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Quantitatively Relating Gene Expression to Light Intensity via the Serial Connection of Blue Light Sensor and CRISPRi

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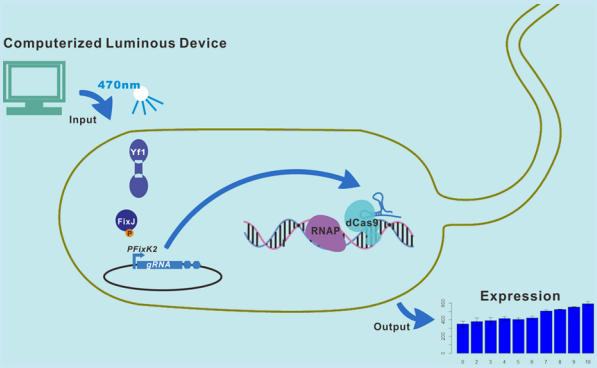
Supporting Information

ABSTRACT: The ability to regulate endogenous gene expression is critical in biological research. Existing technologies, such as RNA interference, zinc-finger regulators, transcription-activator-like effectors, and CRISPR-mediated regulation, though proved to be competent in significantly altering expression levels, do not provide a quantitative adjustment of regulation effect. As a solution to this problem, we place CRISPR-mediated interference under the control of blue light: while dCas9 protein is constitutively expressed, guide RNA transcription is regulated by YF1-FixJ-P_{FixK2}, a blue light responding system. With a computer-controlled luminous device, the quantitative relationship between target gene expression and light intensity has been determined. As the light intensifies, the expression level of target gene gradually ascends. This remarkable property enables sensor-CRISPRi to accurately interrogate cellular activities.



The way that researchers reprogram expression pattern has been revolutionized by sequence-specific gene-targeting technologies, including RNA interference (RNAi), zinc-finger (ZF) regulators, transcription-activator-like effectors (TALE), and CRISPR-mediated regulation. However, to apply these technologies in precision projects (e.g., in medical therapy), their regulation effect must be quantitatively adjusted. Unfortunately, although several switches have been reported to successfully turn on or off the regulation,[1,2] a quantitative adjustment remains absent.

In order to remedy this flaw, the regulation process must be guided by some sort of real-time signals, among which light stands out for its high resolution on both spatial and temporal scale, and for its readiness to be produced by digital instruments. Given these advantages, light can be a most favorable signal for molecular biology manipulations and the only feasible signal for high-throughput projects. In our project, YF1-FixJ-P_{FixK2},[3] a synthetic blue light sensing system, is utilized.

CRISPR-mediated interference (CRISPRi)[4] is chosen as the genome regulation tool. The system of CRISPRi consists of only two biobricks: dCas9, a mutant of Cas9 protein in type II CRISPR/Cas that lacks endonuclease activity; and guide RNA (gRNA), a 108 nt RNA with a 20 nt base-pairing region that determines the sequence-specificity. dCas9:gRNA complex binds its target region and blocks RNA polymerase (RNAP) in transcription. This simplicity leads to its transferability far beyond RNAi, which is limited to particular organisms. In addition, RNA-guided interference is cost-effective in designing, compared with DNA binding proteins, such as zinc-fingers and TALEs.

Here, we coupled the blue light sensor with CRISRPi in *Escherichia coli*. Then, we inspected the performance of sensor−CRISPRi on a plasmid mRFP (monomeric red fluorescent protein) reporter[5] and on *fadD*, a gene on *E. coli* genome that expresses acyl coenzyme A synthetase (EC 6.2.1.3).[6] A computerized light-producing instrument has been created to assist with the experiment.

