Multimodal Control of Bacterial Gene Expression by Red and Blue Light

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Abstract

By applying sensory photoreceptors, optogenetics realizes the light-dependent control of cellular events and state. Given reversibility, noninvasiveness, and exquisite spatiotemporal precision, optogenetic approaches enable innovative use cases in cell biology, synthetic biology, and biotechnology. In this chapter, we detail the implementation of the pREDusk, pREDawn, pCrepusculo, and pAurora optogenetic circuits for controlling bacterial gene expression by red and blue light, respectively. The protocols provided here guide the practical use and multiplexing of these circuits, thereby enabling graded protein production in bacteria at analytical and semi-preparative scales.

Key Words

ANTAR; bacteriophytochrome; gene expression; histidine kinase; light-oxygen-voltage; optogenetics; RNA binding; sensory photoreceptor; synthetic biology; two-component system

1. Introduction

Sensory photoreceptors mediate the sensation of light across manifold organisms ***(1, 2)***. In optogenetics, photoreceptors double as genetically encoded actuators and enable the light-dependent perturbation of cellular traits and processes in reversible, noninvasive, and spatiotemporally acute manner ***(3)***. Protein engineering has introduced a wealth of artificial photoreceptors that serve as custom-tailored optogenetic implements for the precision control of

ever new and more complex biological processes ***(4, 5)***. Although optogenetics originates in the neurosciences where it predominantly relies on rhodopsin photoreceptors acting as light-activated ion pumps and channels ***(6–9)***, the general concept and benefits of light-based cellular control extend to diverse organisms and applications in cell biology and biotechnology. Within bacteria, gene expression is a particularly frequent and versatile subject of optogenetics ***(10)***. Optogenetic gene regulation is mainly exerted at the level of transcription initiation, but other control points also exist. Light-dependent gene expression affords spatial and temporal definition, precise dosing, scope for automation, noninvasiveness, and reversibility. These benefits set the optogenetic approach apart from conventional chemical induction, e.g., via L-arabinose or isopropyl-β-thiogalactoside, and may aid in addressing challenges of irreversibility and poor tunability.

Depending on the underlying photoreceptor, optogenetic circuits for the regulation of bacterial gene expression are sensitive to distinct bands of the electromagnetic spectrum ranging from near-UV to near-infrared light ***(11)***. Sensory photoreceptors usually exhibit a bipartite architecture consisting of a chromophore-binding photosensor and an effector module. Optogenetic applications in bacteria often harness sensory photoreceptors of the light-oxygen-voltage (LOV) ***(12–17)*** and bacteriophytochrome (BphP) classes ***(18–20)*** that sense blue and red/far-red light, respectively. Cyanobacteriochromes (CBCR) ***(21)***, belonging to the phytochrome superfamily, as do the BphPs, also find frequent use in bacterial optogenetics ***(19)***, with individual CBCRs possessing different light sensitivities, for instance red and green in the case of CcaS from *Synechocystis* sp.

In this chapter, we describe the pREDusk and pREDawn systems ***(20)*** for down- and upregulating, respectively, bacterial gene expression under red light, and the pCrepusculo and pAurora setups that mediate similar effects but in response to blue light ***(17)***. All four systems are realized as single plasmids and allow the light-dependent expression of target genes, included on the same plasmid backbone (Fig. 1). The pREDusk and pREDawn plasmids (Fig. 1a-b) descend from the earlier, blue-light-responsive pDusk and pDawn systems ***(12, 22)*** which are widely used in

bacterial optogenetics ***(10)***. Sensitivity to red light in pREDusk and pREDawn is conferred by the chimeric sensor histidine kinase *Dr*F1 ***(20)*** which derives from the fusion of the photosensory core module (PCM) of the *Deinococcus radiodurans* BphP ***(23)*** and the effector moiety of the *Bradyrhizobium japonicum* sensor histidine kinase FixL ***(24)***. Immediately upstream of *Dr*F1, a heme oxygenase (HO), also from *D. radiodurans*, is included in the same operon to supply the chromophore biliverdin via oxidative cleavage of heme. *Dr*F1 and its cognate response regulator FixJ, placed downstream of *Dr*F1 also within the same operon, form a red-light-responsive two-component system ***(25, 26)***. In the case of pREDusk ***(20)***, *Dr*F1 phosphorylates FixJ in darkness, thus enabling its binding to the FixK2 promoter and ramping up gene expression (Fig. 1a). By contrast, red light strongly reduces the net kinase activity of *Dr*F1 and essentially shuts off target-gene expression. In pREDawn, the signaling output is inverted by a genetic circuit comprising the λ phage cI repressor and its associated target promoter pR ***(12, 27)***. Red light thus strongly upregulates target-gene expression in pREDawn rather than inhibiting it (Fig. 1b). Both the pREDusk and pREDawn plasmids possess a ColE1 origin of replication (ori), and separate versions of the vectors are available with ampicillin (Amp), kanamycin (Kan), and streptomycin (Strep) resistance markers ***(20)***.

pCrepusculo and pAurora differ from the above systems not only in their color sensitivity, i.e., blue instead of red and far-red, but also in that light-dependent regulation is achieved at the mRNA level ***(17)*** (Fig. 1c-d). The two systems leverage the RNA-binding LOV receptor PAL from *Nakamurella multipartita* ***(28)*** which upon photoactivation by blue light sequence-specifically interacts with small RNA hairpins with up to several hundredfold enhanced affinity ***(17)***. By interspersing the RNA hairpin with the ribosome-binding site of a target gene, light-induced binding by PAL can modulate the expression of this gene. This concept is realized in pCrepusculo (Fig. 1c) which affords downregulation of target genes under blue light. As described for pREDawn above, a λ-cI-based gene cassette inverts and amplifies the light-dependent signal output in pAurora (Fig. 1d).

