Supplementary Information for:

Rapid, Low-Cost Detection of Water Contaminants Using Regulated *In Vitro* Transcription

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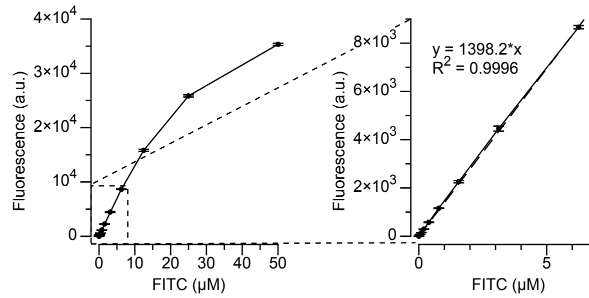
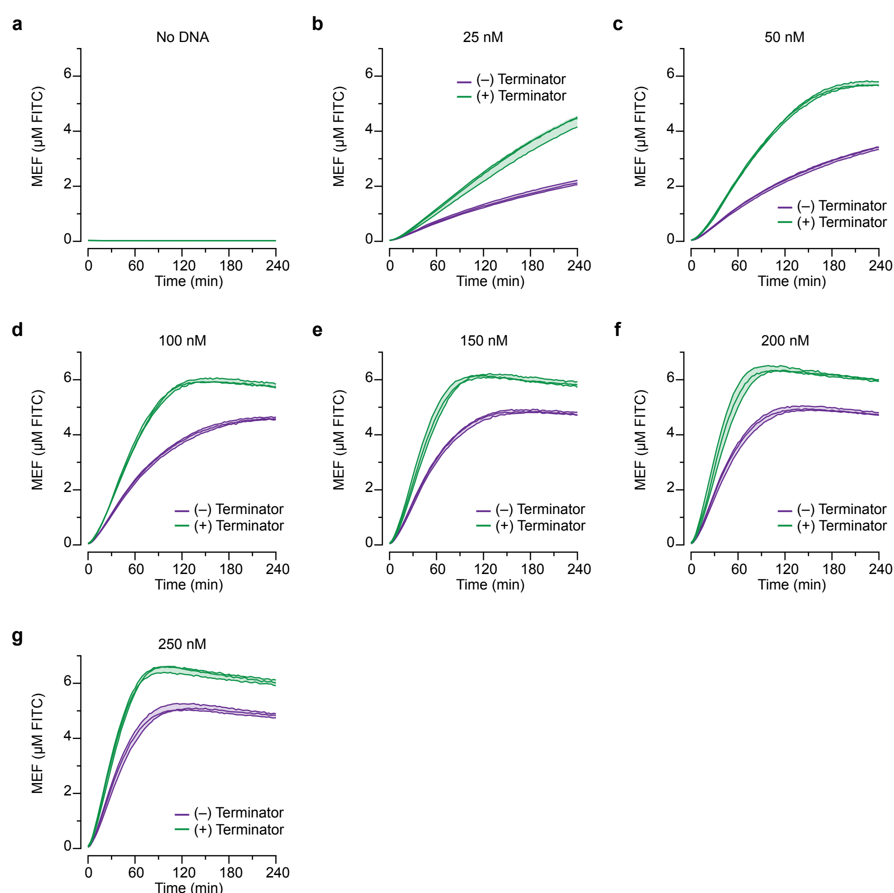
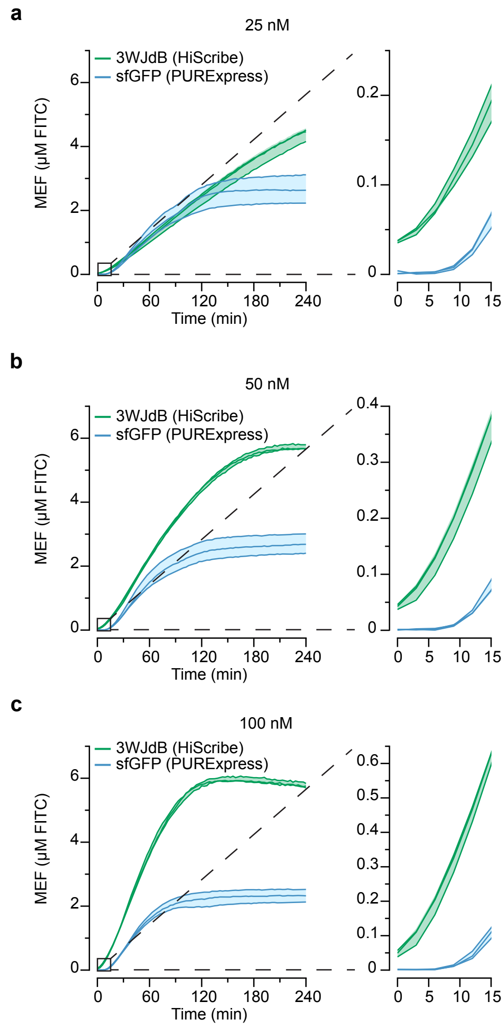