RESULTS AND DISCUSSION

The serial connection of blue light sensor and CRISPRi is illustrated in Figure 1. YF1 is a blue-light-sensitive (excitation maximum at wavelength 470 nm) histidine kinase. In the absence of blue light, YF1 phosphorylates its cognate response regulator, FixJ. In turn, FixJ drives gene expression from

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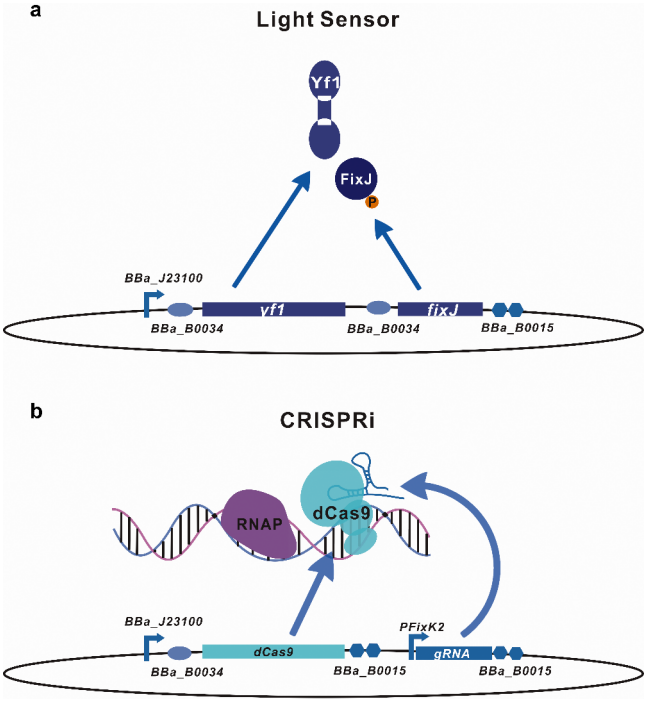

Figure 1. Serial connection of blue light sensor and CRISPRi. (a) YF1 is a blue-light-sensitive histidine kinase which, in the absence of blue light, phosphorylates its cognate response regulator, FixJ. FixJ in turn drives (b). gRNA transcription down from promoter P_{FixK2}. gRNA directs dCas9 to bind the target region and block RNAP in transcription. P_{FixK2}, gRNA gene and BBa_B0015 are assembled without biobrick scars to ensure that no extra nucleotides would appear in the base-pairing region of gRNA.

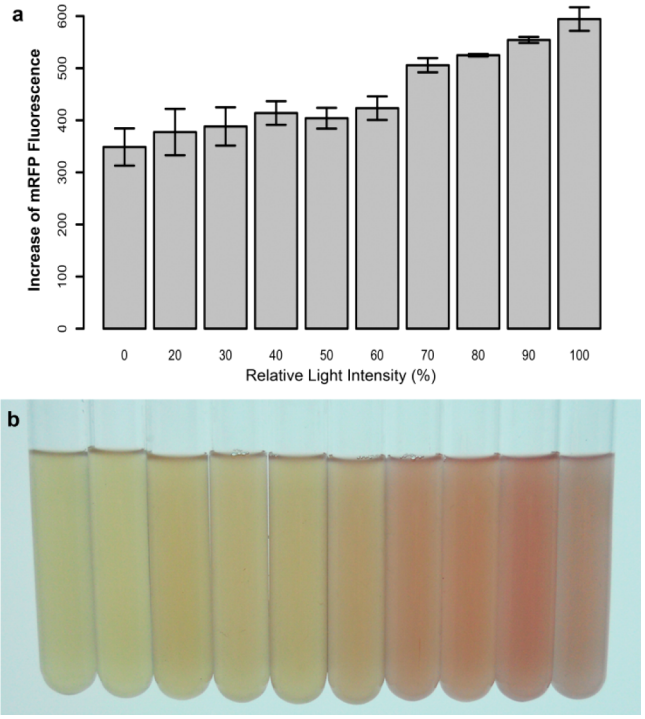

Figure 2. Relationship between mRFP expression and light intensity. (a) Quantitative measurement of mRFP production under different light intensities. Bar height represents mRFP production in 15 h under different light intensities. Error bars shows the standard error (s.e.) of parallel groups. mRFP production gradually increases to about 2-fold. (b) A photo of the experiment result in part a.

promoter P_{FixK2}.[7] We placed gRNA gene downstream P_{FixK2}, where its transcription is controlled by this promoter. P_{FixK2} and gRNA gene are assembled without spacing nucleotides (e.g., restriction scars) to ensure that no extra nucleotides would emerge in the base-pairing region of gRNA. *E. coli* strain BL21 (DE3) is transformed to host this system.

