Human Single Nucleotide Polymorphism (SNP) Determination

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Abstract

SNPs are single nucleotide polymorphisms; they represent the simplest type of genetic variation between individuals. A SNP is a location in the genome where at least 1% of individuals in the population have a different nucleotide. In this laboratory exercise, students determine which allelic form of a particular SNP they have. Students isolate their own DNA, perform PCR to amplify a region surrounding this SNP, and use restriction fragment length polymorphism analysis to determine their genotype. This lab is suitable for introductory students and can illuminate PCR, gel electrophoresis, human genetics, and evolution (population data are available for this SNP).

Introduction

Objectives

- Students will understand the importance of SNPs as medical diagnostic indicators and their applicability to studies of human evolution.
- Students will gain an understanding of using PCR to amplify a specific segment of DNA.
- Students will enhance their understanding of DNA replication by learning about the technique of PCR.
- Students will use RFLP analysis to detect different forms of a SNP in their own DNA.

Background Information

Single nucleotide polymorphisms (SNPs) in the human genome have the potential to be powerful diagnostic indicators in human health, for predicting both disease susceptibility and drug effectiveness. SNPs are also being used in studies of human migration and evolution. This activity investigating one particular human SNP is broken up into two lab sessions as described below.

First lab session*:*

- Students isolate their own cheek cell DNA using a kit (60 minutes).
- Students amplify a particular 311 base pair segment of DNA which contains one SNP (an A/T pair on the chromosomes of some people and a G/C pair in other individuals). Students set up a PCR reaction using ready-made master mix in beads (15 minutes). The PCR reaction runs in a thermal cycler after the students leave the lab (this takes between 2 and 3 hours but does not need to be attended), and an instructor moves the tubes to the freezer until the next lab session.

Second lab session:

- Students perform a restriction enzyme digest of their PCR product (20 minutes, including a 5 minute incubation). The restriction enzyme leaves the A/T variant of the SNP intact but cuts the C/G variant, leaving fragments of 94 and 217 nucleotides.
- Students run both their cut and uncut product on a gel along with a known control (90 minutes, including 55 minutes to run the gel).

This activity is a part of an introductory-level undergraduate biology course on genes, evolution, and development. Students should understand basic DNA structure and base pairing. The activity is used to help students understand PCR and SNPs. It can also be used to introduce restriction enzymes and gel electrophoresis or to reinforce students' prior understanding of these tools. This lab would also be appropriate to a mid-level genetics course.

This lab is designed to support a SNP case study (Walser-Kuntz *et al.*, 2005) which is an important part of our course. In the case study, students role play positions on a bill concerning genetic privacy. The lab stands alone nicely, but can also be used with the case study to firmly connect lab and course work together with a thoughtful discussion of ethical implications of human SNP analysis. Students are excited by the relevance both these activities have to their lives. This lab activity ties into the course in numerous smaller ways as well:

- SNP selection. The SNP we chose to focus on in the lab is found in the intron region of a cell cycle protein we discuss in class.
- PCR and DNA replication. We have found that teaching the concept of PCR after we have introduced DNA replication, and asking students to compare and contrast the processes, cements their understanding of both: they start thinking less abstractly about what elements are required for DNA to be copied.
- Genetics and evolution. Throughout our particular course, we connect the fields of genetics and evolution. We strengthen that connection by pointing out that SNPs are used to study human evolutionary history. The Genographic Project <https://genographic.nationalgeographic.com> is collecting SNP information from multiple populations around the world (as well as volunteers, who can pay to get a DNA collection kit and participate). This information is being used to map early routes of human migration.
- Human biology. We often use examples from human biology in our course, and the SNP lab gives us a chance to put that into practice. SNPs are being extensively analyzed as markers for disease susceptibility. Particular SNPs have been associated with diseases, Crohn's Disease being one example (Libioulle *et al.*, 2007). In addition, broader patterns of SNPs (haplotypes) are being analyzed as possible indicators of disease susceptibility. The International HapMap Project <http://www.hapmap.org/thehapmap.html.en> is collecting data from multiple populations for this purpose. They published "A haplotype map of the human genome" in *Nature* (The International HapMap Consortium, 2005). A good description of their efforts and potential applications can be found in the accompanying summary "Genomics: Understanding human diversity" (Goldstein and Cavalleri, 2005). Students find it intrinsically interesting that SNPs can be used to help in the field of medicine. While we encourage this interest, we have been very careful in the lab activity to select a particular SNP which has no known link to disease, drug susceptibility, or any other trait. We want this lab to focus the students on the process of SNP determination, and to help them understand the possibilities SNP data contain, but we do not want to expose the students to information about themselves (or their peers) which would be better discussed with a genetic counselor.

We chose this SNP by searching the SNPper web site <snpper.chip.org>, which allows multiple types of searches for SNPs, including by gene name. We selected a SNP using three main criteria:

- The SNP was in a gene coding for a cell cycle protein we talked about in class (CDK3).
- The SNP was in an intron region with no known diagnostic value.
- The SNP had known allele frequencies in multiple populations, and the two alleles were both common. (We did not want a SNP with few heterozygotes, or with one allele only present in some identifiable subset of the students in our course.)

Student Outline

Project Goals, Week 1

For the first part of this two-week lab project, you will isolate your own DNA from cheek cells and set up a PCR reaction. The PCR will amplify a region of DNA which contains a SNP; the SNP we are investigating is from an intron in the *CDK3* gene. Next week, you will attempt to digest

your PCR product with a restriction enzyme which recognizes one variant of this SNP but not the other variant. You will gain an understanding of using PCR to amplify a specific segment of DNA and using RFLP to detect different forms of a SNP.

The *CDK3* SNP

SNPs are single nucleotide polymorphisms; they represent the simplest type of genetic variation between individuals. For additional background information about SNPs, see Appendix A. A SNP refers to a specific location in the genome where different people have been shown to have a different nucleotide. For example, the SNP we will begin investigating in lab this week is on chromosome 17, and it is located at base pair 71,511,227 (chromosome 17 has approximately 80,000,000 base pairs). Some people have an "A" in this position, and other people have a "G" in this position.

If more than one percent of the population has a different base at a particular location, that location is considered a SNP. If less than one percent of the population has a different base, it is considered a mutation.

The SNP we are investigating is located in intron 6 of the *CDK3* gene. The CDK3 protein is one specific type of CDK (cyclin dependent kinase); CDKs help regulate a cell's progression through the phases of the cell cycle. CDK function is dependent on binding to other proteins called cyclins. CDK3 is one of the CDKs involved in allowing the cell to move into the S phase of the cell cycle.

The particular polymorphism we are investigating is highly unlikely to have any phenotypic effect (think about why). We will refer to the two different variations at this position as the "A allele" and the "G allele."

Testing for the *CDK3* SNP

You will actually be testing your own DNA to see what your genotype at this SNP is. There will be four major steps in this project:

1. Isolate DNA from your cells.
2. Amplify the region of DNA containing the SNP.
3. Digest the DNA with a restriction enzyme.
4. Run the product(s) of the digest on a gel.

Today, you will perform the first two parts of the project; next week, you will complete the project.

DNA Isolation

You will use a kit to isolate your DNA. You will begin with cells you have scraped (gently!) from the inside of your cheeks. You will break these cells open (chemically) and use a spin column containing a special membrane which traps the DNA from the cells.

Amplification of the SNP Region Using PCR

Next, you will set up a Polymerase Chain Reaction (PCR) designed to amplify the region of DNA surrounding the SNP (Fig. 1). For more information about PCR, please refer to this excellent animation which explains the process: <http://www.dnalc.org/ddnalc/resources/pcr.html>. We need to use PCR so we will have many copies of the particular DNA we are interested in; we do not want to try to experiment with the entire chromosome (remember how big it was!). We need many copies in order to be able to visualize the DNA on a gel.

If we tried to load the entire genomic DNA on a gel, it would mostly be stuck in the well; chunks of DNA which had been inadvertently cut during the DNA isolation procedure might move through the gel and create a smear in the lane, but it would be quite difficult to interpret the gel.

