# Human VNTR Polymorphism Kit *AT*

| | |
|---|---|
| 21-1233 & 21-1233A | VNTR DNA Extraction and Amplification Kit |
| 21-1234 & 21-1234A | VNTR DNA Extraction, Amplification and Electrophoresis Kit with Ethidium Bromide Stain |
| 21-1235 & 21-1235A | VTNR DNA Extraction, Amplification and Electrophoresis Kit with *Carolina* BLU™ Stain |

FOR TEACHING PURPOSES ONLY

Instructor's Manual

(For automatic thermal cycling)

For technical assistance call 800-227-1150 x4381

Upon receipt of the kit, store proteinase K, pMCT118 Primer/Loading Dye Mix, and pBR322/*Bst*N I markers in freezer (approximately –20°C). Other materials may be stored at room temperature (approximately 25°C).

CAROLINA
World-Class Support for Science & Math

This kit was developed in cooperation with the DNA Learning Center of Cold Spring Harbor Laboratory. The experiment was adapted with permission from *Laboratory DNA Science: An Introduction to Recombinant DNA Technology and Methods of Genome Analysis,* by Mark V. Bloom, Greg A. Freyer, and David A. Micklos, ©1996, Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

Individuals should use this kit only in accordance with prudent laboratory safety precautions and under the supervision of a person familiar with such precautions. Use of this kit by unsupervised or improperly supervised individuals could result in serious injury. This product is sold under distribution arrangements with the Perkin-Elmer Corporation.

* Polymerase chain reaction (PCR) is protected by patents owned by Hoffmann-La Roche, Inc.

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# VNTR Polymorphism AT

Although DNA from various individuals is more alike than different, many regions of human chromosomes exhibit a great deal of diversity. Such variable sequences are termed *polymorphic* (meaning many forms) and are used for diagnosis of genetic disease, forensic identification, and paternity testing. Many polymorphisms are located in the estimated 95% of the human genome that does not encode proteins. A special type of polymorphism, termed a VNTR (variable number of tandem repeats), is composed of repeated copies of a DNA sequence that lie next to one another on the chromosome.

In this experiment, students will amplify a non-coding region of chromosome 1 containing the VNTR designated pMCT118, which has a repeat unit of 16 base pairs (bp). Most individuals have between 14 and 40 copies of the repeat on each of their copies of chromosome 1. An individual's two copies of chromosome 1 usually have different numbers of copies, as do the chromosomes from two different individuals. The different versions of the pMCT118 polymorphism are referred to as alleles and are inherited in a Mendelian fashion on the maternal and paternal copies of chromosome 1. The pMCT118 locus has a high degree of heterozygosity, meaning that most people have different numbers of repeats on the chromosomes they inherit from their mother and father.

The source of template DNA for this procedure is a sample of several thousand squamous cells obtained from either hair sheaths or cheek cells. Either procedure is bloodless and noninvasive. Hairs are pulled from the scalp, eyebrow, or arm, and the root ends are mixed with Chelex®/proteinase K. With incubation at 37°C, the proteinase K digests the membrane that contains the sheath cells; vortexing then releases cells in small clumps. Alternatively, cheek cells are obtained by a saline mouthwash, collected by centrifugation, and re-suspended in Chelex®.

In either case, the samples then are boiled to lyse the squamous cells and liberate the chromosomal DNA. The Chelex® binds metal ions, released from the cells that inhibit the PCR reaction. A sample of the clear supernatant, containing chromosomal DNA, is combined with a buffered solution of heat-stable *Taq* polymerase, oligonucleotide primers, the four deoxynucleotide (dNTP) building blocks of DNA, and the cofactor magnesium chloride ($MgCl_2$). The PCR mixture is placed in a DNA thermal cycler and taken through 30 cycles, each consisting of

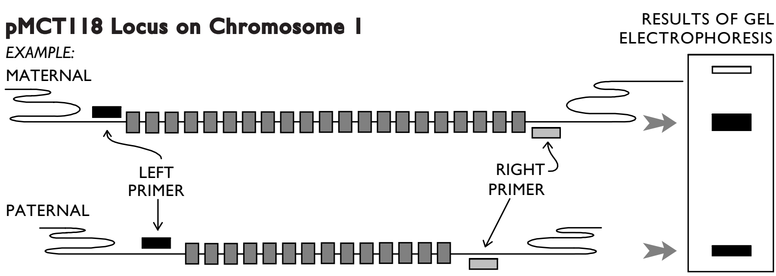
- a 30-second incubation at 94°C, to denature the chromosomal DNA into single strands,
- a 30-second incubation at 65°C, for the primers form hydrogen bonds

with their complementary sequences on either side of the pMCT118 locus, and

- a 30-second incubation at 72ºC, for the *Taq* polymerase to make complementary DNA strands that begin with each primer.

The primers used in this experiment bracket the pMCT118 locus and result in selective *amplification,* or copying, of that region of chromosome 1. Following PCR amplification, student alleles are separated according to size, using agarose or polyacrylamide gel electrophoresis. After staining with ethidium bromide, one or two bands are visible in each student lane, indicating whether an individual is homozygous or heterozygous at the pMCT118 locus. Different alleles usually appear as distinct bands, each composed of several billion copies of the amplified allele. A band's position in the gel indicates the size, and thus number of repeat units, of a pMCT118 allele. During electrophoresis, smaller alleles, with fewer repeats, move further through the gel than larger alleles.

