Supporting Information

Comprehensive profiling of diverse genetic reporters with application to whole-cell and cell-free biosensors

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Experimental Procedure

Plasmid circuit construction

Compared to previously studied plasmids pBW316J101-luxR[1] and pXWJ109Hg-gfp[2], the constitutive promoters with receptors were inverted and oriented in the opposite direction to the inducible promoters and reporters by PCR and BioBrick™ assembly. For tuning the intracellular receptor densities, the constitutive promoters that drive the expression of the mercury receptor MerR and the quorum sensing molecule receptor LuxR were replaced via PCR.

mCherry was amplified from BioBrick™ part BBa_J06504 by PCR with addition of a ribosome binding site (RBS) BBa_B0030. *mScarlet-I* with BBa_B0030 was synthesized through Integrated DNA Technologies (gBlocks® Gene Fragments) and was amplified by PCR. *gfp* was amplified from a previously studied plasmid pXWJ109Hg-gfp[2] by PCR. *deGFP* was amplified from plasmid Pr-deGFP (#67743, Addgene) by PCR with addition of BBa_B0030. *lacZ* was amplified from *E. coli* MG1655 genome by PCR with addition of BBa_B0030. *NanoLuc* was amplified from plasmid pCDNA-NanoLuc[3] with addition of BBa_B0030. *lux* operons from *P. luminescens* and *A. fischeri* were amplified from the gift plasmids provided by Prof Belkin Shimshon (Hebrew University of Jerusalem).

Reporter gene expression assay *in vivo*

For reporter gene characterization, the engineered *E. coli* were first inoculated from a single colony on a freshly streaked solid LB plate to 5 mL LB medium, and cultured overnight at 37°C with shaking (160 rpm). Then the cells were diluted 100-fold from the overnight culture into fresh LB medium. For liquid culture induction, the diluted culture was loaded into a 96-well 2.0 mL deepwell plate with round bottom (E2896-2110, Starlab), and induced with 40 μL inducers to a final volume of 1.6 mL per well. The microplate was sealed with an air permeable film (AXY2006, SLS), and incubated in a shaker incubator (MB100-4A, Allsheng) with continuous shaking (1,000 rpm, 37°C). After 30, 60, 90,120, 180, 240, 300 and 360 min incubation, 200 μL of induced culture were dispensed in 96-well microplates with clear-bottom (655096, Greiner Bio-One). A plate reader (BMG FLUOstar) was used to measure fluorescence (bottom reading), absorbance and bioluminescence (top reading). 485 nm excitation and 520 ± 10 nm emission wavelengths were used for measuring green fluorescent reporters with Gain = 1,000. For red fluorescent reporters measurement, 584 nm excitation and 620 ± 10 nm emission wavelengths with Gain = 2,000 were used. Bioluminescent signal was acquired with 0.1 s of signal integration for each well and Gain = 1,500. Colorimetric signal was acquired by absorbance measurement of A_{650}. NanoLuc-conducted bioluminescent and LacZ-conducted colorimetric measurements were acquired in lysing and non-lysing conditions using the same concentration of substrates (X-gal = 0.04 mg mL^{-1} from 2% X-gal stock solution and 0.2 μL of furimazine stock solution per 200 μL culture). B-PER™ Bacterial Protein Extraction Reagent (78243, ThermoFisher Scientific) and PBS (K813-500ML, VWR) were used in lysing and non-lysing

conditions respectively to dilute the substrate. A 50 μL-volume of diluted substrate was added in each well with a final volume of 250 μL. NanoLuc-derived bioluminescent kinetics were measured for 30 min after substrate addition and the highest signal was chosen for data analysis. Colorimetric signal measurement was preceded by 30 min incubation at 37°C, with orbital shaking at 300 rpm in the plate reader. To determine the cell density, absorbance (A_{600}) was also read prior to each reporter measurement. For *lux* operon reporter, the bioluminescent signal was measured immediately after absorbance measurement without the addition of substrates.

The plate reader data acquired using Omega MARS 3.20 R2 were exported to Microsoft Excel 2013 and GraphPad Prism 6.01 for data analysis and presentation. The medium backgrounds of fluorescence, absorbance or luminescence were determined from blank wells loaded with LB medium and were subtracted from the readings of other wells. The relative fluorescence, absorbance or luminescence unit (RFU, RAU or RLU) at different time points for a sample culture was determined by the blank corrected output signal divided by A_{600}, and after subtracting its triplicate-averaged counterpart of the negative control cultures (reporter-free) at the same time. Unless indicated otherwise, each reporter within different sensors was tested with three biological replicates. All the data shown are mean values with standard deviation as error bars.

Fluorescent, bioluminescent and colorimetric signals were also acquired with a cell phone (OnePlus5) integrated camera (1/2.8" 16MP Sony IMX 398 sensor, 1.12 μm pixel size and F1.7 aperture). The microplates with cultured cells after each measurement were placed onto the surface of a Safe Imager™ (S37102, Invitrogen) blue-light transilluminator, and were covered with an amber filter in a dark environment. Fluorescent signals were acquired with the blue light on while the bioluminescent signals were acquired with the light off. The images with fluorescent or bioluminescent signals were captured with 30 s integration time.

Reporter gene expression assay *in vitro*

E. coli S30 Extract System for Circular DNA (L1020, Promega) was used for *in vitro* characterization of different reporters. Engineered *E. coli* with different reporters were first inoculated from a single colony on a freshly streaked solid LB plate to 2 mL terrific broth (TB) medium (12 g L^{-1} peptone, 24 g L^{-1} yeast extract, 12.54 g L^{-1} K_2HPO_4, 2.31 g L^{-1} KH_2PO_4, 4 mL L^{-1} glycerol), and cultured for 8 h at 37°C with shaking (160 rpm). Then 75 μL of each culture was diluted into 30 mL of fresh TB, and was incubated overnight at 37°C with shaking (160 rpm). The cultured cells were used for plasmid extraction. The plasmids were purified using ZymoPURE II™ Plasmid Midiprep Kit (D4201, Zymo Research) following the manufacturers' protocols, and then were further purified using Monarch® PCR & DNA Cleanup Kit (T1030S, NEB). The plasmids were eluted in nuclease free H_2O.

All cell-free reactions were prepared in a black 384-well microplate with clear bottom (for fluorescence and absorbance measurement, 788096, Greiner Bio-One) or a white 384-well microplate with clear bottom (for bioluminescence measurement, 788095, Greiner Bio-One) on ice, with 4 μL cell-free mixture topped with 5 μL of Chill-Out Liquid Wax (CHO1411, Bio-Rad) in each well. The plate was sealed with a transparent EASYseal plate sealer (676001, Greiner Bio-One) for fluorescence (bottom reading) and absorbance measurement, or covered with a transparent lid for bioluminescence measurement (bottom reading) to ease the addition of the substrate furimazine. The plate was incubated and measured continuously by BMG FLUOstar plate reader at 37˚C without shaking. The settings for measuring the fluorescence and bioluminescence were the same as for the *in vivo* characterization. Absorbance (A_{670}) was used for measuring the colorimetric reporter.

The plate reader data were processed using Omega MARS 3.20 R2, Microsoft Excel 2013 and GraphPad Prism 6.01. To calculate the RFU, RAU and RLU at different time points, the background of output signals was subtracted from each cell-free reaction by using its triplicate-averaged counterpart of the negative control (reporter-free) at the same time. All the data shown are mean values with standard deviation as error bars.

Calculation of sensor detection limit

The limit of detection (LOD) is the lowest analyte concentration likely to be reliably distinguished from the basal signal and at which detection is feasible.[4] The calculation has been described previously.[2]

Mathematical modelling and data fitting

Biochemical models were developed for individual transcription factor receptor modules to abstract their ligand-dependent dose response behaviors. The ordinary differential equation-based deterministic model was used for accurately modelling the gene regulation and expression across the full input or output range of the sensor systems.[5] It has been described previously.[1]

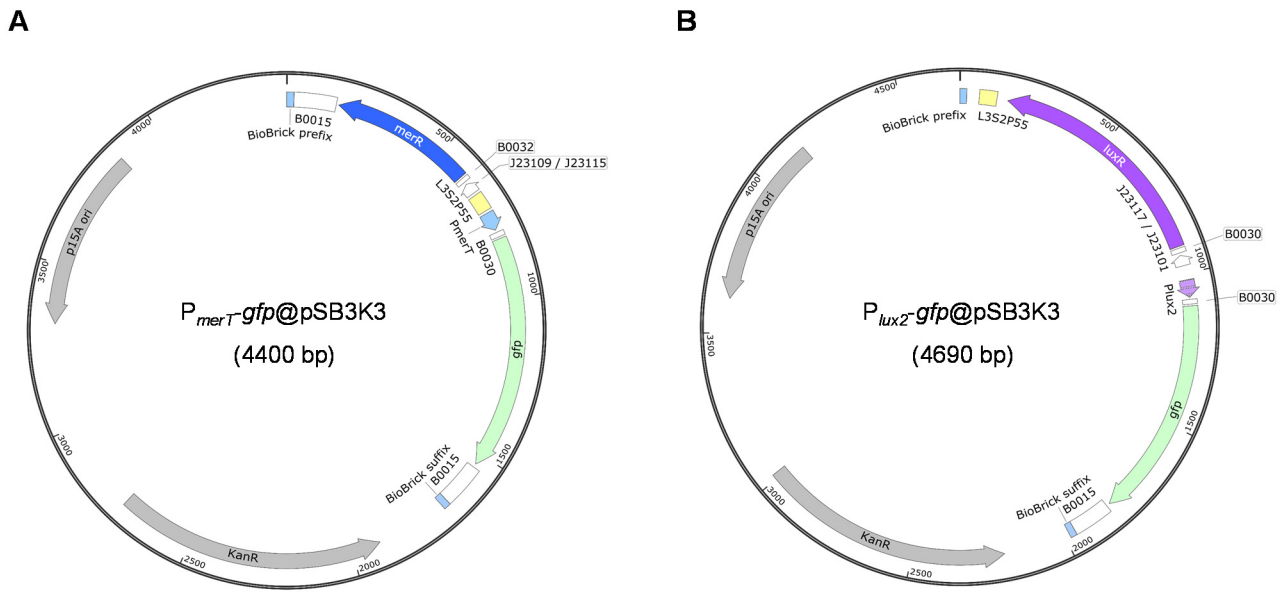


Figure S1: Representative plasmid maps for the sensor genetic circuits constructed and tested *in vivo* and *in vitro* in this study.

