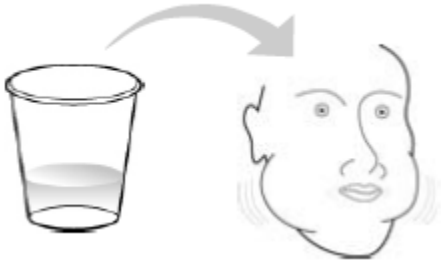
Lab station protocol sheets

ALU & You Lab Activity

Part I: Cheek Cell DNA Template Preparation (Lab Protocol)

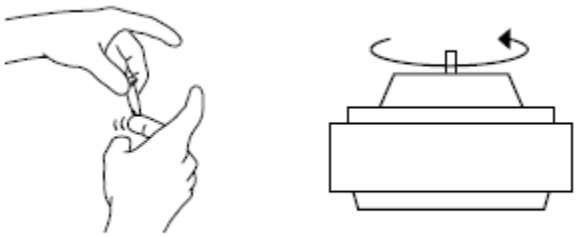
1. Your team of **two students** should have **1 screwcap tube** containing 200 µl InstaGene™ matrix, 1.5 ml **microcentrifuge tube**, and a cup containing 10 ml of **0.9% saline solution.** Label each tube and a cup with your initials.

2. **Do not throw away the saline after completing this step**. Pour the saline from the cup into your mouth. Rinse vigorously for 30 seconds. **Expel (spit) the saline back into the cup**.



3. Carefully use a disposable transfer pipet to place 1 ml of your swished saline into the microcentrifuge tube (use the markings on the side of the microcentrifuge tube and disposable transfer pipette to estimate 1 ml).

4. Spin your tube in a balanced centrifuge for 2 minutes at full speed. When the centrifuge has completely stopped, remove your tube. You should be able to see a pellet of whitish cells at the bottom of the tube. Ideally, the pellet should be about the size of a match head. If you can't see your pellet, or your pellet is too small, pour off the saline supernatant, add more of your saline rinse, and spin again.



Centrifuge

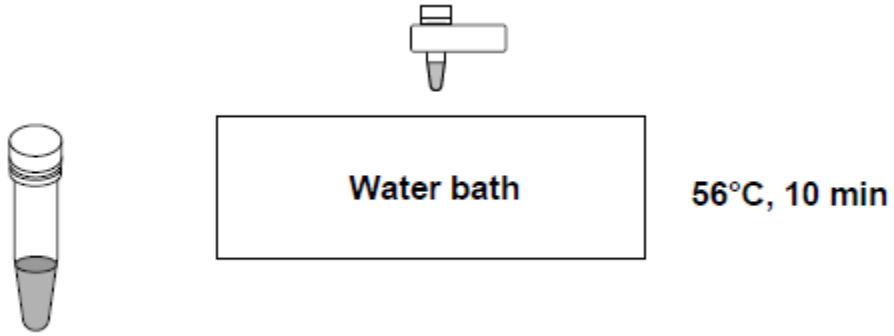
5. Pour off the supernatant (liquid) and discard. Taking care not to lose your cell pellet, carefully blot your microcentrifuge tube on a paper towel. It's OK for a small amount of saline (about 50 µl, about the same size as your pellet) to remain in the bottom of the tube.

6. Resuspend the pellet thoroughly by vortexing or flicking the tubes until no cell clumps remain.

7. Using an adjustable volume micropipette set to 20 µl; transfer your resuspended cells into the screwcap tube containing the InstaGene with your initials. You may need to use the pipet a few times to transfer all of your cells.

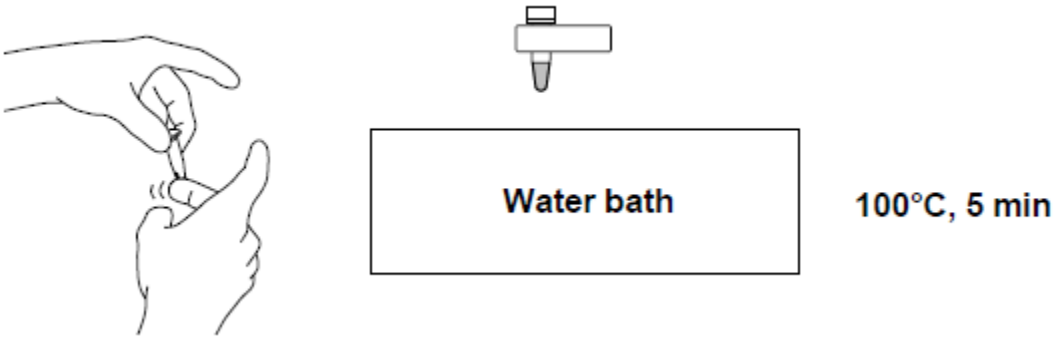
8. Screw the caps tightly on the tubes. Shake or vortex to mix the contents.

