TECHNICAL NOTE

Recording Bacterial Growth Curves in the Cytomat 2 C Incubators

Key Words
bacterial growth, Cytomat, tower shaker, true orbital shaking, Thermo momentum, R

Introduction

Monitoring and optimization of bacterial and yeast growth is important for many applications:

- biochemistry, such as production of proteins, whole cell bio-assays, etc.
- microbiology, such as metabolic pathway studies
- biotechnology, such as metabolite production, upscaling of cell production

We demonstrate here, a method to easily monitor cellular growth in microtiter plates (96 or 384 well) of various resolution (5 minutes to several hours). This method is fully automatable and, if completely setup, does not need human interaction to do the final growth plot visualization. This is highly desirable with multiple growth curves being generated by a lab automation system.

Fig. 1: University-Greifswald Robotic High.Throughput Protein Screening Platform LARA (lara.uni-greifswald.de)

Fig. 2: Thermo Cytomat 2 C1550-LiN ToS equipped with two Tower Shakers.

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Materials and Methods

The LARA system described here is capable of numerous biological assays. For the purposes of this note we focus on the devices critical to monitoring **bacterial growth** and capturing that data.

- **Cytomat** 2C1550-LiN ToS Automated Incubator equipped with 2x True Orbital **Tower Shaker**, 2mm orbital amplitude *(Thermo Fisher Scientific, Langenselbold, Germany)*
- Momentum Automation Scheduling Software v5 or greater *(Thermo Fisher Scientific, Burlington, Canada)*
- F5 Robotic Arm *(Thermo Fisher Scientific, Burlington, Canada)*
- Thermo Varioskan™ LUX Multimode plate reader *(Thermo Fisher Scientific, Waltham MA)*
- Standard microtiter plates (SBS format, round wells, clear flat bottom, polystyrene, e.g. Greiner BioOne 655001) with unstructured/plain lids
- ***R****/R-Studio* (cran.r-project.org / www.rstudio.com - all free & open source)
- *LARA-R* (gitlab.com/larasuite/lara-R)

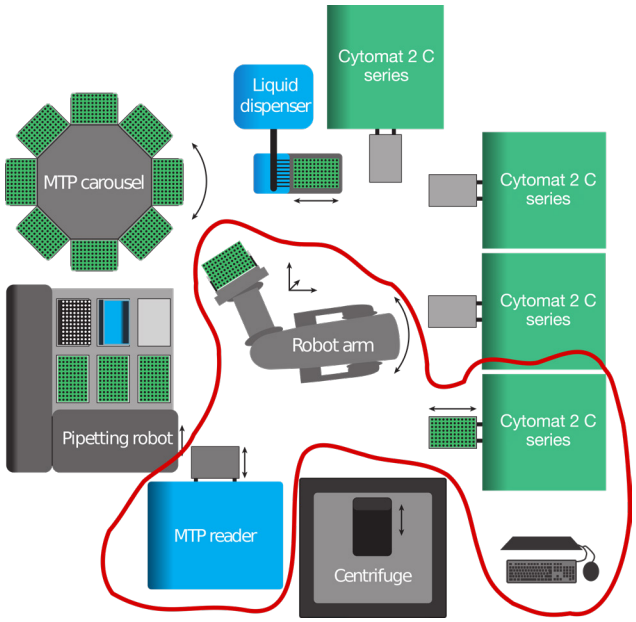

Fig.3: 2D top view of the Greifswald Robotic Protein Screening Platform LARA. Encircled in red are the minimum required devices for the measurements described in this application note.

Plates containing *E. coli* in 200 µL LB media per well begin in the automated incubator equipped with two orbital shaking towers and are stored here over the course of the experiment. Inoculation of the wells should be done with very small cell amounts (< 0.1 µl), (e.g. with the needles of a picking robot), to have even starting conditions. The plates are lidded to prevent contamination and the lids should not completely seal the wells so that oxygenation of the growing cells is not prohibited. The growth experiments can extend from several hours to several days depending on the assay. In this case, the system is programmed to automatically retrieve plates and deliver them to the reader at designated time points for data capture on the plate reader.

For bacterial growth we found that shaking at 700 rpm and maintaining a temperature of 37 °C, and approximate rH of 80% gave good results. The Cytomat 2 series with True Orbital Shaking was our choice of device.

Using absorbance readings to measure the turbidity of the solution, we calculate the cell growth and easily monitor this throughout our experiments. We create a single end-point absorption measurement at the desired wavelength. We used 600 nm for our data here. Common wavelengths for **bacterial growth** measurements are 600 and/or 660 nm, but this also depends on the application/ organism. It should be noted to also set the reader temperature to the cultivation temperature, e.g. 37 °C.

