Quick Guide

Lesson 1: Setting up the PCR Reactions

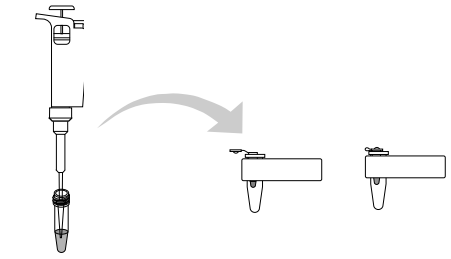
1. Label 5 PCR tubes CS, A, B, C, or D, and include your group name or initials as well. Place each PCR tube into a capless micro centrifuge tube in the foam float on ice.

PCR tube **Capless tube**

2. Using the chart below as a guide, transfer 20 µl of the appropriate template DNA into the correctly labeled tube. **Important: use a fresh aerosol barrier pipet tip for each DNA sample.**

Label PCR tubes	Add DNA template	Add Master mix + primers
CS + your initials	20 µl Crime Scene DNA	20 µl MMP (blue)
A + your initials	20 µl Suspect A DNA	20 µl MMP (blue)
B + your initials	20 µl Suspect B DNA	20 µl MMP (blue)
C + your initials	20 µl Suspect C DNA	20 µl MMP (blue)
D + your initials	20 µl Suspect D DNA	20 µl MMP (blue)

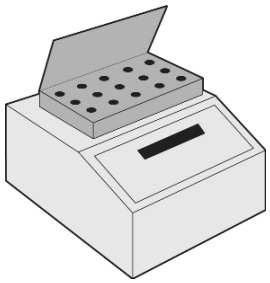
3. Transfer 20 µl of the blue MMP (master mix + primers) into each of the 5 PCR tubes containing template DNA. Pipet up and down to mix. Cap each tube after adding blue MMP. **Important: use a fresh aerosol barrier pipet tip each time. Immediately cap each tube after adding MMP.**



Master mix + primers

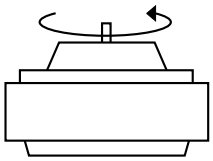
4. Place your capped PCR tubes in their adaptors on ice.

5. When instructed to do so, place your tubes in the thermal cycler. Your instructor will program the thermal cycler for PCR.



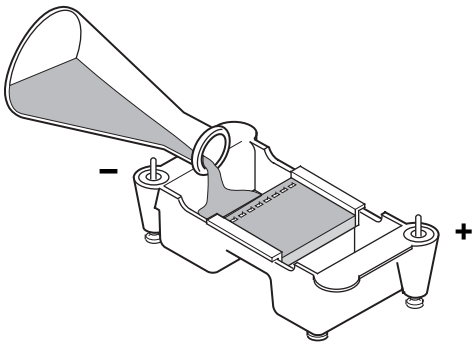
Lesson 2: Electrophoresis of PCR Products

1. Set up your gel electrophoresis equipment as instructed.
2. Obtain your 5 PCR tubes from the previous lesson. Place your PCR tubes in capless tubes and pulse-spin in a balanced microcentrifuge for a few seconds to collect all liquid to the bottom of the tube.



Centrifuge

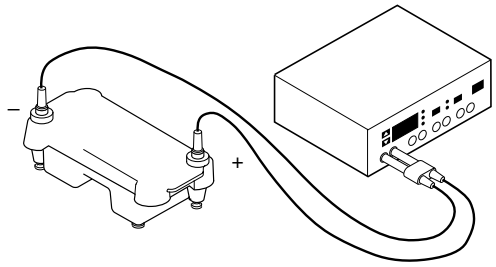
3. Transfer 10 µl of Orange G loading dye (from the tube labeled 'LD') into each of your PCR tubes. Pipet up and down to mix, and pulse-spin to collect liquid in the bottom of the tube.
4. Place an agarose gel in the electrophoresis apparatus. Check that the wells of the agarose gel are near the black (–) electrode and the base of the gel is near the red (+) electrode.
5. Fill the electrophoresis chamber with enough 1x TAE buffer to cover the gel. This will require ~275 ml of 1x TAE buffer.



6. Using a clean tip for each sample, load 20 µl of the samples into 6 wells of the gel in the following order:

Lane	Sample	Load volume
1	Allele Ladder	20 µl
2	Crime Scene	20 µl
3	Suspect A	20 µl
4	Suspect B	20 µl
5	Suspect C	20 µl
6	Suspect D	20 µl

7. 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.



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

9. Stain in Fast Blast DNA stain. Refer to the Student Manual for specific instructions.

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