APPLICATION NOTE

Multitask kinetic measurements of IPTG's effects on protein expression and cell growth

Introduction

Measuring several different signal outputs simultaneously over time is especially useful for studying a protein or compound's effect on cellular growth or gene expression. In this application note, we measure cell growth (absorbance) and protein expression (fluorescence) using SoftMax® Pro 7 Software.

In bacteria, the *lac* operon is a cluster of genes under the control of one promoter, which encodes ß-galactosidase. After ligating a protein's sequence into a plasmid containing the *lac* operon and transfecting bacteria with the plasmid, one can use this transformed bacteria to express the protein of interest. Normally, the *lac* promoter is allosterically repressed, but in the presence of isopropyl ß-D-1-thiogalactopyranoside (IPTG), the repressor is released from the promoter sequence causing expression of the protein of interest. Conversely, if there is excessive IPTG, the cells will divert disproportionate cellular resources into protein expression and their growth rate will be stunted[1].

We demonstrate how *E. coli* responds to a dilution series of IPTG by measuring cell density and protein expression simultaneously.

Materials

- SpectraMax® M2 Multi-Mode Microplate Reader (Molecular Devices)
- Luria Broth (LB) Media (Teknova cat. #L8080)
- Ampicillin Sodium Salt (Teknova cat. #A9525)
- 1 M IPTG (Teknova cat. #I3431)
- 96-well clear, flat-bottom polystyrene microplate (Greiner cat. #655-161)
- *E. coli* containing pBbE5-RFP plasmid (Keasling Lab)

Benefits

- **Measure several different signals simultaneously over time**
- **Utilize a diverse set of algorithms and curve fits for comprehensive data analysis**
- **Set up a simple protocol using the Workflow Editor**

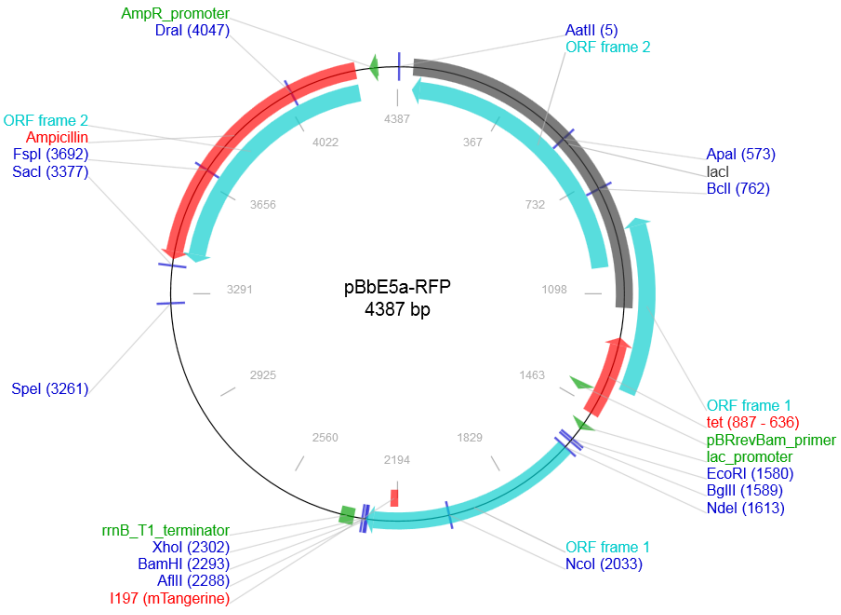

Figure 1. pBbE5a-RFP plasmid map.

Methods

E. coli containing the pBbE5-RFP plasmid (Figure 1) was supplied by Professor Jay D. Keasling[2]. The *E. coli* strain was grown to 0.3 OD600 in LB media containing 100 µM ampicillin. Afterwards, 100 µL of *E. coli* was transferred to a clear 96-well microplate. A two-fold serial dilution of IPTG starting from 500 µM was used to treat the *E. coli*. 0 µM IPTG and a media-only control were assayed as well.

The microplate was then placed in a SpectraMax M2 Multi-Mode Microplate Reader and incubated at 32°C for the duration of the kinetic assay. Using the SoftMax Pro 7 Workflow Editor, a kinetic cycle containing an absorbance read and a fluorescence read, with a 10-second plate shake between reads, was created with the settings shown in Table 1. The kinetic cycle was set to repeat once every 10 minutes for a total of 24 hours. The resulting dual read mode kinetic data was analyzed using SoftMax Pro 7 Software.

Results

The kinetic traces for absorbance and fluorescence measurements are shown in Figures 2 and 3 respectively. In Figure 2, increasing IPTG concentration caused a decrease in OD600 values indicating an adverse effect on bacterial growth. In Figure 3, increasing IPTG concentration did not result in an increase of red fluorescent protein (RFP) expression. In contrast, increasing IPTG concentration caused a decrease in overall RFP expression. This decrease in RFP expression was most likely due to plateauing bacterial growth.

In Figure 4, a reduction was applied to both the absorbance and fluorescence data sets to calculate the area under the curve (AUC). This analysis was performed using SoftMax Pro 7 Software and summarizes IPTG's effects on cell growth and protein expression. IPTG's effect on fluorescence was similar throughout all concentrations with the exception of 500 µM, where increasing IPTG concentration caused a decrease in bacterial growth (absorbance).