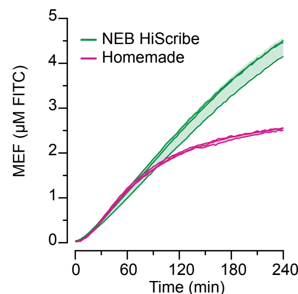
Supp. Fig. 1 | Mean Equivalent Fluorescence (MEF) standardization. Arbitrary units of fluorescence were standardized to μM concentrations of fluorescein isothiocyanate (FITC) using a NIST traceable standard (see Methods). In the representative example shown here, a dilution series of FITC was prepared in buffer (100 mM sodium borate, pH 9.5) and measured on a plate reader using the same settings for measuring 3WJdB signal (472 nm excitation, 507 nm emission). The resulting curve, calculated over the linear range 0-6.25 μM, was then used to standardize fluorescence measured from ROSALIND reactions. The standard curve was generated at regular intervals for each plate reader and each measurement setting. Data shown are for 3 experimental replicates with 3 technical replicates each (n = 9). Error bars indicate standard deviation.



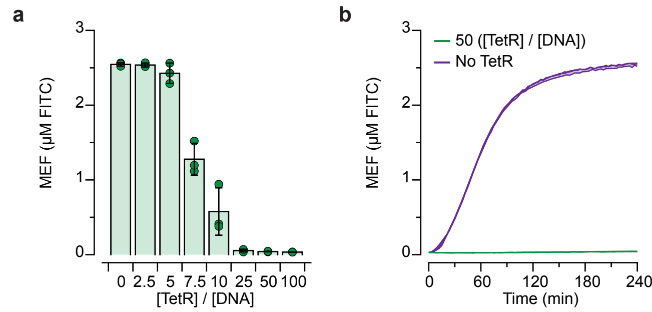
Supp. Fig. 2 | Fluorescence-activation of 3WJdB during run-off *in vitro* transcription reactions is improved by additionally encoding a T7 terminator. *In vitro* transcription reactions using a commercially available kit (NEB HiScribe T7 Quick High Yield RNA Synthesis Kit) were performed across a range of DNA transcription template concentrations, with and without an additional T7 terminator sequence. **a,** Reactions containing no DNA failed to generate signal, while all other DNA containing reactions (**b-g**) generated measurable fluorescence that improved with the addition of a template-encoded T7 terminator. We hypothesize that the terminator-dependent increase in fluorescence could be due to the presence of the terminator structure encouraging faster recycling of T7 RNAP from the elongation phase, to the termination phase, and then to initiation phase. Although less likely due to the highly-structured design of 3WJdB, the terminator-dependent increase could be due to the additional terminator structure stabilizing the productive, fluorescence-activating fold of 3WJdB. All data shown for 3 experimental replicates as lines with raw fluorescence values calibrated to µM FITC. Shading indicates the average value ± standard deviation.



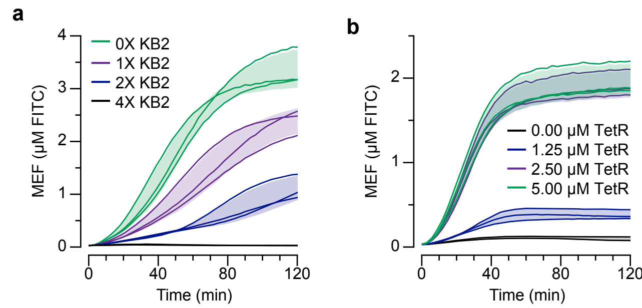
Supp. Fig. 3 | Fluorescence-activation from 3WJdB transcription reactions is faster and brighter than fluorescence from transcription-translation (PURE) reactions of sfGFP. All data shown for 3 experimental replicates as lines with raw fluorescence values calibrated to µM FITC. Shading indicates the average value ± standard deviation.



