BioPharmaceutical Technology Center
I N S T I T U T E

PCR for Genetic Screening

Protocol

Set-up PCR

1. Label a 0.5ml, thin-walled PCR tube with a color dot and the DNA template that you are using: Positive control **(+),** Negative control **(-),** Sample **A**, Sample **B,** or Sample **C**.
2. Each group prepares a master 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 positive control + 1 = 6x) 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 Taq buffer, and the $MgCl_2$ before adding.

6X Master Mix What you actually prepare	**Components**	**PCR Reaction** What amount is in each reaction
172.8µl	Nuclease-Free H_2O	28.8µl
60.0µl	5x Taq PCR Buffer	10.0µl
18.0µl	$MgCl_2$	3.0µl
6.0µl	dNTPs (2.5mM each)	1.0µl
6.0µl	Forward Primer (100pmol/µl)	1.0µl
6.0µl	Reverse Primer (100pmol/µl)	1.0µl
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 master mix and store on ice.
4. Add 45.0µl of the master mix to each sample tube and the control tubes. 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 reaction mix so the DNA template is mixed with the Master Mix in the reaction tube. Keep the tubes on ice.
6. If needed, tap the sample tubes to bring the liquid down to the bottom. Return the tubes to ice.
7. Add a drop of mineral oil to the top each sample, then give all samples a five second spin in the minicentrifuge.
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
10 cycles	Denature	94°C	45 sec
	Anneal	50°C	30 sec
	Extend	72°C	1 min

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Prepare for Electrophoresis

1. Prepare a 2% agarose gel by adding 2g of agarose to 100ml of 1X Na borate in a flask. Microwave the flask for about 2 minutes, until agarose is completely dissolved. 100mls of agarose should be enough for 2-3 gels.

   Allowing agarose to hydrate in the buffer before the mixture is microwaved may make it easier to fully dissolve the agarose. The agarose is then cooled to about 55°C.

2. 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.
3. Place the 6-well comb into the slots at the top of the gel.
4. 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.).
5. Add 325-350ml sodium borate running buffer to the electrophoresis chamber.
6. 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.
7. 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
8. Fill in the blanks (--------) on the table below with the label of the DNA template in 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
10µl Negative Control Sample	**10µl** --------	**10µl** --------	**10µl** --------	**10µl** 100bp Marker in loading dye	**10µl** --------

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Sample Preparation & Loading

1. Label a 0.5ml tube with the name of the template DNA (**+, - , A, B,** or **C**).
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 EZ-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 and 10µl of each sample with dye into the appropriate wells of the gel.
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.