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BNG 501

Joberry: Low-cost, scalable, and customizable optogenetic stimulation system for 24-well plate cell culture

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I. Introduction

Optogenetics is a technique that uses light to control the biological activity of cells via photosensitive proteins; it has proved immensely valuable for fields like neuroscience, medicine, synthetic biology, and metabolic engineering as illumination is a stimulus that can be precisely controlled spatially and temporally [1]. Optogenetics is often used as a means of simplifying and analyzing complex networks of cell signaling and subcellular reactions. For metabolic engineering in particular, optogenetics has the potential to enable co-culture fermentations by regulating the relative growth of mixed microbial consortia populations [2]. Traditional optogenetic methods—such as their use in single-sample microscopy—have poor throughput and may contribute to poor experimental reproducibility due to human error. Recent work to design optogenetic stimulation systems for multi-well plates has proven valuable for parallelizing treatment between replicates, reducing costs for experiments, and comprehensively exploring stimulation settings and their effect on cell growth and dynamics [3]. Here, we share a refined DIY programmable and ultra-low-cost 24-well plate optogenetic stimulation box (informally named “Joberry”) and demonstrate its use for modulating cell growth with the mazE/mazF genetic circuit (called “optoTA”) as developed in [2].

II. Results

Box Design

Our optogenetic stimulation box for a 24-well plate is built using an opaque, fully-enclosed 4-part 3-D printed housing, an array of six diffused LED panels each illuminating 3 wells, and a programmable Arduino Nano microcontroller and corresponding circuitry using a simple breadboard. 3-D printing and electronic prototyping were chosen as the primary fabrication methods for their simplicity, low cost, and reproducibility, as opposed to other 3-D manufacturing methods (i.e., laser cutting or molding) or embedded systems design methods (i.e., a PCB or soldered circuit board).

The design of our device was guided and informed by a set of constraints requisite for its reliable and beneficial use in a laboratory. For example, our system fits on and is durable enough to withstand the vibrations of a typical cell culture shaker plate for at least 24 hours; includes three replicates per stimulation condition at a peak illumination wavelength of approximately 465 nm (blue light) as shown in **Figure 1**, which is optimal for many optogenetic circuits including mazE/mazF; and includes negative (no light) and positive (full light) control conditions in

addition to five experimental conditions. Furthermore, it uses a standard USB power supply, tightly holds a 24-well plate, and is built with low-cost readily available and 3-D printed parts. However, our device is limited in its output illumination power of only 0.962 W/m^2, which falls short of our goal of 21.8 W/m^2 based on prior research, as measured with a Thorlabs power meter. Images and renderings of our device are included in the **Supplementary Information** section below.

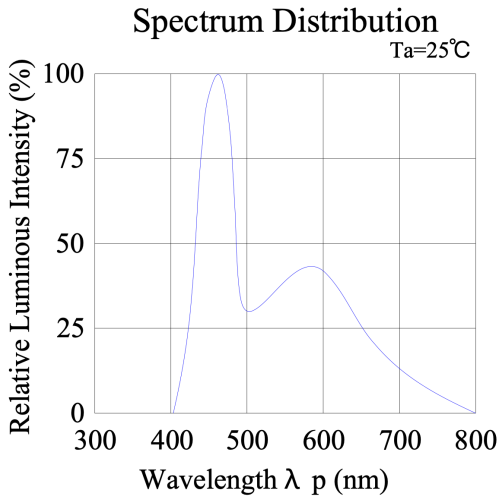

Figure 1. Emission spectrum for the diffused LED panel used for optogenetic stimulation, from [4]. Note the peak in relative luminous intensity around 450-480 nm (blue) wavelengths.

Altogether, our optogenetic stimulation box can be fabricated and assembled in less than a day for less than $100 (excluding the cost of a 3-D printer and a 24-well plate), making our design scalable and cost-effective. The device's housing features an opaque 3-D printed mask layer that lies between the LED panels and the well plate, mitigating light leakage and crosstalk between wells under different experimental illumination conditions. In addition, the electronics are fully enclosed to improve the durability and lifespan of the device. The system also features a straightforward software script to set the illumination period and duty cycle for each of the six individually addressable LED modules. By making use of diffusive LED panels, we reduce the power consumption of the device to less than 5 W and simultaneously eliminate the need for a heatsink (as in [3]) by decreasing the thermal footprint. The diffusion layer also allows for even light distribution across an entire well, unlike some prior optogenetic stimulation devices [3]. Lastly, the three replicates for each of the six illumination conditions allow for more precision and more breadth in a given experiment as a user can program multiple illumination patterns at once.

