How-To-Do-It

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[1]) 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.

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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

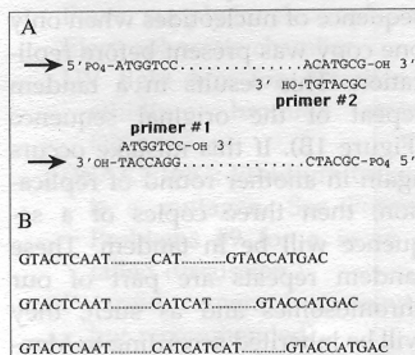

Figure 1. Panel A is a diagram illustrating how PCR primers are complementary to opposite strands of DNA and have their 3′ termini facing towards each other. The arrows point to the two strands of the original template DNA; the dots represent an unspecified length of DNA that is bounded by the primers. Primers are usually longer than those shown here. Panel B illustrates a repeat unit (CAT) mistakenly replicated two and three times which resulted in a variable number of tandem repeats (VNTR). Dots represent the connecting DNA of an unspecified length.

[1] PCR is patented by Hoffmann-La Roche and has been licensed to Promega Corporation.

isolated from a thermophilic bacterium that lives in hot springs. Since the cycle is repeated 30 times, the original copy of DNA will be replicated over one billion (2^{30}) times, which is enough DNA for visualization 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 *v*ariable *n*umber of *t*andem *r*epeats (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 separated 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 easiest 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 polymerase, and the 5′ and 3′ orie tions of DNA strands.
2. By their very existence, VN are a good example of m tions that occur naturally our DNA. From this star point, you could discuss o mutations that might h physiological or evolution repercussions.
3. Mendelian genetics can b lustrated if students can b in hair follicles of fa members. They can obse the genotypes of their par and determine which all they have inherited. (T should work even if the follicles were sent through mail, though we have tried this.)
4. If all the class data are c piled, they can be used to cuss specific areas of pop tion genetics, such as frequencies of different alle pedigree analysis, and the of the Hardy-Weinberg eq tion.
5. Biotechnology can be in duced by using this exp ment as a launching pad discuss forensic DNA u bioengineering, Jurassic P etc. With the incessant ex ple of the O.J. Simpson t the use of DNA profiles criminal cases is an obvi application. An amazing ample can be found in Ho meister et al. (1991) where analysis was conducted DNA isolated from skin c deposited on cigarette bu The possibilities for disc sion continue to increase are often quoted/misquo in daily newspapers.
6. We have our students cal late the molecular weights all the alleles using the mol ular weight markers as st dards (see the accompany paper by Williamson a Campbell for details). D1S80 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 & dePamphilis 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 D1S80. 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

