PCR using Q5® High-Fidelity 2X Master Mix

Introduction

Protocol for PCR using the Q5 2X master mix 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
  - Q5 2X Master Mix

Procedure

PCR Amplfication of DNA Fragments

1. Prepare PCR reaction (see table)

PCR Components

	A	B	C
1	**Component**	**25 µL reaction**	**50 µL reaction**
2	Q5 High-Fidelity 2X Master Mix	12.5 µl	25 µl
3	10 µM Forward Primer	1.25 µl	2.5 µl
4	10 µM Reverse Primer	1.25 µl	2.5 µl
5	Template DNA*	variable (0ften 0.5 µL)	variable (0ften 1 µL)
6	Nuclease-Free Water	to 25 µl	to 50 µl
7	**Total**	**25 µL**	**50 µL**

*Template DNA:
For a 50 µL reaction, 1 ng-1 µg is recommended for genomic DNA and 1 pg-10 ng is recommended for plasmid or viral DNA.

2. 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	30 seconds/kb	
6	Final extension	72 C	2 minutes	1 cycle
7	Hold	4 C	-	1 cycle

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