PCR with Q5® Polymerase (M0491)

New England Biolabs

Abstract

This protocols is for PCR using Q5® High-Fidelity DNA Polymerase (M0491)

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Guidelines

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

Reaction Setup:
We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed prior to use. Q5 High-Fidelity DNA Polymerase may be diluted in 1X Q5 Reaction Buffer just prior to use in order to reduce pipetting errors.

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 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	

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

Transfer PCR tubes to a PCR machine and begin thermocycling.

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
	98°C	5–10 seconds
25–35 Cycles	*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 Tm Calculator is highly recommended.

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:

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 High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil-containing primers or templates.

5. Q5 High-Fidelity DNA Polymerase concentration:
We generally recommend using Q5 High-Fidelity DNA Polymerase at a final concentration of 20 units/ml (1.0 unit/50 µl reaction). However, the optimal concentration of Q5 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 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.

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

For high Tm 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 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 High-Fidelity DNA Polymerase will degrade any overhangs generated.

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

Materials

Q5 High-Fidelity DNA Polymerase - 100 units M0491S by New England Biolabs

Protocol

Step 1.
Set up the following reaction on ice:

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 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	

PROTOCOL

. Mixture for M0491 Q5 PCR

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Step 1.1.
5X Q5 Reaction Buffer

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for 25µl reaction

Step 1.2.
10 mM dNTPs

REAGENTS
Deoxynucleotide Solution Mix - 8 umol of each N0447S by New England Biolabs

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25µl reaction: 0.5µl

Step 1.3.
10 µM Forward Primer

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25µl reaction: 1.25µl

Step 1.4.
10 µM Reverse Primer

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25µl reaction: 1.25µl

Step 1.5.
Template DNA

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Variable, record amount of DNA added and adjust the amount of nuclease-free water added to create a total volume of 25µl.

Step 1.6.
Q5 High-Fidelity DNA Polymerase

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25µl reaction: 0.25µl

Step 1.7.
Optional: 5X Q5 High GC Enhancer

Step 1.8.
Nuclease-Free Water

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Added to create an end volume of 25µl

If no enhancer (step 7) is added, negative control will have 16.76µl of nuclease-free water.

Step 2.
Gently mix the reaction.

Step 3.
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.

Step 4.
Quickly transfer PCR tubes to a PCR machine preheated to the denaturation temperature (98°C) and begin thermocycling.

Warnings

Please note that protocols with Q5® High-Fidelity DNA Polymerase may differ from protocols with

other polymerases. Conditions recommended below should be used for optimal performance.