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 T_mCalculator** 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** 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 T_m 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 T_m of the lower T_m primer. 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 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**) or Klenow exo⁻ (**NEB #M0212**).

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** is highly recommended.