For the statistical evaluation and visualization of the recorded data it is very important to select the right plate reader output format, so that *LARA-R* can read the generated files. *LARA-R* reads plain text file (ASCII) outputs. Please refer to the documentation of *LARA-R* for the details of the reader output format. For the data output, it is highly recommended to generate one directory for each run and export all absorption measurements belonging to the run into that directory.

Install the free statistics software ***R*** according to the instructions at https://cran.r-project.org. It is highly recommended (but not required) to also install the free ***R*** development environment R-Studio from www.rstudio.com.

The *LARA-R* scripts should be installed according to the documentation at github.com/LARAsuite/lara-R and/or gitlab.com/LARAsuite/lara-R.

The growth curves are recorded using **Thermo Momentum** Automated Scheduling software for controlling the hardware and **LARAsuite** to control the data collection and evaluation. **Fig. 4** shows a schematic flow chart of the Momentum process. It should be noted here, this details only the cell growth monitoring and data capture. The intent is that this is documented for implementation into any number of larger scale workflows.

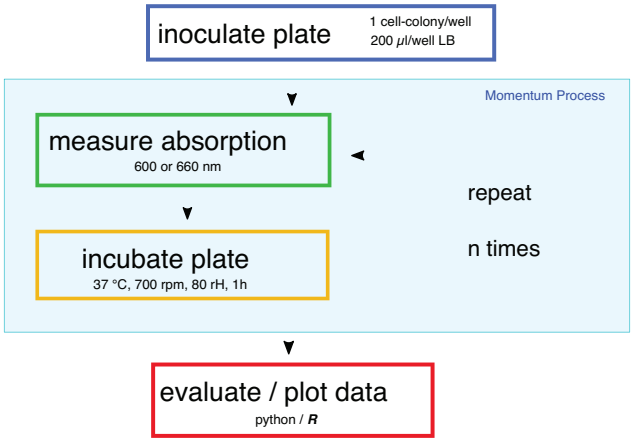

Fig.4: Data recording flow chart. This process can be implemented by Thermo Momentum Scheduling Software to record the growth data.

Results

Loading all the absorption data files and plotting the growth data can be achieved with four powerful commands in ***R*** (# is a comment in ***R***) :

```
library("laraDataReader")
library("laraEvalVis")

# reading multiple single point absorption measurement files generated
# by a Thermo Varioskan microtiter plate reader with two wavelengths
# (600nm and 660nm)
# the import method further specifies,
# how the data is interpreted during the reading phase.
# PLC stands for "PathLength Correction"

# data import
curr_barcode = "3276" # please adjust to your barcode number
filename_pattern = paste0("BC_", curr_barcode ,".*varioskan_Spabs.*")

growth_df = LA_ImportData(structure(filename_pattern, class="varioskan"),
                          method='SPabs', barcode=curr_barcode,
                          layoutBarcode=layout_barcode, useTime=FALSE,
            layout=FALSE, PLC=TRUE)

class(growth_df) <- "data.frame"
head(growth_df ,32)  # printing the first 32 rows

# growth plot of the whole plate
xlim=c(0,18)
ylim=c(0,1.5)
numGrowth = 4
wavelength = 600

# 2D plot of the complete plate
LA_Plot(plot_data_df=structure(comb_df,"growth"), numGrowth=numGrowth, xlim=xlim,
                          ylim=ylim, timeUnit="hours",
                                    errorBars=T, markBest=T, description=TRUE,
                          overview=T,  barcode=curr_barcode,
filename="growthExprPlot_plateView")
```

These commands will result in a 2D plot like Fig.4.

