# Characterization

**Time :** 3h

## I. Principle

Characterization allows to check if a construction is able to produce the expected result and if the yields are correct.

## II. Material

- Transformed bacteria with the plasmids of interest
- LB medium
- Appropriate antibiotics
- COSTAR® 3603 96 well plate from Corning Inc
- CLARIOstar® (BMG LABTECH) plate reader (kindly lent by the Institute of Systems and Synthetic Biology, Evry, France)

## III. Method

**These experiments have to be done in sterile conditions (Bunsen burner or MSC) to avoid contaminations.**

### a. Culture preparation

- Set a liquid culture of transformed bacteria in LB supplemented with 35 µg/mL chloramphenicol
- Incubate overnight at 37°C and 200 rpm
- 100X dilution of the culture in LB supplemented with 35 µg/mL chloramphenicol
- Incubate at 37°C and 200 rpm for 1h
- Dispatch 120 µL per well in a 96 well plate (see Figure 1 for plate map)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Water | Water | Water | Water | Water | Water | Water | Water | Water | Water | Water | Water |
| B | Water | 0 IPTG - 0 psicose | 0 IPTG - 0,1 µM psicose | 0 IPTG - 1 µM psicose | 0 IPTG - 10 µM psicose | 0 IPTG - 100 µM psicose | 0 IPTG - 1 mM psicose | 0 IPTG - 10 mM psicose | 0 IPTG - 100 mM psicose | 0 IPTG - 200 mM psicose | 0 IPTG - 300 mM psicose | Water |
| C | Water | 0 IPTG - 0 psicose | 0 IPTG - 0,1 µM psicose | 0 IPTG - 1 µM psicose | 0 IPTG - 10 µM psicose | 0 IPTG - 100 µM psicose | 0 IPTG - 1 mM psicose | 0 IPTG - 10 mM psicose | 0 IPTG - 100 mM psicose | 0 IPTG - 200 mM psicose | 0 IPTG - 300 mM psicose | Water |
| D | Water | 0 IPTG - 0 psicose | 0 IPTG - 0,1 µM psicose | 0 IPTG - 1 µM psicose | 0 IPTG - 10 µM psicose | 0 IPTG - 100 µM psicose | 0 IPTG - 1 mM psicose | 0 IPTG - 10 mM psicose | 0 IPTG - 100 mM psicose | 0 IPTG - 200 mM psicose | 0 IPTG - 300 mM psicose | Water |
| E | Water | 0 IPTG - 0 psicose | 0 IPTG - 0,1 µM psicose | 0 IPTG - 1 µM psicose | 0 IPTG - 10 µM psicose | 0 IPTG - 100 µM psicose | 0 IPTG - 1 mM psicose | 0 IPTG - 10 mM psicose | 0 IPTG - 100 mM psicose | 0 IPTG - 200 mM psicose | 0 IPTG - 300 mM psicose | Water |
| F | Water | 0 IPTG - 0 psicose | 0 IPTG - 0,1 µM psicose | 0 IPTG - 1 µM psicose | 0 IPTG - 10 µM psicose | 0 IPTG - 100 µM psicose | 0 IPTG - 1 mM psicose | 0 IPTG - 10 mM psicose | 0 IPTG - 100 mM psicose | 0 IPTG - 200 mM psicose | 0 IPTG - 300 mM psicose | Water |
| G | Water | 0 IPTG - 0 psicose | 0 IPTG - 0,1 µM psicose | 0 IPTG - 1 µM psicose | 0 IPTG - 10 µM psicose | 0 IPTG - 100 µM psicose | 0 IPTG - 1 mM psicose | 0 IPTG - 10 mM psicose | 0 IPTG - 100 mM psicose | 0 IPTG - 200 mM psicose | 0 IPTG - 300 mM psicose | Water |
| H | Water | Water | Water | Water | Water | Water | Water | Water | Water | Water | Water | Water |

Figure 1: Example of a plate map used for the biosensor characterization.

### b. Plate preparation

The experiment is conducted in a 96 well plate, the COSTAR® 3603 from Corning Inc. These plates allowed us to characterize two different biosensors at a time. Before dispatching the culture in each well, the plate is filled according to the plate map (Figure 1) with 30 µL solution containing Psicose and IPTG at a 5X concentration (thus, upon addition of the 120 µL of culture, the Psicose and the IPTG will be at the right final concentration). Immediately after complete loading, the plate is inserted in the plate reader.

Peripheral wells are filled with water to avoid evaporation during the incubation time.

### c. Measurments

The plate reader is programmed to assess the fluorescence at mCherry optimal wavelength (587 nm for excitation and 610 nm for emission) and the $OD_{600}$. Those two parameters are measured every 7 minutes for 150 cycles. The plate is incubated in the device at 37°C under constant shaking at 200 rpm.

***All tests were done in technical duplicates and biological triplicates. Fluorescence measurements (mCherry) have been normalized on cell density ($OD_{600}$).***