WeCanFigureThisOut.org: A Hands-on Introduction to Nanoscience

VNTR PCR DNA Fingerprinting Lab Manual

(revision 2 March 2020)

This DNA fingerprinting manual is designed for an early undergraduate lab involving students of any major. The lab consists of two one and a quarter hour student sessions (with the instructors unloading the PCR thermal cycler and casting gels between sessions). To prepare for the lab, students study lecture note sets explaining both “DNA Self-Assembly” and “DNA fingerprinting,” on which they are quizzed at the beginning of the lab. These note sets are available on WeCanFigureThisOut.org's Nanoscience homepage:

https://wecanfigurethisout.org/NANO/Nano_home.htm

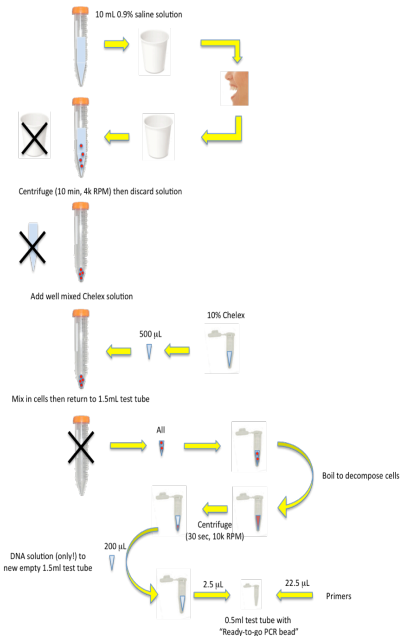
This manual is derived from the "Human VNTR Polymorphism Kit AT" manual from Carolina Scientific (which, in turn, was based on a manual developed by Mark V. Bloom at the Dolan DNA Learning Center of the Cold Spring Harbor Laboratory).

However, to accommodate our lab schedule & non-biology majors we:

- Included instructions only for the analysis of cheek cells
- Assumed the use of a thermal cycler with a heated lid, and Carolina BLU stain
- Substituted an alternate buffer (SBE) permitting faster higher voltage electrophoresis
- Added comments explaining the intent of *all* lab instructions
- Inserted parenthetical definitions for specialized terms (bold red highlights)
- Reordered the manual to clarify its presentation

The original Carolina lab manual was found at:

www.carolina.com/category/teacher+resources/instructions+and+buying+guides/biotech+kit+instruction+manuals/human+vntr+polymorphism+kit+teacher%27s+manual.do



Materials

Carolina Human VNTR Polymorphism Kits:

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 the proteinase K, pMCT118 Primer/Loading Dye Mix, and pBR322/BstNImarkers in a freezer (approximately –20°C). Other materials may be stored at room temperature.

Included in the ***Kit #21-1235***:

1.5 g Chelex®100 resin	mineral oil, 5 mL
5mL 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/BstNImarkers, .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.

5g agarose	4 latex gloves
250 mL CarolinaBLU™ final stain	6 staining trays
7.0 mL CarolinaBLU™ gel/buffer stain	150 mL 20X TBE

Or purchased separately to accommodate our larger UVA class & procedures:

Description	Qty. - Vendor P/N	Qty. for 36 student lab
20x SBE Buffer	1l - Excellgen EG-1002	1 Bottle*
puReTaq Ready to Go PCR Beads 407513D (100)	GE Healthcare 27-9558-01	1/3 Pack
Sodium Chloride Crystal	500g - Carolina 888880	4.5 g + 500 ml DI water
100 Mesh Chelex Powder	25g – Carolina 217310	~ 1/12 Container
Agarose	5g – Carolina 217075	1 container
Carolina BLU gel-buffer stain / final DNA stain	30ml/250ml – Carolina 217300	2 packs
pMCT118 PCR Primer	Carolina 211506	2 vials*
PBR322/BstNI Marker	130 ul - Carolina 211479	2 vials*

*Being cautious, we do not retain any opened liquid chemicals for later use

Additional Required Equipment (numbers are for back-to-back labs of nine students):

