Polymerase Chain Reaction (PCR) Standard Protocol

1. Prepare for PCR

Carefully choose your extracted DNA samples, the appropriate primer for the target genes and the PCR reaction cycles you will need to set.

Be sure to use a PCR worksheet to organize the materials you need for PCR. You will need to locate:

0.5 mL thin-walled PCR tubes (or strip PCR tubes, or a 96 well plate, depending on the thermocycler and how many samples you are working with)
PCR-quality water
Promega GoTaq Green master mix (2x concentration)
Forward Primer (10 uM)
Reverse Primer (10 uM)

Write down which samples you will use as templates for PCR. Be sure to obtain a positive control and remember to create a negative control that uses PCR-quality water as the template.

Calculate the amounts of water, GoTaq Green, and primers you will need to mix. Allow for your total reactions, plus 10% for error.

Put on your gloves to keep your skin's cells and oils away from your PCR tubes.

Label your tubes before you start mixing. Once they are labeled, close them until you are ready to add the reaction mix to them.

This material is based upon work supported by the National Science Foundation under Grant Nos. EF-2025121, EF-2025067, EF-2025009, and EF-2150107. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

2. Prepare the GoTaq Green

You MUST vortex the GoTaq Green tube for 2 minutes!

The reaction master mix tends to stratify, even in the freezer, with heavier components on the bottom of the tube. You need to be sure it is thoroughly mixed, because otherwise you might not be adding any polymerase to your tubes.

3. Make your Reaction Mix

Label a clean 1.5 mL tube as your "reaction mix" tube

Add components 1 to 4 to this reaction mix tube, in order.

1. PCR water
2. GoTaq Master Mix
3. Forward Primer
4. Reverse Primer

4. Mix

Use a pipette set to 180 uL to mix up and down at least 20 times. Pipette slowly to avoid bubbles. This step is important because you need to make sure all the components are well mixed to be equally distributed among your reaction tubes.

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5. Aliquot 48 uL of your Reaction Mix to each of your reaction tubes.

You want to pipette carefully so that each tube receives 48 uL. Hopefully, you should have some of your reaction mix left over from the extra 10% error that you added to your reaction mix.

6. Add 2 uL of the correct template (your DNA sample) to the correct reaction tube.

BE ABSOLUTELY SURE TO CHANGE YOUR PIPETTE TIPS EACH TIME!

7. Place your reactions into the thermocycler. Select the appropriate temperature profile for the primers you are using.

8. Store your reagents in the freezer (-20℃). After the thermocycler program concludes, store your PCR products in the refrigerator (4℃).

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