

PCR Phusion

Water	to a total of 50ul
5X Buffer*	10.0ul
10mM dNTPs	1.0ul
Template	0.3ul-5ul (about 10-50ng total)
5' Primer	1ul
3' Primer	1ul
Phusion Polymerase	0.5 ul
	= 50ul reaction

*Be sure to use GC rich buffer when necessary.

TIPS

- Keep reactions on ice.
- Add Phusion last. Please keep Phusion on ice/freezer box and return to freezer as soon as possible!
- If doing several PCRs, make a master mix. Hint: Make a master mix with a little more volume than you need (+1 reaction).
- DMSO can be added for troublesome PCRs (0.25-1ul).

Cycling Protocol

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	30 s	98°C	30 s	1
Denaturation	98°C	5-10 s	98°C	5-10 s	25-35
Annealing*	-	-	X°C	10-30 s	
Extension	72°C	15-30 s/kb	72°C	15-30 s/kb	
Final extension	72°C	5-10 min	72°C	5-10 min	1
	4°C	hold	4°C	hold	

*usually 55 degrees C for annealing

*under "3-step protocol", the Time is usually 10s for denaturation and 20s for annealing

"2- Step Backbone" PCR Protocol

- 1) 98°C for 30s
- 2) 98°C for 10s
- 3) 55°C for 20s
- 4) 72°C for 1min 20s
- 5) Repeat 2-4 X10

- 6) 48°C for 10s
- 7) 65-67°C for 20s
- 8) 72°C for 1min 20s
- 9) Repeat 6-8 X25
- 10) 72°C for 5min
- 11) 4°C for ∞