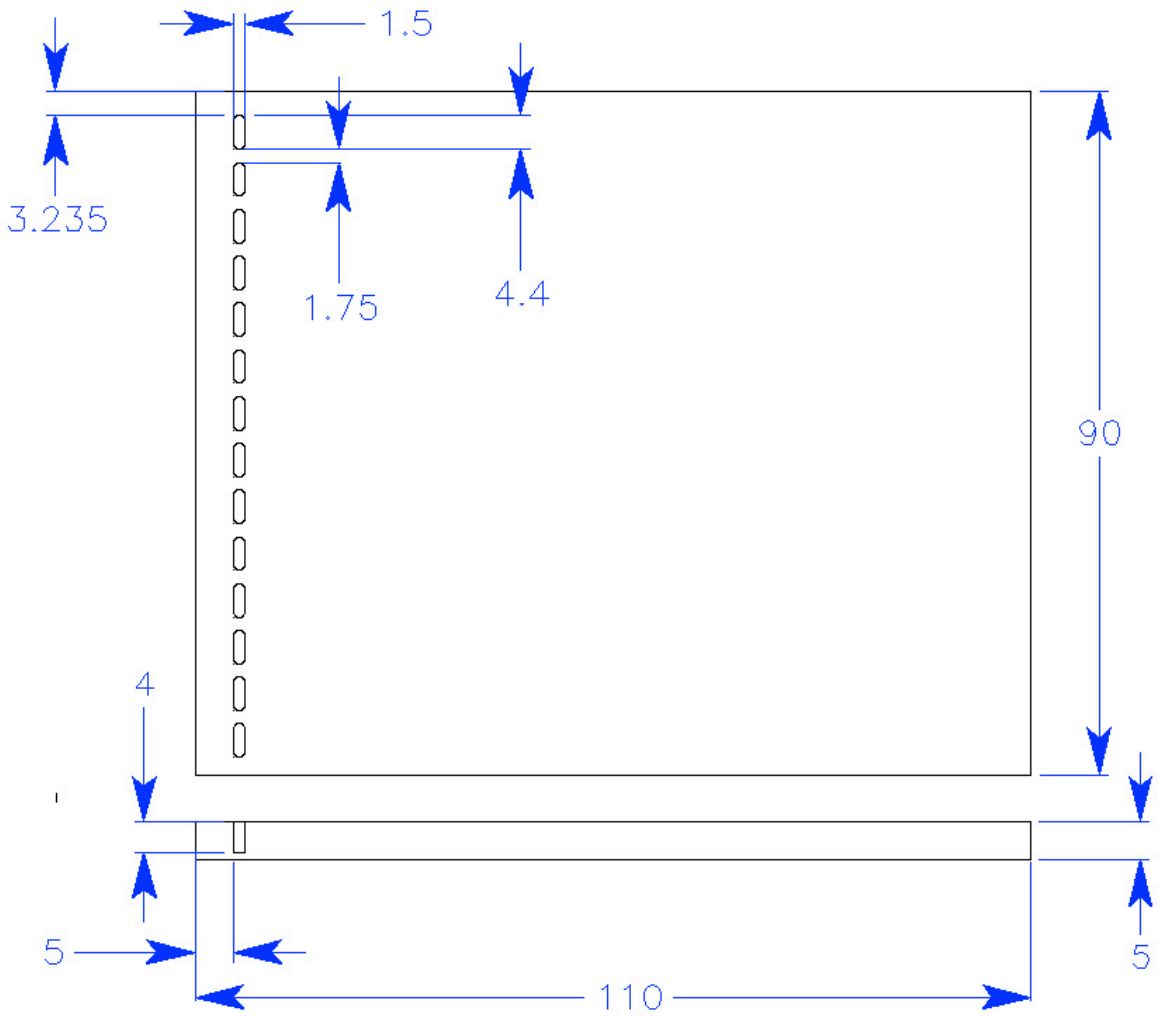
Item:	Qty.	Model used in UVA lab:
Centrifuge for 15-mL tubes	2	Labnet Z100 A
Centrifuge for 1.5-mL tubes	1	Revolutionary Science Microcentrifuge RS-102
Centrifuge for 0.5-mL PCR tubes	1	Denville Scientific Mini Centrifuge
Vortexer	2	Fisher MiniRoto
Microtube racks (at least 1 per student)		
DNA thermal cycler, programmable	2	Labnet MultiGene Mini
Micropipets: 2-1000 μL total range	4	Fisher Finnpipette II 2-20 μL (red button)
	4	Fisher Finnpipette II 5-50 μL (orange button)
	4	Fisher Finnpipette II 100-1000 μL (blue button)
Electrophoresis system	1	Owl EasyCast B1 Horizontal System, with two extra gel trays, and the gel casting fixture
Staining trays	1 per agarose gel	
Electrophoresis power supplies	1	IBI Scientific SH-300 dual output power supply
Water bath, boiling	1	Fisher Isotemp #15-462-2SQ
Floating microtube rack	1	Fisher #1412744
Oscillating mixing table	1	Blotrocker 3D
For CarolinaBLU™ staining: White light box		
Microwave oven		Lab scale
Forceps		DI water container and dispensers
Waste receptacles and liners		

Additional Required Supplies:

Innovita 20X SBE electrophoresis buffer (labsupplymall.com / Excellgen)

Micropipette tips (2 per student of each size plus those used by instructor / TAs)
- Fisher 0.5-20 μL (# 21-377-353)
- Fisher 0.5-100 μL (# 21-377-170)
- Fisher 100-1000 μL (# 21-377-604)

Disposable transfer pipettes (Fisher # 13-711-9D)
Fisher 15-ml polypropylene centrifuge tubes, 1 per student (Fisher # 05-538-59A)
1.5-mL polypropylene centrifuge microtubes, 2 per student (Fisher # 05-402-94)
Paper cup, 1 per student, in dispenser to avoid contamination (Fisher # 02-544-120)
Saline solution, 0.9% NaCl in water, 10 mL per student (Fisher # NC0 047105)
Beakers containing ice
Laboratory markers, 1 per student



SURROGATE EP GEL:

Clear plastic rectangle

110 x 90 x 5 mm

fourteen wells along one edge

each well 4.4 x 1.5 x 4 mm

separated by 1–3/4 mm

(use 1.5 mm or 1/16" end mill)

All dimensions nominal

If 5 mm thick plastic is not available substitute 1/4" stock or machine down thicker sheet from back (non–well) side

Introduction

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** (surface layer) cells obtained from either hair sheaths or cheek cells. Cheek cells are obtained by a saline mouthwash, collected by centrifugation, and re-suspended in Chelex.®

The samples then are boiled to **lyse** (break apart) the squamous cells and liberate the chromosomal DNA. The Chelex® binds metal ions, released from the cells that inhibit the **polymerase chain reaction** (PCR). A sample of the clear **supernatant** (solution above the precipitate), 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 aDNA thermal cycler and taken through 30 cycles, each consisting of

• a 30-second incubation at 94°C, to **denature** (separate) 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** (location), 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.

