

PCR protocol (for different types of Polymerases)

Introduction

This protocol is subdivided into 3 sub-protocols each describing the required components and procedures for a PCR reaction. The sub-divided protocols use the following polymerases:

- 1) PfuX7 DNA Polymerase
- 2) OneTaq DNA Polymerase
- 3) Q5® High-Fidelity DNA Polymerase

Materials that are needed for all the different protocols

Consumables

- PCR tubes

Equipment

- Thermocycler

1) PCR using Pfu X7 DNA Polymerase

Materials

- 10X CXL buffer
- dNTPs (10 mM from Ampliqon PCR Enzymes and Reagents)
- Forward Primer (10 µM)
- Reverse Primer (10 µM)
- Pfu X7 polymerase
- DMSO
- Template DNA
- MilliQ water

Procedure

1. Prepare PCR reaction (see table).

Component	25 µL reaction***[µL]	50 µL reaction***[µL]	Final Concentration
10X CXL buffer	5	10	1X
10 mM dNTPs	0.5	1	200 µM
10 µM Forward Primer	1.25	2.5	0.2 µM
10 µM Reverse Primer	1.25	2.5	0.2 µM
Pfu X7 Polymerase	0.25	0.5	1.25 units/50 µl PCR**
DMSO	0.75	1.5	
Template DNA**	variable	variable	< 1,000 ng
MilliQ water	to 25	to 50	

**For plasmids or viral DNA, use 1 pg–10 ng DNA for a 50 µL reaction.

***It can be advantageous to pool some of the parts into a master mix, as some labs cannot dispense 0.125 µL accurately.

2. Alternatively, a master mix can be prepared.

Reactant	Per reaction (25 µL) [µL]	Mastermix [µL]
Number of reactions	1	4
CXL buffer	5	20
10 mM dNTPs	0.5	2
10 µM Forward Primer	1.25	5
10 µM Reverse Primer	1.25	5
Template	0.5	2
Pfu X7 DNA Polymerase	0.5	2
DMSO	0.75	3
MilliQ	16	64

3. Run reaction in a thermocycler using the following settings.

Step	Temperature	Duration	Number of Cycles
Initial denaturation	95 °C	2 minutes	1 cycle
Amplification	95 °C	30 seconds	25-30 cycles
	45-68°C	30 seconds	
	72 °C	around 1 min/kb*	
Final extension	72 °C	5 minutes	1 cycle
Hold	4 °C	-	1 cycle

*Some Pfu polymerases require 1-2 minutes/kb

- The PCR products can then be stored at -20 °C, used directly, or purified using PCR purification or gel extraction.

2) PCR using OneTaq DNA polymerase

Materials

- 5X OneTaq Standard Reaction Buffer
- dNTPs (10 mM from Ampliqon PCR Enzymes and Reagents)
- Forward Primer (10 µM)
- Reverse Primer (10 µM)
- OneTaq DNA polymerase
- Template DNA
- MilliQ water

Procedure

- Prepare PCR reaction (see table).

Component	25 µL reaction***[µL]	50 µL reaction***[µL]	Final Concentration
5X OneTaq Standard Reaction Buffer*	5	10	1X
10 mM dNTPs (#N0447)	0.5	1	200 µM
10 µM Forward Primer	0.5	1	0.2 µM
10 µM Reverse Primer	0.5	1	0.2 µM
OneTaq DNA Polymerase	0.125	0.25	1.25 units/50 µl PCR**
Template DNA**	variable	variable	< 1,000 ng
MilliQ water	to 25	to 50	

*OneTaq GC Reaction Buffer and High GC Enhancer can be used for difficult amplicons

**For plasmids or viral DNA, use 1 pg–10 ng DNA for a 50 µL reaction.

***It can be advantageous to pool some of the parts into a master mix, as some labs cannot dispense 0.125 µL accurately.

- Alternatively, a master mix can be prepared.

Reactant	50 µL REACTION [µL]	Mastermix [µL]
Number of reactions	1	10
5X OneTaq Standard Reaction Buffer*	10	100
10 mM dNTPs (#N0447)	1	10
10 µM Forward Primer	1	Added individually
10 µM Reverse Primer	1	Added individually
OneTaq DNA Polymerase	0.25	2.5
Template DNA	1	Added individually
Nuclease-free water	34.75	347.5

- Run reaction in a thermocycler using the following settings:

Step	Temperature	Duration	Number of Cycles
Initial denaturation	94 °C	30 seconds	1 cycle
Amplification	94 °C	15-30 seconds	25-30 cycles
	45-68°C	15-60 seconds	
	68°C	around 1 min/kb*	
Final extension	68°C	5 minutes	1 cycle

Hold	4 °C	-	1 cycle
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4. The PCR products can then be stored at -20 °C, used directly, or purified using PCR purification or gel extraction.

3) PCR using Q5® High-Fidelity DNA Polymerase

Materials

- 5X Q5 Reaction buffer
- dNTPs (10 mM from Ampliqon PCR Enzymes and Reagents)
- Forward Primer (10 µM)
- Reverse Primer (10 µM)
- Q5 High-Fidelity DNA polymerase
- Template DNA
- MilliQ water
- 5X Q5 High GC Enhancer (optional)

Procedure

1. Prepare PCR reaction (see table).

Component	25 µL reaction***[µL]	50 µL reaction***[µL]	Final Concentration
5X Q5 Reaction Buffer*	5	10	1X
10 mM dNTPs (#N0447)	0.5	1	200 µM
10 µM Forward Primer	1.25	2.5	0.2 µM
10 µM Reverse Primer	1.25	2.5	0.2 µM
Q5 DNA Polymerase	0.25	0.5	0.02 units/µl
Template DNA**	variable	variable	< 1,000 ng
MilliQ water	to 25	to 50	
5 X Q5 High GC Enhancer (optional)	5	10	1x

**For plasmids or viral DNA, use 1 pg–10 ng DNA for a 50 µL reaction.

***It can be advantageous to pool some of the parts into a master mix, as some labs cannot dispense 0.125 µL accurately.

2. Alternatively, a master mix can be prepared.

Reactant	Per reaction (50µL) [µl]	Mastermix [µl]
Number of reactions	1	10
5 x Q5 buffer	10	100
10 mM dNTPs	1	10
10 µM Forward Primer	1	Added individually
10 µM Reverse Primer	1	Added individually
Template	1	Added individually
Q5 High-Fidelity DNA Polymerase	0.5	2.5
MilliQ	34.75	347.5

3. Run reaction in a thermocycler using the following settings.

Step	Temperature	Duration	Number of Cycles
Initial denaturation	98 °C	30 seconds	1 cycle
Amplification	98 °C	10 seconds	25-30 cycles

	45-68°C	20 seconds	
	72°C	30 min/kb*	
Final extension	72°C	2 minutes	1 cycle
Hold	4 °C	-	1 cycle

4. The PCR products can then be stored at -20 °C, used directly, or purified using PCR purification or gel extraction.