Red light responsive Cre recombinase for bacterial optogenetics

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Abstract
Optogenetic tools have been used in a wide range of microbial engineering applications that benefit from the tunable, spatiotemporal control that light affords. However, the majority of current optogenetic constructs for bacteria respond to blue light, limiting the potential for multichromatic control. In addition, other wavelengths offer potential benefits over blue light, including improved penetration of dense cultures and reduced potential for toxicity. In this study, we introduce OptoCre-REDMAP, a red light inducible Cre recombinase system in *Escherichia coli*. This system harnesses the plant photoreceptors PhyA and FHY1 and a split version of Cre recombinase to achieve precise control over gene expression and DNA excision in bacteria. We optimized the design by modifying the start codon of Cre and characterized the impact of different levels of induction to find conditions that produced minimal basal expression in the dark and full activation within four hours of red light exposure. We characterized the system's sensitivity to ambient light, red light intensity, and exposure time, finding OptoCre-REDMAP to be reliable and flexible across a range of conditions. The system exhibits robust light-sensitive behavior, responding to red light while remaining inactive under blue light, making it suitable for future applications in synthetic biology that require multichromatic control.

Keywords
Bacterial optogenetics; red light; Cre recombinase; multichromatic control

Introduction
Optogenetic approaches can be used to manipulate cellular processes for light-mediated control, offering tunable and precise modulation of gene expression[1–9]. Unlike traditional chemical inducers, light is straightforward to program, it can be added and removed rapidly, and it can be spatially patterned, providing the potential for spatiotemporal control and dynamic perturbations without the need for media alterations or system disruptions. Light is also orthogonal to many endogenous cellular processes, allowing optogenetic tools to control cellular functions with minimal cross-talk or off-target effects[10]. Moreover, as light is easily programmable, optogenetic tools can also interface with dynamic computer-based control and feedback[11–14]. These benefits have led to the use of cellular optogenetic tools in a wide range of applications, from basic research elucidating fundamental biological mechanisms[15,16] to advanced biotechnological and therapeutic interventions[17,18].

Recent efforts in microbial optogenetics have capitalized on the precise and programmable nature of light. For example, light-sensitive proteins have been used to modulate enzyme expression for biofuel production[19,20], to coordinate bacterial growth via metabolic regulation[21,22], and to inhibit the formation of biofilms[23]. In addition, optogenetic techniques have enabled light-induced drug release from hydrogels[24], precise positioning of *E. coli* on substrates[25], and the production of biomaterials[26–28].

Optogenetic systems exist that respond to wavelengths ranging from near-infrared to ultraviolet light[29–32]. The majority of existing bacterial optogenetic systems are blue light responsive, and one advantage of this is that the chromophores required for their light-mediated response are flavin nucleotides, which are

endogenous to all bacteria[10]. However, blue light is more prone to phototoxicity than other wavelengths, introducing the potential for cellular damage[33]. It is also more susceptible to scattering and absorption by biological molecules, leading to less precise spatial targeting of specific cell populations[33]. We chose to focus here on developing a red light responsive system. Red light has superior penetration capabilities compared to blue light, allowing for deeper stimulation within biological tissues[34] or within bioreactors. Moreover, having both blue and red light inducible systems could allow the design of circuits with orthogonal light inputs for multichromatic control.

In plants, the capacity to perceive and respond to red/far-red light relies on proteins from the phytochrome photoreceptor family[35]. For example, PhyA is a photoreceptor from *Arabidopsis thaliana*[35] that binds covalently to the chromophore phycocyanobilin (PCB)[36], resulting in a photochemically functional photoreceptor that is responsive to red light. In the native plant context, after receiving red light PhyA is translocated into the nucleus through interactions with shuttle proteins, including FHY1[37]. Upon red light illumination (λ_{max} = 660 nm), PhyA and FHY1 associate, whereas under far-red light (λ_{max} = 730 nm), they dissociate. Taking advantage of this red light inducible protein interaction, Zhou et al.[38] developed and optimized a compact and sensitive red light mediated photo switch, denoted 'REDMAP', for control of transcription in mammalian cells. Using this design, they fused PhyA to the yeast Gal4 DNA-binding domain and FHY1 to a tetrameric repeat of the minimal herpes simplex virus-derived transcription activator (VP16) to form a light-responsive transcription factor. They demonstrated that after red light induction, PhyA and FHY1 associate, resulting in the formation of a functional transcription factor complex (Gal4-VP16). They tested different REDMAP system compositions and observed that a truncated version of PhyA comprised of amino acids 1–617 (which they denote 'ΔPhyA'), resulted in a 13-fold increase in red light induced gene expression via Gal4–VP16[38].

