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Microplate characterisation

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Protocol status: Working

We use this protocol and it's working.

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Abstract

To characterise OD600 and GFP levels using a microplate reader.

Preparing pre-culture

- 1 Inoculate PGAL-GFP glycerol stock into a 50 mL falcon tube containing 5 mL Yeast Nitrogen Base (YNB) media containing 2% glucose (without leucine).
- 2 Incubate the sample overnight at 30 °C, with shaking.

Microplate characterisation workflow

- 3 After 22-24 h, measure the OD600 of the overnight pre-culture in a cuvette using Nanodrop OneC UV-Vis Spectrophotometer.
- 4 Refresh the overnight pre-culture with fresh YNB media containing 2% glucose (without leucine) to prepare four eppendorf tubes of 1 mL sample with final OD600 of 1.6.
- 5 Spin down the eppendorf tubes at 6,200 g for 1 min.
- 6 In each tube, resuspend the cell pellet with 1 mL of YNB media (without leucine) containing different galactose inducer concentrations:
A) 0.1% glucose (no galactose)
B) 0.1% glucose + 10 mM galactose
C) 0.1% glucose + 50 mM galactose
D) 0.1% glucose + 80 mM galactose
- 7 Plate 300 uL of the sample as triplicates in a clear 96-well plate and include well for media blank.
- 8 Load the plate into microplate reader (Cytation 5) to measure the OD600 and GFP level every 30 mins for 24 h.