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PCR Using Q5U Hot Start High-Fidelity DNA Polymerase (NEB #M0515): *General PCR, USER®Cloning, dUTP incorporation/Carryover prevention* V.1

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We use this protocol and it's working

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Abstract

Q5U Hot Start High-Fidelity DNA Polymerase is a modified version of Q5®High-Fidelity DNA Polymerase, a novel thermostable DNA polymerase that possesses 3′ to 5′ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5U contains a mutation in the uracil-binding pocket that enables the ability to read and amplify templates containing uracil and inosine bases.

Guidelines

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. USER DNA Engineering
Target DNA molecules and cloning vector are generated by PCR with 8 – 12 bases of homology between two fragments. PCR primers start with a 5′ A and contain a single deoxyuracil residue (dU) flanking the 3′ end of the homology region, and can be designed to accommodate multiple-fragment assembly, nucleotide substitutions, insertions and/or deletions. We recommend using the GeneDesign (http://genedesign.thruhere.net/gd/) software to design primers for USER junctions. The best results are typically seen when using each primer at a final concentration of 0.5 µM.

4. $Mg^{++}$and additives:
Typically, the Mg^{++}concentration for Q5U Hot Start High-Fidelity DNA Polymerase should be 2.0 mM. When used at a final concentration of 1X, the Q5U Reaction Buffer provides this optimal Mg^{++}concentration. The addition of common PCR additives such as DMSO may improve amplification of certain difficult or long targets. In these cases, we recommend the addition of up to 2 % DMSO.

5. Deoxynucleotides:
The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

6. dUTP Incorporation/Carryover Prevention
Q5U Hot Start High-Fidelity DNA Polymerse is a dUTP-tolerant DNA polymerase that efficiently incorporates dUTP and amplifies uracil-containing substrates. To prevent carryover contamination, dUTP and Antartic Thermolabile UDG (NEB #M0372) can be added to the reaction. dTTP can be fully replaced by dUTP in the amplification of

certain targets. For best results, we recommend adding dUTP at a final concentration of 200 µM. For UDG activation, a 10 minute, 25 °C incubation step should be added before the initial denaturation step. Typical cycling parameters can be used thereafter.

7. Q5U Hot Start High-Fidelity DNA Polymerase concentration:
We generally recommend using Q5U 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 Q5U Hot Start High-Fidelity DNA Polymerase may vary from 10 – 40 units/ml (0.5 – 2.0 units/50 µl reaction) depending on amplicon length and difficulty. It is rarely helpful to exceed 2.0 units/50 µl reaction, especially for amplicons longer than 5 kb.

8. Buffers:
The 5X Q5U Reaction Buffer provided with the enzyme is recommended as the first-choice buffer for robust, high-fidelity amplification. The 5X Q5U Reaction Buffer contains 2.0 mM Mg^{++}at a final (1X) concentration.

9. Denaturation:
Q5U 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 targets being amplified from pure DNA templates. Longer initial 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.

10. Annealing:
Optimal annealing temperatures for Q5U 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. 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).

11. Extension:
The recommended extension temperature is 72 °C. Extension times are generally 20 – 30 seconds per kb for complex, genomic samples. Extension time can be increased to 1 minute per kb for long, complex templates, if necessary.

A final extension of 5 minutes at 72 °C is recommended.

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

13. 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.

14. Amplification of long products:
When amplifying products > 6 kb, it is often helpful to increase the extension time to 1 minute /kb.

15. PCR product:
The PCR products generated using Q5U 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 Q5U Hot Start 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

MATERIALS

Q5U® Hot Start High-Fidelity DNA Polymerase **New England Biolabs Catalog #**M0515

Troubleshooting

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Before start

Please note that protocols with *Q5U Hot Start High-Fidelity DNA Polymerase* may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

Reaction Setup:
Q5U Hot Start High-Fidelity DNA Polymerase is inhibited at room temperature, allowing flexible reaction setup (room temperature or ice).

All components should be mixed prior to use.

General PCR, USER®Cloning, dUTP incorporation/Carryover prevention

1 Set up the reaction using the following table:

	Component	25 µl Reaction	50 µl Reaction	Final Concentration
	5X Q5U 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
	Q5U Hot Start High-Fidelity DNA Polymerase	0.25 µl	0.5 µl	0.02 U/µl
	Nuclease-Free Water	to 25 µl	to 50 µl	

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.

2 Transfer PCR tubes to a PCR machine and begin thermocycling.

Note

Q5U Hot Start High-Fidelity DNA Polymerase does not require a separate activation step.

	STEP	TEMP	TIME
	Initial Denaturation	98 °C	30 seconds

	30 Cycles	98 °C	5 – 10 seconds
		*55 – 72 °C	20 seconds
		72 °C	20 – 30 seconds/ kb
	Final Extension	72 °C	5 minutes
	Hold	4 – 10 °C	

Thermocycling Conditions for a Routine PCR

*Use of the **NEB T_m Calculator** is highly recommended.