	Read mode	
	Absorbance	Fluorescence
Wavelength (nm)	600	Ex/Em = 530/625
Read type	Bottom read	Bottom read
Interval	1 read/10 minutes	1 read/10 minutes
Optical settings	—	10 flashes/read
PMT gain	—	High

Table 1. Instrument settings for measuring cell density and protein expression.

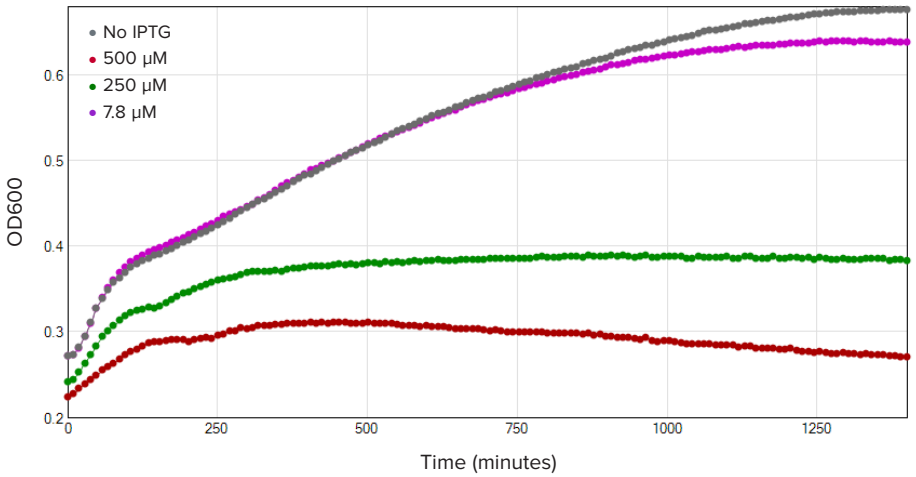

Figure 2. OD600 kinetic trace. OD600 readings were recorded every 10 minutes over the course of 24 hours (n=3). As IPTG concentration increased, bacterial growth rate decreased.

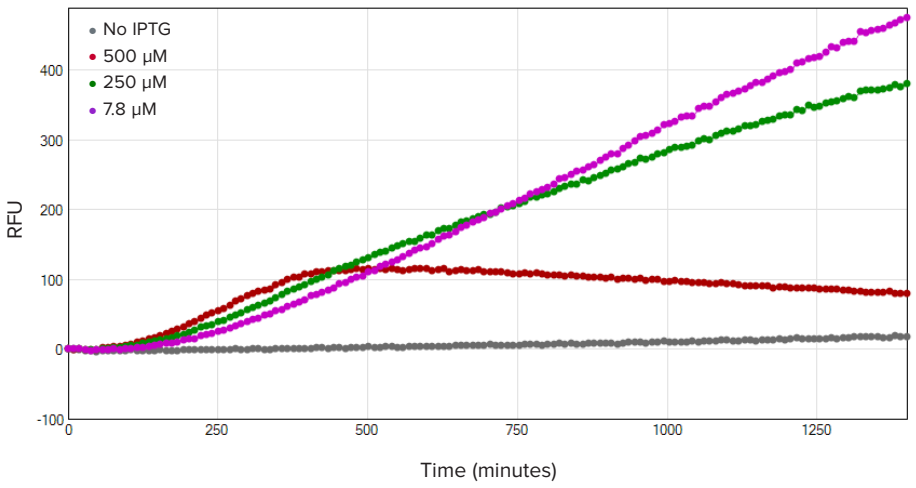

Figure 3. Fluorescence kinetic trace. Fluorescence was recorded alongside OD600 measurements (n=3). With the exception of the 500 µM IPTG-treated wells, fluorescence output was relatively similar for all concentration ranges.

Calculating the RFP/OD600 values allowed for measuring protein expression relative to population density. Using SoftMax Pro 7 Software, we calculated the RFP/OD600 ratio (Figure 5). Based on the kinetic trace, the 250 µM IPTG- treated bacteria expressed the most RFP per cell.

Conclusion

We demonstrated how Molecular Devices SoftMax Pro 7 Software and multi-mode microplate readers can measure several biological events simultaneously over a course of time. The experimental concepts shown in this application note, although simple, can be applied to more complex experiments.

References

1. Malakar, P. and Venkatesh, K.V. (2012) Effect of substrate and IPTG concentrations on the burden to growth of Escherichia coli on glycerol due to the expression of Lac proteins. *Applied Microbiology and Biotechnology,* 93(6), 2543-2549.
2. Lee, T.S., Krupa, R.A., Zhang, F., Hajimorad, M., Holtz, W.J., Prasad, N., Lee, S.K., and Keasling, J.D. (2011) BglBrick vectors and datasheets: a synthetic biology platform for gene expression. *Journal of Biological Engineering,* 5, 12.

