PCR using X7 polymerase

Introduction

This protocol described the required components for the X7 polymerase to be used in a PCR

Materials

Chemicals

- CXL buffer: 10 uL
- dNTPs (10 mM from NEB): 1 uL
- Primer Fwd (10 uM): 2,5 uL
- Primer Rev (10 uM): 2,5 uL
- *Pfu* X7 polymerase 0,5 uL
- DSMO 1,5 uL
- Template 1 uL
- MilliQ to a total of 50 uL

Procedure

PCR Amplfication of DNA Fragments

1. Prepare PCR reaction (see table)

Table1

	A	B
1	**COMPONENT**	**50 µl REACTION**
2	CXL buffer	10 µl
3	10 mM dNTPs	1 µl
4	10 µM Forward Primer	2.5 µl
5	10 µM Reverse Primer	2.5 µl
6	Template DNA	Variable (often 1 µl)
7	Pfu X7 Polymerase	0.5 µl
8	DMSO	1.5 µl
9	Nuclease-Free Water	to 50 µl

2. Alternatively, a master mix can be prepared

Master Mix

	A	B	C	D	E
1		1 reaction	Master mix	number of reactions	Volume
2	CXL buffer	5	400	80	25
3	10 mM dNTPs	0.5	40	X	
4	10 µM Forward Primer	1.25	100	X	
5	10 µM Reverse Primer	1.25	100	X	
6	Template DNA	0.5	40		
7	Pfu X7 Polymerase	0.5	40		
8	DMSO	0.75	60	X	
9	Nuclease-Free Water	16	1280	X	

3. Run reaction in a thermocycler

Thermocycler PCR regimen

	A	B	C	D
1	Step	Temperature	Duration	Number of Cycles
2	Initial denaturation	95 C	2 minutes	1 cycle
3	Amplification	95 C	30 seconds	25-30 cycles
4		Primer Tm	30 seconds	
5		72 C	around 1 min/kb*	
6	Final extension	72 C	5 minutes	1 cycle
7	Hold	4 C	-	1 cycle

*Some Pfu polymerases require 1-2 minutes/kb

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