

Table 6-4. Organization of PCR ingredients.

Part one: Design	PCR Tube #	1	2	3	4	5	
	Name of Sample/Control	positive control	negative control	NTC	unknown #1 (student sample)	unknown #2 (student sample)	
Template DNA (insect sample ID/type of control/none)	positive control						
Primer Mix? (yes/no)	yes						
Expected Result (+/-)	Insect CO1 Gene	+					
	<i>Wolbachia</i> 16S rRNA Gene	+					
Part two: Preparation	Volumes (in μL)	Template DNA (+ control/ - control/ none/ student sample)	μL	μL	μL	μL	μL
		PCR-Grade Water	μL	μL	μL	μL	μL
		Primer Mix	μL	μL	μL	μL	μL
		Final Volume	μL	μL	μL	μL	μL

What reagent volumes should you add to each reaction?

1. You should complete Part two of Table 6-4 (above) by writing the appropriate volumes of each reagent in the columns at the bottom of the table. This table will help you during the preparation of the tubes. (Directions for this process are on the next page.) Some things to consider:
- Each tube should contain a final volume of 25 μL .
 - You should add 23 μL of Primer Mix to any tubes that require the Primer Mix.
 - You should add PCR-grade water instead of template DNA to any tubes that do not require template DNA.
 - Your instructor should verify that the volumes in your chart are correct before you begin to prepare your samples.

Cleared for sample preparation _____

EXERCISE 6

Notes

Part two: Preparing PCR samples

How should you prepare your samples?

Once given permission from your instructor, work with your partner and carefully follow these instructions and use Table 6-4 to prepare your samples at your bench. Use the check boxes to monitor your progress.

- 1. Disinfect your working bench area with the appropriate blue disinfectant.
- 2. Collect your tube of the Primer Mix (one per group of two students) and PCR-grade water (one per group of two students).
- 3. Obtain your insect genomic DNA samples from a previous lab exercise. Both should be found at the instructor's bench. (These are your "template" DNAs for your "unknown" samples.)
- 4. Gently mix each tube by vortexing briefly or by flicking the bottom while holding tightly to the top.
- 5. Use the minicentrifuge to briefly centrifuge all tubes for a few seconds to force down the contents to the bottoms of the tubes. Remove the tubes and place them in the rack at your bench. Make sure that the tubes are balanced appropriately. You may need to work with another student group for balancing.
- 6. Number your five PCR Ready tubes 1-5 on the tops and sides of the tubes. Also, include some symbols or marks, such as your initials, to uniquely identify your tubes within the class set.
- 7. Organize your microcentrifuge tubes in the microcentrifuge tube rack, your PCR Ready tubes in the PCR tube rack, and your pipettes, pipette tips, and waste container on your bench. Remove any unnecessary items. (Your instructor will keep the positive and negative control template DNA at the front podium.)
- 8. Referring to Table 6-4, add the Primer Mix to the appropriate PCR Ready tubes. You may use the same tip for all samples.
- 9. Refer to Table 6-4 to determine which tube should receive PCR-grade water instead of template DNA. Add the correct volume of PCR-grade water to this tube. Tip: When adding small amounts of volume (like 2 μ L) to a tube, directly add the small volume of liquid to the larger volume of liquid (if possible) to ensure that the small volume is indeed added to the mix. This means that you should place your pipette tip directly into the larger volume of liquid when dispensing the smaller volume.
- 10. Add the correct volume of each student template DNA to the respective PCR Ready tubes. Change pipette tips between samples. Refer to Table 6-4 to determine which template DNA should be added to which tube.

- 11. Your instructor has the positive and negative control template DNA, and you should approach the instructor bench to pipette these DNAs into the appropriate PCR Ready tubes only after you have added the other templates to the other tubes. *Remember to change pipette tips between each template!*
- 12. Ensure that all tubes are firmly closed, then gently mix the PCR Ready tubes by flicking the bottoms while holding tightly to the tops.
- 13. Use the minicentrifuge to briefly centrifuge your PCR Ready tubes to force the contents to the bottom of the tubes. Be sure that you only insert your tubes into rotor slots that contain adaptors specifically for PCR (0.2 mL) tubes, and be sure that these tubes are balanced! Most likely, you will need to pair with another group to balance your PCR tubes in the centrifuge with their tubes.

