



## PCR with Phusion High-Fidelity PCR Master Mix with GC Buffer

- ◆ 25  $\mu$ L reaction mixture:
  - ◇ 1.25  $\mu$ L Forward Primer
  - ◇ 1.25  $\mu$ L Reverse Primer
  - ◇ Template DNA
  - ◇ (optional: DMSO 0,75  $\mu$ L)
  - ◇ 12.5  $\mu$ L 2x Phusion Master Mix
  - ◇ To 25  $\mu$ L ddH<sub>2</sub>O
- ◆ 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	0:15 – 0:30 s/kb
	Final extension	72.0	5:00-10:00
	Hold	4.0 – 10.0	PAUSE

\*primer annealing temperature: 45 – 72 °C

From: [NEB](#)