Given that pCrepusculo and pAurora use a CDF ori and a Strep resistance marker, they can be readily combined inside the same bacterial cell with pREDusk and pREDawn (that is, with the Amp- and Kan-conferring plasmid versions). In this manner, the multiplexed control of two separate target genes is achieved ***(20)***.

We here provide protocols detailing the deployment of the pREDusk, pREDawn, pCrepusculo, and pAurora plasmids for light-regulated gene expression in *Escherichia coli*. The performance of each light-responsive genetic circuit can be assessed separately using fluorescent reporter genes and flow cytometry (see section 3.1). Due to different replication origins, light color sensitivities, and regulatory mechanisms, pREDusk and pREDawn lend themselves to combinations with pCrepusculo and pAurora which we describe in section 3.2. Multiplexed gene expression using combinations of these plasmids is scalable to the semi-preparative scale and supports the graded production of two individual proteins, as controlled by red and blue light (see section 3.3). Taken together, these protocols guide the implementation and, as desired, the multiplexing of the plasmids for light-regulated bacterial expression. Given the increasing relevance of pertinent approaches for biotechnological, biomedical, and material-science use cases ***(10)***, the pREDusk, pREDawn, pCrepusculo, and pAurora plasmids appear versatile and widely applicable.

2. Materials

2.1 Bacterial strains and cultivation

The cultivation of bacteria underlies all experiments described in sections 3.1-3.3 and requires these materials:

1. lysogeny broth (LB) liquid medium supplemented with 50 µg mL^{-1} kanamycin (Kan) and/or 100 µg mL^{-1} streptomycin (Strep)
2. LB agar plates supplemented with 50 µg mL^{-1} Kan and/or 100 µg mL^{-1} Strep

3. an *E. coli* strain of choice, e.g., the CmpX13 strain ***(29)*** (see note 1)
4. plasmids pREDusk-MCS (Addgene identifier 188970), pREDawn-MCS (188971), pCrepusculo-MCS (190156), and pAurora-MCS (190158) (MCS, multiple cloning site) (see note 2)
5. microtiter plate (MTP) shaker (e.g., PMS-1000i Grant-bio)
6. incubator (e.g., Lucky Reptile), protected from ambient light by covering the windows with tin foil or cardboard
7. incubator shaker (e.g., New Brunswick Innova 42R) with window covered for light protection
8. light power meter to measure and adjust light intensities (e.g., Newport model 842-PE equipped with a 918D-UV-OD3 silicon detector)

2.2 Implementation and Analysis of Individual Plasmid Systems

The following materials are required for the experiments in section 3.1:

2.2.1 pREDusk and pREDawn

1. plasmids pREDusk-*Ds*Red and pREDawn-*Ds*Red bearing the red-fluorescent reporter *Ds*Red Express2 ***(30)*** and a Kan resistance marker (see note 3)
2. empty-vector control pREDusk-MCS (see above)
3. phosphate-buffered saline pH 7.4 (Fisher Scientific)
4. 10 mM phosphate buffer pH 7.4
5. 50 mg mL^{-1} chloramphenicol in 100% ethanol
6. 10 mg mL^{-1} tetracycline in water
7. Arduino-controlled red LED spotlight (e.g., Mightex SLS-0310-C, 656 nm) with 3D-printed holders for illumination of Erlenmeyer flasks (Fig. 2a)
8. 500 mL baffled Erlenmeyer flasks
9. microcentrifuge tubes

10. flow cytometer (e.g., NovoCyte Quanteon 4025 or BioRad S3e Cell sorter)

2.2.2 pCrepusculo and pAurora

1. plasmids pCrepusculo-*Ds*Red and pAurora-*Ds*Red bearing the red-fluorescent reporter *Ds*Red Express2 ***(30)*** (see note 3)
2. empty-vector control pCDF-Duet (Novagen)
3. panel with blue-light-emitting diodes (e.g., Winger WEHBL01-D1M, 470 nm)
4. ProFlow sheath fluid (BioRad) containing 1 mM Na EDTA, 1.9 mM K phosphate, 3.8 mM KCl, 16.6 mM Na phosphate, 139 mM NaCl, pH 7
5. 96-deep-well clear MTPs (Axygen P-DW-11-C)
6. 96-well clear MTPs (ThermoFisher 269620)
7. gas-permeable sealing membrane (Corning BF-410400-S)
8. flow cytometer, e.g., NovoCyte Quanteon 4025 or S3e Cell sorter (BioRad)

2.3 Multiplexing of Systems

The following materials are required for the experiments in section 3.2:

1. plasmids pREDusk-YPet and pREDawn-YPet bearing the yellow-fluorescent YPet ***(31)*** reporter and a Kan resistance marker (see note 3)
2. pCrepusculo-*Ds*Red and pAurora-*Ds*Red bearing the red-fluorescent reporter *Ds*Red Express2 ***(30)*** and a Strep resistance marker (see note 3)
3. empty-vector controls pREDusk-MCS, pREDawn-MCS, pCrepusculo-MCS, and pAurora-MCS (see above)
4. panel with blue-light-emitting diodes (e.g., Winger WEHBL01-D1M, 470 nm)
5. panel with red-light-emitting diodes (e.g., Kingbright L53SRCJ4, 660 nm)

