

Lab 9 – CSI PCR Lab

See handout for background

Purpose: To improve the students' ability to conduct a PCR, interpret the results, and better understand the implications of PCR.

Day 1 Procedure

1. Label 5 PCR tubes CS, A, B, C, and D, and include your initials as well. Place each PCR tube into a PCR tube rack that is on ice.
2. Using the chart below as a guide, transfer 20 μ L of the appropriate template DNA into the correctly labeled tube. Important: use a clean pipette tip for each sample.

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

3. Transfer 20 μ L 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 clean pipette tip for each sample.
4. Place your capped PCR tubes in their rack on ice.
5. When instructed, place your tubes in the thermocycler. The instructor will program the thermocycler for PCR.

Day 2 Procedure

1. Calculate how much agarose is needed to prepare a 50 mL 3% agarose gel. Have the instructor verify and initial your calculations before proceeding to the next set.
2. Obtain your 5 PCR tubes from Day 1. Pulse-spin with a microfuge your PCR tubes to collect all the liquid to the bottom of the tube.
3. Obtain PCR tube labeled "AL" for allele ladder from the instructor's table.
4. Transfer 10 μ L of Orange G loading dye into each of the tubes in step 2 and 3. Pipet up and down to mix, and pulse-spin in a microfuge to collect liquid in the bottom of the tube.
5. Prepare DNA electrophoresis apparatus with a 3% agarose gel supplied by the instructor and TEA buffer.
6. Load 20 μ L of each sample into the gel in the following order:

Lane	Sample	Load Volume
1	Allele Ladder	20 μ L
2	Crime Scene	20 μ L
3	Suspect A	20 μ L
4	Suspect B	20 μ L
5	Suspect C	20 μ L
6	Suspect D	20 μ L

7. Run at 100 mV until the dye front has moved half the distance of the gel.
8. When approved by the instructor, remove the gel and image using the Bio-Doc imager.
9. Tape picture of gel into lab book as data. Label lanes and sizes of the allele ladder.
10. Clean up lab bench and throw away all used samples and tubes.

Data: Tape a copy of the gel picture in your lab book and label all lanes and values of allele ladder.

Conclusion:

Which suspect is linked to the crime scene and how did you determine the indicated suspect?