe allelic differences between individuals. Consequently, this preliminary experiment, when performed as a class, provides a rich introduction to molecular genetic analysis and allelic polymorphism and serves as a useful positive control for individual students.

For this control, we use only the pMCT118 primers from Carolina (not the complete PCR kit) in a PCR reaction having the same reagents and conditions used for subsequent PCR reactions. In this way, variables are minimized and the pMCT118 primers can be used as a positive control for all subsequent PCR experiments. Such a positive control is useful in the event that student-designed primers fail to amplify the gene of interest. Using the pMCT118 primers, all students successfully obtained a PCR product using their genomic DNA as a template. Most student reactions yielded either one or two bands, as expected for homozygosity or heterozygosity at this locus, respectively (Figure 4). In other cases, additional bands were observed.

Reaching the Summit: The PCR Experiment

Student excitement about the project peaks at this stage, particularly since students are about to perform an experiment that has never been done by anyone previously. Although their particular gene may be fairly well-studied, it is highly unlikely that anyone has ever designed the identical primers to theirs, and certainly the experiment has never been performed on their own genomic DNA as a template. Students are reminded that this project is truly exploratory.

When the primers arrive from the manufacturer, dehydrated, the quantity is indicated in picomoles. Students calculate the volume of water needed to rehydrate each primer to a concentration of 50 µM. For example, 73,087 picomoles of primer should be rehydrated in 1.46 ml water.

When all the primers are rehydrated, students are ready to perform their first PCR reaction to identify their gene of interest using the outer primer pair. We used the JumpStart RedTaq DNA Polymerase reagent (Sigma #D8187) for all PCR reactions, which contains the Taq polymerase, dNTPs, buffer, $MgCl_2$, and the loading dye for subsequent gel electrophoresis (Figure 3). The product also utilizes a hot start reagent so that PCR reactions can be set up at room temperature without concern of false priming.

The positive control should yield the same amplification profile that was obtained earlier with the pMCT118 primers, indicating good student technique, integrity of genomic DNA and PCR reagent, and proper functioning of PCR instrument. No amplification should occur in the negative control, indicating that amplification is dependent on added template. The presence of a band in the negative control reaction suggests false priming or that contamination occurred somewhere in the process. In our class, 16/17 positive controls produced consistent PCR products, and in no case did product appear without the addition of genomic DNA.

Students in our laboratory set up their experimental reactions using four different annealing temperatures in a gradient PCR machine in order to optimize specificity and yield. They chose a range of annealing temperatures above and below the calculated T_m. Following an initial heating at 94˚C for 4 minutes, 30 cycles of the following are performed: 94˚C denaturation for 30 seconds, annealing (temperatures varied) for 30 seconds, and 72˚C synthesis for 30 seconds. When the cycles are complete, the reactions are held at 4˚C until the samples are removed for analysis. Students analyze a 10μl aliquot of their sample by electrophoresis and save the remainder for subsequent use in the semi-nested PCR reaction.

Students discovered that at lower annealing temperatures, the specificity was sometimes reduced, leading to multiple products (Figure 5, Lane 4). Although specificity was increased at higher annealing temperatures, the yield was substantially reduced (Figure 5, Lane 6). If it is not possible for students to optimize the annealing temperature, it is better to choose a lower annealing temperature to facilitate product formation. We suggest using an annealing temperature that is 5˚C below the calculated T_m, or about 62˚C.

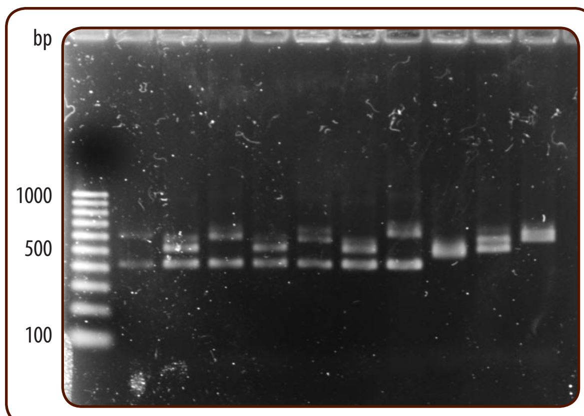

Figure 4. PCR results from one class using pMCT118 VNTR primers.
Each lane represents a different student genomic DNA used as a template. PCR products were separated by 1.5% agarose gel electrophoresis in 1XTBE buffer, with a size standard in the First Lane. Gels were stained with ethidium bromide and photographed on a BioRad VersaDoc.

