

## Textual Description of the Automated Experiment – Part II

### 1. System Overview

The automated system to perform Part II must be able to perform a variety of tasks based on molecular biology kits by third party providers. General features:

- Fully enclosed
- Not sterile but decontamination via UV light must be possible
- Space for an additional automated phase contrast microscope
- Suitable for NGS and other molecular biological work
- Options for cooling of plates and reaction tubes (0.5, 1.5 and 2 mL)

### 2. Data Management Principles

It is important to link sampling IDs of the Sanger sequencing approach with the wells the samples originate from (Part I). Once Sanger Data are processed, the human will decide which wells to select that then change the logical state in the database to “selected”. These wells receive a stain identifier of the format “strain\_experiment code\_#column\_#row”.

### 3. Tasks and Reaction systems (Kits)

Each task should be run as a separate experiment. The human provides input, like how many reactions will be performed. For example, how many 96 well PCR plates are provided for the 16S rRNA gene PCR

#### 16S PCR

The PCR is performed with one primer pair for all reactions and a Mastermix prepared by a human including these primers (DreamTaq from ThermoFisher). The Master Mix (about 5.5 mL) is provided by the human and must be cooled either in one or several tubes.

#### PCR Purification

Macherey-Nagel NucleoSpin Gel and PCR Clean-up Kit (<https://www.mnnet.com/de/nucleospin-gel-and-pcr-clean-up-mini-kit-fuer-gelextraktion-undpcr-clean-up-740609.50>) or equivalent magnetic bead-based method.

#### Qubit Measurements

1x dsDNA BR Kit (<https://www.thermofisher.com/order/catalog/product/Q33265>)

#### Whole Genome Amplification

Qiagen REPLI-g Single Cell Kit (<https://www.qiagen.com/deus/products/discovery-and-translational-research/pcr-qpcrdpcr/preamplification/repli-g/repli-g-single-cell-kit?catno=150343>); until Debranching (T7-digestion), only ¼ reaction volume is used, afterwards full volume

#### Oxford Nanopore Sequencing Library preparation

Native Barcoding Kit 24 V14 (SQK-NBD114.24) <https://store.nanoporetech.com/eu/native-barcoding-kit-24-v14.html>