Visit the DNA Learning Center at http://DNALC.org. Click on **Resources**, then **[Biology] Animation Library** to view or download animations on PCR and DNA fingerprinting using VNTRs.




# Materials

The materials in the Human VNTR Polymorphism Kit *AT* are sufficient for 25 reactions. Prior knowledge of basic methods of gel electrophoresis and staining of DNA is presumed. The materials are supplied for use with the exercise described in this kit only. Carolina Biological Supply Company disclaims all responsibility for any other use of these materials.

Upon receipt of the kit, store proteinase K, pMCT118 Primer/Loading Dye Mix, and pBR322/*Bst*N I markers in freezer (approximately –20°C). Other materials may be stored at room temperature (approximately 25°C).

*Included in the kit*

*For extraction and amplification (all kits)*

| | |
|---|---|
| 1.5 g Chelex® 100 resin | mineral oil, 5 mL |
| 5 mL proteinase K, 100 µg/mL | Instructor's Manual |
| 25* Ready-to-Go PCR Beads™ | Student Guides |
| 700 µL pMCT118 primer/loading dye mix | |
| 130 µL pBR322/*Bst*N I markers, .075 µg/µL | |

*Ready-to-Go PCR Beads incorporate *Taq* polymerase, dNTPs, and $MgCl_2$. Each bead is supplied in an individual 0.5-mL test tube.

*For* Carolina *BLU™ electrophoresis (Kits #21-1235 & 21-1235A)*

| | |
|---|---|
| 5 g agarose | 4 latex gloves |
| 250 mL *Carolina* BLU™ final stain | 6 staining trays |
| 7.0 mL *Carolina* BLU™ gel/buffer stain | 150 mL 20× TBE |

*For ethidium bromide electrophoresis (Kits #21-1234 & 21-1234A)*

| | |
|---|---|
| 5 g agarose | 6 staining trays |
| 250 mL ethidium bromide, 1 µg/mL | 150 mL 20× TBE |
| 4 latex gloves | |

**Note**: For 21-1233 & 21-1233A, electrophoresis agents will need to be purchased separately.

*Needed but not supplied*

aluminum foil

beakers containing ice

camera for photographing gels (optional)

centrifuge, clinical, for 15-mL tubes, minimum 500 × g (CC)

centrifuge, micro- or nano-, for 1.5-mL tubes (optional)

DNA thermal cycler, programmable

electrophoresis chambers for agarose gels

electrophoresis power supplies

forceps

laboratory markers, 1 per student

micropipets, 1–10 µL or 1–20 µL, one for instructor or several shared by students

tips, 3 per student

micropipets, 100–1000 µL (or 1-mL transfer pipets)
  tips, 3 per student

microtube racks, may be shared

1.5-mL polypropylene microtubes, 3 per student

paper cup, 1 per student (CC)

razor blade or scalpel, 1 per student (HS)

saline solution, 0.9% NaCl in water, 10 mL per student (CC)

6 staining trays, 1 per agarose gel

UV transilluminator with UV-blocking screen or glasses (for ethidium bromide staining)

vortexer (HS) (optional)

water bath, boiling, per 12 students

water baths, 37°C and 65°C (used at different times)

white light box (for *Carolina* BLU™ staining)

(HS) = Needed for DNA isolation from hair sheaths (procedure A1)

(CC) = Needed for DNA isolation from cheek cells (procedure A2)

# Scheduling

VNTR Polymorphism Kit *AT* requires several different activities. Plan your time as follows:

| Day | Time | Activity | |
|---|---|---|---|
| 1 or more days before lab | 30 min. | Pre-lab: | Mix and aliquot Chelex®/proteinase K (HS) |
| | | | Mix and aliquot saline solution (CC) |
| | | | Aliquot Chelex® (CC) |
| | | | Aliquot pMCT118 primer/loading dye mix |
| Lab period 1 | 30 min. | Pre-lab: | Set up workstations |
| | 30 min. | | Isolate squamous cell DNA |
| | 10 min. | | Set up PCR reactions |
| | 60–180 min. | Post-lab: | Amplify DNA in thermal cycler |
| Lab period 2 | 40 min. | Pre-lab: | Prepare gel solution and cast gels |
| | 15 min. | | Load DNA samples into gels |
| | 20+ min. | | Electrophoresis |
| | 20 min. | Post-lab: | Stain gels |
| | 20 min. | Post-lab: | De-stain gels |
| | 10 min. | Post-lab: | Photograph gels |
| Lab period 3 | 40 min. | Results and Discussion | |

# Pre-Lab Preparation

**Laboratory Period 1**

Make up a 10% Chelex solution: 1.5 g Chelex + 15 mL distilled or deionized water.

*For DNA Isolation from Hair Sheaths (A1)*

1. On the day of the lab, prepare a 1/1 mixture Chelex® solution and proteinase K, by adding 5 mL of each component into a 15-mL test tube. Be sure to shake the Chelex stock tube to resuspend the Chelex beads before adding to the mixture. Store refrigerated (4°C) until use.

