

Blue light-mediated transcriptional activation and repression of gene expression in bacteria.

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SupplementaryTable S1: Sequence of promoters and rbs used in this study. EL222 binding region, -35 hexamer, -10 hexamer and spacer.

Promoters and RBS used in this study	Description	Sequence
P _{Blind} (v1)	Blue-light inducible promoter (version 1)	GGTAGCCTTTAGTCCATGtagcgaagaaaatggttgtTATAGTcgaataaa
P _{Blind} (v2)	Blue-light inducible promoter (version 2)	GGTAGCCTTTAGTCCATGttacgcaagaaaatggttgtTATAGTcgaataaa
P _{BLrep} (v1)	Blue-light repressible promoter (version 1)	TTGACAGGTAGCCTTTAGTCCATGTATAAT
P _{BLrep} (v2)	Blue-light repressible promoter (version 2)	ttgacggctagctcagtcctaggtacagtgctagcTATGTCTAGAGGGTAGCCTTTAGTCCATG
P _{LasI}	LasR-AHL inducible promoter	AAATCTATCTCATTTGCTAGTTATAAAATTATGAAATTTGCATAAATTCTTCA
rrnBp1	Constitutive promoter	atttcctctgtcaggccggaataactccctataatgcg
BBa_B0034 RBS (rbs34)	Strong	AAAGAGGAGAAA

Default RBS (rbsD)	Very strong	TTTAAGAAGGAGATATACAT
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Supplementary Table S2: Parameters used for curve fitting the dose-response curves.

Promoter/Dose	k (a.u.)	n	K_1	α	R^2
P _{BLind-v1} /illumination pulse	1545	2	6.554 %	444.6	0.975
P _{BLind-v1} /intensity	1577	2	1.052 W/m ²	441.6	0.989
P _{BLrep-v1} /illumination pulse	2.471e6	2	27.93 %	1514	0.991
P _{BLrep-v1} /intensity	4.11e4	2	3.4467 W/m ²	1346	0.997

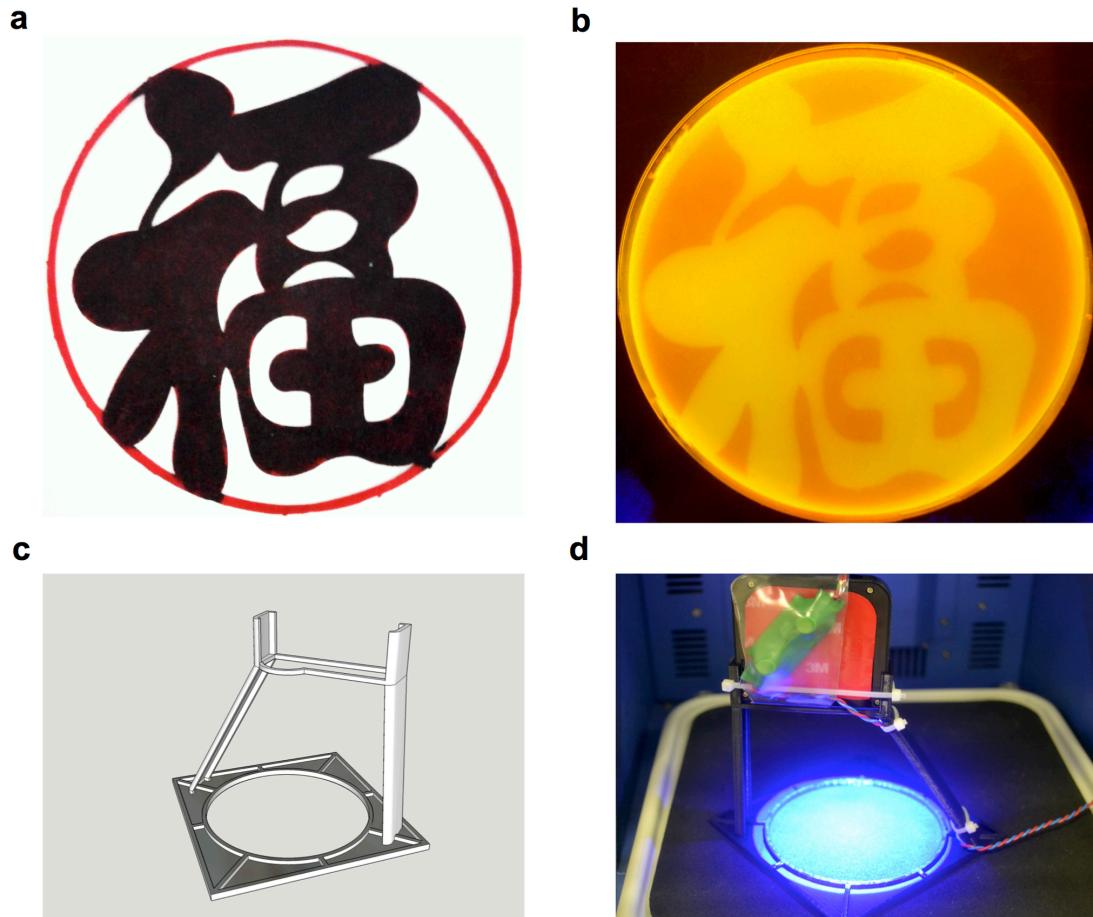
*Hill function model: $f([L]_{ind}) = \alpha + \frac{k([L]^n)}{K_1^n + [L]^n}$ (1), $f([L]_{rep}) = \alpha + \frac{k}{K_1^n + [L]^n}$ (2). Where, α is the basal expression level of the promoter, k is the maximum expression due to induction or repression, $[L]$ is the dose of the blue-light; K_1 and n are the Hill constant and co-efficient.