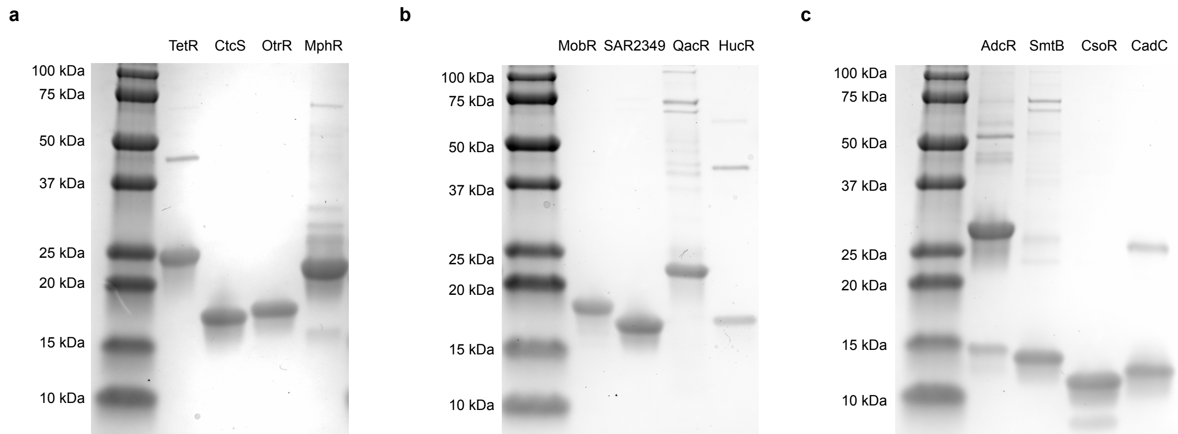
Supp. Fig. 4 | Homemade transcription reactions can be optimized to perform as well as commercial kits. Homemade *in vitro* transcription reactions were compared to a commercially available high yield transcription kit (NEB HiScribe T7 Quick High Yield RNA Synthesis Kit). 0.5 pmol of DNA encoding T7-3WJdB-T was added to each reaction in a total reaction volume of 20 µL (25 nM transcription template). Over the course of 1 hour the data show similar fluorescence activation. However, the homemade reaction begins to saturate after 1 hour, likely due to the exhaustion of NTPs in the homemade reaction (11.4 mM) when compared to the commercial kit (40 mM). All data shown for 3 experimental replicates with raw fluorescence values calibrated to µM FITC. Shading indicates the average value ± standard deviation.



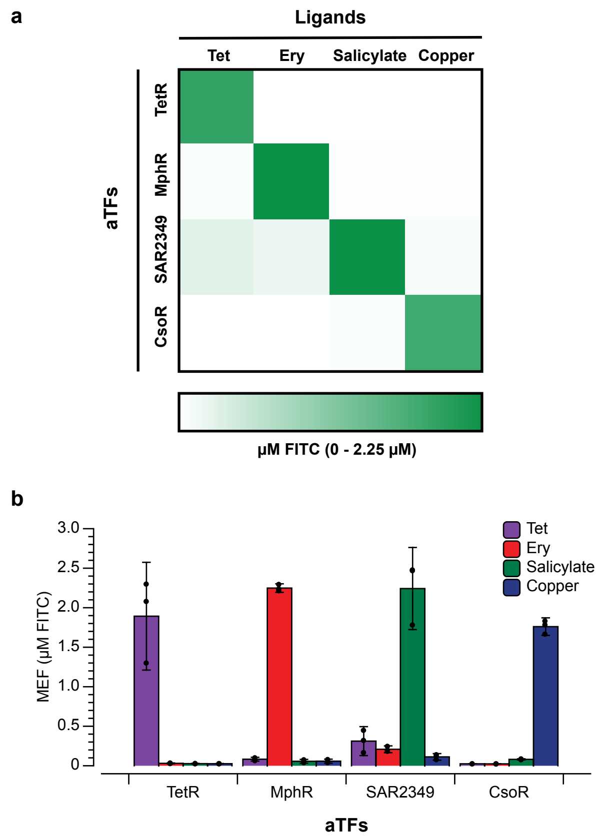
Supp. Fig. 5 | Titration of the TetR aTF into a regulated *in vitro* transcription. TetR protein was titrated as a function of DNA concentration to optimize for low fluorescence-activation. **a**, Data taken 4 hours after initiating reactions with T7 RNAP. Fluorescence-activation is substantially repressed at 25-fold excess or greater of TetR dimer over DNA template. **b**, Time course comparing the 0-fold and 50-fold [TetR]/[DNA] ratio experiments. All data shown for 3 experimental replicates as points or lines with raw fluorescence values calibrated to µM FITC. Colored bars and shaded regions indicate the average value ± standard deviation. All data shown for 3 experimental replicates with raw fluorescence values calibrated to µM FITC.