Plasmids maps showing the mercury (A) and quorum sensing molecule (B) sensor circuits with *gfp* as the output reporter. For the sensors with other reporters (except *lux* operon), *gfp* was replaced by *mCherry*, *mScarlet-I*, *degfp*, *lacZ* or *NanoLuc*. For the sensors using *lux* operon as output reporter, *gfp* with its RBS was replaced by the *lux* operon with RBS. Sequence details are listed in Table S1.

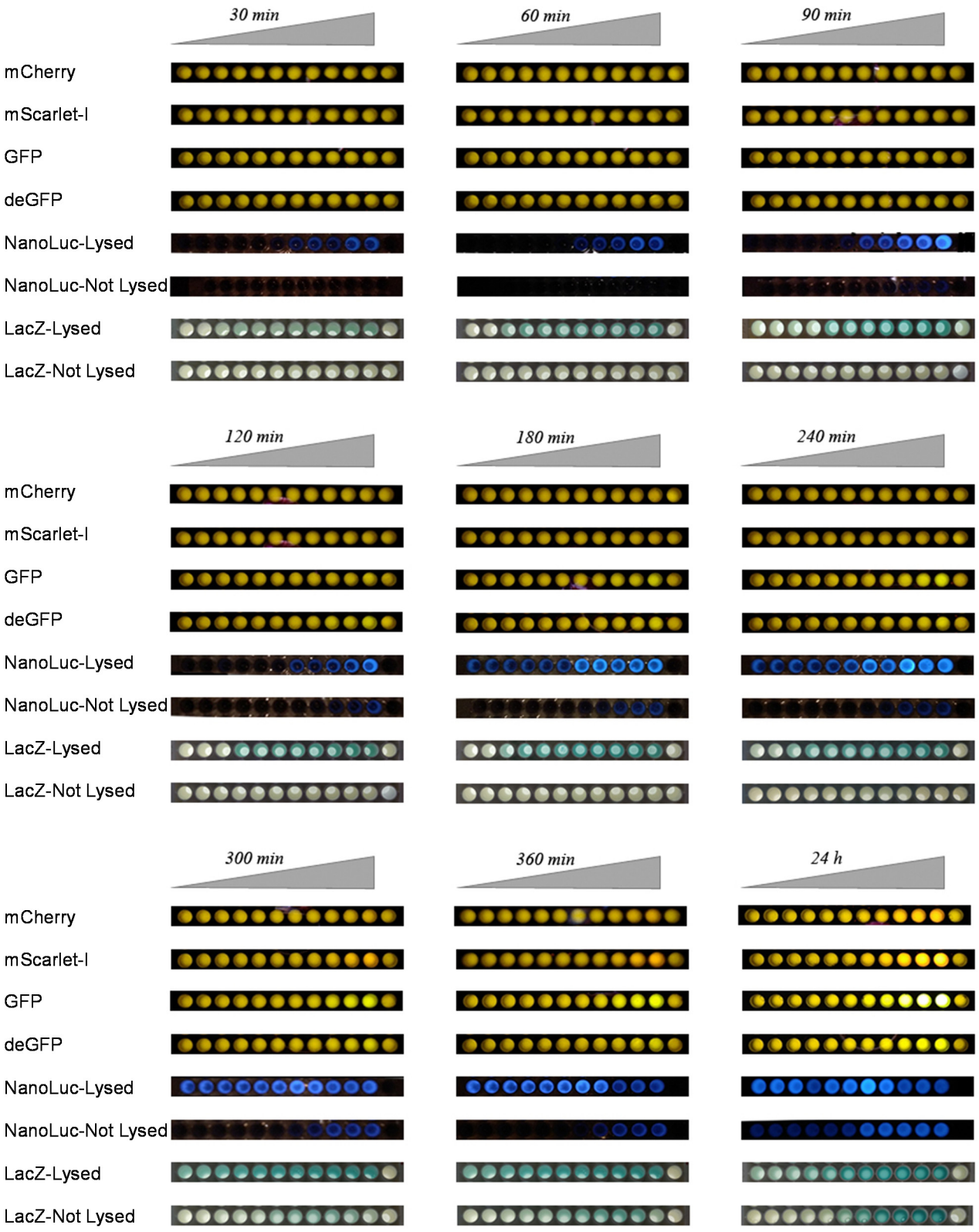


Figure S2: Cell phone images of diverse reporters within whole-cell mercury sensor. Related to Figures 2 and 3B–C.

The last well in each cell phone image shows the reporter-free negative control cultures.

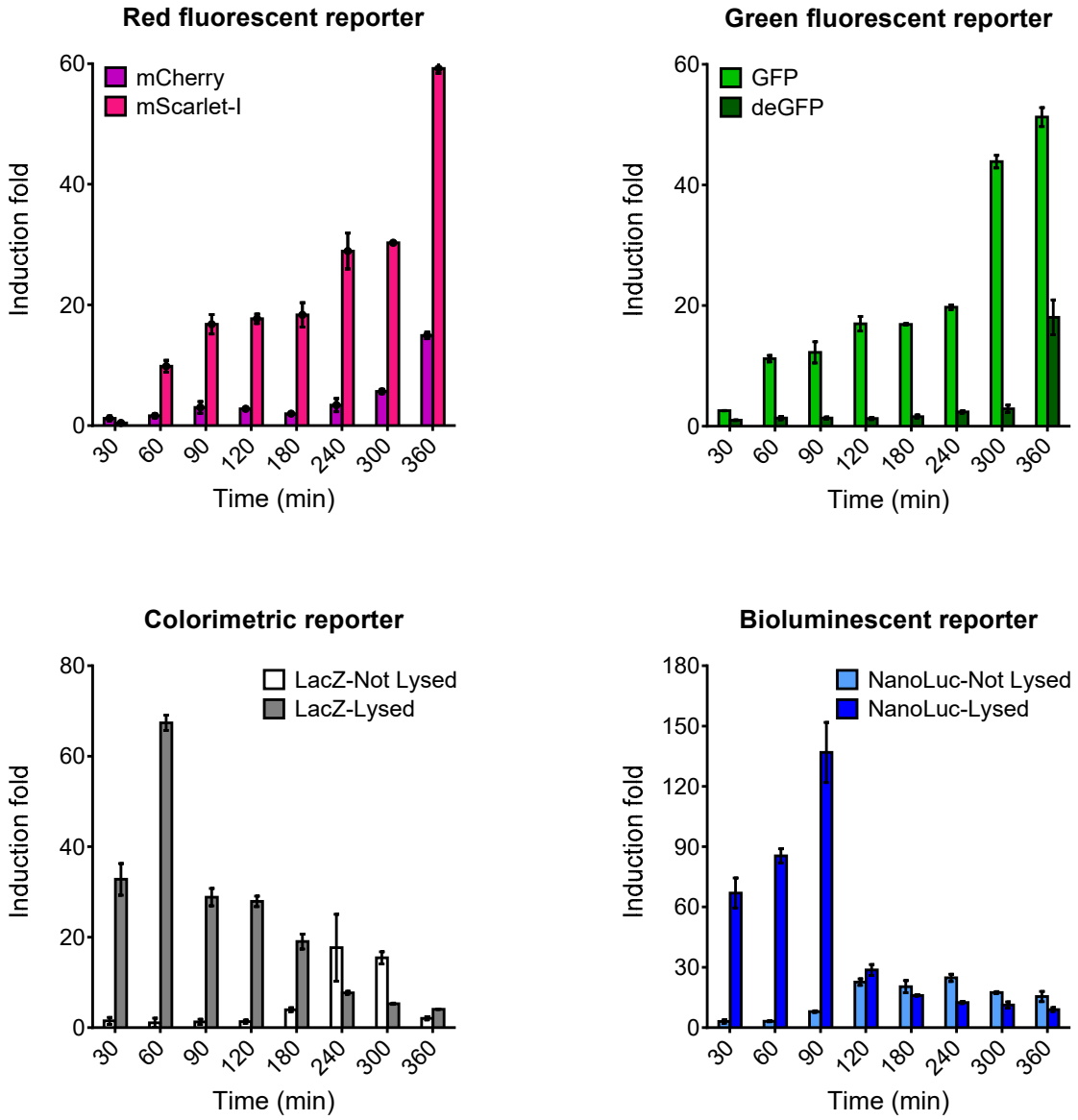
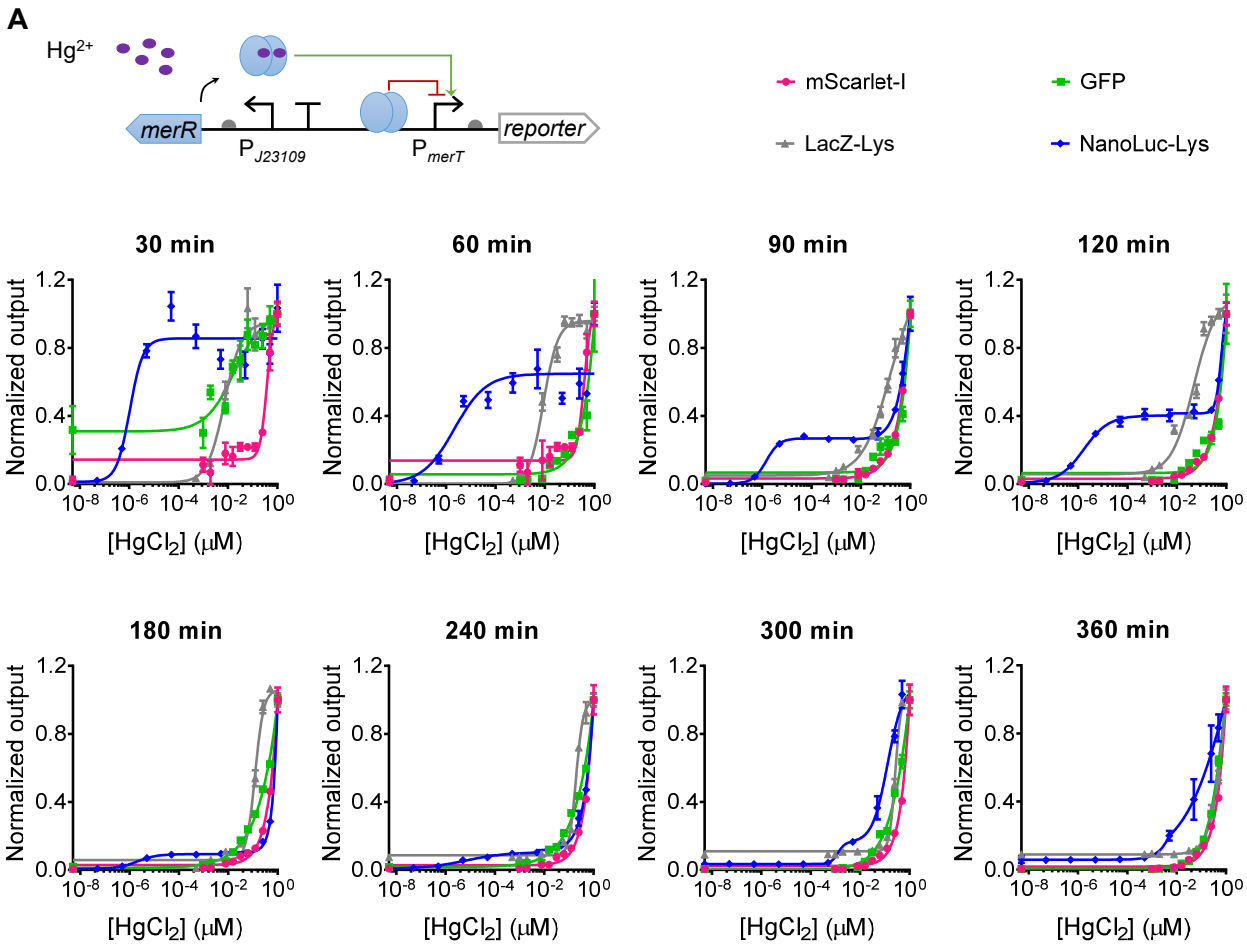