Fine Points of Lab Procedure

1. Disclosure and Confidentiality

The pMCT118 insertion polymorphism was specifically selected for use in this laboratory because it is **phenotypically neutral** (i.e., it does not contribute to observable inherited characteristics). 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. 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.

3. 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).

4. Setting Up PCR Reactions

The **lyophilized** (freeze dryed) 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.

5. 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.

6.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.

7. DNA Size Markers

Plasmid pBR322 digested with the restriction endonuclease BstNI 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.

8. 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.

Lab Day 1

Instructor Pre-Lab Preparation

1. At least 3 hours before the labs, set up one boiling water bath per 12 students. This water bath (the Isotemp 220S) should be filled with ½ gallon of water, and set to 100C.

2. Check the thermal cyclers to make sure they contain a step file program named "VNTR":

- Turn on thermal cycler power (rocker switch on back).
- Wait for unit to boot (completion of the horizontal progress bar)
- Select FILE (button F1)
- Use arrow keys to move the blinking cursor to the program "VNTR"

This program runs in the "Block" (low thermal overshoot) mode and consists of:
Thirty cycles of: 94°C – 30 sec / 65°C – 30 sec / 72°C – 30 sec
Linking to a soak at 72°C for ten minutes, followed by a hold at 4°C

3. Number pieces of paper for students to draw (students will label samples with their number)

4. Prepare student stations (for 3 students each), each with the following materials:

1- Student Guide
1 - laboratory marker
1 - waste container
1 - micropipette, 2-20 µL
1 - micropipette, 5-50 µL
1 - micropipette, 100–1000 µL
3 - **Ready-to-Go PCR Bead™** in 0.5 mL test tube
3 - microtube racks
6 - microtubes, 1.5 ml
3 - 15-mL tubes
micropipette tips (labeled)

The entire class will share the following:
Centrifuges
Forceps
Boiling water bath
pMCT118 primer/loading dye mix
Timer
DNA thermal cycler

5. Make up a 10% **Chelex solution**: 2.5 g **Chelex** + 25 mL **deionized water**.

6. For each student, **aliquot** (repeatedly dispense) 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.

7. Make up a **0.9% saline solution** by adding 4.5 g NaCl to 500 ml **distilled water** water

8. For each student, pour 10 mL of 0.9% saline solutuon into a 15-mL polypropylene culture tube.

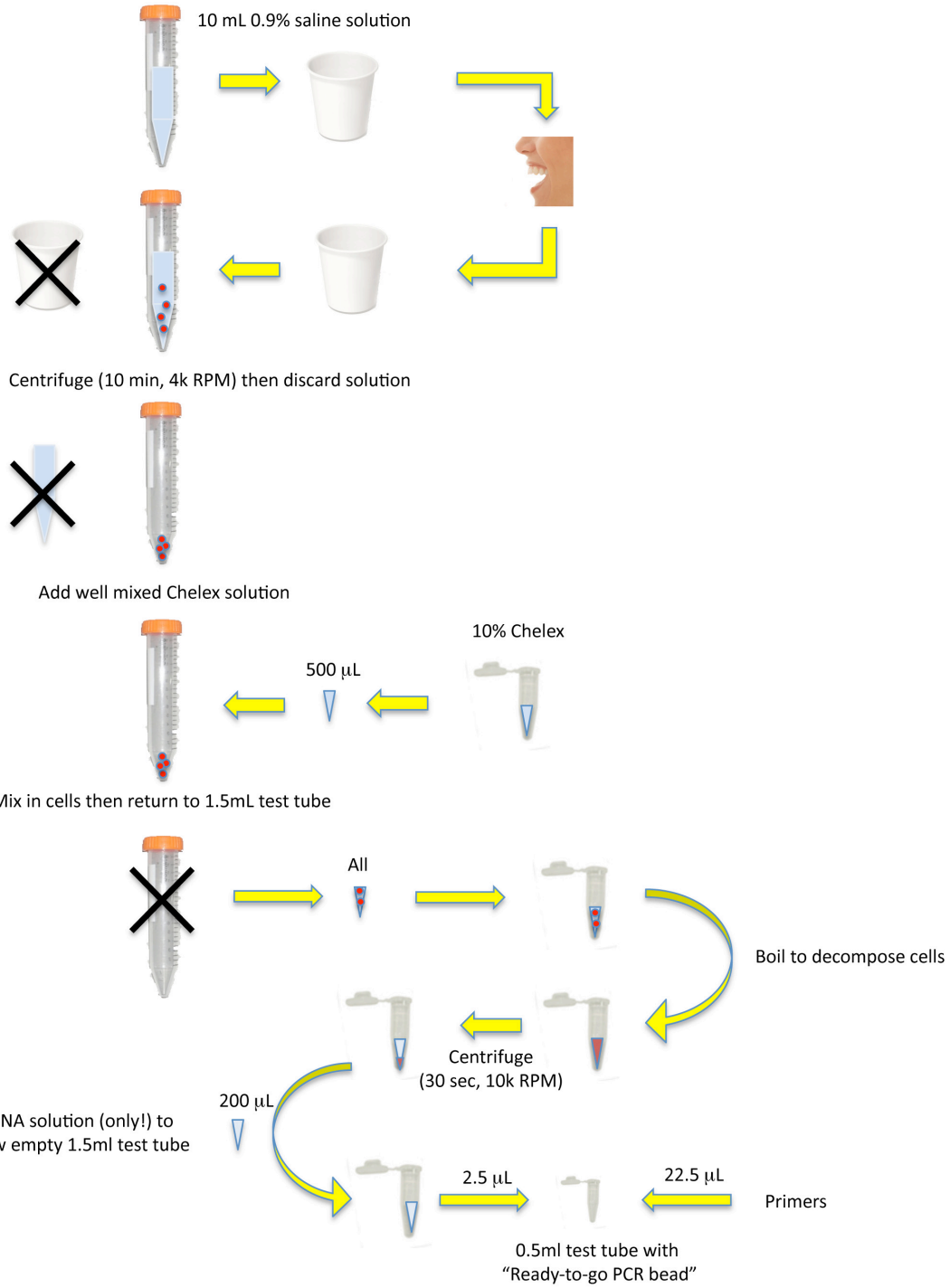
9. Submerge the plastic mockup of the electrophoresis gel beneath about 5 mm of water in a plastic staining tray (first squirt alcohol in wells to eliminate trapped bubbles). Label a plastic container "mock DNA sample solution." In that container mix 400mL **distilled water** + 120mL **glycerol** + 5mL primer/loading dye mix.