Experimental Performance

To demonstrate the performance of our optogenetic stimulation box, we used the MazE/MazF genetic circuit as described in [2]. In summary, this circuit modulates the function of the mRNA-degrading endoribonuclease toxin MazF using an unstable antitoxin MazE, which binds to and inhibits MazF homodimers. Naturally, under cellular stress, MazE is degraded by ClpP

protease to induce programmed cell death or inhibit growth. By coupling MazE expression with the optogenetic pDusk and pDawn system [5], cell growth can be precisely controlled. Accordingly, the optoTA construct contains the MazE/MazF systems and pDusk and pDawn promoters. With enough light exposure, the MazE gene is expressed with pDawn, inhibiting MazF and allowing cell proliferation. In contrast, without light exposure, the MazE gene is not expressed and the MazF gene is expressed and uninhibited with pDusk, mitigating cell growth or inducing apoptosis.

To demonstrate the light-sensitive growth of *E. coli* cells transformed with the optoTA construct, we prepared two plates left for 24 hours in full darkness or full blue light (~465 nm wavelength) stimulation, shown in **Figure 2**. Visually, there was significant growth for all replicates in the lighted condition compared to minimal growth in the dark condition as summarized in **Table 1**.

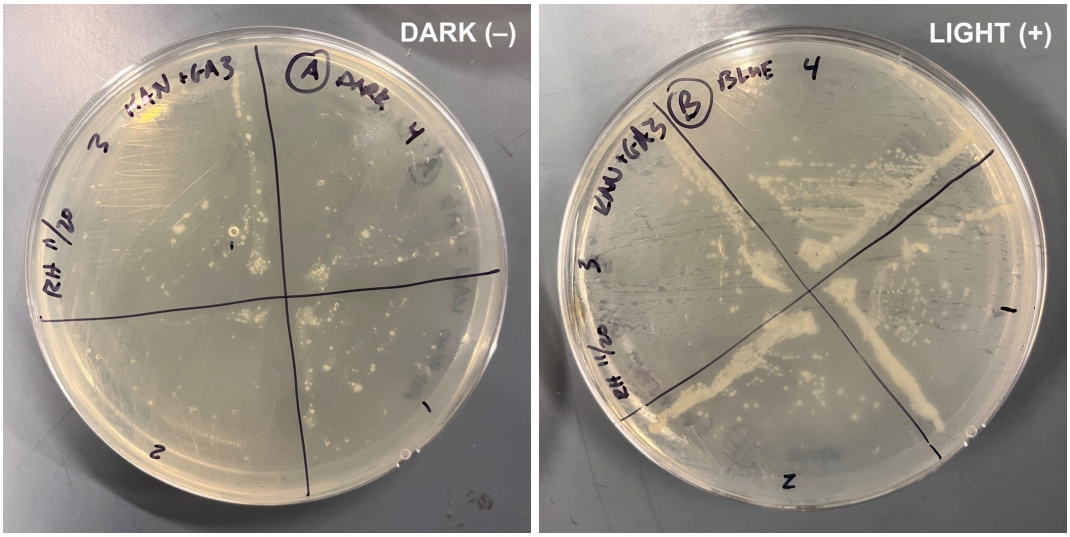

Figure 2. (Left) Plates of *E. coli* cells with the optoTA plasmid after 24 hours with no stimulation (left) and full stimulation (right) in ~465 nm blue light.

Table 1. Qualitative observations of *E. coli* cells with the optoTA plasmid construct after 24 hours in no stimulation and full stimulation.

Test condition	**Observations**
No stimulation (dark)	Minimal growth for all replicates.
Full stimulation (~465 nm blue light)	Significant growth for all replicates.

