

## PCR (For 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  
10, 20, 200  $\mu$ l pipettes  
Pipette Tips  
PCR Tubes, Rack  
Calculator, Pen  
PCR Worksheet  
Waste Jar  
Microcentrifuge

### Ingredients

Extracted Samples (templates)  
Positive Control (a sample which works)  
Molecular Biology Grade Water  
10X PCR Buffer  
8mM dNTPs (deoxynucleotide triphosphates)  
25mM Magnesium Chloride  
10uM heavy strand primer  
10uM light strand primer  
Taq Enzyme (*Thermus aquaticus* DNA Polymerase, usually 5units/uL)

### **Procedure**

1. Take out the following reagents from the freezer to thaw:

Water  
dNTPs  
PCR Buffer  
MgCl<sub>2</sub>  
Primer 1  
Primer 2



Date: _____		PCR FORM	
Reagents			
Primer	Vol	Template	Total Volume
1			
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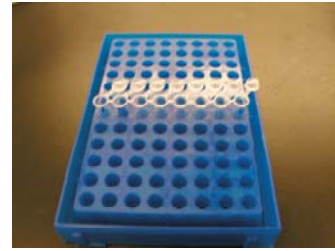
Sample No. \_\_\_\_\_  
Date: \_\_\_\_\_  
Operator: \_\_\_\_\_  
Lab: \_\_\_\_\_

Sample No.	Sample Name	Sample Type	Sample Volume	Sample Date	Sample Time
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2. Fill out PCR sheet with date, sample numbers, type of extraction, other notes.
3. Multiply out volumes of standard protocol by X (where X = # samples + pos. PCR control + neg. Chelex control + neg. PCR control + 1 extra).
4. Label and number PCR tubes in rack. Set up samples so that they are in a pattern corresponding to labeled PCR tubes. Set up extra tubes for controls.
5. Once ingredients are thawed, flick each tube with your finger to mix, and shake contents to bottom of tube. When you are almost out of a reagent, centrifuge tube to get remaining liquid.
6. Make Master Mix 1: Using **NO DNA pipette**, add ingredients at calculated volumes in the order listed on the PCR sheet to a 1.5mL tube. Use a **separate tip** for each reagent addition. Pipette up and down to mix reagents thoroughly.
7. Using **NO DNA pipette**, make Master Mix 2 in a separate 1.5mL tube, but **DO NOT** add the Taq. Use a **separate tip** for each reagent addition. Pipette up and down to mix reagents thoroughly.



8. Using **NO DNA pipette**, dispense 14  $\mu\text{l}$  of MM1 into each of your PCR tubes. Use **same tip** throughout. Pipette directly into the bottom of the tubes, and take care not to introduce bubbles into your tubes by pipetting too far.



9. Remove Chelex DNA extractions from the fridge and if necessary, centrifuge briefly to remove condensation and/or concentrate chelex beads. Using **LOW DNA pipette**, add 1  $\mu\text{l}$  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 for each sample.**



10. Take Taq from freezer. Pause to contemplate how this tube relates to your monthly rent. **Using ENZYME" pipetteman**, aspirate the desired amount 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). Put Taq back in freezer. Carefully add Taq to MM2. Pipette up and down to mix. Watch to see the glycerol dissipate in solution.

11. Move PCR tubes, waste jar, pipette tips and **LOW DNA pipette**, pipette to PCR bench.



12. Select and start PCR hot-start program. Enable heated lid and pause when temperature reaches 80°.

13. Place a strip of tubes into PCR machine (make sure all lids are closed to prevent evaporation of your PCR reaction).

14. With the **LOW DNA pipette**, pipetman set on 10ul, pipette up and down in MM2 to further mix the master mix. Then, one at a time, open lid, add MM2, pipette up and down, and close lid. Repeat for each tube. You must **change tips** between each sample.

15. Double check to make sure all lids are closed. Unpause program and watch the display to ensure that the PCR machine is running.

16. Clean up your workspace, put away reagents in the freezer and DNA extractions in the fridge.

#### TIPS:

1. Wear gloves.
2. Put reagent tubes in order, then move each ingredient's tube to another spot in the rack to keep track of which one you've used.
3. 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.
4. Keep lids on whenever possible.
5. Reagents must be completely thawed and mixed prior to use.
6. Pipettes have two stops. To aspirate, use the first stop. To expel, use the second.
7. Do not touch tips or lids of reagents to anything.
8. Do not lean over samples or reagents, or look into tubes from the top, especially Taq.
9. Go back and make sure that no reagents are laying out and the PCR machine is running.
10. 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.