Q5 Hi Fi 2X Master Mix Protocol

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

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- › Q5 High-Fidelity 2X Master Mix
- › 10 µM Forward Primer
- › 10 µM Reverse Primer
- › Template DNA
- › Nuclease-Free Water

Procedure

Assembly Overview

Table1

	A	B	C	D
1	**Component**	**25 µL Reaction**	**50 µL Reaction**	**Final Concentration**
2	Q5 High-Fidelity 2X Master Mix	12.5 µL	25 µL	1X
3	10 µM Forward Primer	1.25 µL	2.5 µL	0.5 µM
4	10 µM Reverse Primer	1.25 µL	2.5 µL	0.5 µM
5	Template DNA	variable	variable	< 1,000 ng
6	Nuclease-Free Water	to 25 µL	to 50 µL	

1. Gently mix reaction. Transfer PCR tubes to PCR machine.

Thermocycling Conditions for a Routine PCR

Table2

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	98°C	30 seconds
3	25–35 Cycles	98°C	5–10 seconds
4		*50–72°C	10–30 seconds
5		72°C	20–30 seconds/kb
6	Final Extension	72°C	2 minutes
7	Hold	4–10°C	

Table3

	A	B
1	**DNA**	**AMOUNT**
2	DNA Genomic	1 ng–1 µg
3	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.

11. Amplification of long products:

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

12. 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.