

## ALU POLYMORPHISM, POLYMERASE CHAIN REACTION & GEL ELECTROPHORESIS

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### Laboratory Kit Contents

<u>Sent Out</u>	<u>Returned</u>	
<input type="checkbox"/>	<input type="checkbox"/>	1 ice container (cooler)
<input type="checkbox"/>	<input type="checkbox"/>	____ tube(s) 10% Chelex solution (keep on ice)
<input type="checkbox"/>	<input type="checkbox"/>	____ tube(s) Proteinase K solution (keep on ice)
<input type="checkbox"/>	<input type="checkbox"/>	TPA-25 Primers/Buffer/Loading Dye solution (keep on ice)
<input type="checkbox"/>	<input type="checkbox"/>	DNA Ladder (keep on ice)
<input type="checkbox"/>	<input type="checkbox"/>	6 small, ice bath containers
<input type="checkbox"/>	<input type="checkbox"/>	small scissors ( ____ pairs)
<input type="checkbox"/>	<input type="checkbox"/>	6 micropipettors (2-20 ul volume)
<input type="checkbox"/>	<input type="checkbox"/>	6 boxes of 2-20 ul micropipettor tips
<input type="checkbox"/>	<input type="checkbox"/>	6 micropipettors (100-1000 ul volume)
<input type="checkbox"/>	<input type="checkbox"/>	3 boxes of 100-1000 ul micropipettor tips
<input type="checkbox"/>	<input type="checkbox"/>	3 vortex mixers (1 per two groups)
<input type="checkbox"/>	<input type="checkbox"/>	3 microcentrifuges with power cord (1 per two groups)
<input type="checkbox"/>	<input type="checkbox"/>	1 Thermocycler
<input type="checkbox"/>	<input type="checkbox"/>	____ (# of students) 0.2 ml PCR Ready-to-Go beads
<input type="checkbox"/>	<input type="checkbox"/>	6 fine-point permanent markers
<input type="checkbox"/>	<input type="checkbox"/>	1 Digital camera
<input type="checkbox"/>	<input type="checkbox"/>	1 Printer
<input type="checkbox"/>	<input type="checkbox"/>	____ FlashGel® power base units with electrical cords
<input type="checkbox"/>	<input type="checkbox"/>	____ FlashGel® 1 .2% agarose gels (place in orange bag)
<input type="checkbox"/>	<input type="checkbox"/>	Several used gels for student micropipette practice
<input type="checkbox"/>	<input type="checkbox"/>	1-2 box(es) of disposable gloves
<input type="checkbox"/>	<input type="checkbox"/>	6 small autoclavable bags for tube and tip disposal
<input type="checkbox"/>	<input type="checkbox"/>	____ orange autoclavable bag for used FlashGels
<input type="checkbox"/>	<input type="checkbox"/>	CCA Science Department DVD
<input type="checkbox"/>	<input type="checkbox"/>	3-ring notebook with protocols and other supplementary materials
<input type="checkbox"/>	<input type="checkbox"/>	____ (# of students) 0.5 ml microcentrifuge tubes (colored)

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### Materials and Supplies Needed

#### Per Student:

- 0.5 ml microcentrifuge tubes (1 per student)
- 0.2 ml tubes with Ready-to-Go PCR bead ®

#### Per table: (class can contain no more than six groups)

- 1:1 mixture of 10% Chelex 100/ 100ug/ml Proteinase K (1 small test tube per lab table)
- permanent lab markers
- small, sharp scissors
- tube racks
- 100-1000 ul micropipettors (1 per lab pair or lab table)
- 2-20 u1 micropipettors (1 per lab pair or lab table)
- box of 100-1000 u1 micropipettor tips
- box of 2-20 u1 micropipettor tips
- 1 or 2 pairs of sharp scissors (1 per lab pair)
- Ice bath containers (square)

#### Per classroom (place in a central location)

- TPA-25 Primer
- small autoclavable bags for used tips and tubes
- clock or timer
- vortexers ( 1 per 2 groups = 3 total)

- microcentrifuge (1 per 2 groups = 3 total)
- Thermocycler
- 1.2% agarose FlashGels ® with 34 lanes (1 gel per class)
- FlashGel ® powerbase units
- disposable gloves (1 box per class)
- exACT Gene PCR DNA Ladder or other size marker (1 tube per class)
- Digital camera and printer