Figure S3: Induction fold of different reporters within whole-cell mercury sensor. Related to Figures 2 and 3B–C.

The mercury sensors with different reporters were induced with 0.1 μM $HgCl_2$. Values are mean ± s.d. (n = 3 biologically independent experiments).



B

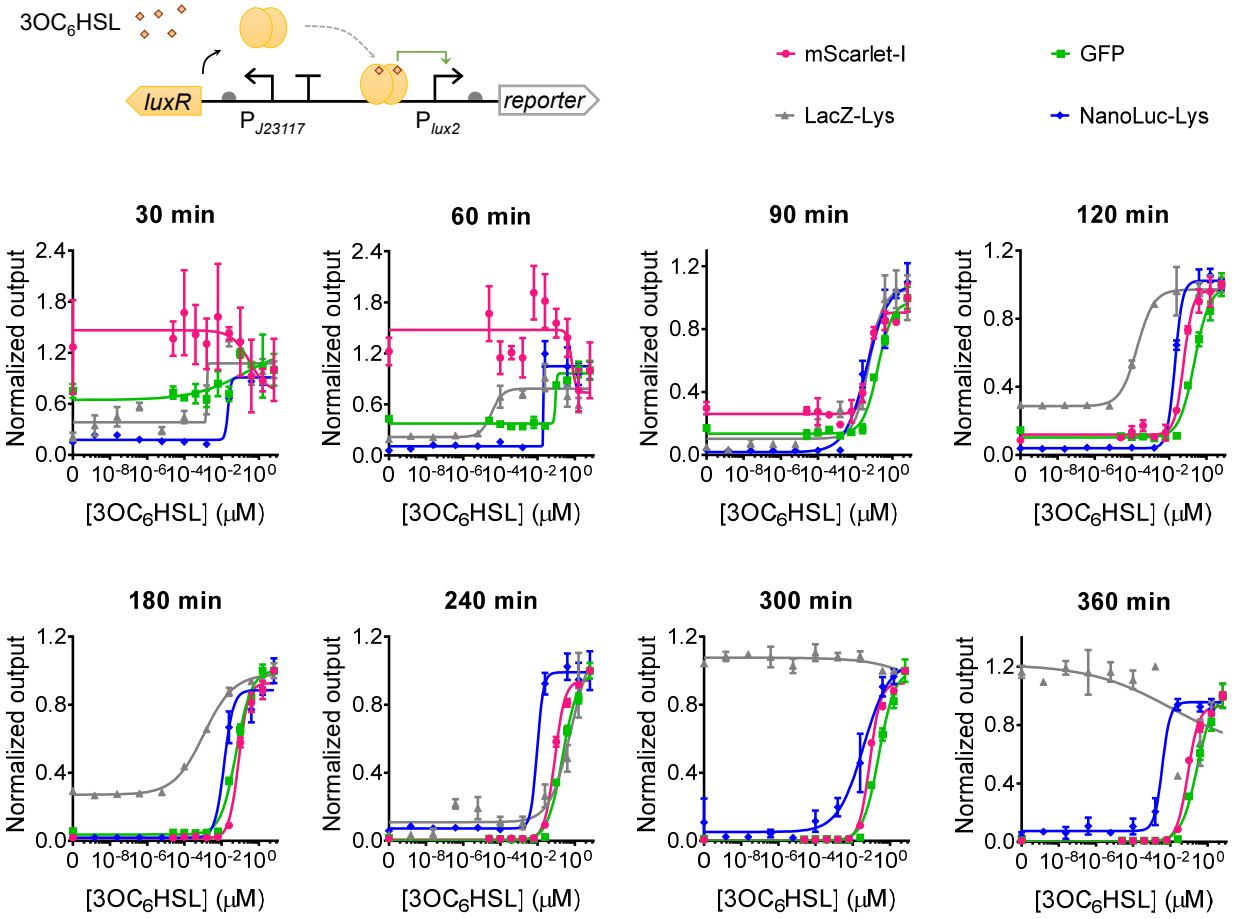
	30 min	60 min	90 min	120 min	180 min	240 min	300 min	360 min
mCherry	–	250.00	250.00	125.00	125.00	125.00	125.00	15.63
mScarlet-I	–	15.63	15.63	7.81	7.81	7.81	7.81	7.81
GFP	–	15.63	15.63	15.63	7.81	7.81	7.81	7.81
deGFP	–	500.00	250.00	250.00	250.00	250.00	250.00	125
NanoLuc-Lysed	5.00×10^{-5}	5.00×10^{-5}	5.00×10^{-5}	5.00×10^{-4}	5.00×10^{-4}	5.00×10^{-4}	0.50	0.50
NanoLuc-Not Lysed	–	0.50	0.50	0.50	0.05	0.50	5.00	5.00
LuxCDABE	5.00×10^{-4}	5.00×10^{-4}	5.00×10^{-4}	5.00×10^{-3}	5.00×10^{-3}	0.05	0.05	0.05
LacZ-Lysed	1.95	0.49	0.49	0.49	0.49	7.81	7.81	31.25
LacZ-Not Lysed	–	–	–	–	250.00	125.00	125.00	7.81

All data shown have the same unit of nM $HgCl_2$.
–: LOD cannot be calculated due to no significant difference observed between the mercury induced and non-induced samples.

Figure S4: Dose-response curves of whole-cell mercury sensor with diverse genetic reporters. Related to Figures 2 and 3B–C.

(A) Dose-response curves of whole-cell mercury sensor with diverse output genetic reporters. Top left, schematic of the mercury sensor. Values are mean ± s.d. (n = 3 biologically independent experiments). (B) Limits of detection (LOD) of whole-cell mercury sensors with different reporters.

