iGEM TU/e 2015
Biomedical Engineering

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Interlab study
Protein Expression Measurement

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Notebook

1 Protein Expression

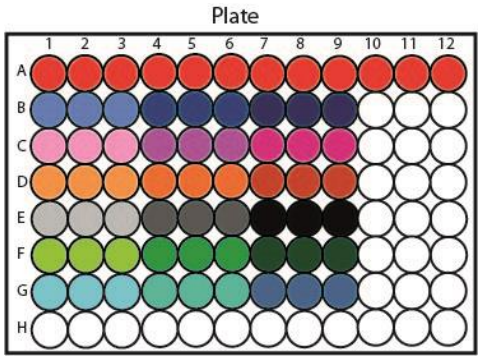
1.1 Measurement of the OD600

1) Fill a 1.5 mL cuvet with 1mL LB and perform a blanc measurement at OD600.
2) Insert 1 mL bacterial culture into another cuvet.
3) Measure the OD at 600 nm.
4) Calculate the correct dilution to fill the well plates with an OD of 0.5.
- X= Measured OD/0.5
- 1/ X = Volume of added bacterial culture (mL)
- LB (pH 7.2) added (mL) = 1 – Volume of added bacterial culture

5) Fill a new 1.5 mL cuvet with the bacterial culture and LB (pH 7.2) in the calculated proportions.
6) Measure the OD at 600 nm again.
7) Add up LB (pH 7.2) until an OD of 0.5 (±0.025) is reached.

1.2 Measurements with the plate-reader

1) Start the Tecan infinite F500 and heat up to 37°C.
2) Fill row A of a 96 black well plate flat bottom with 200µL LB (pH 7.2).
3) Fill the plate with 200 µL of the diluted constructs and the various controls.
- In our measurements the lay-out was as follows:
  - Row A: LB medium
  - Row B: Colony 1,2 and 3 of construct 1
  - Row C: Colony 1,2 and 3 of construct 2
  - Row D: Colony 1,2 and 3 of construct 3
  - Row E: Colony 1, 2 and 3 of construct 4: Negative control – Empty BL21 bacteria
  - Row F: Colony 1, 2 and 3 of construct 5: Positive control – GFP with working promotor.
  - Row G: Colony 1, 2 and 3 of construct 6: Negative control – BL21 without GFP and with B0062 promotor.



4) Measure the plate with the following settings:
   a. Excitation filter: 485nm (20)
   b. Emission filter: 535nm (25)
   c. Heated to 37°C
   d. Orbital shaking at 2.5mm amplitude and frequency 247.6 orbital for 180 seconds.
   e. GFP gain 30

5) Safe the results