

Q5 High-Fidelity DNA Polymerase from NEB

Master Mix (50 µl):

	Stock concentration	Volume	Final concentration
5X Q5 Reaction Buffer	5x	10 µl	1x
H ₂ O	-	32,5 µl	-
Primer I	10µM (10 pmol/µl)	2,5 µl	0,4 µM
Primer II	10µM (10 pmol/µl)	2,5 µl	0,4 µM
dNTPs	10 mM of each	1 µl	200 µM each
template	-	1 µl	-
Q5 High-Fidelity DNA Polymerase	2 U/µl	0,5 µl	0,02 U/µl
5X Q5 High GC Enhancer (optional)	5 x	(10 µl)	1x

The 5X Q5 Reaction Buffer provided with the enzyme is recommended as the first-choice buffer for robust, high-fidelity amplification. For difficult amplicons, such as GC-rich templates or those with secondary structure, the addition of the Q5 High GC Enhancer can improve reaction performance. The 5X Q5 Reaction Buffer is detergent-free and contains 2.0 mM MgCl₂ at the final (1X) concentration.

Programme:

98°C	0:30	
98°C	0:05-0:10	} 35x
X°C	0:10-0:30	
72°C	Y:YY	
72°C	02:00	
4-10°C	∞	

X°C – annealing temperature

Y:YY –Extension times are generally 20–30 seconds per kb for complex, genomic samples, but can be reduced to 10 seconds per kb for simple templates (plasmid, *E. coli*, etc.) or complex templates < 1 kb. Extension time can be increased to 40 seconds per kb for cDNA or long, complex templates, if necessary.