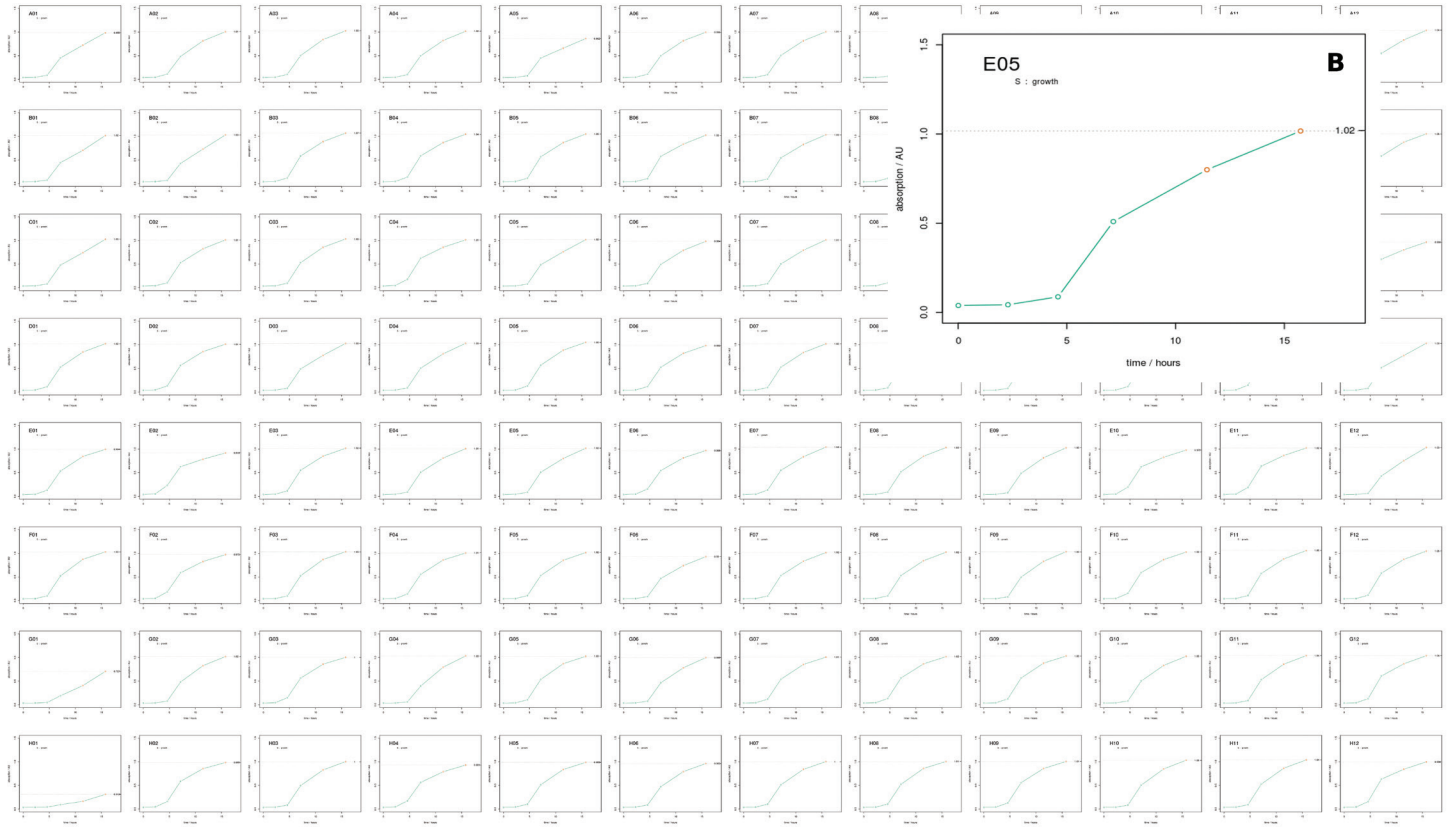

Fig. 5: Example of 96 bacterial growth curves 600 nm. Green circles denote absorption during growth phase, red circles represent absorption after protein expression inductor addition (0.1 mM IPTG).

It is also possible to generate a 3D plot of the same data: simply add the following line to the above script:

```
# 3D plot
LA_Plot(plot_data_df=structure(comb_df,"barplot3d"),
wavelength=wavelength,
        preview=TRUE, fillCol="bluered", plotWidth=1201,
plotHeight=1024,
                barcode=curr_barcode, filename="well_
                plot3D")
```

Which will result in a figure like **Fig. 6.**

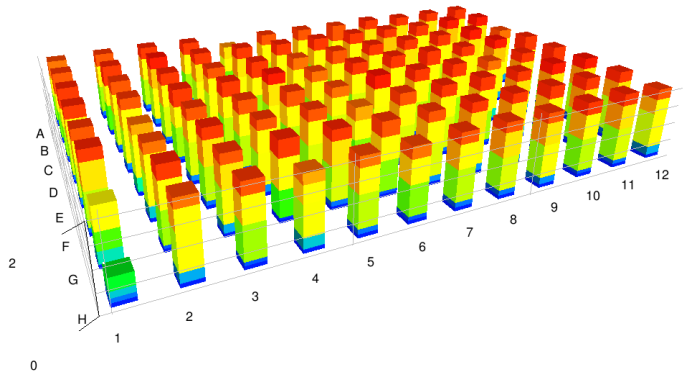

Fig. 6: 3D plot of growth measurements in 96 wells. Same data as in Fig. 5, but in 3D representation. Each bar represents the change in absorption measured at 600 nm (corresponds to cellular growth) in a single well of a standard 96 well microtiter plate (E.coli, 200 µL LB medium/per well) at 37 °C and 700 rpm shaking. Note the even growth and lack of edge effects across the plate

The results show highly consistent values across the plate (mean(OD600, 96wells)=1.03+/- 0.09 AU, median(OD600, 96 wells) = 1.04 AU) with a very low standard deviation of 0.09 AU.