These transformed cells, when added to a 24-well plate, were stimulated with different illumination patterns using our new optogenetic stimulation box as summarized in **Tables 2A, 2B**. OD600 was used as a proxy for measuring cell growth in the well plate. **Table 2A** and its corresponding graph in **Figure 3** show a clear increase in cell density with an increase in the stimulation duty cycle with a fixed 100 s period; a factor of 100 increase in the duty cycle from 1% to 100% corresponds to an approximately four-fold increase in optical density. Biologically, a longer duty cycle implies a longer stimulation exposure in a fixed time span (i.e., 10 hours—the

total length of the incubation here), resulting in greater MazE expression, greater MazF inhibition, and ultimately greater cell proliferation/density. This trend appears to be approximately linear; a tenfold increase in duty cycle between 1% and 100% roughly corresponds to a doubling in optical density. The exact relation between light flux or intensity and cell growth rate is likely determined by the specific kinetics of the optogenetic system and is worth further study.

In contrast, **Table 2B** and its corresponding graph in **Figure 3** do not show a clear correlation between cell density and stimulation period with a fixed 10% duty cycle; a factor of 200 increase in stimulation period does not produce a statistically significant change in optical density over 10 hours. Biologically, this would indicate that the kinetics of the optogenetic system (i.e. MazE/MazF binding/unbinding, promotion/expression, and degradation) are much faster than the illumination time scale. In other words, the optogenetic mechanism responds to a change in stimulation nearly instantaneously (relative to the scale of the stimulation periods tested). As a result, because the cells under a fixed duty cycle experience the same effective total stimulation time over the course of the experiment (10 hours) regardless of the stimulation period length, they have similar levels of growth and transient changes in MazE/MazF concentration take negligible time. An investigation using shorter stimulation periods may help shed light on this hypothesis.

Table 2A. Stimulation patterns with duty cycle modulation and fixed period (100 s). Average OD600 values with their standard deviation are noted for the wells under each condition after 10 hours.

Condition *≥3 replicates*	**Duty cycle (time on [s])** *variable*	**Period [s] (time off [s])** *fixed*	**OD600 (SD)**
A (+ control)	**100%** (100)	100 (0)	**0.550** (0.02)
B	**10%** (10)	100 (90)	**0.256** (0.03)
C	**1%** (1)	100 (99)	**0.139** (0.03)
D (– control)	**0%** (0)	100 (100)	**0.135** (0.01)

Table 2B. Stimulation patterns with period modulation and fixed duty cycle (10%). Average OD600 values with their standard deviation are noted for the wells under each condition after 10 hours.

Condition *≥3 replicates*	**Duty cycle (time on [s])** *fixed*	**Period [s] (time off [s])** *variable*	**OD600 (SD)**
E	10% (0.1)	**1** (0.9)	**0.272** (0.02)
F	10% (1)	**10** (9)	**0.299** (0.02)
B	10% (10)	**100** (90)	**0.256** (0.03)
G	10% (20)	**200** (180)	**0.292** (0.02)