We will use DNA primers which are 24 nucleotides long. Using what you know about PCR, what primers will we use to amplify this region of DNA? Write the sequence in the box below; indicate the 5' and 3' ends.

```
5' AAGGGCGTGT AGCACAGCAT AAAGACAGAG CTAACTCAAT GAGCGCCACT TTCACAGGGA
   AGATAAATAC TGCACTTATC CTGGGGGAGG CTTCCAGGTT GAACAATCAG TATACCCAAG
   CCAGTTGTGT ACAAAGGTCA GGAAAGAGAC CCTGGCCTTG GACTCAGAAA GTGCCAGGGT
   TATGTAAGAG GCTGGCTGAT GAGGGGAAAC TGTAGTCGGA GCAGCAGCTG GAGCCCACAT
   GCACCTACCA TGAGCAGGTC CCTGCCCTCT GGCTCCAGAT TGGGCACAAT CTCTTCCAGT
   CCCTTCCTGG T 3'
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Figure 1. The DNA Sequence we will amplify with PCR. Note that only one strand of DNA is shown. The SNP is indicated in a box; it is denoted as an "A" but could also be a "G." The spaces have been inserted only to make counting bases simpler; they have no function in the sequence other than to show the bases in groups of ten.

Primers for PCR:

DNA Digestion with the Restriction Enzyme Hpa II

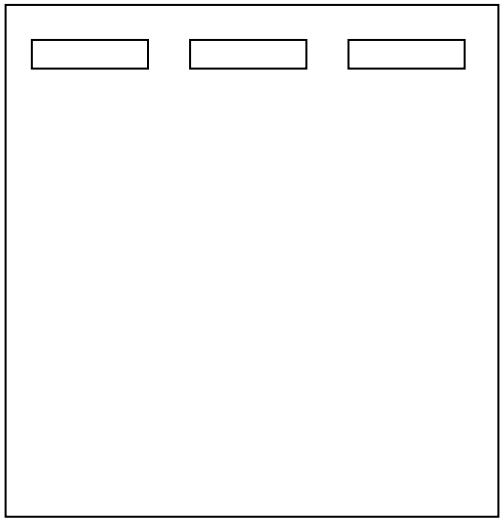
Next week, once you have plenty of copies of the region of DNA we're interested in, you will set up a restriction enzyme digest. How will this tell you what SNP you have? Restriction enzymes are quite specific about what DNA sequences they will cut. If the sequence is different, *even by one base*, then they will not cut. We have made use of this fact by choosing a restriction enzyme which will cut one variety of the SNP, but not the other. For additional background about restriction enzymes, see Appendix B.

The site which the restriction enzyme Hpa II recognizes is 5' CCGG 3'. Hpa II cuts the sugar-phosphate backbone between the two C's at the 5' end of the sequence: 5' C|CGG 3'. Using the position of the SNP in the sequence above, which allele will be cut by Hpa II? How many nucleotides will the resulting DNA fragments be? Write your answers below.

Allele	Will Hpa II cut?	Fragment Length(s)
A		
G		

Running the Digest Products on a Gel

By now you should have some idea of how a gel will help you determine your genotype with regard to this SNP. For more information on gel electrophoresis, see Appendix C. In the space below, draw a picture of a gel with three lanes: one lane for someone homozygous for the "A" allele, a "G" homozygote, and a heterozygote. Indicate (in nucleotides) what size you expect each band to represent.



This general type of analysis is called "RFLP," which stands for Restriction Fragment Length Polymorphism. In RFLP analysis, you cut DNA with restriction enzymes, determine the sizes of the resulting DNA fragments using a gel, and infer something about the original DNA based on the lengths of these fragments. In our case, we can determine the allele of our SNP just by looking at the size and number of fragments after cutting.

Experimental Procedures, Week 1

You should wear gloves for the procedures in lab today, in order to prevent contamination of your DNA sample. In addition, you should only work with your own DNA sample, not your lab partners' samples.

Cheek Cell Collection

1. Scrape a sterile swab against the inside of each cheek for approximately 20 seconds. Air-dry the swab for 15 minutes after collection, by placing the handle of the swab in a 15-mL plastic, conical tube at your bench. **Do not consume food or drink for 30 minutes before collection.**
2. While the swab is drying, label a microfuge tube with your initials and pipet 400 µL of PBS into the tube.
   PBS is phosphate-buffered saline.
3. When the swab has dried for 15 minutes, eject the tip containing your cheek cells into the PBS in the microfuge tube. To eject the tip, press the stem end toward the swab.

DNA Isolation

4. Add 20 µL of proteinase K to the microfuge tube.

Your lab instructor or TA will have the tube of Proteinase K. Take your tube to them for pipetting. Proteinase K is an enzyme which lyses (breaks open) the cells and breaks down proteins. Some of the proteins broken down by proteinase K include enzymes which might otherwise break down your DNA.

5. Add 400 µL Buffer AL and immediately mix by vortexing for 15 seconds. Make sure the lid on your tube is tightly closed before vortexing.
   This buffer contains a high salt concentration, which will help your DNA bind to the silica-gel-membrane of the spin column (below).
6. Incubate your tube at 56°C for 10 minutes.
   This incubation is to give the cells time to lyse.
7. Microfuge your tube briefly (around 15 seconds) to remove any drops of condensation from inside the lid. (Don't forget to balance your tube with someone else's tube.)
8. Add 400 µL of ethanol to the microfuge tube. Mix thoroughly by vortexing for 15 seconds. Briefly microfuge to prevent your solution from sticking to the inside of the lid.
9. Locate a DNeasy spin column and three collection tubes. Remove 700 µL of your sample and apply to the DNeasy spin column (while the column is in one of the collection tubes). Microfuge at 8000 rpm for 1 minute.
   These spin columns will only hold about 700 µL of fluid, so you will repeat this process in step 11 in order to move all your DNA onto a single spin column.
10. Remove the spin column and discard the flow-through by dumping the collection tube into your liquid waste beaker.
11. Place the spin column back into the same collection tube (which should now be empty). Add the remainder of your sample to the spin column. Microfuge at 8000 rpm for 1 minute.
    If you have any precipitate which has formed in your original tube by this stage, pipet all of this (along with the liquid) onto the spin column. The DNA from your cells is now binding to the silica-gel-membrane in the spin column.
12. Remove the spin column and discard both the flow-through and the collection tube into the appropriate waste beakers. Place the spin column in a new, clean collection tube.
13. Open the spin column and add 500 µL of Buffer AW1. Microfuge at 8000 rpm for 1 minute.
14. Discard the collection tube and the flow-through. Place the column in a new collection tube.
15. Open the spin column and add 500 µL of Buffer AW2. Close the cap and microfuge at maximum speed for 3 minutes. When removing your tube from the microfuge, be careful not to let the bottom of the spin column come into contact with the flow-through.
16. Remove the spin column from the collection tube and place the spin column in a labeled microfuge tube. Discard the collection tube.
17. Add 150 µL Buffer AE to the spin column. Let the column sit at room temperature for 1 minute. Then, microfuge for 1 minute at 8000 rpm. When you microfuge, the cap of your tube will be open; check the direction the microfuge rotor goes in, and make sure your cap is trailing the spin column and tube (ask your lab instructor or TA if you have questions). The DNA will be eluted (pulled off) the membrane and be in the microfuge tube.

Buffer AE has a very low salt concentration, which helps pull the DNA off the silica-gel-membrane of the spin column. Congratulations! You have now isolated your own DNA into a microfuge tube!

18. Store your DNA on ice until you are ready to set up the PCR reaction.

Prepare Your DNA for PCR

19. Locate a PCR tube containing a master mix bead.
    Marvel at the size of this tube. "Master mix" is the commonly used term for this subset of the PCR components: it contains TAQ polymerase, dNTPs, and buffer. In this case, the components have been dried into a bead, so you'll dissolve the bead into solution using your primers and DNA.
20. Pipet 24 µL of primers into your PCR tube.
    This tube contains a combination of the two primers necessary to replicate both strands of your DNA. The final concentration of each primer in the PCR tube will be 0.2 µM.