10.Where necessary, add balancing vials to centrifuges

Lab Day 1

Schematic of first day student procedures:

University of Virginia DNA Fingerprinting Lab: Extraction of cheek cell DNA for PCR



www.virlab.virginia.edu/Nanoscience_class/labs/materials/UVA_DNA_lab_poster.pdf (J.C. Bean)

Lab Day 1

Student Step-by-Step Procedures

WARNING: Use gloves and other precautions to avoid DNA cross-contamination

Collect Cheek Cells:

1. Draw an identification number from the pile circulated by the TA. Remember this number for both labs - but keep it to yourself (to protect the anonymity of your sample).

2. Use a permanent marker to write the number you drew on the tops of the:

- 15-mL (large) test tube with 10 mL saline (0.9% NaCl) solution:

- 1.5-mL (small) test tube - containing the Chelex solution:
- 1.5-mL (small) test tube - clean and empty (at this point):

- 0.5-ml (tiny) test tube containing "Ready-to-Go PCR bead:"

3. Get a paper cup from the dispenser.

4. Pour the saline solution from the 15 mL test tube into your cup.

5. Take the saline solution from the cup into your mouth and vigorously rinse your mouth for 10 seconds.

6. Expel saline solution into the paper cup.

7. Carefully pour saline solution from the paper cup **back** into the original 15 mL test tube and close the tube's cap tightly (it helps to bend paper cup's rim into a funnel). Dispose of the paper cup.

Concentrate cells (discarding excess solution):

8. Place your 15 mL test tube, together with other student samples, in a balanced configuration into one of the larger centrifuges and spin for 10 min at 4,000 rpm.

While we are waiting:

The instructor will explain our plastic mockup of the electrophoresis gel into which you will load your DNA during the second lab.

9) Practice use of the micropipettes: Including: Setting quantity / loading a tip / load vs. expel stops on plunger / ejecting a tip. ***Always*** load new tip for each fluid transfer, discarding it immediately after use.

10. Carefully pour off the supernatant (clear overlying liquid) into a liquid waste container. Be careful not to disturb the cell pellet at the bottom of the test tube.

Add "Chelex" solution (to which metals & polar cellular components will bind)

11. Thoroughly mix your 1.5 mL (small) test tube containing 10% Chelex resin beads by pressing the bottom end of this tube into the top of the "vortexer."

12. Before the resin settles, rapidly pour ALL of Chelex suspension into the 15 mL (large) test tube containing your cell pellet.

Mix the Chelex solution and cells, transfer back to 1.5 ml test tube:

13. Using the **disposable pipette**, withdraw and expel the solution several times to re-suspend the cells and resin. Then transfer the full volume **back** into your first 1.5-mL (small) test tube (the one that contained the Chelex resin).

14. Discard both the disposable pipette and your 15 mL (large) test tube.

Boil to decompose cells and release DNA

15. Place your 1.5 mL sample test tube in a floating tube rack in the boiling water bath. Do not submerge or drop the tube into the water. Set a timer for 10 minutes

*While you are waiting, for the second DNA lab practice loading **17 µL** of mock DNA sample solution into a specific sample well in the submerged mock electrophoresis gel.*

INSTRUCTOR: Prepare primer/loading dye mix

16. Thaw and then spin the pMCT118 Primer/loading dye mix (balanced by a vial of comparable mass) briefly in the microcentrifuge to drive any solution down out of the vial's cap.

17. After 10 minutes, carefully open the bath's cover using the tabs on the lid's corners (beware of exiting steam). Then use forceps to remove your tube from the boiling water bath and allow it to cool for 2 min.

18. Place your 1.5 mL sample test tube, with others, in a balanced configuration in the microcentrifuge and spin for 30 sec at 10,000 rpm.

Save solution containing your DNA (leaving behind cell debris + chelated metals)

19. Set the 100-1000 µL micropipette (pale blue plunger button) to **200 µL**.

20. With this micropipette, transfer 200 µL of the clear supernatant into your *OTHER* (still clean and empty) 1.5-mL test tube. Be careful not to remove or disturb the Chelex/cell debris at the bottom of the tube.

