Test 6

Description: Fluorescence estimation in green (GFP)
Purpose: estimation of fluorescence in coltures of T9002 induced with different amounts of 3OC6HSL. We want to characterize the green filter to set the gain value
Methods: A flat-bottom non sterile plate is used. 96 wells are filled with:

- 200 ul LB+Amp,
- 200 ul T9002 induces with different amounts of 3OC6HSL
- 200 ul J100-GFP (A1 2008), positive control
- 200 ul BBa_E0240 (negative control)

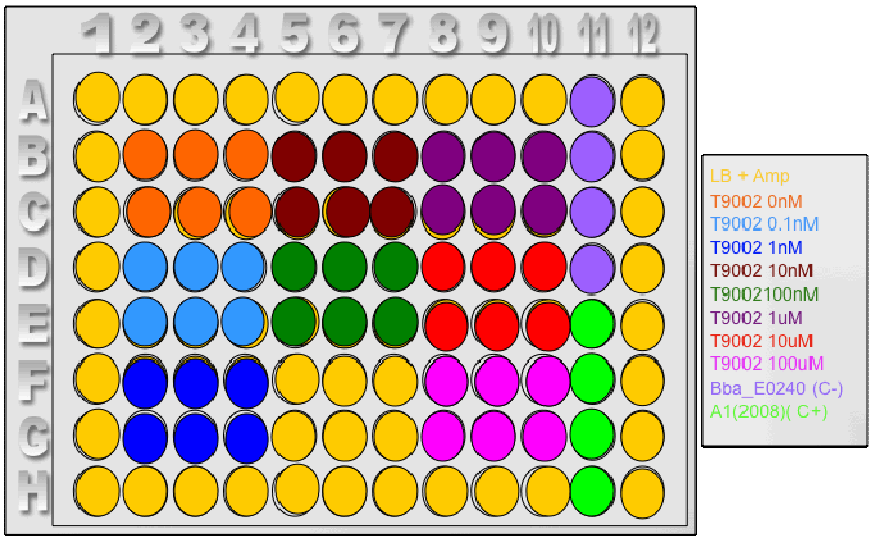
Dilution: coltures of BBa_E0240, T9002, A1(2008) were incubated in 5ml LB+Amp 37°C 220 rpm overnight and then diluted 1:1000 (2 steps: 1:100 dilution, then 1:10 dilution)
T9002 was tested with 8 different concentration of 3OC6HSL:

- 0nM (nothing added to the colture)
- 250 ul of 2mM -> 100uM in 5ml LB+bacteria
- 50 ul of 1mM-> 10 uM in 5ml LB+bacteria
- 50 ul of 100uM-> 1 uM in 5ml LB+bacteria
- 50 ul of 10uM-> 100 nM in 5ml LB+bacteria
- 50 ul of 1uM-> 10 nM in 5ml LB+bacteria
- 50 ul of 100nM-> 1 nM in 5ml LB+bacteria
- 50 ul of 10nM-> 0.1 nM in 5ml LB+bacteria

After dilution, 26 falcons (3 for every 3OC6HSL, one for the other coltures) were incubated 37°C 220 rpm. LB is infected in 96-wells plate by dipping pipette in infected colture and then in LB.

Protocol:

- The plate is filled as described in Methods
- The instrument temperature was set at 37°C
- A first absorbance and fluorescence (green, yellow?) measure is performed to have the static induction curve.
- The dynamic cycle starts uo to see saturation of green filter:
    - cycle: shaking 15 s linear 3mm, waiting 30s and absorbance , GFP and YFP measurement.



Convenzione:
La prima riga di ogni colore è 200ul da coltura, la seconda riga è ottenuta intingendo