21. Very carefully, pipet 1 µL of your isolated cheek cell DNA into your PCR tube.
    You may use a P20 pipettor even though it is generally used only to 2 µL.
22. Mix the contents of your PCR tube well by gently flicking the bottom of the tube. Then, vortex the tube gently (at a vortex speed of 3) until the bead has gone completely into solution. The contents of the tube should be clear.
23. Microfuge the tube briefly (5 seconds) to get all the contents of the tube to the bottom.
    You will need to use adapters to microfuge these tiny tubes; your lab instructor and TA will have information about how to do this.
24. Follow the instructions of your lab instructor and TA for loading your sample into the PCR machine (called a "thermal cycler"); you can mark your tube, but you cannot rely completely on the mark staying on during the temperature cycles. Your instructor or TA will tell you how to record the location of your tube in the thermal cycler.
    Here is the cycle we will use for your samples:
    First, there is an initial step for 5 minutes at 95°C, to make sure all the DNA strands are disassociated to begin with.
    This is followed by 35 cycles with the following pattern:
        1 minute at 95°C, for "melting" the DNA
        1 minute at 55°C, for reannealing the DNA
        1 minute at 72°C, for building new DNA
    There is then a 10 minute stage at 72°C, to complete whatever building has been started.
    Finally, the tubes are chilled at 4°C until someone moves them to the freezer for longer term storage.
25. Your lab instructor or TA will return to lab after the PCR cycles are complete. They will store your PCR tubes in a box in the freezer.

Project Goals, Week 2

Last week in lab, you isolated your own DNA from cheek cells and set up a PCR reaction. The PCR amplified a region of DNA which contains a SNP; the SNP we are investigating is from an intron in the *CDK3* gene. This week, you will attempt to digest your PCR product with a restriction enzyme which recognizes one variant of this SNP but not the other variant. You will gain an

understanding of using PCR to amplify a specific segment of DNA and using RFLP to detect different forms of a SNP.

Additional Reading

Review the background information presented in last week's handout. If you didn't have time to answer the questions throughout the background information, you will find that helpful before lab this week.

Experimental Procedures, Week 2

Prepare to Run a Gel

1. Making an agarose solution:
   a. Each lab group should make a 40 mL solution of 2% agarose as described below.
   b. Calculate the amount of agarose you should weigh out to make your solution (you can do this before lab):
   40 mL 2% agarose:

   weigh out _____ g of agarose
   dilute in _____ mL of TBE buffer

   Remember, use the fact that 1 g is equal to 1 mL to do this calculation. Measuring volume to the nearest milliliter is fine when you are making agarose for a gel.
   c. Check your calculations with your lab instructor or your TA before proceeding.
   d. Using one of the balances in lab, carefully weigh out the appropriate amount of agarose, and mix it in the proper amount of TBE buffer (not water).
   It will not go into solution until the next step.
   e. Microwave your agarose solution, checking the flask often to prevent boiling over. Use the "hot hands" to handle the flasks, since the glass will get very hot.
   The agarose should be completely dissolved and the solution should be uniformly clear when you swirl it around.
   f. Take your flask back to your bench and let it cool until you can comfortably touch the glass flask.
   You can do step 3a while you are waiting.
2. Adding GelStar® DNA stain to your agarose:
   a. After your flask of agarose has cooled enough that you can touch it near the top comfortably, it is time to PUT ON GLOVES.
   Any time you work with your agarose or gel for the remainder of the lab, you will need to be wearing gloves.
   b. Ask your TA to add 4 µL of GelStar® stain to your flask. Wearing gloves, swirl the flask gently to mix, and proceed to the next step.
3. Casting a gel:
   a. Wearing gloves, raise the sliding gates of your gel-casting tray (Fig. 2) and fasten them in place by *gently* tightening the plastic screws at either end. Place the 8-well plastic comb into the slots nearest the end of the gel casting tray.

Place the prepared tray on a level, undisturbed location on your bench so that when you pour the liquid agarose solution into the gel-casting tray it can solidify unperturbed.

b. Wearing gloves, pour the agarose solution into the prepared casting tray, watching to make sure the agarose does not overflow. You may not need to use all the agarose you have prepared

If you pour the agarose while it is too hot, you can crack or warp the casting tray, which may cause leaking.

c. After pouring, check to make sure that there are no large bubbles in the molten agarose. If there are bubbles, use a clean pipet tip to move the bubbles to the side of the tray.

d. Leave the gel alone to harden for several minutes while you go on to the next section.

Restriction Enzyme Digest

4. Locate your PCR tube from last week, and set it out at your bench to thaw. Your lab group (three students) will need to thaw one tube of 10X restriction buffer ("10X RB").
5. Each person at your lab bench will set up a restriction enzyme digest with their own PCR product; in addition, as a group, you will set up one digest of cheek cell DNA from a PCR tube your TA set up last week.

   Your TA performed PCR using cheek cell DNA previously isolated from a heterozygous cheek cell donor; this sample will serve as a "known" lane on your gel.
6. Get a clean microfuge tube and label it with your name or initials, and some indication that you are digesting the DNA in the tube. Your lab group should label one tube for the sample your TA will share with you.
7. Pipet 8 µL of your PCR product into your microfuge tube. Someone in your lab group should pipet 8 µL of your TA's PCR sample into the proper labeled tube.

   Be very careful not to pipet more than 8 µL; you will need most of the remainder of your PCR product to run uncut on your gel.
8. Add 1 µL of 10X Restriction Buffer to your tube. Mix well by pipetting up and down. Someone in your group should repeat with the sample from your TA.

   You may use a P20 pipettor even though it is generally used only to 2 µL.
9. Your TA will add 1 µL of the restriction enzyme Hpa II to each of the microfuge tubes from your group.
10. Find a floater for your microfuge tubes, and put all four tubes from your group (one for each member of your group plus one tube with the TA's sample) in one floater. Incubate your tubes in the 37°C water bath for 5 minutes (longer is okay).

    DO NOT THROW AWAY YOUR PCR TUBE: you will run uncut DNA on your gel along with the product of your restriction enzyme digest.

Preparing Samples

11. Preparing your undigested samples:

    You can do this during the digest.

    a. Label a microfuge tube with your initials and indicate that it contains undigested DNA.

    b. Pipet 10 µL of your PCR product from your PCR tube into the microfuge tube.

       If you do not have 10µL left, add as much of your PCR product as you have.

c. Add 2 µL of loading dye to the microfuge tube. Mix thoroughly by pipetting up and down. Don't forget to use a clean, new tip for each tube.
Do not throw the stock tube of loading dye away when you clean up after this lab.

12. Preparing the marker lane:
a. Your lab group should acquire one tube of marker lane DNA from your lab instructor or TA.
