

# POLYMERASE CHAIN REACTION PROTOCOL page 32

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modified from the protocol of Sonia Wallman

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[http://biology.clc.uc.edu/fankhauser/Labs/Genetics/PCR/PCR\\_Protocol.htm](http://biology.clc.uc.edu/fankhauser/Labs/Genetics/PCR/PCR_Protocol.htm)

Wear latex gloves at all times when handling DNA to avoid contamination.

1. **Label a 0.5mL PCR tube** and the assigned cap with your initials. (They come in strips of 8.)
2. **Prepare reaction mix** which contains the following to be run per each student sample, assuming DNA sample of 2  $\mu\text{L}$  (adjust the  $\text{dH}_2\text{O}$  for other volumes of DNA):

**Per tube,  $\mu\text{L}$  :** **(I will probably prepare it for you: for a class of 20,  $\mu\text{L}$ )**

18.37 $\mu\text{L}$	sterile $\text{dH}_2\text{O}$	460
2.5 $\mu\text{L}$	10x PCR buffer + 25 mM $\text{MgCl}_2$	62.5
1.25 $\mu\text{L}$	10 mM dNTP (contains all four nucleotide triphosphates)	32
0.75 $\mu\text{L}$	10 mM D1S80 primer set	19
0.125 $\mu\text{L}$	Taq polymerase, 5 units/ $\mu\text{L}$ (temp resistant polymerase from <i>Thermus aquaticus</i> )	3.125

3. **Deliver 23  $\mu\text{L}$  of reaction mix to each tube on ice.** Include a control with no DNA.
4. **Add 2  $\mu\text{L}$  of your buccal cell DNA** (from the previous DNA isolation).
5. Close top of tubes securely (feel the “click”) and mix reagents by vortexing the assembled strip.
6. Store strips of prepared tubes on crushed ice until all samples have been prepared.
7. **Program the thermocycler** with the prescribed step file (see conditions required for given probe set). For D1S80 primers (primers which have worked for us), the step file will be:

<u>SEGMENT</u>	<u>TEMPERATURE</u>	<u>TIME</u>	<u>FUNCTION</u>
1	95° C	1 minute	melt DNA
2	65° C	1 minute	anneal primer
3	72° C	1 minute	polymerize

Repeat the 1, 2, and 3 steps for 30 cycles. At the end of the 30 cycles:

TIME DELAY FILE	72° C	10 minutes
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8. Warm up the thermocycler
9. **Load thermocycler** with prepared 0.5mL PCR reaction tubes.
10. **[For hot start, run at 95 C for five minutes, then add 0.15  $\mu\text{L}$  Taq polymerase, 5 units/ $\mu\text{L}$ ]**
11. **Run the thermocycler for prescribed number of cycles** so that the polymerase chain reaction amplifies the probed segment of DNA. (This usually requires coming back to the lab 2.5 hours later for this D1S80 program)
12. **Add loading dye** to the PCR sample (**5  $\mu\text{L}$  of 6x, 3  $\mu\text{L}$  if 10x**).
13. **Vortex** to mix.
14. **Freeze the amplified samples** at  $-20^\circ\text{C}$  until you are ready to cast agarose gel and electrophorese . (Run the gel for 1  $\frac{1}{2}$  hours)

\*To initiate hot start PCR, denature the DNA by incubating the tubes for 5 minutes at  $95^\circ\text{C}$  (segment 1), maintain the tubes at  $95^\circ\text{C}$  while you add 0.4  $\mu\text{L}$  Taq DNA polymerase to each tube. Do not allow the tubes to cool and do not take time to mix the reaction mixture after adding the Taq polymerase.