PCR

Introduction

PCR for the amplification of Insert-DNA.

Materials

- Polymerase
- Polymerase-Buffer
- dNTPs
- Primer
- Template
- nuclease free Water

Procedure

1. Pipette the following volumes:

	Volume [µl]
Q5-Buffer	10
Q5-Polymerase	0.5
dNTPs, 2mM	5
Primer fw, 10µM	5
Primer rv, 10µM	5
Template-DNA (10 ng/Probe)	1
nuclease-free H2O	23.5
Complete Volume	**50**

2. Distribute 50µl of the PCR-Mix to one PCR-Tube. Amplify the DNA in the Thermocycler, use the following programme:

Step	**Temperature**	**Time in se**

		c
Initial Denaturation (25-35 cycles)	98°C	120
Denaturation	98°C	20
Annealing	64°C	30
Extension	72°C	60
Final Extension	72°C	600
Cooling	4°C	forever

3. Purify with pRC-CleanUp or Prep-Gel.