Assessing Promoter Activities

To follow the growth and development of the luminescence signal as readout, perform Plate Reader experiments in a 96-well plate format in your choice of Medium:

1. Müller-Hinton Medium (MH-Medium) for antibiotic testing.
2. MNGE for Quorum sensing studies
3. MCSE (Modified chemically defined medium)

- The day before: 3mL overnight cultures of the strains of interest in Test Medium containing antibiotics (1:1000)
- incubate overnight at 37˚C with 200rpm agitation
- The next morning: set up 10mL day cultures without antibiotics -> inoculate 1:500 and incubate at 37˚C with 200rpm agitation to an OD_{600} between 0.2 and 0.4
- Dilute cultures to an OD_{600} of 0.01 and distribute 100µl of the dilution per well into the 96 well plate
- Fill at least 3 wells with pure Medium to serve as your Blank, always carry a positive and negative control with you
- incubate the plate at 37˚C with agitation in a Plate Reader (here Model Synergy Neo) and measure growth and luminescence for 1 hour
- After 1 hour: Induce your samples (substance that potentially induces your promoter activity) by adding 5µl of the inducer (concentration of interest)
- Put back in the Plate Reader and measure growth and Luminescence for 15 to 18 hours every five minutes