This marker lane DNA is a 100 bp ladder. It contains DNA fragments of the following sizes: 1517 bp, 1200 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, and 100 bp. Using what you know about the SNP region we amplified with PCR and the location of the Hpa II cutting site, you should be able to predict where you might see bands with respect to the marker lane bands.

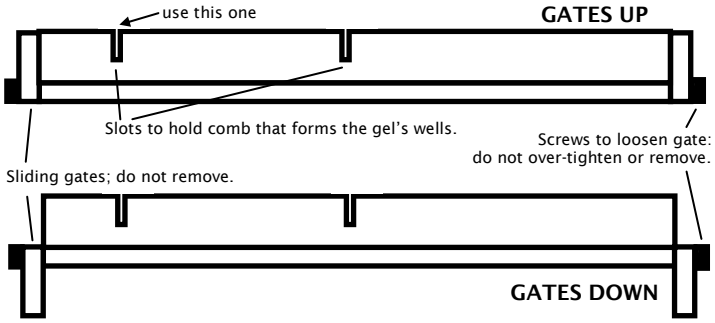


Figure 2. Gel casting tray, side view. Top: gates up. Bottom: gates down.

b. Add 2 µL of loading dye to the marker lane DNA tube. Mix thoroughly by pipetting up and down.

13. Preparing your digested samples:
a. After your restriction enzyme digest is complete, microfuge your tubes briefly (15 seconds) to get any condensation from the lid into the bottom of the tube.
b. Add 2 µL of loading dye to each tube of digested DNA (one tube for each member of your lab group, plus the tube containing your TA's sample).
You should now (assuming you have three members in your group) have eight tubes which contain DNA and loading dye: one digested tube for each group member, one undigested tube for each group member, one digested tube of sample from your TA, and one tube of marker lane DNA.

Loading and Running the Gel

14. Transferring your gel to the gel box:
a. Once your gel has solidified, remove the comb carefully.
b. Make sure the gates on your tray are down, and place the casting tray, still containing the gel, into the gel rig with the proper orientation: the comb end of the gel should be closest to the black (negative) electrode.
If there is already buffer in the rig, you can leave it there and re-use it (as long as the rig has been covered to prevent evaporation).
c. Add enough TBE buffer to completely cover the gel.
Do not fill the gel rig completely (it will run too slowly); just make sure the gel is completely submerged.

15. Load the contents of each tube into the gel. Be careful not to let any bubbles into the pipet tip when you are drawing the sample into the tip.
Check your tubes before loading: microfuge briefly (around 15 seconds) if necessary. The digest tubes may be particularly bubbly; tap them on the bench top to reduce the bubbles,

or try microfuging. You may not be able to get rid of all the bubbles; when you pull up the sample into your pipette, just try to avoid the bubbles which remain. In your notebook, make a record of which sample is loaded into which well.

16. When all of the samples have been loaded, re-check the orientation of the gel with respect to the electrical leads. Remember that DNA, having a net negative charge, will migrate toward the positive (red) electrode.
17. Placing the lid on the box connects the leads to the gel box. Connect the other ends of the leads to the power supply. Check that the power supply is set at 100-105 volts, and then turn on the "juice." The voltage will be applied to the gel for 55 minutes.

Viewing the Gel

18. When the gel has run for 55 minutes, disconnect the leads from the power supply and remove the lid from the gel rig.
19. Carefully, wearing gloves, remove your casting tray (still containing the gel) from the gel rig and set it on your benchtop.
20. Remove the gel from the casting tray and transfer it to the plastic sandwich container at your bench. Do not allow the gel to flip over as you make the transfer. Carry the gel over to the sink, and rinse it gently with distilled tap water (black knobs at the sinks) to remove any unbound stain from the gel. When this destaining has been completed, you will view your gel on the transilluminator (UV light box). Your TA or lab instructor will help you with this.
21. Ascertain whether your enzymes cut your DNA. How can you tell? How many fragments do you see for each person in your lab group?

Recording Your Data

22. Determine what genotype you are: AA, AG, or GG. Be sure to check your interpretation of the gel with your lab instructor or TA. Once you are sure what your genotype is, write this on a scrap of paper and put it in the envelope specified by your instructor or TA. Do not write your name on the paper.

    Class results will be compiled and presented to protect anonymity. You are not required to share your personal data with anyone else in lab (though you are free to do so if you wish).

Materials

Supplier addresses can be found in Appendix D. Additional instructions for the preparation of solutions can be found in Appendix E.

For the first lab session, "DNA Isolation and PCR"

For students in each lab section (20-24 students) to share

- 1 thermal cycler for doing PCR
- 1 waterbath set to 56 degrees C
- 2 microfuges which will run at 8000 rpm (1 microfuge would be okay)
- adaptors for spinning PCR tubes in the microfuge (or use 0.6 mL tubes with lids removed placed inside 1.6 mL tubes with lids removed)
- 1 box of gloves in each size (S, M, L)
- 4 vortexers
- 4 boxes each of stuffed/aerosol pipet tips, for P20 or P10 micropipets and for P200 micropipets (these will get used only for the final steps when students set up their PCR reactions)
- 1 DNeasy Blood and Tissue Kit per 50 DNA isolations (from QIAGEN, catalog number 69504)
- 1 extra bottle of Buffer AL for DNA extraction per 200 DNA isolations (from QIAGEN, catalog number 19075
- 1 box of PuReTaq Ready-To-Go PCR Beads per 96 PCR reactions (order enough to have students run a known sample of heterozygote DNA if you'd like) (from GE Healthcare, catalog number 27-9559-01)
- PCR tube labels (1 label per student, plus 4 extras for control DNA)
- 1 clean, used 96-well plate to store PCR tubes in after they are taken out of the thermal cycler (any PCR tube rack will do, though)

Per lab group (2-3 students)

- 2-3 sterile cheek swabs (Whatman OmniSwabs, available from VWR, catalog number 12000-611) (1 swab per student)
- 1 microfuge tube floater capable of holding 3-4 tubes (1 tube per student + 1 for heterozygote)
- 1 P1000 micropipet
- 1 P200 micropipet
- 1 P20 (or P10 if available) micropipet
- 1 box sterile yellow pipet tips
- 1 box sterile blue pipet tips
- 1 box lab tissues (like Kimwipes)
- 1 jar sterile 1.5 mL or 1.6 mL microfuge tubes (each student in the group needs a minimum of 2 microfuge tubes for the DNA isolation)
- 1 microfuge tube rack
- 2-3 test tubes no taller than 5 inches (we use used 15 mL conical plastic tubes, but you could substitute whatever you have that is approximately the right size--their cheek swabs just need a place to dry upright for 15 minutes)
- 1 rack to hold 15 mL conical plastic tubes (or other test tube as described above)
- 1 rack for PCR tubes
- 1 ice bucket

Aliquots for each group of 3 students

- 1.5 mL sterile PBS (see Appendix E)
- 1.5 mL 200 proof ethanol
- 1.5 mL Buffer AL (from QIAGEN kit above, plus extra needed (we use more than the kit calls for, in order to submerge the cheek swabs))
- 1.6 mL Buffer AW1 (from QIAGEN kit above)
- 1.6 mL Buffer AW2 (from QIAGEN kit above)
- 700 µL Buffer AE (from QIAGEN kit above)
- 85 µL primer stock (see notes below)

Other aliquots

- 1 tube Proteinase K per 50 PCR reactions (from QIAGEN kit above; we have the TA or lab instructor pipet this for the students as they need it, since the volume used is small)
- Heterozygote DNA: We identified a heterozygote for this SNP in our department and perform a DNA isolation prior to the lab sections. Then during each lab, we ask the TA's to set up four PCR reactions using this heterozygote DNA. The second week of labs, each lab group then digests a sample of this and runs a lane on their gel with the cut known heterozygote DNA as a control and additional marker lane for comparison. For the purpose of aliquotting, we provide each TA with enough heterozygote DNA to run a PCR reaction for each pair of lab groups (in our course, each lab section has 8 lab groups, so the TA runs 4 PCR reactions). Each PCR reaction requires 1 µL of DNA.