INSTRUCTOR: ARM the PCR reaction tubes by adding primer/loading die mix:

Note: The PCR reaction tubes are pre-loaded with a "Ready-To-Go PCR Bead" *containing buffered solution of heat-stable Taq polymerase, the four deoxynucleotide (dNTP) building blocks of DNA, and the cofactor magnesium chloride ($MgCl_2$).*

21. Set the 5-50 μL micropipette (orange plunger button) to **22.5 μL**.

22. With this micropipette add 22.5 μL of pMCT118 primer/loading dye mix into the 0.5 mL (tiny) PCR reaction tubes already loaded with a "Ready-To-Go PCR Bead" (this includes all student PCR tubes **plus** the PCR tube awaiting the "Student Sample X" DNA sample). NOTE: On this step ONLY, the pipette tip can be reused for loading all of the tubes.

STUDENTS: To the now fully loaded PCR tubes, add your DNA sample:

23. With its lid closed, tap the PCR reaction tube on the table to help dissolve the chemical bead

24. Set the 2-20 μL micropipette (red plunger button) to **2.5 μL**.

25. With this micropipette add 2.5 μL of your DNA solution to the 0.5 mL PCR reaction tube.

IMPORTANT: *The 2.5 μL DNA sample is so tiny that the droplet will not fall off the tip of the micropipette!* Therefore, bring the tip of the micropipette down so that it is only slightly above the colored reactant solution in the PCR tube. Then, as you depress the plunger, move the pipette to the side transferring the DNA droplet to the wall of the PCR tube. Close the top of the tube and immediately centrifuge (along with other PCR tubes for balance) for 10 seconds in the smallest centrifuge. This should drive the DNA sample down into the PCR reactants.

26. Place your sample in the PCR thermal cycler. IMPORTANT: Samples should be spread evenly over the cycler's 18 pockets to ensure uniform heating and lid pressure.

INSTRUCTOR: Prepare secret "Student Sample X"

- Collect labeled 1.5 mL DNA sample test tubes from students.
- Secretly select one of these tubes (and record its student number).
- Micropipette 2.5 μL of solution from this chosen tube into the final 0.5 mL PCR reaction tube.
- Label the top of that PCR reaction tube with "X"
- Load that final PCR reaction tube into the thermal cycler.

Load the thermal cycler:

- With the thermal cycler still open, turn the knob on its lid counterclockwise until it click
- Close the lid.
- Turn knob clockwise until it clicks (this applies proper pressure to the samples)

Program "VNTR" should already have been selected (page 7) and be blinking on screen.
- Select RUN (button F5) to execute the "VNTR" program.

The unit will take about 5 minutes warming the lid, and will then start cycling. The thermal cycling program will run for ~ 1.5 hours. Time remaining in the program is displayed at the lower right of the cycler's screen. The cycler beeps continuously when its program is complete. ***When PCR thermal cycling program is complete, store samples in freezer until 2nd lab session.***

Lab Day 2

Instructor Pre-Lab Preparation

In this lab prep, quantities are for two labs of nine students, plus a spare gel. Begin by inspecting electrophoresis unit and gel casting trays (clean with water or dilute detergent if necessary)

1. Place the electrophoresis unit on top of the leveled light table, and set up the power supply in a convenient but protected location.

Dilute the buffer solution:

2. Prepare 1.5 liters of **1X SBE buffer** (sodium borate electrophoresis) solution by mixing 75 mL of the **Innovita/Excellgen 20X SBE buffer** solution with 1425 mL of **deionized water** (to be used for three gels and two changes of electrophoresis solution).

> **ALTERNATIVE**: Prepare one liter of 1X **TBE** (tris borate EDTA buffer) solution by mixing 50 mL of the kit's 20X TBE solution with 950 mL of deionized water. Substitute **TBE** for **SBE** in steps to follow.

Prepare the agarose and die mixture:

3. Weigh out 2.25g **agarose** and add it to 150 mL of **1X SBE** in a glass Erlenmeyer flask to produce a 1.5% solution. This will make three gels approximately 5 mm in depth (one for each of the day's labs, plus a spare).

4. Place the **agarose and buffer solution** into the microwave and heat on high for 90 seconds. Take out every 30 seconds or so to swirl the solution. Continue until agarose powder has completely dissolved. Take care not to spill or burn yourself with hot agarose. Agarose, a polysaccharide derived from seaweed, is a common food additive requiring no special handling procedures.

5. Remove the heated **agarose solution** and check its final volume. If it has fallen significantly below 150 ml, top off with **1X SBE solution** and swirl to mix.

6. To the **agarose solution** now add 240 μL of **Carolina BLU gel/buffer stain** (from 7 mL bottle). Swirl the solution to mix.

7. Allow the agarose solution to cool approximately five minutes.

Cast the agarose electrophoresis gels:

8. To produce gels for two classes plus a spare: Install three gel casting trays in the gel-casting fixture (figure at left below).

9. Make sure that the trays are well leveled.

10. When the agarose has cooled to below 60°C, pour it into the casting trays. Use a glass graduated cylinder to measure out 50 mL of agarose solution for each gel tray. (The graduated cylinder will retain less agarose residue if it is first heated via microwaving with some DI water).

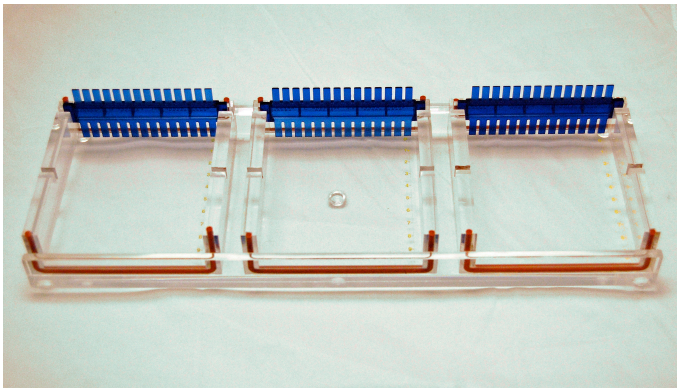
This should produce 0.5 cm deep gel layers. Should the agarose solution have congealed, simply

reheat in the microwave swirling to dissolve the gel, then try again. (A glass cylinder is used in this step to facilitate its later cleaning using hot/boiling water).