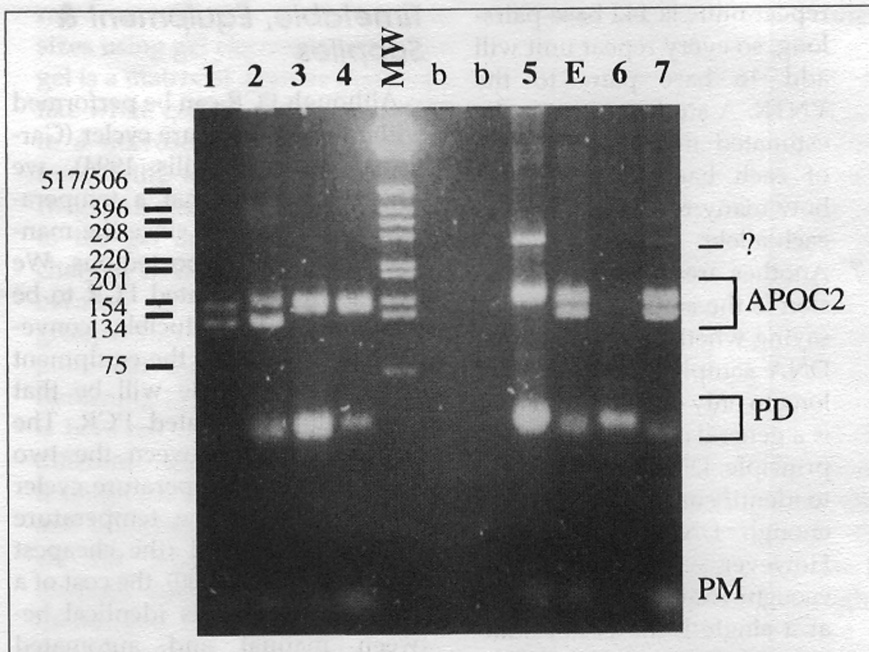

Figure 2. Photograph of a 3% agarose gel, stained with ethidium bromide, showin students' results for the APOC2 locus. Lanes: 1–7 are from seven different student PCRs; MW is the 1 kb molecular weight marker lane and the sizes of the majc bands are indicated in base pairs on the left side of the photograph; b indicate blank lanes; E is the evidence DNA we used for a fabricated crime. The sampl from Lane 7 evaporated when the lid popped open during the PCR, and an extr tube of evidence DNA was substituted for the student's sample (see Potenti Problem #6). Lane 6 produced very faint bands of the expected size and is a indication of a pipetting error or that a "bad" hair follicle was used. The labels o the right side of the photograph, beginning at the bottom, note the positions of: PM primer monomers; PD, primer dimers; APOC2, the APOC2 alleles; ?, a band foun in Lanes 5 and 7 that are unidentified. The white spots scattered across the gel ar dust and powder from our gloves.

new 500-ul microfuge tube by adding the following:

Reagent	*Volume*	*Final Concentration*
extracted DNA	7.5 *ul*	~50 ng of DNA
reaction mixture	17.5 *ul*	See below.**

**The reaction mixture contains the following cocktail:

Reagent	*Volume*	*Final Concentration*
H_2O	11.55 *ul*	
10× PCR buffer (without Mg)	2.50 *ul*	0.75 mM $MgCl_2$
20× dNTPs	1.25 *ul*	200 uM each
#1 primer	1.00 *ul*	100 ng primer
#2 primer	1.00 *ul*	100 ng primer
Taq DNA polymerase	0.20 *ul*	1 unit
Total Volume	17.50 *ul*	

PCR

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 complete the tubes are removed an stored at 4° C until the ne lab meeting.

Second Lab Meeting

1. Add 2.5 *ul* of 10× loading dy to your PCR products an load 25 *ul* of each sample in a separate well.
2. Electrophorese 25 *ul* of the r actions + loading dye on 3.0% (w/v) agarose gel i 0.5× TBE + ethidium br mide (200 ng/ml) at 90 vol for 1.75 hours (see Potenti Problems section below).

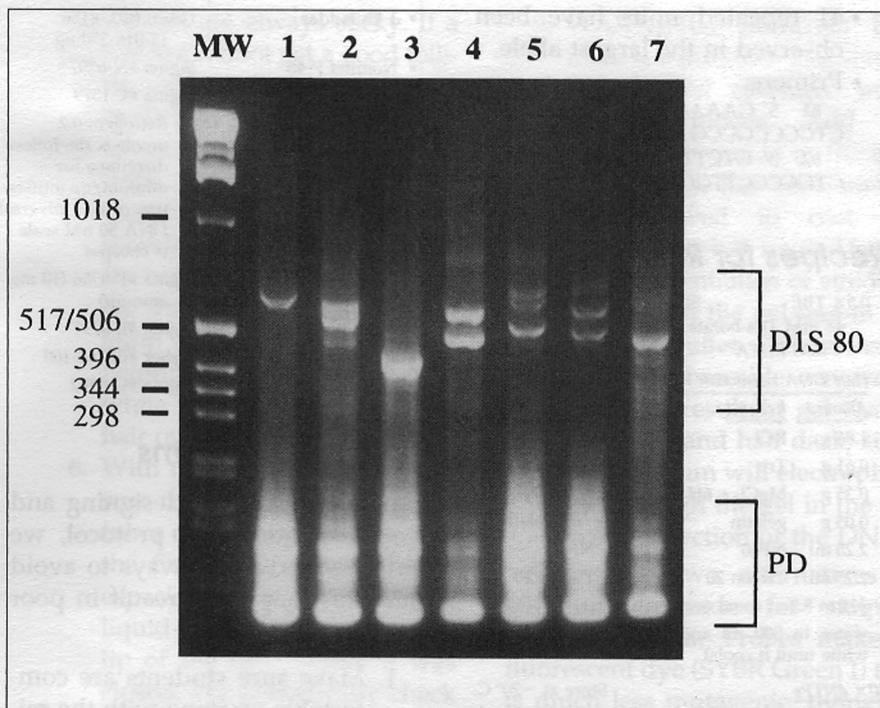

Figure 3. Photograph of a 1.5% agarose gel, stained with ethidium bromide, showing the results for the D1S80 locus using seven different sources of DNA (Lanes 1–7). Examples were chosen to illustrate the wide range of alleles found in a class of 17 students, with a bias to highlight apparently homozygous individuals. The DNA used in Lane 5 was extracted from five "bad" follicles (see Potential Problems #4). Labels: MW, the 1 kb molecular weight markers with the size in base pairs of relevant bands indicated to the left of the photograph; PD, primer dimers; D1S80, the D1S80 alleles.