A



B

	30 min	60 min	90 min	120 min	180 min	240 min	300 min	360 min
mScarlet-I	–	100.00	100.00	25.00	25.00	6.25	6.25	6.25
GFP	–	100.00	100.00	10.00	25.00	25.00	25.00	6.25
NanoLuc-Lysed	3.81×10^{-4}	3.81×10^{-4}	6.10×10^{-3}	9.77×10^{-2}	1.56	1.56	25.00	25.00
LacZ-Lysed	1.56	6.10×10^{-3}	9.77×10^{-2}	9.77×10^{-2}	1.56	1.56	1.56	–

All data shown have the same unit of nM $3OC_6HSL$.
–: LOD cannot be calculated due to no significant difference observed between the $3OC_6HSL$ induced and non-induced samples.

Figure S5: Dose-response curves of whole-cell quorum sensing molecule sensor with diverse output genetic reporters. Related to Figure 3E–F.

(A) Dose-response curves of whole-cell quorum sensing molecule sensor with diverse output genetic reporters. Top left, schematic of the quorum sensing molecule sensor. Values are mean ± s.d. (n = 3 biologically independent experiments). (B) Limits of detection (LOD) of whole-cell quorum sensing molecule sensors with different reporters.

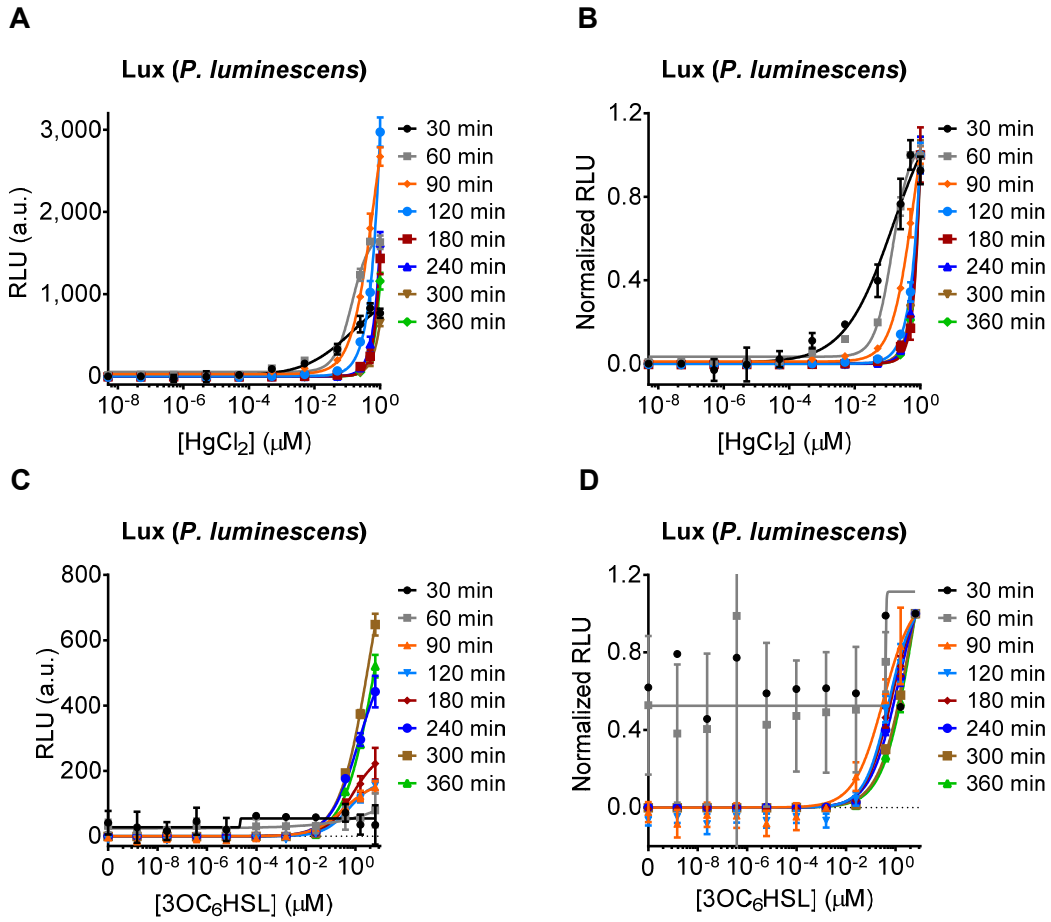


Figure S6: Dose-response curves of sensors *in vivo* with *lux* operon as reporter.

(A,B) Dose response curves (A) and normalized response curves (B) of a mercury sensor with *lux* operon from *P. luminescens* as output reporter. (C,D) Dose response curves (C) and normalized response curves (D) of a quorum sensing molecule sensor with *lux* operon from *P. luminescens* as output reporter. Values are mean ± s.d. (n = 3 biologically independent experiments). a.u., arbitrary units.

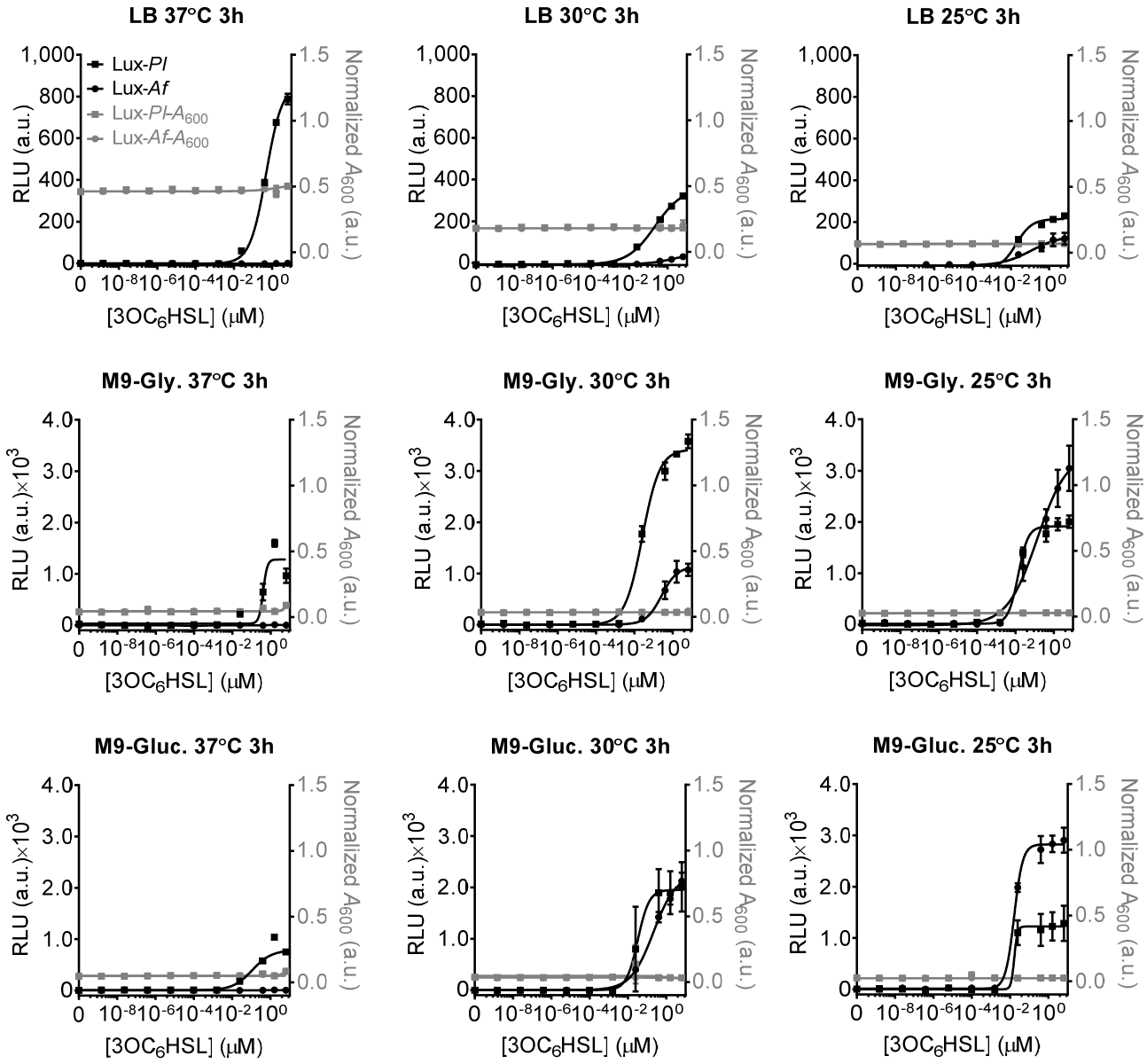


Figure S7: Characterization of *lux* operon within quorum sensing molecule sensor using different media and incubation temperature.

Lux-*Pl*, LuxCEABE from *P. luminescens*. Lux-*Af*, LuxCEABE from *A. fischeri*. M9-Gly., M9 medium with glycerol as carbon source. M9-Gluc., M9 medium with glucose as carbon source. All data were collected 3 h post induction and incubation. Values are mean ± s.d. (n = 2 biologically independent experiments). a.u., arbitrary units.

