PCR

Introduction

Polymerase chain reaction (**PCR**) is a laboratory technique used to make multiple copies of a segment of DNA. **PCR** is very precise and can be used to amplify, or copy, a specific DNA target from a mixture of DNA molecules.

Materials

- Phusion DNA Polymerase, 2 U/µL
- 5X Phusion HF Buffer
- 5X Phusion GC Buffer
- 50 mM MgCl2 solution
- DMSO

Procedure

1. CR reactions should be set up on ice.
2. Prepare a master mix 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 forstandard DNA polymerases.
5. Due to the high salt concentration in the reaction buffer, the Phusion™ High–Fidelity DNA Polymerase tends to work better at elevateddenaturation and annealing temperatures.

Table1

	A	B	C	D
1	Component	20 µL rxn	50 µL rxn	Final conc.
2	H2O	add to 20 µL	10 µL	1X
3	5X Phusion™ HF Buffer	4 µL	1 µL	200 µM each
4	Forward primer	X µL	X µL	0.5 µM
5	Reverse primer	X µL	X µL	0.5 µM
6	Template DNA	X µL	X µL	
7	(DMSO, optional)	(0.6 µL)	(1.5 µL)	(3%)
8	Phusion™ High–Fidelity DNA Polymerase	0.2 µL	0.5 µL	0.02 U/µL
9	10 mM dNTPs	0.4 µL	1 µL	200 µM each

6.

Table2

	A	B	C	D	E	F
1	Cycle step	2–step protocol		3–step protocol		Cycles
2		Temp.	Time	Temp.	Tiime	
3	Initial Denaturation	98°C	30 s	98°C	30 s	1
4	Denaturation	98°C	5–10 s	98°C	5–10 s	25-35
5	Annealing	-	-	X°C	10–30 s	
6	Extension	72°C	15–30 s/kb	72°C	15–30 s/kb	
7	Final extension	72°C	5–10 min	72°C	5–10 min	1
8	Hold	4°C	Hold	4°C	Hold	Hold

7.