Fluorometric real-time quantification of in vivo fluorescence in E. coli

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Abstract

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Materials

- Microplate Reader Synergy Mx View by Contributed by users
- Costar 96 clear bottom black side plates by Contributed by users

LB Broth (Lennox) vegetal 0155 by Carl Roth

Mineral oil for Molecular Biology HP50 by Carl Roth

Protocol

Overnight culture preparation

Step 1.

Prepare o/n culture of each 3 independent clones

- add 5 mL of LB medium to a sterile culture tube
- add the appropriate amount of antibiotic(s)
- use a sterile inoculation needle, pipette tip or toothpick to pick a colony from the Agar plate
- gently whirl the tip in the medium (or drop it)
- incubate at 37 °C and 230 rpm

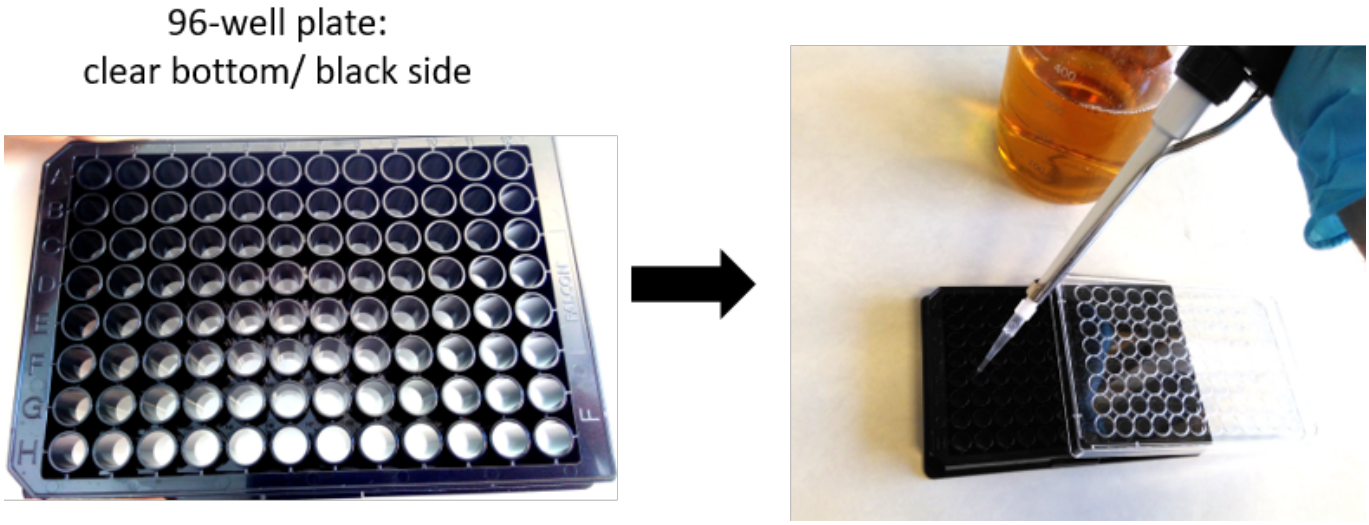
TEMPERATURE
37 °C Additional info: 230 rpm
LINK:
https://www.addgene.org/protocols/inoculate-bacterial-culture/

Inoculation of 96-well plates

Step 2.

1:100 dilution of o/n cultures in microtiter (96-well) plate

- pipet 99 µL fresh LB medium (incl. the appropriate antibiotic) into each well of the 96-well plate
- add 1 µL of each o/n culture to the medium
- add 100 µL LB medium to 6 wells as blank
- overlay each well with 40 µL of mineral oil (to prevent evaporation)



NOTES

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• pipet in a sterile or semi-sterile environment, i.e. in sterile hood, since also contaminants would have the chance to grow during the following o/n incubation

• due to the autofluorescence of LB medium, you might prefer to use a mineral medium like M9 medium - note that the choice of media will significantly influence the growth characteristics and might require longer cultivation periods

Plate reader settings and measurement

Step 3.

- remove the lid from the microtiter plate
- insert microtiter plate into plate reader device (e.g. Synergy Mx, Biotek)

Settings

Gain: 50

Absorbance: **OD_{600}** (8 measurements per data point)

Fluorescence **GFP**: Excitation λ - **480/ 9 nm**

Emission λ - **520/ 20 nm**

Fluorescence **YFP**: Excitation λ - **500/ 9 nm**

Emission λ - **535/ 20 nm**

Frequence of measurements: **5 min** (241 reads, 10 measurements per data point)

Temperature: **37 °C**

Agitation: **continuous** 'medium mode'

Time: **20 h**

TEMPERATURE

37 °C Additional info: 'medium mode'

Data collection and basic analysis

Step 4.

- export data to .csv/.xlsx file
- substract blank (LB) values from both the fluorescence and the OD_{600} values
- estimate relative fluorescence by dividing GFP/YFP-fluorescence by the corresponding OD_{600}
- the figure below depicts exemplary curves of relative YFP (mVenus) fluorescence accumulation over time in *E. coli* strain W3110
    - the unit of measurement is

relative mVenus fluorescence [Ex.:Em. 500:535nm * OD_{600}^{-1}]

- as a quantitative proxy for the expression dynamics calculate the slopes of relative fluorescence within the linear phase of relative fluorophor accumulation

- divide single values of relative fluorescence by the corrsponding value of the control group at a given time point, or a given OD_{600}