PCR Amplification Protocol
Created April 18, 2017
Ajay Arya

Polymerase Chain Reactions (PCR) are a means to selectively copy a fragment of DNA through the use of primers with nucleotide sequences complementary to the 5' and 3' ends. Within the sequence added to a primer one may add restriction sites for the assembly of DNA fragments.

Allow reagents to thaw completely on ice before using. When handling tubes, hold near the top not at the bottom where the reagent is, as the heat from your hand could destroy enzymes and reagents. Use long (10 µL) tips because it prevents contamination.

Timeline

Reaction set-up:	5-10 min
PCR:	1-2 h

Add the below reagents to a PCR tube:

Component	Volume (µL)	Final Concentration
nuclease-free water	50 – below V	
5× polymerase buffer	10	1×
10 mM dNTPs	1.0	200 µM
10 µM forward primer	2.5	0.5 µM
10 µM reverse primer	2.5	0.5 µM
template DNA	2.0[A]	< 250 ng total
DMSO (optional[B])	1.5	≤ 3 %
DNA polymerase[C]	0.5	1.0 unit/50 µL PCR

A: For Phusion Polymerase, NEB recommends the following amounts of template DNA per 50 µL PCR:

genomic DNA: 50 – 250 ng
plasmid or viral DNA: 1 pg – 10 ng

In my experience, the concentration of DNA from an overnight culture is ~50 ng/µL with either the Zymo Plasmid miniprep kit (for a medium copy plasmid, ~20/cell) or the Wizard Genomic DNA purification kit. Keep in mind, the concentration of plasmid DNA you purify is greatly affected by the plasmid copy number.

B: DMSO relieves supercoiling in DNA. I always use it when using Phusion DNA Polymerase, it does not seem to be necessary for GoTaq DNA Polymerase.

C: Use the appropriate DNA Polymerase for the job at hand. For PCR verification, use GoTaq (cheaper, more error-prone); for amplification of DNA to be used in vectors or genomic insertions, use Phusion (costs more, low error rate). Always add the polymerase to the reaction mixture last; this is for several reasons including the possibility of an error in adding the appropriate reagents to the mixture (perhaps you realize you used the wrong primers) but mostly the fact that DNA polymerase is most stable in a buffered solution.

Standard PCR Cycle

Step	Temperature (°C)	Time (s)
Initial denaturation	98	30
	98	5-10
25-35 cycle extension	Tm (45-72)	10-30
	72	15-30 per kb
Final extension	72	5-10 min
Hold	4-10	∞

When designing primers, make sure their Tm is within 5 °C of each other so your PCR will amplify only your DNA of interest. Run the annealing stage at the lower Tm of the two primers.