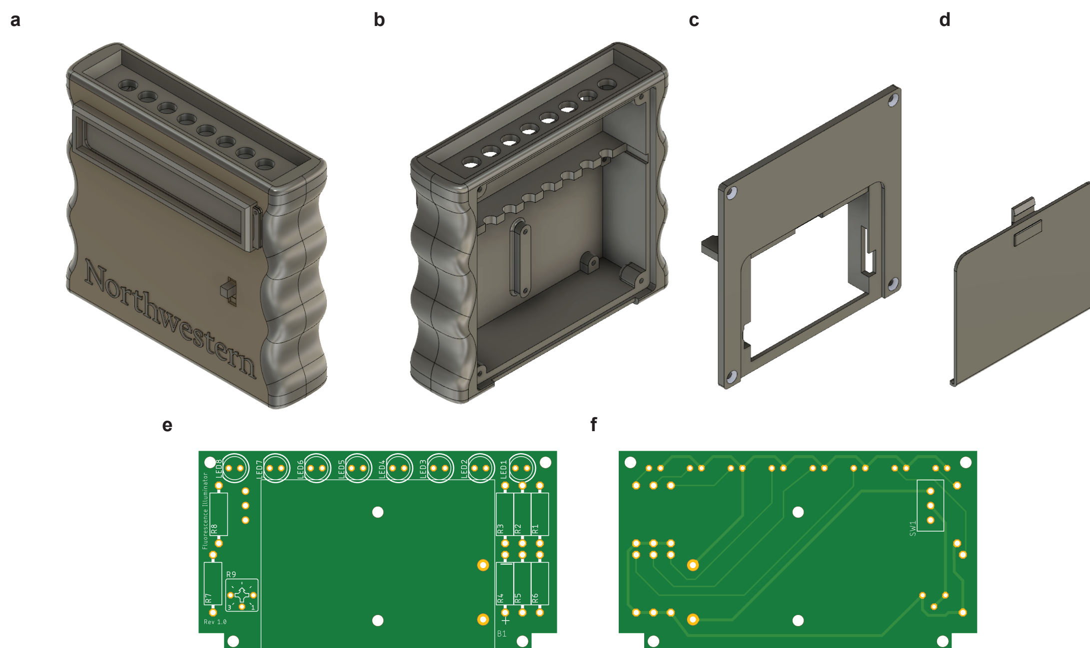
Supp. Fig. 6 | RNA kleptamers are used to invert transcription factor responses. a, Titration of the KB2 DNA template into an *in vitro* transcription of 3WJdB decreases the fluorescence from 3WJdB and **b,** Titration of TetR protein into an *in vitro* transcription of 3WJdB and a TetR regulated KB2 expression template restores the fluorescence from 3WJdB.