6. Arduino-controlled LED illumination setup detailed in ***(32, 33)*** (Fig. 2c-d)
7. 3D-printed housing for LED illumination device (templates available for download at http://www.moeglich.uni-bayreuth.de/en/software/index.html)
8. 96-well MTPs with black walls and clear bottom (µclear plates, Greiner 655906)
9. 96-well clear MTPs (ThermoFisher 269620)
10. 96-well black MTPs (ThermoFisher 237108)
11. multimode MTP reader (e.g., Tecan Infinite M200pro)

2.4 Multiplexed Optogenetic Control of Semi-preparative Protein Production

The following materials are required for the experiments in section 3.3:

1. plasmid pREDusk-YPet bearing the yellow-fluorescent YPet ***(31)*** reporter and a Kan resistance marker (see note 3)
2. pAurora-*Ds*Red bearing the red-fluorescent reporter *Ds*Red Express2 ***(30)*** and a Strep resistance marker (see note 3)
3. empty-vector controls pREDusk-MCS and pAurora-MCS (see above)
4. 35 mg mL^{-1} chloramphenicol in 100% ethanol
5. 15 mg mL^{-1} tetracycline in 70% ethanol
6. 96-well clear MTPs (ThermoFisher 269620)
7. 96-well black MTPs (ThermoFisher 237108)
8. panel with blue-light-emitting diodes (e.g., Winger WEHBL01-D1M, 470 nm)
9. panel with red-light-emitting diodes (e.g., Kingbright L53SRCJ4, 660 nm)
10. 250 mL baffled Erlenmeyer flasks
11. PCR tubes
12. multimode MTP reader (e.g., Tecan Infinite M200pro)

3. Methods

3.1 Implementation and Analysis of Individual Plasmid Systems

Section 3.1.1 describes the use of the pREDusk and pREDawn plasmids for red-light-controlled bacterial gene expression. Likewise, section 3.1.2 covers the blue-light-responsive pCrepusculo and pAurora setups.

3.1.1 pREDusk and pREDawn

1. Streak bacteria carrying the plasmids pREDusk-*Ds*Red, pREDawn-*Ds*Red, or pREDusk-MCS on LB/Kan agar plates.
2. Incubate the plates overnight (~ 16 h) at 37°C under non-inducing conditions, i.e., red light (660 nm, 100 µW cm^{-2}) or darkness for pREDusk and pREDawn, respectively.
3. Prepare five 100 mL LB/Kan cultures in 500-mL Erlenmeyer flasks and inoculate them with single colonies from the agar plates (2× pREDusk-*Ds*Red, 2× pREDawn-*Ds*Red, and 1× pREDusk-MCS).
4. Incubate the cultures at 37°C and 220 rpm agitation under non-inducing conditions, i.e., red light (660 nm, 100 µW cm^{-2}) or darkness for pREDusk and pREDawn, respectively.
5. At an optical density of the cultures at 600 nm (OD_{600}) of 0.5, transfer one flask each of pREDusk-*Ds*Red and pREDawn-*Ds*Red to inducing conditions, i.e., darkness or red light (660 nm, 100 µW cm^{-2}) for pREDusk and pREDawn, respectively (Fig. 2a). Keep the other flasks under non-inducing conditions, and the pREDusk-MCS in darkness (see note 4).
6. Continue with the incubation at 37°C and 220 rpm agitation for 20 h.
7. Following incubation, pipet 200 µL of the cultures to microcentrifuge tubes and supplement them with antibiotics by adding 14 µL chloramphenicol solution and 8 µL tetracycline solution (see note 5).

8. Keep the samples on ice in darkness for 2 h to allow *Ds*Red maturation.
9. Pellet the bacteria by centrifugation at 1,845 × *g* for 2.5 min. Resuspend the pellet in 500 µL PBS and store on ice.
10. For analysis by flow cytometry, dilute the samples 10-fold in PBS.
11. Record single-cell fluorescence of approximately 100,000 cells on a flow cytometer using a 561-nm excitation laser and a (586 ± 20)-nm bandpass emission filter. (see note 6)
12. Fit the frequency distribution of the logarithm of the single-cell fluorescence to a skewed Gaussian probability density function (Fig. 3a-b), e.g., using the Fit-o-mat software ***(34)***.

3.1.2 pAurora and pCrepusculo

1. Streak bacteria harboring the plasmids pCrepusculo-*Ds*Red, pAurora-*Ds*Red, or the pCDF control on LB/Strep agar plates.
2. Incubate the plates overnight (~ 16 h) in darkness at 37°C.
3. Inoculate triplicates of 500-µL LB/Strep medium for every sample in a 96-deep-well plate by picking single bacterial clones from the respective agar plates.
4. Seal the plates with a gas-permeable membrane and incubate for 16 h in darkness at 37°C and 600 rpm agitation.
5. Transfer 2× 2 µL of each culture to 198 µL LB/Strep medium within two separate clear MTPs and seal them with a gas-permeable membrane.
6. Incubate one plate in darkness and the other under blue light (470 nm, 60 µW cm^{-2}) at 37°C and 800 rpm agitation for 24 h (see note 7).
7. For flow-cytometry measurements, dilute the cells ten- to thirtyfold in sheath fluid.

8. Analyze single-cell fluorescence on a flow cytometer [excitation lasers at 488 and 561 nm, emission at (585 ± 15) nm] by collecting approximately 200,000 events per sample (see note 6).
9. Fit the data to log-normal functions (Fig 3c-d), e.g., by using the Fit-o-mat software ***(34)***.