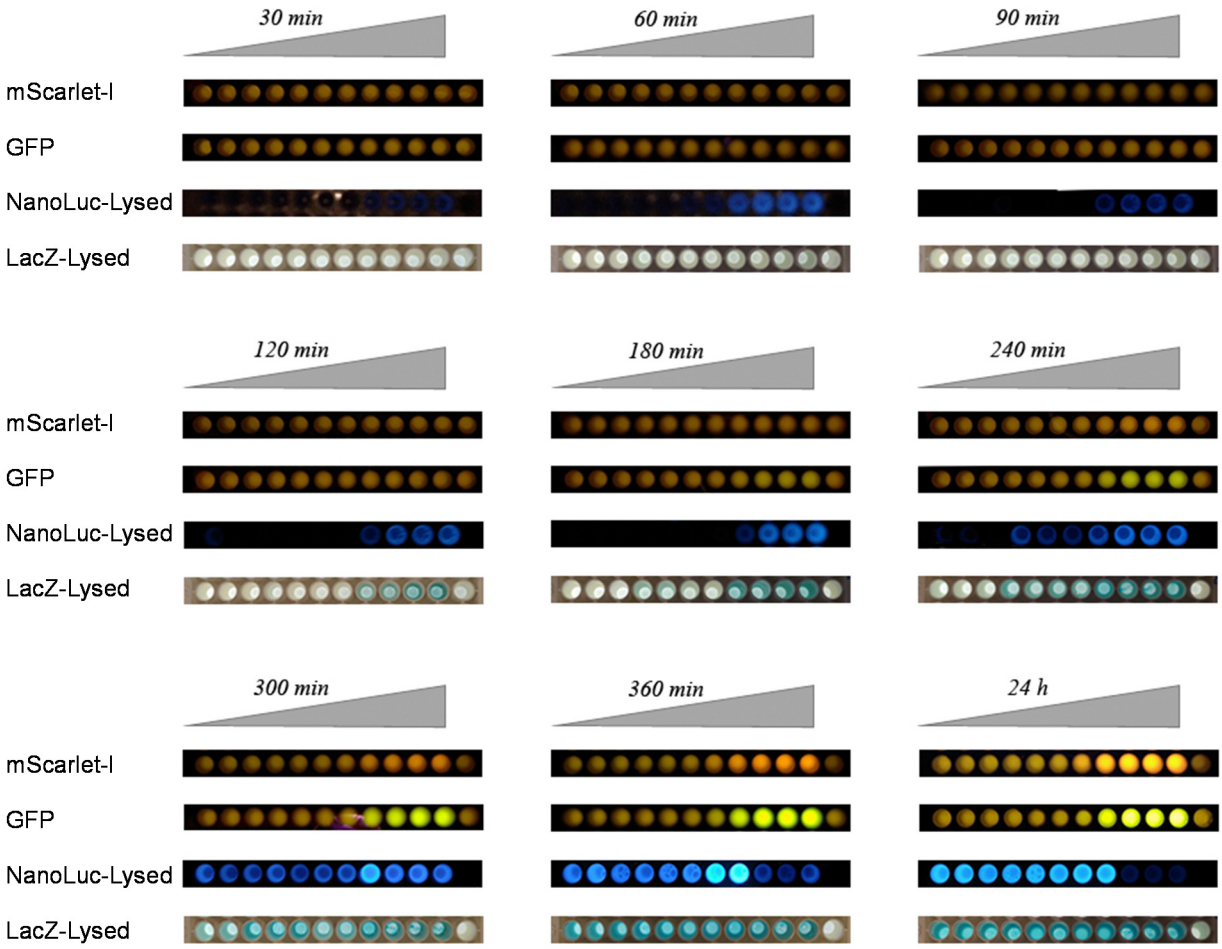
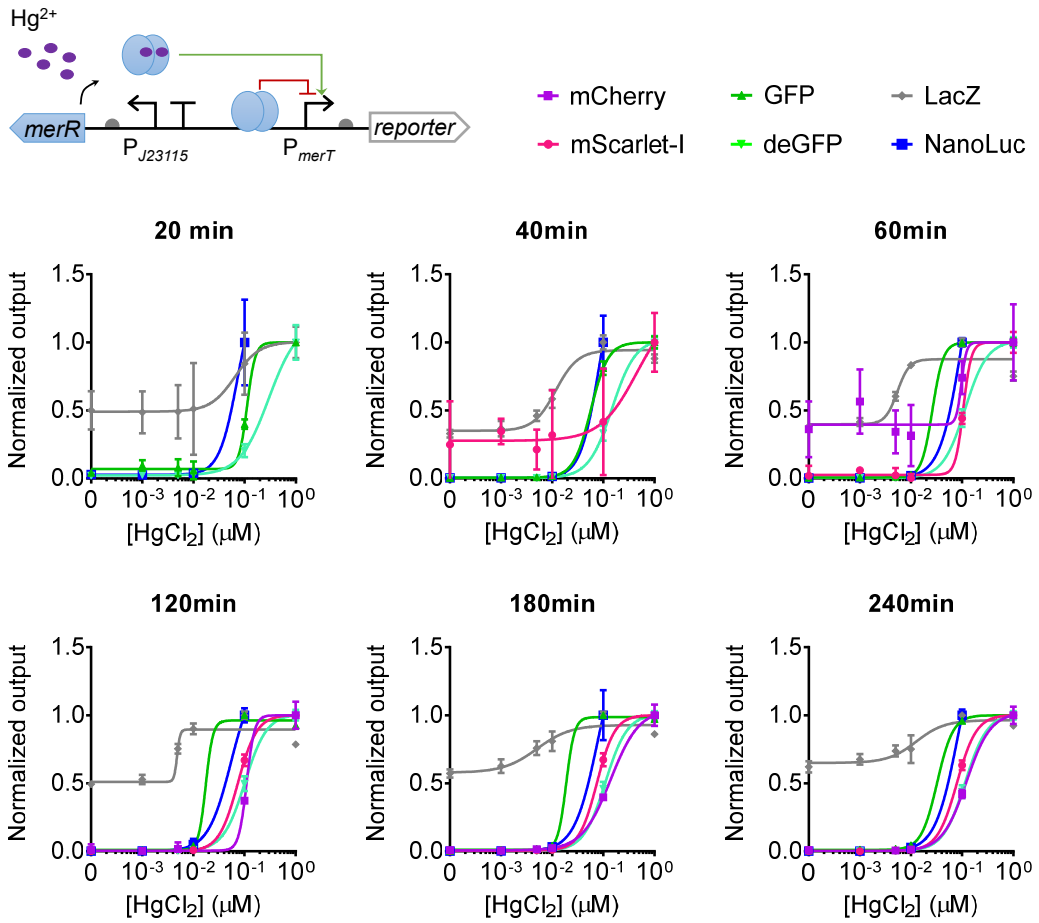


Figure S8: Cell phone images of diverse reporters within whole-cell quorum sensing molecule sensor. Related to Figure 3E–F.

The last well in each cell phone image shows the reporter-free negative control cultures.

A



B

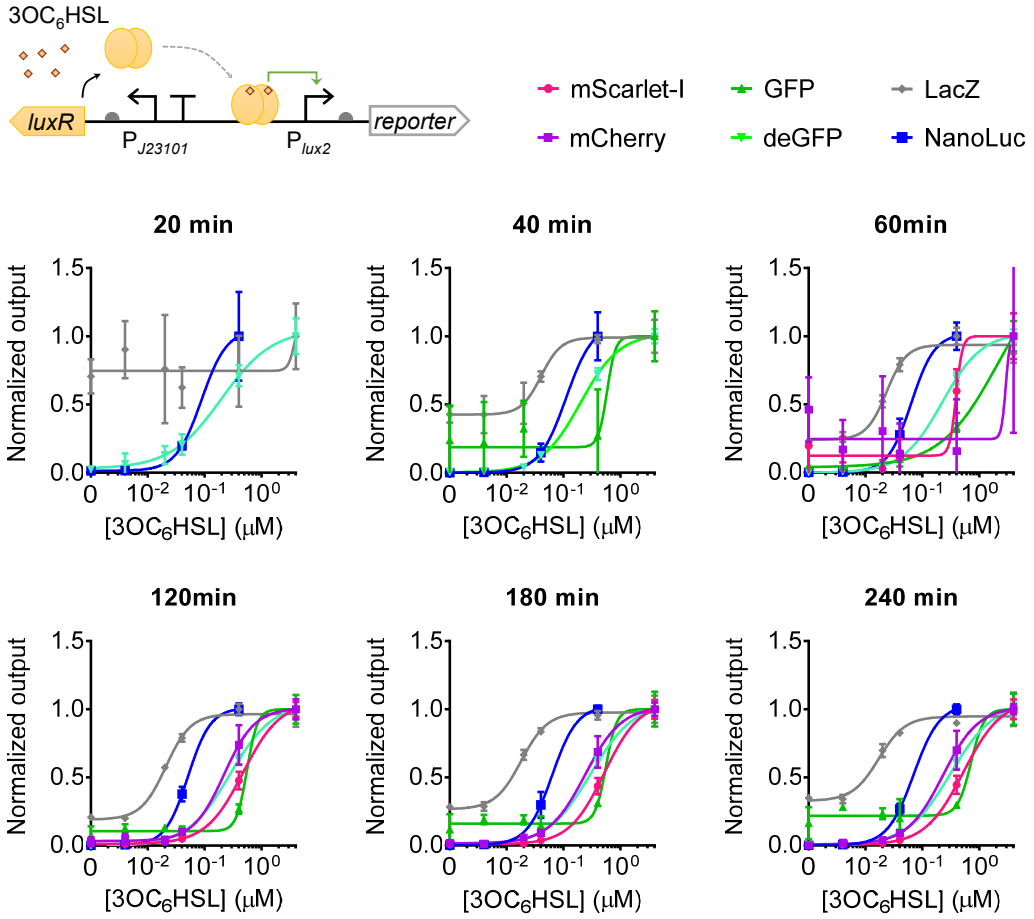
Reporters	20 min	40 min	60 min	120 min	180 min	240 min
mCherry	–	–	–	0.01 – 0.10	0.01 – 0.10	0.01 – 0.10
mScarlet-I	–	–	0.01 – 0.10	0.01 – 0.10	0.01 – 0.10	5.00×10^{-3} – 0.01
GFP	0.01 – 0.10	5.00×10^{-3} – 0.01	1.00×10^{-3} – 5.00×10^{-3}	1.00×10^{-3} – 5.00×10^{-3}	$< 1.00 \times 10^{-3}$	$< 1.00 \times 10^{-3}$
deGFP	0.01 – 0.10	5.00×10^{-3} – 0.01	1.00×10^{-3} – 5.00×10^{-3}	5.00×10^{-3} – 0.01	1.00×10^{-3} – 5.00×10^{-3}	1.00×10^{-3} – 5.00×10^{-3}
LacZ	0.10 – 1.00	1.00×10^{-3} – 5.00×10^{-3}	1.00×10^{-3} – 5.00×10^{-3}	1.00×10^{-3} – 5.00×10^{-3}	1.00×10^{-3} – 5.00×10^{-3}	0.01 – 0.10
NanoLuc	0.01 – 0.10	1.00×10^{-3} – 0.01	1.00×10^{-3} – 0.01	0.01 – 0.10	1.00×10^{-3} – 0.01	1.00×10^{-3} – 0.01