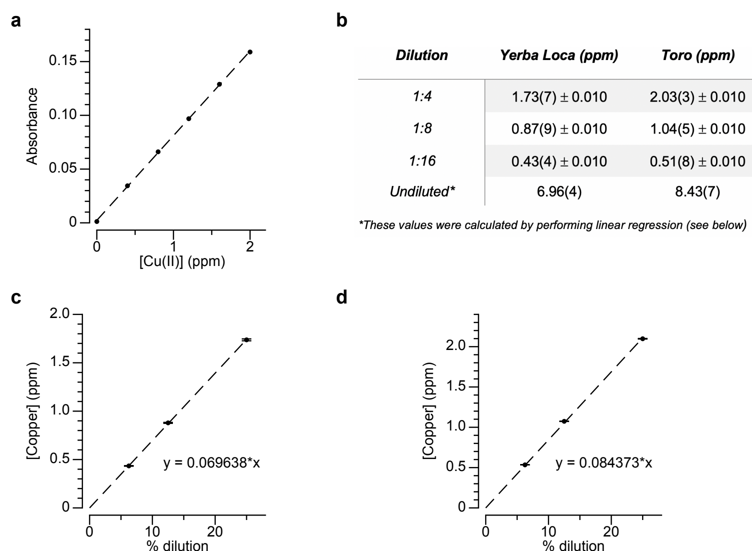
Supp. Fig. 7 | SDS-PAGE protein gel of all aTFs. a, antibiotics sensing aTFs (TetR – 24.9 kDa, CtcS – 18.7 kDa, OtrR – 19.5 kDa, MphR – 23.2 kDa), **b,** small molecule sensing aTFs (MobR – 20.6 kDa, SAR2349 – 18.9 kDa, QacR – 23.8 kDa, HucR – 19.8 kDa), and **c,** metal sensing aTFs (AdcR – 16.6 kDa, SmtB – 15.2 kDa, CsoR – 11.5 kDa, and CadC – 13.8 kDa). The molecular weights listed are calculated from aTFs amino acid sequences (**Supp. Data File 1**). The gel was run at a denaturing and non-reducing condition. In the AdcR lane, the most intense band corresponds to AdcR dimer (~33.2 kDa) formed by a disulfide bond under this condition.



Supp. Fig. 8 | Orthogonality of ROSALIND across different families of ligands. Four different sensors (one representative from each family) were tested for crosstalk with different ligands and determined to be orthogonal with each other as shown in the **a**, 4X4 orthogonality matrix and **b**, corresponding bar graph. Concentrations of ligands tested are 25 µM of tetracycline, 50 µM of erythromycin, 12 mM of salicylate, and 10 µM of $CuSO_4$. Reactions were configured as described in Figure 5.



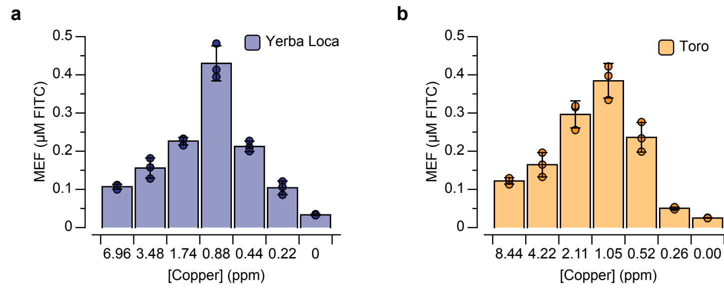
Supp. Fig. 9 | Handheld fluorescence illuminator design. a, A low-cost handheld fluorescence illuminator enables point-of-use functionality for ROSALIND. 3D printed components include a **b,** front case, **c,** back case, and **d,** battery cover. A printed circuit board (**e,** front view facing back case, **f,** rear view facing front case) mounts in the front case and connects LEDs, resistors, a trimmer potentiometer, a power switch, and a battery holder. Full CAD files, 3D print files (.STL), PCB design files, and assembly instructions are provided in the **Supporting Data File 4**.



Dilution	Yerba Loca (ppm)	Toro (ppm)
1:4	1.73(7) ± 0.010	2.03(3) ± 0.010
1:8	0.87(9) ± 0.010	1.04(5) ± 0.010
1:16	0.43(4) ± 0.010	0.51(8) ± 0.010
Undiluted*	6.96(4)	8.43(7)

These values were calculated by performing linear regression (see below)

Supp. Fig. 10 | Flame atomic absorption spectroscopy (FAAS) measurements of Chilean field samples. FAAS was calibrated with a $Cu(NO_3)_2$ standard solution as shown in **a**. Serial dilution was performed on each sample to create three separate diluted samples that are in the operating ppm range of FAAS, and their ppm values were measured as reported in Table **b**. Using these measurements, linear regression on the averages of each dilution was performed to calculate the ppm value of the undiluted sample from **c**, the Yerba Loca Creek and **d**, the Toro River.



Supp. Fig. 11 | Titrations of Chilean field samples into lyophilized ROSALIND copper sensing reactions. 1:1 serial dilution was performed on the field samples from **a**, the Yerba Loca Creek and **b**, the Toro River. The diluted field samples and laboratory-grade water (0.0 ppm) were used to rehydrate lyophilized ROSALIND copper sensing reactions. 4-hour end-point fluorescent values are reported over three replicates (points) calibrated to µM FITC. Bars represent averages over the replicates and error bars standard deviations. The copper concentrations indicated were taken from Supp. Fig. 10, or calculated from the extrapolated ppm value of the undiluted sample.