3.2 Multiplexing of Systems

1. Streak bacteria harboring either pREDusk-YPet or pREDawn-YPet combined with either pCrepusculo-*Ds*Red or pAurora-*Ds*Red on LB/Kan+Strep agar plates. As empty-vector controls, streak bacteria harboring the pREDusk-MCS plasmid on LB/Kan plates (see note 8).
2. Incubate the agar plates overnight (~ 16 h) at 37°C in darkness.
3. Inoculate 5-mL LB cultures supplemented with antibiotics for each bacterial clone carrying different plasmid combinations by picking a single colony from the respective agar plates.
4. Incubate for 24 h at 30°C and 225 rpm agitation under non-inducing conditions, i.e., darkness for the pREDawn/pAurora combination, red light (660 nm, 100 µW cm^{-2}) for pREDusk/pAurora, blue light (470 nm, 60 µW cm^{-2}) for pREDawn/pCrepusculo, and both red and blue light for pREDusk/pCrepusculo.
5. After incubation, dilute each of the cultures 100-fold into 5 mL LB medium supplemented with antibiotics. Transfer 200 µL of the dilution into wells A1-H8 (i.e., 64 wells in total) of a black-walled, clear-bottom MTP and seal with a gas-permeable membrane.
6. Configure the Arduino LED illumination setup such that each of the desired 64 wells (A1-H8) is illuminated with an individualized combination of blue and/or red light at varying intensities (Fig. 2d) (see note 9)
7. Place the MTP on top of the programmable Arduino illumination setup (Fig. 2b-c) ***(32, 33)***.

8. Incubate at 37°C and 750 rpm agitation for 18 h in an incubator protected against ambient light.
9. After incubation, transfer 50 µL from each well into wells of a clear MTP containing 200 µL H_2O each. Measure the absorbance at 600 nm (OD_{600}) of the resulting 5-fold dilution using a multimode MTP reader.
10. Next, transfer 50 µL from each well of the first, 5-fold dilution into wells of a back MTP containing 200 µL H_2O each. Measure the YPet and *Ds*Red fluorescence (*F*) using excitation wavelengths of (500 ± 9) nm and (530 ± 20) nm, and emission wavelengths of (554 ± 9) nm and (591 ± 20) nm, respectively (see note 10).
11. Determine F/OD_{600} ratios for each well and calculate the mean and standard deviation of the biological replicates. Correct the data for background fluorescence of the empty-vector control.
12. Normalize the data and plot as a three-dimensional surface diagram (Fig. 4), e.g., by using Python/matplotlib.

3.3 Multiplexed Optogenetic Control of Semi-preparative Protein Production

1. Streak bacteria harboring the plasmids pREDusk-YPet and pAurora-*Ds*Red on LB/Kan+Strep-agar plates. As an empty-vector control, also streak bacteria containing the pREDusk-MCS and pAurora-MCS plasmids (see note 8).
2. Incubate the agar plates overnight (~ 16 h) at 37°C in darkness.
3. Inoculate 5 mL LB/Kan+Strep medium with single bacterial clones from the plates in triplicate.
4. Incubate overnight (~ 16 h) at 37°C and 225 rpm agitation at non-inducing conditions, i.e., red light (660 nm, 100 µW cm^{-2}).

5. After incubation, for each replicate use 2× 1 mL culture to inoculate two Erlenmeyer flasks containing 100 mL LB/Kan+Strep (see note 11).
6. Incubate flasks at 37°C and 225 rpm agitation at non-inducing conditions, i.e., red light (660 nm, 100 µW cm^{-2}).
7. At an OD_{600} of ~ 0.5, transfer one flask for each of the replicates of the pREDusk-YPet/pAurora-*Ds*Red combination or the empty-vector control to red light (660 nm, 100 µW cm^{-2}), blue light (470 nm, 10 µW cm^{-2}), or both red and blue light. The remaining flasks are kept in darkness.
8. Incubate at 37°C and 225 rpm agitation for 24 h.
9. After incubation, take 200-µL aliquots from each flask and add 23 µL chloramphenicol and 6 µL tetracycline (see note 5).
10. Keep the samples on ice in darkness for 2 h to allow *Ds*Red and YPet maturation.
11. Measure the optical density at 600 nm (OD_{600}), and the *Ds*Red and YPet fluorescence (*F*) of the samples with a multimode MTP reader as described in section 3.2.
12. Determine the F/OD_{600} ratio and calculate the mean and standard deviation across the biological replicates. Correct the data for background fluorescence of the empty-vector control.
13. Normalize the data and plot (Fig. 5).

4. Notes

1. To the extent tested, pREDusk, pREDawn, pCrepusculo, and pAurora widely apply to different *E. coli* bacteria. The use of a specific strain is hence not required.
2. For the subsequent experiments, fluorescent reporters must be introduced into these plasmids, for instance via restriction or Gibson cloning ***(35)***.

3. The reporter may be substituted with another fluorescent protein of choice.
4. For keeping cell cultures in darkness, the flasks can be for instance covered with aluminum foil.
5. The mix of chloramphenicol and tetracycline arrests cell growth and translation.
6. The settings for the excitation laser and the emission filter chosen here apply to *Ds*Red. If another fluorescent protein is used, these values require adjustment.
7. For adjusting the light intensity, take into account the attenuation by the sealing membrane.
8. The combined plasmid systems can be introduced to the bacteria by transformation either simultaneously or sequentially.
9. Rather than continuous illumination, intermittent light with a duty cycle of, e.g., 1:10 may be used, i.e., 20 s illumination, followed by 180 s in darkness.
10. The settings for the excitation laser and the emission filter chosen here apply to *Ds*Red and YPet, respectively. If other fluorescent proteins are used, these values require adjustment.
11. To prevent inadvertent induction by ambient light, this step should be performed under red light (e.g., by using LED stripes).

