Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is commonly used to amplify a certain DNA fragment. In our project we mainly used the PCR for the creation of our inserts.

In a first step the reaction mix was prepared in PCR-tubes. Every sample also needed a negative control, entailing the same ingredients but ddH_2O instead of the template DNA.

1.1. Reaction Mix (for 1 reaction):

- 10µL 5x Q5 Reaction Buffer
- 1µL 10mM dNTPs
- 10µL 5x Q5 High GC Enhancer (optional)
- 0,5µL Q5 High-Fidelity DNA Polymerase
- 1µL 10µM Primer Solution (1µL of each used primer was added, at least one forward and one backward primer are needed for a successful PCR reaction)
- 1µL template DNA 1pg/µL-1ng/µL (1µL of each used template was added, some reactions, only consisting of matching primers, did not need a template DNA)
- ddH_2O to a total volume of 50µL

The PCR-tubes were transferred to a thermocycler to go through the following temperature program.

1.2. Q5 Program:

- 30sec 98°C (initial denaturation)
- 30x:
    - 10sec 98°C (denaturation)
    - 40sec 72°C (primer annealing and extension)
- 2min 72°C (final extension)