

Basic PCR Protocol

CGLab, 7/2002

- 1) Wipe down the bench area with bleach and a new paper towel.
- 2) Take the PCR components out of the freezer to thaw – place them into a tube rack to thaw, and then into a "chiller box" to stay cold while assembling your PCR. The components are: water, 10X buffer, dNTPs, MgCl₂, and primers .
- 3) Take DNA extracts out of freezer (or fridge).
- 4) While components are thawing:

Fill out a PCR worksheet with the sample identifiers of all samples to be used. Following the master mix template sheet, calculate the amount of each component needed for the total number of samples + 2. Remember to include a tube at the end for the "negative control" (a tube with no template added to check for contamination).

Label 200 µl tubes/strip tubes with sample identifier numbers from the PCR worksheet, so that the numbers can be cross-referenced with identifiers and each PCR reaction tube can be individually identified. PCR tubes can be placed in a freezer rack to keep cold.
- 5) When all the components have thawed, prepare the Master Mix (MM) in a 1.5 ml tube, adding each component in the order listed on the PCR form: water, 10x buffer, dNTPs, MgCl₂, then primers. **Be sure to mix all tubes well** (by shaking , then shake contents down into the bottom of the tube) before pipetting them into the MM.
- 6) Take out taq chiller box from freezer and add the correct amount of Taq to the MM using the P2 "enzyme" pipet (pump up and down to rinse taq into tube), then close the MM tube. It is not necessary to shake up the Taq before pipetting, but you may want to spin down the taq tube to force contents to the bottom. Replace the taq into freezer immediately after use.
- 7) Mix the MM solution by shaking, then shake the liquid into the bottom of the tube.
- 8) Distribute the MM into your labelled 200 µl PCR tubes/strip tubes (usually 40 µl per tube) with the pre-PCR 200 µl pipet
- 9) Put the pre-PCR pipets away in their rack, and take out the template addition pipet (10 µl).
- 10) Take each DNA extract tube in order following the worksheet, add 1 µl of the proper extract into the matching tube (with the 10 µl template addition pipet. Replace the extract tube 1 slot over in the rack, so you can keep track of which tubes have already been used. (If liquid is not well-settled in the bottom of the tubes, spin down all extract tubes before opening.)
- 11) After template has been added to all tubes, close caps on all tubes firmly and place the tubes into the thermal cycler.
- 12) Start the program. Most PCR programs take about 2 hours. Be sure to place a labelled (your name and date, minimum) tube-box near the PCR machine so the next user has a place to put your tubes when they empty the machine.
- 13) After the PCR is complete, run out samples on a mini-gel (separate protocol) or store in the PCR refrigerator until the minigel is run.