GFP Fluorescence Measurement

Introduction

How we measured GFP fluorescence dynamics in E.coli transformants, over 44h time period.

Materials

- LB Media/Agar
  - 10 g/L Tryptone
  - 5 g/L yeast extract
  - 10 g/L NaCl
  - for agar plates:
    - + 15 g/L agar
- Fluorescence spectrophotometer
- Photometer

Procedure

Day 1

1. Make 15 mL over night cultures (ONC) with:
   - GFP transformed strains
   - Non GFP transformed strains (control)
2. Incubate overnight, 140 rpm, 37°C

Day 2

1. Measure the bacterial density OD_{600} and inoculate 50 mL LB liquid culture so it has a final concentration of $OD6_{00}$=0.1
2. Incubate and measure OD_{600} and GFP fluorescence of the 50 ml cultures (GFP strains + control)
   - GFP Excitation wavelength: 488 nm
   - GFP Fluorescence absorbance: 510 nm

- incubate your cultures in the morning: (8:00 am)
    - measure at 8 AM [0h]
    - measure at 12 AM [4h]
    - measure at 4 PM [8h]
    - measure at 8 PM [12h]

- next day:
    - measure at 12AM, [28h]
    - measure at 4 PM, [32h]
    - measure at 8 PM, [36h]

- incubate another probe at (16 pm)
    - measure at 8 AM, [16h]
    - measure at 12 AM, [20h]
    - measure at 4 PM, [24h]

- next day:
    - measure at 6 AM [40h]
    - measure at 10 AM [44h]
    - measure at 2 PM [48h]