Interlab

THURSDAY, 19/07/2018

Calibration 1 (2018-06-11)

Added 100 µl of MQ-water to wells A-D2 of a black 96-well plate with transparent bottom.
Added 100 µl of Ludox-CL-X to wells A-D1.
Made measurements with FLUOStar Omega.
Results are shown in table 1.

Table1

	A	B
1	0.024	0.0070
2	0.030	0.0240
3	0.020	0.017
4	0.025	0.035

High variability in the results. In addition, the volume seemed to differ between samples.
Remade the experiments using wells A-D12 and A-D11 (or using a different plate??). Results are shown in table 2.
(Note: is this the results we used??)

Table2

	A	B
1	-0.011	-0.037
2	-0.017	-0.027
3	-0.021	-0.024
4	-7.0e-4	-0.039

Calibration 1 new measurements (2018-06-12)

Made new measurements according to previous experiments using the NUNC_96 plate. The results are shown in table 3. The Ludox was stored on ice for 5 min and vortexed before added it to the wells.

Table3

	A	B
1	0,084	0,059
2	0,059	0,072
3	0,056	0,078
4	0,086	0,002

Calibration 2 (2018-06-13)

Silica beads were vortexed for 30 seconds before 96 µl of the solution was added to an eppendorf tube. MQ-water was added to the solution to get a final volume of 1 ml and the mixture was vortexed.

100 µl MQ-water was added to wells A2-D12 in a 96-well plate with black walls and transparent bottom. A serial dilution of the silica beads were made by adding 200 µl of silica beads to well A1 and then transferring 100 µl from well A1 to well A2 and then 100 µl from well A2 to well A3 and so on until well A11. 100 µl from well A11 was discarded. For each well, the liquid was pipetted up and down before transferring to the next well. The same procedure was repeated for rows B, C and D. Deviation: well A12 got microspheres in it so the well was emptied and water was readded to it.

Before the measurements in the plate reader, the fire alarm went of and the well plate was left next to the plate reader for about 45 min. The wells were remixed before measurements. The absorbance measurements are shown in table 4.

Table4

	A	B	C	D	E	F	G	H	I	J	K	L
1	0,839	0,39	0,226	0,088	0,01	0,005	0,007	0,021	0,008	0,013	0,023	0,035
2	0,874	0,444	0,217	0,126	0,092	0,024	0,011	0,014	-0,002	0,0001	0,013	0,028
3	0,821	0,419	0,256	0,122	0,061	0,026	0,086	0,033	0,011	0,043	-0,022	0,033
4	0,872	0,417	0,254	0,136	0,079	0,05	0,032	0,016	0,03	0,018	0,016	0,053

Calibration 3 (2018-06-13)

The fluroescein kit tube was spun down. Fluorescein was resuspeneded in 1 ml 1xPBS to get a final concentration of 100 µM. The solution was mixed by pipetting up and down. A dilution series was made with fluorescein as for the silica beads but using fluorescein and 1xPBS instead of silica beads and MQ-water,

Fluorescence measurements were performed with top optics, excitation at 485 nm, emission at 520 nm and gain 500.

Table5

	A	B	C	D	E	F	G	H	I	J	K	L
1	13172	7135	3785	1965	977	494	259	145	80	50	39	21
2	14283	7199	3707	1872	973	496	262	139	79	50	36	23
3	14274	7264	3735	1915	989	499	271	147	83	53	36	19
4	14360	7429	3618	1888	965	494	264	142	80	51	38	22

Preparation of LB agar plates with chloramphenicol (2018-07-19)

Added 5 g peptone from caseine 5 g NaCl and 2.5 g yeast extract to a 1 L container. Mixed with 125 ml MQ-water. Adjusted pH with NaOH to 7.0. Added 8 g of agar powder and water up to 0.5L. The mixture was autoclavated. 25 mg of chloramphenicol salt was added to 1ml ethanol- Added 0.5 ml of suspeneded chloramphenicol to LB agar. The LB agar was spred on 27 petri dishes.

Note that the plates were stored in freezer which was not ideal.

Transformation of DH5alpha cells (2018-07-19)

DNA from plate 7 provided by iGEM head quarters wells 2B-P (every other) was resuspended in 10 µl MQ-water. Competent cells (DH5alpha) were thawed on ice for 30 min and eppendorf tubes were pre-chilled on ice. DNA was added to 50 µl cells each. The cells were incubated on ice for 30 min and then put in water bath at 42 degrees celsius for 45 s before put on ice for 5 min. 950 µl of recovery media was added to each sample and the cells were put in heat shaker at 37 degrees celsius, 200 rpm for 60 min.

