Polymerase chain reaction

Adapted from: https://www.neb.com/protocols/2012/08/29/protocol-for-q5-high-fidelity-2x-master-mix-m0492. Use NEB Tm Calculator to calculate the annealing temperature of the primers.

Aim of the Experiment

This experiment can be used for exponential amplification of a 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 3 or 5 point end, insertion of point mutation etc.)

Materials

- Q5-High-Fidelity 2x Master Mix (NEB, USA)
- Forward primer (See list of sequences)
- Reverse primer
- template DNA

Procedure

1. To a PCR tube add following reagents:

Table 1: PCR-Mix

Concentration	Chemicals
1x	Q5 HF 2x Master Mix
0.5 μM	Forward primer
0.5 μM	Reverse primer
1 ng to 1 μg	Template DNA (for genomic DNA)
1 pg to 1 ng	Template DNA (for plasmid or viral DNA)
fill up to 50 μl	H_2O

2. Transfer tube to a Thermocycler and run following program:

Table 2: Thermocycling conditions

Step	**Temperature** (°C)	**Time** (s)
Initial denaturation	98	30
25-35 cycles	98	10
	annealing temperature	20
	72	30/kb
Final extension	72	300
Hold	4	forever

Possible follow-up protocols

The following protocols are the next steps of a possible cloning cycle after a Polymerase Chain Reaction (PCR):

1. Restriction digest
2. Agarose-Gel-electrophoresis
3. PCR clean-up