**Plate reader assay – Conductive curli module**

*Objective*

To see how *Escherichia coli* biofilm is produced on a 96-well plate. The biofilm is caused due to the presence of the curli genes CsgA and CsgB. The biofilm detection is possible because the cells are carrying constitutively expressed eGFP.

*Details*

- Rhamnose concentrations to be studied: 0, 0.5 and 1%
- Negative controls:
    - LB alone: to check for autofluorescence of the media)
    - *E. coli* ΔcsgB strain with no construct: this strain has csgB gene neither on its genome nor on a plasmid. Therefore, it cannot produce biofilm by means of curli.
    - *E. coli* carrying the BBa_K1316016 BioBrick (constitutive eGFP) but not the curli-producing BioBrick.
- Cells to test:
    - *E. coli* ΔcsgB strain carrying BBa_K1316013 and BBa_K1316016
    - *E. coli* ΔcsgB strain carrying BBa_K1316014 and BBa_K1316016
    - *E. coli* ΔcsgB strain carrying BBa_K1316015 and BBa_K1316016
    - *E. coli* ΔcsgB strain carrying BBa_K1316016 alone
    - *E. coli* ΔcsgB strain carrying no BioBrick

*Protocol*

1. Take O/N cultures of the cells to test
2. Dilute them to OD 0.2 with LB medium
3. For each Rhamnose concentration to be studied, put 100 µL of each culture on a well of a 96-well plate (do in duplicate)
4. Place the 96-well plate in the stove and let the cells grow for 24 hours (no agitation)
5. Measure fluorescence signal at the plate reader. For eGFP, Excitation wavelength: 488nm; Emission wavelength: 508nm
6. Wash the cells out of the 96-well plate and add 100 µL of LB onto each well
7. Measure again fluorescence signal at the plate reader