

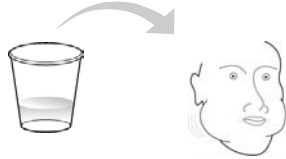
Quick Guide

Lesson 1 Cheek Cell DNA Template Preparation

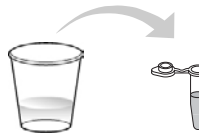
1. Label one 1.5 ml micro test tube with your initials. Label one screwcap tube containing 200 μ l of InstaGene matrix with your initials.



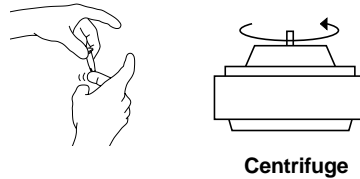
2. Obtain a cup containing saline solution from your instructor. Pour the saline into your mouth and rinse vigorously for 30 seconds. Expel the saline back into the cup.



3. Transfer 1 ml of your saline rinse into the micro test tube (NOT the screwcap tube) with your initials. If a P-1000 micropipet is not available, **carefully** pour ~1 ml of your saline rinse into your micro test tube (use the graduations on the side of the micro test tube to estimate 1 ml).



4. Spin your tube in a balanced centrifuge at full speed for 2 minutes. When the centrifuge has completely stopped, remove your tube. You should see a match-head sized pellet of whitish cells at the bottom of the tube. If you don't see a pellet of this size, decant the saline, refill your tube with more of your oral rinse, and repeat the spin.



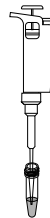
5. After pelleting your cells, pour off the saline. Being careful not to lose your pellet, blot your tube briefly on a paper towel or tissue. It's OK for a small amount of saline (< 50 μ l, about the same size as your pellet) to remain in the bottom of the tube.



6. Resuspend the pellet by vortexing or flicking the tube so that no clumps of cells remain.

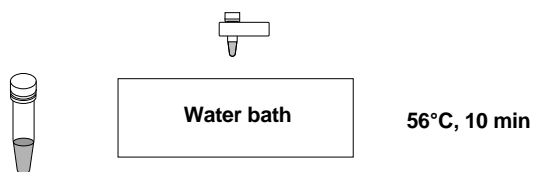


7. Using a 2–20 μ l adjustable-volume micropipet set to 20 μ l, transfer all of your resuspended cells to the screwcap tube containing InstaGene.

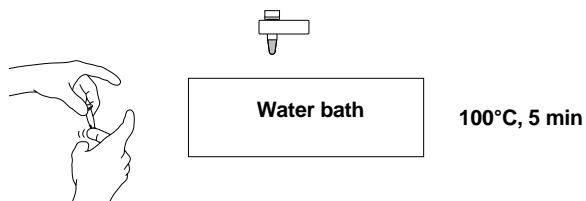


8. Screw the cap tightly on the tube. Shake or vortex to mix the tube contents.

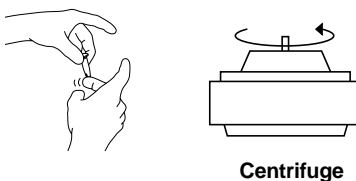
9. When all members of your team have collected their samples, place the tubes in the foam micro test-tube holder, and incubate at 56°C for 10 minutes in a water bath. At the halfway point (5 minutes), shake or vortex the tubes gently, then place back in the 56°C water bath for the remaining 5 minutes.



10. Remove the tubes, shake or vortex, and place the tubes in a boiling water bath (100°C). Incubate at 100°C for 5 minutes.



11. Remove the tubes from the boiling water bath and shake or vortex the contents to resuspend. Pellet the matrix by spinning at 6,000 x g for 5 minutes (or 2,000 x g for 10 minutes) in a centrifuge.