9. Incubate the tube for 10 min in the 56°C water bath. At the halfway point (5 minutes), shake or vortex the tubes several times. Place the tubes back in the water bath for the remaining 5 minutes.

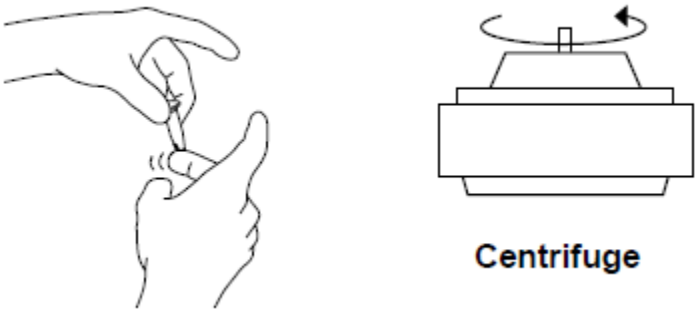


10. Remove the tubes from the water bath and shake them several times.

11. Incubate the tubes for 5 min at 100°C in the water bath (boiling) for 5 minutes.



12. Remove the tubes from the 100°C water bath or dry bath and shake or vortex several times to resuspend the sample. Place the eight microcentrifuge tubes in a balanced arrangement in a centrifuge. Pellet the matrix by spinning for 5 minutes at 6,000 x g (or 10 minutes at 2,000 x g).



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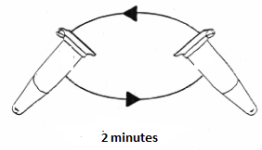
13. Store your screwcap tube in the refrigerator until the next laboratory period, or proceed to step 2 of Lesson 2 if your teacher instructs you to do so.

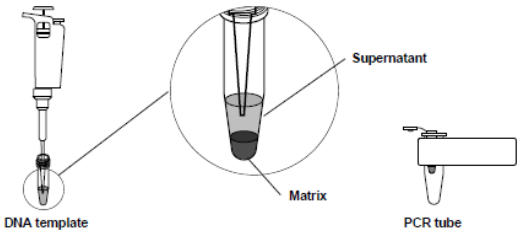
Part II: PCR Amplification (Lab Protocol)

1. Obtain your screwcap tube that contains your genomic DNA template from the refrigerator. Centrifuge your tubes for 2 minutes at 6,000 x g or for 5 minutes at 2,000 x g in a centrifuge.

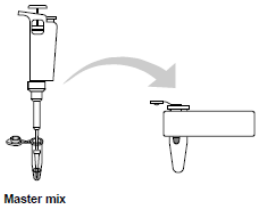


2. Each member of the team should obtain a PCR tube and capless micro test tube. Label each PCR tube on the side of the tube with your initials and place the PCR tube into the capless micro test tube as shown. Place the PCR tube in the foam micro test tube holder.

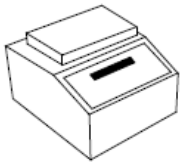
3. Transfer 20 µl of your DNA template from the supernatant in your screwcap tube into the bottom of the PCR tube. **Do not transfer any of the matrix beads into the PCR reaction because they will inhibit the PCR reaction.**



4. 4. Locate the tube of yellow PCR master mix (labeled “Master”) in your ice bucket. Transfer 20 µl of the master mix into your PCR tube. Mix by pipetting up and down 2–3 times. Cap the PCR tube tightly and keep it on ice until instructed to proceed to the next step. Avoid bubbles, especially in the bottom of the tubes.



5. Remove your PCR tube from the capless micro test tube and place the tube in the MyCycler thermal cycler.



6. When all of the PCR samples are in the thermal cycler, the teacher will begin the PCR reaction. The reaction will undergo 40 cycles of amplification, which will take approximately 3 hours.

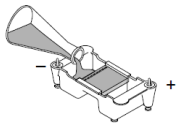
Part III Gel Electrophoresis (Lab protocol)

1. Remove your PCR samples from the thermal cycler and place in the micro test tube holder. If a centrifuge is available, place the PCR tubes in the capless micro test tubes and pulse-spin the tubes (~3 seconds at 2,000 x g) to bring the condensation that formed on the lids to the bottom of the tubes.