2. For each student, aliquot 300 µL of Chelex/proteinase K into a 1.5-mL tube. Be sure to shake the stock tube (or draw liquid in and out of pipet tip several times) to re-suspend the Chelex beads each time before pipetting a student aliquot.

*For DNA Isolation from Cheek Cells (A2)*

1. For each student, prepare and aliquot 10 mL 0.9% saline solution into a 15-mL polypropylene culture tube. The formula for 0.9% saline solution is 0.9 g NaCl per 100 mL distilled or deionized water.
2. For each student experiment, aliquot 500 µL 10% Chelex solution into a 1.5-mL polypropylene tube. Be sure to shake the stock tube (or draw liquid in and out of pipet tip several times) to re-suspend the Chelex beads each time before pipetting a student aliquot.

*Remaining Preparation is Common to Both Isolation Methods (A1 and A2)*

3. The pMCT118 Primer/loading dye mix may collect in the tube cap during shipping. To have the full volume available for student use, pool reagents by spinning tubes briefly in a microcentrifuge or tapping the tube end on a desktop.
4. Set up one boiling water bath per 12 students. This water bath should consist of a beaker and test tube rack to allow 1.5-mL centrifuge tubes to be suspended with the reaction in the boiling water. The entire tube should not be submerged. Alternatively, affix a double layer of aluminum foil to a beaker, and use pencil or other object to punch holes for 1.5-mL tubes.
5. Prepare student stations, each with the following materials:

Chelex, 500 µL (CC)
Chelex/proteinase K, 300 µL (HS)
Student Guide
laboratory marker
micropipet tips
micropipet, 1–10 µL or 1–20 µL
micropipet, 100–1000 µL (or 1-mL transfer pipet)
microtube rack
microtubes, 1.5 ml
paper cup (CC)
razor blade or scalpel (HS)
Ready-to-Go PCR Bead™ and test tube
saline solution, 0.9% (CC)
15-mL tube (CC)

6. Students will share the following materials:

| | |
|---|---|
| centrifuge, micro- or nano- (optional) | mineral oil |
| centrifuge, clinical (CC) | pMCT118 primer/loading dye mix |
| beakers containing ice | |
| DNA thermal cycler | water bath, boiling |
| forceps | vortexer (HS)(optional) |

7. Program thermal cycler with a step file:

94°C – 30 sec

65°C – 30 sec

72°C – 30 sec

(30 cycles) link to soak file at 4°C (if your machine has this option)

**Laboratory Period 2**

1. Dilute the 20× TBE to 1× by adding 150 mL 20× TBE to 2850 mL of distilled or deionized water. Prepare 1.5% agarose solution sufficient for the number of gels needed to hold all student samples, by adding 5 g agarose to 333 mL of 1× TBE. Each gel will need to be approximately 8 mm in depth to accommodate samples of up to 25 µL. Cover agarose solution with aluminum foil and keep warm in a 65°C water bath until ready for use.

2. Students will share the following materials:

| | |
|---|---|
| agarose or polyacrylamide gels | laboratory markers |
| camera system | loading dye |
| centrifuge, micro- or nano- | 1 micropipet, 10-µL or 1 20-µL |
| pBR322/*Bst*N I size markers | microtube rack |
| electrophoresis apparatus | staining trays |
| electrophoresis buffer | UV transilluminator with safety screen |
| ethidium bromide staining solution (Kit 21-1234) | *Carolina* BLU™ staining solution (Kit 21-1235) |

***For Kit 21-1235:***

*Add Carolina BLU® Stain to Agarose and Buffer*

The concentration of stain added to the agarose/buffer is dependent on the voltage used for electrophoresis. If electrophoresing at voltages less than 50 volts, a slightly lower concentration is used than if running at voltages greater than 50 volts. The stain may be added to the entire volume of agarose and distributed, or the agarose may be distributed to each lab station and the stain added by the students in the amounts listed below.

| **Voltage** | **Agarose Volume** | **Stain Volume** |
|---|---|---|
| <50 Volts | 30 mL<br>60 mL<br>400 mL | 40 µL (1 drop)<br>80 µL (2 drops)<br>520 µL (13 drops) |
| >50 Volts | 50 mL<br>400 mL | 80 µL (2 drops)<br>640 µL (16 drops) |

After addition of the stain to the agarose, swirl to mix and immediately pour the gel. Gels may be prepared one day ahead of the lab day, if necessary. Gels stored longer tend to fade and lose their ability to stain bands during electrophoresis. Store covered with a small amount of buffer (leaving masking tape in place), or store covered in the gel box. Don’t try using more stain than recommended, in your gel. This leads to precipitation of the DNA in the wells and can create artifactual aggregated DNA bands in the agarose gel.

Use the chart below for addition of the stain to 1× TBE electrophoresis buffer:

| **Voltage** | **Buffer Volume** | **Stain Volume** |
|---|---|---|
| <50 Volts | 500 mL<br>2.6 L | 480 µL (12 drops)<br>2.6 mL (65 drops) |
| >50 Volts | 500 mL<br>2.6 L | 960 µL (24 drops)<br>5 mL (125 drops) |

The dropper bottle provided delivers 40 µL/drop. If a calibrated pipet is available, the dropper tip can be removed for quicker addition of larger volumes of stain. The volume of buffer and agarose required for some gel box options are listed below:

| Type Gel Box Required | Volume Buffer Required | Volume Agarose Required |
|---|---|---|
| Mini Gel System Box | 200 mL | 30 mL/casting tray |
| Carolina Gel Box, 1 tray | 250 mL | 50 mL |
| Carolina Gel Box, 2 trays | 450 mL | 100 mL |

While *Carolina* BLU™ is not toxic, we recommend that the students wear gloves to prevent staining the skin. TBE buffer containing *Carolina* BLU™ stain may be reused if the reuse occurs within a day or so. If left longer, the dye loses its ability to stain DNA during electrophoresis. If reusing the buffer for several days is important, we recommend using *Carolina* BLU™ in the gel and as a final stain only.