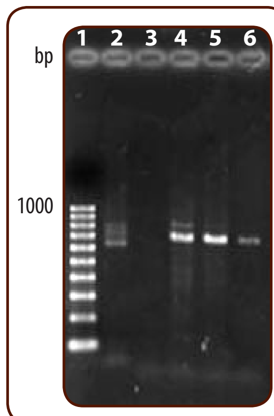

Figure 5. Temperature optimization using outer PCR primers.
Results from one student using primers designed to the MC1R gene. Lane 1: size standard; Lane 2: pMCT118 positive control; Lane 3: negative control; Lanes 4-6: outer primer experimental reactions. Annealing temperatures were 62˚C (Lane 4), 64˚C (Lane 5), 68˚C (Lane 6).

Semi-nested Experimental Reaction	
DNA from outer PCR reaction	1 μl
Primer FP2	1 μl
Primer RP3	1 μl
PCR Reagent	20 μl
Nanopure Water	17 μl
Total =	**40** μl

Figure 6. Setup of semi-nested PCR reaction.

Students can determine the sizes of their PCR products from the DNA standard and then compare this to the predicted size, obtained by counting the number of bases between the 5' ends of the primer pair in the published sequence. The size of the specific product should closely match what was predicted, and the specificity can be further verified by the subsequent semi-nested primer reaction.

In order to verify the specificity of their primers and further amplify any weak products, students perform the second, semi-nested PCR reaction (Figure 6). For this reaction, the PCR product from the previous reaction is used as the template for amplification with the inner primers (FP2 and RP3).

For a side-by-side comparison, students include the product from the original outer primer reaction as well as the positive and negative controls (Figure 7). Again, students should compare the size of the actual product obtained with that predicted for the semi-nested primer pair based on the published sequence. Nearly all students in the class obtained specific PCR product from the primers they designed using their own DNA as a template, verified by the semi-nested PCR reaction. In most cases, the semi-nested reaction produced substantially greater quantities of product than the original reaction (Figure 7, compare Lane 4 to Lane 3), although nonspecific bands often appeared.

The Media Reports

Students in the class were eager to discuss their projects and report their laboratory findings. We used two different formats for the communication of their projects: a formal oral presentation on the gene and corresponding trait or disease that was the foundation for the research, and a poster presentation of their specific laboratory results. The former took place early in the project before their laboratory work was completed. This gave students the opportunity to share their topics based on what they had learned from their literature research. We had students present background

information on their trait or disease as well as an analysis of a current primary research article on their gene. In the poster, students presented their laboratory findings in the context of how they had developed an assay to detect a specific gene in an individual's genome, potentially leading to a better understanding of how this gene contributes to the trait or disease if further analysis of allelic sequence variation in the population were to be conducted.

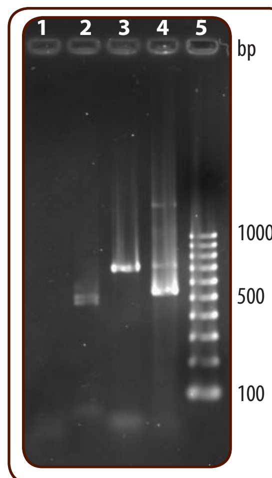

Figure 7. Semi-nested PCR reaction. Results from one student using primers designed to the BRCA2 gene. Lane 1: negative control; Lane 2: pMCT118 positive control; Lane 3: outer primer reaction; Lane 4: semi-nested primer reaction; Lane 5: size standard.

Conclusion

In conclusion, we have described an effective genomics research project that is driven by the students' personal interest, that provides a firsthand experience with the challenges and rewards of deriving information from the Human Genome Project, and that exposes students to basic techniques in biotechnology. Students reported in a course survey that the "personalization" of their genome trek, both in the choice of topic and in the use of their own DNA, substantially increased their motivation and curiosity.

Although this project was initially conducted in an upper-level Molecular Genetics course, it could be adapted to the introductory or high school level since the databases are accessible to anyone and the laboratory techniques are relatively straightforward. The laboratory project could be streamlined by performing the outer PCR experiment using a single annealing temperature and/or omitting the semi-nested experiment. In our class, students continued their project during several subsequent weeks by cloning and sequencing their PCR product, but this second stage of the research is beyond the scope of this report.

Acknowledgment

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