In this study, we sought to use light to control recombinase activity. Recombinases are enzymes capable of targeting specific 30−50 base pair sequences within DNA and modifying the designated DNA flanked by these recognition sites. For example, Cre is a widely used tyrosine recombinase that is derived from the P1 bacteriophage. Cre can recognize DNA flanked by loxP sites and, depending on the orientation of the loxP sites, will excise, invert, or translocate the DNA sequence[39]. The proficiency of recombinases in DNA manipulation has been widely recognized for synthetic biology applications, where they have been used to construct complex cellular logic circuits[40] and engineer gene circuits with memory[41,42]. Researchers have also recognized the potential of combining optogenetic control with recombinase function. For example, light-responsive recombinases have been used in mammalian systems[40,43–46] and yeast[47]. Optogenetic recombinases have also been developed for bacteria[3,48,49], where they have been used to regulate the expression of antibiotic resistance genes[48,50] and to directly capture spatial information and input signals via light using DNA as a means to store digital information[51]. Inducible recombinase designs typically employ a split protein strategy, where the recombinase is rendered light-sensitive by splitting the gene into N-terminal and C-terminal fragments, each linked to a light-responsive photodimer or photoreceptor sequence[2,3,40]. Upon light stimulation, conformational changes in the photodimer or association of photoreceptor proteins promote the reassembly of the two recombinase fragments to form a functional enzyme.

In this work, we developed and optimized a red light inducible Cre recombinase in *E. coli*. We began our efforts with OptoCre-VVD, a blue light inducible Cre variant that we developed in a previous project[3]. By integrating red light responsive FHY1 and ΔPhyA photoreceptors[46] into our design to replace the blue light responsive VVD photo dimers, we engineered a novel red light responsive Cre. Here, we introduce this optimized design, which we call 'OptoCre-REDMAP'. First, we investigated the robustness of the system in response to different split Cre induction levels. Our initial design exhibited undesirable basal activity in the dark, which we reduced by optimizing the Cre start codon. The new construct showed robust activation with negligible basal expression. Then, we characterized sensitivity of the OptoCre-REDMAP construct to ambient light exposure, variations in red light intensity, and light exposure time. We also measured the

activation levels of the system in response to blue light to show its potential for applications in multichromatic genetic regulatory networks.

Results

To render Cre recombinase red light activatable, we started with the blue light inducible OptoCre-VVD[3], which is comprised of N-terminal and C-terminal fragments of Cre fused with VVD photo dimers. Blue light induces a conformational change in VVD to allow dimerization, bringing the Cre fragments together[3]. We constructed OptoCre-REDMAP by replacing the VVD photo dimers with the FHY1 and ΔPhyA photoreceptors, attaching FHY1 to the N-terminal fragment of the Cre sequence, and ΔPhyA to the C-terminal fragment (Fig. 1). We placed the two components of OptoCre-REDMAP together on an operon, with a ribosome binding site separating nCre-FHY1 and cCre-ΔPhyA. We employed an IPTG-inducible P_{lacUV5} promoter to regulate the expression of split Cre to prevent premature target DNA excision prior to IPTG induction (Fig. 1). Additionally, ΔPhyA requires the PCB chromophore for photochemical activity, thus we included the *ho1-pcyA* gene that produces PCB in the circuit design (Methods).

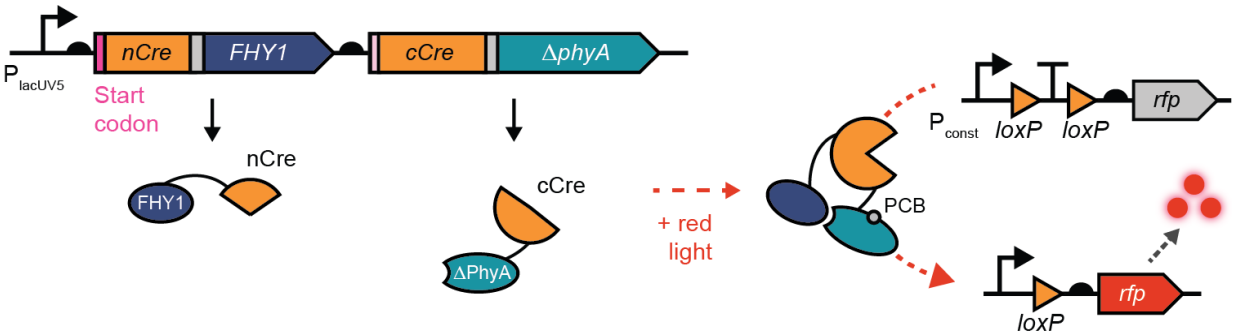

Figure 1. Red light induced recombination in *E. coli*. Split Cre fragments are linked to FHY1 and ΔPhyA photoreceptors and expressed under the control of an IPTG-inducible promoter (P_{lacUV5}). ΔPhyA must be covalently bound to the chromophore phycocyanobilin (PCB) to be photochemically active. When exposed to red light, FHY1 and ΔPhyA associate, forming a functional Cre protein. Cre can then act on the reporter plasmid, excising the loxP-flanked transcriptional terminator and allowing expression of RFP under the control of a constitutive promoter (P_{const}).

Cre recognizes and excises DNA flanked by loxP sites that are oriented in the same direction. To test the function of OptoCre-REDMAP, we used a reporter where a transcriptional terminator flanked by loxP sites is placed between a constitutive promoter and the gene encoding red fluorescent protein (RFP). In the absence of functional Cre, the terminator effectively halts the transcription process and prevents the expression of RFP. With functional Cre, the terminator is excised, allowing transcription and subsequent production of RFP (Fig. 1).

