**Experimental setup for metabolite screening experiments (Anne-Flore Deton-Cabanillas)**

Taken from:

S:\512-PCV\512.4-Signal\512.4.6-AD260936\Anne-Flore data\Luminescence measurements on TECAN\Nano-Glo measurements\Biolog plates

Strain

T1-N80. This is a CC-125 WT strain expressing ectopically the Nano-luciferase, NanoLuc {Crozet:2018bq} under the control of the endogenous promoter of *LHCSR3.1* (1480 bp upstream of the atg)

Culture conditions

17/09 (day -5): T1-N80 grown in 25mL TAP (from plates), LL

20/09 (day -2): refresh in 25mL TAP, LL

22/09 (day 0): culture set at 1 million cells/mL in 40mL **HSM** + carbenicillin 100µg/mL

Experimental plan

**Day 0**

Just after putting T1-N80 in HSM + carb @ 1 million cells/mL

- distribute **100µL/well** in a **96-well plate - Biolog Phenotyping microplate PM1**

- distribute **100µL/well** in a **96-well plate - Biolog Phenotyping microplate PM2A**

- incubation on shaker under LL (15 µE) for 24h, at 23oC.

**Day1**

for each Biolog plate :

1)      Mix by pipetting meticulously

2)      Measure OD750

3)      Mix by pipetting meticulously

4)      Take from each well 25µL of culture and put them in a low volume plate

5)      Transfer the Biolog plate from **LL to HL (300 µE)** on a shaker for 4h with lid on the plate

6)      During the 4 hours incubation, prepare a mix of HSM and Nano-Glo Reagent for 108 wells (96 wells + 12 extra) : 2700 µL HSM + 54 µL Nano-Glo Buffer + 1.08 µL Nano-Glo Substrate

7)      Put this mix in a “gouttière”

8)      Add 25.5µL of “HSM/Nano-Glo Reagent” mix per well into the low volume plate (with the Thermo multichannel)

9)      Mix by pipetting

10)   Incubate 5min RT

11)   Measure luminescence

12)   After the incubation of 4 hours in HL, repeat step 1 to step 11 for each Biolog plate.

Measure OD750 as a probe for cell density directly on the plate (without the cover lid).

Normalize all luminescence values with the OD750 (basically luminescence/OD750).

This experiment was repeated three times for every plate. The results are summarized in the excel table.

An additional experiment was performed where the cells were transferred in an empty microwell plate (no metabolites). This dataset could serve as negative control.