

Amplicon PCR

1. **Preparations:** Take out of the freezer and thaw all reagents on ice:

1. Primers (5 μ M) – the stock solution comes with 100 μ M, if you need to dilute more primer solution take nuclease free water.
2. OneTaq HS Quick-Load 2XMM w/Std Buffer from Biolabs
3. DNA templates (1 ng/ μ L)

2. **Prepare PCR reactions:**

1. Prepare MM in 1,5 mL reaction tubes:

Mastermix Component	Final [c]	1 X reaction
5 μ M Forward Primer	0,2 μ M	1 μ L
5 μ M Reverse Primer	0,2 μ M	1 μ L
OneTaq HS Quick-Load 2X MM	1X	12,5 μ L
Nuclease-free water		9,5 μ L
total		24 μ L

2. Pipette 24 μ L MM in a PCR reaction tube
3. Add 1 μ L of the prepared DNA template (1 ng/ μ L)

3. **Run PCR program:** ~ 1h, PCR products can be stored at -20°C or o/n at 4°C

Cycles	Temperature	Time
1 cycle	94 °C	30 seconds
25 cycles	94 °C	15 seconds
	56°C	30 seconds
	68°C	45 seconds
1 cycle	68°C	5 minutes
1 cycle	4°C	open end

4. **Measure PCR product concentration with 1xdsDNA high sensitivity Qubit assay:**

1. Measure DNA Concentration with Qubit™ 1x dsDNA HS Assay Kit. (stored at +4°C)
2. Use special Qubit™ assay tubes
3. Use 1 μ L extracted DNA solution + 199 μ L Qubit™ 1X dsDNA HS Working Solution
4. Vortex the tubes for 2 - 3 seconds
5. Incubate the tubes for 2 minutes at room temperature in the dark