Quick Start Protocol

Bio-Rad's MyCycler

Before initializing the thermal cycler

1) Do not mark the tops (caps) of the PCR tubes as the ink will be transferred to the heated lid of the thermal cycler. Instead, keep track of the samples using the grid reference system indicated on the green, plastic tube holder, e.g. A1, A2, B1, etc. The PCR tubes can be marked on the shoulder of each tube, below the cap.

2) After adding 10 µL of master mix 1 and 5 µL of the student's genomic DNA to the PCR tube, place all of the tubes in ice. You can use the green, plastic sample loading tray to help keep the tubes upright in the ice.

Initializing the Thermal Cycler

1) Attach the power cord to the thermal cycler.

2) Press the **STANDBY** button, on the front panel, to start the thermal cycler.
The thermal cycler will run through a self-test.

3) Press the **F1** key to access the "Protocol Library."
The "tPA alu" program should be highlighted by default. If it is not highlighted, use the arrow keys on either side of the ENTER key to highlight the tPA alu program.

4) Press **ENTER** to select the "tPA alu" program.

5) Select "Run Protocol," this should be the highlighted default, and press **ENTER**.

6) The screen will change to RUN SETUP. Press **F5** to "Begin Run."

Preparing Samples for Thermal Cycling

1) Add 10 µL of master mix 2 to each tube and return them to the ice. When you've added master mix 2 to all of the tubes, open the lid of the thermal cycler and carefully align the green, plastic sample holder tray over the reaction block.

2) Close and lock the lid.

3) Press the **F1** key to "Resume Run." When the tPA alu program is completed- about 3 hours- the thermal cycler will hold the tubes at 4ºC indefinitely.

4) When the program is completed, press **F4** and return to the "Home Screen."

5. Turn off the thermal cycler by holding down the **STANDBY** key for 2 or 3 seconds.

Preparing PCR Products for Gel Electrophoresis

1) Add 3 µL of Orange-G loading dye to each PCR sample.
2) Load 15 µL of each sample into a 2% Agarose gel. *Note: Prepare 25 mL of a 2% Agarose solution using 1x SB buffer. You may want to double-comb this gel.