# Student Lab Briefing

Students should be familiar with the polymerase chain reaction and electrophoresis. The introductory material assumes students are familiar with basic Mendelian genetics.

It is highly recommended that students be given a chance to view their own cells under a light microscope—preferably before beginning the experiment. **For cheek cells,** use a toothpick to gently scrape inside of mouth or to pick up some of the pellet formed in Step A2-6. Smear cell debris on a microscope slide, add a drop of 1% methylene blue (or other stain), and add a coverslip. **For hair sheath,** cut hair just above sheath material and place on microscope slide. Add a drop of methylene blue, a drop of proteinase K (100 µg/mL), and a cover slip. Observe at several time points, to see the effect of enzyme digestion. Gently smashing the hair under the coverslip will aid in disrupting the sheath membrane.

### Additional Background Resources

Kreuzer, H., and A. Massey. 2000. *Recombinant DNA and Biotechnology: A Guide for Teachers.* American Society for Microbiology (Carolina catalog #21-2218), Washington, DC.

Bloom, M., G. Freyer and D. Micklos. 1996. *Laboratory DNA Science: An Introduction to Recombinant DNA Technology and Methods.* Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

A videotape explaining the molecular mechanism of PCR is also available in the Carolina catalog (#21-2734).

An animation of the PCR process can be viewed or downloaded at the DNA Learning Center website (http://DNALC.org/Shockwave/pcranwhole.html).

## Fine Points of Lab Procedure

Be alert to the following cautions when performing the experiment. Where appropriate, discuss fine points with students and have them make notes in their student guides.

*1. Disclosure and Confidentiality*

The pMCT118 insertion polymorphism was specifically selected for use in this laboratory because it is phenotypically neutral. pMCT118 alleles have no known relationship to disease states, sex determination or any other phenotype. Even though there is no chance of disclosing phenotypic information about the experimenters, the confidentiality of student pMCT118 genotypes can be maintained by identifying student samples only by numbers.

The pMCT118 alleles are inherited in a Mendelian fashion and can give indications about family relationships. To avoid the possibility of discovering inconsistent pMCT118 inheritance, it is best not to generate genotypes from siblings or other family members. However, a diallelic polymorphism has an inherently low information content—usually there are at least several parental genotypes that could account for an observed student genotype. In a formal sense, a single experiment with a single-locus polymorphism cannot definitively prove or disprove relatedness for several reasons:

- Student samples can be mixed up when isolating DNA, setting up PCR reactions, and loading electrophoresis gels. A forensic laboratory would use approved methods for maintaining “chain of custody” of samples and for tracking samples.
- There is a finite chance that recombination during gamete formation has altered an allele inherited from either parent.

*2. Comparison of DNA Isolation from Check Cells and Hair Sheaths*

**Saline mouthwash** is the most effective method of cell collection for PCR. Cells are gently loosened from the inside of the cheek, yielding small groups of several cells each. This maximizes the surface area of cells, allowing for virtually complete lysis during boiling. Experience has shown that the mouthwash procedure produces interpretable PCR results in 85%–95% of samples. Surprisingly, food particles rinsed out with the mouthwash have little effect on PCR amplification, but may obstruct passage of fluid through pipet tips and make pipetting difficult. So, it is not advisable to eat immediately before the experiment—especially fruits.

The mouthwash method does generate liquid waste; however, the risk of spreading an infectious agent is much less likely than from natural atomizing processes, such as coughing or sneezing. Several elements further minimize any risk of spreading an infectious agent that might be present in mouthwash samples:

- Each experimenter works only with his or her sample.
- The sample is sterilized during a 10-min boiling step.
- There is no culturing of the samples that might allow growth of pathogens.

**Hair sheaths** are the safest source of human cells from which to extract DNA for PCR amplification. Risk of spreading an infectious agent is minimized by “dry” collection, which does not involve any body fluid or generate any supernatant. This method also stresses the power of PCR in forensic cases—even one good sheath provides enough DNA for excellent amplification. As in the mouth wash method, squamous cells are liberated from the sheath singly or in small clusters and a high percentage are lysed by boiling.

Successful amplification is closely correlated to presence of a sheath. Unfortunately, most people find sheaths only on some hairs, and some people are unable to find any sheaths at all. Hair roots usually yield little DNA, because the cell mass is not digested by proteinase K and only cells at the edge of the mass are lysed by boiling. Although amplification success from sheaths probably rivals the mouthwash method, DNA isolated from a typical mixture of sheaths and roots produces 65%–85% interpretable results.

Each method requires one piece of relatively inexpensive equipment. The mouthwash method requires a clinical centrifuge (for 15-mL tubes) that develops 500–1000 x g. DNA isolation from hair sheaths requires a vortexer.