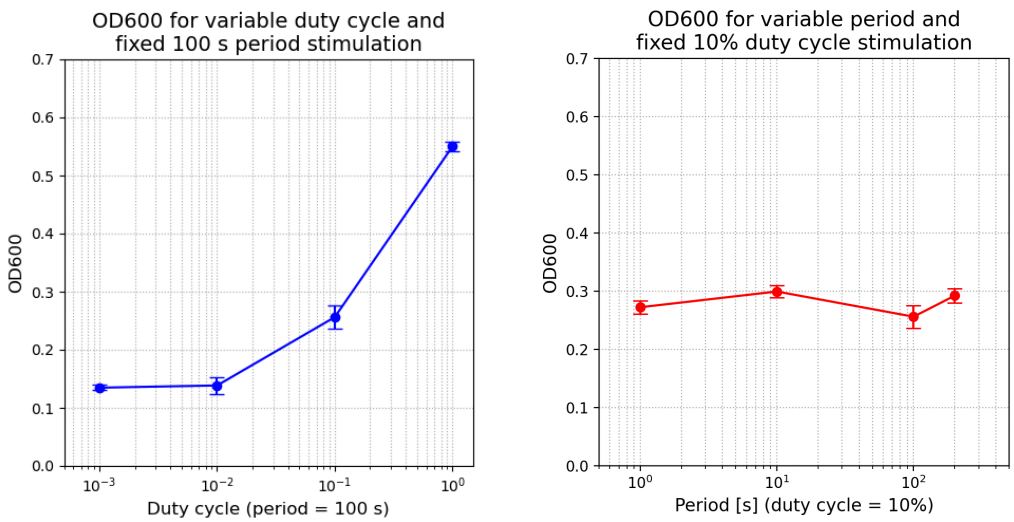

Figure 3. (Left) OD600 values after 10 hours for stimulation patterns with duty cycle modulation and fixed period (100 s). (Right) OD600 values after 10 hours for stimulation patterns with period modulation and fixed duty cycle (10%). Error bars on both graphs are the standard error of the mean (SEM) for the ≥3 replicates per condition.

III. Discussion

Our stimulation box demonstrates optogenetic regulation of the optoTA construct in *E. coli* to modulate cell growth with fine control over the duty cycle and period of well illumination, but our experiment leaves many questions unanswered and room for improvement. While our results appear to show clear trends in cell growth with reasonable biological explanations, this platform does not directly explain the underlying kinetics of the optoTA circuit; modeling the dynamics of this system and fitting rate parameters to this experimental data would provide a more thorough understanding of the optogenetic mechanism and validate our findings. Further work can also be done to characterize the performance limits of our design, including quantifying the extent of light leakage or crosstalk between illumination conditions, determining the lower limits to illumination duty cycle and period length—i.e., how briefly the microprocessor can stimulate the wells—and formally testing the durability of our box for repeated use. This experiment could also be repeated to probe a broader range of stimulation duty cycles and periods with better resolution (note that our prior conclusions were only based on four experimental conditions). Furthermore, our system was designed around a specific 24-well plate (VisiPlate-24 Black from Revvity Health Sciences); to accommodate slight dimensional differences between brands, the plate mounting system should be redesigned to be adjustable. Lastly, the base of the box could be improved to robustly and reversibly attach to a shaker incubator instead of using tape.

More complex improvements include support for multiple precise wavelength stimulation (similar to taborlab.github.io/LPA-hardware/index.html), which would better support other optogenetic circuits, and integrated stimulation and spectroscopy in a single apparatus to study cell growth temporally by automatically measuring optical density at regular intervals (see [6]). Altogether, however, our stimulation box is an integrated, simplistic, easily programmable and customizable, and low-cost tool for performing 24-well plate experiments with optogenetic

circuits. On a personal note, this project was a valuable opportunity to learn about the intersection of optogenetics in synthetic biology, rapid prototyping of embedded systems, and 3-D printing.

IV. Methods

A. Hardware Fabrication

As previously mentioned, the optogenetic stimulation box was fabricated using a 3-D printed housing, light panels, and circuitry for a 24-well plate. The housing was printed with an A1 Mini (Bambu Lab) with black PETG filament (PolyLite) and was composed of a base for the electronics (Arduino Nano, breadboard, resistors, and wires), a frame to hold six diffused LED light panels (Adafruit Industries, part #1622 [4]), a frame to hold the black-walled 24-well plate (VisiPlate-24 Black, Revvity Health Sciences) via friction fit and mask the light panels, and an opaque lid to mitigate stimulation from external light sources and secure the well plate. Opaque black electrical tape was used along the lateral faces of each light panel to eliminate crosstalk between adjacent panels. The six panels were glued to the light frame. Six GPIO outputs from the Arduino Nano were wired to the anode (+) of each light panel with a 220 Ω current-limiting resistor in series for each. The cathode (–) of each light panel was wired to the ground pin on the Arduino. A USB cable was used to program and power the Arduino. With the electronics assembly complete, the base, light frame with the LED panels, and well plate frame were glued or taped together. The entire assembly was adhered to a shaker plate with double-sided and masking tape. A 5V/1A USB adapter was used to power the system during use.