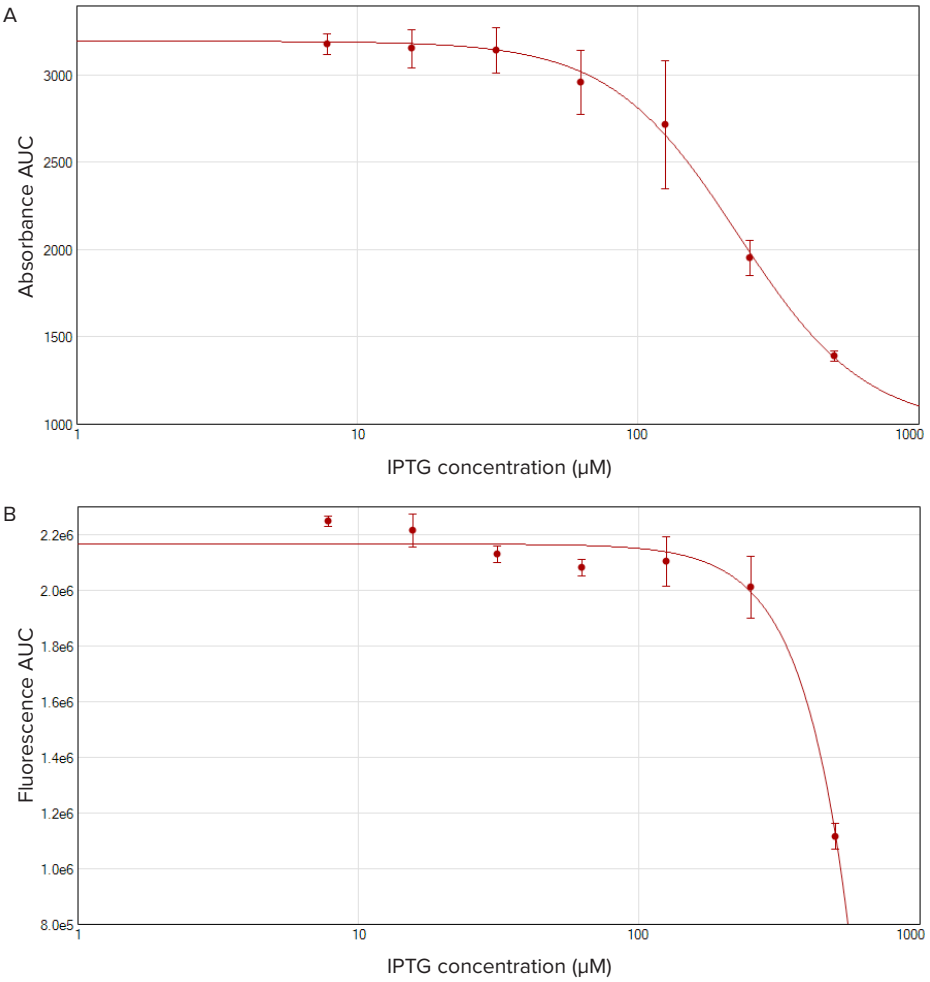

Figure 4. Area under the curve (AUC) of absorbance and fluorescence measurements. The AUC for absorbance **(A)** and fluorescence **(B)** were calculated and plotted using SoftMax Pro 7 Software. A 4-parameter curve was applied to demonstrate the effect of IPTG on cell growth (absorbance) and protein expression (fluorescence).

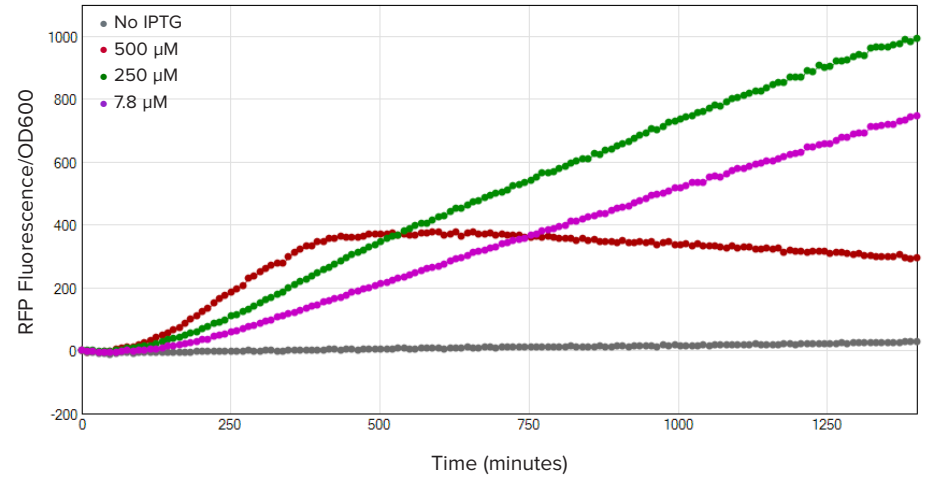

Figure 5. RFP/OD600 ratios. The ratio of fluorescence to cell density was calculated by dividing fluorescence values by OD600 values. After normalizing the fluorescence to cell density, the 250 µM IPTG-treated bacteria (green) demonstrated the highest RFP concentration per cell.

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