# MATERIALS

## PROTOCOL FOR CHARACTERIZATION OF THE SENSING MODULE

**1X PBS**

**AHL SOLUTION**

**9 DIFFERENT PARTS LB+CHL**

**96 DEEP WELL PLATE**

**96 WELL PLATE**

DAY 1

1. Transform the followings into DH10B E.Coli:
    a. pSB1C3_BBa_F2620 (-)
    b. pSB1C3_BBa_J23110_E0240 (+)
    c. pSSB1C3_BBa_J23117_E0240 (+)
    d. pSB1C3_BBa_T9002
    e. pSB1C3_BBa_F2620-C0261-E0240
    f. pSB1C3_BBa_F2620-C0261-E0240-Anti1
    g. pSB1C3_BBa_F2620-C0261-E0240-Anti2
    h. pSB1C3_BBa_F2620-ABR1-C0261-ABR1-E0240-Anti1
    i. pSB1C3_BBa_F2620-ABR2-C0261-ABR2-E0240-Anti2

DAY 2

2. Pick a colony on each plate and streak the following:
    a. pSB1C3_BBa_F2620 (-)
    b. pSB1C3_BBa_J23110_E0240 (+)
    c. pSSB1C3_BBa_J23117_E0240 (+)
    d. pSB1C3_BBa_T9002
    e. pSB1C3_BBa_F2620-C0261-E0240
    f. pSB1C3_BBa_F2620-C0261-E0240-Anti1
    g. pSB1C3_BBa_F2620-C0261-E0240-Anti2
    h. pSB1C3_BBa_F2620-ABR1-C0261-ABR1-E0240-Anti1
    i. pSB1C3_BBa_F2620-ABR2-C0261-ABR2-E0240-Anti2

DAY 3

1. Pick 3 colonies from each streaked plates for inoculation in 5mL LB+CHL (there should be a total of 9x3=27 tubes):
    a. pSB1C3_BBa_F2620 (-)
    b. pSB1C3_BBa_J23110_E0240 (+)
    c. pSSB1C3_BBa_J23117_E0240 (+)
    d. pSB1C3_BBa_T9002
    e. pSB1C3_BBa_F2620-C0261-E0240
    f. pSB1C3_BBa_F2620-C0261-E0240-Anti1
    g. pSB1C3_BBa_F2620-C0261-E0240-Anti2
    h. pSB1C3_BBa_F2620-ABR1-C0261-ABR1-E0240-Anti1
    i. pSB1C3_BBa_F2620-ABR2-C0261-ABR2-E0240-Anti2

DAY 4

1. Sample dilution:
    a. Measure the OD600 of each culture.
    b. Dilute the overnight culture so that it becomes 8ml 0.1 OD600 culture.
    c. Incubate them inside the shaker until it become 0.6 (around 2 hours, but start measuring OD600 after 1hr 30mins).

Meanwhile,

2. AHL Preparation:
    a. Make $4.38 \times 10^{-4}$ M AHL stock solution by AHL powder and DMSO.
    b. Prepare 20 tubes of Eppendorf.
    c. Transfer 60ul $4.38 \times 10^{-4}$M AHL into the first Eppendorf, and make 19 tubes of 54ul 1XPBS.
    d. Make a serial dilution from $4.38 \times 10^{-4}$ M to $4.38 \times 10^{-23}$ M by transfer 6ul previous AHL solution into 54µl 1XPBS. *Make sure in each dilution, pipette up and down several times to mix well.

3. Sample loading after OD600 reaches 0.6:
    a. For 96 Deep Well plate: (3 plates)
        i. pSB1C3-BBa_T9002: transfer 11 x 650ul of cell culture into for A1-11
        ii. pSB1C3-BBa_F2620-C0261-E0240: transfer 11 x 650ul of cell culture into for B1-11
        iii. pSB1C3-BBa_F2620-C0261-E0240-R0063-Anti1: transfer 11 x 650ul of cell culture into for C1-11
        iv. pSB1C3-BBa_F2620-C0261-E0240-R0063-Anti2: transfer 11 x 650ul of cell culture into for D1-11
        v. pSB1C3-BBa_F2620-C0261-E0240(ABR1)-R0063-Anti1: transfer 11 x 650ul of cell culture into for E1-11

vi. pSB1C3-BBa_F2620-C0261-E0240(ABR2)-R0063-Anti2: transfer 11 x 650ul of cell culture into for D1-11
vii. pSB1C3-BBa_J23110-E0240: transfer 1 x 650ul of cell culture into for H1
viii. pSB1C3-BBa_J23117-E0240: transfer 1 x 650ul of cell culture into for H2
ix. pSB1C3-BBa_F2620: transfer 1 x 650ul of cell culture into for H3
x. LB+CHL: transfer 1 x 650ul of cell culture into for H4

b. For each part and each colony, transfer 100ul of cell culture into each well and make triplicate (3x100ul) at 0min. (See Appendix 1) (3x3=9 plates)

c. Then from part ai) to avi), add 8ul of corresponding AHL in the 350ul culture to make 11 different condition solution ( 0M, 10-5M,10-7M,...,10-23M).