To test the performance of OptoCre-REDMAP, we exposed cultures to red light for 4 hours. We kept another set of samples in the dark during the same period (Fig. 2a). For comparison, we also included a negative control strain that only contained the reporter plasmid and positive control strain consisting of the reporter plasmid with the transcriptional terminator excised, allowing full expression of RFP. We found that OptoCre-REDMAP was functional, with clear differences in fluorescence between the dark and red light conditions (Fig. 2a). We conducted experiments using a range of IPTG concentrations, finding that full activation was achieved for IPTG concentrations at or above 50 μM (Fig. 2a). However, our construct exhibited significantly higher basal expression than the negative control for most levels of IPTG induction, indicative of leaky OFF-state behavior in the dark. First, we attempted to decrease the leaky activity of the system by reducing the duration of light induction to 2 hours. This led to a modest decrease in the leaky activity, in addition to a slight decrease in the red light induced levels of RFP (Fig. 2b). However, we ultimately deemed this approach insufficient given the residual leaky activity in the dark conditions. We also tried to further reduce the red light exposure time, but with shorter red light induction the system did

not reach full activation (Fig. S1a). Next, we tried to decrease the red light intensity rather than its duration. However, we found that the red light intensity did not significantly impact the behavior of the system and thus was not a viable strategy for decreasing the basal expression (Fig. S1b).

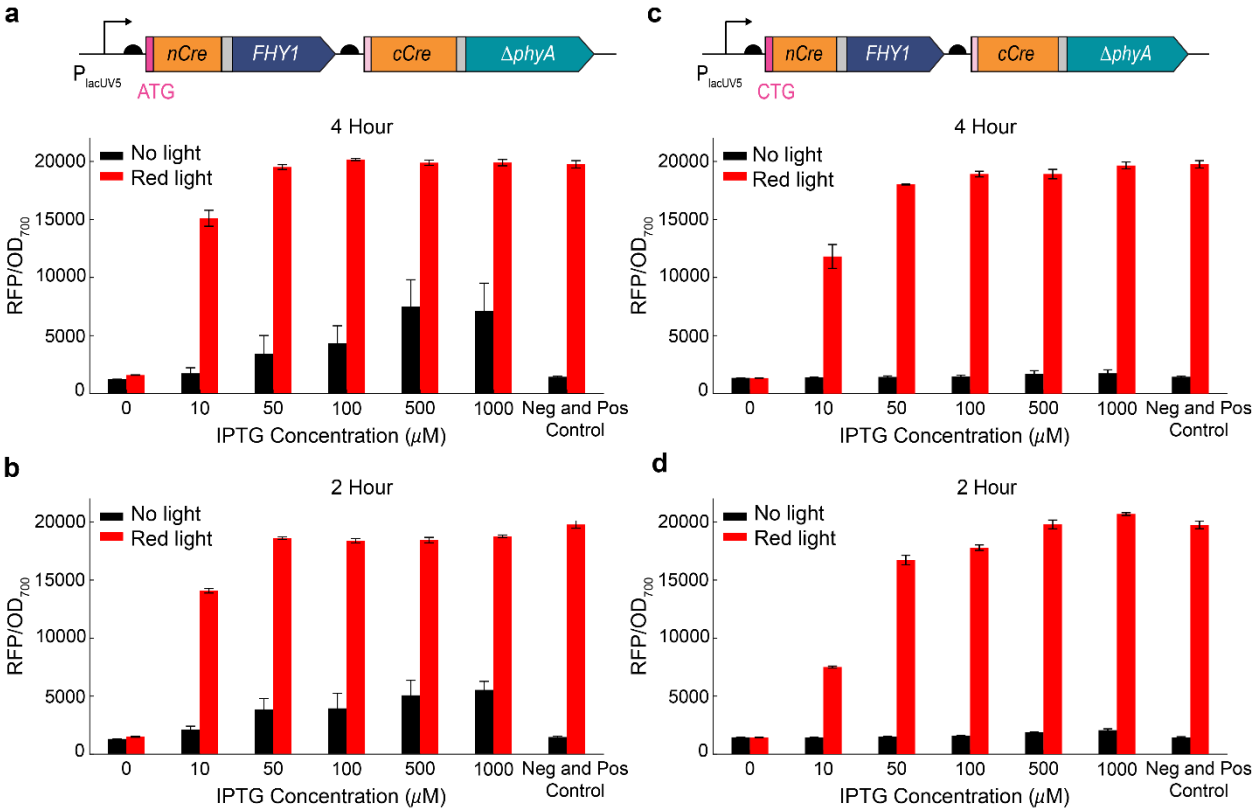

Figure 2. Comparison of two different designs for OptoCre-REDMAP. (a) RFP expression levels of OptoCre-REDMAP (ATG version) at different IPTG concentrations with 4 hours and (b) 2 hours of red light exposure. (c) RFP expression levels of OptoCre-REDMAP (CTG version) at different IPTG concentrations with 4 hours and (d) 2 hours of red light exposure. In all cases, error bars show standard deviation around the mean (n=3 biological replicates).

