Fluorescence intensity quantification protocol

To measure fluorescence intensity in whole bacterial cells.

Adapted from iGEM Wageningen 2019
(https://www.protocols.io/view/gfp-rfp-plate-reader-assay-784hryw)

Materials

- Bacteria
    - From plates or glycerol stocks
- Luria-Bertani (LB) medium
- Antibiotics (if required)
- Phosphate buffered saline (PBS) 1X, pH 7.4
- Eppendorf tubes
- 96-well plate, clear bottom
- CLARIOstar Plus Microplate Reader

Procedure

1. Calibrate the microplate reader to obtain a standard curve:
    a. For OD600 measurement, calibrate with silica beads.
    b. For GFP fluorescence intensity measurement, calibrate with fluorescein.
    c. For RFP fluorescence intensity measurement, calibrate with Texas Red.
2. Set the following protocols for CLARIOstar Plus Microplate Reader:
    a. To measure OD600:

        Measurement type: absorbance, endpoint

        Excitation wavelength: 600 nm

        Top optic

        Temperature: 37°C

    b. To measure GFP fluorescence intensity:

        Measurement type: fluorescence (FI), endpoint

        Excitation wavelength: 483 nm

        Emission wavelength: 530 nm

        Gain: 680

        Dichroic filter: 502.5

        Focal height: 10 mm

Top optic

Temperature: 37°C

c. To measure RFP fluorescence intensity:

Excitation wavelength: 560 nm

Emission wavelength: 615 nm

Gain: 910

Dichroic filter: 582.5

Focal height: 10 mm

Top optic

Temperature: 37°C

3. Prepare bacteria cultures (from plates or glycerol stocks) in 10 mL LB media supplied with antibiotics (if required).
4. Incubate cultures overnight at 37°C, 150 rpm.
5. Transfer 1 mL of overnight culture into an Eppendorf tube.
6. Centrifuge at 13000 rpm for 5 minutes to pellet cells.
7. Resuspend the cell pellet in 1 mL PBS.
8. Repeat centrifuging and washing steps.
9. In a clear bottom 96-well plate, load 160 µL PBS and 40 µL bacteria suspension.
    a. As negative control, load 160 µL PBS and 40 µL non-fluorescent bacteria suspension.
    b. As blank, load 200 µL PBS.
10. Measure OD600 and fluorescence intensity of the samples using the protocols above.
11. Adjust fluorescence intensity measurements for OD600 values.