AND-Gate With Quorum Sensing

Abstract

We incorporated a module into our system that would allow our engineered bacteria to autonomously decide if they are in tumor tissue or not . This decision is taken upon AND-logic integration of two inputs: AHL and lactate (Figure 1). Only if both these chemicals are present, the downstream modules are activated. To achieve such behaviour, we designed a synthetic promoter consisting of operators taken from BBa_K1847007, part of the Lactate sensing system [4] and the pLux promoter, part of the quorum sensing system. This promoter is regulated by the two proteins LldR and LuxR. LldR binds to the operators O1 and O2, whereby a loop in the DNA is formed that "hides" the sequence in between the operators from regulatory proteins. When lactate is present and binds to LldR, the protein undergoes a conformational change leading to release of the loop. When LuxR binds to AHL, it also undergoes a conformational change which leads to formation of LuxR homo-dimers that bind to the pLux sequence and recruit RNA polymerase whereby transcription is initiated.

In this experiment we aimed determining the behaviour of our AND-gate when induced with different amounts lactate and let grow to different final population densities.

Samples

- *E. Coli* TOP10, transformed with the regulator plasmid piG17-2-004, coding for constitutive expression of LuxR, LldP and LldR, and the actuator plasmids piG17-1-012a/b/v, coding for the three AND-gate designs driving sfGFP and LuxI expression.
- As controls served for AHL a AHL-responsive (TOP10, transformed with plasmids piG17-1-002 and piG17-2-001) and a lactate-responsive strain (provided by a researcher at the department)

Materials

- Luria Broth (LB) growth medium
- M9 Minimal Medium
- Round bottom culture tubes 12 mL with breathable cap
- Kanamycin and chloramphenicol
- 1.5 mL microcentrifuge tubes
- Deep-well 96-well plates
- Flat bottom 96-well transparent microtiter plate

Equipment

- Tecan M1000 plate reader
- Thermo Fisher NanoDrop One Microvolume UV-Vis Spectrophotometer
- Incubator with shaking function
- Kuhner Deep-well Shaker

Procedure

Day 1

- Streak out TOP10 with versions of the AND gate with LuxI
- Incubate at 37° C over night

Day 2

- Pick
  - 3 colonies each
  - 1 colony of each control
  - with 10 uL pipette suck in a bit of the colony. Pipet up- and down in the media. Media: 3 mL LB

- Let grow at 37 C for 8 hours
- Inoculate 10 uL into 5 mL M9 (0.4 % glucose) in tube and let grow over night for at least 18 hours

Day3

- Prepare media in deep well plates:
    - Mix 10 uL of 50x glucose/lactate of each with 470 uL M9
- Wash cultures in PBS:
    - Spin down at 3000 g for 10 min
    - Discard supernatant
    - Resuspend in 5 mL PBS
    - Spin down at 3000 g for 10 min
    - Discard supernatant
    - Resuspend in 3 mL PBS
- Measure OD's
- Dilute each culture to OD=2 with PBS
- Mix 10 uL diluted culture with 490 uL prepared conditional M9 in deep-well plate
- Let grow in deep-well shaker over night at 37° C and 300 rpm

Day4

- Transfer 200 uL of each culture in 96-well microtiter plate
- Measure A600 and GFP in plate reader
    - A600: absorbance at wavelength 600 nm
    - sfGFP
        - excitation wavelength: 488 nm, bandwidth 9 nm
        - emission wavelength: 530 nm, bandwidth 20 nm