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:

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