Mixture for M0530 Phusion PCR

New England Biolabs

Abstract

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Protocol

Step 1.
Nuclease-free water

Step 2.
5X Phusion HF or GC Buffer

NOTES

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GC buffer should be used in experiments where HF buffer does not work. Detergent-free reaction buffers are also available for applications that do not tolerate detergents (e.g. microarray, DHPLC).

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5X Phusion HF Buffer and 5X Phusion GC Buffer are provided with the enzyme. HF buffer is recommended as the default buffer for high-fidelity amplification. For difficult templates, such as GC-rich templates or those with secondary structure, GC buffer can improve reaction performance.

Step 3.
10 mM dNTPs

REAGENTS

Deoxynucleotide Solution Mix - 8 umol of each N0447S by New England Biolabs

NOTES

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Phusion cannot incorporate dUTP.

Step 4.
10 µM Forward Primer

Step 5.
10 µM Reverse Primer

Step 6.
Template DNA

Step 7.
DMSO (optional)

NOTES

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It is important to note that if a high concentration of DMSO is used, the annealing temperature

must be lowered as it decreases the primer Tm (2).

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Amplification of difficult targets, such as those with GC-rich sequences or secondary structure, may be improved by the presence of additives such as DMSO (included). A final concentration of 3% DMSO is recommended, although concentration can be optimized in 2% increments.

Step 8.

Phusion DNA Polymerase