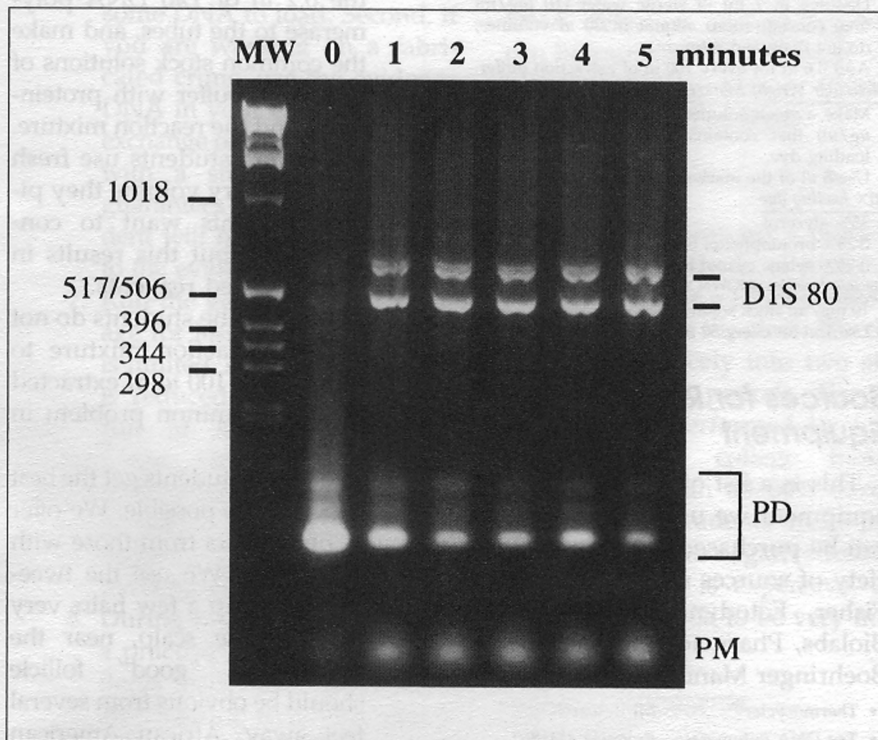

Figure 4. Photograph of a 1.5% agarose gel, stained with ethidium bromide, showing the need for hotstart PCR. One DNA sample was used in all lanes and the Taq DNA polymerase was added to the appropriate tube at the indicated minutes (from 0 to 5) after being heated to 95° C. The labels are the same as in Figure 3.

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′ GGGAGAGGGCAAAGATCGAT 3′

Advanced Protocol

D1S80 (Figure 3; Nakamura et al. 1988; Budowle et al. 1991; Skowasch 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-ul microfuge tube by adding the following:

Reagent	*Volume*	*Final Concentration*
extracted DNA	15.0 ul	~100 ng of DNA
reaction mixture	10.0 ul	See below.**

**The reaction mixture contains the following cocktail:

H_2O	4.00 ul	
10× PCR buffer (without Mg)	2.50 ul	1.5 mM $MgCl_2$
DMSO	1.25 ul	5% v/v
20× dNTP's	1.25 ul	200 uM each
#1 primer	0.50 ul	100 ng primer
#2 primer	0.50 ul	100 ng primer
Total Volume	10.00 ul	

PCR

5. To initiate hotstart PCR, denature the DNA by incubating the tubes for 5 minutes at 95° C (Step 1), maintain the tubes at 95° C while you add 0.2 *ul* Taq DNA polymerase to each tube. Do not allow the tubes to cool and do not take time to mix the reaction mixture after adding the Taq polymerase.
6. Resume the following PCR program with the heated lid enabled:

Step 2 1 min 95° C
Step 3 1 min 65° C
Step 4 1 min 72° C
Step 5 Repeat Steps 2–4 29 more times.
Step 6 Hold at 15° C.

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

Second Lab Meeting

Add 2.5 *ul* of the 10× loading dye to each tube and electrophorese the DNA on a 1.5% agarose gel using 0.5× TBE and 200 ng/ml ethidium bromide. We usually run these gels at 90 volts for 1–1.5 hours. The exact time and voltage will depend on the gel box configuration and appropriate conditions can be refined accordingly.