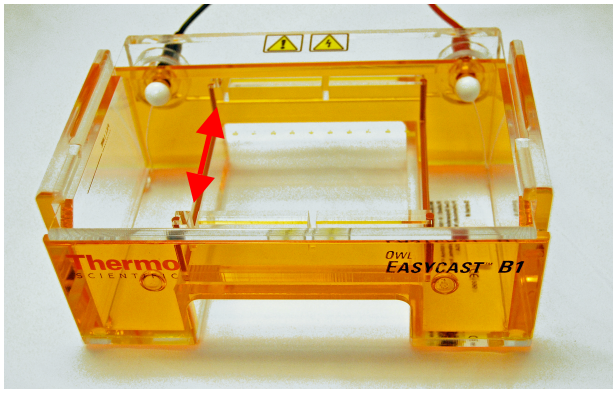
11. Use one of the blue plastic combs to carefully pop or push any bubbles in the gel to the sides.

12. Place a plastic comb in the notches at the end of each tray to create the fourteen sample loading wells in the gel (with the **thicker** teeth of the double-sided combs downward into the cooling gel).

13. When the gels have cooled for at least 30 minutes, gently remove the combs by wiggling them upwards. *While gels are cooling, clean glassware used with hot/boiling water.*



Three gel casting trays in casting fixture. Sample well combs set into tray notches at far end.



Electrophoresis unit with single gel tray. Notch in tray / wells in gel to left (red arrow).

Add buffer & stain to the electrophoresis unit:

14. Pour 450 mL of the **1X SBE buffer** into the electrophoresis unit.

15. Then add 1150μL of **Carolina BLU gel/buffer stain** (from 7 mL bottle). Stir until color is uniform.

For each lab, load the gels into the electrophoresis unit:

16. Place a gel tray in the electrophoresis unit. Tray should be on the raised center area, between the raised stops. *The wells in the gel must be to the left*, adjacent to the negative black electrode so that negatively charged DNA fragments will be driven rightward when voltage is applied (figure above, at right).

17. The gel should now be submerged by 1-5mm **1X SBE buffer solution**. If not, carefully add a small amount of 1X SBE buffer solution.

Thaw Student DNA samples:

18. About 30 minutes before the class, remove the PCRed student DNA samples (and sample X) from the freezer and allow them to thaw.

For second class replace electrophoresis solution:

19. Pour 450 mL of the **1X SBE buffer** into the electrophoresis unit.

20. Then add 1150μL of **Carolina BLU gel/buffer stain** (from 7 mL bottle). Stir until color is uniform.

Lab Day 2

Student Lab Procedures

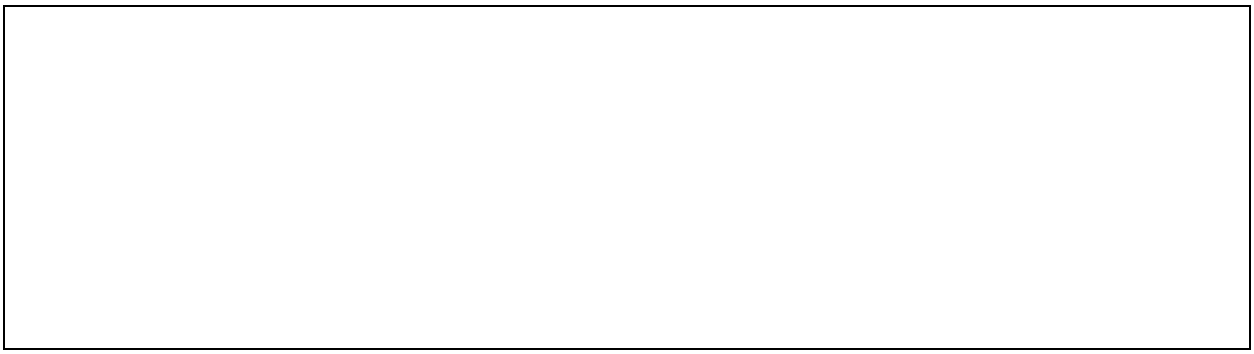
INSTRUCTOR: Review pre-lab gel preparation procedures (above) with the students.

STUDENTS: At one end of the gel, there are fourteen tiny rectangular pockets. Theses pockets will be filled with your PCR amplified samples. The PCR solution is heavier than the buffer solution in which the gel is submerged. It is thus ONLY necessary to inject a sample into the TOP of a well, after which it will float down and stay trapped (at least until the electrophoresis voltage is applied to drive it sideways through the gel.

But, to keep all of your samples separate, it is imperative that: a) You carefully pipette your sample into ONLY your assigned well through the loading template we've prepared; b) That your pipette NOT penetrate through the gel at the bottom of your well (which would then liberate your sample to flow everywhere).

As a group, load sample "X," DNA marker lanes, and agree upon lane assignments:

1. Decide on lane assignments for student samples. Lanes 1, 7, 8, and 14 will be used for markers and "sample X." From the remaining ten empty lanes (2-6, 9-13) indicate your lane by penciling your sample number into that lane on this representation of the gel:



2. Set a 5-50 μL micropipette (orange plunger button) to **17 μL**.

3. Carefully watch the TA's actions as he/she uses this pipette to load sample "X" into lane 8.

4. Load a new tip (~6 cm long) onto the 5-50 μL micropipette and fill the outer and center lanes (1,7 and 14) with the reference DNA marker solution.