Supplementary Table S3: Parameter values used in the ODE model.

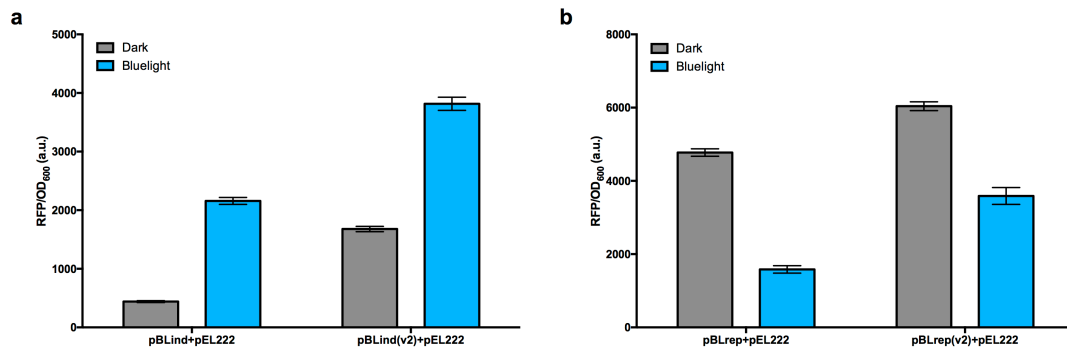
S.No	Parameter Abbreviation	Parameter Name	Parameter Value	Units	Reference
1	$K_{S_{Ind}}$	Increase in mRNA synthesis rate (inducible system)	8.4e-7	$\mu\text{M}/\text{min}$	Estimated
2	$\delta_{S_{Ind}}$	Decrease in mRNA synthesis rate (inducible system)	0.003	/min	Estimated
3	$K_{S_{Rep}}$	Increase in mRNA synthesis rate (repressible system)	6.4e-6	$\mu\text{M}/\text{min}$	Estimated
4	$\delta_{S_{Rep}}$	Decrease in mRNA synthesis rate (repressible system)	0.032	/min	Estimated
5	$initial K_{RFPmRNA}$	Initial synthesis rate of mRNA RFP	2.22e-14	$\mu\text{M}/\text{min}$	Estimated
6	$initial K_{GFPmRNA}$	Initial synthesis rate of mRNA GFP	2.22e-14	$\mu\text{M}/\text{min}$	Estimated
7	$K_{basalmRNARFP}$	Basal synthesis rate of mRNA RFP	3.22e-7	$\mu\text{M}/\text{min}$	Estimated
8	$K_{basalmRNAGFP}$	Basal synthesis rate of mRNA GFP	8.62e-7	$\mu\text{M}/\text{min}$	Estimated
9	$\delta_{mRNARFP}$	Decay rate of mRNA RFP	0.346	/min	Bio-number 110055
10	$\delta_{mRNAGFP}$	Decay rate of mRNA GFP	0.346	/min	Bio-number 110055
11	K_{RFP}	Translation rate of RFP	0.001	/min	Estimated
12	K_{GFP}	Translation rate of GFP	0.001	/min	Estimated
13	δ_{RFP}	Decay rate of RFP protein	0.006	/min	Dilution rate
14	δ_{GFP}	Decay rate of GFP protein	0.006	/min	Dilution rate