5. References

1. Hegemann P (2008) Algal sensory photoreceptors. Annu Rev Plant Biol 59:167–189
2. Möglich A, Yang X, Ayers RA, et al (2010) Structure and function of plant photoreceptors. Annu Rev Plant Biol 61:21–47
3. Deisseroth K, Feng G, Majewska AK, et al (2006) Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci 26:10380–10386
4. Losi A, Gardner KH, and Möglich A (2018) Blue-Light Receptors for Optogenetics. Chem Rev 118:10659–10709
5. Tang K, Beyer HM, Zurbriggen MD, et al (2021) The Red Edge: Bilin-Binding Photoreceptors as Optogenetic Tools and Fluorescence Reporters. Chem Rev 121:14906–14956

6. Nagel G, Ollig D, Fuhrmann M, et al (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. Science 296:2395–2398
7. Nagel G, Szellas T, Huhn W, et al (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci USA 100:13940–13945
8. Boyden ES, Zhang F, Bamberg E, et al (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8:1263–1268
9. Zhang F, Wang L-P, Brauner M, et al (2007) Multimodal fast optical interrogation of neural circuitry. Nature 446:633–639
10. Ohlendorf R and Möglich A (2022) Light-regulated gene expression in Bacteria: Fundamentals, advances, and perspectives. 10
11. Ziegler T and Möglich A (2015) Photoreceptor engineering. Front Mol Biosci 2:30
12. Ohlendorf R, Vidavski RR, Eldar A, et al (2012) From dusk till dawn: one-plasmid systems for light-regulated gene expression. J Mol Biol 416:534–542
13. Baumschlager A, Aoki SK, and Khammash M (2017) Dynamic Blue Light-Inducible T7 RNA Polymerases (Opto-T7RNAPs) for Precise Spatiotemporal Gene Expression Control. ACS Synth Biol 6:2157–2167
14. Han T, Chen Q, and Liu H (2017) Engineered Photoactivatable Genetic Switches Based on the Bacterium Phage T7 RNA Polymerase. ACS Synth Biol 6:357–366
15. Ding Q, Ma D, Liu G-Q, et al (2020) Light-powered Escherichia coli cell division for chemical production. Nat Commun 11:2262
16. Dietler J, Schubert R, Krafft TGA, et al (2021) A Light-Oxygen-Voltage Receptor Integrates Light and Temperature. Journal of Molecular Biology 433:167107
17. Ranzani AT, Wehrmann M, Kaiser J, et al (2022) Light-Dependent Control of Bacterial Expression at the mRNA Level. ACS Synth Biol 11:3482–3492
18. Levskaya A, Chevalier AA, Tabor JJ, et al (2005) Synthetic biology: Engineering Escherichia coli to see light. Nature 438:441–442
19. Tabor JJ, Levskaya A, and Voigt CA (2011) Multichromatic control of gene expression in Escherichia coli. J Mol Biol 405:315–324
20. Multamäki E, García de Fuentes A, Sieryi O, et al (2022) Optogenetic Control of Bacterial Expression by Red Light. ACS Synth Biol 11:3354–3367
21. Fushimi K and Narikawa R (2019) Cyanobacteriochromes: photoreceptors covering the entire UV-to-visible spectrum. Curr Opin Struct Biol 57:39–46

22. Diensthuber RP, Bommer M, Gleichmann T, et al (2013) Full-length structure of a sensor histidine kinase pinpoints coaxial coiled coils as signal transducers and modulators. Structure 21:1127–1136
23. Davis SJ, Vener AV, and Vierstra RD (1999) Bacteriophytochromes: Phytochrome-Like Photoreceptors from Nonphotosynthetic Eubacteria. 286:2517–2520
24. Möglich A, Ayers RA, and Moffat K (2009) Design and signaling mechanism of light-regulated histidine kinases. J Mol Biol 385:1433–1444
25. Buschiazzo A and Trajtenberg F (2019) Two-Component Sensing and Regulation: How Do Histidine Kinases Talk with Response Regulators at the Molecular Level? 73:null
26. Möglich A (2019) Signal transduction in photoreceptor histidine kinases. Protein Science 28:1923–1946
27. Elowitz MB and Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. Nature 403:335–338
28. Weber AM, Kaiser J, Ziegler T, et al (2019) A blue light receptor that mediates RNA binding and translational regulation. 15:1085–1092
29. Mathes T, Vogl C, Stolz J, et al (2009) In vivo generation of flavoproteins with modified cofactors. 385:1511–1518
30. Strack RL, Strongin DE, Bhattacharyya D, et al (2008) A noncytotoxic DsRed variant for whole-cell labeling. Nat Methods 5:955–957
31. Nguyen AW and Daugherty PS (2005) Evolutionary optimization of fluorescent proteins for intracellular FRET. Nat Biotechnol 23:355–360
32. Hennemann J, Iwasaki RS, Grund TN, et al (2018) Optogenetic control by pulsed illumination. 19:1296–1304
33. Dietler J, Stabel R, and Möglich A (2019) Pulsatile illumination for photobiology and optogenetics, In: Methods in enzymology, pp. 227–248 Elsevier
34. Möglich A (2018) An open-source, cross-platform resource for nonlinear least-squares curve fitting, ACS Publications
35. Gibson DG, Young L, Chuang R-Y, et al (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 6:343–345

