

PCR Standard Protocol (with Taq polymerase)

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WARNING: Contamination can be a major problem, unless you are careful. Common routes for contamination include using the same pipette to set up a reaction and load products on a gel, or using the same water for PCR and other activities such as restriction digests. Reagents used for PCR should be pipetted using filtered tips to reduce contamination. Aliquotting reagents will help ensure that they are "clean."

STEP 1: Create primer sequences based on the template, taking note of the melting temperatures (Tms). Program the PCR machine (see step 4).

Typically, PCR is done with annealing temperatures 5C below the Tm, but the optimal temperature must be determined empirically. Most annealing temperatures are between 50 and 60C, for oligos that are 18-22 nt. Be careful: some oligo programs show Tms for several salt concentrations, not all of which are used for PCR (which is typically done at 50-60 mM depending on the enzyme system).

STEP 2: Prepare the PCR stock solutions. If not already done, dissolve the two primers with pristine, sterile, non-contaminated, diH₂O at 1 mg/ml.* Use a filter-tip pipette in a clean location, i.e. unlikely to contain contaminating DNA.

**To avoid confusion, be consistent in your stock concentrations. I use 1 mg/ml since it is easy to remember, but another good choice is 100 μM. It is common to make a working stock that is 10-times diluted, i.e. 10 μM.*

For routine experiments, use the Perkin-Elmer buffer recipe shown below. Note: Other enzymes may require other buffers.

Perkin-Elmer buffer minus Mg (10X stock)

500 mM KCl
100 mM Tris-Cl pH 8.3 (at 25C)
1 mg/ml gelatin

(autoclave and freeze aliquots; add gelatin just before autoclaving; it will not dissolve until the solution is heated).

STEP 3: Set up PCR reaction by the following recipe, adding Taq polymerase last:

Water	25 μl final volume
2.5 μl	10X Perkin-Elmer buffer
2 μl	1.25 mM dNTPs***
0.375 μl*	0.1 M MgCl ₂ (for 1.5 mM)
XX μl	template DNA (2-20 ng)
0.05 μl**	each primer at 1 mg/ml
0.2 μl	Taq polymerase (5 units/μl)

Or: 0.5 μl of each primer at 0.1 mg/ml
Or: 1 μl of each primer at 10 μM
Or: 0.1 μl of each primer at 100 μM

**Some primers require higher or lower Mg concentrations for optimal performance, usually between 0.75 and 2.5 mM.*

*** If you are only setting up a few tubes, you should make a 10-fold dilution of your oligos, i.e. a 0.1 mg/ml stock, to ensure pipetting accuracy. Each primer should be between 0.25 and 0.5 μ M, which equals 6.25 to 12.5 picomoles per 25 μ l reaction. Using the recipe shown with the 1 mg/ml stock, you are adding 7.5 pg of a 20-nt oligo (if your oligo is larger, you should increase the oligo volume). Alternatively, set up your stocks in μ M, using the guidelines shown in the box; then you won't have to adjust for oligo size.*

****Note that the 1.25 mM stock of dNTPs means that the final concentration of each dNTP (dATP, dCTP, dGTP, and dTTP) is 1.25 mM; all dNTPs together are 5 mM.*

Master mixes improve the accuracy of PCR. If several reactions are being run side by side, which differ only in the template DNA, it is sensible to make a master mix that includes everything but the template DNA by multiplying the volumes in the protocol by the number of reactions (plus one or two extra to manage cumulative pipetting error).

Normally, 2-20 ng of template DNA is used per 25 μ l reaction. If the volume to be added to each reaction is small (<1 μ l), the template DNA can be in a solution such as standard TE (10 mM Tris 8.0, 1 mM EDTA). However, if a larger volume is to be used, the DNA should be in water or "low TE" (10 mM Tris 8.0, 0.1 mM EDTA).

Assemble the reactions on ice for maximum specificity. The order in which the components are added is not important as long as the Taq polymerase is added last. Before starting the reactions, make sure all of the liquid is in the bottom of the tube. Either flick the tube or spin.

ALWAYS run a no-DNA control to check for contamination!!!!!!

STEP 4: Put the tubes into the thermocycler. This should have been pre-programmed with the desired parameters. Here is a typical program, but note the some primers may work better at other annealing temperatures.

92°C Melt for 30 seconds	1 cycle
92°C Melt for 30 seconds 55°C Anneal for 30 seconds 72°C Extend for 60 seconds	35 cycles
72°C Extend for 120 seconds	1 cycle
10°C Cool down	1 cycle
Room temperature/shut off	hold

Note: leaving the instrument at 10°C or 4°C at the end of the program is not required and is bad for the instrument (it must work too hard). After PCR is done, the DNA will be stable at room temperature for a long time.

Most published protocols use a 94°C melting temperature, but I have found that this works just as well and is faster.

STEP 5: Run out PCR fragments on a agarose or polyacrylamide gel. Typically, only 1/2-3/4 of the reaction is run on the gel.

COLONY PCR

(for screening *E. coli* colonies)

- Using the same recipes as above, fill tubes with 25 μ l PCR mix.
- Pick up a TINY part of the colony using a toothpick or pipette tip and place in the PCR tube.
- Remove the toothpick and seal tube
- In PCR block, heat to 92C for 1 minute (this helps break open the cells and kills DNAses).
Then proceed with standard protocol, 30 cycles.