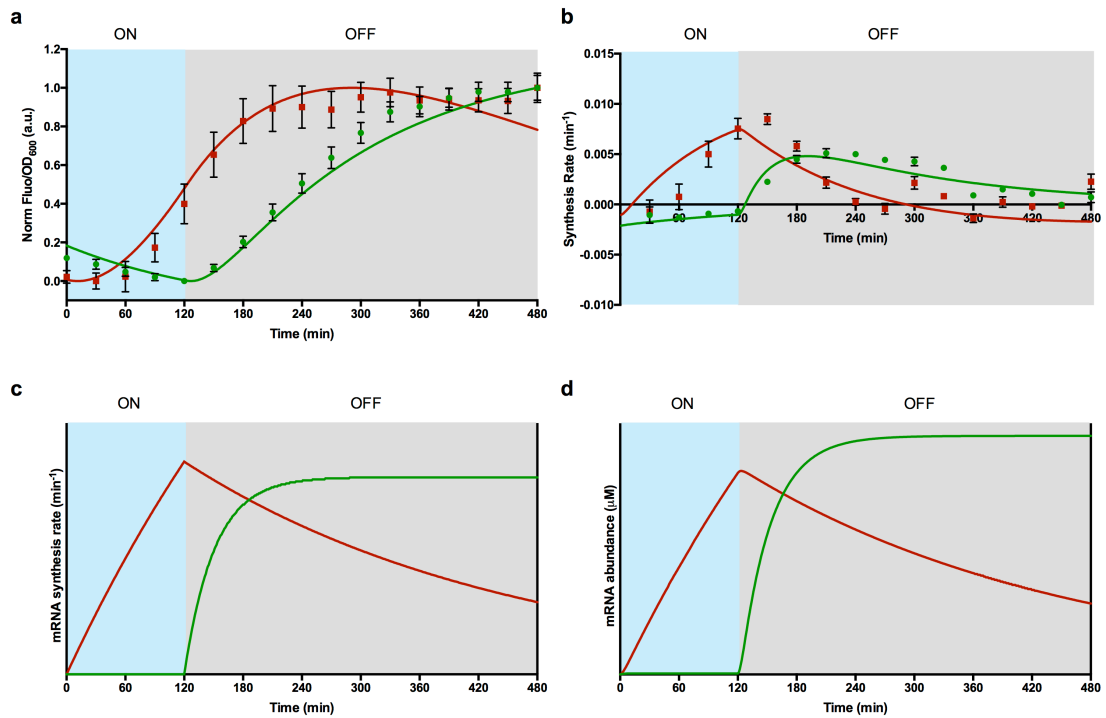
Supplementary Figure S1: 12-LED Blue Light Source setup. (a) The 12-LED blue light source is designed to provide 465nm light for the 12-well culture plate, with adjustable on/off time and light intensity on individual wells. This design incorporates a sandwich structure, where light & circuit enclosure sits at the bottom, followed by a whitespacer in the middle, and a 12-well culture plate on top. (b) The spacer holds and aligns the light & circuit enclosure with the culture in position. The tube structure inside the spacer helps to diffuse and isolate the 12 light channels for individual wells on the plate. (c) Micro controller and LED driver circuit sit inside the bottom casing, with through-hole LEDs mounted directly on top.