**Optional:**

- \* tubes of colored water for micropipettor practice
- \* used Flash-Gels ® for well-loading practice
- \* Small forceps (1 per table or several available in classroom)

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### Pre-Lab Preparation

#### Student Skills/Knowledge Required for Success

- Understanding of metric units of measurement (e.g. ml, ul, etc)
- Use of micropipettor to measure small volumes in ul using sterile tips
- Handling of microcentrifuge tubes
- Use of **microcentrifuge (balance of tubes, setting parameters, etc.)**
- Use and purpose of Vortexer
- Loading of wells in FlashGel ® agarose gels
- Safety issues (e.g. wearing gloves, handling gels, waste disposal, etc.)

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### *Teacher Guide Lab Set-up:*

1. Make sure the Thermocycler is placed on a firm surface in a location that will allow the ventilation slit at the upper back of the-machine to remain uncovered and to allow air to flow under the machine for cooling purposes. No paper should be under the machine. Plug in the Thermocycler and make sure it is turned on with the button on the back of the machine.

**Practice using the Thermocycler prior to class time.** For this experiment, you will start the Thermocycler three different times, using three different programs:

1. For the incubation phase, the program is called “WARM”
2. For the boiling phase, the program is called “BOIL”
3. For the PCR phase, the program is called “PCR”

Note: there is a factory loaded program called ‘incubate’ – DO NOT USE THIS ONE.

\*To start a program: go to the “**main menu**” on the left hand side of the screen on the Thermocycler. **Start should be highlighted on the left hand side. Push the Enter button. You should see the three programs on the right-middle are of the screen. Use the arrow keys to go to the proper program “WARM,” “BOIL,” or “PCR.” Then press ENTER.** Your program should now say started and will run for the allotted time while flashing “RUNNING”. Do not abort the program before it is finished because this will influence your results. Once your program is finished it will say FINISHED flashing at the bottom of the screen just like it did for running.

\*\*\*\*\* IF you don’t see the main menu, push the EXIT button until it comes up. **If it says a program is running** (flashing Warm or any other program running), stop the program. To stop the program, push the “STOP/START button, at the bottom if it says program: stop... then push ENTER. Then it will say “WARM aborted” and the screen will become blank for a moment and the screen will return to where you can go to the main menu and scroll down to start on the left side and run the programs like normal.

2. Each small, colored test tube in this kit (1 tube for each lab table for a total of 6 tubes per class session) already contains 1.0 ml (1000 ul) of a 10% Chelex solution. [Note the very small beads at the bottom of the tubes]. **Right before the first lab session you will need to thaw the frozen tube containing the 100ug/ml Proteinase K solution and add 1.0 ml (1000 ul) of Proteinase K (the entire small bottle) to each of the tubes containing Chelex (the big bottle) that will be at each lab table. \*If you do not see any Proteinase K bottles, then they are already mixed into the Chelex!**

Label these tubes Chelex/ Proteinase K. Each tube should contain 2.0 ml (2000 ul) of this solution which should be plenty for each student at each table to use 300 ul for this lab activity.

Between uses keep all tubes containing Proteinase K on ice or in the refrigerator.

3. Place in a central accessible location the following lab supplies/equipment

First lab session (Part A)

- Thermocycler
- vortexers
- microcentrifuges

\* **ice bath in bucket (must be provided by instructor)!!**

\* several small forceps

\* additional 0.5 ml microcentrifuge tubes

Second lab session (Part B):

- 1-2 1.2% agarose FlashGels ® with 34 lanes each
- 1 FlashGel ® power base unit
- disposable gloves (for handling gels)
- one microcentrifuge tube containing DNA ladder
- Printer

- Digital camera

4. Set out supplies at each table of two lab pairs. Each lab table should have:

First Lab Session (Part A):

- small test tube containing 2.0 ml of Chelex/Proteinase K solution
- one permanent lab marker
- two pairs of small sharp scissors
- four 0.5 ml microcentrifuge tubes
- one 100-1000 ul micropipettor
- one box of sterile tips for 100-1000 ul micropipettor
- two 2-20 ul micropipettors
- one box of sterile tips for 2-20 ul micropipettor
- one microcentrifuge tube containing 120 ul TPA-25 Primer/loading dye/buffer