Ordering and preparing primers

We order the following two primers for this lab (from Eurofins MWG Operon):

- FORWARD: 5' AAGGGCGTGTAGCACAGCATAAAG 3'
- REVERSE: 5' ACCAGGAAGGGACTGGAAGAGATT 3'

We then dilute each of these to 5 µM stocks. For the lab, we mix the primers together with sterile water so that each primer has a concentration of 0.2083 µM. This means that the primers will be at a final concentration of 0.2 µM when we mix it with the DNA in the PCR tube.

For the second lab session, "Restriction Enzyme Digest and Gel Electrophoresis"

For students in each lab section (20-24 students) to share

- 1 waterbath set to 37 degrees C
- 2 microfuges just to spin fluid to the bottom of the tube (1 microfuge, or low-speed table-top models, would be okay)
- adaptors for spinning PCR tubes in the microfuge (or use 0.6 mL tubes with lids removed placed inside 1.6 mL tubes with lids removed)
- 1 box of gloves in each size (S, M, L)
- 1 large carboy of TBE for making and running gels (see Appendix E for recipe)
- 2 balances capable of weighing 0.8 g of agarose (1 balance would be okay)
- 2 spatulas or other implements to scoop and weigh agarose
- weigh boats for agarose (1 per lab group, or have students re-use)
- access to a microwave to melt agarose
- agarose near the balances; need a minimum of 0.8 g per gel (1 gel per lab group of 3 students)
- GelStar® DNA stain, available from Lonza, catalog number 50535; enough for each lab group to use 4 µL
- 1 UV transilluminator and camera for observing and photographing gels

Per lab group (2-3 students)

- 1 potholder or equivalent for handling hot flasks of agarose

- 1 microfuge tube floater capable of holding 4 tubes (1 tube per student plus 1 tube for heterozygote DNA)
- 1 50 mL or 100 mL graduated cylinder for measuring TBE used in making agarose
- 1 250 mL flask for making up 40 mL of agarose
- 1 agarose gel casting tray
- 1 gel comb with eight teeth to make an eight-well gel
- 1 gel rig
- 1 power supply for the gel rig
- 1 P20 (or P10 if available) micropipet
- 1 box sterile yellow pipet tips
- 1 square plastic sandwich box for transporting gel
- 1 box lab tissues (like Kimwipes)
- 1 jar sterile 1.5 mL or 1.6 mL microfuge tubes (each student in the group needs 2 microfuge tubes for their samples, plus 1 tube for the heterozygote DNA)
- 1 microfuge tube rack
- 1 rack for PCR tubes
- 1 ice bucket

Aliquots for each group of 3 students

- 8 µL of 100 bp DNA marker (available at NEB, catalog number N3231), which has been diluted with water from 0.5 mg/mL to 0.0625 mg/mL (an 8x dilution)
- 4 µL of FastDigest® Hpa II (available at Fermentas, catalog number FD0514); we usually put enough for the whole lab section in a tube (40 µL for 8 groups of three) and ask the TAs to dispense it
- 8 µL of 10X FastDigest® Buffer, which comes with the FastDigest® Hpa II enzyme
- 1 tube containing loading dye, at least 16 µL; for this gel we use 50% glycerol and 0.4% xylene cyanol in water (see Appendix E for recipe); bromophenol blue (which we otherwise use at 0.4% along with xylene cyanol in our loading dye) can get in the way of viewing the smaller DNA bands in which we're interested.

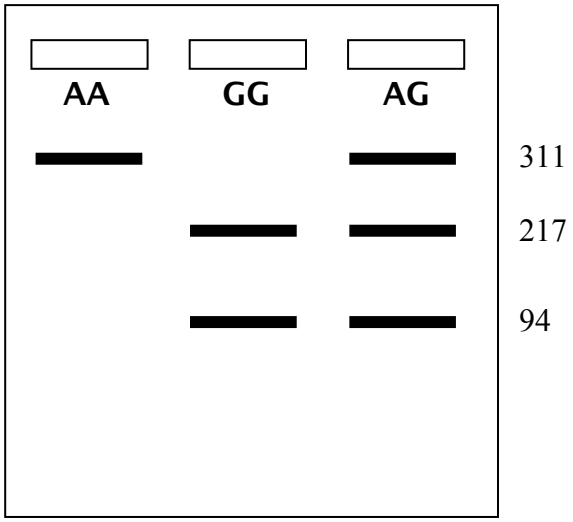
Notes for the Instructor

Answers to Questions in Student Handout

Primer sequences

Forward Primer: 5’ AAGGGCGTGTAGCACAGCATAAAG 3’
Reverse Primer: 5’ ACCAGGAAGGGACTGGAAGAGATT 3’

Allele	Will Hpa II cut?	Fragment Length(s)
A	no	311
G	yes	94 and 217 OR 96 and 215 (because of sticky ends)



Cheek cell DNA isolation

Do not let students eat or drink for 30 minutes before they begin the cheek cell isolation procedure; there is a 15 minute air-drying period right after they scrape their cheek cells when it will be fine for them to eat. Tell the students to swab gently—they do not need blood cells in their samples!

Every student should isolate their own DNA. Each stock tube of buffers contains enough for three students to isolate DNA.

There is only one tube of Proteinase K: TAs or instructors should dispense as students are ready, to be sure we have enough for all the students in the course. This tube can be kept at room temperature (look for the blue box).

PCR

The primers are in the freezer. The PCR master mix (includes dNTPs, TAQ, and buffer) is already in the PCR tubes, in the form of a dehydrated bead in each tube. These PCR tubes (with beads) are at room temperature; have students check to make sure there is a bead present before using the PCR tube.

Warn the students that the PCR tubes are specially designed with thin walls in order to prevent the tubes from insulating the reaction mixture. This makes the tubes pretty flimsy, so they should be quite careful.

You can have students can set up the PCR reactions on ice or using the racks at their bench—each lab table will have a rack with smallish holes for the PCR tubes (normal microfuge tube rack holes are too large). Students should pipet the primers and their DNA into the PCR tubes using the special pipet tips which are stuffed with cotton; these tips are expensive, so there will be one box for P20 tips and one box for P200 tips at each lab bench. Groups across the bench will need to share, and they should NOT use these tips for the rest of the lab protocol.

TAs: please set up FOUR additional PCR reactions using DNA which has already been isolated. Follow the set-up instructions in the lab manual. You can find the DNA in the same box which contains the primers (labeled “DNA for TA’s”). Next week, students in your lab section will cut & run this DNA in a lane on their gel so they have at least one sample that we know should work and give them heterozygous results.

Using the PCR Machine

When you set up the PCR tubes in the PCR machine, ask your students to do two things:

1. Print (neatly) their names in the appropriate circle on the "PCR Machine Sign Up Sheet" (see Appendix F) for your lab section.
2. After they have done that, they should print their name on a PCR tube label along with the row & column designation corresponding to where they signed the PCR Machine Sign Up Sheet. These labels should stay on the label sheet for now.

To run the PCR Machine: (This will need to be modified for your thermal cycler. The temperature profile we use is available in the student lab handout.)

1. Make sure all the tubes from your lab section are in the machine. Close the lid of the machine all the way, including bringing the arm forward to lock the lid down.
2. Turn on the power switch on the back right side of the PCR machine.
3. Go to "USER" (push the F5 button).
4. Arrow down to "bio125."
5. Press "ACCEPT" (F1).
6. Press "RUN" (F1).
7. Arrow down to "bio125-beads1."

   To show students what the cycles look like, you can press "VIEW" (F2) before going on.
8. Press "START" (F1).
9. Enter 25 μL as the volume of the reaction, using the number pad keys.
10. Press "START" (F1).

After running the PCR machine, someone (TA or instructor) should come back and do the following:

1. Remove the PCR tubes from the machine, and as you do so, wrap the appropriate label around each PCR tube (e.g. the tube coming out of the top left-hand corner should get the "A1" label). The labels may need to stick out like flags in order to be read later.
2. Put the PCR tubes into the cardboard box labeled for your lab section. These boxes contain 96-well plates, which can be used as PCR tube racks; it helps if you can put the tubes in the box in the same arrangement as in the PCR machine.
3. Put the cardboard box into the freezer.

