Use PCR & a Single Hair To Produce a “DNA Fingerprint”

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As biology teachers, we are aware of the ever growing amount of information and new methods that we would like to incorporate into our classes. One area of particular growth has been in the field of DNA manipulations, or molecular biology. All of us want to share with our students these new and exciting techniques that are driving the revolution in biotechnology in addition to the fundamentals of population genetics, DNA structure, replication and mutations.

We have developed a laboratory procedure that draws upon all of these topics and is appropriate for introductory level college biology courses (for majors and/or nonmajors), as well as advanced high school biology classes. Using the protocols described in this article, in two lab periods of two hours each, every student extracts his or her own DNA from a single hair follicle, uses polymerase chain reaction (PCR) to amplify a polymorphic locus, electrophoreses the PCR products on an agarose gel, and visualizes the alleles to generate a “DNA fingerprint.” These single-locus fingerprints can be used to generate population genetics data or to solve a fabricated crime.

**Theoretical Background**

A few fundamental concepts of biology are critical for students to understand in order to appreciate this laboratory exercise. We use “DNA fingerprinting” as a motivational tool to make relatively abstract concepts seem more tangible, exciting and easier to learn. In this paper, we describe the basics of PCR, variable number of tandem repeats (VNTRs), and electrophoresis that are integral to this laboratory procedure.

Kary Mullis developed the Nobel Prize winning technique of PCR that has been described previously (Garrison & dePamphilis 1994; Mullis 1990). PCR allows you to start with one molecule of double-stranded DNA and replicate a selected portion of that DNA over a billion times within three hours. The portion that is replicated is defined by two primers, short stretches of single-stranded DNA, which are used to prime the DNA polymerase. The sequences of the two primers are complementary to opposite strands of the double helix and have their 3' ends facing towards each other (Figure 1A). The power behind PCR is the chain reaction component; replication is expanded exponentially because after each replication, the resulting DNA is unzipped, or denatured, by raising the temperature to 95°C. After a newly polymerized segment of DNA is denatured, it becomes the template for the next round of replication. The DNA polymerase used in this process can withstand such extreme temperatures because the enzyme was...
isolated from a thermophilic bacterium that lives in hot springs. Since
the cycle is repeated 30 times, the original copy of DNA will be re-
licated over one billion (2^{30}) times, which is enough DNA for visual-
ization on an agarose gel.

The evolutionary principle of variation within a population is a cornerstone in biology. This variation results from subtle differences in the DNA sequence in individuals of a given species, and the DNA of *Homo sapiens* is no exception. Variation commonly originates by the mistaken duplication of a small sequence of nucleotides when only one copy was present before replication. This results in a tandem repeat of the original sequence (Figure 1B). If this mistake occurs again in another round of replication, then three copies of a sequence will be in tandem. These tandem repeats are part of our chromosomes and as such, they will be inherited according to Mendelian genetics. Over the centuries, the number of tandem repeat units has increased, therefore each of us has inherited a variable number of tandem repeats (VNTRs) at many loci scattered throughout our genomes. A VNTR can be thought of as a locus with each particular number of repeated units being analogous to different alleles. Therefore, each human (except for identical twins) carries a unique combination of VNTRs; and these alleles can be used in population studies or to identify a particular individual.

PCR can be used to amplify portions of human DNA that are known to contain VNTRs. By the end of the first lab period, each student will have generated PCR fragments that contain his or her own VNTR alleles. Next, the students need to be able to see their DNA “fingerprint,” more appropriately referred to as a DNA profile. In order to visualize a DNA profile from a given locus, the DNA fragments need to be sepa-
rated according to their variable sizes using gel electrophoresis. The gel is a matrix of agarose that looks like white gelatin and is analogous to a microscopic thicket of small trees with lots of branches and twigs. An electric current is applied to the gel and all the negatively charged DNA molecules (negative because of all the phosphate groups) race towards the positive pole. But this is not a fair race. Returning to our analogy, the race is between children and adults of different sizes trying to run through the thicket of agarose trees and branches. Of course, it is easier for small children to run through narrow passages in the thicket and so they move the fastest, the oldest children and the smallest adults run through at a medium speed, and the largest adults come in last place. Like people running through a thicket, DNA molecules of different sizes migrate through the gel at different speeds, depending on their size. By this process, students can separate their different sized VNTR alleles with the smallest alleles migrating the fastest and the largest alleles migrating the slowest. Once the DNA is stained in the gel, it can be visualized to reveal the DNA profile of each student.

**Teaching Applications**

The results from this experiment can be integrated into a biology curriculum at several points throughout the semester plus the experiment exposes students to several techniques used in molecular biology. Below we have compiled a list of some areas of the curriculum that can be enhanced by this laboratory experiment.

