IGEM EPFL - Bioluminescence assay

Materials :

- LB with appropriate antibiotic (25ug/mL)
- L-Arabinose
- Phosphate buffer saline (PBS, pH 7)
- Coelenterazine (10mM dissolved in 100% ethanol, stored at -80°C) for Renilla Luciferase
- D-Luciferin (10mM dissolved in H2O, stored at -20°C) for Firefly Luciferase

Procedure :

1. Grow a starter culture overnight in 3mL of LB with the appropriate antibiotic

2. Dilute 50 ul overnight culture into 5mL fresh LB containing 5mM arabinose and grow for 3h at 37°C on a rotary shaker to an OD ~1. For negative controls, do the same with glucose.

3. Wash the culture twice with PBS, adjust to an OD 0.4-0.5

4. Thaw aliquots of clz or d-luc in the dark for 30 minutes

5. Aliquot PBS-washed cells in an opaque 96-well plate (with transparent bottom) in triplicates and add clz or d-luc o a concentration of 10uM into 200uL final volume

6. Take measurements of the luminescence (preferably from the bottom) with the plate reader every minutes for one hour