RBS characterization protocol

This protocol aims for the characterization of the constructs we built during our partnership project. Analysis is made using a plate reader with timecourse measurements of OD600 for growth and fluorescence for RBS strength (sfGFP).

Prior to following this protocol, a fluorescence calibration of the OD reader should be done.

- Start with a 5mL overnight culture of the cells with the constructs to be characterized in LB and the appropriate antibiotic.
- Transfer 1.8mL to a microcentrifuge tube and centrifuge at max speed for 30sec.
- Discard the supernatant and resuspend in 900uL PBS.

The cells are now 2 times concentrated.

- Measure OD600.
- Dilute the cells to an OD of 0.05 in 20mL of M9 0.8% glucose.
- Plate 50uL onto a 384well plate, a column for each strain allowing for 16 replicates to be made.

In our case, 19 strains + a negative control (NEB Turbo).

- Add 10uL of mineral oil to each well.
- Place the plate in a plate reader for a 9h timecourse experiment.

Settings to use for the Tecan Spark

Temperature: 37°C
Kinetic loop: over 9h with measurements every 10 min
Measurements:

- OD600 (quantify growth)
- fluorescence: excitation: 480nm (quantify GFP production)
  emission: 520nm