# PCR Using Q5 High-Fidelity DNA Polymerase

**Prepare MasterMix:**

Per PCR-Tube:

- 5 µL 5x Q5 Reaction Buffer
- 0,5 µL 10 mM dNTPs
- 0,25 µL High-Fidelity Q5 Polymerase
- 15,75 µL MQ (to get to 25 µL with DNA and Primers)

  -> snip then spin down

  -> transfer 21,5 µL to each PCR-tube

**Add DNA and Primers:**

Per PCR tube:

- 1 µL Template DNA
- 1,25 µL 10µM forward Primer
- 1,25 µL 10µM reverse Primer

**PCR-Program:**

1. Initial Denaturation : 30 s at 98°C
2. Annealing and Extension: -> 10 s at 98°C

   (25-35 cycles) -> 30 s at 50-72°C*

   -> 20-30 s at 72°C per 1000 bp
3. Final Extension : 30 s at 72°C
4. Hold : 8°C

* use  NEB Tm Calculator