This fact is also illustrated by the growth distribution histogram, **Fig. 7.**

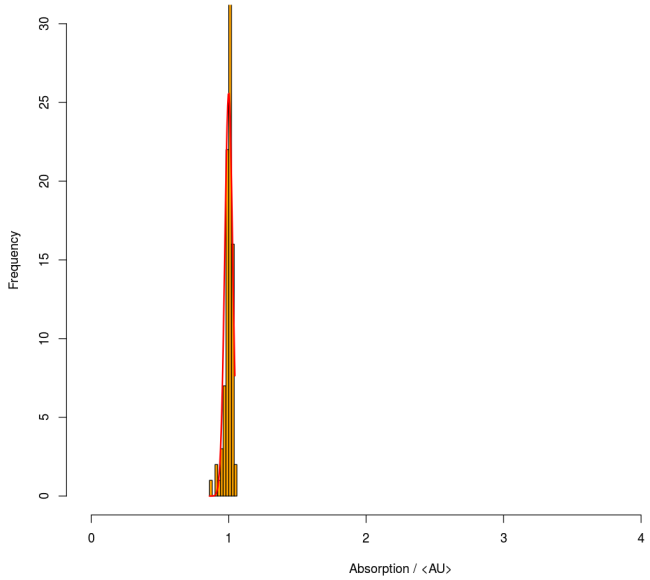

Fig. 7: Histogram plot of the data shown in Fig. 5, all 96 wells.

The noted absence of any edge effects over the course of a 16-hour time course study suggests a consistent environment with steady moisture distribution in the **Cytomat** 2 series. Even longer growth experiments over four days did not show any liquid loss in the plate edge wells (data not shown). There is also no detectable difference in cell growth rates between the plates stored in various positions of the **Cytomat** 2 C series. The **Cytomat** True Orbital **Tower Shakers** are equipped with motors on the top and bottom of each stack, thus providing synchronized shaking patterns and eliminating the possibility of plates being subject to varying conditions that might lead to inconsistent growth patterns.

It is also possible to do statistic plots over the complete plate, as illustrated in **Fig. 8.**

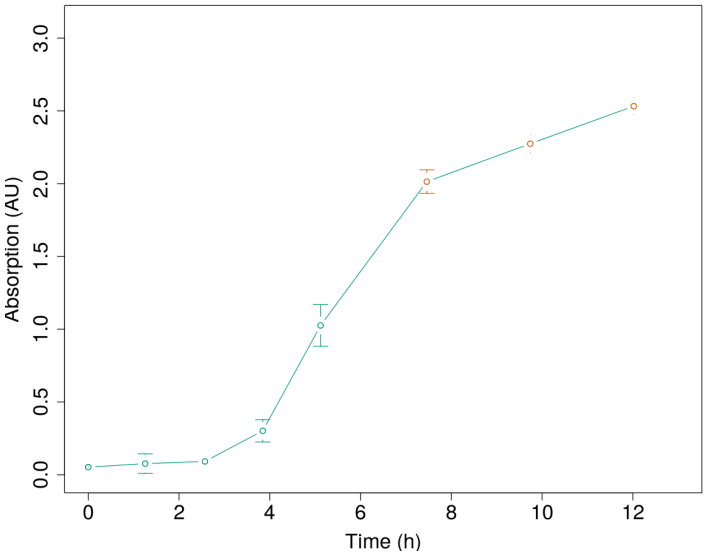

Fig. 8: Example of 96 bacterial growth curves 600 nm. Each dot represents the mean of 96 wells. Green circles denote absorption during growth phase, red circles represent absorption after protein expression inductor addition (0.1 mM IPTG) (this data is from different experiment).

Fig. 5 to **8** illustrate the versatility of the **LARA-R** scripts. This data can be generated without any manual interference.

Final Remarks

This procedure is highly extensible, since the software used is very flexible. In case of the **LARAsuite/R** scripts, everything is open source and can be adjusted to specific individual needs, e.g. storage in a database, multiple visualisations, automatic statistical evaluation of thousands of datasets - the only limit is creativity.

Cell cultures of eukaryotic cells should be similarly treatable - with the right adjustments (no shaking, CO_2 and humidity levels, temperature) in a **Cytomat** series automated incubator.

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