Restriction Enzyme Digest

TAs/instructors should be prepared to aliquot the HpaII into each student's microfuge tube for the digest. Be sure to put the pipet tip into the bottom of the student's tube, and pipet up and down to ensure mixing. This is the best way to ensure a good, complete digest. Use a clean tip for each student.

Gel Electrophoresis

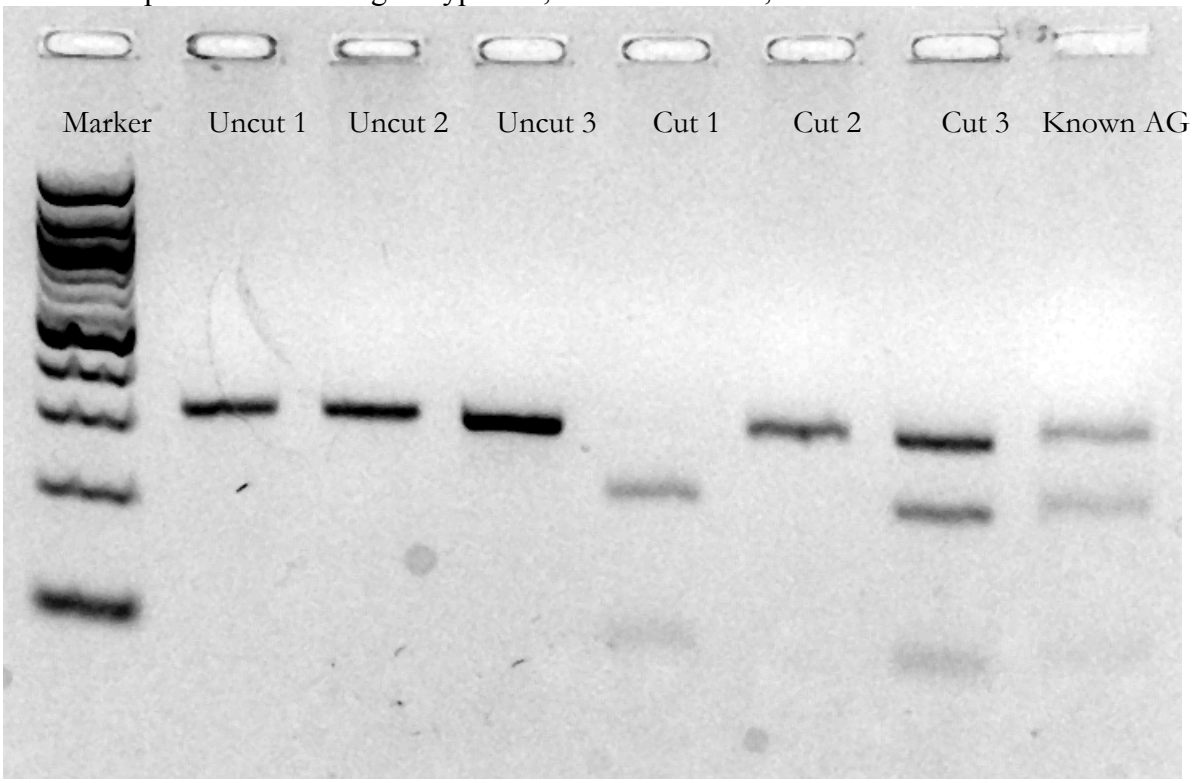
TAs/instructors should add the GelStar® DNA stain to each group's molten agarose. Be aware that the GelStar® may refreeze on ice, so check it periodically and re-thaw as needed.

Interpreting Results

The gels are usually very straightforward to interpret. Talk to the students about what an incompletely digested sample looks like, compared to a heterozygote (AG) sample. In a true heterozygote, the band closest to the well will be the brightest, and the band furthest from the well will be the faintest. This is because more of the GelStar® can bind to longer pieces of DNA. If the top band (nearest the well) is fainter than the band below it, this is a good indication that the digest was incomplete for some reason, and that if the digest had gone to completion, all the DNA would have been cut (a GG homozygote). We have not had difficulty in the past with partial cutting.

Sample Gel

Below are actual student data from Bio 125, Carleton College, Winter 2009 (IRB 08-09 045 sdeel). "Uncut" indicates undigested PCR product; "Cut" indicates PCR product which has been digested with HpaII. Student 1 is genotype GG, Student 2 is AA, and Student 3 is AG.



Published Data and Sample Class Data

Listed below are published data for our SNP, rs363796, as of October 2008. To further investigate this SNP, you can search <http://www.ncbi.nlm.nih.gov/>. Choose the "SNP" database and type "rs363796" into the search box. Each line represents the results of a different study; "Individual Group" indicates broadly who was in the study. "Chrom. Sample Cnt." gives the sample size, and is double the number of individuals sampled. The other numbers are the proportion of individuals in the study with that genotype or allele. According to the NCBI database, the A allele is thought to be the ancestral allele.

Individual Group	Chromosome Sample Count	A/A	A/G	G/G	A	G
Nigerian (Yoruba)	120	0.950	0.050	0	0.975	0.025
African American	90				0.900	0.100
African American	124	0.758	0.194	0.048	0.855	0.145
European	120	0.200	0.450	0.350	0.425	0.575
Caucasian	92				0.420	0.580
Han Chinese	90	0.133	0.400	0.467	0.333	0.667
Japanese	90	0.133	0.356	0.511	0.311	0.689
Japanese	1464				0.327	0.673
Global	178	0.191	0.461	0.348	0.421	0.579
Unknown	54	0.111	0.481	0.407	0.352	0.648

The following data are actual, unpublished student data from Bio 125, Carleton College, Winter 2009 (IRB 08-09 045 sdeel). Out of 72 students, 63 had interpretable results on their gels. At the ABLE conference in Delaware in 2009, all 21 participants had interpretable results.

Individual Group	Chromosome Sample Count	A/A	A/G	G/G	A	G
Bio 125 Winter 2009	126	0.175	0.365	0.460	0.357	0.643

A geographic representation of published results for this SNP is available through the Human Genome Project Diversity Selection Browser <http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP/>. If you type "rs363796" into the search box, it will show up under the "Genotyped SNPs" category; clicking the yellow triangle next to the SNP's number will link to a map showing allele frequencies (in pie chart form) distributed across a map of the world.

Acknowledgements

Special thanks to Julia VanderMeer, Lydia McClure, and Heidi Mullen for their work developing the lab protocol. I appreciate the comments from ABLE attendees, and in particular would like to thank Michael Stone. He suggested using the FastDigest restriction enzyme, which saves students 45 minutes of waiting time in lab; we tested it in October 2009 and it worked very well. In addition, thanks to all the students in Bio 125 who agreed to share their results with us.

Literature Cited

Goldstein, D. B., and G. L. Cavalleri. 2005. Genomics: Understanding human diversity. Nature, 437:1241-1242.

Gore, R. 2004. Who were the Phoenicians? National Geographic Magazine, 206:26-49.

The International HapMap Consortium. 2005. A haplotype map of the human genome. Nature, 437:1299-1320.

Libioulle, C., E. Louis, S. Hansoul, C. Sandor, F. Farnir, *et al.* 2007. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. Public Library of Science Genetics, 3: e58.

<http://www.plosgenetics.org/article/info:doi/10.1371/journal.pgen.0030058> Accessed 12 March 2009.

Moody, G. 2004. Googling the genome. The Guardian, 15 April 2004:23. <http://www.guardian.co.uk/technology/2004/apr/15/genetics.science> Accessed 13 March 2009.

Walser-Kuntz, D., S. Deel, and S. Singer. 2005. "SNPs and snails and puppy dog tails, and that's what people are made of…" A case study on genome privacy. National Center for Case Study Teaching in Science. <http://www.sciencecases.org/snp/snp.asp> Accessed 12 March 2009.

About the Author

Sarah Deel received her B.A. in Biology from Grinnell College in 1991, and her M.S. in Zoology from Oregon State University in 1996. She is a Lecturer in Biology at Carleton College, where she coordinates and teaches introductory labs for "BIOL 125 Genes, Evolution, and Development." She is interested in methods of teaching introductory biology, particularly those which encourage traditionally underrepresented groups, such as women and minorities, to continue in biology.

APPENDIX A: SNP Background

Text used with permission from "'SNPs and snails and puppy dog tails, and that's what people are made of...' A Case Study in Genome Privacy" by Debby Walser-Kuntz, Sarah Deel, and Susan Singer, Carleton College; published by the National Center for Case Study Teaching in Science, University at Buffalo, State University of New York (Walser-Kuntz *et al.*, 2005).

What are SNPs?

SNP (pronounced "snip") stands for single nucleotide polymorphism. Polymorphism refers to the presence of more than one allele of a gene in a population. This allele must be present in more than 1% of the population to distinguish it from a mutation. A SNP is a specific type of allele caused by a small genetic change, or variation, that occurred generations ago within a DNA sequence. The replacement of one single nucleotide with any one of the other three nucleotides resulted in a SNP. A SNP is, therefore, the simplest kind of polymorphism because it involves only one nucleotide change.

The following is one example of a mutation that may have occurred over evolutionary time, persisted, and resulted in a SNP. Originally, one DNA segment on a chromosome reads GGTAAC. The replacement of the second G with a C created a novel DNA segment that reads GCTAAC. This variation is referred to as a G/C SNP. Each individual in the population inherits a version of the SNP on the chromosome donated from each parent. Therefore, each SNP variant that occurs at a particular site on a chromosome is shared by some fraction of the population.

There are probably five to 10 million SNPs in the human genome and it is estimated that about 60,000 of them are found within regions of DNA that code for proteins. Because codons are like words made of three nucleotides (or letters), a single nucleotide change in the DNA sequence of a chromosome alters the codon at that site. The new codon has the potential to direct the cell's machinery to add a different amino acid at this site during protein synthesis. The substituted amino acid may alter either the protein's stability or function. In this manner, SNPs may be responsible for many of the phenotypic differences between humans. The majority of SNPs, however, occur in noncoding regions of the DNA and are not responsible for any protein changes.