1. Since PCR is based on DNA replication, you may want to discuss the mechanism of the DNA polymerase activity, the semiconservative nature of replication, the need for primers to initiate a DNA poly-

2. By their very existence, VNTRs are a good example of mutations that occur naturally in our DNA. From this starting point, you could discuss other mutations that might have physiological or evolution repercussions.

3. Mendelian genetics can be illustrated if students can build models in hair follicles of family members. They can observe the genotypes of their parents and determine which alleles they have inherited. (They should work even if the models were sent through mail, though we have tried this.)

4. If all the class data are compiled, they can be used to discuss specific areas of popula-
tion genetics, such as frequencies of different alleles and determination of Hardy-Weinberg equilibrium.

5. Biotechnology can be introduced by using this experiment as a launching pad for design and bioengineering, Jurassic Park etc. With the incessant ex-
peditions of the O.J. Simpson trial, the use of DNA profiles in criminal cases is an obvious application. An amazing example can be found in Ho-

6. We have our students calculate the molecular weights of all the alleles using the molecular weight markers as standards (see the accompanying paper by Williamson and Campbell for details). The D18S80 PCR product with z
repeat units is 142 base-pairs-long, so every repeat unit will add 16 base pairs to the VNTR. A student can use the estimated molecular weights of each band to determine how many repeat units are in each allele.

7. Another area ripe for discussion is the statistical basis for saying whether or not a given DNA sample is likely to belong to only one person. There is a general agreement that, in principle, DNA could be used to identify any given person if enough DNA is examined. However, how much is enough? Examining the VNTRs at a single locus is not sufficient to distinguish individuals within a large population since the number of possible alleles is small compared to the number of people. The number of VNTR loci examined is a minor problem compared to the statistical analysis of a given set of data. There are several different methods to analyze the data and these methods are eloquently discussed in the National Research Council’s *DNA Technology in Forensic Science* (1992).

8. In conjunction with this quantitative analysis, a fun approach is to fabricate a crime where the criminal has left behind a single hair follicle. The students are mentally prepared to find a guilty party; and in our experience, they always do, even if the hair did not come from someone in the class. This is a good opportunity to discuss objective evaluation of data, the handling of evidence, the number of loci necessary to identify the source of the DNA, and statistical methods to calculate the odds of this DNA profile belonging to more than one person.

### Timetable, Equipment & Supplies

Although PCR can be performed without a temperature cycler (Garrison & defPamphilis 1994), we strongly suggest that a temperature cycler be used since the manual procedure is too tedious. We have found automated PCR to be much more reproducible, convenient and faster, so the equipment listed in this article will be that needed for automated PCR. The only difference between the two methods is the temperature cycler itself, and once the temperature cycler is purchased (the cheapest one costs about $2500), the cost of a given experiment is identical between manual and automated PCR. If you buy a thermocycler, we recommend you buy one with a heated lid. This enables you to perform PCR on small volumes without having to use an oil overlay. We have found that students have a difficult time loading their samples when oil is used in the PCR. Although we have noticed that reactions conducted with oil overlays produced fewer extraneous bands and gave “cleaner” results, students find the samples too difficult to pipet. However, there is a simple trick to facilitate pipetting PCR products that have an oil overlay. Pipet an aliquot onto wax paper or parafilm before dispensing the sample into the well. The hydrophobic surface of the paper will reduce the amount of oil carried over to the gel which will make loading the well much easier.

### Student Timetable

**Day #1**

- Pluck hairs (5–10 minutes).
- Add hair to DNA extraction buffer (5 minutes).
- Incubate hair at 55°C (1 hour).
- Incubate hair at 95°C (10 minutes).
- Set up PCR mixtures in clean tubes (10 minutes).
- Allow PCR to automatically cycle 30 times (less than 3 hours).

**Day #2**

- Mix PCR sample with loading dye (5 minutes).
- Load gel (10 minutes).
- Electrophorese fragments (1–2 hours).
- Photograph gel (5–10 minutes).
- Discuss results.

### Equipment

- One temperature cycler (a programmable heating block)
- Micropipettors (variety of volumes)
- Microcentrifuge (optional, but convenient)
- Power supply for electrophoresis
- Electrophoresis chamber
- UV light box for detection of ethidium bromide stained DNA

(Warning: Ethidium bromide is a mutagen. See Potential Problems #9 for a more detailed discussion.)

- Polaroid camera (not required but recommended)
- Tweezers for hair plucking
- Ice bucket
- Scissors

### Consumable Supplies

- Marking pens
- Microcentrifuge tubes (500 microliter size)
- Agarose
- Ethidium bromide (or other DNA stain)
- Micropipet tips
- Gloves (Disposable are easier but reusable dishwashing gloves are OK.)
- Ice water bath or crushed ice
- Distilled water (Grocery store quality is acceptable.)
- Toothpicks
- PCR reagents (See below.)

