WCSB, Wageningen University	EPP-SOP-WU05
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Making a growth curve of a bacterial or yeast strain

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Experimenter 1	Rita Volkers		25/9/2018

Instruction

Making a growth curve of a bacterial or yeast strain

1. Introduction / Purpose

In this protocol a method to make a growth curve of a bacterial or yeast strain is described. To make a growth curve of a microorganism, the growth of a liquid culture needs to be followed through time by measuring the optical density at 600 nm with a spectrophotometer.

Keywords: Growth curve – bacteria – yeast – *P. putida* – *E. coli*

2. Equipment and chemicals

2.1. Equipment

- Spectrophotometer for cuvettes OR plate reader
- Shaking incubator

2.2. Special Consumables

- Sterile tube or erlenmeyer flask for overnight culture
- Sterile erlenmeyer flasks that can contain at least 25 ml medium or [96 or less]-wells plate
- Cuvettes when using a spectrophotometer

2.3. Other materials

- Plate with strain of interest (not older than a few days)

3. Media and Buffers

- Appropriate medium for strain of interest

4. Procedures

Day 1

- Start overnight culture of strain of interest. Volume depends on the cell density that you expect to have next morning and the number and volume of the cultures that you need to inoculate with it.

Day 2

- Start the cultures that you want to analyse at an optical density at 600 nm between 0.1 and 0.5. In the case of Erlenmeyer flasks, start at least at 0.2, in the case of a –wells plate start lower. Do this first thing in the morning.
- Measure the optical density at 600 nm regularly during the day by either taking samples every hour (flasks) or by putting the –wells plate in the plate reader and measure.

Day 3

- Take one last sample and analyse your data.

Data analysis:

Make a growth curve by putting the optical density on the y-axis and the time on the x-axis. Do this for the average of the replicates. Add error bars to the graph. Typically the standard deviation is used for the error bars. Standard deviation is the typical difference between each replicate and their average. More information about error bars can be found in Cumming *et al.* (2007).

5. Remarks / troubleshooting

- Growth can be measured in Erlenmeyer flasks or in a [96 or less]–wells plate. When only a few cultures need to be analyzed Erlenmeyer flasks are fine, otherwise the wells plate is more practical.
- Biological triplicates (or more) should be made to allow for proper analysis of growth.

6. Biosafety

No biosafety issues were associated with this protocol.

7. References

Cumming G, Fidler F, Vaux DL, 2007, *Error bars in experimental biology*, The Journal of Cell Biology 177(1):7-11, doi:10.1083/jcb.200611141

8. Acknowledgements

IBISBA (grant agreement No 730976) and EmPowerPutida (grant agreement No 635536) projects have received funding from the European Union's Horizon 2020 research and innovation programme.