2. Add 10 µl of PV92 XC loading dye to each PCR tube and mix gently.

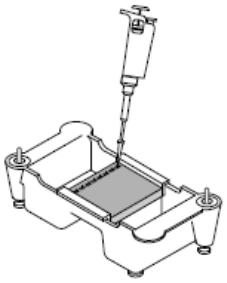
3. Obtain an agarose gel (either the one you poured or one pre-poured by your teacher). Place the casting tray with the solidified gel in it, onto the platform in the gel box. The wells should be at the cathode (–) end of the box, where the black lead is connected.

4. Pour ~275 ml of electrophoresis buffer into the electrophoresis chamber, until it just covers the wells.



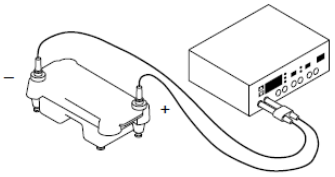
5. Using a clean tip for each sample, load the samples into the 8 wells of the gel in the following order:

Lane	Sample	Load Volume
1	MMR (DNA standard)	10 µl
2	Homozygous (+/+) control	10 µl
3	Homozygous (–/–) control	10 µl
4	Heterozygous (+/–) control	10 µl
5	Student 1	20 µl
6	Student 2	20 µl
7	Student 3	20 µl
8	Student 4	20 µl



6. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.

7. Turn on the power supply. Set it to 200 V and electrophorese the samples for 20 minutes.



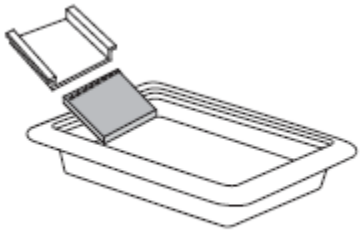
8. When electrophoresis is complete, turn off the power and remove the lid from the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, the gel is very slippery. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.

Directions for Using Fast Blast DNA Stain

WARNING:
Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes.

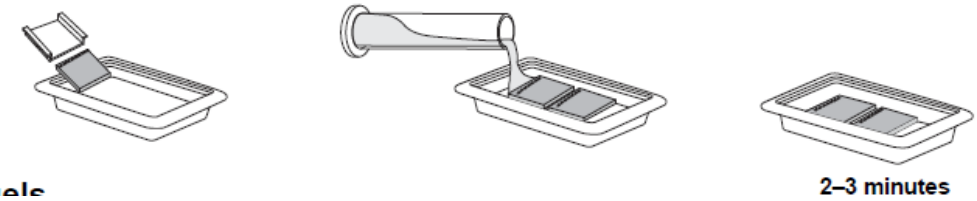
Protocol 1: Quick Staining of Agarose Gels in 100x Fast Blast DNA Stain

1. **Mark the staining tray with your initials and class period.** You will stain 2 gels per tray.



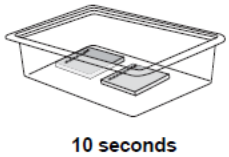
2. **Stain gels (2–3 minutes)**

   - Remove each gel from the gel tray and carefully slide it into the staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gels. Stain the gels for 2–3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100x stain into storage bottle and save it for future use. **The stain can be reused at least 7 times.**



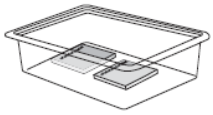
3. **Rinse gels**

   - Transfer the gels into a large container containing 500–700 ml of clean, warm (40–55°C) tap water. Gently shake the gels in the water for ~10 seconds to rinse.



4. **Wash gels**
   - Transfer the gel into a large container with 500–700 ml of clean, warm tap water. Gently rock or shake the gels on a rocking platform for 5 minutes. If no rocking platform is available, move the gels gently in the water once every minute.

5. **Wash gels**
    - Perform a second wash as in step 4.



5 minutes

6. Record and analyze results

- Examine the stained gels for expected DNA bands. The bands may appear fuzzy immediately after the second wash, but will begin to develop into sharper bands within 5–15 minutes after the second wash. This is due to Fast Blast dye molecules migrating into the gel and binding more tightly to the DNA molecules.

- To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not wash the gels in water overnight. If you cannot complete the destaining in the allocated time, you may transfer the gel to 1x Fast Blast stain for overnightstaining.

Dry the agarose gel

To create a permanent record of the experiment place the gel directly upon the hydrophilic size of a piece of gel support film. (Water will form beads on the hydrophobic side of a piece of gel support film.) Center the gel on the film and remove bubbles that may form between the gel and film. Place the film on a paper towel and let the gel dry in a well-ventilated area, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but will not shrink. If left undisturbed on the support film, the gel will dry completely at room temperature after 2–3 days. The result will be a flat, transparent, and durable record for the experiment. Tape the dried gel into your laboratory notebook.

Note: Avoid extended exposure of dried gels to direct light to prevent band fading.
However DNA bands will reappear if the dried gels are stored in the dark for 2–3 weeks after fading.