Because straightforward changes based on IPTG or red light induction were insufficient to reduce basal expression while maintaining light induced activity of OptoCre-REDMAP, we next considered whether aspects of our construct design could be changed to improve performance. We specifically focused on reducing basal expression of OptoCre-REDMAP. To accomplish this, we changed the start codon of nCre-FHY1 from ATG to CTG. Hecht et al.[52] measured the translation initiation rate corresponding to 64 different start codons in *E. coli* and observed that CTG exhibits 100 times lower expression than ATG. Therefore, we hypothesized that changing the start codon from ATG to CTG would help reduce RFP expression in the dark. By switching the start codon, the total number of split Cre fragments in the cell will decrease, ideally resulting in conditions where the number of split Cre proteins capable of association will be insufficient to activate in the dark, yet adequate for achieving full activation when fragments combine in the presence of red light. We constructed the new variant and quantified RFP expression at different IPTG concentrations. The new design exhibited substantially reduced basal expression relative to the ATG version, while maintaining high RFP levels when exposed to red light (Fig. 2c). The basal expression was minimal for all IPTG concentrations we tested, remaining at levels comparable to the negative control. We found that full activation by OptoCre-REDMAP can be achieved with IPTG concentrations at or above 50 μM with 4 hours of red light illumination. However, if a shorter red light illumination period, i.e. 2 hours, is desirable, increasing the IPTG concentration to 500 μM will result in full activation (Fig. 2d). Given the minimal

basal expression and robust activation under red light, we proceeded with this optimized construct for subsequent experiments.

We tested the impact of red light intensity and exposure time on the performance of the CTG variant of OptoCre-REDMAP. First, we confirmed that light induction did not impact growth. When conducting experiments to optimize the behavior of OptoCre-REDMAP in Fig. 2, we used red light with full intensity (155 μW/cm^2) to activate the system. By analyzing both the optical density values (Fig. 3a) and microscopy images of individual cells (Fig. S2) at 155 μW/cm^2, we observed no notable disparities in growth rates or cell morphology between experimental conditions with and without light exposure, indicative of minimal phototoxicity effects with this exposure level. Next, we assessed whether results were sensitive to light induction levels by testing a range of intensities, spanning 15.5 μW/cm^2, a very dim lighting condition, to 155 μW/cm^2, bright lighting (Fig. 3b-c). We found that the light intensity did not significantly affect the behavior of our system with 4 hours of light exposure (Fig. 3b). With 2 hours of light, we observed modest decreases in fluorescence levels for the low light intensity values (Fig. 3c), but overall, the system responds robustly to many levels of red light.

