

## PCR with Q5 High-Fidelity Polymerase

- ◆ 25  $\mu$ L reaction:
  - ◇ 5  $\mu$ L 5x Q5 Reaction Buffer
  - ◇ 0.5  $\mu$ L dNTPs (10 mM)
  - ◇ 1.25  $\mu$ L Forward Primer
  - ◇ 1.25  $\mu$ L Reverse Primer
  - ◇ Template DNA
  - ◇ 0.25  $\mu$ L Q5 High-Fidelity DNA Polymerase
  - ◇ To 25  $\mu$ L ddH<sub>2</sub>O
- ◆ Thermocycling Conditions:

	Steps	$^{\circ}$ 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
	Final extension	72.0	2:00
	Hold	4.0 – 10.0	PAUSE

\*primer annealing temperature: 50 – 72  $^{\circ}$ C

\*\* 20 – 30 s/kb

From: [NEB](http://www.neb.com)