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PCR Preparation & Gel Electrophoresis

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We use this protocol and it's working

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Our protocols are constantly evolving and old versions will be deleted.
The documents here are not intended to be cited in publications

Abstract

How to set up a PCR reaction (Sanger sequencing only).

Guidelines

If you need to prepare primer stocks (see **Primer Preparation** protocol for help), do that before beginning this protocol.

Materials

- personal primer stocks (forward and reverse), 1:10 dilution from lab primer stocks
- Taq polymerase (Apex as default, Conquest for special applications)
- water aliquot
- DNA extractions
- PCR strip tubes
- 1.6mL eppendorf tube (for master mix)

Before start

Use 75% Ethanol to clean the lab bench, tube racks, and pipettes before taking out any reagents and starting the protocol.

Scale Protocol

1 Optionally, follow along with this supplementary tutorial video as you perform *PCR Preparation*:

https://www.youtube.com/embed/w9jhjG9G6C8

Double check that you have appropriately scaled this protocol. After clicking "run" above, you will need to enter your number of samples + 1-2 to account for the negative (e.g. if you are running 6 samples, scale this protocol for 7).

Tube and Master Mix Prep

2 Prepare your PCR tubes. To do this, use an ethanol resistant pen to label the top of each tube (use a short, 1-2 letter or number code) and the sides of each tube (repeat the short code or use a slightly longer one; also add the gene or primers that you used if there is space). Don't forget to label your negative. *Be sure to note the names of each sample and the negative in your lab notebook.*

Remember to thoroughly clean your workstation with 70% ethanol and 10% bleach before and after this protocol.

3 *Make sure all reagents are completely thawed and mixed (vortex mixer) before using; these reagents should all be stored in the molecular lab freezer.*
To create your **PCR Master Mix**, combine 8.5 µL Water + 12.5 µL Taq Polymerase + 1.0 µL Forward Primer + 1 µL Reverse Primer (volumes are scaled; amount for a single sample would be 8.5 uL water, 12.5 uL Taq, 1 uL fwd. primer, and 1 uL rev. primer) into a 1.6 mL eppendorf tube (lab counter, right side).

Vortex mix the **PCR Master Mix** well (~15 sec).

Be sure to note each reagent used and the date it was opened in your lab notebook.

4 Pipette **23 uL** of your **PCR Master Mix** into each of your PCR tubes, including the negative. Close all of the tubes.

Sample Prep & Final Mixing

5 Locate each of the DNA extractions you planned to run and double check that they are in the same order as the tubes you set up in step 2. Vortex mix each of your DNA extractions for 10-15 seconds. *Assure that any frozen extractions are completely thawed.* Check again that the extractions are in the same order as the PCR tubes set up in step 2.

6 Open all PCR tubes except for the negative (remains closed). One at a time, open an extraction tube and pipette **2 uL** of your **DNA extraction** into its corresponding PCR tube. Close the PCR tube as soon as you finish to keep track of your place.

Note

In a troubleshooting scenario, you may need to increase the concentration of the DNA in an individual PCR tube. You can do this easily by increasing the volume of DNA extraction used in this step, e.g. 4 uL instead of 2 uL. However, you will need to decrease the amount of water in the PCR Master Mix for this sample (6.5 uL instead of 8.5 uL in this scenario)--be careful of this when you set up your Master Mix. If you change the concentration of DNA in a tube, be sure to note it in your lab notebook.

7 Once all PCR tubes have received the appropriate amount of extraction and are closed, flip them upside down and flick a few times on either side to mix. Use the mini-tube centrifuge to spin them down briefly (5-10 sec) before placing the tubes into one of the thermal cyclers.

Starting a Thermal Cycler Program

8 Once you've loaded your tubes and closed the cycler, note the number/name posted on the cycler in your lab notebook. On the cycler menu screen, log in to the folder you need to access (no password necessary), then choose the program you would like to use. *Be sure to note the program and folder names in your lab notebook.*

Once you click **start**, you'll see a screen that asks you to confirm the vessel being used for the reaction (tubes). Click **start** again, and you should see a status screen that shows that your program has begun.

Gel Electrophoresis & ExoSAP-IT

9 Optionally, follow along with this supplementary tutorial video as you perform *Gel Electrophoresis & ExoSAP-IT*:

https://www.youtube.com/embed/mzcaQvxNb4s

After your PCR program has terminated, follow the agarose gel electrophoresis instructions posted on the wall in the ***Ethidium Bromide Contaminated Work Station***.

Note

To each row of wells, add 1 uL of **Bioline HyperLadder** to the first well, followed by 2 uL of PCR product for each sample (including your "negatives") to the remaining wells.

The **Bioline HyperLadder** is stored in the molecular lab fridge.

10 Once gel electrophoresis has finished running at 120 V for 25 min, place the gel inside the UV chamber. Turn on the UV light, and take note of (in your lab notebook) which PCR samples amplified the corresponding genes you targeted.

Note

You must ensure that there are no amplicons in the wells associated with your "negative" PCR reactions! If there is a band visible under the UV light for a "negative", you must redo the corresponding PCR protocol with new reactions to avoid contamination again.

11 Place the PCR tubes that amplified correctly (thus need to be shipped out for Sanger sequencing) inside a frozen PCR tube rack, which can be found in the molecular lab freezer along with the **ExoSAP-IT** (inside the frozen 2-mL tube rack with a lid).

Note

"ExoSAP-IT™ PCR Product Cleanup Reagent is used for enzymatic cleanup of amplified PCR product. It hydrolyzes excess primers and nucleotides in a single step. ExoSAP-IT-purified samples are ready for use in downstream applications such as DNA sequencing or single nucleotide polymorphism (SNP) analysis."

CITATION

ThermoFisher Scientific. ExoSAP-IT PCR Product Cleanup Reagent.
LINK
https://www.thermofisher.com/order/catalog/product/78205.10.ML#/78205.10.ML

At the ***Amplified PCR Product Work Station***, pipette 2 uL of **ExoSAP-IT** from the cap into each of the PCR tubes inside the frozen PCR tube rack. *You must change pipette tips in between each sample!*

Note

It is very important to pipette the **ExoSAP-IT** from the cap, because there is a higher risk of contaminating the entire **ExoSAP-IT** stock if you insert the pipette tip deep inside the tube each time.

Spin down the PCR tubes in the mini centrifuge, and insert them into an Eppendorf thermal cycler. Run the program within Jose's ***Favorites*** folder titled ***exosapit protocol***.