PCR Using Phusion® High-Fidelity DNA Polymerase

Aim

Amplification of a DNA fragment of interest for further experiments.

Procedure

Note: All reaction components should be assembled on ice and the reaction mix should be quickly transferred to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed and centrifuged prior to use. It is important to add Phusion DNA Polymerase last in order to prevent any primer degradation caused by the 3´→ 5´ exonuclease activity.

Thaw 5X Phusion HF or GC Buffer, dNTPs, primers, DNA sample, DMSO, and Phusion DNA Polymerase on ice. Let thaw completely and mix before adding to reaction mix to avoid localized concentration differences.

Prepare a primer working solution containing 1 µl of the forward primer (100 µM) and 1 µl of the reverse primer (100 µM), and 8 µl of RNase-free water for a final volume of 10 µl. This achieves a final primer concentration of 200 nM.

Set up the following reaction on ice:

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Phusion HF or GC Buffer	4 µl	10 µl	1X
10mM dNTPs	0.4 µl	1 µl	200 µM
Primer mix	0.6 µl	1.2 µl	200 nM
Template DNA	variable	variable	<250 ng

Lab protocol

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iGEM Stockholm

DMSO	0.6 µl	1.5 µl	3%
Phusion DNA Polymerase	0.2 µl	0.5 µl	1
RNase-free water	To 20 µl	To 50 µl	0.5 U/ 1.0 U

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary.
Quickly transfer PCR tubes to a PCR machine and begin thermocycling.

STEP	TEMP	TIME
Initial Denaturation	98°C	30s
25-35 cycles: Denaturation Annealing Extension	 98°C *45-72°C 72°C	 5-10s 10-30s 15-30s/kb
Final Extension	72°C	5-10min
Hold	4°C	-

*Use of the **NEB Tm Calculator** is highly recommended.

When the program is finished, the PCR product may be removed and stored at 4°C.

Note:
The above protocol could also be used for colony PCR reactions.
The colony PCR reaction set-up follows the standard PCR protocol described above.
The DNA is obtained by picking a single colony from the plate using a sterile pipette tip and thoroughly swirling it in 20 µl of RNase-free water.
1 µl of this water will be used for the PCR reaction.
The rest could be plated or inoculated in a flask overnight.

Sources

This protocol is a modified version of the original Phusion® High-Fidelity DNA Polymerase PCR amplification protocol provided by NEB®.