

# PCR

## Aim of the experiment

Polymerase chain reaction is an in vitro technique to exponentially amplify DNA of interest. There are different existing variations and applications of the reaction which can be used for special functions (i.e. addition of certain short sequences at the 3' or 5' prime end, insertion of point mutation etc.)

## Materials

- Template DNA
- Forward Primer (see list of sequences)
- Reverse Primer (see list of sequences)
- Phusion® High-Fidelity PCR Master Mix with HF Buffer

## Procedure

1. Mix the following reagents.

Concentration	Reagents
1x	Phusion Master Mix
0.5µM	Forward Primer
0.5µM	Reverse Primer
1ng – 1µg	Template (genomic DNA)
1pg – 1ng	Template (plasmid DNA)
Fill to 50µl	ddH <sub>2</sub> O

2. Transfer tube to a thermocycler with the following program.

Step	Temperature (°C)	Time (s)
Initial denaturing	95	300
25 – 35 cycles	95	10
	Annealing Temperature	30
	72	30/kb
Final extension	72	300
Hold	4	forever