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Figure Captions

Figure 1

Architecture and light responses of the pREDusk, pREDawn, pCrepusculo, and pAurora plasmids. **(a)** pREDusk relies on the chimeric photoreceptor *Dr*F1 which consists of the photosensory core module of the bacterial phytochrome from *Deinococcus radiodurans* and the histidine kinase effector from *Bradyrhizobium japonicum* FixL. Together, with the response regulator FixJ, also from *B. japonicum*, *Dr*F1 forms a two-component system and regulates the expression of a gene of interest (GOI), with strong expression in darkness but not under red light. A heme oxygenase (HO) enzyme provides the biliverdin chromophore of *Dr*F1. **(b)** The pREDawn architecture extends pREDusk by a gene cassette comprising the λ phage cI repressor and its promoter pR. The system response is thereby inverted and gene expression is induced by red light. **(c)** pCrepusculo is based on the photoreceptor PAL from *Nakamurella multipartita* which sequence-specifically binds to small RNA hairpins upon blue-light exposure. By embedding said hairpins in the ribosome-binding site upstream of the GOI, its expression is subjected to light control. The expression in darkness is higher than under blue light. **(d)** The response of pAurora is inverted by the λ-cI-based gene-inversion cassette and affords gene expression that is activated by blue light rather than being repressed.

Figure 2

Illumination setups for optogenetic control. **(a)** Schematic of illumination setup for Erlenmeyer flasks. **(b)** Setup for illuminating bacterial cells during incubation comprising: (1) incubator protected from ambient light; (2) Arduino microcontroller; (3) programmable LED matrix embedded in 3D-printed housing (see panel (b) for details); (4) shaker. **(c)** Schematic of device depicted in panel (b) that allows illumination of bacterial cultures in MTPs from below. (1) Spring clip; (2) gas-permeable membrane; (3) black-wall clear-bottom 96-well MTP; (4) bacterial cultures; (5) 3D-printed adapter; (6) 8×8 LED array. **(d)** Top view of the assembled setup with individual wells exposed to varying intensities of red [(634 ± 8) nm] and blue light [(463 ± 12) nm].

Figure 3

Characterization of the light responses of the pREDusk, pREDawn, pCrepusculo, and pAurora systems. The fluorescence of the red-fluorescent reporter *Ds*Red was measured at the single-cell level by flow cytometry. **(a)** Bacteria harboring pREDusk-*Ds*Red were incubated in darkness (gray curves) or under red light (red curves). The empty-vector control pREDusk-MCS is shown in orange. Note that the abscissa is split with fluorescence values below 100 a. u. shown on a linear scale and those above on a logarithmic scale. **(b)** As in panel a but for bacteria carrying pREDawn-*Ds*Red. **(c)** Single-cell fluorescence of bacteria harboring pCrepusculo-*Ds*Red and cultivated in darkness or under blue light (blue curves), with a pCDF empty-vector control displayed in orange. **(d)** As in panel c but for bacteria equipped with pAurora-*Ds*Red. For each bacterial clone and light condition, data for three biologically independent replicates are shown. For each replicate, at least 10^5 cells were analyzed.

Figure 4

Multiplexed optogenetic control of bacterial expression by red and blue light. Bacteria harboring either pREDusk-YPet or pREDawn-YPet paired with either pCrepusculo-*Ds*Red or pAurora-*Ds*Red were cultivated under different intensities of red and blue light. The left panels (a, c, e, g) show the YPet fluorescence in the bacterial cultures following incubation, and the right panels (b, d, f, h) report the *Ds*Red fluorescence in these cultures. The individual panels show data for bacteria carrying **(a-b)** pREDusk-YPet and pCrepusculo-*Ds*Red; **(c-d)** pREDusk-YPet and pAurora-*Ds*Red; **(e-f)** pREDawn-YPet and pCrepusculo-*Ds*Red; and **(g-h)** pREDawn-YPet and pAurora-*Ds*Red. Data represent mean ± s.d. of three biologically independent replicates.

Figure 5

Multiplexed optogenetic control of protein expression at the semi-preparative scale. Bacteria containing the pREDusk-YPet and pAurora-*Ds*Red plasmids were incubated either under red light (i.e., non-inducing conditions), in darkness (i.e., activation of pREDusk), under red and blue light (i.e., activation of pAurora), or under blue light (i.e., activation of both pREDusk and pAurora). The *Ds*Red and YPet fluorescence in the cultures after incubation is shown in pink and green, respectively. Data represent mean ± s.d. of three biologically independent replicates.

