PCR Using Q5® High-Fidelity 2X Master Mix

Aim

Amplification of a DNA fragment of interest for further experiments.

Procedure

Thaw Q5 High-Fidelity 2X Master Mix, primers, and DNA sample 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
Q5 High-Fidelity 2X Master Mix	12.5 µl	25 µl	1X
Primer mix	0.6 µl	1.2 µl	200 nM
Template DNA	variable	variable	<1000 ng
RNase-free water	To 25 µl	To 50 µl	

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.

Lab protocol

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iGEM Stockholm

STEP	TEMP	TIME
Initial Denaturation	98°C	30s
25-35 cycles: Denaturation Annealing Extension	 98°C *50-72°C 72°C	 5-10s 10-30s 20-30s/kb
Final Extension	72°C	2-5min
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 Q5® High-Fidelity 2X Master Mix PCR amplification protocol provided by NEB®.