pLac kinetics analysis

Aim:

pLac is a promoter induced by IPTG. In order to follow its behavior, bacteria are cultured in a 96 well plate for 24h. During this test OD(600nm) and Fluorescence are measured with a micro plate reader TECAN INFINITE 200.

Protocol :

Day 1:

- A culture of NM522 containing the plasmid P_{lac}**(*B. subtilis*)-RBS(*E. coli*)-GFP** BBa_K802002 is made by inoculation of a clone in 5 mL LB medium with Chloramphenicol (20µg/mL). This culture is replicated with a second clone.
- A culture of NM522 is also made in the same condition. This culture will be the control.

Then cultures are incubated overnight in a 37°C shaking water bath.

Day 2:

Different solutions of IPTG are prepared in a LB medium:

- IPTG 1mM: 0.1mL from a 100mM solution is diluted in 9.9mL of LB.
- IPTG 0.5mM: 5mL from the previous solution is diluted in 5mL of LB.
- IPTG 0.1mM: in the same conditions 1mL is diluted in 4mL of LB.

Put 200 µL of the IPTG solutions in the A,B and C row of the 96 wells plate according to the following picture.

200 µL of LB is put in the D row.

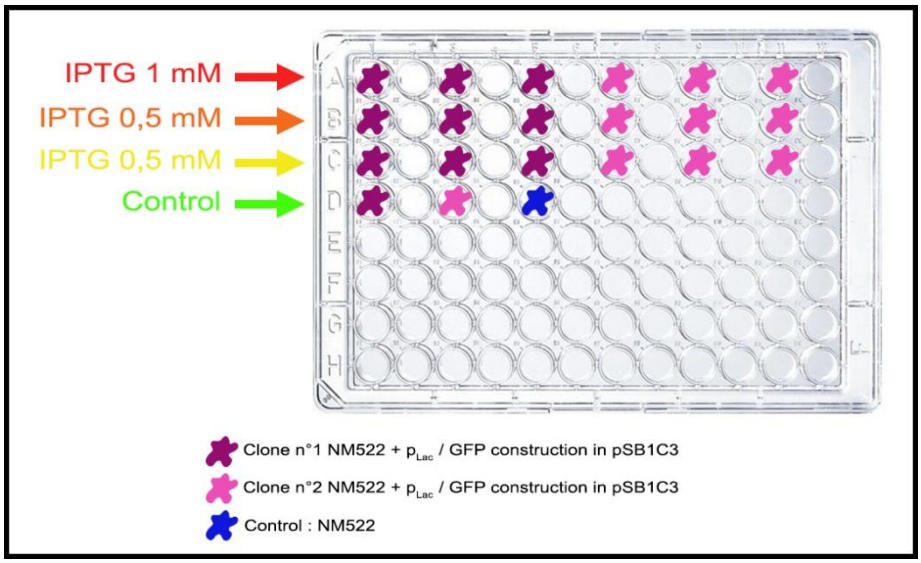

Figure 1: Representation of different culture conditions in the 96 well plate

Afterwards 2µL of bacteria are inoculated (1/100 dilution) the same way as previously described in figure 1.

The OD and fluorescence of each well of the plate are then measured by a reader for 24h at 30°C with a 10 second agitation every 10min. To be more exactly the OD is recorded at 600nm, the excitation wavelength is 485 nm and the emission wavelength is 530nm in 500µm cuve.