How Are SNPs Being Used?

SNPs are being identified that serve as genetic markers for disease. In order to establish a link between a SNP and a specific disease, the genomes of many different individuals need to be scanned for SNPs. Several SNPs are identified within the individual and a SNP profile is constructed. The identified SNPs are also recorded in web-based databases. To determine whether a particular SNP is associated with a disease, the frequency of the SNP pattern found in individuals affected with a disease is compared to the SNP pattern found in unaffected individuals. A SNP may confer disease susceptibility if one pattern is found to be significantly more common in the affected population than in the control group. In some cases, a disease-linked SNP has been identified and a screening test for the disease based on the SNP has been developed. Information about an individual's SNP profile may indicate whether one is at an increased risk, for example, of developing heart disease. The individual may then be able to modify their lifestyle or take medications to prevent the disease rather than waiting for symptoms to occur. However, a SNP profile could also identify other diseases, such as Huntington's, for which there is no effective prevention or treatment. It is important to note that although SNPs may serve as genetic markers for a disease, the majority are not responsible for causing the disease.

It may theoretically become possible to scan one's entire genome for all SNPs. A complete genome SNP profile could indicate a whole range of diseases to which one is predisposed. Currently, the cost of sequencing every individual's genome is prohibitive. However, as Glyn Moody, author of *Digital Code of Life: How Bioinformatics is Revolutionizing Science, Medicine, and Business*, writes, "with a dozen companies racing towards the goal of the sub-$1000 genome, the day when your DNA is sequenced and burnt on to a CD-ROM for roughly the cost of a conventional health checkup is not far off" (2004:23).

SNP profiles can be used in medicine beyond identifying disease risk. One hot area in pharmaceutical research is the design of personalized drug treatments based on a patient's SNP profile. SNP information could allow drug therapy to be customized. Individuals always vary in their response to medication both in terms of effectiveness and side effects. SNPs may provide information about the most appropriate drug to prescribe or the optimal dose. One example is the SNP that occurs in the morphine receptor. Individuals homozygous for one SNP allele are known to need much higher levels of morphine-derived pain relieving drugs. Development of a SNP screening test will allow treatment of those individuals with the appropriate dose of morphine.

In addition to medical uses, SNPs are proving useful in mapping the migrations of human populations. SNPs provide information about human evolution and the descent from ancestral populations (see, for example, "Who Were the Phoenicians?" in the October 2004 issue of National Geographic Magazine (Gore, 2004)).

APPENDIX B: Restriction Enzyme Background

In order to make new combinations of DNA (for example, if you wanted to stick your favorite gene into a plasmid), you need a tool to help you cut and paste DNA together. Luckily, there is a large class of enzymes called **restriction endonucleases**, or **restriction enzymes**. Each restriction enzyme recognizes a specific, short (typically 4-8 bp) sequence of DNA and makes a cut wherever that sequence is found. The resulting pieces of DNA are typically called "fragments." What makes these enzymes particularly useful is that the cut they make is often staggered, rather than blunt; on each side of the cut, it will leave one strand of the DNA a little longer than the other (the difference is typically just a handful of nucleotides). Think about what this means--the resulting ends of DNA are "sticky" because there are unpaired bases (Figure 1).

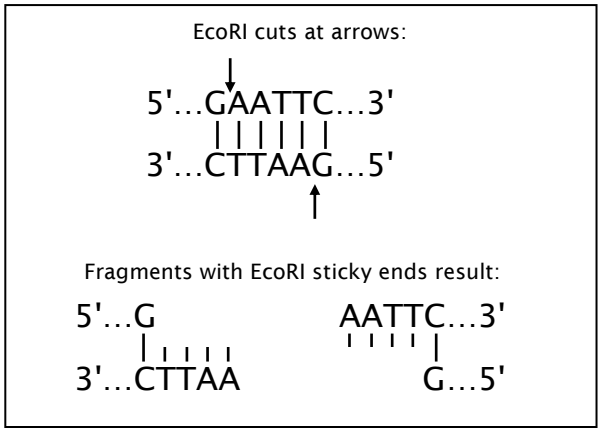


Figure 1. Example of restriction enzyme digestion.

Another very cool feature of restriction enzymes is that they cut at palindromic sequences. In English, a palindrome refers to a word or sentence that reads the same forwards and backwards: "dud" is a very short palindrome, and "A man, a plan, a canal, Panama!" is one of the most famous palindromes. (You can find huge archives with a search on the web.) An example of a palindromic DNA sequence (for the restriction enzyme Xho I) is read "CTCGAG" in the 5'-3' direction. Note that this strand does not read the same backwards (CTCGAG ≠ GAGCTC). The palindrome here lies in the fact that the *other,* complementary (3'-5') strand reads backwards: GAGCTC.

In other words, **both strands are identical in the 5'-3' direction**. So why is this cool? Beyond being interesting, it means that any Xho I sticky end can bind with any other Xho I sticky end. Therefore, if you cut on either side of your favorite gene with the same restriction enzyme you cut a plasmid with, the sticky ends will sometimes pair up correctly to splice your gene into the plasmid. Cool, no?

Because the sequence of bases that Xho I recognizes is not the same as that for a different restriction enzyme like EcoR I, the bases in their sticky ends will not pair with each other. In other words, the stickiness of the cut ends is very specific.

A note on restriction enzyme names: most are named after the scientific name of the species they were isolated from; Xho I is from *Xanthomonas holcicola*, and EcoR I is from *E. coli*. Pronunciation is a little funny and sometimes varies by lab; for example, in some labs, EcoR I is pronounced "eek oh are one." In other places, you may hear "eck oh are one."

APPENDIX C: Agarose Gel Electrophoresis Background

We want you to understand how gel electrophoresis works. Electrophoresis is an important general technique, used in many different circumstances (separation of DNA molecules is only one application), and we want you to get a sense of the circumstances when it would be a useful technique to use.

Gel electrophoresis is a technique used to separate various types of molecules based on size and charge. Today, you will use gel electrophoresis to separate pieces (commonly called "fragments") of DNA based on their size, which we'll refer to in terms of the number of base pairs.

The Gel

The "gel" part of gel electrophoresis is a gelatinous matrix (it might help to think of it as a mesh or a complex system of tunnels) which molecules can move through at different rates depending on their physical characteristics. In this lab, you'll be using 1% or 2% agarose to make your gel.

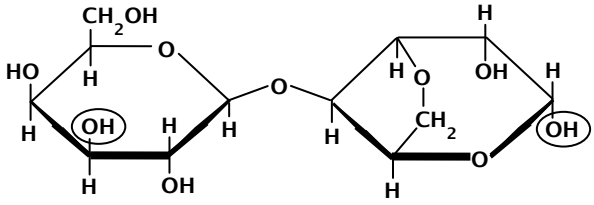


Figure 1. Repeating unit of agarose. Long chains are formed by linking units at the circled groups.

Agarose (Fig. 1) is a polysaccharide (for you inquisitive folks, agarose is a linear polymer of alternating D-galactose and 3,6-anhydro-L-galactose.). When solidified, agarose provides an ideal matrix for separating DNA molecules on the basis of their lengths.

Agarose comes from seaweed, where it helps prevent desiccation. It consists of long chains of sugars which pair up and wind around each other in a double helix. Generally, we make up a gel by boiling agarose in water to dissolve it (a lot like JELL-O®); as it cools, different chains of sugars crosslink, forming a network of sugar molecules in the gel. The gels we'll be making are flat and rectangular, or slab-like, in shape.

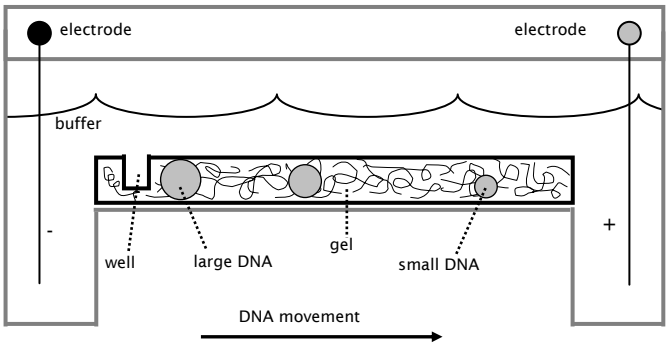