**96 deep wells plate:**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | $10^{-5}$M | $10^{-7}$M | $10^{-9}$M | $10^{-11}$M | $10^{-13}$M | $10^{-15}$M | $10^{-17}$M | $10^{-19}$M | $10^{-21}$M | $10^{-23}$M | 0M | |
| B | $10^{-5}$M | $10^{-7}$M | $10^{-9}$M | $10^{-11}$M | $10^{-13}$M | $10^{-15}$M | $10^{-17}$M | $10^{-19}$M | $10^{-21}$M | $10^{-23}$M | 0M | |
| C | $10^{-5}$M | $10^{-7}$M | $10^{-9}$M | $10^{-11}$M | $10^{-13}$M | $10^{-15}$M | $10^{-17}$M | $10^{-19}$M | $10^{-21}$M | $10^{-23}$M | 0M | |
| D | $10^{-5}$M | $10^{-7}$M | $10^{-9}$M | $10^{-11}$M | $10^{-13}$M | $10^{-15}$M | $10^{-17}$M | $10^{-19}$M | $10^{-21}$M | $10^{-23}$M | 0M | |
| E | $10^{-5}$M | $10^{-7}$M | $10^{-9}$M | $10^{-11}$M | $10^{-13}$M | $10^{-15}$M | $10^{-17}$M | $10^{-19}$M | $10^{-21}$M | $10^{-23}$M | 0M | |
| F | $10^{-5}$M | $10^{-7}$M | $10^{-9}$M | $10^{-11}$M | $10^{-13}$M | $10^{-15}$M | $10^{-17}$M | $10^{-19}$M | $10^{-21}$M | $10^{-23}$M | 0M | |
| G | | | | | | | | | | | | |
| H | 110 | 117 | F | LB | | | | | | | | |

pSB1C3-BBa_T9002: Blue
pSB1C3-BBa_F2620-C0261-E0240: Green
pSB1C3-BBa_F2620-C0261-E0240-R0063-Anti1: Light Blue
pSB1C3-BBa_F2620-C0261-E0240-R0063-Anti2: Blue
pSB1C3-BBa_F2620-C0261-E0240(ABR1)-R0063-Anti: Purple
pSB1C3-BBa_F2620-C0261-E0240(ABR2)-R0063-Anti2: Light Pink
pSB1C3-BBa_J23110-E0240: Red
pSB1C3-BBa_J23117-E0240: Orange
pSB1C3-BBa_F2620: Dark Red
LB+CHL: Pink

c. Incubate the 96 deep wells plate into 37C shaker for 3 hrs.
d. For each part, again, transfer 100ul of cell culture into each well and make triplicate at 200min. (See Appendix 1) (3x3=9 plates)

*All procedures in step 3a) to 3c) and 3e) should be performed on ice.

4. Measurements:
   Program Plate Reader:
   a. Shaking: 15 seconds of orbital shaking (6mm amplitude) followed by 10 seconds waiting before the measurement ( to ensure consistency within culture sample)
   b. Measure fluorescence(RFU) and OD595
   c. Calculate the Fluroescence/OD
   d. Provide the data with all Raw data to us.