*3. Ready-To-Go PCR Beads™*

Each PCR bead contains reagents so that when brought to a final volume of 25 µL the reaction contains 1.5 units of *Taq* polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP.

*4. pMCT118 Primer/Loading Dye Mix*

This mix includes pMCT118 primers (.25 pmol/µL), 13.9% sucrose, and 0.0082% cresol red in tris-low EDTA (TLE) buffer (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA).

*5. Storing Squamous Cell DNA Samples*

Student DNA samples isolated in Procedure A1 or A2 are unstable and must be kept on ice prior to setting up PCR reactions. Samples may be stored at –20°C for several weeks without significant DNA degradation.

*6. Setting Up PCR Reactions*

The lyophilized *Taq* polymerase in the Ready-To-Go PCR Bead becomes active immediately upon addition of the pMCT118 primer/loading mix. In the absence of thermal cycling, "nonspecific priming" allows the polymerase to begin generating erroneous products, which can show up as extra bands in gel analysis. Therefore, work quickly, and initiate thermal cycling as soon as possible after mixing PCR reagents. Be sure the thermal cycler is set and have all experimenters set up PCR reactions coordinately. Add primer/loading dye mix to all reaction tubes, then add each student sample, and begin thermal cycling immediately.

The repeated structure of the VNTR polymorphism makes it more difficult to amplify. So, this experiment greatly benefits from a "hot start," where one reagent is withheld from the reactions until the samples are cycled to the initial denaturing temperature. You can perform a hot start by adding the student samples during the first denaturation step. Either program an extended first denaturation of 10 minutes, or stop cycling and restart after adding the samples. A simpler alternative is to set up reactions on ice, start the thermal cycler, and then place the tubes in the machine as the temperature approaches the denaturing set point. Keep all tubes on ice until placed in thermal cycler.

*7. Thermal Cycling*

PCR amplification from crude cell extracts is biochemically demanding and requires the precision of automated thermal cycling. The recommended amplification times and temperatures will work adequately for all types of thermal cyclers.

*8. Electrophoresis Options and Limitations*

This kit is provided with electrophoresis-grade agarose, which is more convenient and inexpensive. While it cannot resolve all alleles in the pMCT118 system, it does provide adequate allele separation to illustrate genetic diversity. When used with care, high-resolution agarose—such as MetaPhor and NuSieve—can resolve virtually all pMCT118 alleles. In fact the first commercial pMCT118 typing kit, designed for forensic use in crime labs, used high-resolution agarose!

Only polyacrylamide gel electrophoresis is capable of reproducibly resolving alleles of small size difference. Polyacrylamide gels, however, are difficult to cast. In addition, polyacrylamide electrophoresis chambers and high-voltage power supplies are comparatively expensive.

*9. DNA Size Markers*

Plasmid pBR322 digested with the restriction endonuclease *Bst*N I produces fragments that are useful as size markers in this experiment: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp (this last band may be faint).

Use 20 µL of the DNA ladder per gel. A ladder of actual pMCT118 repeats, though more expensive, should be used in polyacrylamide systems capable of definitively determining pMCT118 genotypes.

*10. Viewing and Photographing Gels*

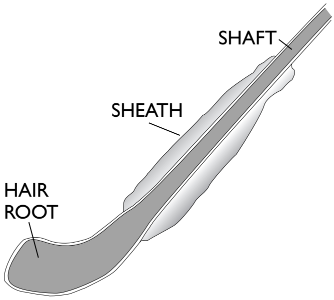
View and photograph gels as soon as possible after appropriate destaining. Over time, PCR products disappear as stained bands because they slowly diffuse through the gel.

# Laboratory Procedure

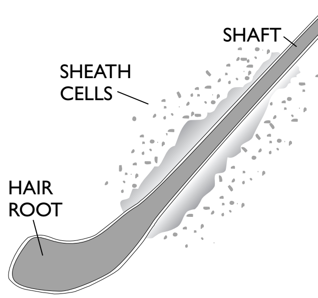
*Procedure A1: Isolate Hair Sheath DNA*

1. Pull out several hairs and inspect for presence of a sheath. The sheath is a barrel-shaped structure surrounding the base of the hair, and can be readily observed with a hand lens or dissecting microscope. The glistening sheath can be observed with the naked

eyes by holding the hair up to a light source. (Sheaths are most easily observed on dark hair.)




**HUMAN HAIR**




**AFTER PROTEINASE K TREATMENT**

2. Select one to several hairs with good sheaths. Alternatively, select hairs with the largest roots.
3. Use a fresh razor blade or scalpel to cut off hair shafts just above the sheath.
4. Use forceps to transfer hairs to a 1.5-mL tube containing 300 µL of Chelex/proteinase K. Make sure sheath is submerged in the solution and not stuck on the test tube wall.
5. Incubate sample tube in 37°C water bath for 10 minutes.
6. Remove sample tube to room temperature. Vortex by machine or vigorously with finger for 15 sec to dislodge cells from hair shaft.
7. Place your sample in a floating tube rack in the boiling water bath for 8 min. Do not submerge or drop the tube into the water. Use forceps to remove your tube from the boiling water bath and allow to cool for 2 min. The tube may be placed on ice for faster cooling.
8. Vortex by machine or vigorously with finger for 15 sec.
9. Put your assigned number on your sample tube and place it, along with others, in a balanced configuration in the microcentrifuge and spin for 30 sec (1 min in nanofuge). Alternatively, let tube set for 5 min to allow debris to settle.
10. Use a fresh tip to transfer 200 µL of the clear supernatant to a clean 1.5-mL tube. Be careful not to remove or disturb the Chelex/cell debris at the bottom of the tube.
11. Store your sample on ice or in the freezer until ready to begin Procedure B.