For each student, load your personal PCR amplified DNA sample:

5. Set a 5-50 μL micropipette (orange plunger button) to **17 μL**.

6. With this micropipette add the entire PCR sample/loading dye mixture (17 μL) into its

assigned well in the gel. Expel any air from the tip before loading, and be careful not to push the tip of the pipette through the bottom of the sample well.

Proceed with electrophoresis (and begin work on lab report):

7. Carefully place the lid (with attached electrical cables) on the electrophoresis apparatus.

8. Turn on electrophoresis power supply to electrophorese at 200 volts for 35-40 min. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

ALTERNATIVE: If **SBE** buffer has been substituted, 130 volts should instead be used and longer electrophoresis times many be required.

While electrophoresis proceeds, begin work on Part I of the "Lab Report" (below).

Stain gel to reveal positions of DNA fragments (and continue work on lab report):

9. Slide the gel out of its plastic casting base and place it in a staining tray (12 x 12 cm^2 clear square plastic box).

10. Add "CarolinaBLU™ final DNA stain" to trays (large bottle), covering the gel. Place on the oscillating mixing table, and mix for 15 min.

While waiting, continue work on Part I of the Lab Report.

11. Decant stain from trays.

12. Add DI water to trays, covering gels to leech out excess stain.

13. Place tray with gel and DI water on light table. Refresh the DI water when it becomes discolored with stain. Continue this "destaining" process for ~ 5-10 min.

While waiting, continue work on Part I of the Lab Report.

14. Examine the "destained" gel on the light table and sketch your lane:

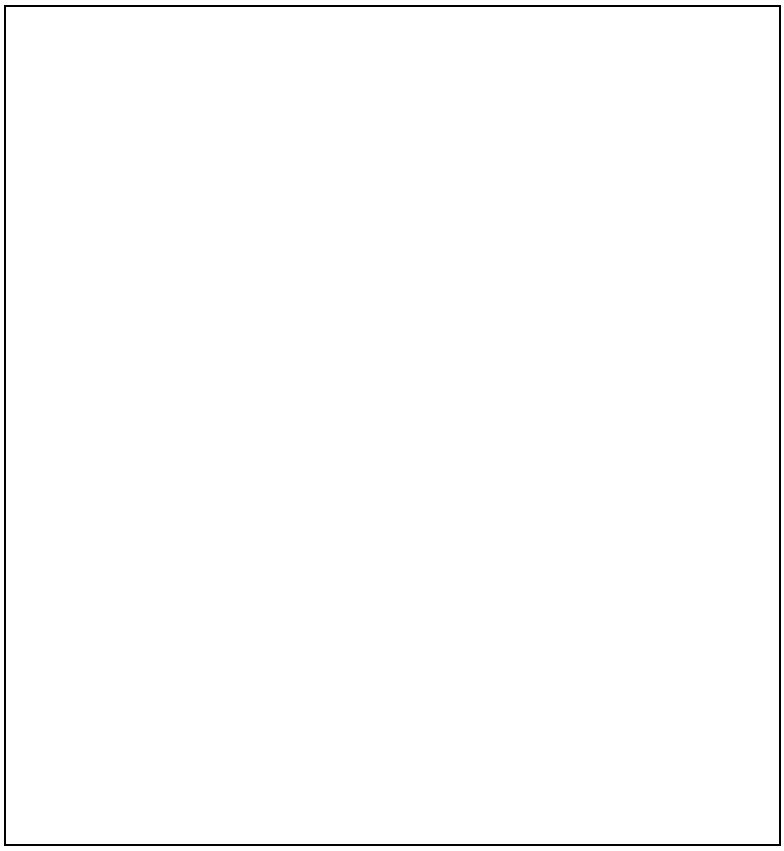
Complete the Lab Report and hand in.

Lab Report

Printed Name: _____ Signature: _____

Part I: Background Information and Analysis

1. The following diagram represents gel electrophoresis results for the pMCT118 genotypes of 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. Identify allele pairs in the remaining lanes of the above gel (*one answer is already entered*):

Lane 2: Lane 3: 18, 18 Lane 4:

Lane 5: Lane 6:

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

c. Which alleles appear to be most common?

2. The table on the following page gives the frequencies of the pMCT118 alleles in several populations.

a. Which three alleles might be the LEAST useful in identifying the population from which a DNA sample originated (i.e. alleles common in *all* populations)?

b. Which three alleles might be the MOST useful in identifying the population from which a DNA sample originated (i.e. alleles that occur in *only* certain populations)?

Allele inheritance probabilities can be quantified by assuming a “Hardy-Weinberg equilibrium” in which ”the alleles for the next generation for any given individual are chosen randomly and independent of each other” (Wikipedia).

