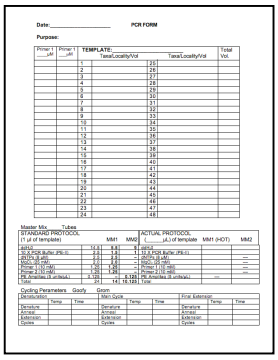
HOT START PCR (For manual Hot-Start, Double-Stranded Amplifications)

PCR will make about 40 Billion copies from a given template. Because of this, it is imperative to maintain excellent sterile technique throughout as any contaminant will be amplified along with (or instead of) your template DNA. Work in the **LOW DNA** portion of the lab.

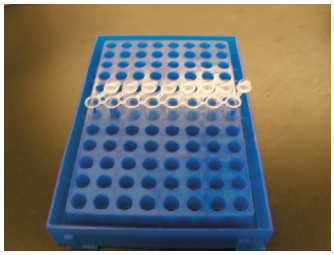
Equipment:
PCR Machine
Gloves
Pipettors
Pipette Tips
PCR Tubes, Rack
Pencil
PCR Worksheet
Waste Tip Box
Microcentrifuge

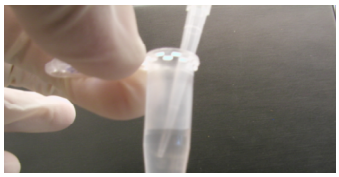
Ingredients
Extracted Samples (templates)
Positive Control (a sample which works)
Master Mix for 18 samples containing:
Water, Buffer, $MgCl_2$, dNTP's, Primers
Amplitaq Gold Enzyme (*Thermus aquaticus* DNA Polymerase, 5units/uL, modified to become active only at 95°C)


Date:_____ PCR FORM
Purpose:
TEMPLATE:
Master Mix Tubes
STANDARD PROTOCOL (1 µl of template)
ACTUAL PROTOCOL (__ µL) of template
MM1 (HOT)
MM2
Cycling Parameters Gooty Goom
Initial Denature
Denature
Anneal
Extension
Cycles
Final Extension

Procedure

1. Take out the master mixes (MM1 and MM2) from the freezer to thaw:
2. Fill out PCR sheet with date, sample numbers, locality, etc.
3. Label and number 16 PCR tubes in rack. Set up 2 extra tubes for positive and negative controls.
4. Once master mixes are completely thawed, flick tubes with your finger to mix, and shake contents to bottom of tube.
5. Aliquot 14µl of MM1 into each of your tubes (16 samples plus 2 controls).
6. Remove Chelex DNA extractions from the fridge and, if necessary, centrifuge briefly to remove condensation and/or concentrate chelex beads. Set up samples so that they are in a pattern corresponding to the labeled PCR tubes. Using **LOW DNA pipette**, add 1 µl of DNA extraction to each tube. These samples will include your DNA extractions, positive PCR control & negative Chelex control. Take sample FROM TOP of Chelex extraction, as addition of Chelex beads will inhibit the PCR. **Change tips between pipetting each chelex sample**.
7. Put the lids onto your tubes. Press gently as you will be opening them again soon. Check your strip tubes for bubbles. If there are any, spin them down briefly in the microcentrifuge.
8. Take Amplitaq (Red Lid) from freezer. Pause to contemplate how this tube relates to your monthly rent. ***Using "ENZYME" pipetteman*****,** aspirate 2.25 µl of Taq from the very TOP of the liquid (Taq is viscous and will stick to the outside of your tip, so you don't want to dip your tip in too far) Carefully add Taq to the master mix. Pipette up and down to mix. Watch to see the glycerol dissipate in solution. ***Put Taq back in freezer.***
9. Set an appropriate **No DNA pipette** to 10 µl, and pipette up and down ~25 times to stir in the Taq. You should no longer see the denser Taq swirling around.
10. Place your samples into the thermal cycler. Double check to make sure all lids are closed, but not pushed down completely.





11. Start th thermal cylcer, select and run the appropriate program (HOTINVT or HOTFISH) depending on the taxa you are amplifying. Enable heated lid.
12. The thermal cycler will ramp up to 80C and pause. Once machine is paused, add 10µl of MM2 to each of the tubes. Only open one tube at a time, and once the MM2 is added, close the lid tightly on the tube. You want to work to minimize evaporation.

13. Once all samples have had MM2 added, unpause the machine. It should start to cycle.
14. Watch the thermal cycler to ensure that it is running the program that you think it should be.
15. Once you confirm that the machine is running with the appropriate cycling parameters, c**lean up your workspace**, put away DNA extractions in the fridge.

TIPS:

1. ALWAYS wear gloves.
2. Set up everything in groups of 8 when possible (e.g. 8 samples, 8 tubes, 8 tips). Use tip one for sample one in tube one. This will help you keep track of which sample you are on.
3. Keep lids on whenever possible.
4. *Master mix must be completely thawed and mixed prior to use*.
5. Pipettes have two stops. To aspirate, use the first stop. To expel, use the second.
6. Do not lean over samples or master mix, or look into tubes from the top, especially Taq.
7. Go back and make sure that no samples are lying out and the PCR machine is running.
8. Always look at the amount of reagent or sample that you are adding to ensure that there are no bubbles in the tip and the amount looks correct.