12. Store your screwcap tube in the refrigerator until the next laboratory period (or proceed to step 2 of Lesson 2).

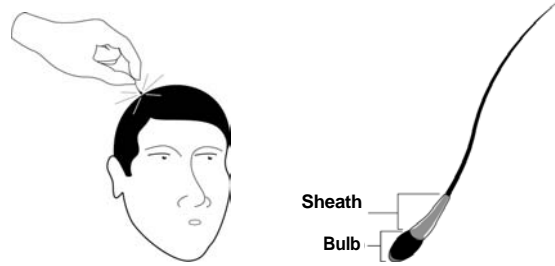
Quick Guide

Lesson 1 Hair Follicle DNA Template Preparation

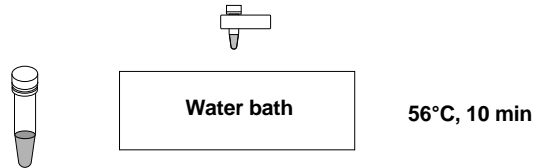
1. Label 1 screwcap tube containing 200 μ l of InstaGene matrix plus protease with your initials.



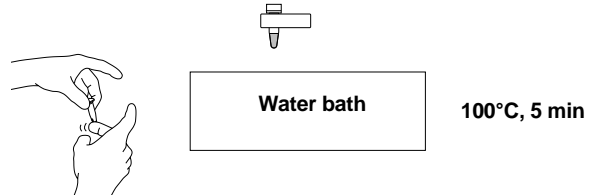
2. Collect 2 hairs from yourself. Select hairs that have either a noticeable sheath (a coating of epithelial cells near the base of the hair), or a good sized root (the bulb-shaped base of the hair). Trim the hair, leaving the last ~2 cm of the base of the hair. Place the trimmed hairs into the screwcap tube with your initials.



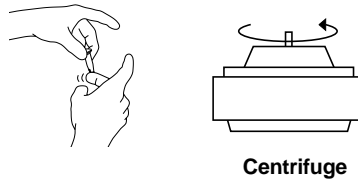
3. Place your tube in the foam micro test-tube holder and incubate at 56°C for 10 minutes in a water bath. At the halfway point (5 minutes), shake or vortex gently, then place it back in the 56°C water bath for the remaining 5 minutes.



4. Remove the tubes, gently shake or vortex, and place them in a boiling water bath (100°C). Incubate at 100°C for 5 minutes.



5. Remove the tubes from the boiling water bath and shake or vortex the contents to resuspend. Pellet the matrix by spinning at 6,000 x g for 5 minutes (or 2,000 x g for 10 minutes) in a centrifuge.

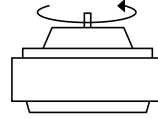


6. Store your screwcap tube in the refrigerator until the next laboratory period (or proceed to step 2 of Lesson 2).

Quick Guide

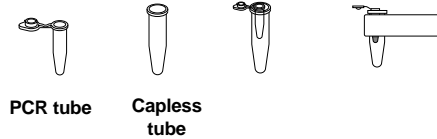
Lesson 2 PCR Amplification

1. Obtain the tube with your DNA template from the refrigerator. Spin the screwcap tube for 2 minutes at 6,000 x g (5 minutes at 2,000 x g) in a centrifuge.



Centrifuge

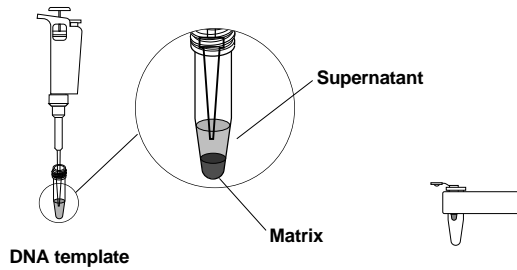
2. Label a PCR tube and a capless micro test tube with your initials, place the PCR tube in the capless tube as shown, and place both in the foam holder.



PCR tube

Capless tube

3. Transfer 20 µl of the DNA template (the supernatant) from the screwcap tube into the bottom of the PCR tube. Be very careful **not** to transfer any of the matrix beads into the PCR tube.

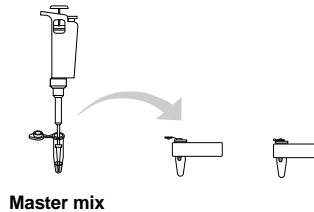


DNA template

Supernatant

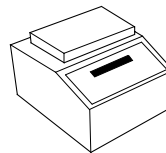
Matrix

4. Locate the tube of yellow master mix on ice and transfer 20 µl of the master mix into the PCR tube. Mix by pipetting up and down 2–3 times. Cap the PCR tube tightly. The mixture should be yellow.