Part three: Using the thermocycler

Check with the instructor to ensure that the thermocycler is programmed and ready to use. Place your PCR Ready tubes into the thermocycler, and note the placement of the tubes here in your lab manual and on the instructor's log. (Labels on PCR tubes often rub off during the temperature cycling, so it is of extreme importance that you record the location of your tubes. Most likely, this will be how you will find and identify your tubes for the next lab exercise.)

My tubes are in thermocycler slots _____.

The following is the thermocycler program that you will use for your reactions. Write the name of the stage on the empty blanks to the right of the temperatures. (Hint: Refer to Figure 6-3 on the next page.) Your instructor will start the thermocycler once all students have loaded their samples.

30 Cycles

30 sec. @ 94 °C _____

45 sec. @ 55 °C _____

1 min. @ 72 °C _____

1 cycle

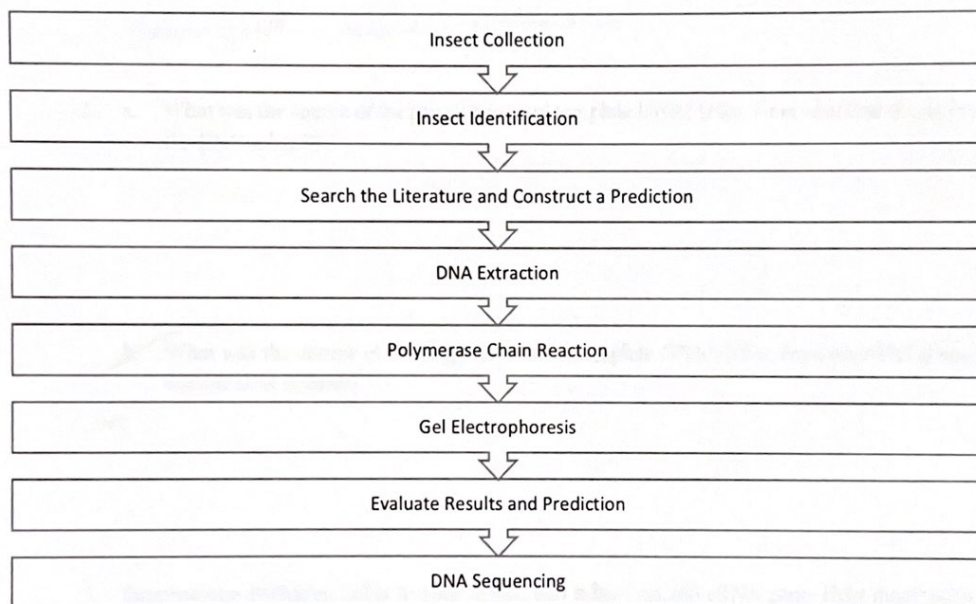
10 min. @ 72 °C final extension

Hold @ 4 °C storage

Your instructor will remove the PCR samples from the thermocycler when the procedure is complete and will store them at -20 °C.

Part four: Stage of *Wolbachia* project

1. Did the work you performed in lab today directly complete one of the steps of the *Wolbachia* project?
 - a. If the work you did today DID NOT directly complete one of the steps of the *Wolbachia* project, how was the work that you did do related to the *Wolbachia* project?
 - b. If the work that you did today DID directly complete one of the steps of the *Wolbachia* project, circle that step in the flowchart below.

**Bibliography**

1. G. Wolfe, PCR Lab, in *Discover the Microbes Within! The Wolbachia Project* (discover.mbl.edu).
2. A. Saradaki and K. Bourtzis, *Wolbachia: more than just a bug in insect genitals*, *Current Opinion in Microbiology* 13(1): 67-72 (2010).
3. G. DeMars and J. Brofft Bailey, Polymerase Chain Reaction. In *Investigations in Biology I Laboratory Manual*, 8th ed., Biology Department, Armstrong Atlantic State University. Hayden-McNeil, pp. 121-148 (2013).