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

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:

<table>
<thead>
<tr>
<th>DNA Genomic</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ng – 1 µg</td>
<td></td>
</tr>
<tr>
<td>Plasmid or Viral</td>
<td>1 pg – 1 ng</td>
</tr>
</tbody>
</table>

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 Polymerase is a dUTP-tolerant DNA polymerase that efficiently incorporates dUTP and amplifies uracil-containing substrates. To prevent carryover contamination, dUTP and Antarctic 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 $\geq$ 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**

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

**SAFETY WARNINGS**

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

**BEFORE START INSTRUCTIONS**

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.

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<table>
<thead>
<tr>
<th>Component</th>
<th>25 µl Reaction</th>
<th>50 µl Reaction</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X Q5U Reaction Buffer</td>
<td>5 µl</td>
<td>10 µl</td>
<td>1X</td>
</tr>
<tr>
<td>10 mM dNTPs</td>
<td>0.5 µl</td>
<td>1 µl</td>
<td>200 µM</td>
</tr>
<tr>
<td>10 µM Forward Primer</td>
<td>1.25 µl</td>
<td>2.5 µl</td>
<td>0.5 µM</td>
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protocols.io | https://dx.doi.org/10.17504/protocols.io.7schnaw  Oct 16 2020
<table>
<thead>
<tr>
<th></th>
<th>10 µM Reverse Primer</th>
<th>1.25 µl</th>
<th>2.5 µl</th>
<th>0.5 µM Template DNA</th>
<th>variable</th>
<th>variable</th>
<th>&lt;&lt;1,000 ng Q5U Hot Start High-Fidelity DNA Polymerase</th>
<th>0.25 µl</th>
<th>0.5 µl</th>
<th>0.02 U/µl Nuclease-Free Water</th>
<th>to 25 µl</th>
<th>to 50 µl</th>
</tr>
</thead>
</table>

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**

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

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<table>
<thead>
<tr>
<th>STEP</th>
<th>TEMP</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>98 °C</td>
<td>30 seconds</td>
</tr>
<tr>
<td>30 Cycles</td>
<td>98 °C</td>
<td>5 – 10 seconds</td>
</tr>
<tr>
<td></td>
<td>*55 – 72 °C</td>
<td>20 seconds</td>
</tr>
<tr>
<td></td>
<td>72 °C</td>
<td>20 – 30 seconds/kb</td>
</tr>
<tr>
<td>Final Extension</td>
<td>72 °C</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Hold</td>
<td>4 – 10 °C</td>
<td></td>
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</tbody>
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