**PCR with Qiagen Multiplex PCR Master Mix**

**Basics:**

* Primers normally used to PCR reaction are in 10 uM/ul concentration.
* We carry out a PCR in 10 ul.
* In case of using few primers pairs in one reaction (multiplex), higher primer concentration might be needed.
* When preparing Master Mix always use more reagents than needed (~10% more).
* Always sign the PCR plate with your name and date.
* Amount of all ingredients used in PCR and reaction volume might be changed freely, but „2x Qiagen Buffer” must always be half the volume.
* Do not have to prepare PCR on ice!

Standard Master Mix preparation for one pair of primers.

|  |  |  |
| --- | --- | --- |
| **Component** | **x 1** | **x100\*** |
| 2x Qiagen Buffer | 5 | 500 |
| **10 uM** Primer F 1 | 1 | 100 |
| **10 uM** Primer R 1 | 1 | 100 |
| dd H2O | 2 | 200 |
| **total** | **9 ul** | **900 ul** |
|  |  |  |
| **+ DNA template** | **1 ul** |  |

\* for 96 samples

Master Mix preparation for multiplex (eg. three primer pairs). Use primers with higher concentration (20 uM or higher).

|  |  |  |
| --- | --- | --- |
| **Component** | **x 1** | **x100\*** |
| 2x Qiagen Buffer | 5 | 500 |
| **20 uM** Primer F 1 | 0.5 | 50 |
| **20 uM** Primer R 1 | 0.5 | 50 |
| **20 uM** Primer F 2 | 0.5 | 50 |
| **20 uM** Primer R 2 | 0.5 | 50 |
| **20 uM** Primer F 3 | 0.5 | 50 |
| **20 uM** Primer R 3 | 0.5 | 50 |
| dd H2O | 1 | 100 |
| **total** | **9 ul** | **900 ul** |
|  |  |  |
| **+ DNA template** | **1 ul** |  |

\* for 96 samples

**Thermocycler program**

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Temp. [oC]** | **Time [s]** | **Cycles** |
| Initial denaturation | 95 | 900 | 1 |
| Denaturation | 94 | 30 | 30 |
| Annealing | 50 | 90 | 30 |
| Extension | 72 | 90 | 30 |
| Final extension | 72 | 600 | 1 |
| Store | 8 | ∞ |  |