All data shown have the same unit of μM $HgCl_2$.
–: LOD cannot be calculated due to no significant difference observed between the mercury induced and non-induced samples.

Figure S9: Dose-response curves of cell-free mercury sensor with diverse output genetic reporters. Related to Figure 4B–C.

(A) Schematic and normalized dose-response curves of cell-free mercury sensors. Values are mean ± s.d. (n = 3 technical replicates). (B) Limit of detection (LOD) of the sensor tested in A. As only a few concentrations of mercury were tested, the LOD were shown as a range.

A



B

Reporters	20 min	40 min	60 min	120 min	180 min	240 min
mCherry	–	–	–	0.04 – 0.40	0.02 – 0.04	0.02 – 0.04
mScarlet-I	–	–	0.40 – 4.00	0.04 – 0.40	0.02 – 0.04	4.00×10^{-3} – 0.02
GFP	–	0.40 – 4.00	0.04 – 0.40	0.04 – 0.40	0.40 – 4.00	0.40 – 4.00
deGFP	0.02 – 0.04	4.00×10^{-3} – 0.02	4.00×10^{-3} – 0.02	4.00×10^{-3} – 0.02	4.00×10^{-3} – 0.02	$< 4.00 \times 10^{-3}$
LacZ	–	0.02 – 0.04	4.00×10^{-3} – 0.02	4.00×10^{-3} – 0.02	4.00×10^{-3} – 0.02	4.00×10^{-3} – 0.02
NanoLuc	4.00×10^{-3} – 0.04	$< 4.00 \times 10^{-3}$	$< 4.00 \times 10^{-3}$	$< 4.00 \times 10^{-3}$	4.00×10^{-3} – 0.04	$< 4.00 \times 10^{-3}$

All data shown have the same unit of μM $3OC_6HSL$.
–: LOD cannot be calculated due to no significant difference observed between the $3OC_6HSL$ induced and non-induced samples.

Figure S10: Dose-response curves of cell-free quorum sensing molecule sensor with diverse output genetic reporters. Related to Figure 4E–F.

(A) Schematic and normalized dose-response curves of cell-free quorum sensing molecule sensors. Values are mean ± s.d. (n = 3 technical replicates). (B) Limit of detection (LOD) of the sensor tested in A. As only a few concentrations of $3OC_6HSL$ were tested, the LOD were shown as a range.

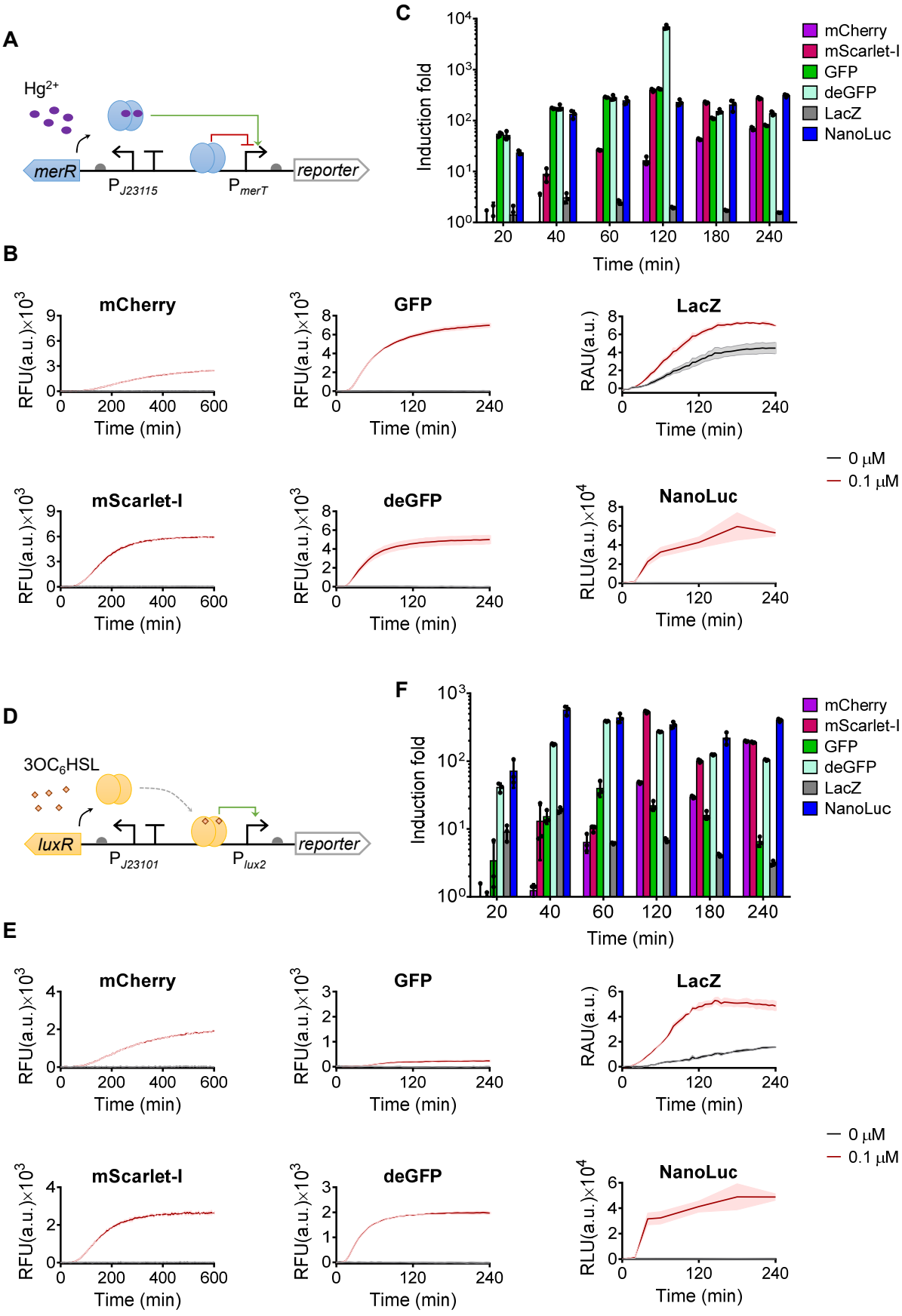

A
Hg2+
merR
P J23115
P merT
reporter
B
mCherry
GFP
LacZ
mScarlet-I
deGFP
NanoLuc
RFU(a.u.)×10^3
RAU(a.u.)
RLU(a.u.)×10^4
Time (min)
0 μM
0.1 μM
C
Induction fold
mCherry
mScarlet-I
GFP
deGFP
LacZ
NanoLuc
Time (min)
D
3OC6HSL
luxR
P J23101
P lux2
reporter
E
F

Figure S11: Characterization of diverse genetic reporters within mercury and quorum sensing molecule cell-free biosensors. Related to Figure 4.

(A,D) Schematics of the mercury or quorum sensing molecule sensor modules (J23115-*merR*-P_{merT} or J23101-*luxR*-P_{lux2}) coupled to diverse genetic reporters. (B,E) Dynamic output responses of the sensors responding to two concentrations of $HgCl_2$ (B) or $3OC_6HSL$ (E). (C,F) Fold of induction over time of the mercury or quorum sensing cell-free sensors with different reporters responding to 0.1 μM $HgCl_2$ (C) and 0.4 μM $3OC_6HSL$ (F) respectively. Induction fold was calculated using the output with induction divided by the output without induction. Values are mean ± s.d. (n = 3 technical replicates). a.u., arbitrary units.

Table S1: List of genetic parts and sequences used in this study

Underlined sequences indicate –35 and –10, or –24 and –12 promoter regions. Sequences in blue are MerR binding sites, sequences in yellow are LuxR binding sites.