100 µl of each sample was spred on 8 LB agar plates with chloramphenicol. The rest of the samples were put in the centrifuge for 3 min at 6800 g. Part of the supernatants were discarded before the cells were resuspended in the remaining liquid and spred on plates.

The plates were incubated for about 15 hours. Colonies were observed on all plates with the higher cell concentration but only a low amount for the cells transformed with DNA from well 2N. Only a few colonies were observed on the other plates, if any. The plates were put in 37 degrees for a few more hours. The plates with the lower cell concentration were put on heat during the weekend.

Preparation of more plates (2018-07-20)

1 L of LB agar was prepared according to the previous protocol. One quarter of the media was lost in the autoclave. The volume of the remaining liquid was estimated to be 750 ml and 750 µl of 25 mg/ml chloramphenicol was added before the media was poured onto petri dishes (in total 54 plates).

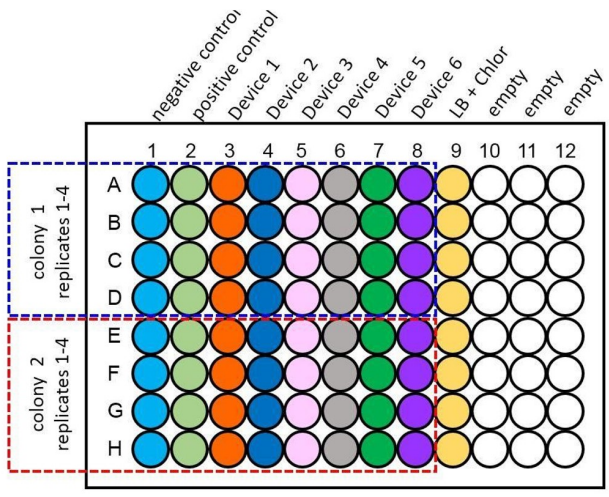
Inoculation of colonies (2018-07-23)

Two colonies were taken from each plate with the higher cell concentration and put in 5 ml LB+chloramphenicol(25 µg/ml). An exception was made for the cells transformed with DNA from well 2N, where the two colonies were taken from the plate with the lower cell concentration (only a few colonies showed up on the other plate). The cells were incubated in heat shaker at 37 °C, 200 rpm overnight (15 hours).

Cell measurements (2018-07-24)

10:1 dilutions were made for all of the overnight cultures by taking 0.5 ml culture and adding it to 4.5 ml LB+chloramphenicol. Absorbance measurements were made at 600 nm in a 96-well plate with black walls and clear bottom (NUNC_96). Samples were loaded according to the figure below.

Plate.png



Based on the absorbance measurements, the cell cultures were diluted to an OD of 0.02 and a final volume of 12 ml. 0.5 ml of each culture was added to a 1.5 ml eppendorf tube and put on ice. Approximately 4 hours later, these samples were loaded on a plate according to the figure above (100 µl in each well) and absorbance measurements were made at 600 nm. The last column was loaded with LB+chloramphenicol. Flourescence measurements were made with excitation 485 nm, emission 520 nm, top optics and gain 1000. The measurments for the cultures at 0h were dicovered to have been measured with too low of a gain (500), new measurements were planned.

Preparation for new cell measurements (2018-07-25)

Prepared for new measurements at friday by autoclaving and marking tubes, preparing media, checking plates etc. Innoculated cultures at 15:20.

New cell measurements (2018-07-26)

The cultures were taken out of the heat shaker were taken out at 9:30. The absorbance and fluorescence measurements on E. coli cells were remade according to the previous protocol, this time using 100 gain for the measurements at 0h. The results are ???.

CFU (2018-07-26)

From the negative and positive control cultures, 1 ml was taken for the purpose of finding cfu/ml. The cultures were split into two samples respectively, diluted to an 1:8 dilution, and measured in a pate reader for OD_{600} using two blanks of LB + Chloramphenicol. The 4 samples were each split into three samples respectively, which were diluted to a target OD_{600} of 0.1, and then measured in a plate reader. A serial dilution were performed for all 12 samples, and the 10^4, 10^5, and 10^6 dilutions for all samples were plated on LB + chloramphenicol plates and left to incubate for 18 hours.

FRIDAY, 27/07/2018

CFU (2018-07-27)

The plates were removed from the incubator and the number of colonies per plate were counted.