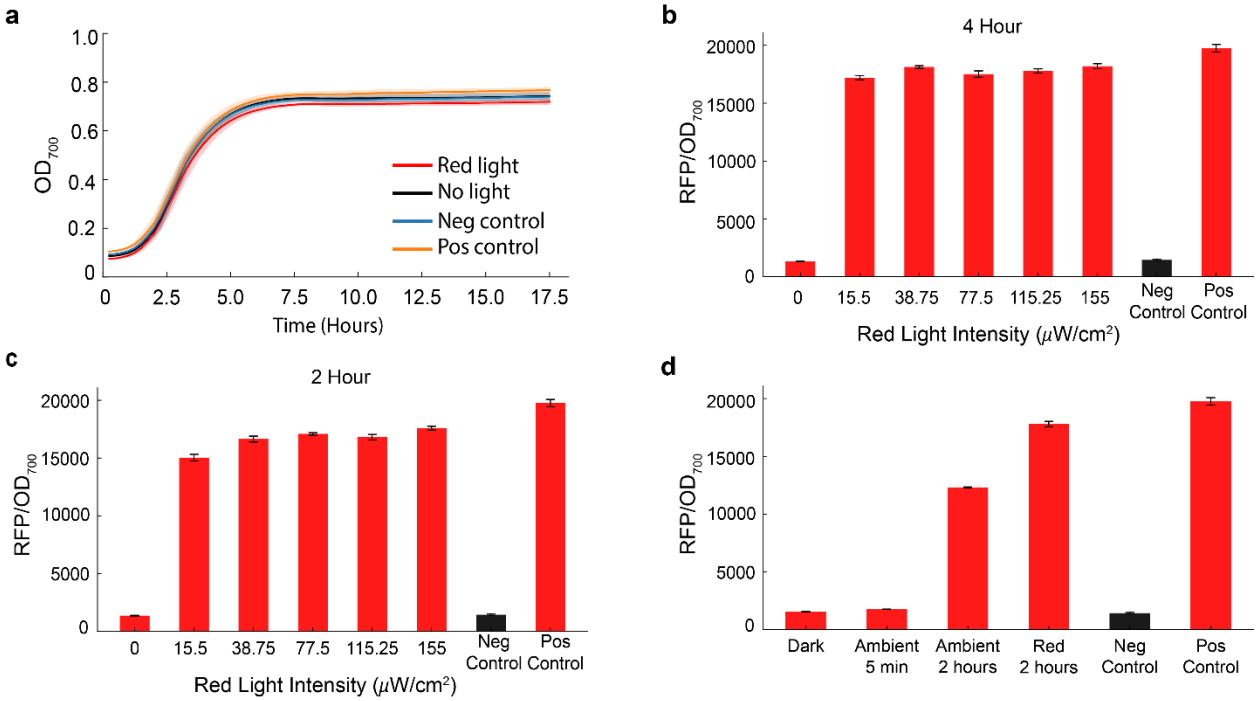

Figure 3. Characterizing the behavior of OptoCre-REDMAP. (a) Optical density at 700 nm, OD700, values for cultures subjected to 4 hours of red light compared to the dark state, negative, and positive control. Shaded error bars represent standard deviation around the mean from plate reader data (n=3 wells). (b) RFP output in response to red light intensities ranging from 15.5 to 155 μW/cm^2 with 4 hours or (c) 2 hours of light exposure. (d) RFP expression of OptoCre-REDMAP in response to 5 minutes and 2 hours of exposure to ambient light compared to the dark state, 2 hours of red light exposure, and the negative and positive control. Error bars show standard deviation around the mean (n=3 biological replicates).

Next, we conducted tests to characterize the sensitivity of OptoCre-REDMAP to ambient light exposure. When working with light-responsive systems, it is important to assess their sensitivity to surrounding light conditions since short-term exposure to ambient light is inevitable in practical experimental conditions where plates are temporarily removed from the dark, as might occur during the transfer of cultures for flow cytometry or microscopy assays. In addition, it would be helpful to know whether keeping the cultures in the dark is necessary for the robustness of the results. Thus, we assessed the performance of OptoCre-REDMAP under a brief, 5-minute exposure to ambient light, and a long, 2-hour exposure to ambient light

(Fig. 3d). We found that OptoCre-REDMAP is not very sensitive to short periods of ambient light, suggesting its reliability in conditions with incidental exposure (Fig. 3d). However, the 2-hour ambient light exposure produced substantial activation of the system, indicating that keeping the cultures in the dark for most portions of the experiment is necessary for reproducibility and robustness of the results (Fig. 3d).

In addition to assessing the ON and OFF states of our system after red light stimulation, we investigated the dynamics of activation in light-inducible cells relative to both negative and positive controls. To achieve this, we conducted an experiment where we diluted the cultures 1:100 and transferred them to a plate reader immediately following light stimulation, as opposed to allowing them grow in the dark within the incubator overnight before measuring their fluorescence. We found that RFP expression in the light-activated cells was comparable to the positive control, indicating that RFP was fully induced at the conclusion of the 4 hour induction period (Fig. 4).

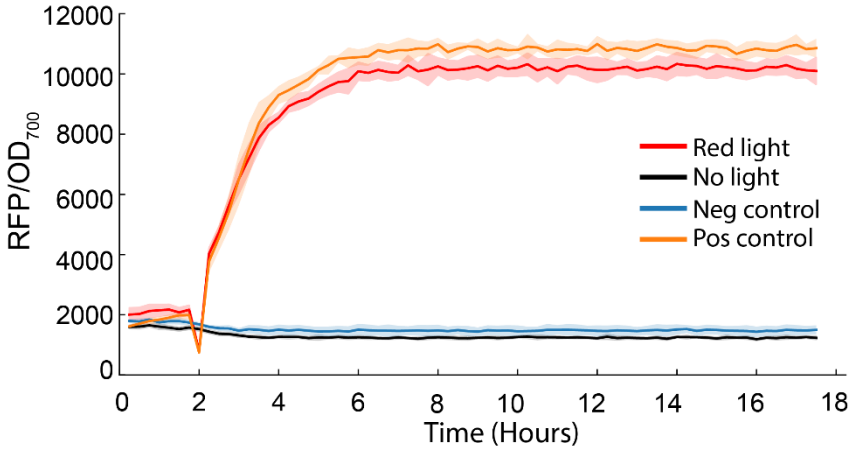

Figure 4. Real-time RFP expression of OptoCre-REDMAP in the dark or after being exposed to red light for 4 hours (t=-4 until t=0 hours), compared to the negative and positive control. Shaded error bars represent standard deviation around the mean from plate reader data (n=3 wells).

Next, we sought to examine the impact of varying durations of red light exposure on cells by characterizing DNA excision and quantifying RFP expression levels in both bulk culture and at the single cell level. To accomplish this, we conducted an experiment wherein we altered the duration of red light illumination, ranging from 5 minutes to 8 hours. In addition to reading the fluorescence levels using a plate reader, we conducted PCR and gel electrophoresis on cultures after light illumination to assess transcriptional terminator excision at the bulk culture level. Consistent with fluorescence data, we observed that red light exposure durations of at least 2 hours resulted in the excision of the transcriptional terminator, enabling the expression of RFP (Fig. 5a). Additionally, our bulk culture data affirmed that within 2 hours of light stimulation, cells exhibited RFP expression levels comparable to those of the positive control (Fig. 5b). We were also interested in investigating the transferability of bulk culture trends to individual cells. Therefore, we conducted readings using single cell microscopy. By observing cells individually on agar pads, we noted consistent trends in activation and RFP expression between single cells and bulk culture studies, where the majority of cells exhibited activation in cultures subjected to red light stimulation for 2 hours or more (Fig. 5c-d). By observing the variation in RFP expression at the single cell scale, we found that while there are cells with lower or higher expression levels in all cultures, the majority of the cells expressed RFP (Fig. S3).