## Appendix 1: (Time: 0, 200 mins)

Plate 1: pSB1C3-BBa_T9002: Blue, pSB1C3-BBa_F2620-C0261-E0240: Green

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | $10^{-5}$M | $10^{-5}$M | $10^{-5}$M | $10^{-7}$M | $10^{-7}$M | $10^{-7}$M | $10^{-9}$M | $10^{-9}$M | $10^{-9}$M | $10^{-11}$M | $10^{-11}$M | $10^{-11}$M |
| B | $10^{-13}$M | $10^{-13}$M | $10^{-13}$M | $10^{-15}$M | $10^{-15}$M | $10^{-15}$M | $10^{-17}$M | $10^{-17}$M | $10^{-17}$M | $10^{-19}$M | $10^{-19}$M | $10^{-19}$M |
| C | $10^{-21}$M | $10^{-21}$M | $10^{-21}$M | $10^{-23}$M | $10^{-23}$M | $10^{-23}$M | 0M | 0M | 0M | | | |
| D | $10^{-5}$M | $10^{-5}$M | $10^{-5}$M | $10^{-7}$M | $10^{-7}$M | $10^{-7}$M | $10^{-9}$M | $10^{-9}$M | $10^{-9}$M | $10^{-11}$M | $10^{-11}$M | $10^{-11}$M |
| E | $10^{-13}$M | $10^{-13}$M | $10^{-13}$M | $10^{-15}$M | $10^{-15}$M | $10^{-15}$M | $10^{-17}$M | $10^{-17}$M | $10^{-17}$M | $10^{-19}$M | $10^{-19}$M | $10^{-19}$M |
| F | $10^{-21}$M | $10^{-21}$M | $10^{-21}$M | $10^{-23}$M | $10^{-23}$M | $10^{-23}$M | 0M | 0M | 0M | | | |
| G | | | | | | | | | | | | |
| H | 110 | 110 | 110 | 117 | 117 | 117 | F | F | F | LB | LB | LB |

Plate 2: pSB1C3-BBa_F2620-C0261-E0240-R0063-Anti1, pSB1C3-BBa_F2620-C0261-E0240-R0063-Anti2,

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | $10^{-5}$M | $10^{-5}$M | $10^{-5}$M | $10^{-7}$M | $10^{-7}$M | $10^{-7}$M | $10^{-9}$M | $10^{-9}$M | $10^{-9}$M | $10^{-11}$M | $10^{-11}$M | $10^{-11}$M |
| B | $10^{-13}$M | $10^{-13}$M | $10^{-13}$M | $10^{-15}$M | $10^{-15}$M | $10^{-15}$M | $10^{-17}$M | $10^{-17}$M | $10^{-17}$M | $10^{-19}$M | $10^{-19}$M | $10^{-19}$M |
| C | $10^{-21}$M | $10^{-21}$M | $10^{-21}$M | $10^{-23}$M | $10^{-23}$M | $10^{-23}$M | 0M | 0M | 0M | | | |
| D | $10^{-5}$M | $10^{-5}$M | $10^{-5}$M | $10^{-7}$M | $10^{-7}$M | $10^{-7}$M | $10^{-9}$M | $10^{-9}$M | $10^{-9}$M | $10^{-11}$M | $10^{-11}$M | $10^{-11}$M |
| E | $10^{-13}$M | $10^{-13}$M | $10^{-13}$M | $10^{-15}$M | $10^{-15}$M | $10^{-15}$M | $10^{-17}$M | $10^{-17}$M | $10^{-17}$M | $10^{-19}$M | $10^{-19}$M | $10^{-19}$M |
| F | $10^{-21}$M | $10^{-21}$M | $10^{-21}$M | $10^{-23}$M | $10^{-23}$M | $10^{-23}$M | 0M | 0M | 0M | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

Plate 3: pSB1C3-BBa_F2620-C0261-E0240(ABR1)-R0063-Anti1, pSB1C3-BBa_F2620-C0261-E0240(ABR2)-R0063-Anti2,

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | $10^{-5}$M | $10^{-5}$M | $10^{-5}$M | $10^{-7}$M | $10^{-7}$M | $10^{-7}$M | $10^{-9}$M | $10^{-9}$M | $10^{-9}$M | $10^{-11}$M | $10^{-11}$M | $10^{-11}$M |
| B | $10^{-13}$M | $10^{-13}$M | $10^{-13}$M | $10^{-15}$M | $10^{-15}$M | $10^{-15}$M | $10^{-17}$M | $10^{-17}$M | $10^{-17}$M | $10^{-19}$M | $10^{-19}$M | $10^{-19}$M |
| C | $10^{-21}$M | $10^{-21}$M | $10^{-21}$M | $10^{-23}$M | $10^{-23}$M | $10^{-23}$M | 0M | 0M | 0M | | | |
| D | $10^{-5}$M | $10^{-5}$M | $10^{-5}$M | $10^{-7}$M | $10^{-7}$M | $10^{-7}$M | $10^{-9}$M | $10^{-9}$M | $10^{-9}$M | $10^{-11}$M | $10^{-11}$M | $10^{-11}$M |
| E | $10^{-13}$M | $10^{-13}$M | $10^{-13}$M | $10^{-15}$M | $10^{-15}$M | $10^{-15}$M | $10^{-17}$M | $10^{-17}$M | $10^{-17}$M | $10^{-19}$M | $10^{-19}$M | $10^{-19}$M |
| F | $10^{-21}$M | $10^{-21}$M | $10^{-21}$M | $10^{-23}$M | $10^{-23}$M | $10^{-23}$M | 0M | 0M | 0M | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

----------END---------