



PCR with Q5 High-Fidelity Polymerase

- ◆ 25 μ L reaction mixture:
 - ◇ 5 μ L 5x Q5 Reaction Buffer
 - ◇ 0.5 μ L dNTPs (10 mM)
 - ◇ 1.25 μ L Forward Primer
 - ◇ 1.25 μ L Reverse Primer
 - ◇ Template DNA
 - ◇ 0.25 μ L Q5 High-Fidelity DNA Polymerase
 - ◇ To 25 μ L ddH₂O
- ◆ Thermocycling Conditions:

	Steps	°C	m:s
	Cell lysis and denaturation	98.0	0:30
25 –30 x	Denaturation	98.0	0:05 – 0:10
	Primer annealing	annealing temperature*	0:10 – 0:30
	Extension	72.0	0:20 – 0:30 s/kb
	Final extension	72.0	2:00
	Hold	4.0 – 10.0	PAUSE

*primer annealing temperature: 50 – 72 °C

From: [NEB](#)