GFP fluorimeter calibration

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Introduction

The purpose of this experiment is to establish a relationship between the Arduino input voltage registered at the photoresistance in response to the fluorescence coming from GFP proteins present in the culture and the concentration of the inductor composite (lactone in our case). Once this transfer function has been obtained, the turbidostat can automatically have a continuous registration of the lactone concentration within a cell culture.

Materials

- LED 485nm
- Photoresistance
- Optical filter
- Electronic circuit (see *electronics module* in *Hardware* page)
- Box
- 3D printed piece
- Reporter cell culture

Procedure

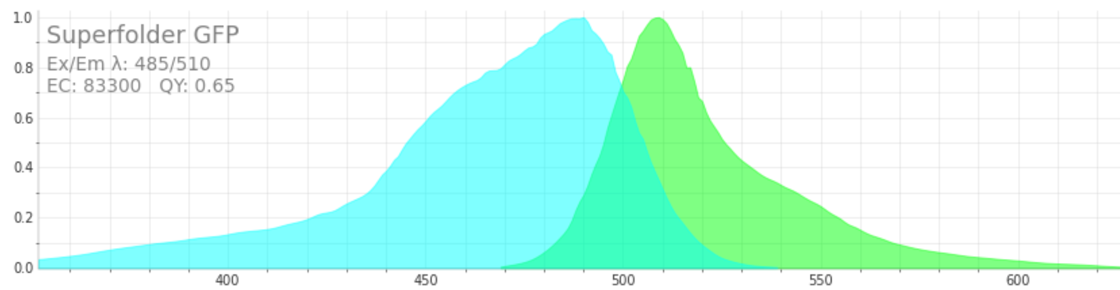
Based on the fact that reporter cells have already been shown in previous experiments to generate GFP luminescence in the presence of the inducing compound (lactone in our case), dilutions with a different lactone concentration will be prepared the day before (going from 10^{-10} to 10^{-4} M) and a control one with no lactone. It is worthwhile to do a second and quick check before starting this experiment. To do so, a sample of control culture and another from 10^{-4} lactone cell culture are taken and placed inside the transilluminator. The control one should not present any fluorescence while the lactone one does.

It is to be taken into account that the experiment should be performed under a controlled value of OD. An interval ranging from 0.4 to 0.5 was chosen, because it corresponds to the value set in the turbidostat. Bearing in mind that sfGFP is excited at 485 nm and it emits light at 510 nm, an optical filter with a bandwidth of ~500-550 nm should be placed in front of the photoresistance. The LED is controlled by the Arduino PWM output and the analogic value of the photoresistance is registered at the analogic input of Arduino (as shown in *Hardware* page).

Before starting the calibration some setup has to be done. A vial containing 5 ml is placed fitting with the 3D printed piece. Note that the 3D printed piece is designed in such a way to generate an angle of 90º between the LED and the photoresistance, avoiding therefore a direct impact of the LED into the photoresistance. It is then covered with its box in order to match the conditions at which it will operate and avoid possible future performance distortions.

Knowing the lactone concentration in each of the cell cultures, the analogic values obtained in the photoresistance are annotated until completing the characterization of the GFP fluorescence with respect to lactone concentration. This experiment is repeated over several times and the average transfer function is computed. So as to do the final checking, it is highly

recommended to compare the obtained experimental characterization with the one obtained in the plate reader, analyse the results and check if they correlate.



Results

Due to the lack of time and several complications during experimental procedures, the obtained results were not accurate enough for a precise characterization of the GFP fluorescence with respect to lactone concentration. A deep troubleshooting was done in which solutions for a future improvement were proposed: the inclusion of an optical filter with specific bandwidth (~500-550 nm), the inclusion of an amplifier to expand the resolution of the target analogic values and the acquisition of more sophisticated electronic components, as for example a led diode. In terms of wetlab, several strategies were contemplated to amplify the expression of GFP protein, such as removing the degradation tag.