Supplementary Figure S2: Blue-light projector structural design. (a) We tried a photomask with a complex pattern (Chinese **character Fú**, meaning “fortune” or “good luck”). (b) Our blue-light biregulatory promoter system was able to precisely reproduce the complex pattern on the photomask (a). (c) The design incorporates a modified car door projection light and a 3D printed holder. The white LED inside the original projection light is replaced by Cree® XLamp® XP-E LED (royal blue) with dominating wavelength of 460nm. The LED is powered by 1W constant current driver module at 350mA, stepped down from 5V 1A power adapter. The holder positions the projector to get a clear image on the surface of the plate. (d) The image of the projector placed inside the incubator.

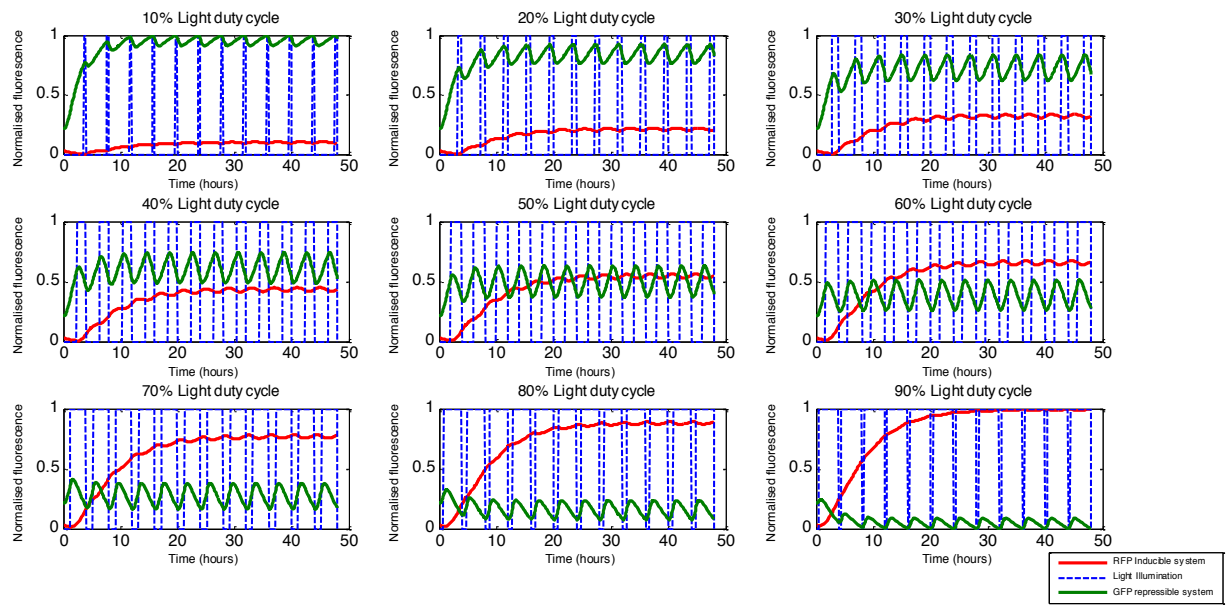


Supplementary Figure S3: Characterization results of both the versions of blue-light inducible (a) and repressible (b) promoters under dark and illumination conditions.