Under that assumption, one can calculate the probability that an individual will have a specific pair of alleles. If the allele P occurs in the population with a probability p, and another allele Q occurs with a probability q, then one can set up a ”Punnet Square” predicting the probability that a child inherits alleles P and Q from its parents:

		Mother	
		Prob. of P on first chromosome = p	Prob. of Q on second chromosome = q
Father	Prob. of P on first chromosome = p	Prob. of yielding P, P in child = p^2	Prob. of yielding Q, P in child = qp
	Prob. of Q on second chromosome = q	Prob. of yielding P, Q in child = pq	Prob. of yielding Q, Q in child = q^2

Yielding net probabilities in the child for these alleles:

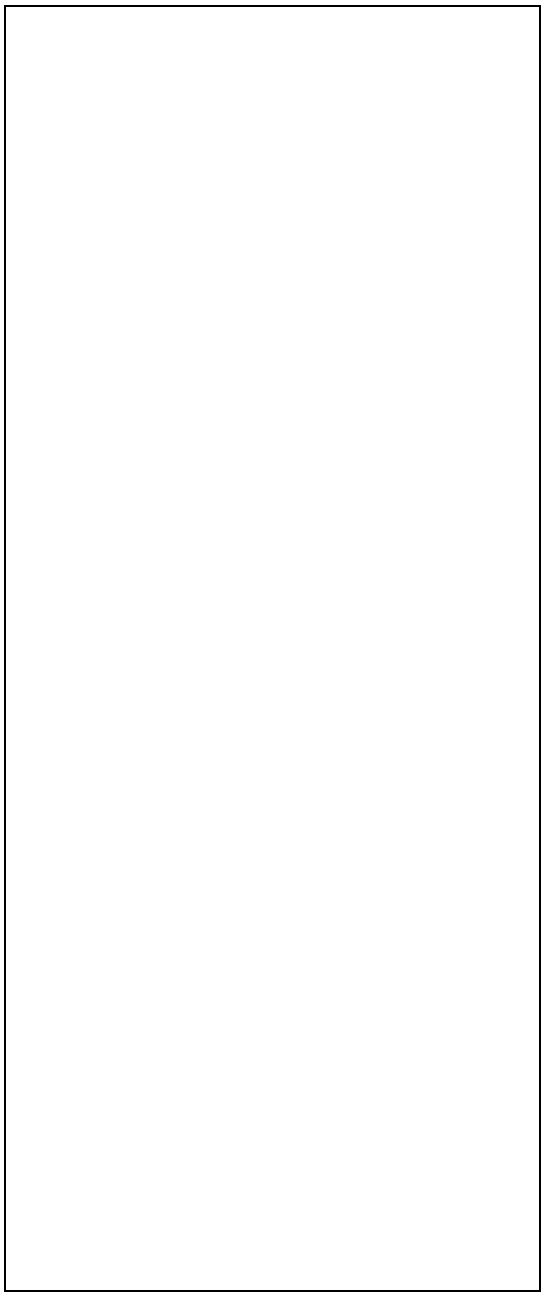
Prob. of P, P = p^2 Prob. of Q, Q = q^2 Prob. of P, Q = 2pq

Using the data below, the frequency of heterozygous genotype 18, 24 in U.S. Caucasians is:

2(0.265)(0.320) = 0.170

The frequency of the homozygous genotype 24, 24 is:

(0.320)(0.320) = 0.102



d. Use the table of allele frequencies to calculate the genotype frequencies, by population group, for allele pairs represented in lanes 2–6 of the gel in Question 1. Fill in your answers on the chart below (*one line already filled in*):

Sample	Genotype	U.S. Caucasian	African American	U.S. Hispanic
2				
3				
4	28, 31	0.006	0.021	0.005
5				
6				

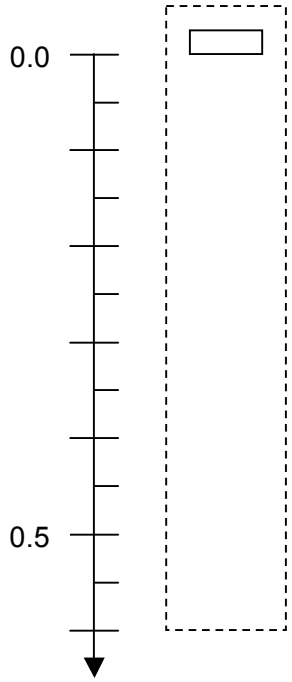
3. In our lab, the marker lanes of the electrophoresis gel will not contain all pMCT118 alleles (as represented in problem #1). Instead, we are using a "pBR322/BstN1" marker solution that contains DNA fragments with base pair lengths of 1857bp, 1058bp, 929bp, 383bp, and a faint final fragment at 121bp.

Shorter DNA fragments will migrate faster through the pores in the gel. This behavior can be approximated by the relationship:

D = 1/ log MW *where D is the distance migrated under electrophoresis, and MW is the DNA fragment's molecular weight*

Defining the mass of the average base pair to be 1, calculate the relative distance our markers fragments should move. Enter results in the following table and plot in the marker lane:

Marker Mass	Distance Moved
1857	
1058	
929	
383	
121	



Part II: Analysis of this lab's results

Observe the stained gel containing your sample and those from other students.

a. Scan across the gel 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. The pMCT118 VNTR alleles in your own DNA range between 929 and 383 base pairs in length. This would place them between the 929bp and 383bp lines of "pBR322/BstN1" marker solution you analyzed in problem 3, above.

c. It is common to see an additional band lower on the gel. This diffuse (fuzzy) band is a "primer dimer" which occurs when the two primers connect by overlapping a few end base pairs. PCR then replicates this ~50 bp long assembly (from Wikipedia):

d. Additional faint bands, at other positions, occur when the primers bind to chromosomal loci other than pMCT118 and give rise to "nonspecific" amplification products.

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

2. 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:

Alleles: 29 Heterozygosity: 72% of people Matches: 1 in 18 people

What reasons can you give for differences?

3. Which student, identified by number, appears to be "sample X"? (Or which students?)

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?