# Confocal microscopy protocol (surfactin test)

The aim of this experience is to prove the surfactant properties of the surfactin produced by the *B. subtilis* transformed with the part BBa_K802009 prevent biofilm formation. The test was performed in a 12 well plate.

Day 1:

→Make liquid cultures with the following strains:

- Bacillus subtilis with BBa_K802000 part;
- Bacillus subtilis with BBa_K802009 part;

→Incubate at 37°C for 48 h;

Day 3:

→ Make a liquid culture with a fluorescent adherent *E. coli* strain.

→ Filter the supernatant of the liquid cultures.

→ Incubate each well with the appropriate supernatant. The assay was done in triplicate. 4 different wells were made with 2mL of one of the following:

- Supernatant extracted from *B.* subtilis transformed with BBa_K802000;
- Supernatant extracted from B. subtilis transformed with BBa_K802009;
- A solution containing purified surfactin ( at 80 µg/mL) in LB media;
- LB media

Day 4:

→ Seed the plate with an E. coli saturated culture diluted 50 times in LB media diluted 2 times after previously having eliminated the supernatant. Incubate the plate at 30°C for 36 hours.

Day6:

→Observation of the biofilm formed on the glass lamellae. Before observation, the lamella is rinsed with distilled water and then it is fixed between a cover slip and a cover glass. It is important to avoid bubble formation between the two slips which could perturb the observation.