B. Opto-Strain Design

Unless otherwise noted, all reactions were performed following a given manufacturer's instructions. Gibson assembly was used to synthesize the optoTA construct with the optogenetic mazE/mazF control mechanism as described in [2]. To summarize, the DNA fragment containing the mazE/mazF genes and corresponding plasmid backbone (Integrated DNA Technologies) were amplified with primers using CloneAmp HiFi PCR premix (Takara Bio). Gel electrophoresis was used to validate this PCR amplification. The products were then incubated with rCutSmart Buffer (New England Biolabs) and DpnI (New England Biolabs) to digest the unwanted methylated wildtype DNA overnight at 37°C and the subsequent products were purified with an E.Z.N.A. Gel Extraction Kit (Omega Biotek). The Gibson reaction was performed with a Gibson Assembly Master Mix (New England Biolabs) using a 1:4 ratio of DNA vector to fragment. A heat shock was used to transform the assembled plasmids into competent DH5α *E. coli* cells, which were then added to plates with LB and 50 µg/mL kanamycin antibiotic and grown overnight in blue light (~465 nm wavelength) at 37°C. Colony PCR was performed on individual colonies using EconoTaq Master Mix (Biosearch Technologies). A gel electrophoresis was performed to identify successful (positive phenotype) mutant colonies, which were grown overnight in tubes with LB with 50 µg/mL kanamycin antibiotic at 37°C. An E.Z.N.A. Plasmid DNA Mini Kit (Omega Biotek) was used to miniprep positive phenotypes, which were sequenced (Plasmidsaurus) for validation. Valid colonies were plated on LB and 50 µg/mL kanamycin antibiotic and grown

overnight in darkness and blue light (~465 nm wavelength) at 37°C to observe the positive light-sensitive phenotype.

C. Experimental Design

Positive light-sensitive phenotypes in LB with 50 µg/mL kanamycin were grown overnight, pelleted, and then resuspended with M9 media. These cells were diluted to an OD600 of 0.05 in the M9 media as measured with a spectrophotometer (Eppendorf BioSpectrometer basic). 1 mL of culture was added to each well on a 24-well plate, save for one blank well with only M9. The 24-well plate was placed in our fabricated optogenetic stimulation system. The light array was programmed with the following conditions (duty cycle [%]/period [s]): 0%/100 (negative/dark control), 100%/100 (positive/light control), 10%/100, 1%/100, 10%/1, and 10%/200. The assembly was shaken and incubated for 10 hours at 37°C. Final OD600 measurements were taken (TECAN plate reader).

V. References

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VI. Supplementary Information

A. Data Availability

All CAD ".stl" files for 3-D printing, all SnapGene ".dna" files for the optoTA construct and Gibson assembly, and the Arduino ".ino" file to run the stimulation system are available on our GitHub repository (github.com/robertheeter/optogenetic-box). This repository also contains supplementary images, data, figures, renderings, and datasheets from this project, a selection of which are shown below in **Figures S1, S2, S3**.

