Time-Course AHL (3-oxo-C12-HSL) Dose-Response Fluorescence

Introduction

The protocol is used to ensure the reporter's time-kinetic response to pure 3-oxo-C12-HSL: how much time it would take for the response of onset, the rate of response, the maximum response intensity, and the dose-response relationship across different concentrations.

Reference:
1. Alba Balletbó 2019. OD and GFP Plate Reader Assay (72 h Measurement). protocols.io. https://dx.doi.org/10.17504/protocols.io.8aihsce
2. Hartmann FSF, Weiß T, Kastberg LLB, Workman CT and Seibold GM (2023) Precise and versatile microplate reader-based analyses of biosensor signals from arrayed microbial colonies. Front. Microbiol. sz14:1187228. doi: 10.3389/fmicb.2023.1187228
3. Chappell, J., Jensen, K., & Freemont, P. S. (2013). Validation of an entirely in vitro approach for rapid prototyping of DNA regulatory elements for synthetic biology. *Nucleic Acids Research*, *41*(5), 3471–3481. https://doi.org/10.1093/nar/gkt052

Materials

- Synthetic 3-oxo-C12-HSL (3mM stock dissolved by DMSO)
- M9 medium
- Bacterial strain
- Chloramphenicol (CM)
- 96-well (black-wall, clear/black bottom)
- M2 plate reader

Procedure

Pre-culture

1. Culture the bacteria overnight in 2ml M9 medium with Cm

Sub-culture

2. Dilute 1:100 of bacteria suspension with pre-warmed M9 medium to 5ml also with Cm
3. Incubate in 37°C; 200–250 rpm for 3 hr
4. Adjust OD_{600} to 0.07

5. inoculate for another 1 hr

Loading sample

6. Using stock solution, dilute 3-oxo-C12-HSL using DMSO or culture medium (a log-step gradient is recommended, e.g., 0, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM)

7. Separate every 199ul bacterial suspension into 96-well

8. Add 1ul of the dilution for every AHL concentration, so the final DMSO concentration is the same in every well (including the solvent control and blank groups)

Kinetic Read

9. Place the plate into the plate reader, incubate at 37°C, and read OD600 (to monitor growth), GFP fluorescence (Ex/Em ≈ 485/520 nm) every hour

10. Continue reading the plate every hour for 6 hours

    Note: During the reading, set brief shaking to promote aeration

Data processing

11. At each time, calculate normalized fluorescence((raw GFP signal − blank background) ÷ OD600)