Textual Description of the Automated Experiment – Part I

1. System Overview

The automated system consists of:

- A levelled housing with an on-site computer enabling the user to operate and monitor the state of the experiment at any time, must be connected to the data base able to visualize data from the data base
- A spot to insert and remove three racks (see rack characteristic below) by the operator without influencing the sterile state of the system, racks are held in place by magnets or other durable holding devices on defined positions to ensure that the robot grips the plates of the racks reliably
- Plate gripper able to grip plates from a variety of vendors in 96- & 384-well formats
- OD600 plate reader suited for 96- & 384 well plates, must be able to store the data in the on-site data base, must be able to heat the lid to remove condensation bubbles on the lid, must be able to perform shaking (linear and orbital)
- A system to un-lid a plate, to keep the lid sterile and to return the lid on the plate
- Multi-channel dispenser (minimum 4 channels, 10–1000 µL range, compatible with 96- and 384-well plates, must be able to dispense liquid sterile / must be cleanable, must be able to dispense silicone oil (*insert product number here*))
- Pipetting head (which aspiration position can be assigned to user-specified position within the well, including bottom-aspiration); must be capable for cherry-picking
- Storage capacity for minimum 40 × 384-well plates with lids; pipet tips and a minimum of 10 × 96 well plates with lids
- A place to collect trash (L2-safe, consumables, etc.)
- 2 removable incubation racks for minimum 40 × 384-well plates in total
- 1 removable output rack for minimum 20 either 384-well or 96-well plates
- Storage and handling capability for 96-well plates
- Persistent, power-shortage-safe, redundant database for full experiment state tracking, accessible from on-site and off-site (e.g. office) locations

Environmental Requirements

- Operation in a fully enclosed, sterile environment (minimum ISO 5) with laminar flow and overpressure if doors are opened.
- Equipped with multiple UV light sources for full decontamination.
- Workspace monitored by one or more remotely controllable cameras.
- Camera data stored for 1 month and accessible for troubleshooting.
- Error messages during the run are sent to an e-mail enabling fast troubleshooting also outside core-working times
- Robot stops working when workspace of robot is intruded by human (safety protection)

2. Data Management Principles

For **every well**, the following data must be persistently stored:

- Unique Plate ID (including experiment ID)
- Well position

- Raw OD600 values (all time points)
- Blank-corrected values (where applicable)
- Logical state:
    - “blank”
    - “keep”
    - “ignore”
    - “ready for cherry-picking”
    - “cherry-picked”
    - “selected”
- Cherry-picking history (source–destination mapping)
- Timestamps and day indices

The database should provide:

- Human-readable reports
- Graphical visualization of growth curves (optional)
- Easy filtering and well tracking
- Export function of the data in .csv files
- 24/7 accessibility (also if no experiment is running)
- A multi-level structure placing data in respective folders/levels specified and changed by the user
- Contain all collected data

All experiment phases must be restart-safe. Every data file is saved after the addition of every new piece of information to the file ensuring data integrity after power-shortages or device failure. All information needs to be saved to allow restart of the experiment that then continues where it stopped before. Backup is preferable realized via a RAID system that can be located on an external drive.

I.1 Setting Up an Experiment

Initial Conditions

A human registers a new experiment in the database by providing:

- Unique experiment identifier
- Experiment code (3 characters and or numbers in upper or lower case)
- Metadata (sample origin, date, operator, etc.)
- Number of 384 well plates that are part of this experiment (1-40)

Dispenser Channel Configuration

- Channel 1: Media + sample mixture
- Channel 2: Medium only
- Channel 3: Silicone oil (Sigma xyz)
- Channel 4+: Optional

Plate Supply

- 1–40 384-well plates provided in supply racks (stored with lid).

- System must support both 384-well and 96-well plates.
- Plates must be kept sterile during storages

Loading Procedure (Performed X times, X = 1–40)

For each plate:

1. Robot retrieves one 384-well plate using the gripper from the supply rack.
2. Plate is transferred to the dispenser deck.
3. Lid is removed and placed in a defined temporary lid position keeping it sterile.
4. Row A is filled with 10–40 µL medium (blank control + sterile control) without introducing air bubbles.
5. Rows B–P are filled with 10–40 µL sample + medium without introducing air bubbles.
6. All wells are topped with 10–20 µL silicone oil without introducing air bubbles.
7. Lid is put back on plate.
8. Plate is transferred to removable incubation rack.
9. Plate state and loading confirmation are written to database.

Procedure repeats until X plates are loaded.

I.2 Initial Two-Week Measurement Phase

Initial Well States

- Row A → state: “blank”
- All other wells → state: “keep”

Daily Measurement Procedure (14 consecutive days)

For each plate (X plates total):

1. Retrieve plate from removable incubation rack.
2. Transfer to plate reader.
3. Remove lid.
4. Measure OD600 for all wells.
5. Calculate mean OD600 of blank wells (row A).
6. IF OD600 of row A > 0.1 pause and inform human: “sterility issue check”
7. For each well with state “keep”:
    - Compare OD600 to 2 × mean blank OD or user-defined value.
    - If OD600 > 2 × blank mean or user-defined value:
        - Update state to “ignore”.
8. Store:
    - Raw OD values
    - raw blank OD and calculated mean blank OD
    - State transitions
    - Timestamp and day index
9. Put lid back on plate.