**to be distributed and monitored by the instructor**

- four 0.2 ml tubes with Ready-to-Go PCR ® beads
- one small autoclavable bag for used tips and tubes

Second Lab Session (Part B):

- two 2-20 ul micropipettors
- one box of sterile tips for 2-20 ul micropipettors
- one small autoclavable bag for used tips and tubes (same bags from 1st lab session)

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### Procedures for Lab Session

#### Part A: Isolation of Hair Sheath DNA and Set Up for PCR Reactions

##### *Student Guide*

**NOTE:** *There are enough reagents for EVERY student to run their own sample!*

1. Label a 0.5 ml microcentrifuge tube on the cap with your name or initials (use three initials because it is possible that other students might have the same two initials e.g. CLB.)
2. Examine the small test tube at your lab table containing a solution of 10% Chelex and 100 ug/ml Proteinase K solution. Notice the small clear beads at the bottom of this tube. Shake this capped tube so that these Chelex beads are suspended throughout the solution.

Before the beads settle to the bottom again, use the 100-1000 ul micropipettor to transfer 300 ul of this Chelex/Proteinase K solution to your labeled microcentrifuge tube. Each student should shake this capped test tube as necessary before making the transfer to make sure that **each 300 ul includes a mixture of beads and liquid.**

Allow the contents of your microcentrifuge tube to settle and then examine it to make sure there are beads at the bottom.

3. Carefully and individually pull out 3-5 hairs from your scalp, and inspect each for the presence of a sheath. The sheath is a thickening of cells at the base of a hair strand and can be observed by holding the hair up to the light or against a dark background. **Note: It is much easier to use eyebrow hairs for this procedure.** Try to get three hairs with thick sheaths so you will have enough cells from which to isolate DNA. If the sheaths are not especially thick, you may need to use additional hairs (5) to get good results.
4. Hold the hairs you have collected directly over your open microcentrifuge tube and use the small scissors to snip these hairs just above the sheaths, so that short hair segments with sheaths drop into the Chelex/Proteinase K solution. If any of the short hair pieces get

stuck on the inner sides of the tube, use a sterile micropipettor tip to push the pieces down into the solution at the bottom of the tube so they are submerged.

5. Close the cap tightly on your microcentrifuge tube, make sure your name or initials are on the cap and place your tube in one of the larger holes in the Thermocycler for 10 minutes. Make sure the Thermocycler is closed and set to the right tube setting with the nob (upper left one). Note the Thermocycler is set to the program "WARM." The samples will be warmed to 37° C. Note the time or set a timer for 10 minutes.

6. After 10 minutes remove your sample tube from the Thermocycler and use the vortex mixers to thoroughly mix the solution for 5-15 seconds to dislodge cells from the hair shafts.

7. Place your sample tube back into the larger hole of the Thermocycler for 8 minutes and note the program is set to "BOIL." Make sure again that the closed nob is set to the larger tube setting (upper left side). Note the time or set the timer for 8 minutes

8. After 8 minutes, the Thermocycler will automatically cool the samples at 4° C. Allow it to cool for 2 minutes.

9. Vortex your sample tube for 5-15 seconds using the vortex mixers.

10. Place your sample tube along with those of other students in one of the microcentrifuges (using special 0.5 mL adaptors) so they are **balanced** for spinning. Create balance by spacing the tubes so that they are evenly distributed in the holes. If needed, use an empty microcentrifuge tube to balance your samples! **IT IS ABSOLUTELY NECESSARY FOR THE MICROCENTRIFUGES TO BE BALANCED BEFORE SPINNING!** Spin for 10 seconds.

After spinning, carefully remove your tube from the microcentrifuge without shaking or tilting it upside down. Observe the Chelex beads and other debris at the bottom of the tube. Set your sample tube in the microcentrifuge rack at your table.

11. Obtain one 0.2 ml tube containing a Ready-to-Go PCR ® bead. Label the top of this tube with a letter/number combination as assigned by your teacher. Record this

letter/number in your lab notebook; you will need to know this number after the PCR process is complete and will also use this letter/number to load your finished sample in an electrophoresis gel along with the samples of other students.

12. Use the 2-20 ul micropipettor to transfer 20 ul of the TPA-25 primer/loading dye/buffer solution at your table to your 0.2 ml PCR tube. The Ready-to-Go PCR ® bead contains all the other reagents necessary for the process of Polymerase Chain Reaction. Close the lid tightly and tap this tube gently on the table to help dissolve the bead and mix the reagents.