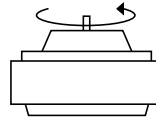
Master mix

5. Place the PCR tube into the thermal cycler. Control reactions prepared by the instructor should also be placed into the PCR machine at this point. The reactions will undergo 40 cycles of PCR amplification.



Lesson 3 Electrophoresis of Amplified PCR Samples and Staining of Agarose Gels

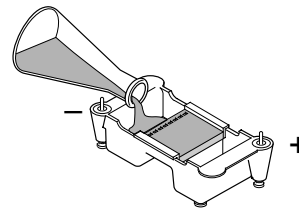
1. Obtain your PCR tube from the thermal cycler and place in the capless micro test tube. Pulse-spin the tube for ~3 seconds at 2,000 x g.



Centrifuge

2. Add 10 μ l of PV92 XC loading dye into your PCR tube and mix gently.

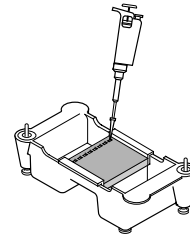
3. Place an agarose gel in the electrophoresis apparatus. Check that the wells of the agarose gels are near the black (-) electrode and the base of the gel is near the red (+) electrode.



4. Fill the electrophoresis chamber and cover the gel with 1x TAE buffer. This will require ~275 ml of 1x buffer.

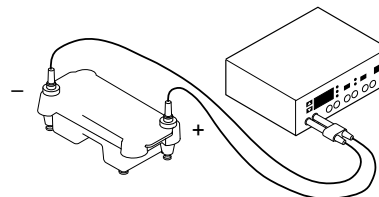
5. Using a clean tip for each sample, load the samples into 8 wells of the gel in the following order:

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



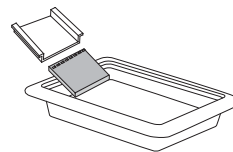
6. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.

7. Turn on the power supply and electrophorese your samples at 100 V for 30 minutes.



Staining of Agarose Gels

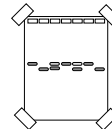
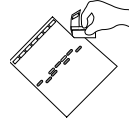
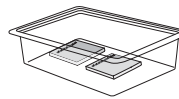
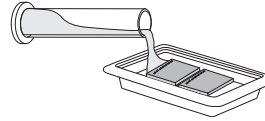
1. When electrophoresis is complete, turn off the power and remove the lid from the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, **the gel is very slippery**. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.



2. There are two protocols for staining your gel. Your instructor will inform you which one you will use.

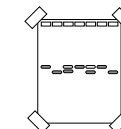
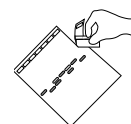
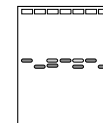
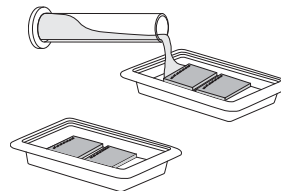
Protocol 1: Quick staining (requires 12–15 minutes)

- a. Add 120 ml of 100x Fast Blast stain into your staining tray (2 gels per tray).
- b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use. Stain can be reused at least 7 times.
- c. Transfer the gels into a large washing container and rinse with warm (40–55°) tap water for approximately 10 seconds.
- d. Destain by washing **twice** in warm tap water for 5 minutes each with gentle shaking for best results.
- e. Place the gel on a light background and record your result. With a permanent marker, trace the wells and band patterns onto a clear sheet of plastic or acetate sheet.
- f. With the help of your instructor, determine whether you are homozygous or heterozygous for the Alu insertion.
- g. Trim away any empty lanes of the gel with a knife or razor blade.
- h. To obtain a permanent record, air-dry the gel on gel support film. Tape the dried gel onto your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.



Protocol 2: Overnight staining

- a. Add 120 ml of 1x Fast Blast DNA stain to your staining tray (2 gels per tray).
- b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.
- c. The next day, pour off the stain into a waste beaker.
- d. Place the gel on a light background and record your result. With a permanent marker, trace the wells and band patterns onto a clear sheet of plastic or acetate sheet.
- e. With the help of your instructor, determine whether you are homozygous or heterozygous for the Alu insertion.
- f. Trim away any empty lanes of the gel with a knife or razor blade.
- g. To obtain a permanent record, air-dry the gel on gel support film. Tape the dried gel into your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.





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