10. Return plate to original rack position.

Repeat daily for 1-14 days.

At end of two-week measurement phase:

- Database contains complete measurement history.
- removable incubation racks will be removed and incubated elsewhere
- Experiment is paused and other experiments can be performed in between.

I.3 Restart After Two Months

Initialization

1. Robot loads stored well states from database.
2. Human provides OD600 selection threshold.
3. Human provides both removable incubation racks

Measurement Procedure

For each plate:

1. Retrieve plate.
2. Transfer to reader.
3. Remove lid if necessary.
4. Measure OD600 for all wells.
5. Store raw OD600 values.
6. Replace lid if necessary.
7. Return plate to rack.

Selection

For each well:

- If state = “keep”
- AND OD600 > human-defined threshold
  → update state to “ready for cherry-picking”

Robot reports number of ready wells to human.

Human decides:

- Start cherry-picking and afterwards continue incubation
- Continue incubation without cherry-picking

I.4 Cherry-Picking Procedure

Preparation of 96-Well Plates

For Y required 96-well plates:

1. Retrieve plate from supply.
2. Remove lid, keep sterile.
3. Fill Row A with 50–100 µL sterile medium (blank control + sterility control).
4. Fill remaining wells with 50–100 µL medium.
5. Store preparation event in database.

Transfer Procedure

For each well marked “ready for cherry-picking”:

1. Retrieve corresponding 384-well plate.
2. Remove lid.
3. Aspirate 30 µL from bottom of source well.
4. Dispense into unique destination well in 96-well plate except row A.
5. Update source well state to “cherry-picked”.
6. Record source–destination mapping in database.
7. Repeat until all wells marked as “ready for cherry picking” are transferred to a unique well in the 96-well plate.
8. Put lid back on 384-well plate.
9. Return 384 plate to removable incubation rack.

After completion:

- Put Lid back on 96-well plate.
- Store 96-well plates in output rack.

I.5 Incubation of Candidate 96-Well Plates

For each candidate plate (1–20 days, human decision):

Daily:

1. Retrieve plate.
2. Transfer to reader.
3. Remove lid.
4. Measure OD600.
5. Calculate mean blank (row A).
6. If mean blank row A >0.1 pause and inform human “potential sterility issue”
7. Subtract blank from all other wells.
8. Store values in database.
9. Put lid back on plate.
10. Return plate to rack.
11. Display data graphically to human: OD600 evolution over time for each well separately.

Human assigns plate status:

- “ready”
- or “continue incubation”

Plates marked “ready” become “master plates”.

I.6 Master Plate Output

For each plate marked “ready”:

1. Retrieve master plate.
2. Retrieve fresh 96-well cultivation plate -> “backup plate”.
3. Remove lid of backup plate.
4. Fill all wells of backup with 100 µL medium.
5. Remove lid from master plate.
6. Transfer 40 µL from each master well to corresponding backup well.
7. Put lid back on backup plate
8. Retrieve 96-well PCR plate.
9. Transfer 5 µL from each master well to PCR plate.
10. Put lid back on master plate.
11. Store mapping in database.
12. Hand over master, backup, and PCR plates to human on removable output rack.
13. Mark master plate as “completed”.

Repeat until no plates marked as “ready” remain.

I.7 Further Restarts (6 Months and 1 Year, adjustable if necessary)

At each restart:

1. Load well states from database.
2. Perform OD600 measurement (as in I.3).
3. Apply new human-defined threshold.
4. Wells that:
    - are “keep”
    - are not marked as “cherry-picked”
    - exceed threshold
      → become “ready for cherry-picking”
5. Perform cherry-picking (I.4).
6. Return plates to rack.
7. Store all updates persistently.

I.8 Final cherry-picking of strains

Once the Sanger 16S rRNA gene sequencing was performed and evaluated (in house AI system), the human selects the wells that get the status “strain_*experiment code*_#colum_#row” in the database. The database changes the logical state of the selected wells to “selected”

- Z 96-well “Master Plates” are transferred to the robot via the removable output rack

1. Retrieve master plate.

2. Retrieve fresh 96-well cultivation plate -> “strain plate”.

4. Remove lid of strain plate.
5. Fill all wells of strain plate with 50 µL medium.
6. Transfer 100 µL from each master well with logical state “selected” to the strain plate. Count from n=1-96
7. Write into the database which history each strain plate well has.
8. If no “selected” wells are left in “Masterplate” and n<96 place master plate back into cultivation rack and retrieve next master plate. Return to 6.
9. If n=96 put lid back on strain plate and move strain plate back to output rack.
10. Return to 1 until no master plate with a single well with the logical status “selected” is left.
11. Hand over strain plates to human via removable output rack.

I.9 General Technical Constraints

12. All volumes must be user-adjustable within plate-specific limits.
13. All pipetting steps must:
    1. Avoid air bubble formation
    2. Use bottom aspiration where required
    3. Include optional slow dispense mode
14. All phases must be restart-safe.