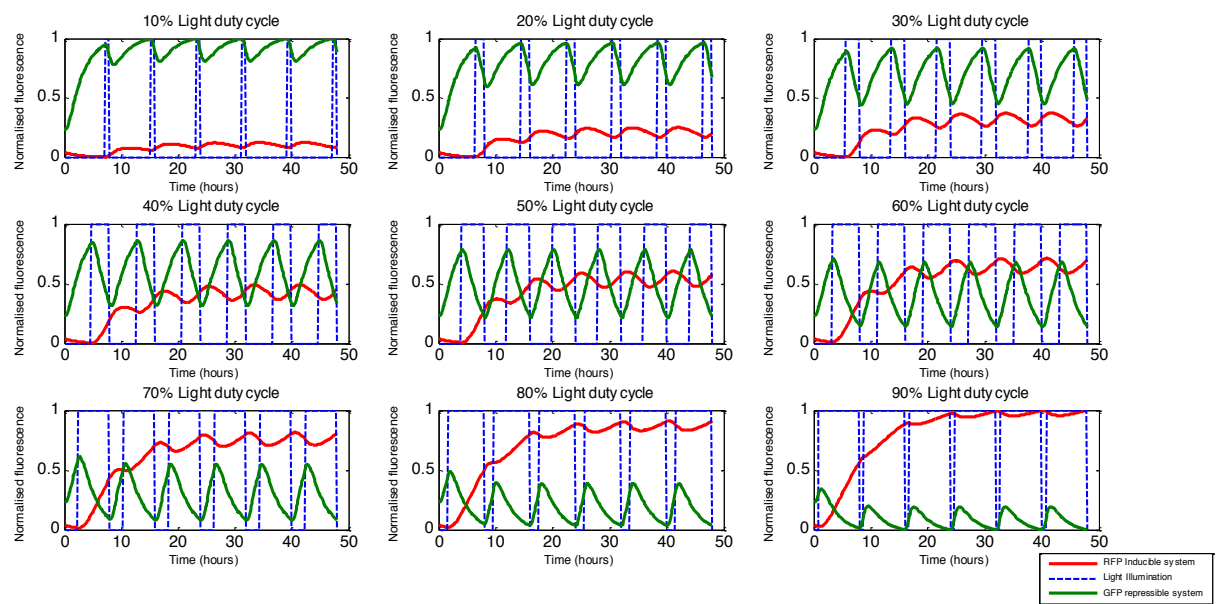


Supplementary Figure S4: Temporal control of gene expression using 2 h ON and 6 h OFF pattern of blue-light exposure. (a) Normalized Fluo/OD₆₀₀ data obtained. Solid lines are the model predictions. (b) Calculated synthesis rates based on the normalized Fluo/OD₆₀₀ data obtained. Model predicted mRNA synthesis rates (min⁻¹) (c) and mRNA abundance (μM) (d). Error bars indicate s.d. (n=3). Grey areas represent dark state (OFF) while the blue regions (illumination ON, 12 W/m²).