*Procedure A2: Isolate Cheek Cell DNA*

1. Use a permanent marker to place your assigned number on two clean 1.5-mL tubes and on the 15-mL test tube containing 10 mL saline (0.9% NaCl) solution.
2. Pour the saline solution into your mouth and vigorously rinse your mouth for 10 sec. Save test tube for later use.
3. Expel saline solution into the paper cup.
4. Carefully pour saline solution from the paper cup back into the original test tube and close cap tightly. Save paper cup for later use.
5. Place your sample tube, together with other student samples, in a balanced configuration in a clinical centrifuge and spin for 10 min.
6. Carefully pour off supernatant into the paper cup. Be careful not to disturb the cell pellet at the bottom of the test tube.
7. Set micropipet to 500 µL. Draw 10% Chelex suspension in and out of the pipet tip several times to suspend the resin beads. Before resin settles, rapidly transfer 500 µL of Chelex suspension to the test tube containing your cell pellet.
8. Re-suspend cells by pipetting in and out several times. Examine against light to confirm that no visible clumps of cells remain.
9. Pipet several times to re-suspend cells and resin, then transfer 500 µL of your cell sample into a clean 1.5-mL tube.
10. Place your sample in a floating tube rack in the boiling water bath for 10 min. Do not submerge or drop the tube into the water. Use forceps to remove your tube from the boiling water bath and allow to cool for 2 min. The tube may be placed on ice for faster cooling.
11. Place your sample tube, with others, in a balanced configuration in the microcentrifuge and spin for 30 sec (1 minute in nanofuge). Alternatively, let tube sit for 5 min to allow debris to settle.
12. Use a fresh tip to transfer 200 µL of the clear supernatant to a clean 1.5-mL tube. Be careful not to remove or disturb the Chelex/cell debris at the bottom of the tube.
13. Store your sample on ice or in the freezer until ready to begin Procedure B.
14. Pour supernatant from Step 6 into the sink and rinse down with water.

*Procedure B: Set Up PCR Reactions*

1. Use a micropipet with a fresh tip to add 22.5 µL of pMCT118 primer/loading buffer mix to a PCR tube containing a Ready-To-Go PCR Bead. Tap tube with finger to dissolve bead.
2. Use a fresh tip to add 2.5 µL of student DNA to reaction tube, and tap to mix. Pool reagents by pulsing in a microcentrifuge or by sharply tapping tube bottom on lab bench.
3. Label the cap of your tube with a number, as assigned by your teacher. In this way, your results will be anonymous.
4. Add one drop of mineral oil to the top of reactants in the PCR tube. Be careful not to touch the dropper tip to the tube or reactants, or subsequent reactions will be contaminated with DNA from your preparation.

   **Note:** Thermal cyclers with heated lids do not require use of mineral oil.
5. Store all samples on ice or in the freezer until ready to amplify according to the following profile.

   Instructor: Program and start thermal cycler with a step file:

   94°C – 30 sec

   65°C – 30 sec

   72°C – 30 sec

   (30 cycles) link to 4ºC soak file (if your machine has this option)

**Note:** The 30th cycle can be followed by a 10-minute extension at 72°C. This extension can increase the amount of DNA present in the sample.

*Procedure C: Load and Electrophorese PCR Products*

The cresol red and sucrose in the primer mix functions as loading dye, so that amplified samples can be loaded directly into gels.

1. Use a micropipet with a fresh tip to add the entire PCR sample/loading dye mixture (25 µL) into your assigned well of a 1.5% agarose gel. Expel any air from the tip before loading, and be careful not to push the tip of the pipet through the bottom of the sample well. Be sure not to get any mineral oil in your tip.
2. Load 20 µL of the pBR322-*Bst*N I size markers into one lane of gel.
3. Electrophorese at 130 volts for 20–30 min. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

**4.** Gels may be stained either with *Carolina* BLU™ for 20 min and destained with distilled or dechlorinated water for 30 min, or stained with1 µg/mL ethidium bromide for 10 min.

## Results and Discussion

**1.** Observe the photograph of the stained gel containing your sample and those from other students. Orient the photograph with the sample wells at the top. Interpret the band(s) in each lane of the gel:

- **a.** Scan across the photograph to get an impression of what you see in each lane. You should notice that virtually all student lanes contain one to three prominent bands.
- **b.** Now locate the lane containing the pBR322/*Bst*N I markers. Working from the well, locate the bands corresponding to each restriction fragment: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp (may be faint).
- **c.** The 29 known pMCT118 alleles range in size from 369–801 bp, so all alleles should fall between the 383-pb and 929-bp marker bands.
- **d.** It is common to see an additional band lower on the gel. This diffuse (fuzzy) band is "primer dimer," an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. Primer dimer is approximately 50 bp, and should be in a position ahead of the 121-bp marker.
- **e.** Additional faint bands, at other positions, occur when the primers bind to chromosomal loci other than pMCT118 and give rise to "nonspecific" amplification products.

