**Preparation of pre-cultures SM091 for kinetics assays**

1. Plated SM091 (Streptococcus mutans ΔcomS PsigX-luc reporter) in TSB agar. Incubatet it overnight at 37 celsius and 5% CO2.
2. On the other day, took the some and added them to 15 mL of CDM.
3. Grew until culture reached OD 0.503 (37 celsius, 5% CO2).
4. Removed from the incubator, added glycerol (15% - 225µL:1275 µL – culture – for each small tube). Place the tubes on ice right away.
5. Added 1,5mL of the mix in each tube and stocked it at -80 celsius.
6. As a control, plated the culture with glycerol afterwards
7. Two drops of **25µL** for each dilution
8. Next day, counted colonies:

-5 dilution:

|  |  |
| --- | --- |
| -5 dilution | -6 dilution |
| 67  69  74 | 11  13  13 |
| **2,7 x 108 CFU/mL** |  |

**Kinetics of sigX expression to different concentration of XIP**

Strains: *S. mutans* SM091 (ΔcomS PsigX-luc reporter)

XIP: *S. mutans* (Stock concentration 100µM)

Pre-cultures: SM091 in CDM

Assay Medium: CDM

Methods:

1. Pre-cultures at OD0.5 wered stored at -80C in 15% glycerol.
2. Prepare a white-bottom 96-well plate according to the planned design. Two wells will serve as blank.
3. Concentrations of XIP to be tested: 2000nM, 1000nM, 500nM, 250nM, 125nM, 62,5nM, 31,25nM, 15,625nM, 7,8nM, 3,9nM, 1,9nM and control (NO XIP).
4. Start in column 12 of the plate, with 200µl of CDM and 4µl of XIP. Take 100µl from this well and add to column 11 previously loaded with 100µl of CDM. Repeat it sequentially until column 2 (lowest concentration). Column 1 will be the control with no XIP.
5. Diluted the pre-cultures 1:5 in CDM (OD600~0.089).
6. Add 100µl of culture to each well (NOT THE BLANK WELLS!).
7. Add 20µl luciferin to all wells. Keep the plate from light exposure as much as possible.
8. Inserted the plate into the plate reader machine (Cytation 3)previously heated to 37C.
9. Started kinetics assay.
10. Made exact the same plate setup on a transparent 96-well plate. Same XIP concentration, culture volume, but without luciferin. This plate served as basis for plating to count CFU.
11. At time 0, prepared serial dilution from -1 to -5 and plate triplicates of each concentration and dilution on TSB agar plates. Repeated the same at times 1h, 2h, 3h, 4h, 5h and 6h.
12. Incubated the plates at 37C 5% CO2 for 24.
13. Next day, counted the colonies in each plate.
14. Next day, exported and analyzed data from Gen5 software.

* Note: The kinetics experiment was run 06.10.2022 and 07.10.2022 – results and raw data are registered in physical lab book and online lab log.