Supplementary Table 1 – Estimated cost per ROSALIND reaction*

Component	Supplier	Catalog Number	Quantity	Price (USD)	[Stock]	Stock Volume (mL)	Amount / Reaction (mL)	# Reactions	Cost / 20 µL Reaction (USD)
DFHBI-1T	Tocris	5610	10 mg	$240.59	40 mM	0.781	0.00113	691	$0.35
ATP	Sigma	A2383-25G	25 g	$466.48	100 mM	453.605	0.00057	795798	< $0.01
CTP	Sigma	C1506-1G	1 g	$448.84	100 mM	18.971	0.00057	33282	$0.01
GTP	Sigma	G8877-1G	1 g	$600.74	100 mM	19.114	0.00057	33533	$0.02
UTP	Sigma	U6625-1G	1 g	$495.88	100 mM	18.179	0.00057	31893	$0.01
Tris	Sigma	RDD009-2.5KG	2.5 kg	$166.56	2 M	7931.5	0.00020	39657500	<< $0.01
$MgCl_2$	Sigma	M2670-1KG	1 kg	$109.81	800 mM	6148.5	0.00020	30742500	<< $0.01
Spermidine	Sigma	S2626-25G	25 g	$498.82	200 mM	860.585	0.00020	4302926	<< $0.01
NaCl	Sigma	746398-25KG	25 kg	$198.50	2 M	213894.6	0.00020	1069473000	<< $0.01
DTT	Gold Bio	DTT500	500 g	$1,425	1 M	3241.5	0.00020	16207500	<< $0.01
Sucrose	Sigma	S0389-5KG	5 kg	$61.53	5 M	2921.4	0.00020	14607000	<< $0.01
Mannitol	Sigma	M4125-5KG	5 kg	$173.01	1 M	27446.9	0.00500	5489380	<< $0.01
TIPP	NEB	M0296L	1250 U	$244.79	2 U/µL	0.625	0.00015	4167	$0.06
T7 RNAP[a]	n/a	n/a	50 mg	$400.00	1 mg/mL	50	0.0002	250000	< $0.01
aTF[a]	n/a	n/a	10 mg	$400.00	1 mg/mL	10	0.0002	50000	< $0.01
DNA Template[b]	n/a	n/a	20 µg	$42.81	0.1 µg/µL	0.2	0.001	200	$0.21
TOTAL									**~ $0.67 / reaction**

[a]Homemade. Estimated for 2 L expression culture using core facility pricing (NU rPPC, March 2019).
[b]Calculated for the cost of a 2 mL PCR and purification using NEB #M3050 ($34.96/prep), NEB #N0447L ($2.45/prep), Qiagen #28106 ($4.40/prep), and PCR primers (< $1/prep).
*Calculated using institutional pricing (March 2019). Not included in calculation: labor, overhead, equipment, consumables (*e.g.* pipette tips) and additional materials (*e.g.* for pH adjustments), laboratory grade water, storage, shipping.

Supp. Table 2 | Plasmid constructs and purification method of aTFs.

aTF	Type of Purification	Tag Location	TEV Cleavage	Columns Used
TetR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
OtrR	His-tag affinity followed by size exclusion	C-terminus TEV followed by 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
CtcR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
MphR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
MobR	His-tag affinity followed by size exclusion	C-terminus TEV followed by 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
SAR2349	His-tag affinity	C-terminus TEV followed by 6XHis-tag	No	Gravity flow column packed with Qiagen Ni-NTA Agarose
QacR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
HucR	His-tag affinity	N-terminus 6XHis-tag and MBP followed by TEV tag	Yes	Gravity flow column packed with Qiagen Ni-NTA Agarose
AdcR	PEI precipitation, $(NH_4)_2SO_4$ precipitation, ion exchange followed by size exclusion	N/A	N/A	SP-Sepharose fast flow column for ion exchange, and Superdex 75 preparative-grade column for size exclusion
SmtB	His-tag affinity	C-terminus TEV followed by 6XHis-tag	No	Gravity flow column packed with Qiagen Ni-NTA Agarose

CsoR	His-tag affinity	N-terminus 6XHis-tag followed by TEV tag	Yes	Gravity flow column packed with Qiagen Ni-NTA Agarose
CadC	PEI precipitation, $(NH_4)_2SO_4$ precipitation, ion exchange followed by size exclusion	N/A	N/A	SP-Sepharose fast flow column for ion exchange, and Superdex 75 preparative-grade column for size exclusion

Supp. Table 3 | Concentrations of ROSALIND components in each sensor.

