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PCR for Genetic Screening

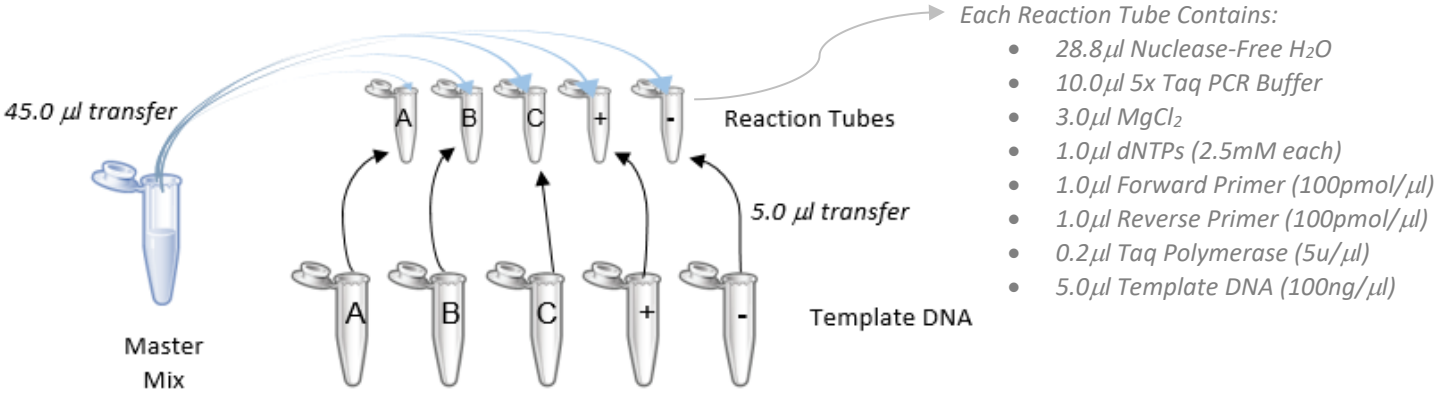
Protocol

Set-up PCR

1. Label the top of a 0.2ml, thin-walled PCR tube with the DNA template that you will be using: Sample **A**, Sample **B,** Sample **C**, positive control **(+)** or negative control **(-)**. Put tubes on ice.
2. Each group prepares a master mix. Label a 0.5ml tube with **MIX**. Prepare enough mix for the number of reactions plus one. (Ex. 3 samples + 1 negative control + 1 positive control + 1 = 6x)
   Keep all components and the master mix on ice.
3. Vortex the 5x Taq PCR buffer and the $MgCl_2$ before adding.
4. Centrifuge your component tubes before you measure them into your master mix.

Components	Volume in 6x Master Mix
Nuclease-Free H_2O	172.8µl
5x Taq PCR Buffer	60.0µl
$MgCl_2$	18.0µl
dNTPs (2.5mM each)	6.0µl
Forward Primer (100pmol/µl)	6.0µl
Reverse Primer (100pmol/µl)	6.0µl
Taq Polymerase (5u/µl)	1.2µl
Total:	**270.0µl**

5. Set pipette to 45.0µl and gently pipette up and down to mix the master mix and store on ice.
6. Add 45.0µl of the master mix to each reaction tube (sample tube and the control tubes). Pipette so that the mix is at the bottom of the tube. Keep the tubes on ice.
7. Centrifuge the template DNA tubes. Add 5.0µl of the appropriate template DNA to each reaction tube. Pipette reaction mix so the template DNA is mixed with the Master Mix in the reaction tube.
   Keep the tubes on ice.



8. If needed, tap or briefly centrifuge the sample tubes to bring the liquid down to the bottom. Return the tubes to ice.

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Set-up PCR (continued)

9. Place tubes firmly into the thermal cycler to ensure good thermal contact and make sure the caps are sealed. Begin cycling according to the following parameters:

Initial Denaturation		96°C	2 min
10 cycles	Denature	94°C	45 sec
	Anneal	50°C	30 sec
	Extend	72°C	1 min

Sample Preparation & Loading

1. Label a 0.5ml tube with the name of the template DNA (**A**, **B**, **C**, **+**, or **-**).
2. Add 2µl of 6x loading dye to the tube.
3. When the reactions have finished cycling, use a P20 pipette to add the 10µl of PCR sample to the labeled tube with 2µl of 6x loading dye and pipette mix. Centrifuge to combine if needed.

Agarose Gel Electrophoresis

1. Obtain a 2% gel from an instructor and set up your MiniOne electrophoresis system.
2. Place the gel tray with the gel in the clear buffer tank.
3. Add ~135ml of 1x sodium borate buffer to the buffer tank. There is a marking on the buffer tank to help guide you. The buffer should just cover the gel. Do not overflow!
4. Fill in the blanks (--------) on the table below with the label of the DNA template on the tube that you plan to load in each lane of the gel. With the wells at the top of the gel, Lane 1 is the well on the left side.

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
10 µl	**10 µl**	**10 µl**	**10 µl**	**6 µl**	**10 µl**
Negative control sample (-)	_____	_____	_____	100 bp marker in loading dye	_____

5. Load 6µl of the DNA marker and 10µl of each sample with dye into the appropriate wells of the gel.
6. Place the amber filter photo hood on top of the MiniOne gel box and push the power button. To see the DNA bands in the gel, push the light bulb button along the right side of the MiniOne unit. Your gel will run for approximately 20 minutes.
7. When the gel has separated far enough to see your PCR bands relative to the DNA ladder (100 bp marker), push the power button to stop the electrophoresis. Use a camera to take a picture of your gel through the window at the top of the photo hood.

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Appendix

PCR Notes

Forward Primer T_m = 59°C 10pmol/µl
5'CGCCAGGGTTTTCCCAGTCACGAC-OH **3'**

Reverse Primer T_m = 50°C 10pmol/µl
5'TCACACAGGAAACAGCTATGAC-OH **3'**

dNTPs are 2.5 mM of each dATP, TTP, dGTP, dCTP for a total of 10mM dNTPs. The final concentration of all four nucleotides in the reaction is 200µM.

Taq DNA Polymerase is a thermal stable enzyme that was isolated from an organism (*Thermus aquaticus*) found living in geyser pools in Yellowstone Park. Taq polymerase, like most polymerases, requires a primer, which is a small piece of DNA ending in a 3' –OH group, bound to the single-stranded template DNA. The enzyme sits down on this small stretch of double-stranded DNA and begins to travel down the single-stranded template adding complimentary nucleotides as it reads the template.