Quantification of bacterial fluorescence

Protocol for Quantification of bacterial fluorescence using independent calibrants

Materials:

- Viable *E. coli DH5*α
- Liquid medium LB
- Chloramphenicol (60 mg/mL)
- Chamber at 37°C
- 15 flask of 125 mL
- Biotek Synergy HTX Multi-mode microplate reader
- Plasmid DNA (100 pg/uL in 10 µl of Buffer EB)

Procedure:

Day 1.

1. From a relatively fresh plate of each genetic variant of *E. coli*, pick a colony and grow O/N at 37 °C in a falcon with 25 mL of LB medium and incubate in a shaker.

Day 2.

1. Centrifuge at 5000 rpm for 10 minutes and resuspended the pellet with LB medium, to a final OD of 0.01.

2. Incubate for 8 h at 37 °C, at slow rpm (120).

3. Take samples every hour.

4. Measure at a wavelength of 600 nm in the Biotek Synergy HTX Multi-mode microplate reader for the OD and measure at 485 nm for excitation and 525 nm for emission to quantity GFP protein.

5. You should use each cell line (with your construct inserted) as a sample and a mother strain with your empty vector as control.