# PCR Protocol

## Phusion High-Fidelity DNA Polymerase

### Reaction setup:

| | | |
|---|---|---|
| **Water** | 13,3 - X µl | 32,5 - X µl |
| **10X DreamTaq Buffer** | 4 µl | 10 µl |
| **dNTP Mix (10mM each)** | 0,5 µl | 1 µl |
| **Forward primer (10 µM)** | 1 µl | 3 µl |
| **Reverse primer (10 µM)** | 1 µl | 3 µl |
| **DreamTaq (5 U/µl)** | 0,2 µl | 0,5 µl |
| **Tempalte DNA** | X µl (0,004 - 100 ng) | X µl (0,01 - 250 ng) |
| Total | **20 µl** | **50 µl** |

Phusion DNA Polymerase exhibits 3’ ⟶ 5’ exonuclease activity that can degrade primers in the absence of dNTPs.

### Thermocycler program:

| **Step** | **Temperature** | **Time** | **Cycles** |
|---|---|---|---|
| Initial denaturation | 98 ˚C | 30 s - 3 min | 1 |
| Denaturation | 98 ˚C | 5-10 s | 25-40 |
| Annealing | Lower Tm + 3 ˚C | 10-30 s | |
| Extension | 72 ˚C | 15-40 s/kb | |
| Final extension | 72 ˚C | 5-10 min | 1 |

# DreamTaq DNA Polymerase

Reaction setup:

| | | |
|---|---|---|
| **Water** | 15,4 - X µl | 37,75 - X µl |
| **10X DreamTaq Buffer** | 2 µl | 5 µl |
| **dNTP Mix (10mM each)** | 0,5 µl | 1 µl |
| **Forward primer (10 µM)** | 1 µl | 3 µl |
| **Reverse primer (10 µM)** | 1 µl | 3 µl |
| **DreamTaq (5 U/µl)** | 0,1 µl | 0,25 µl |
| **Tempalte DNA** | X µl (0,004 - 400 ng) | X µl (0,01 - 1000 ng) |
| Total | **20 µl** | **50 µl** |

Thermocycler program:

| **Step** | **Temperature** | **Time** | **Cycles** |
|---|---|---|---|
| Initial denaturation | 95 ˚C | 1-3 min | 1 |
| Denaturation | 95 ˚C | 30 s | 25-40 |
| Annealing | Lower - 5 ˚C | 30 s | |
| Extension | 72 ˚C | 1 min/kb | |
| Final extension | 72 ˚C | 5-15 min | 1 |

# PrimeSTAR HS DNA Polymerase

Reaction setup:

| | | |
|---|---|---|
| **Water** | 13,3 - X µl | 32,5 - X µl |
| **10X DreamTaq Buffer** | 4 µl | 10 µl |
| **dNTP Mix (10mM each)** | 0,5 µl | 1 µl |
| **Forward primer (10 µM)** | 1 µl | 3 µl |
| **Reverse primer (10 µM)** | 1 µl | 3 µl |
| **DreamTaq (5 U/µl)** | 0,2 µl | 0,5 µl |
| **Tempalte DNA** | X µl (0,004 - 100 ng) | X µl (0,01 - 200 ng) |
| Total | **20 µl** | **50 µl** |

Thermocycler program:

| **Step** | **Temperature** | **Time** | **Cycles** |
|---|---|---|---|
| Initial denaturation | 98 ˚C | 30 s - 3 min | 1 |
| Denaturation | 98 ˚C | 10 s | 25-40 |
| Annealing | Tm | 5-15 s | |
| Extension | 72 ˚C | 1 min/kb | |
| Final extension | 72 ˚C | 5-10 min | 1 |