**2.** How would you interpret a lane in which you observe primer dimer, but no other bands?

*The presence of primer dimer confirms that the reaction contained all components necessary for amplification, but that there was insufficient template to amplify the target sequence.*

**3.** Determine the number of different alleles represented among your classmates, the number of people whose DNA fingerprints "match," and the percentage of heterozygous individuals. Compare your class data with the following pMCT118 data from scientific studies of large populations:

pMCT118 alleles: 29

pMCT118 heterozygosity: 72% of people

pMCT118 matches: 1 in 18 people

What reasons can you give for differences?

*Class results typically show 6–8 different alleles and 50% heterozygosity. Apparent matches will likely be greater than 1 in 18. The most frequent reason for these discrepancies is the inability of the agarose gel system to resolve similarly-sized alleles. A person with two different alleles that are close in size may appear to have only one allele (homozygous). Different alleles that are close in size may be interpreted as a match between people. Less likely causes of discrepancies are the small sample size, the racial/ethnic makeup of the class, and the inefficient amplification of large alleles.*

**4.** Considering your results, do you think this protocol alone could be used to link a suspect with a crime or establish a paternity relationship? Why or why not? How could you modify the experiment to improve its ability to identify individuals?

*No, there are not a large enough number of allele combinations to identify a specific individual within a population. Forensic scientists typically examine alleles from four to six areas of the genome. The ability of this experiment to identify individuals could be improved by amplifying several other VNTR regions in addition to the pMCT118 locus.*

**5.** Polyacrylamide gel electrophoresis is used to resolve VNTR polymorphisms in identity testing. The following diagram shows the pMCT118 genotypes for 6 individuals. The marker lanes (L) show most of the known pMCT118 alleles, each differing by one repeat unit. A person's genotype is "scored" by comparing the bands in the genotype to those in the marker lane. For example, person #1 has one 18-repeat allele and one 31-repeat allele, and is scored as 18, 31.

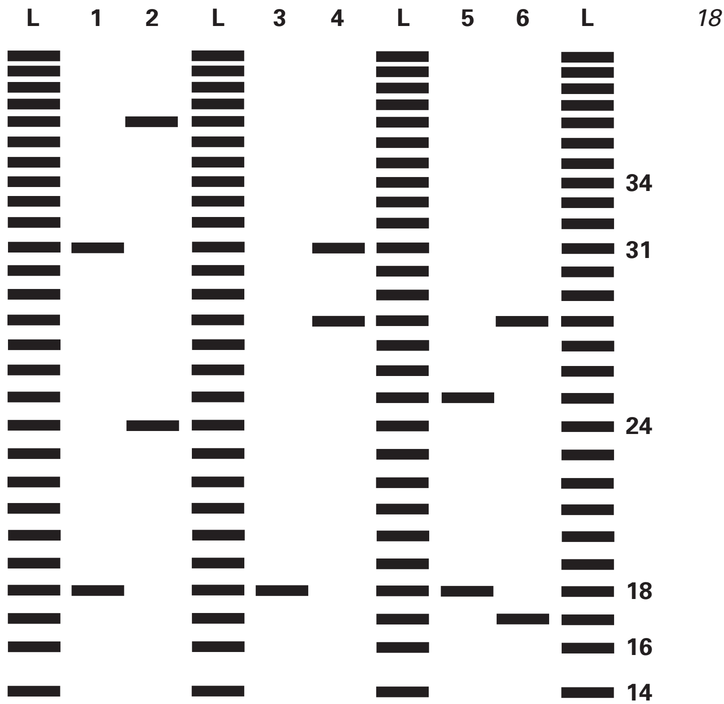
**a.** Score the remaining individuals.

*2 (24, 37); 3 (18, 18); 4 (28, 31); 5 (18, 25); 6 (17, 28)*

**b.** What percentage of individuals are heterozygous—having two different alleles?

*83%*

**c.** Which alleles appear to be most common?




**6.** The table on the following page shows the frequencies of the pMCT118 alleles in several populations.

**a.** Which are the three most common alleles in all populations?
*18, 24, 31*

**b.** Which allele frequencies differ substantially between populations?
*Most do.*

**c.** If we assume that each population is in Hardy-Weinberg equilibrium, then the following formula is used to calculate genotype frequencies in a population:

$p^2 + 2pq + q^2 = 1$

For example, the frequency of the heterozygous genotype 18, 24 in U.S. Caucasians is:

