

How to run the PCR

Standard setup:

| <u>ingredient</u> | <u>volume per rxn</u> | <u>mix for 5</u> |
|-------------------------------|-----------------------|------------------|
| H ₂ O | 15-16 μ l | 75 μ l |
| 10x buffer | 2 μ l | 10 μ l |
| dNTP (2 mM each) | 1 μ l | 5 μ l |
| forward primer (12.5 μ M) | 0.5 μ l | 2.5 μ l |
| reverse primer (12.5 μ M) | 0.5 μ l | 2.5 μ l |
| Taq polymerase (5u/ μ l) | 0.2 μ l | 1.0 μ l |
| template DNA | 0.1-1.0 μ l | -- |
| total | 20 μ l | 95 μ l |

It is usually convenient to set up a master tube, where all common ingredients (except for the template) are mixed.

Always calculate the mix for one more reaction than you will actually run (i.e., the example above is good for 4 reactions).

Template volume varies with concentration and complexity (i.e., you will need more of a genomic template than of a plasmid template, because the same amount of DNA brings in different numbers of template copies).

PCR machine:

Load the reactions into 0.2 ml PCR tubes (use Eppendorf tubes to ensure good fit in machine)

Make sure to use small holes in the PCR machine.

You can load up to 25 tubes at a time.

Close lid and turn knob until it stops.

Turn on PCR machine (switch on back).

The menu should point at "START" (if not use arrows up and down).

Press "ENTER".

Use arrow keys to select the program you want to run.

Press "ENTER".

The program will ask you what kind of tube you're using and the rxn volume; select the tube with the "SELECT" button, enter the volume on the number keypad; hit "ENTER".

-- While the program is running, you can use the "OPTION" key to check how much longer you have to wait.

-- This is a great time to pour a gel!

Program Naming Convention:

All programs use a 15 s denaturation at 94°C, followed by 15 s annealing, extension at 72°C.

Programs are identified by their annealing temperature (A##), extension time (E##), and cycle number (letter code: A=20, B=25, C=30, D=35, E=40).

For example, "A60E120C" means: Annealing at 60°C, Extension for 120 s, 30 cycles.