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PCR Using Q5® Hot Start High-Fidelity DNA Polymerase (M0493) V.2

New England Biolabs[1]

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Q5®Hot Start High-Fidelity DNA Polymerase is a high-fidelity, thermostable, hot start DNA polymerase with 3´→ 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. The addition of an aptamer-based inhibitor allows room temperature reaction setup. With an error rate ~280-fold lower than that of *Taq*DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase is ideal for cloning and can be used for long or difficult amplicons. Q5 Hot Start High-Fidelity DNA Polymerase is supplied with an optimized buffer system that allows robust amplification regardless of GC content. The 5X Q5 Reaction Buffer contains 2 mM Mg^{++}at final (1X) reaction concentrations and is recommended for most routine applications. For GC-rich targets (≥ 65% GC), amplification can be improved by the addition of the 5X Q5 High GC Enhancer. Q5 Hot Start High-Fidelity DNA Polymerase is unlike typical, lower fidelity PCR enzymes. To determine the optimal annealing temperatures for a given set of primers, use of the NEB T_mCalculatoris highly recommended.

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General Guidelines:

1. Template:
Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

A	B
DNA	**AMOUNT**
DNA Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–1 ng

2. Primers:
Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 can be used to design or analyze primers. The best results are typically seen when using each primer at a final concentration of 0.5 µM in the reaction.

3. Mg^{++} and additives:
Mg^{++} concentration of 2.0 mM is optimal for most PCR products generated with Q5 High-Fidelity DNA Polymerase. When used at a final concentration of 1X, the Q5 Reaction Buffer provides the optimal Mg^{++} concentration.

Amplification of some difficult targets, like GC-rich sequences, may be improved by the addition of 1X Q5 High GC Enhancer. The Q5 High GC Enhancer is not a buffer and should not be used alone. It should be added only to reactions with the Q5 Reaction Buffer when other conditions have failed.

4. Deoxynucleotides:
The final concentration of dNTPs is typically 200 µM of each deoxynucleotide. Q5 Hot Start High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil-containing primers or templates.

5. Q5 Hot Start High-Fidelity DNA Polymerase concentration:
We generally recommend using Q5 Hot Start High-Fidelity DNA Polymerase at a final concentration of 20 units/ml (1.0 unit/50 µl reaction). However, the optimal concentration of Q5 Hot Start High-Fidelity DNA Polymerase may vary from 10–40 units/ml (0.5–2 units/50 µl reaction) depending on amplicon length and difficulty. Do not exceed 2 units/50 µl reaction, especially for amplicons longer than 5 kb.

6. Buffers:
The 5X Q5 Reaction Buffer provided with the enzyme is recommended as the first-choice

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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 contains 2.0 mM Mg^{++} at the final (1X) concentration.

7. Denaturation:
Q5 Hot Start High-Fidelity DNA Polymerase does not require a separate activation step.

An initial denaturation of 30 seconds at 98°C is sufficient for most amplicons from pure DNA templates. Longer denaturation times can be used (up to 3 minutes) for templates that require it. During thermocycling, the denaturation step should be kept to a minimum. Typically, a 5–10 second denaturation at 98°C is recommended for most templates.

8. Annealing:
Optimal annealing temperatures for Q5 Hot Start High-Fidelity DNA Polymerase tend to be higher than for other PCR polymerases. The NEB T_m Calculator should be used to determine the annealing temperature when using this enzyme. Typically, use a 10–30 seconds annealing step at 3°C above the T_m of the lower T_m primer. A temperature gradient can also be used to optimize the annealing temperature for each primer pair.

For high T_m primer pairs, two-step cycling without a separate annealing step can be used (see note 11).

9. Extension:
The recommended extension temperature is 72°C. 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.

A final extension of 2 minutes at 72°C is recommended.

10. Cycle number:
Generally, 25–35 cycles yield sufficient product. For genomic amplicons, 30-35 cycles are recommended.

11. 2-step PCR:
When primers with annealing temperatures ≥ 72°C are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

12. Amplification of long products:
When amplifying products > 6 kb, it is often helpful to increase the extension time to 40–50 seconds/kb.

13. PCR product:
The PCR products generated using Q5 Hot Start High-Fidelity DNA Polymerase have blunt ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, the DNA should be purified prior to A-addition, as Q5 Hot Start High-Fidelity DNA Polymerase will degrade any overhangs generated.

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Addition of an untemplated -dA can be done with *Taq* DNA Polymerase (NEB #M0267) or Klenow exo– (NEB #M0212).

MATERIALS

Q5 Hot Start High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0493L**

Q5 Hot Start High-Fidelity DNA Polymerase - 100 units **New England Biolabs Catalog #M0493S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Please note that protocols with Q5® Hot Start High-Fidelity DNA Polymerase may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

1

Set up the following reaction:

Q5 Hot Start High-Fidelity DNA Polymerase is inhibited at room temperature, allowing flexible reaction setup (RT or ice).

All components should be mixed prior to use. Q5 Hot Start High-Fidelity DNA Polymerase may be diluted in 1X Q5 Reaction Buffer just prior to use in order to reduce pipetting errors.

A	B	C	D
Component	**25 µl Reaction**	**50 µl Reaction**	**Final Concentration**
5X Q5 Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
Template DNA	variable	variable	< 1,000 ng
Q5 Hot Start High-Fidelity DNA Polymerase	0.25 µl	0.5 µl	0.02 U/µl
5X Q5 High GC Enhancer (optional)	(5 µl)	(10 µl)	(1X)
Nuclease-Free Water	to 25 µl	to 50 µl	

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Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary and overlay the sample with mineral oil if using a PCR machine without a heated lid.

3

Transfer PCR tubes to a PCR machine and begin thermocycling:

Q5 Hot Start High-Fidelity DNA Polymerase does not require a separate activation step. Standard Q5 cycling conditions are recommended.

Thermocycling Conditions for a Routine PCR:

A	B	C
STEP	**TEMP**	**TIME**
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
	*50–72°C	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

*Use of the NEB T_m Calculator is highly recommended.

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