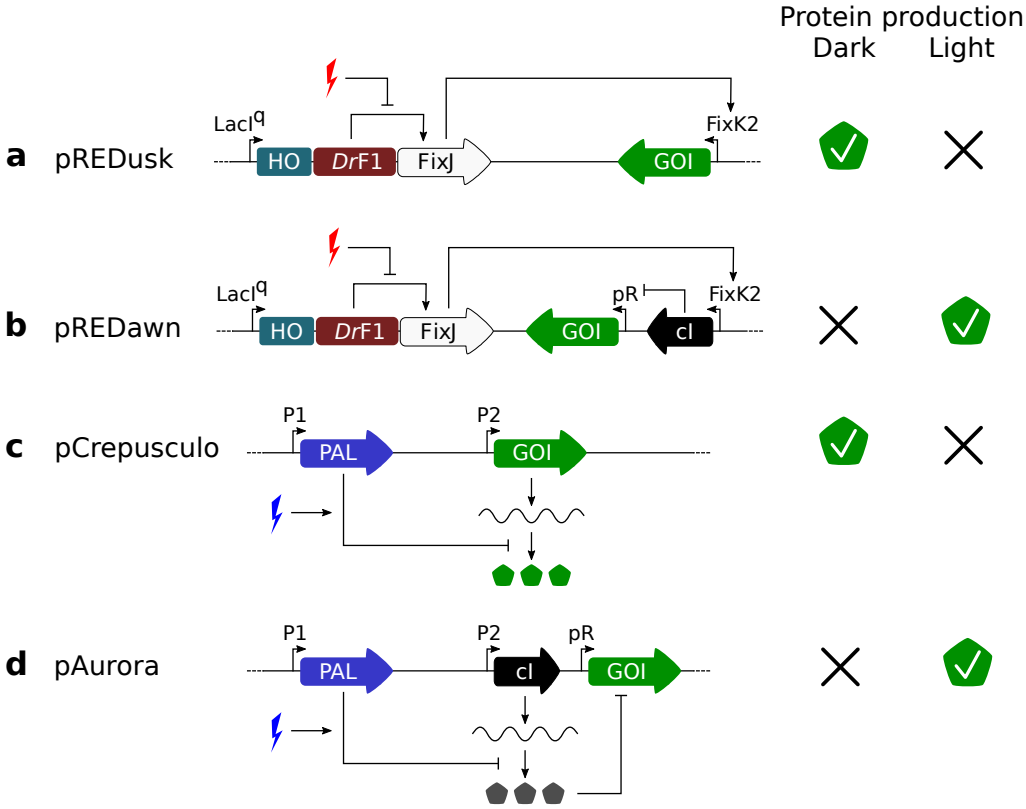
Protein production
Dark
Light
a
pREDusk
LacIq
HO
DrF1
FixJ
FixK2
GOI
b
pREDawn
LacIq
HO
DrF1
FixJ
GOI
pR
cI
FixK2
c
pCrepusculo
P1
PAL
P2
GOI
d
pAurora
P1
PAL
P2
cI
pR
GOI

Figure 1

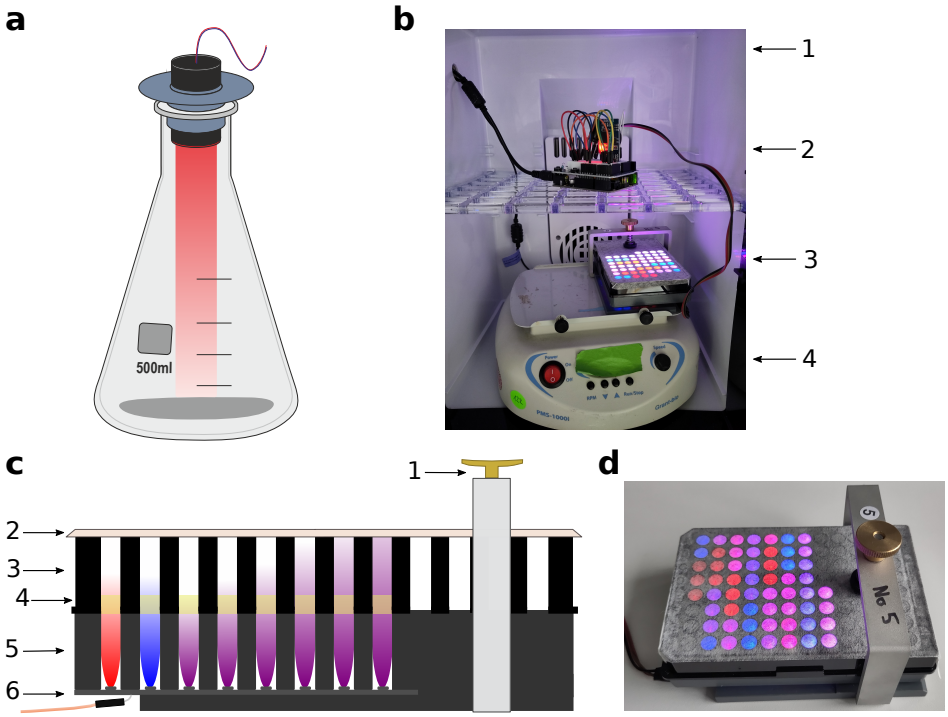

Figure 2

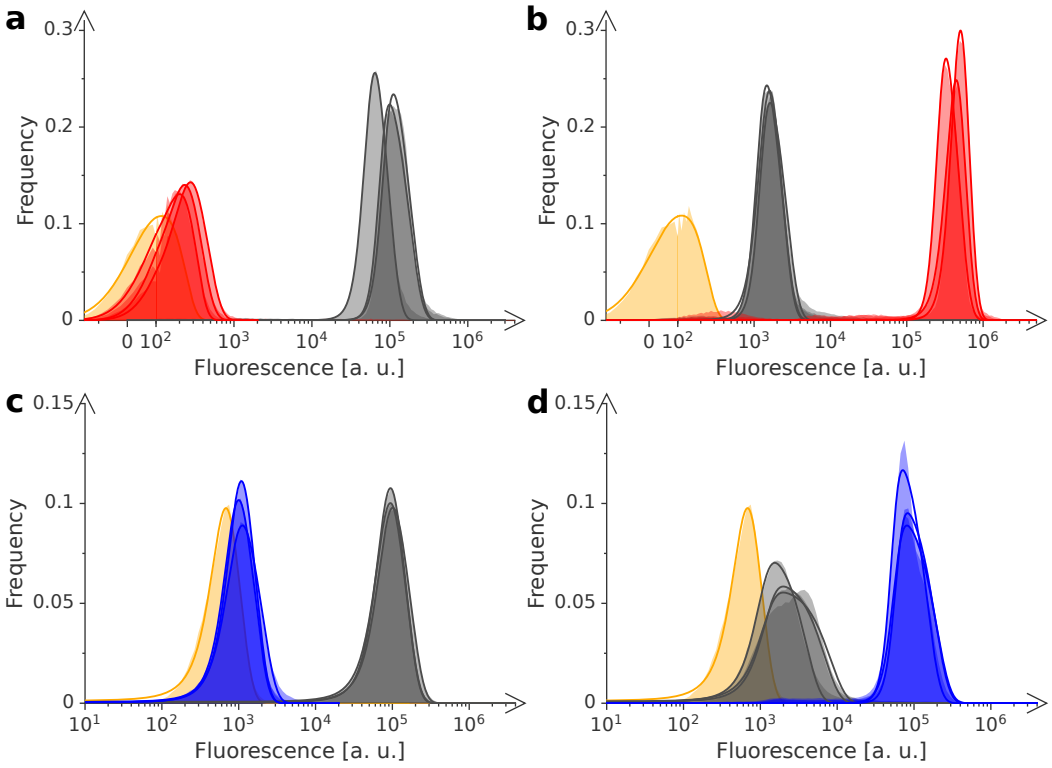
a
b
c
d
Frequency
Fluorescence [a. u.]
0
0.1
0.2
0.3
0.05
0.15
0 10²
10¹
10³
10⁴
10⁵
10⁶