The expression of target gene is expected to be positively correlated with light intensity. When blue light gets stronger, the sensor will be less active. Consequently, gRNA expression is weakened. With a smaller quantity of gRNA, CRISPRi will repress target gene less effectively.

We first tested our sensor-CRISPRi on plasmid mRFP reporter. An appropriate gRNA will direct the system to regulate *mrfp* gene. A gradient of 10 light intensities was established within the sensing range of YF1-FixJ-P_{FixK2}, from zero to saturation.[3] However, to avoid the discrepancy of growing phase among different experiment groups, bacteria are cultured in darkness to stationary phase (OD600 ≈ 2.2) before they are divided. This procedure takes approximately 24 h. The fluorescence intensity reaches 28 au. Then, after another 15 h culture under different blue light intensities, fluorescence intensities are measured again. The result is shown in Figure 2a. Bar height represents the increase in fluorescence intensity between two successive measurements, which corresponds to the production rate of mRFP. With the increase of light intensity, we observed a gradual ascend in the expression strength.

Two properties make our sensor-CRISPRi suitable for precise regulation. For the first property, squared Pearson coefficient (R^2) of linear fit is calculated to be 0.901. So, the expression is stably accelerated as we lift up light intensity (even if the relationship is not strictly linear), making it easier for researchers to adjust for an optimal regulation. For the second property, the variance (standard errors are represented as error bars in Figure 2a) is relatively small. So our system is stable, and a predetermined working curve can be referenced in later experiments.

We next examined how sensor-CRISPRi acts on *fadD*. Even though favorable results have already been acquired in mRFP tests, we still need to verify that our system also works for genome-residing genes. Unlike plasmid genes, genome-residing genes are generally single-copied, thus may behave differently under regulation. We replaced the base-pairing region of gRNA by inverse PCR to redirect sensor-CRISPRi onto this *fadD*. From this redirection, a bonus of CRISPRi can be observed: in case that researchers change their targets, all they need is to substitute a 20-nt sequence.

Bacteria are cultured in darkness to stationary phase (OD600 ≈ 2.0) before they are divided into different experiment groups. After another 15 h culture under different blue light intensities, cell bodies are collected for RNA extraction. Real-Time PCR (RT-PCR) is applied to assay the amount of *fadD* mRNA. *gapA*, the *E. coli* house-keeping gene for glyceraldehyde-3-phosphate dehydrogenase (GADPH, EC 1.2.1.12), served as the internal reference.[8] In relative quantitation (comparative threshold method), we took wild-type *E. coli* strain BL21 (DE3) as the control. The result is presented in Figure 3.

The eligibility of sensor-CRISPRi in precise regulation is confirmed on this genome-residing gene. mRNA amount of *fadD* increases continuously and steadily when blue light exposure is enlarged. Both of the two properties revealed in

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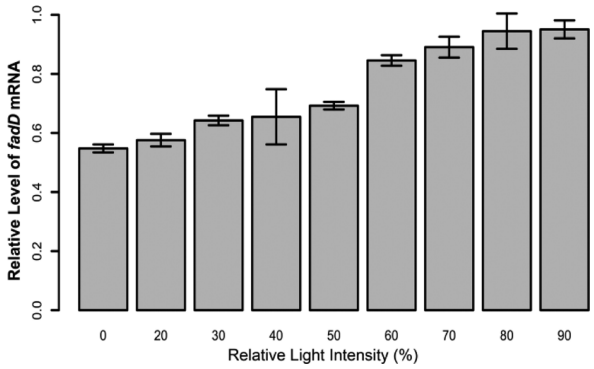

Figure 3. Relationship between *fadD* transcription and light intensity. Bar height represents the relative amount of *fadD* mRNA. Error bars shows the standard error (s.e.) of parallel groups. Transcription level gradually increases to about 2-fold.

plasmid mRFP test are repeated here: the increase is steady (R^2 = 0.924); and the system performance is relatively robust.