13. Put a clean tip on the micropipettor. Transfer 5 ul of the supernatant in your 0.5 ml sample tube to the mixture in the 0.2 ml PCR tube. Tap this 0.2 ml PCR tube gently on the table to mix the reagents and make sure they are at the bottom of the tube. If 0.2 ml adaptors are available, you may also use the microcentrifuge to pool the reagents at the tube bottom. As when you spun the previous tubes, make sure the microcentrifuge is balanced for spinning.

14. Place your labeled tube (along with those of the other students) on ice or in the freezer until your teacher can begin the Polymerase Chain Reaction process in the Thermocycler. **DO NOT** ever put tubes into the fridge during any time! When ready to use, make sure the PCR tubes are in the **smaller** holes inside the Thermocycler and the nob is closed on the smaller tube setter (upper RIGHT this time). Make sure the Thermocycler is set to the program “PCR,” and begin the procedure. Depending on the total number of tubes in each class performing this lab activity, your teacher may choose to run all of them at once (limit of 96 reactions per run) at the end of the day. The entire PCR process takes approximately 3 hours to complete.

15. Once the process is complete, the tubes will remain at 4°C in the metal well plate. However, it is recommended that they be removed and placed in the refrigerator until the next lab session. If a power failure occurs while the tubes remain in the machine, the samples will heat up which may damage the DNA.

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### Procedures for Part B — Gel Electrophoresis

#### *Student Guide*

You will work with your classmates to share the agarose FlashGels used to display your PCR results. These FlashGels are quite expensive so it is necessary to share them. Each gel has 34 small wells (holes) – one for each student's DNA sample. One class can use a single gel effectively. A DNA ladder/size marker will be placed in a center lane (#6 on top and bottom) for comparison with the student results on either side.

Your teacher will open the gel and prepare it for a small amount of your sample to be loaded into the FlashGel. This procedure requires concentration and a minimal amount of disturbance to the gel.

1. Carefully load **5ul** of your sample into one of the wells. It sometimes works best if you hold the micropipettor in one hand and steady it by bracing with the other hand. Gently lower the micropipettor tip with your sample into the well and release.
2. Make a note of the number your teacher has written above the well you used so that you can check on the progress of your sample as it is developed!