Figure 3

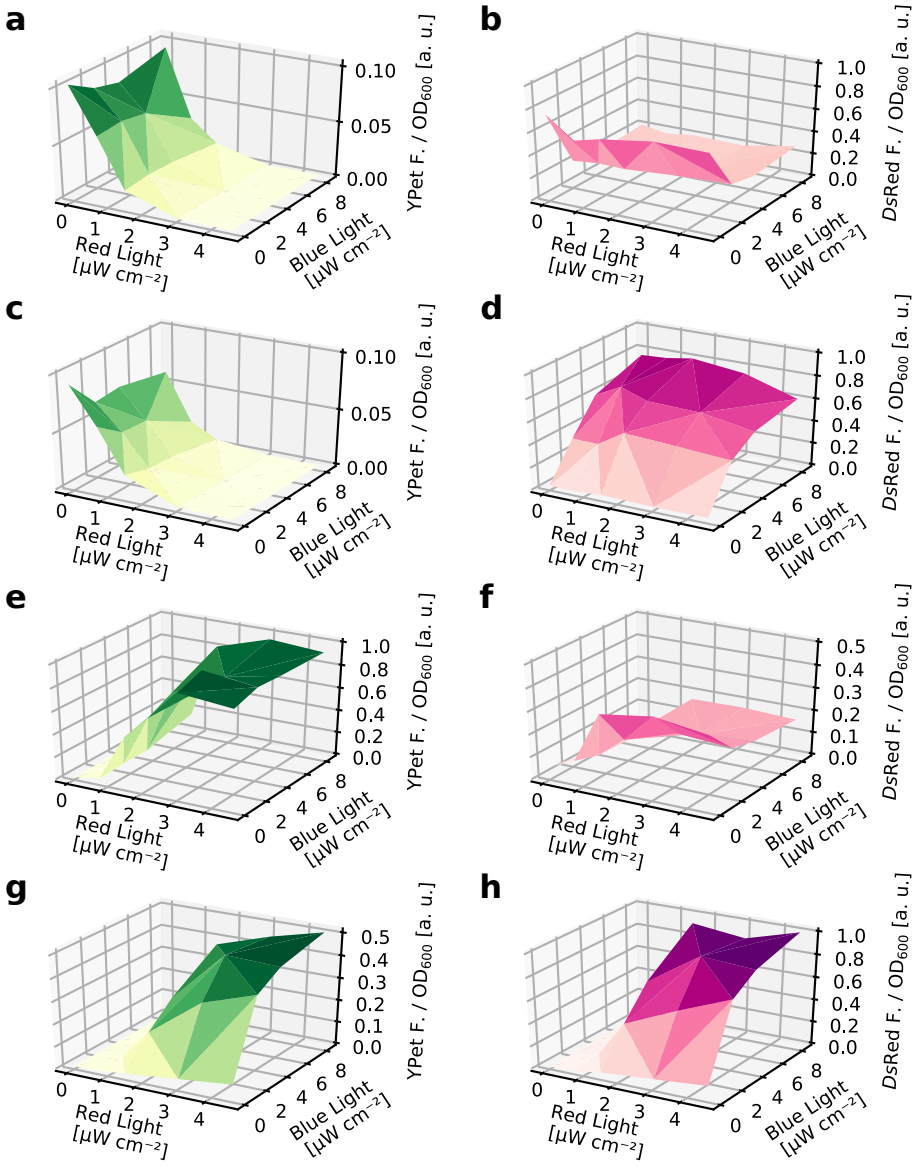
a
b
c
d
e
f
g
h
Red Light [μW cm⁻²]
Blue Light [μW cm⁻²]
YPet F. / OD600 [a. u.]
DsRed F. / OD600 [a. u.]

Figure 4

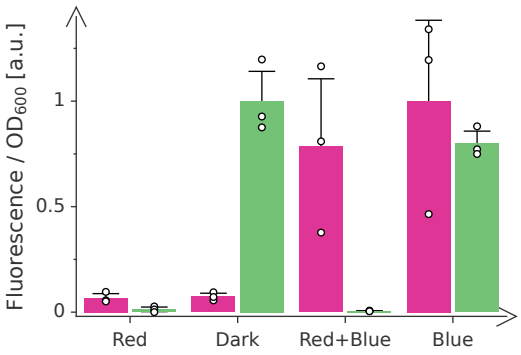

Figure 5