### Collecting the Hair Sample

The most critical step is plucking a substantial hair follicle. We do this with our fingers and have found that most people can harvest good follicles if they pull between 3 and 20 hairs out at a time. For those with fragile hair, we use tweezers.
to grip the hair shaft at its base, pull quickly, and harvest the follicle. A “good” follicle is comprised of many cells and is easily identified because it has a black bulbous base that is sticky and a shiny white layer of cells surrounding the lowest portion of the hair shaft. Many follicle cells mean more template DNA, which means better student results, since the number of DNA molecules replicated equals the initial number of template molecules raised to the thirtieth power. We cut off most of the hair shaft and use a toothpick to transfer one follicle to a labeled microfuge tube since a good follicle will stick to the toothpick. If a student cannot get a “good” follicle, we have used as many as 10 “bad” follicles in one tube to extract as much DNA as possible.

Beginner’s Protocol

We have used two different loci: APOC2 and D15S80. We have found the APOC2 locus to be easier to execute successfully because it is technically easier to amplify than the other locus. Therefore, we suggest that you attempt PCR amplification with this locus first. APOC2 (Figure 2, Weber & May 1989) is a locus that encodes apolipoprotein C2 (which is involved in cholesterol transportation in the blood).

DNA Extraction (adapted from Erlich 1992):

1. Pluck a hair so that a follicle root is removed from your head.
2. Cut off most of the hair shaft but keep the follicle (~5 mm). Be careful, sometimes the follicle jumps away when you cut the hair.
3. With the heated lid disabled, incubate the follicle in 100 ul extraction buffer (which contains 6 ug of proteinase K) for 1 hour at 55°C, 10 minutes at 95°C, then cool the samples to room temperature.
4. When the DNA extraction cools, vortex and then set up a new 500 ul microfuge tube by adding the following:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>extracted DNA</td>
<td>7.5 ul</td>
<td>50 ng of DNA</td>
</tr>
<tr>
<td>reaction mixture</td>
<td>17.5 ul</td>
<td>1.8 ng/ml of DNA</td>
</tr>
</tbody>
</table>

**The reaction mixture contains the following cocktail:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>11.55 ul</td>
<td></td>
</tr>
<tr>
<td>10× PCR buffer</td>
<td>2.50 ul</td>
<td>0.75 mM MgCl2</td>
</tr>
<tr>
<td>(without Mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20× dNTPs</td>
<td>1.25 ul</td>
<td>200 uM each</td>
</tr>
<tr>
<td>#1 primer</td>
<td>1.00 ul</td>
<td>100 ng/ul primer</td>
</tr>
<tr>
<td>#2 primer</td>
<td>1.00 ul</td>
<td>100 ng/ul primer</td>
</tr>
<tr>
<td>Taq DNA polymerase</td>
<td>0.20 ul</td>
<td>1 unit</td>
</tr>
<tr>
<td>Total Volume</td>
<td>17.50 ul</td>
<td></td>
</tr>
</tbody>
</table>

5. Start the following PCR program with the heated lid enabled.

Step 1  5 min 95°C
Step 2  1 min 95°C
Step 3  1 min 55°C
Step 4  1 min 72°C
Step 5  Repeat Steps 2–4 29 more times
Step 6  Hold at 15°C

6. When the PCR is completed, the tubes are removed and stored at 4°C until the next lab meeting.

Second Lab Meeting

1. Add 2.5 ul of 10× loading dye to your PCR products and load 25 ul of each sample into a separate well.

2. Electrophorese 25 ul of the reactions + loading dye on 3.0% (w/v) agarose gel + 0.5× TBE + ethidium bromide (200 ng/ml) at 90 vol for 1.75 hours (see Potenti Problems section below).
**APOC2 Facts**
- 80% of the American population is heterozygous.
- 11 alleles have been published.
- The repeated sequence is two nucleotides long.
- The VNTR occurs within an intron.
- There are 30 dinucleotide repeats in the largest allele.
- Primers:
  - #1: 5' CATAGCGAGACTCCATCTCC 3'
  - #2: 5' GCCGAGGGCCAAAGATCATG 3'

**Advanced Protocol**

D1S80 (Figure 3; Nakamura et al. 1988; Budowle et al. 1991; Skow- asch et al. 1992) is located on the distal portion of the short arm of Chromosome 1. It is not a part of any gene, and is used in several countries for forensic analysis of DNA samples (Sajantila et al. 1992). This locus requires hotstart PCR which means that the Taq DNA polymerase is not added to the PCR mixture until it has been heated to 95°C. The hotstart is necessary because the D1S80 primers have a tendency to anneal to each other rather than the template while the mixture is heating up for the first time, which allows the DNA polymerase to generate "primer dimers" (Figure 4). If addition of the DNA polymerase is delayed, then inappropriately annealing primers are denatured as the kinetic energy increases. Therefore, no replication occurs until the temperature is lowered later in the procedure, allowing the primers to anneal to the proper portion of the template DNA. DMSO has been included in the reaction mixture to enhance the specificity of the primers (Filikin & Gelvin 1992).