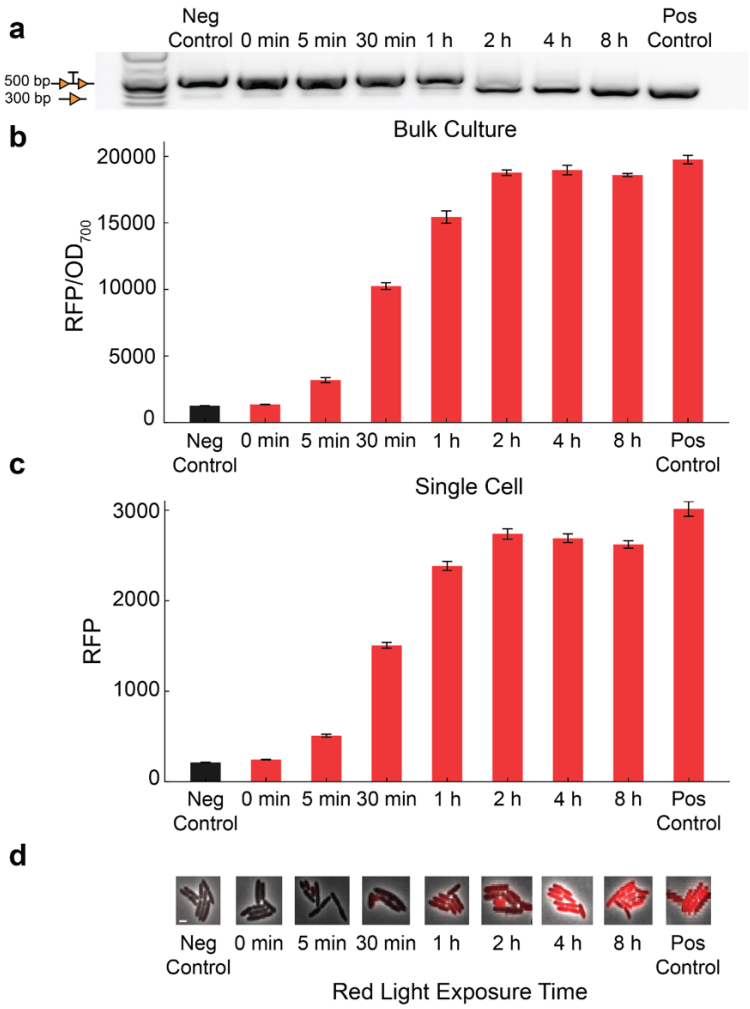

Figure 5. Light exposure duration necessary to induce excision by OptoCre-REDMAP and comparison of bulk culture and single cell results. (a) DNA gel image showing reporter bands with and without transcription terminator excision. (b) Bulk culture fluorescence measurement of RFP after red light exposure with different durations. For illumination durations shorter than 8 hours, the cultures were kept in the dark for the remainder of the 8 hours. The error bars show standard deviation around mean value (n=3 biological replicates). (c) Single cell fluorescence microscopy averages of RFP values. Error bars show standard error around the mean value (n ≈ 800 single cells) (d) representative microscopy images (scale bar = 2 μm).

Finally, to support future applications employing multichromatic control, we characterized the performance of OptoCre-REDMAP and OptoCre-VVD to red and blue light inputs. To achieve this, we subjected each system to red or blue light stimulation for 4 hours across a range of IPTG concentrations. Our aim was to determine the optimal experimental conditions conducive to activating both constructs under their respective light stimuli. Both constructs remained consistently inactive across all IPTG concentrations when subjected to opposite light stimulation, with OptoCre-REDMAP responding to red light and not blue and OptoCre-VVD to blue but not red (Fig. 6a-b). These results support the feasibility of employing these systems in tandem in subsequent genetic circuit designs, allowing for robust multichromatic control.

We were also interested in determining the shortest light stimulation pulse required to activate both systems. This is particularly useful in setups where prolonged constant light stimulation is not feasible, and the systems can only be stimulated in a pulse-like manner. For example, with time-lapse microscopy multiple fields of view may need to be illuminated sequentially and optogenetic stimulation must also be OFF during intervals when fluorescent protein levels are measured. To investigate the light stimulation requirements, we exposed the OptoCre-REDMAP and OptoCre-VVD systems to pulses of red or blue light of varying

durations (e.g., 0-120s) delivered every 5 minutes over a total period of 18 hours. We found that a 6-second pulse of red light every 5 minutes was sufficient to activate OptoCre-REDMAP, while OptoCre-VVD required a 60-second pulse of blue light for reliable activation (Fig. 6c). These findings help refine the parameters for optimal use of the systems in applications where light stimulation must be limited.

