lysis A15 TEST 1/10/09

Description: Lysis test: we test the dynamic of death of A15
Purpose: estimation of death time at different induction of 3OC6HSL.
Methods: A flat-bottom non sterile plate is used. 96 wells are filled with:

- 200 ul LB+Amp,
- 200 ul A15-3
- 200 ul A15-1
- 200 ul BBa_F2620 (negative control)
- 200 ul A15-3 picked from colony

Dilution: coltures of BBa_F2620, A15-3, A15-1 were incubated in 5ml LB+Amp 37°C 220 rpm overnight and then diluted 1:000 (50ul culture in 5ml fresh medium), A15-3 has been picked from colony in the morning and incubated with the others for the further 5 hours. Then O.D. has been measured and the usual phormula has been used to dilute cultures to start from O.D. 0,02.

All the parts were tested with 3 different concentration of 3OC6HSL:

- 0nM (2ul ddH20)
- 1nM
- 100nM

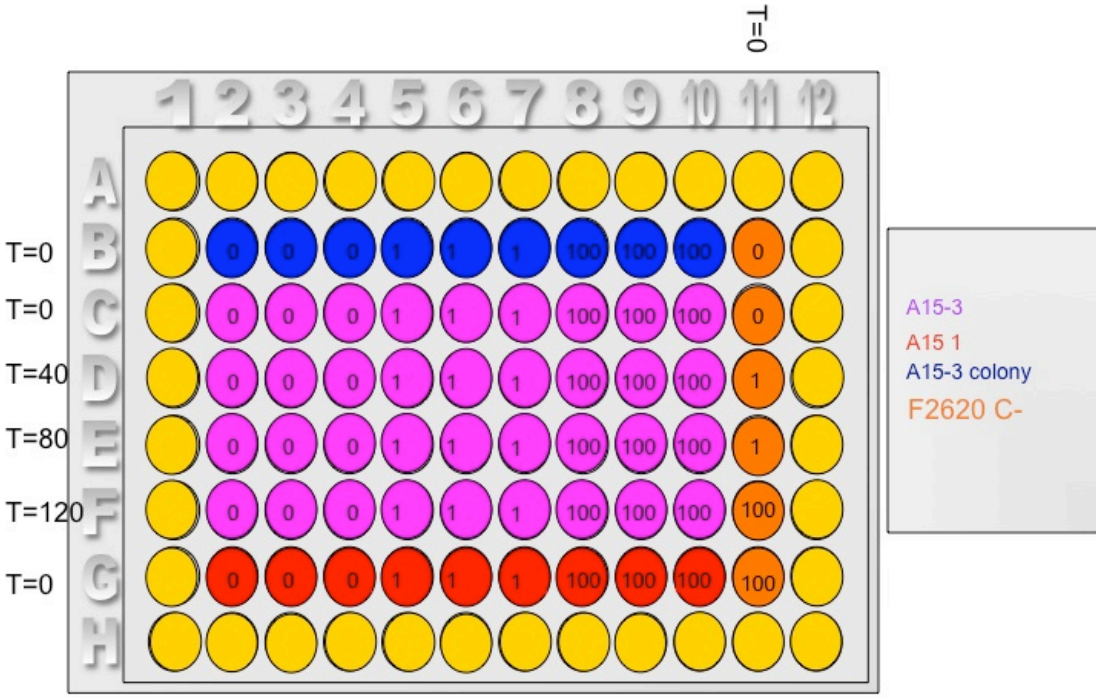
Dilutions have been prepared as follows. Original concentration: 2mM
Wanted concentrations usually are:

- 0nM (20 ul ddH20)
- 0,1nM (2ul in 200ul = 1:100 -> 10uM) diluizione 1:10 1nM: 1ul 1nM + 19ul ddH20
- 1nM (2ul in 200ul = 1:100 -> 100nM) diluizione 1:10 10nM: 1ul 10nM + 19ul ddH20
- 10nM (2ul in 200ul = 1:100 -> 1uM) diluizione 1:10 100nM: 1ul 100nM + 19ul ddH20
- 100nM (2ul in 200ul = 1:100 -> 10uM) diluizione 1:10 1uM: 1ul 1mM + 19ul ddH20
- 1uM (2ul in 200ul = 1:100 -> 100uM) diluizione 1:20 2mM: 1ul 2mM + 19ul ddH20

Minimal volume needed to fill the plate: 50ul (USED CONCENTRATIONS)

- 0nM 50 ul ddH20
- 0,1nM (2ul in 200ul = 1:100 -> 10uM) diluizione 1:10 1nM: 1ul 1nM + 19ul ddH20
- 1nM (2ul in 200ul = 1:100 -> 100nM) diluizione 1:10 10nM: 6ul 10nM + 54ul ddH20
- 10nM (2ul in 200ul = 1:100 -> 1uM) diluizione 1:10 100nM: 1ul 100nM + 19ul ddH20
- 100nM (2ul in 200ul = 1:100 -> 10uM) diluizione 1:10 1uM: 6ul 1mM + 54ul ddH20
- 1uM (2ul in 200ul = 1:100 -> 100uM) diluizione 1:20 2mM: 1ul 2mM + 19ul ddH20

After dilution, the plate has been filled as shown in figure:



Induzione: 0min, 40min, 80min, 120min

Protocol:

- The plate is filled as described in Methods
- The instrument temperature was set at 37°C
- Kynetic cycle: 4 hours with interval of 5 minutes
    - Shaking 3mm linear 15 s
    - Wait 10 s
    - Absorbzance read
    - Fluorescence read
- At cycle 8 (40 minutes after), 16(80 minutes after) and 24 (120 minutes after) plate is moved out to induct the wells indicated in the picture
    - At cycle 8: D2-D10
    - At cycle 16: E2-E10
    - At cycle 24: F2-F10
- All others lines and cultures have been induced at t=0.