B. Box Renderings

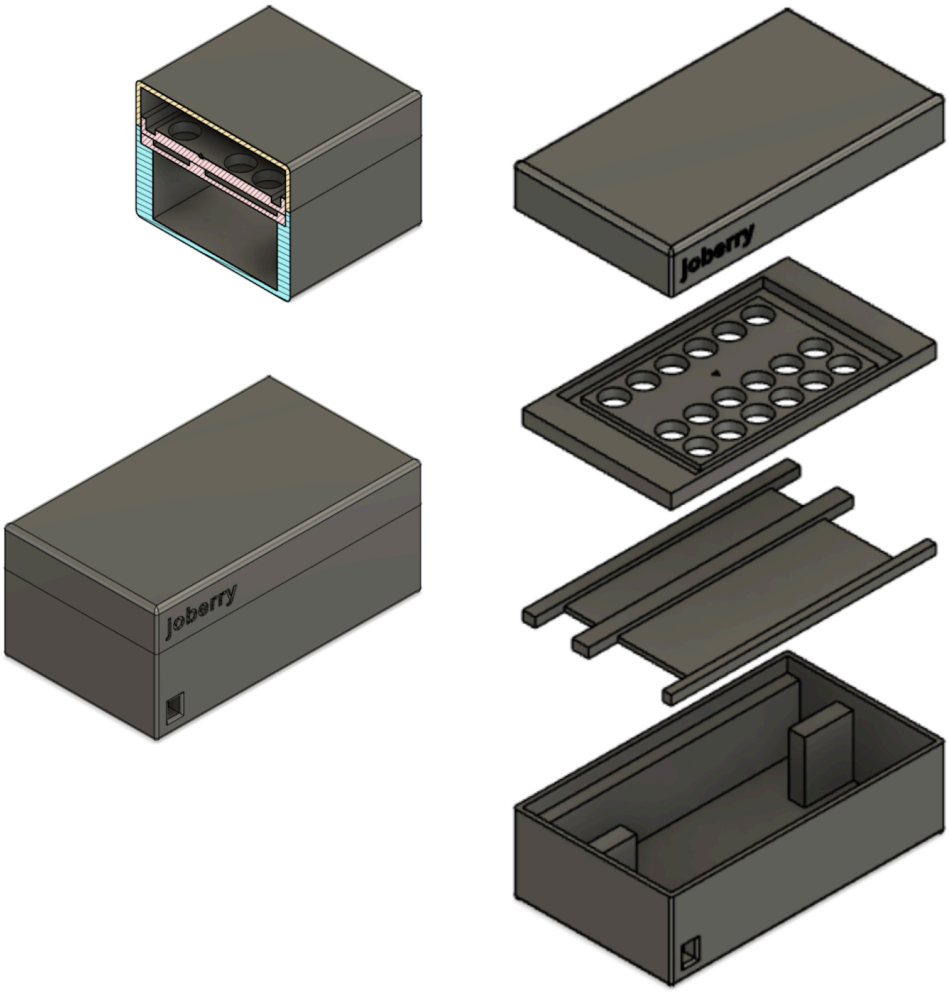

Figure S1. Renderings of the optogenetic stimulation box housing made in Fusion 360 showing an assembled cross-section view (top left), an assembled view (bottom left), and a disassembled stacked view (right) showing the lid, well plate frame, light panel frame, and electronics housing from top to bottom.

C. Box Images

Figure S2. Images of the complete optogenetic stimulation box from an assembled top view (left) and a disassembled top view (right) showing the interior electronics.

D. OptoTA Plasmid Map

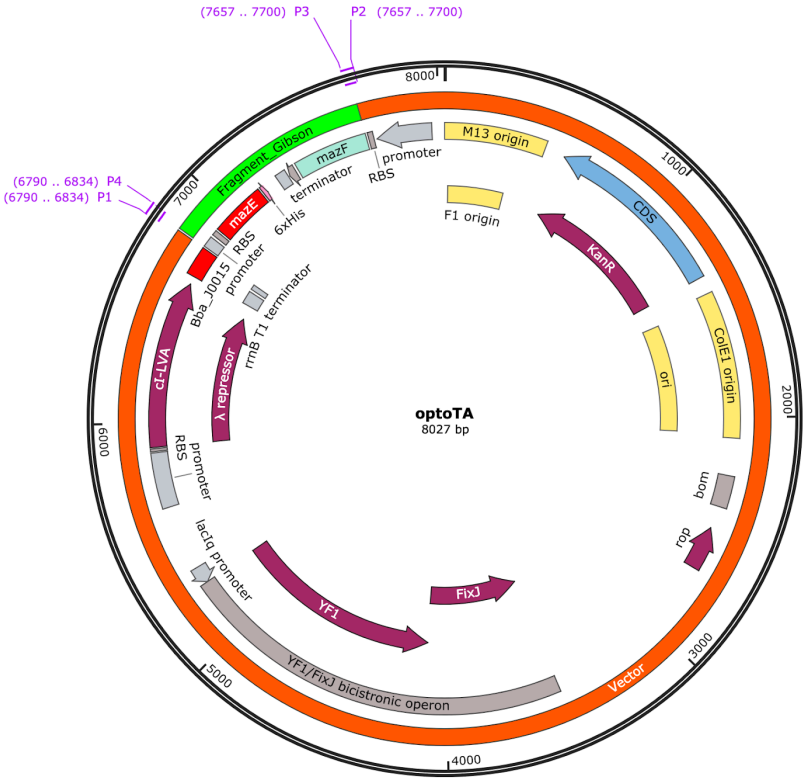

Figure S3. The complete optoTA plasmid map from SnapGene created from Gibson assembly. Note the mazE/mazF genes in the fragment region used for the optogenetic control mechanism.