Figure 2. Gel box, side view. DNA (represented by circles) is loaded into the well of the gel at the end near the negative electrode. The DNA is pulled toward the positive electrode at the other end of the gel; how fast it moves depends on how big it is (bigger pieces of DNA make their way through the gel more slowly).

Getting DNA Into the Gel

There are wells at one end of the gel (Figs. 2 and 3); these small, rectangular depressions provide a place to introduce a sample. Glycerol is mixed with the DNA sample before adding it to the gel, which causes the sample to sink into the well (glycerol is about the same texture as thick honey).

Moving DNA Through the Gel

The wells in a gel are located near a negative electrode, and there is a positive electrode at the far end of the gel (Fig. 2). All DNA is negatively charged (remember all those oxygen atoms in the phosphate groups of the backbone?), so when a charge is applied to the system, the DNA moves towards the

positive electrode.

In order to apply a current to move the DNA in a gel, the gel is placed in a gel box under a liquid (1XTBE buffer in this exercise) that supplies salts (for example, Na^+ and Cl^-) that help to provide electrical current that will move the DNA through the gel. This process of applying a current to a gel is typically referred to as "running a gel."

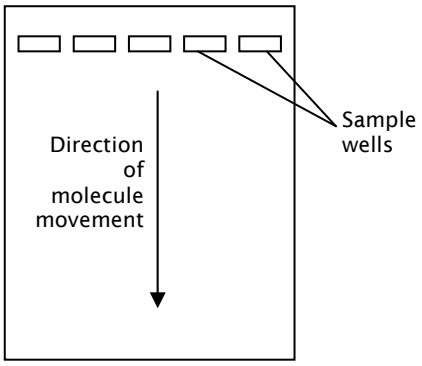


Figure 3. View of agarose gel from above, showing multiple wells. Once DNA is stained, bright bands will appear like ladder rungs, parallel to each well. Each rung will represent DNA of a certain size: rungs near the top represent large pieces of DNA and rungs near the bottom represent shorter bands of DNA.

Why DNA Fragments Separate Based on Size

In the absence of a supporting medium (like a gel), all DNA molecules move in an electric field at essentially the same rate. However, longer DNA fragments (that is, larger DNA molecules) become tangled in the agarose matrix and as a result move much more slowly than the shorter (smaller) fragments that are able to slither easily through the matrix. A 1.0% agarose gel can be used to separate DNA fragments that range in length from 300 bp (base pairs) to approximately 10 kb (10,000 base pairs). As a reference point, you have 3 billion base pairs of DNA divided amongst 46 chromosomes in each of your cells, and an *E. coli* bacterium contains 4.6 million base pairs.

Knowing When to Stop Running a Gel

How long you run a gel depends on many things, including what the gel is made of (and at what concentration) and the sizes of the DNA molecules you are interested in. Since very small DNA molecules separate from each other fairly quickly, it will not take as long to run a gel with them as it will if your DNA fragments are quite large.

The DNA is too small to see, so we can't monitor how fast it is moving directly. To prevent us from running all of the DNA off the end of the gel (and there's really nothing to stop it), we will add a blue dye to the DNA sample. (The dye mixture also contains glycerol, mentioned above.) This dye, xylene cyanol, moves through a 1% agarose gel with the same speed as a DNA molecule 4,000 base pairs long. Keep in mind that the xylene cyanol is *not* binding to the DNA--it is merely a useful marker while we run the gel.

Seeing the DNA

In order to visualize the DNA after the completion of the electrophoresis, we will need to stain it. we will use a fluorescent stain called GelStar® to stain our gels. GelStar® fluoresces when exposed to ultraviolet light. We will add this stain to our agarose solution before we pour our gel. After we run the gel, we'll then view it under ultraviolet light and photograph the stained gel. GelStar®, because it binds to DNA, is treated as a carcinogen. Be sure to wear gloves when working with the gels once the GelStar has been added.

In Fig. 3, note that the wells in a gel are positioned at the top; this is a standard orientation for reporting results. You may have seen photos of gels like this, with dark bands parallel to the wells. Bands representing larger DNA molecules will appear near the wells, and bands representing smaller DNA molecules will appear closer to the bottom of the gel.

Knowing How Big Your DNA Is

Since you are separating DNA based on size, and all pieces of DNA that are the same size migrate through the DNA at the same rate, it is useful to include a marker lane on your gel. A **marker lane**, or **ladder**, contains a set of DNA fragments which are of known sizes. You can actually graph the relationship between the distance a band of DNA moves from the well and the number of base pairs in that band of DNA. If you do this for each of the bands in the marker lane, you will see a relationship emerging. The relationship between size and distance is not linear; as the length of the DNA decreases, the distance traveled increases exponentially.

How does this relationship in the marker lane help us determine the size of DNA in other lanes? It should be easy for you to believe that if we have a band of DNA in one of our "unknown" lanes that is the same distance from its well as, say, an 8000 bp band in our marker lane is from its well, then the band of unknown DNA is also 8000 bp in length. Having a graphical relationship between the marker lane bands and distances allows us to determine the size of unknown bands which fall between two of the marker lane bands (for example, at 7500 bp).

Appendix D: Supplier Addresses

Fermentas
798 Cromwell Park Drive, Suites R-S
Glen Burnie, MD 21061-2596
Phone: 800-340-9026 Web: http://www.fermentas.com

GE Healthcare
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
Phone: 800-526-3593 Web: http://www.gelifesciences.com

Lonza
90 Boroline Road
Allendale, NJ 07401
Phone: 201-316-9200 Web: http://lonza.com

New England Biolabs (NEB)
240 County Road
Ipswich, MA 01938-2723
Phone: 800-632-5227 Web: http://www.neb.com

Eurofins MWG Operon
2211 Seminole Drive
Huntsville, AL 35805
Phone: 256-704-8200 Web: http://www.operon.com

Qiagen
27220 Turnberry Lane
Suite 200
Valencia, CA 91355
Phone: 800-426-8157 Web: http://www.qiagen.com

VWR
VWR International
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Appendix E: Instructions for Preparing Solutions

PBS (Phosphate Buffered Saline) (50 mM potassium phosphate, 150 mM NaCl, pH 7.2)
Recipe:

71.7 mL 0.5 M K_2HPO_4
28.3 mL 0.5 M KH_2PO_4
8.8 g NaCl
dilute to 1 L with water

We autoclave to allow longer term storage. Can be stored at room temperature or refrigerated.

10X TBE (Tris-Borate-EDTA)

We usually make up 1 L of 10X TBE and then dilute it down to a 1X working concentration in a large carboy. You'll need enough to make the 2% agarose gels and to use as a running buffer in your gel rigs. Here's the recipe for the 10X TBE:

Put 800 mL of Nanopure water and a stirbar in a beaker. Add the following ingredients:
100 g of Tris base (MW = 121.1)
55 g of Boric Acid (MW = 61.83)
10 g of EDTA, disodium salt, dihydrate (MW = 372.2)

Stir to dissolve. Transfer liquid to a 1 liter graduated cylinder, add nanopure water to bring to final volume of 1 liter. Pour into a screw-cap bottle, cap, and invert to mix. Dilute to 1X concentration before use. Store at room temperature.

Loading Dye (50% glycerol + 0.4% xylene cyanol in water)
Make 500 µL in a microfuge tube:

250 µL glycerol
2 mg xylene cyanol
250 µL water

Mix well (I'd guess vortexing might even be a good idea because of the glycerol). We usually make up larger batches. Can be stored long term in the freezer.

APPENDIX F: PCR Machine Sign Up Sheet

Print your name inside the circle corresponding to the placement of your PCR tube. (Top row = back of machine.)