**DNA Extraction**

Steps 1 through 3 are identical to the steps described above.

4. When the DNA extraction cools, set up a new 500-μl microfuge tube by adding the following:
**Reagents for Reagents & Equipment**

- **1 kb ladder**: Gibco BRL #1516 15-016 250 δg
- **Nonidet P-40**: Sigma N-6507
- **Twee 20**: Sigma P-1397
- **Protease K**: by Retrogen 0.2 umele scale, follow directions for dilution/quantification or by Univer DNA 50 umele scale is cheaper
- **Ehdi bromide**: Sigma #D-6556 (10 umele
- **Agorase (low EEO)**: Fisher BPI160-100
- **Gelatin**: Sigma G-9382

**Potential Problems**

In the process of designing and troubleshooting this protocol, we have learned a few ways to avoid mistakes that could result in poor results.

1. Make sure students are comfortable working with the micropipet. In our introductory labs, we have the students pipet only three solutions: 10 umele of the extraction buffer, 1 umele of extracted DNA, and 10 umele of the reaction buffer. We add the 0.2 umele of Taq DNA polymerase to the tubes, and make the common stock solutions cDNA extraction buffer with proteinase K and the reaction mixtures.

2. Make sure students use fresh tips for every volume they pipet. Students want to conserve tips, but this results in contaminated reagents.

3. Make sure the students do not add the reaction mixture to their entire 100 umele of extracted DNA, a common problem in our labs.

4. Make sure students get the best hair follicles possible. We offer to pluck hairs from those with brittle hair. We use the tweezers and grip a few hairs very close to the scalp, near the crown. A “good” follicle should be obvious from several feet away. African American hair follicles are more difficult to distinguish visually since the hair and its follicle are equally pigmented. However, “good
follies are always sticky. If a student cannot get a good follie, we have them use as many as 10 "bad" follies in one tube to extract as much DNA as possible.

5. Care should be taken by working close (about 5 cm) to the bench top when cutting off the shaft, since follies tend to jump when cut free. We find it helpful to cut dark hair over white paper and light colored hair over dark surfaces.

6. With the AP02C5 locus, make sure the lids of the microfuge tubes do not pop open during the first three cycles. This is especially a problem if any liquid is dispersed on the lip of the tube before it was closed. We periodically check the microfuge tubes during this time by quickly lifting the heated lid of the temperature chamber. We recommend that you set up a few extra tubes for two reasons. First, it ensures that every student will have some DNA to load. Second, if you are working on a fabricated crime, use the evidence DNA in your extra tubes and exchange one of the extra tubes with a student’s tube. This guarantees that at least one student will have identical bands to the evidence (Figure 2).

7. Run the gels at a higher voltage for less time, only if time is limited, since the resolution is reduced compared to gels run at lower voltage for longer times. You will get more distinct bands if the gels are run at 90 volts rather than higher voltages (e.g. 120 volts maximum).

8. During the first lab, we make a practice gel into which students load water mixed with 10X loading dye. This allows them to make mistakes when there is no harm; for the next lab, they get only one chance to load their PCR samples.

9. **WARNING**: Ethidium bromide is a mutagen and should be handled carefully while wearing gloves. Add the ethidium bromide to the agarose after it has been melted and allowed to cool—just warm enough to touch. Use the same concentration of ethidium bromide in the gel and in the running buffer. Differences in ethidium bromide concentration will result in gel that is half light and half dark, since the ethidium will electrophores through the gel in the opposite direction of the DNA.

There are two alternatives to ethidium bromide for staining DNA. Molecular Probes makes a fluorescent dye (SYBR Green I) that is much less mutagenic, though it requires a different filter for photography than the orange one used with ethidium bromide. Alternatively, you can use methylene blue which is non-toxic and can be seen with visible light, though it is a less sensitive staining method. Kits for DNA detection and photography can be purchased from many suppliers including Fisher, Carolina Biological, and Fotodyne.

**Conclusion**

This article presents a powerful way to use methods of molecular biology to define a DNA profile for every student in the class. The time required fits nicely into two standard laboratory periods. The protocol can be performed by introductory level college biology students with an 80–90% success rate. (A few students make mistakes that lead to negative results.) Although PCR is expensive, students find this lab to be very interesting and fun.

**Acknowledgments**

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**References**