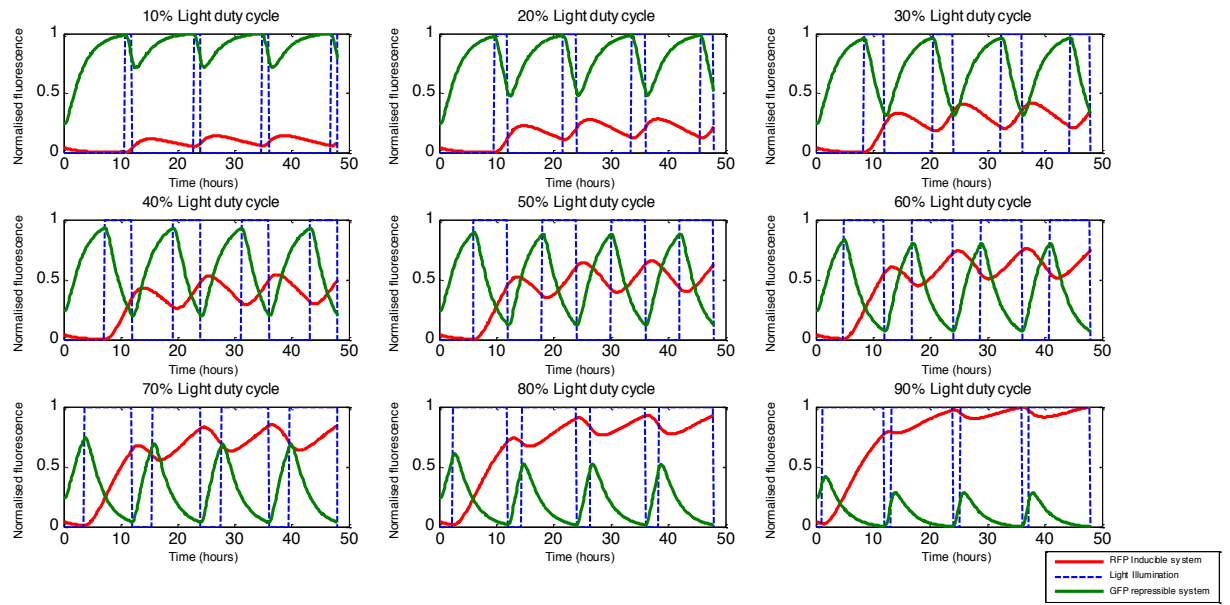
a



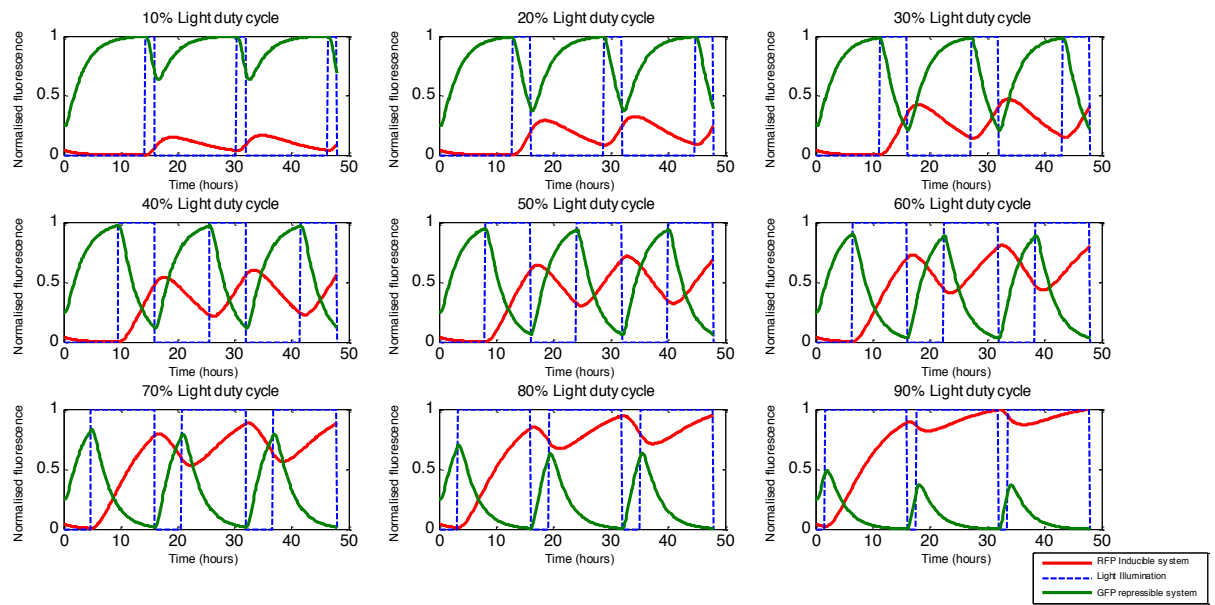
b



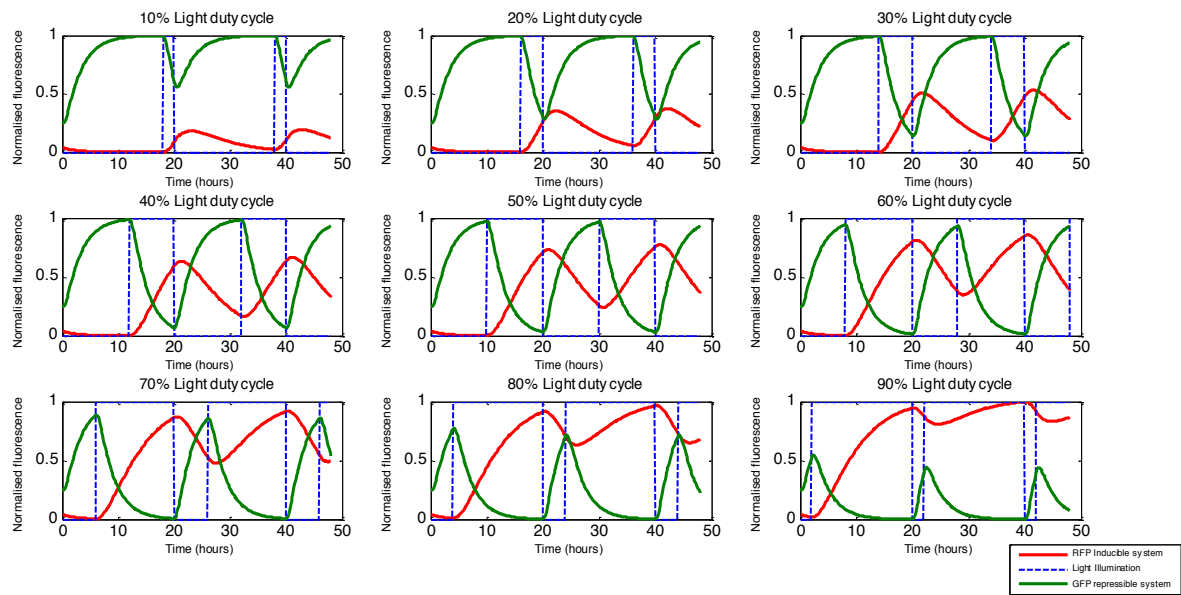
c.



d.



e.



Supplementary Figure S5: Model predicted 48 h simulation results of the oscillations (assuming constant growth conditions) for varying time periods (**4, 8, 12, 16 and 20 h**) and duty cycles (**10% to 90%**). **a. 4 h time period.** Duty cycle: 10%-24min ON; 216 min OFF, 20%-48 min ON; 192 min OFF, 30%-72 min ON; 168 min OFF, 40%-96 min ON; 144 min OFF; 50%-120 min ON; 120 min OFF, 60%-144 min ON; 168 min OFF, 70%-168 min ON; 72 min OFF, 80%-192 min ON; 48 min OFF, 90%-216 min ON; 24 min OFF. **b. 8 h time period.** Duty cycle: 10%-48 min ON; 432 min OFF, 20%-96 min ON; 384 min OFF, 30%-144 min ON; 336 min OFF, 40%-192 min ON; 288 min OFF, 50%-240 min ON; 240 min OFF, 60%-288 min ON; 192 min OFF, 70%-336 min ON; 144 min OFF, 80%-384 min ON; 96 min OFF, 90%-432 min ON; 48 min OFF. **c. 12 h time period.