PCR Protocol for Phusion® High-Fidelity DNA Polymerase

Introduction

Protocol for PCR using the Phusion polymerase adapted by Jacob Mejlsted from the NEB protocol for the same product.

Materials

- DNA
- Consumables
  - PCR tubes (1 per reaction + 1 for positive control)
- Chemicals
  - Forward primers
  - Reverse primers
  - Nuclease-free water
  - Phusion DNA Polymerase
  - 5X Phusion HF or GC Buffer
  - 10 mM dNTPs
  - DMSO (optional)

Procedure

PCR Amplfication of DNA Fragments

1. Prepare PCR reaction (see table)

Table1

	A	B	C	D
1	**Component**	**20 µl Reaction**	**50 µl Reaction**	**Final Concentration**
2	Nuclease-free water	to 20 µl	to 50 µl	
3	5X Phusion HF or GC Buffer	4 µl	10 µl	1X
4	10 mM dNTPs	0.4 µl	1 µl	200 µM
5	10 µM Forward Primer	1 µl	2.5 µl	0.5 µM
6	10 µM Reverse Primer	1 µl	2.5 µl	0.5 µM
7	Template DNA	variable	variable	< 250 ng
8	DMSO (optional)	(0.6 µl)	(1.5 µl)	3%
9	Phusion DNA Polymerase	0.2 µl	0.5 µl	1.0 units/50 µl PCR

2. Alternatively, a master mix can be prepared

Homemade master mix

	A	B	C
1	**Reactant**	**Per reaction (50uL) [µl]**	**Mastermix [µl]**
2	Number of reactions	1	10
3	5X Phusion HF or GC Buffer	10	100
4	10 mM dNTPs	1	10
5	10 µM Forward Primer	2.5	Added individually
6	10 µM Reverse Primer	2.5	Added individually
7	Template DNA	variable	Added individually
8	Phusion DNA Polymerase	0.5	5
9	DMSO (optional)	0	0
10	MilliQ	33.5	335

3. Run reaction in a thermocycler

Thermocycler PCR regimen

	A	B	C	D
1	Step	Temperature	Duration	Number of Cycles
2	Initial denaturation	98 C	30 seconds	1 cycle
3	Amplification	98 C	10 seconds	25-30 cycles
4		Primer Tm	20 seconds	
5		72 C	15-30 seconds/kb*	
6	Final extension	72 C	5-10 minutes	1 cycle
7	Hold	4 C	-	1 cycle

*15 seconds/kb works for most reactions. 30 seconds/kb can be used for more complex reaction, such as cDNA.