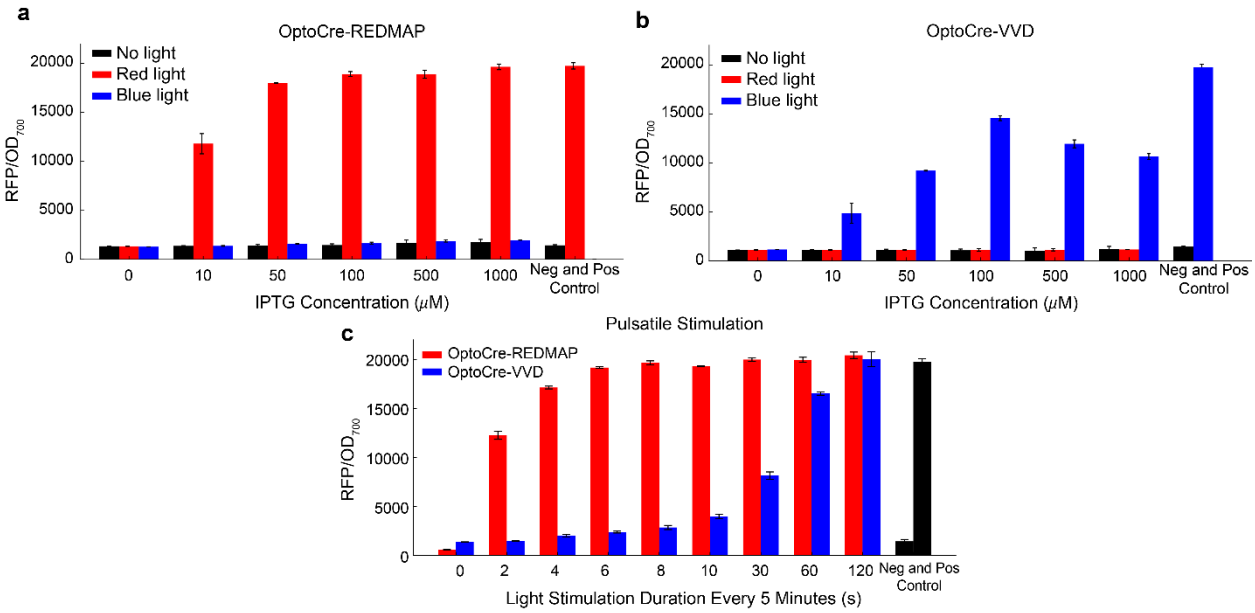

Figure 6. RFP expression with OptoCre-REDMAP and OptoCre-VVD. (a) RFP levels of OptoCre-REDMAP subjected to 4 hours of constant dark, red light, or blue light. (b) RFP levels of OptoCre-VVD subjected to 4 hours of constant dark, red light, or blue light. (c) RFP levels of OptoCre-REDMAP and OptoCre-VVD subjected to pulses of red or blue light with varying durations. To make the RFP levels comparable to each other, we used the same reporter plasmid construct for both OptoCre-REDMAP and OptoCre-VVD. Thus, in all cases, the negative control is a strain with only the reporter plasmid with the terminator unexcised and the positive control is a strain with only the reporter plasmid wherein the terminator is fully excised. In all cases, the error bars show standard deviation about the mean (n=3 biological replicates).

Discussion

In this study, we developed, optimized, and characterized a red light activatable Cre recombinase for *E. coli*. Leveraging the FHY1-ΔPhyA light mediated protein interaction[38], we engineered Cre recombinase to respond to red light stimuli. After testing the resulting construct, OptoCre-REDMAP, at various IPTG concentrations we observed that while photochemically active, the construct showed high basal expression, limiting its robustness. Following attempts to mitigate basal expression using straightforward methods such as adjusting exposure time and light intensity, we ultimately modified the genetic circuit design by changing the start codon of nCre-FHY1 from ATG to CTG. This modification led to a substantial reduction in basal expression while preserving red light induced activity. Importantly, our new construct demonstrated robust and reproducible performance across varying light durations, IPTG concentrations, and red light intensities. Notably, red light exposures of 2 to 4 hours with IPTG induction were sufficient to achieve full activation. Finally, we demonstrated the potential for utilizing the OptoCre-REDMAP and OptoCre-VVD constructs in tandem to multiplex gene regulatory networks using red and blue as orthogonal light inputs. The ability to selectively activate OptoCre-REDMAP with red light while remaining unresponsive to blue light suggests potential for the development of multichromatic control in genetic circuits. This could pave the way for more complex and dynamic regulatory networks in synthetic biology.

While OptoCre-REDMAP shows great promise, there are areas for further research and improvement. For instance, exploring different recombinase variants, such as Flp may enhance the system's performance and expand its applications[40]. In addition, it would be interesting to test how light wavelengths for other bacterial

optogenetic designs that do not use blue light as an input, such as CcaS-CcaR[31,53] and UirS−UirR[29], impact OptoCre-REDMAP. Further, we have demonstrated that activation kinetics differ between OptoCre-REDMAP and OptoCre-VVD and engineering variants that change the response times could help to alter the sensitivity of the new designs.