Name	Type	DNA sequence (5'-3')
J23101	Constitutive promoter[1]	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC
J23109	Constitutive promoter[2]	TTTACAGCTAGCTCAGTCCTAGGGACTGTGCTAGC
J23115	Constitutive promoter[1]	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC
J23117	Constitutive promoter[1]	TTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGC
P_{merT}	Inducible promoter[6]	TTCCATATCGCTTGACTACGTACATGAGTACGGAAGTAAGGTTACGCTATCCAATCC
P_{lux2}	Inducible promoter[1]	AGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTACTTTCGAATAAA
B0030	RBS[5]	ATTAAAGAGGAGAAA
R0032	RBS[5]	TCACACAGGAAAG
B0015	Terminator[7]	CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA
L3S2P55	Terminator[8]	CTCGGTACCAAAGACGAACAATAAGACGCTGAAAAGCGTCTTTTTTCGTTTTGGTCC
merR	Gene[6]	ATGGAAAATAATTTGGAAAACCTGACCATTGGCGTTTTTGCCAAGGCGGCCGGGGTCAACGTGGAGACAATCCGCTTCTATCAGCGCAAGGGCCTGTTGCGGGAACCGGACAAGCCTTACGGCAGCATCCGCCGCTATGGGGAGGCGGACGTGGTTCGGGTGAAATTCGTGAAATCGGCACAGCGGCTGGGGTTCAGTCTGGACGAGATTGCCGAGCTGTTGCGGCTCGACGATGGCACCCACTGCGAGGAGGCCAGCAGCCTGGCCGAACACAAGCTCAAGGACGTGCGCGAGAAGATGGCCGACTTGGCGCGCATGGAAACCGTGCTGTCTGAACTCGTGTGCGCCTGCCATGCACGAAAGGGGAATGTTTCCTGCCCGTTGATCGCGTCACTACAGGGCGAAGCAGGCCTGGCAAGGTCAGCTATGCCTTAG
luxR	Gene[1]	ATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCTTGTAGAAGCAATAATGATATTAATCAATGCTTATCTGATATGACTAAAATGGTACATTGTGAATATTATTTACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATCCTAGATAATTACCCTAAAAAATGGAGGCAATATTATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTATTCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAAAACAATGCTGTAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCACTGGGTTTAGTTTCCCTATTCATACGGCTAACAATGGCTTCGGAATGCTTAGTTTTGCACATTCAGAAAAAGACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATACCATTAATTGTTCCTTCTCTAGTTGATAATTATCGAAAAATAAATATAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAAAGAATGTTTAGCGTGGGCATGCGAAGGAAAAAGCTCTTGGGATATTTCAAAAATATTAGGTTGCAGTGAGCGTACTGTCACTTTCCATTTAACCAATGCGCAAATGAAACTCAATACAACAAACCGCTGCCAAAGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATACTTTAAAAATTAATAA
mCherry	Gene*	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCTTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACAT

		CAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAA CGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAA
mScarlet-I	Gene[9]	ATGGTGAGTAAAGGAGAAGCTGTGATTAAAGAGTTCATGCGCTTCAAAGTTCACA TGGAGGGTTCTATGAACGGTCACGAGTTCGAGATCGAAGGCGAAGGCGAGGGCCG TCCGTATGAAGGCACCCAGACCGCCAAACTGAAAGTGACTAAAGGCGGCCCGCTG CCTTTTTCCTGGGACATCCTGAGCCCGCAATTTATGTACGGTTCTAGGGCGTTCA TCAAACACCCAGCGGATATCCCGGACTATTATAAGCAGTCTTTTCCGGAAGGTTT CAAGTGGGAACGCGTAATGAATTTTGAAGATGGTGGTGCCGTGACCGTCACTCAG GACACCTCCCTGGAGGATGGCACCCTGATCTATAAAGTTAAACTGCGTGGTACTA ATTTTCCACCTGATGGCCCGGTGATGCAGAAAAAGACGATGGGTTGGGAGGCGTC TACCGAACGCTTGTATCCGGAAGATGGTGTGCTGAAAGGCGACATTAAAATGGCC CTGCGCCTGAAAGATGGCGGCCGCTATCTGGCTGACTTCAAAACCACGTACAAAG CCAAGAAACCTGTGCAGATGCCTGGCGCGTACAATGTGGACCGCAAACTGGACAT CACCTCTCATAATGAAGATTATACGGTGGTAGAGCAATATGAGCGCTCCGAGGGT CGTCATTCTACCGGTGGCATGGATGAACTATACAAA
gfp	Gene[7]	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAG ATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGC AACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTT CCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGAT ACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTA TGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCT GAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTG ATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTC ACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTC AAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAAC AAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTC CACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTT CTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAAT AA
degfp	Gene[10]	ATGGAGCTTTTCACTGGCGTTGTTCCCATCCTGGTCGAGCTGGACGGCGACGTAA ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC CTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACA TGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCG CACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGG ACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTA TATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCAC AACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA TCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGC CCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTG ACCGCCGCCGGGATCTAA
NanoLuc	Gene[11]	ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGCGACAGACAGCCGGCTACA ACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGTTTCAGAATCTCGG GGTGTCCGTAACTCCGATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAAG ATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGGCGACCAAATGGGCC AGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGT GATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGATCGAC TATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG TAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCAACCC CGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCTG TGCGAACGCATTCTGGCGTAA
LacZ	Gene (Amplified from *E. coli* MG1655 by PCR)	ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAA ACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTG GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTG AATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCT GGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCA GATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGGTC AATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTA ATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGT TAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGAC

		AGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACC GCCTCGCGGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGGA TATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACT ACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCG CTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGT AACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTC GGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTC TGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGC GGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGAT GTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGC CGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGT CATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTT AACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCG ACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGT GCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGC GTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGC TGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAA ATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCACG GCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCC CGGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCG CCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGCGGTTTC GCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCT GGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTC GGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATGAAC GGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCAAAACACC AGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGA ATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGAT GGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTAAAC AGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCT CACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATC AGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCG CGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCT GGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGG ATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCAC CGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTG GGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAG TGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCACGCGTGGC AGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTACCGGATTGATGGTAG TGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCG GCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGC TCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTTGA CCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAA AACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCG GCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAG CCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTC CATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAGTTTC AGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAATAA
lux operon (*P. luminescens*)	Gene with RBS**	AGGAGGGGCAAATATGACTAAAAAAATTTCATTCATTATTAACGGCCAGGTTGAA ATCTTTCCCGAAAGTGATGATTTAGTGCAATCCATTAATTTTGGTGATAATAGTG TTTACCTGCCAATATTGAATGACTCTCATGTAAAAAACATTATTGATTGTAATGG AAATAACGAATTACGGTTGCATAACATTGTCAATTTTCTCTATACGGTAGGGCAA AGATGGAAAAATGAAGAATACTCAAGACGCAGGACATACATTCGTGACTTAAAAA AATATATGGGATATTCAGAAGAAATGGCTAAGCTAGAGGCCAATTGGATATCTAT GATTTTATGTTCTAAAGGCGGCCTTTATGATGTTGTAGAAAATGAACTTGGTTCT CGCCATATCATGGATGAATGGCTACCTCAGGATGAAAGTTATGTTCGGGCTTTTC CGAAAGGTAAATCTGTACATCTGTTGGCAGGTAATGTTTCATTATCTGGGATCAT GTCTATATTACGCGCAATTTTAACTAAGAATCAGTGTATTATAAAAACATCGTCA ACCGATCCTTTTACCGCTAATGCATTAGCGTTAAGTTTTATTGATGTAGACCCTA ATCATCCGATAACGCGCTCTTTATCTGTTATATATTGGCCCCACCAAGGTGATAC ATCACTCGCAAAAGAAATTATGCGACATGCGGATGTTATTGTCGCTTGGGGAGGG CCAGATGCGATTAATTGGGCGGTAGAGCATGCGCCATCTTATGCTGATGTGATTA AATTTGGTTCTAAAAAGAGTCTTTGCATTATCGATAATCCTGTTGATTTGACGTC

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lux operon (*A. fischeri*)	Gene with RBS**	AGGAGGGGCAAATATGAATAAATGTATTCCAATGATAATTAATGGAATGATTCAA GATTTTGATAATTATGCATATAAAGAAGTTAAACTAAATAATGATAATAGAGTAA AATTATCTGTCATTACTGAAAGTTCAGTTTCAAAAACATTAAATATCAAAGATAG AATTAATCTAAATTTAAATCAGATTGTGAATTTTTTATATACCGTTGGTCAACGA TGGAAAAGTGAAGAATATAATCGGCGACGAACCTATATCCGTGAGTTAAAAACAT ATCTTGGTTATTCTGATGAAATGGCAAGATTAGAAGCGAATTGGATTGCAATGTT ATTGTGCTCTAAAAGTGCTTTGTATGACATTGTTAATTATGATTTGGGCTCTATA CACGTATTAGATGAATGGCTTCCACGTGGTGATTGCTATGTTAAAGCACAACCGA AAGGTGTTTCTGTTCACTTGTTAGCTGGTAATGTTCCATTATCAGGAGTGACATC TATTTTGCGTGCTATTTTAACAAAAAATGAGTGCATTATTAAAACTTCGTCTTCA GATCCTTTTACTGCAAACGCTTTAGTTTCCAGTTTTATTGATGTTAATGCAGACC ATCCAATAACCAAATCAATGTCTGTTATGTATTGGCCGCATGATGAAGATATGAC TCTATCTCAAAGAATAATGAATCATGCCGACGTGGTTATTGCTTGGGGTGGAGAC GAGGCGATTAAATGGGCGGTAAAATATTCACCACCGCATGTCGATATTCTGAAAT TTGGACCAAAGAAAAGCTTAAGTATTATTGAAGCTCCTAAAGATATAGAAGCCGC AGCAATGGGGGTTGCTCATGATATTTGTTTCTATGACCAGCAAGCCTGCTTCTCT ACTCAAGACGTTTATTATATAGGAGATAATTTACCTTTATTTTTAAATGAACTTG AAAAACAGCTAGATCGATACGCGAAAATTTTACCAAAAGGTTCAAATAGTTTTGA TGAAAAAGCGGCGTTTACTCTTACTGAAAAAGAAAGTCTATTTGCTGGATATGAA GTGAGAAAGGGAGATAAGCAAGCTTGGTTAATAGTCGTATCACCTACAAATAGCT

