Overview of PCR process

All reagents must be kept **on ice** at all times. Assemble and mix **on ice** as well.
We are using the X7 DNA Polymerase (Common stock) and Phusion buffer 5x HF (Sotirios' lab)

Thermocycler setup

1. **Initialization (hot start PCR):** Heat chamber to respective temperature (98 °C for X7) in order to activate the DNA polymerase.
2. **25-35 Cycles:**
    i. **Denaturation:** Heat chamber to denature DNA (98 °C for 5-10 sec. for X7 - Can go up to 30 sec.).
    ii. **Annealing:** Re-annealing of DNA strings/primers. Too hot and no hybridization, too cold and hybridization becomes unspecific (Use NEB Tm Calculator or similar to determine annealing temperature. 45-72°C for 10-30 sec. Typically ~3°C above the lowest Tm). Alternatively, do a temperature gradient test, where different annealing temperatures are tested to find the optimal one.
    iii. **Elongation:** dNTPs are linked by the polymerase (For X7: 72 °C, 15-30 sec per kb).
3. **Final elongation:** Makes sure all single strands are fully elongated (For X7: 72 °C for 5-10 min).
4. **Hold:** Cool down and use for short-term storage at 10 °C.

Reagents:

Depending on the use the PCR mixtures is assembled in an appropriate volume typically between 10 and 50 µL.

- For testing if a given insert is there, use 10 µL.
- When amplifying something (e.g. a plasmid or gene insert) for later use or gel extractions, do PCR in 50 µL aliquots.

Protocol:

1. Dilute the primers 10x in an Eppendorf tube (or similar) using nuclease free H_2O.
2. Dilute plasmid 30x in an eppendorf tube (or similar) using nuclease free H_2O.
3. Mix reagents in the listed order.
4. Run the PCR.

Component	10 uL reaction	50 uL reaction
MQ H2O	6.7 uL	33.5 uL
5x Phusion HF or GC Buffer	2.0 uL	10.0 uL
dNTPs	0.2 uL	1.0 uL
10x diluted Forward Primer	0.25 uL	1.25 uL
10x diluted Reverse Primer	0.25 uL	1.25 uL
30x diluted Template DNA (Plasmid)	0.4 uL	2.0 uL
X7 DNA Polymerase	0.2 uL	0.5 uL

N.B. Generally if ssDNA is used as template, use 1/4th the reaction volume.