D1S80 Facts

- ≥80% of all populations tested are heterozygous.
- 28 alleles have been published.
- Repeat unit is 16 nucleotides long.
- PCR products range from 430 to 814 base-pairs-long.
- 41 repeated units have been observed in the largest allele.
- Primers:
  #1 5′ GAAACTGGCCTCCAAACA-CTGCCCGCCG 3′
  #2 5′ GTCTTGTTGGAGATGCAC-GTGCCCCTTGC 3′

Recipes for Reagents

0.5× TBE Store at room temperature.
45 mM Tris-borate pH 8.0
1 mM EDTA

Hair DNA Extraction Buffer Store at +4° C

Amount	*Reagent*	*Final Concentration*
1.86 g	KCl	50 mM KCl
0.61 g	Tris	10 mM Tris
0.25 g	$MgCl_2 \cdot 6H_2O$	2.5 mM $MgCl_2$
0.05 g	gelatin	0.1 mg/ml gelatin
2.25 ml	NP40	0.45% NP40
2.25 ml	Tween 20	0.45% Tween 20

pH to 8.3 at room temperature
water to 500 ml and autoclave (will look white until it cools)

20× dNTPs Store at −20° C.
Final concentration of this stock is 4 mM of each nucleotide.

Primers Store at −20° C.
dilute each primer to 200 ng/*ul*

Taq DNA polymerase Store at −20° C.
Do not alter concentration, use as supplied by manufacturer.
Very temperature sensitive, do not leave out except to pipet.

Proteinase K Store at −20° C.
Dissolve in 1 ml of sterile water (10 mg/ml final concentration) aliquot in 20 *ul* volumes; do not thaw and refreeze
Add 0.6 *ul* for every 100 *ul* of extraction buffer.

Molecular Weight Markers Store at −20° C.
Make a stock solution of the 1 kb ladder (0.1 *ug/ul*) that contains a 1× concentration of loading dye.
Use 5 *ul* of the marker per lane.

10× loading dye Store at +4° C.
35% glycerol
0.25% bromophenol blue
0.25% xylene cyanol FF

Ethidium bromide KNOWN MUTAGEN Store at +4° C.
10 mg/ml stock solution
Use 1 *ul* for every 50 ml of buffer or gel.

Sources for Reagents & Equipment

This is a list of the reagents and equipment we use, but substitutes can be purchased from a wide variety of sources such as: Amresco, Fisher, Fotodyne, New England Biolabs, Pharmacia, Promega, and Boehringer Mannheim.

- Thermocycler — MJ Research
- Taq DNA polymerase — Promega #M1862 (in storage buffer A)
- 10× reaction buffer — free from Promega
- Deoxynucleotides (dNTPs) — Promega #U1240 (40 *u*moles each)
- 1 kb ladder — Gibco BRL #156 15-016 250 *ug*
- Nonidet P-40 — Sigma #N-6507
- Tween 20 — Sigma #P-1379
- Primers — by Retrogen: 0.2 umole scale, follo directions for dilution/quantific tion or by Univer DNA 50 nM scale is cheaper
- Proteinase K — Sigma #P-6556 (10 m amount)
- Ethidium bromide — Sigma #E-8751
- Agarose (low EEO) — Fisher #BP160-100
- Gelatin — Sigma #G-9382

Potential Problems

In the process of designing and troubleshooting this protocol, w have learned a few ways to avoi mistakes that could result in poo results.

1. Make sure students are com fortable working with the mi cropipets. In our introductor labs, we have the students pi pet only three solutions: 10 *ul* of the extraction buffer, 1 *ul* of extracted DNA, and 10 *u* of the reaction buffer. We ad the 0.2 *ul* of Taq DNA poly merase to the tubes, and mak the common stock solutions o extraction buffer with protein ase K and the reaction mixture
2. Make sure students use fresh tips for every volume they pi pet. Students want to con serve tips, but this results i contaminated reagents.
3. Make sure the students do no add the reaction mixture t their entire 100 *ul* of extracte DNA, a common problem i our labs.
4. Make sure students get the bes hair follicles possible. We offe to pluck hairs from those wit brittle hair. We use the twee zers and grip a few hairs ver close to the scalp, near th crown. A "good" follicl should be obvious from severa feet away. African America hair follicles are more difficul to distinguish visually since th hair and its follicle are equall pigmented. However, "good

follicles are always sticky. If a student cannot get a good follicle, we have them use as many as 10 "bad" follicles 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 follicles 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 APOC2 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 was dispensed 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 cycler. 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 10× 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 electrophorese 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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