In addition to multiplexing, we anticipate that OptoCre-REDMAP will offer benefits for use cases that require deeper light penetration than what can be achieved with blue light, or applications that are very sensitive to phototoxicity. These characteristics makes the system particularly suitable for applications involving dense cultures or complex biological environments. Overall, OptoCre-REDMAP offers a robust response across a range of red light intensities and exposure times while remaining insensitive to blue light, making it a promising candidate for future optogenetic studies in bacteria.

Methods

Strains and Plasmids

In this study, we used *E. coli* strain MG1655. All recombinase constructs use a plasmid with a low copy pSC101 origin of replication and an ampicillin resistance gene. The recombinase genes are under the control of the IPTG-inducible P_{lacUV5} promoter. The construct was derived from the pBbS5a BglBrick plasmid[54]. All reporter plasmids use a medium copy p15A origin of replication with a kanamycin resistance gene and the gene for red fluorescent protein (mCherry) under the control of a constitutive, medium strength promoter (P_{W4}). The P_{W4} promoter is modified from the phage T7 A1 promoter (ATCGTTTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGA GCGGATAACAAT)[55]. The PCB encoding gene, *ho1-pcy*, is on the reporter plasmid under the control of a constitutive promoter (J23108) from Anderson promoter library[56]. Plasmids were constructed using the Golden Gate[57] and Gibson assembly[58] methods. Primers are listed in Table S1. Original OptoCre-VVD plasmids are from Sheets et al.[3], where we use the OptoCre-VVD2 variant (Addgene #160400). The original FHY1 and ΔPhyA plasmids were constructed using the red light domains developed in Zhou et al.[38]. The *ho1-pcy* gene was derived from the pNO286-3 plasmid (Addgene #107746) that was deposited by Ong et al.[59]. We express Cre split with a photoreceptor pair as an operon (Fig. 1). The N-terminal fragment of Cre (nCre), AA(1-43), is followed by a 10 AA glycine-serine linker and FHY1 photoreceptor. A separate RBS is used to express ΔPhyA with a 10 AA glycine-serine linker to the C-terminal fragment of Cre (cCre).

Light Exposure Assays and Recombinase Efficiency Characterization

In all experiments working with liquid cultures, cultures were maintained at 37 °C with continuous shaking at 220 rpm. On day one, an overnight culture was inoculated from a single colony and was incubated in LB medium supplemented with 100 μg/mL carbenicillin and 30 μg/mL kanamycin for plasmid maintenance. All cultures were shielded from surrounding light, except during designated red, blue, or ambient light exposure periods. The following day, cultures were diluted 1:100 in selective LB medium and induced with 100 μM IPTG, unless otherwise stated, for 2 hours prior to exposure to red light. Light exposure was conducted using an OptoPlate-96 device[60] equipped with LEDs emitting light at a wavelength of 630 nm (red) or 465 nm (blue) with an intensity of 155 $\mu W/cm^2$ per well. Unless stated otherwise, cultures were subjected to red light exposure for a period of 4 hours. Following exposure, samples were transferred to selective LB medium devoid of IPTG for overnight growth. The following day, the fluorescence levels were measured using a BioTek Synergy H1m plate reader. For optical density (OD), absorbance was conducted at 700 nm to avoid overlap with the RFP spectra, and the fluorescence read was conducted with an excitation of 584 nm and emission of 610 nm.

In experiments testing the impact of light intensity, cultures were exposed to red light for a total of 4 hours. Ambient light exposure involved exposing samples to laboratory lighting in a shaking incubator for either

5 minutes or 2 hours after IPTG induction, followed by continued incubation in the dark for the remainder of the 4 hours.

In experiments involving blue light exposure, the same device and protocol were used. After 2 hours of IPTG induction, samples were exposed to blue light for a period of 4 hours. Then they were diluted 1:100 in selective LB media for overnight growth.

In experiments involving pulsatile red or blue light illumination, the OptoPlate-96 device was programmed to turn on red or blue LEDs for only 2-120 seconds every 5 minutes in an overnight experiment for a total period of 18 hours. The next day, all cultures were diluted 1:100 in selective LB medium and were incubated in the dark overnight. The following day, the fluorescence levels were measured using a BioTek Synergy H1m plate reader.

We confirmed target DNA excision genetically using PCR analysis and gel electrophoresis. We used a forward primer about 200 base pairs upstream of the first loxP site and a reverse primer about 100 base pairs downstream of the second loxP site to check for differences in band length before and after recombination. The primers are listed in Table S1.

Microscopy and Image Analysis

We conducted single cell microscopy using a 100x objective and a Nikon Ti2-E microscope to analyze the bacteria containing OptoCre-REDMAP. After light stimulation, samples were diluted 1:100 for overnight growth in selective LB medium to allow full RFP expression. The next day, samples were diluted 1:100 2 hours prior to imaging in minimal media consisting of M9 salts supplemented with 2 mM MgSO4, 0.2% glycerol, and 0.01% casamino acids. After 2 hours, samples were placed on 1.5% low melting agarose pads made with minimal medium. Images were segmented and analyzed using DeLTA 2.0[61].

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Author Contributions

FJ and MJD conceived and designed the experiments. FJ performed the experiments and analyzed the data. FJ and MJD wrote the manuscript.

Conflict of Interest

The authors declare no competing financial interest.

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