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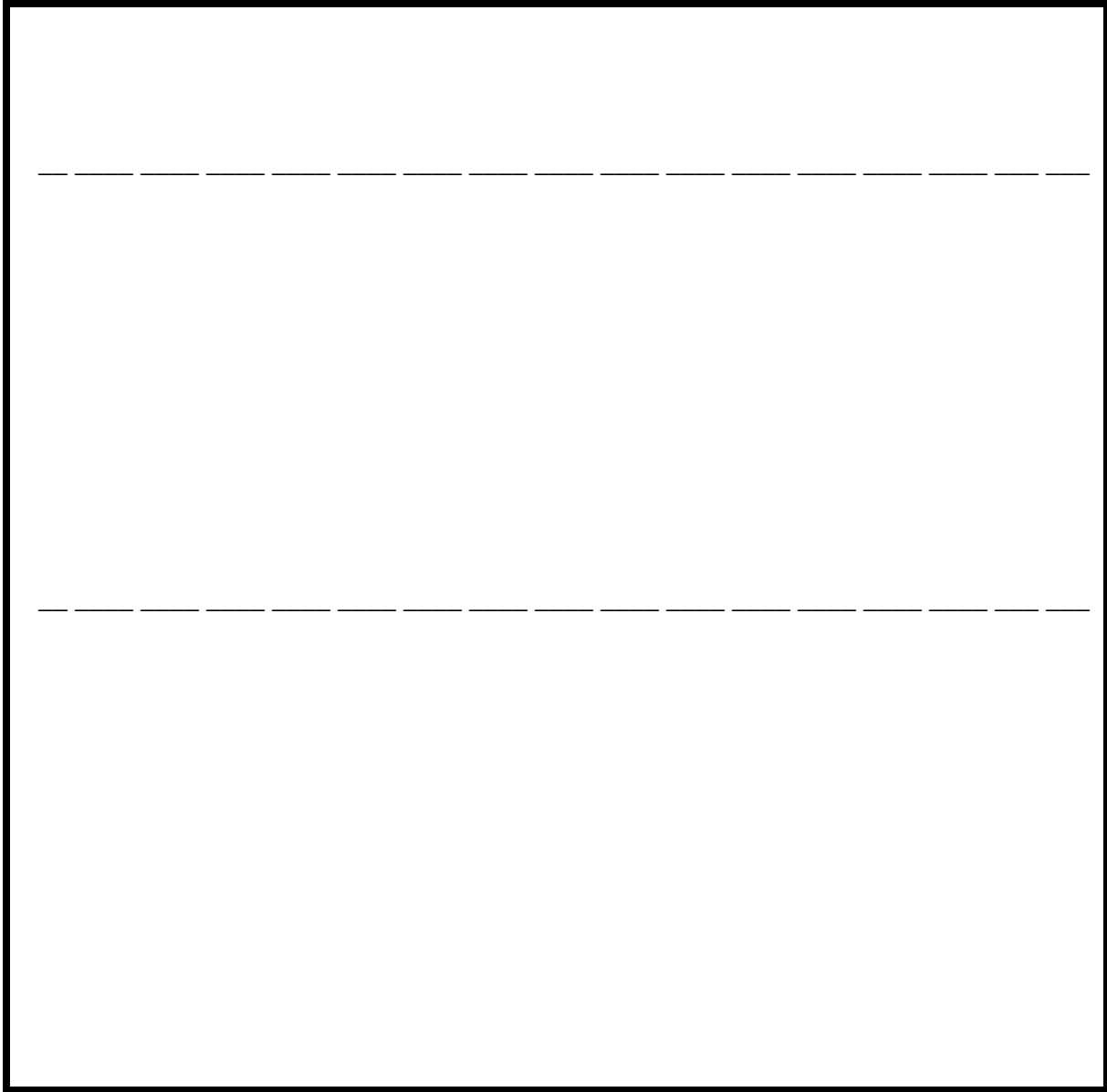
### Procedures for Part B — Gel Electrophoresis

#### *Teacher Guide*

1. Wearing a pair of disposable gloves, open a package containing a 1.2% agarose FlashGel. It is very important that anyone handling these gels wear gloves and handle the gels only at their edges. These prepared gels contain a chemical which will allow the DNA fragments to be seen with an ultra violet light source, but should not come into contact with your skin or be ingested. Avoid rubbing your eyes or face after handling these gels and wash your hands thoroughly after completing this part of the lab activity. Once the cassette has been removed from the bag, remove the white well seals. Remove the elongated thin seal first, followed by the larger seal. Use caution when removing the larger seal by pulling forward (towards yourself) and not straight up. This reduces the amount of air bubbles in the gel. **DO NOT remove the clear, circular side vent seals.**
2. Insert the cassette into the dock by sliding it in gently. Use the FlashGel mask strip in place, underneath the wells of the bottom (or second) row. It is a flexible strip, so it can be moved to block the glowing light from the wells if it is not in place correctly.
3. Mark each well with a number, using the fine-point permanent marker provided
4. Flood the sample wells with distilled water. Tilt the cassette to move excess fluid to the edge and blot off the excess with a kimwipe. **DO NOT** blot the wells.
5. If there are still large amounts of air bubbles in the gel, gently press on the cassette with your fingers and manipulate the bubbles towards the wells for escape. Do not touch the wells or push on the cassette too hard.
6. Carefully **load 5ul of the DNA ladder into the first well(s).**

7. Once there are only a few air bubbles left, supervise students as they load **5ul** of their sample in the wells and make note of the number on the well they use.
8. Plug in the voltage cords to their corresponding outlet in the power supply, i.e. black to black and red to red. Plug in the lower voltage light supply to an available outlet. Set the voltage to **275V**
9. Turn on the light and the voltage and **watch the DNA travel down the gel** with the aid of the built in light source.
10. Run the gel for **2-5 minutes** or until separation of desired fragments is complete.
11. Take digital photographs of each FlashGel using the digital camera.
12. Print pictures using the printer included with the camera.
13. While wearing gloves, remove the FlashGel ® from the docking unit and dispose of in the provided waste bag or box to be returned to CCA.

## Flash Gel Wells



\* Please initial and label your samples for each well on this paper. The first well(s) will be saved for the DNA ladder.