2(0.265)(0.265) = 0.170

The frequency of the homozygous genotype 24, 24 is

(0.320)(0.320) = 0.102

| | Allele Frequency (n = number of alleles typed) | | |
|---|---|---|---|
| Allele | U.S. Caucasians (n = 200) | African Americans (n = 214) | Hispanic Americans (n = 210) |
| 14 | — | — | — |
| 15 | — | — | — |
| 16 | — | — | — |
| 17 | — | 0.051 | 0.005 |
| 18 | 0.265 | 0.079 | 0.281 |
| 19 | 0.005 | 0.005 | — |
| 20 | 0.045 | 0.033 | 0.014 |
| 21 | 0.010 | 0.108 | 0.033 |
| 22 | 0.030 | 0.108 | 0.029 |
| 23 | 0.005 | 0.028 | 0.005 |
| 24 | 0.320 | 0.168 | 0.300 |
| 25 | 0.045 | 0.014 | 0.038 |
| 26 | 0.025 | 0.009 | 0.010 |
| 27 | 0.010 | 0.005 | 0.010 |
| 28 | 0.050 | 0.173 | 0.038 |
| 29 | 0.075 | 0.051 | 0.062 |
| 30 | 0.005 | — | 0.071 |
| 31 | 0.060 | 0.061 | 0.062 |
| 32 | 0.015 | 0.005 | 0.005 |
| 33 | — | 0.005 | — |
| 34 | 0.005 | 0.089 | 0.010 |
| 35 | — | — | — |
| 36 | 0.005 | 0.005 | — |
| 37 | 0.015 | — | 0.005 |
| 38 | — | — | 0.010 |
| 39 | — | — | — |
| 40 | 0.005 | 0.005 | 0.010 |
| 41 | — | — | — |
| >41 | 0.005 | — | 0.005 |

**d.** Use the table of allele frequencies to calculate the genotype frequencies, by population group, for DNA samples in lanes 1–6 of the gel in Question 5. Fill in your answers on the chart below:

| Sample | Genotype | U.S. Caucasian | African American | U.S. Hispanic |
|---|---|---|---|---|
| 1 | *18, 31* | *0.032* | *0.010* | *0.035* |
| 2 | *24, 37* | *0.010* | *0.0* | *0.003* |
| 3 | *18, 18* | *0.070* | *0.006* | *0.079* |
| 4 | *28, 31* | *0.006* | *0.021* | *0.005* |
| 5 | *18, 25* | *0.024* | *0.002* | *0.021* |
| 6 | *17, 28* | *0.0* | *0.018* | *0.0* |

**7.** When there is a match between a forensic DNA sample collected at a crime scene and DNA subpoenaed from a suspect, the genotype frequency can be interpreted in two ways. In one sense, it is the probability of finding that specific genotype in the population. In another sense, it is the probility that the match is due to chance. How does the information in the chart you constructed in Question 3 affect this sort of analysis.

*Genotype frequencies can differ substantially between population groups. A genotype that is common in one group would be of less use in proving identity. The same genotype might be rare in another population and of greater use in proving identity.*

**8.** For identification purposes, crime laboratories now have settled on a panel of six VNTR loci, which are amplified together in a single PCR reaction and electrophoresed together in a polyacrylamide gel. Since the loci are located on different chromosomes, they are thought to be unlinked—that is, they are inherited independently of each other. Thus, the probability of inheriting an observed set of alleles at the six loci is the product of their individual probabilities. Assuming Hardy-Weinberg equilibrium, calculate the occurrence probability for the following genotype:

| | Allele 1 | Allele 2 | | Allele 1 | Allele 2 |
|---|---|---|---|---|---|
| Locus A | 0.25 | 0.15 | Locus D | 0.05 | 0.20 |
| Locus B | 0.10 | 0.05 | Locus E | 0.20 | 0.20 |
| Locus C | 0.05 | 0.10 | Locus F | 0.15 | 0.05 |

*2 (0.25 x 0.15) x 2 (0.10 x 0.15) x 2 (0.05 x 0.20) x (0.20 x 0.20) x 2 (0.15 x 0.05) =* $2.7 \times 10^{-10}$

**9.** The sizes of alleles run on polyacrylamide gels can be determined directly by comparison with a ladder of all pMCT118 alleles. A close estimate of allele sizes in your experiment can be obtained by graphing the function that determines the migration of linear DNA fragments through an agarose gel:

$$D = \frac{1}{\log_{10} MW}$$

where *D* equals the distance migrated and *MW* equals the molecular weight of the fragment. For simplicity, biologists substitute bp length for molecular weight in this calculation.

a. Working from the well, label the bands corresponding to each of the pBR322/*Bst*N I markers: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp (this last band may be faint). Carefully measure the distance, in mm, that each marker fragment migrated from the sample well. Measure from the front edge of the well to the front edge of each band.

**b.** Set up semi-log graph paper with distance migrated as the x (arithmetic) axis and bp length as the y (logarithmic) axis. Plot distance migrated versus bp length for each marker fragment. Connect data points with a line. Because you are plotting bp length, and thus molecular weight, on the logarithmic axis, it is not necessary to take the log of fragment sizes before graphing.

**c.** Measure and record distances migrated by the various alleles. To determine the base-pair size of an allele, first locate the distance it migrated on the x axis. Then, use a ruler to draw a vertical line from this point to its intersection with the marker data line. Now, extend a horizontal line from this point to the y axis. The number on the y axis is the calculated base-pair size of the allele.

**d.** Compare the largest and smallest allele observed in your class with the known size range of most pMCT118 alleles.

# Further Reading

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National Research Council. 1996. *The Evaluation of Forensic DNA Evidence.* National Academy Press, Washington, DC.

**Carolina Biological Supply Company**
2700 York Road, Burlington, North Carolina 27215
Phone: 800.334.5551 • Fax: 800.222.7112
Technical Support: 800.227.1150 • www.carolina.com
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