PCR with Phusion

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Abstract

This is the PCR protocol for Phusion Polymerase, adapeted from NEB to match the protocol followed by Northeastern_Boston.

Citation: Joshua Timmons PCR with Phusion. **protocols.io**
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Before start

Annealing temperatures should be determined. We used Benchling for all Tm calculations.

Protocol

PCR Prep

Step 1.
Set up the following reaction on ice.

Component	20 µl Reaction
Nuclease-free water	to 20 µl
5X Phusion HF or GC Buffer	4 µl
10 mM dNTPs	0.4 µl
10 µM Forward Primer	1 µl
10 µM Reverse Primer	1 µl
Template DNA	variable
DMSO (optional)	(0.6 µl)
Phusion DNA Polymerase	0.2 µl

PROTOCOL

. Mixture for M0530 Phusion PCR

CONTACT: New England Biolabs
Step 1.1.
Nuclease-free water

Step 1.2.
5X Phusion HF or GC Buffer

NOTES

New England Biolabs 16 Oct 2014
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).

New England Biolabs 16 Oct 2014
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 1.3.
10 mM dNTPs

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

NOTES
New England Biolabs 16 Oct 2014
Phusion cannot incorporate dUTP.

Step 1.4.
10 µM Forward Primer

Step 1.5.
10 µM Reverse Primer

Step 1.6.
Template DNA

Step 1.7.
DMSO (optional)

NOTES
New England Biolabs 16 Oct 2014
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).

New England Biolabs 16 Oct 2014
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 1.8.
Phusion DNA Polymerase

PCR Prep

Step 2.
Gently mix the reaction.

PCR Prep

Step 3.
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.

Thermocycling

Step 4.
Quickly transfer PCR tubes from ice to a PCR machine with the block preheated to 98°C and begin thermocycling.

The following thermocycling settings were standard:

STEP	TEMP	TIME
Initial Denaturation	98 C	30 sec
32 Cyles	98 C 45-72 C 72 C	5 sec 15 sec 15 sec/kb
Final Extension	72 C	10 min
Hold	4 C	