** Duty cycle: 10%-72 min ON; 648 min OFF, 20%-144 min ON; 576 min OFF, 30%-216 min ON; 504 min OFF, 40%-288 min ON; 432 min OFF, 50%-360 min ON; 360 min OFF, 60%-432 min ON; 288 min OFF, 70%-504 min ON; 216 min OFF, 80%-576 min ON; 144 min OFF, 90%-648 min ON; 72 min OFF. **d. 16 h time period.** Duty cycle: 10%-96 min ON; 864 min OFF, 20%-192 min ON; 768 min OFF, 30%-288 min ON; 672 min OFF, 40%-384 min ON; 576 min OFF, 50%-480 min ON; 480 min OFF, 60%-576 min ON; 384 min OFF, 70%-672 min ON; 288 min OFF, 80%-768 min ON; 192 min OFF, 90%-864 min ON; 96 min OFF. **e. 20 h time period.** Duty cycle: 10%-120 min ON; 1080 min OFF, 20%-240 min ON; 960 min OFF, 30%-360 min ON; 840 min OFF, 40%-480 min ON; 720 min OFF, 50%-600 min ON; 600 min OFF, 60%-720 min ON; 480 min OFF, 70%-840 min ON; 360 min OFF, 80%-960 min ON; 240 min OFF, 90%-1080 min ON; 120 min OFF.