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GAAGAGCAAAAAGAAATACGGGATAAATTAGTTCGTGAGGCTTTTTATTTTCACT
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TGAAAGAAAACAATATTGAATTTAAAGGAGGAGATAGAGTACATATTATTACTGG
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TTATTAATGGATACTTTCCAACTCGACAAGATTAATCAAATTAGAGATACCTTTA
ATCAAGTTGAGCTTAATACTTGTTTTTTTGAAGATGAATTTCAAAGAAAACATGT
TCCACCGTGGGTATATGCTCGGGCTCTTGATCCTGAAACCTTGAAACCCGTAGCA
GATGGTGAGATCGGGTTGTTAAGTTATATGGACGCCTCATCAACTGCTTACCCTG
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TTACCCAGGGGTAACTGTTGAGATTGTTCGGCGCTTAAATACACGTGCGCAAAAA
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TGATTGTTGATGGTAGAGTTTCAAAAATAGTATTAGCGTCAATAAGAAATAATAT
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CAAAAAATTACGAAATAGAATTGCATATTGGTAGTTCGAATAGAGACTGCTCATT
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GATGCTCCCCATGGAAACGCTTGGTTACGGTCTGAAAGTAATAACCCATTGCTAT
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GTTTGTGGACCCTTCATGATGGCTAAAACAGCAAAAGAAAAATTAATTGAAGAGA
AAAAAGCAAAGTCAGAACAGATGTTTGCCGATGCTTTTGCATACGTATAA

*: http://parts.igem.org/Part:BBa_J06504

**: gift from Prof Belkin Shimshon (Hebrew University of Jerusalem)

Table S2: List of oligonucleotides used in this study

Underlined sequences are the enzyme cutting site and the sequences in bold are the coding sequence regions of the reporter genes.

Primer (set)	Sequence (5'– 3')	Usage
mCherry_F	CGTCTAGAGATTAAAGAGGAGAAATACTAG**ATGGTGAGCAAGG**	To add XbaI cutting site and B0030 to the front of *mCherry*
mCherry_R	GCACTAGTATTA**TTACTTGTACAGCTCGTCCATGCCGC**	To add SpeI cutting site to the end of *mCherry*
ScarLet-I_F	CGTCTAGAGATTAAAGAGGAGAAATACTAG**ATGGTGAGTAAAGG**	To add XbaI cutting site and B0030 to the front of *mScarlet-I*
ScarLet-I_R	GCACTAGTATTA**TTAGTATAGTTCATCCATGCCACCTTAGTATAGTTCATCCATGCCACC**	To add SpeI cutting site to the end of *mScarlet-I*
GFP_F	CGGAATTCGCGGCCGCTCTAGAGATTAAAGAGGAGAAATACTAG**ATGGAGCTTTTCACTGGCGTTGTTCCC**	To insert BioBrick prefix and B0030 to the front of *gfp*
GFP_R	GCACTAGTATTA**TTAGATCCCGGCGGCGGTCACG**	To add SpeI cutting site to the end of *gfp*
deGFP_F	CGGAATTCGCGGCCGCTCTAGAGATTAAAGAGGAGAAATACTAG**ATGGAGCTTTTCACTGGCGTTGTTCCC**	To insert BioBrick prefix and B0030 to the front of *degfp*
deGFP_R	GCACTAGTATTA**TTAGATCCCGGCGGCGGTCACG**	To add SpeI cutting site to the end of *degfp*
NanoLuc_F	CGGAATTCGCGGCCGCTCTAGAGATTAAAGAGGAGAAATACTAG**ATGGTCTTCACACTCGAAGATTTCGTTGGG**	To insert BioBrick prefix and B0030 to the front of *NanoLuc*
NanoLuc_R	GCACTAGTATTA**TTACGCCAGAATGCGTTCGCACAGC**	To add SpeI cutting site to the end of *NanoLuc*
Rbs30_spacer_F	CTGGAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACTAG	To introduce B0030
Rbs30_spacer_R	ATTACCGCCTTTGAGTGAGC	
PmerT_J23115_F	GCTACTAGTATTTATAGCTAGCTCAGCCCTTGG	To introduce P_{merT}
PmerT_R	GGAATTCGCGGCCGCTTCTAGAGATATAAACGCAGAAAGGCCC	
PmerT_J23109_F	GCTACTAGTATTTACAGCTAGCTCAGTCCTAGG	
Additional B0015 Terminator	GCTTCTAGAGCTCGGTACCAAAGACGAACAATAAGACGCTGAAAAGCGTCTTTTTTCGTTTTGGTCCTACTAGAGTTCCATATCGCTTGACTACG	To introduce the additional B0015 terminator
mCherry_F	CGTCTAGAGATTAAAGAGGAGAAATACTAG**ATGGTGAGCAAGG**	To add XbaI cutting site and B0030 to the front of *mCherry*

mCherry_R	GCACTAGTATTA**TTACTTGTACAGCTCGTCCATGCCGC**	To add SpeI cutting site to the end of *mCherry*
J23101_fromJ23117_F	CCTAGGACTGAGCTAGCTGTAAATCACACTGGCTCACCTTC	To mutate J23117 to J23101 promoter by point mutation
J23101_fromJ23117_R	GCTAGCTCAGTCCTAGGTATTATGCTAGCTACTAGAGATTAAAGAGG	To mutate J23117 to J23101 promoter by point mutation
E_X_r30_lacZ_fwd	CCGGAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACTAG**ATGACCATGATTACGGATTCACTGGCC**	For amplifying *lacZ* from *E. coli* MG1655 genome
S_lacZ_rev	GCTACTAGTATTA**TTTTTGACACCAGACCAACTGGTAATGGTAGCGACCGGCGCTCAGCTGAAACTCCGCCGATACTGACGGGCTCC**	
GB_ORF8-B15_fwd2	**GCCCTATAG**TAATACTAGAGCCAGGC	For amplifying the sensor circuits and *lux* operon for AQUA cloning[12]
GB_Plux2_rev	ATTTGCCCCTCCTCTCTAGTTTTATTCGAAAG	
GB_PmerT_rev	ATTTGCCCCTCCTCTCTAGTAGGATTGGATAGCGTAACC	
GB_LuxC_fwd	ACT**AGAGAGGAGGGGCAAATATGACTAAAAAAATTTC**	
GB_ORF8_rev	CTCTAGTAT**TACTATAGGGCGAATTCCTTTAATC**	
GB_VFLuxC_fwd	ACTAGAGAGGAGGGGCAAAT**ATGAATAAATGTATTCCAATG**	
GB_VFLuxG_rev	CTCTAGTATTATTA**TACGTATGCAAAAGCATC**	
GB_VFLuxG-B15_fwd	**CATACGTA**TAATAATACTAGAGCCAGGC	
-XbaI_luxD_fwd	**TGCCGGATAACCTAGATTTTGAAGGC**	For removing the XbaI and EcoRI sites in the *lux* operon (*P. luminescens*) using AQUA cloning[12]
-XbaI_luxD_rev	**TGGCCTTCAAAATCTAGGTTATCCGGC**	
-EcoRI_ORF8_rev	**CTATAGGGCAAATTCCTTTAATCCC**	
-EcoRI_ORF8_fwd	**GGATTAAAGGAATTTGCCCTATAG**	

Table S3: List of abbreviations used in this study

Abbreviation	Full name / explanation
$3OC_6HSL$	N-(β-ketocaproyl)-L-homoserine lactone
CFS	cell-free system
deGFP	a GFP derived from an enhanced GFP with optimized translation ability in CFS
DMSO	dimethyl sulfoxide
fM	femtomolar
GFP	green fluorescent protein
GFPmut3	a GFP derived from *Aequorea victoria* with improved fluorescence emission at excitation 488 nm
$HgCl_2$	mercury (II) chloride
LacZ	β-galactosidase
LB	lysogeny broth
LOD	limit of detection
LucFF	firefly luciferase
LuxCDABE/LuxAB	bacterial luciferase operon
LuxR	quorum sensing molecule-responsive transcription activator
mCherry	a monomeric RFP derived from *Discosoma sp.*
MerR	mercury (II)-responsive transcription activator-repressor
mScarlet-I	a monomeric RFP derived from synthetic construct based on mCherry and multiple other naturally occurring RFPs and chromo proteins
NanoLuc	luciferase engineered by directed evolution from the deep sea shrimp *Oplophorus gracilirostris*
PBS	phosphate-buffered saline
PURE	purified recombinant elements
RFP	red fluorescent protein
TX-TL	transcription-translation
X-gal	5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside

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