**Reaction Conditions:** 1X Q5 Reaction Buffer, DNA template, 0.5 µM primers, 200 µM dNTPs (not included), 1X Q5 High GC Enhancer (optional) and 1 unit of Q5 High-Fidelity DNA Polymerase in a total reaction volume of 50 µl.

**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.

**Unit Assay Conditions:** 25 mM TAPS-HCl (pH 9.3 @ 25°C), 50 mM KCl, 2 mM MgCl₂, 1 mM β-mercaptoethanol, 200 µM dNTPs including [β-³²P]-dTTP and 400 µg/ml activated Calv Tafmy DNA.

**Heat Inactivation:** No

**Endonuclease Activity:** Incubation of a 50 µl reaction in NEBuffer 2 containing a minimum of 10 units of Q5 High-Fidelity DNA Polymerase with 200 µM dNTPs and 1 µg of supercoiled pUC19 DNA for 4 hours at either 37°C or 72°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Physical Purity:** Purified to >97% homogeneity as determined by SDS-PAGE analysis using Cooomassie Blue detection.

**qPCR DNA Contamination (E.coli Genomic):** A minimum of 4 units of Q5 High-Fidelity DNA Polymerase is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is less than 1 E. coli genome.

**PCR**
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.

**Amplification of a variety of human genomic amplicons from low to high GC content using Q5 High-Fidelity DNA Polymerase.** All reactions were conducted using 30 cycles of amplification and visualized by microfluidic LabChip® analysis.

**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</th>
<th>AMOUNT</th>
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</thead>
<tbody>
<tr>
<td>Genomic</td>
<td>1 ng-1 µg</td>
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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 (http://frodo.wi.mit.edu/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.

**Certificate of Analysis**
Transfer PCR tubes to a PCR machine and begin thermocycling.

**Thermocycling Conditions for a Routine PCR:**

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</thead>
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<tr>
<td>Initial Denaturation</td>
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<td>30 seconds</td>
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*Use of the NEB Tm Calculator is highly recommended.

**Applications:**
- High-fidelity PCR
- Cloning
- Long or difficult amplification
- High-throughput PCR

**Reagents Supplied with Enzyme:**
- 5X Q5 Reaction Buffer
- 5X Q5 High GC Enhancer

**Quality Control Assays**
7 kb Genomic DNA PCR: 30 cycles of PCR amplification in a 50 µl reaction containing 20 ng genomic DNA with 1.0 unit of Q5 High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs and 0.5 µM of each primer in Q5 Reaction Buffer result in the expected 7 kb product.

20 kb Lambda DNA PCR: 22 cycles of PCR amplification in a 50 µl reaction containing 10 ng Lambda DNA with 1.0 unit of Q5 High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs and 1.0 µM of each primer in Q5 Reaction Buffer result in the expected 20 kb product.

**Enhancer-Dependent High GC (65% GC) PCR:**
30 cycles of PCR amplification in a 50 µl reaction containing 20 ng genomic DNA with 1.0 unit of Q5 High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs, 1X Q5 High GC Enhancer and 0.5 µM of each primer in Q5 Reaction Buffer result in the enhancer-dependent production of the 452 bp high GC product.

**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.

**APPLICATIONS:**
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- Cloning
- Long or difficult amplification
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**Certificate of Analysis**
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.

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 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:

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 enzyme concentration to 2 units/50 μl and 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).

Companion Products Sold Separately:
Q5 Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units
Q5 High-Fidelity 2X Master Mix
#M0492S 100 reactions
#M0492L 500 reactions
Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
Q5 Reaction Buffer Pack
#B9027S 6.0 ml
Deoxynucleotide Solution Set
#N0446S 25 μmol of each
Deoxynucleotide Solution Mix
#N0447S 8 μmol of each
#N0447L 40 μmol of each
Magnesium Chloride (MgCl2) Solution
#B9021S 6.0 ml

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