PCR - Phusion™ High–Fidelity DNA Polymerase

Introduction

PCR stands for polymerase chain reaction which is a method used to amplify a specific sequence. Here follows the description of doing PCR for Thermo Fisher Phusion™ High–Fidelity DNA Polymerase.

Link to Thermo Fisher product information sheet of Phusion™ High–Fidelity DNA Polymerase and the original protocol:
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012393_Phusion_HighFidelity_DNAPolymerase_UG.pdf

Materials

- Phusion™ High–Fidelity DNA Polymerase
- 5X Phusion™ HF Buffer
- Template DNA
- Forward Primer
- Reversed Primer
- dNTPs
- DMSO (optional - if GC-rich amplicons are made)

Procedure

Preparations

- Read the following important notes (more info is found in Thermo Fishers product information sheet):
- Use 98°C for denaturation (see “Initial denaturation“ on page 3 and “Denaturation“ on page 3).
- The annealing rules are different from many common DNA polymerases (such as Taq DNA polymerases). Read “Primer annealing“ on page 3 carefully.
- Use 15–30 s/kb for extension. Do not exceed 1 min/kb (see “Extension“ on page 4).
- Use Phusion™ High–Fidelity DNA Polymerase at 0.5–1.0 U per 50 µL reaction volume. Do not exceed 2 U/50 µL (see “Enzyme“ on page 3).
- Use 200 µM of each dNTP. Do not use dUTP (see “Mg2+ and dNTP“ on page 3).
- Phusion™ High–Fidelity DNA Polymerase produces blunt end DNA products.

1. PCR reactions should be set on ice
2. Prepare a mastermix for the appropriate number of samples to be amplified.
3. The Phusion™ High–Fidelity DNA Polymerase should be pipetted carefully and gently as the high glycerol content (50%) in the storage buffer may otherwise lead to pipetting errors
4. Due to the nature of the Phusion™ High–Fidelity DNA Polymerase, the optimal reaction conditions may differ from PCR protocols for standard DNA polymerases.

5. Due to the high salt concentration in the reaction buffer the Phusion™ High–Fidelity DNA Polymerase tends to work better at elevated denaturation and annealing temperatures.

6. Follow the conditions listed in "Notes about cycling conditions" on page 3 when running your reactions

The PCR

1. Prepare PCR reactions. Add the following components in the order listed in the following table.

Component	20 µL rxn	50 µL rxn	Final conc.
H_2O	add to 20 µL	add to 50 µL	
5X Phusion™ HF Buffer[1]	4 µL	10 µL	1X
10 mM dNTPs	0.4 µL	1 µL	200 µM each
Forward primer[2]	X µL	X µL	0.5 µM
Reverse primer [2]	X µL	X µL	0.5 µM
Template DNA	X µL	X µL	
(DMSO[3], optional)	(0.6 µL)	(1.5 µL)	(3%)
Phusion™ High–Fidelity DNA Polymerase	0.2 µL	0.5 µL	0.02 U/µL

[1] Optionally 5X GC Buffer can be used. See "Buffers" on page 3 for details.
[2] The recommendation for final primer concentration is 0.5 µM, but it can be varied in a range of 0.2–1.0 µM, if needed.
[3] Addition of DMSO is recommended for GC-rich amplicons. DMSO is not recommended for amplicons with very low GC % or amplicons that are > 20 kb.

Notes

· It is critical that the Phusion™ High–Fidelity DNA Polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3´→5´ exonuclease activity that can degrade primers in the absence of dNTPs.

· Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery.

2. Run the following program:

Cycle step	2–step protocol		3–step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial Denaturation	98°C	30 s	98°C	30 s	1
Denaturation	98°C	5–10 s	98°C	5–10 s	25–35
Annealing [1,2]	–	–	X°C	10–30 s	
Extension [3,4]	72°C	15–30 s/kb	72°C	15–30 s/kb	
Final extension	72°C	5–10 min	72°C	5–10 min	1
Hold	4°C	Hold	4°C	Hold	Hold

[1] See "Primer annealing" on page 3.
[2] For the 2–step protocol, there is no annealing step.
[3] See "Extension" on page 4.
[4] For the 2–step protocol, annealing and extansion are performed at the same temperature.