

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

- 25 μ L reaction mixture: $\diamond$ 1.25 μ L Forward Primer
 - 1.25 μ L Reverse Primer
 - Template DNA
 - (optional: DMSO 0,75 μ L)
 - 12.5 μ L 2x Phusion Master Mix
 - 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	0:15 – 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: iGEM Bielefeld-CeBiTec