Ligand – aTF Pair	[DNA] (nM)	[aTF] (µM)	[Ligand] (µM)	Ligand Solvent	[Ligand Stock] (µM)	Ligand Purchased From
Anhydrotetracycline – TetR	25	1.25	0.1-50	Ethanol	5 – 500	Sigma-Aldrich (Cat#: 37919 – 100MG – R)
Tetracycline – TetR	25	1.25	25	Laboratory-grade H_2O	46.2	Gold Biotechnology (Cat#: T – 101 – 25)
Doxycycline – TetR	25	1.25	12.5	Laboratory-grade H_2O	45.8	Gold Biotechnology (Cat#: D – 500 – 1)
Oxytetracycline – OtrR	25	2.5	100	Laboratory-grade H_2O	159	Gold Biotechnology (Cat#: O – 410 – 10)
Chlortetracycline – CtcS	25	1.25	50	Laboratory-grade H_2O	500	Gold Biotechnology (Cat#: C – 840 – 5)
Erythromycin – MphR	25	0.625	50	Ethanol	68.126	Sigma-Aldrich (Cat#: E5389 – 1G)
Azithromycin – MphR	25	0.625	50	Ethanol	500	Sigma-Aldrich (Cat#: PHR1088 – 1G)
Clarithromycin – MphR	25	0.625	50	Ethanol	500	Sigma-Aldrich (Cat#: PHR1038 – 500MG)
Roxithromycin – MphR	25	0.625	250	Ethanol	3488.5	Sigma-Aldrich (Cat#: R4393-1G)
3-Hydroxy Benzoic Acid – MobR	10	100	2,000	Tris-base Buffer	13,153	Sigma-Aldrich (Cat#: H20008 – 5G)
Salicylate – SAR2349	10	50	12,000	Laboratory-grade H_2O	236,102	Sigma-Aldrich (Cat#: S2679 – 100G)
Benzalkonium Chloride – QacR	25	5	100	Laboratory-grade H_2O	387.5	Sigma-Aldrich (Cat#: 12060 – 5G)
Uric Acid – HucR	17.5	2.15	303	30mM NaOH	1011	Sigma-Aldrich (Cat#: U0881 – 10G)
$ZnSO_4$ – AdcR	7.5	1.5	30	Laboratory-grade H_2O	200	Sigma-Aldrich (Cat#: 83265 – 250mL – F)
$ZnSO_4$ – SmtB	25	5	10	Laboratory-grade H_2O	202	Sigma-Aldrich (Cat#: 83265 – 250mL – F)
$CuSO_4$ – CsoR	25	2.5	10	Laboratory-grade H_2O	0.1 – 1,000	Sigma-Aldrich (Cat#: 209198 – 5G)

$CdCl_2$ – CadC	20	1.5	10	Laboratory-grade H_2O	360	Sigma-Aldrich (Cat#: 202908 – 10G)
$PbCl_2$ – CadC	10	1.5	51.8	Laboratory-grade H_2O	0.2 – 200	Sigma-Aldrich (Cat#: 203572 – 10G)

Supporting Data File Descriptions

Alam_Jung_ROSALIND_SuppVideo_4kUHD.mp4 – Supplementary Video showing illumination of an unregulated ROSALIND reaction.

Alam_Jung_ROSALIND_Supp_Data_File1_Sequences.xlsx – DNA and Protein sequences used in this study.

Alam_Jung_ROSALIND_Supp_Data_File2_Experiment_Mastersheet.xlsx – Template excel worksheet for setting up a ROSALIND reaction.

Alam_Jung_ROSALIND_Supp_Data_File3_Images.zip – Raw illuminator and SDS-PAGE gel images included in figures.

Alam_Jung_ROSALIND_Supp_Data_File4_Handheld_Fluorescence_Illuminator_Instructions.zip – Instructions for 3D printing and assembling a hand-held illuminator.

Alam_Jung_ROSALIND_Supp_Data_File5_All_Source_Data.xlsx – Calibrated plate reader data for all figures.