Instead of such astonishing CRISPRi repression of 10- to 300-fold reported in previous publications,[4] we observed a maximal repression of approximately 2-fold for our sensor-CRISPRi. This is because P_{FixK2} and its downstream gRNA gene are held by a low-copy-number [20−40 copies per cell] vector, pCDFDuet. Therefore, though dCas9 proteins are always abundant (constitutively expressed from a high-copy-number vector, pRSFDuet [>100 copies per cell]), only a small fraction of them form complex with gRNA, even in absolute darkness. According to recent analysis on dCas9 (Cas9) structure, gRNA-mediated conformational activation is indispensable for dCas9 target binding.[9,10] As a result, dCas9 repression potential is not fully exploited by our sensor-CRISPRi coupling.

Despite this incomplete utilization of CRISPRi competence, our design is able to provide the highest light-sensitivity. Since dCas9 is constantly overdosed, newly transcribed gRNA will always be complexed by them instantly. So, we have tuned CRISPRi to a condition that finely accommodates the entire sensing range of YF1-FixJ-P_{FixK2}. On the expense of partial CRISPRi effectiveness, we have harnessed the most competence of light sensor. As a result, whenever researchers adjust light intensity, even by a small amount, our sensor-CRISPRi will respond to that immediately.

On the contrary, suppose we used high-copied gRNA gene, then dCas9 would not always be overdosed compared with gRNA, especially under weak light. Hence, the sensing ability of YF1-FixJ-P_{FixK2} could not be manifested completely in CRISPR interference, particularly in dim-light range.

However, certainly, the coupling between light sensor and CRISPRi, especially the respective amount of dCas9 and gRNA, is worth of further exploration. We will continue working on this, to the end of reaching a best balance between light-sensitivity and the width of regulation range. Basically, we further raise the amount of dCas9, on condition that ordinary cell activity is not disrupted. Then, we experiment different expression amounts for light sensor and gRNA, measuring sensitivity, and regulation range. We expect to find out an ideal set of parameters according to these trials.

In conclusion, by serially connecting blue light sensor (YF1-FixJ-P_{FixK2}) and CRISPRi, the expression of target gene can be quantitatively related to light signals. Therefore, sensor-CRISPRi can be applied where it is necessary to precisely regulate endogenous genes, for example, in medical therapies and in metabolic optimization.

METHODS

Plasmid Assembly. pRSFDuet [>100 copies per cell] harbors the constitutively expressed dCas9 gene; pCDFDuet [20−40 copies per cell] harbors YF1-FixJ-P_{FixK2} and the gRNA gene (targeting either *mrfp* or *fadD*); pETDuet [~ 40 copies per cell] harbors *mrfp* report gene.

P_{FixK2}, gRNA gene and BBa_B0015 are assembled without restriction scars, in order to ensure the quality of gRNA. Inverse PCR and overlap extension PCR are used to accomplish this goal. In addition, BBa_B0015 is selected to reliably terminate gRNA transcription.

Additional methods. Described in the Supporting Information.

Computerized Luminous System. PC (Personal Computer) User Interface (UI) is written in C#. Microcontroller Unit (MCU) freescale MC9S12DG128 MPVE stores computerized program from UI and commands each LED according to the program. The wavelength range of LEDs (Geesled YY-BH5272UC) used to produce light is 465 to 470 nm.

ASSOCIATED CONTENT

Supporting Information

Bacteria strains, fluorescence measurement, RNA extraction, and real-time PCR. gRNA sequences used in this study. Information about luminous system and executives of computer User Interface (UI). Characterization of YF1-FixJ-P_{FixK2}, the Blue Light Sensing System. This material is available free of charge via the Internet at http://pubs.acs.org. Team Wiki of SJTU-BioX-Shanghai: http://2013.igem.org/Team:SJTU-BioX-Shanghai.

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Author Contributions

Y.W., H.Y.W., L.H., and G.M. conceived the project and designed the experiments. H.Y.W., Y.S.W., X.A.C., Y.F.W., Z.F.M., Z.Y.W., S.Y., W.J.X., Q.S., S.Y.L., P.C., and J.X.W. coperformed the experiments. H.Y.W., Y.S.W., X.A.C., Y.F.W., Z.F.M., Z.Y.W., S.Y., W.J.X., Q.S., and G.M. analyzed the data. H.Y.W., Y.S.W., M.R.I.K., L.H., and G.M. contributed reagents/materials/analysis tools. H.Y.W., Y.S.W., L.H., and G.M. wrote the article.

Notes

The authors declare no competing financial interest.

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