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PCR for Genetic Identity

Protocol

Set-up PCR

1. Label four 0.5ml, thin-walled PCR tubes with the names of the DNA templates that you are using: Paternal Parent **(PP),** Maternal Parent **(MP),** Offspring #1 **(O1)**, or Offspring #2 **(O2).**

2. Each group prepares a reaction mix. Label a blue 0.5ml tube with **MIX.** Prepare enough mix for the number of reactions plus one. (Ex. 3 samples + 1 negative control + 1 = 5x) Keep all of the components and the master mix on ice.

   Centrifuge your component tubes before you measure them into your Master Mix. Vortex the PCR buffer, and the $MgCl_2$ before adding to the mix.

6X Reaction mix What you actually prepare		**Components**	**PCR Reaction** What amount is in each reaction
172.8µl		Nuclease-Free H_2O	25.8µl
60.0µl		5x Taq PCR Buffer	10.0µl
18.0µl		$MgCl_2$	3.0µl
6.0µl		dNTPs (2.5mM each)	1.0µl
12.0µl		Forward & Reverse Primer Mix (50pmol/µl each)	5.0µl
1.2µl		Taq Pol (5u/µl)	0.2µl
N/A		Template DNA (100ng/µl)	5.0µl
270.0µl total			50.0µl
45.0µl/reaction			

3. Pipet gently to mix the reaction mix and store on ice.

4. Add 45.0µl of the master mix to each labeled PCR tube. Pipet so that the mix is at the bottom of the tube. Keep the tubes on ice.

5. Centrifuge the DNA tubes and then add 5.0µl of the appropriate template DNA to each reaction tube. Pipet-mix each reaction so that the DNA template is mixed well with the reaction mix in the PCR tube. Keep the tubes on ice.

6. Add a drop of mineral oil to the top each sample.

7. Spin all PCR tubes before continuing.

8. Place tubes firmly into the thermal cycler to ensure good thermal contact and begin cycling according to the following parameters:

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

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Prepare for Electrophoresis

To prepare a 2% agarose gel, add 2g of agarose to 100ml of buffer (TBE, TAE or sodium borate). The agarose can be allowed to hydrate in the buffer before the mixture is microwaved to dissolve the agarose. The agarose is cooled to 55°C. (*For BTC Institute field trips, this is already done by the instructors*.)

1. Prepare the gel tray by bringing up the dams on the ends of the tray and carefully tightening the screws snuggly, but not too tight. If the gel tray does not have attached dams, then firmly tape the ends to create dams.

2. Place the 6-well comb into the slots at the top of the gel.

3. Pour the agarose into the middle of the tray until it is about half way up the teeth of the comb and has filled the tray to the corners. Do not disturb while the agarose is solidifying (about 20 min.).

4. Add 325-350ml sodium borate running buffer to the electrophoresis chamber.

5. After the gel has set, lower the dams, or carefully remove the tape. Hold the tray on the high side, with the comb closest to the black electrode, and slip it into the electrophoresis chamber on top of the platform. The dams, if present, should hang down over the ends of the platform. If the level of the running buffer in the electrophoresis chamber does not cover the gel, then add more so that the gel is covered.

6. Rock the comb very gently, back and forth in the gel, to allow a little buffer into the well around the teeth. Gently remove the comb and rinse it with ddH_2O**.**

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Sample Preparation and Loading

1. Label a 0.5ml tube with the name of the template DNA (**PP, MP, O1** or **O2**).

2. Add 2μl of EZ-Vision 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 FOTO/Vision™ dye and pipette up and down several times to mix. Be sure that the pipet tip is underneath the top layer of oil when you draw your DNA!

4. Load 10μl of the DNA Marker, Allelic Ladder and of each PCR reaction with dye into the appropriate wells of the gel.

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
10μl 100bp **DNA Marker** in loading dye	**10μl** (**PP**) Paternal Parent	**10μl** **(MP)** Maternal Parent	**10μl** **(O1)** Offspring #1	**10μl** **(O2)** Offspring #2	**10μl** **Allelic Ladder** in loading dye

5. Place the lid on the gel box; connect the electrodes to the power supply. Make sure that the black wire goes into the black plug and the red into red.

6. Turn on the power supply and set it at 220 V. Check the milliamps to make sure that the current is running. Bubbles at the electrodes also indicate that the current is running. The gel will run for approx. 20 to 25 minutes.

7. After the gel has run, remove it from the gel box. Drain off as much buffer as possible. Place the gel on the UV light box to visualize the DNA and to photograph it.

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.