PCR with Q5® High-Fidelity 2X Master Mix (M0492) V.2

**Protocol status:**Working

**We use this protocol and it’s working**

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Abstract

The Q5 High-Fidelity 2X Master Mix offers robust, high-fidelity performance in a convenient master mix format. The Q5 High- Fidelity 2X Master Mix features a high-fidelity, thermostable DNA polymerase with 3´→ 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. With an error rate ~280-fold lower than that of *Taq* DNA Polymerase, Q5 High-Fidelity DNA Polymerase is ideal for cloning and can be used for long or difficult amplicons. The convenient master mix formulation is supplied at a 2X concentration. The mix contains dNTPs, Mg++ and a proprietary broad-use buffer requiring only the addition of primers and DNA template for robust amplification regardless of GC content. When used at the recommended 1X final concentration, the Q5 High-Fidelity Master Mix contains 2 mM Mg++. Q5 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 TmCalculator**](https://www.neb.com/external-links/tm-calculator) is highly recommended.

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:

|  | A | B |
| --- | --- | --- |
|  |  |  |
|  | 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**](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:

The Q5 High-Fidelity Master Mix contains 2.0 mM Mg++ when used at a 1X concentration. This is optimal for most PCR products generated with this master mix.

4. Deoxynucleotides:

The final concentration of dNTPs is 200 μM of each deoxynucleotide in the 1X Q5 High-Fidelity Master Mix. 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:

The concentration of Q5 High-Fidelity DNA Polymerase in the Q5 High-Fidelity 2X Master Mix has been optimized for best results under a wide range of conditions.

6. 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.

7. Annealing:

Optimal annealing temperatures for Q5 High-Fidelity DNA Polymerase tend to be higher than for other PCR polymerases. The [**NEB Tm Calculator**](http://tmcalculator.neb.com/#!/main) 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 10).

8. 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.

9. Cycle number:

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

10. 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.

Amplification of long products:

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

11. PCR product:

The PCR products generated using Q5 High-Fidelity 2X Master Mix 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**](https://www.neb.com/products/m0267-taq-dna-polymerase-with-thermopol-buffer) ) or Klenow exo– ([**NEB #M0212**](https://www.neb.com/products/m0212-klenow-fragment-3-5-exo) ).

Materials

MATERIALS

Q5 High-Fidelity 2X Master Mix - 500 rxns**New England BiolabsCatalog #**M0492L

Q5 High-Fidelity 2X Master Mix - 100 rxns**New England BiolabsCatalog #**M0492S

Safety warnings

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

Before start

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.

1

Set up the following reaction On ice:

|  | A | B | C | D |
| --- | --- | --- | --- | --- |
|  | **Component** | **25 µl Reaction** | **50 µl Reaction** | **Final Concentration** |
|  | Q5 High-Fidelity 2X Master Mix | 12.5 µl | 25 µl | 1X |
|  | 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 |
|  | Nuclease-Free Water | to 25 µl | to 50 µl |  |

2

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

Quickly transfer PCR tubes to a preheated (98°C) PCR machine and begin thermocycling.

**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 Tm Calculator**](http://tmcalculator.neb.com/#!/) is highly recommended.