Supplemental Information for

At-Home, Cell-Free Synthetic Biology Education Modules for Transcriptional Regulation and Environmental Water Quality Monitoring

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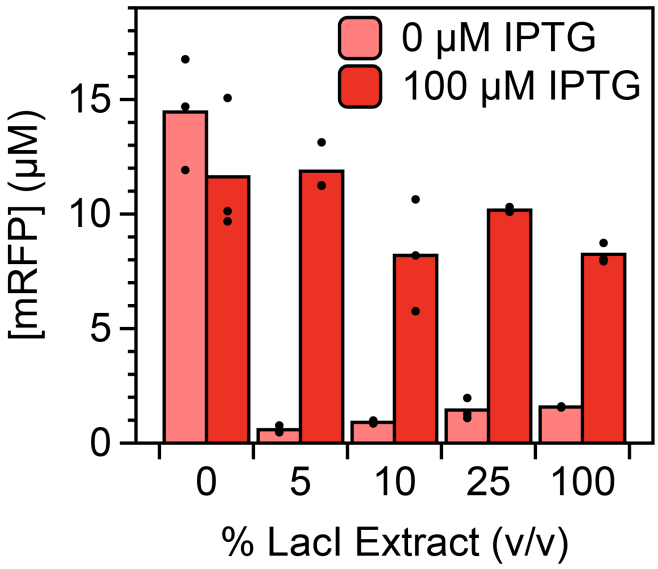

Figure S1. LacI-enriched extract titration. CFE reactions with 20 nM pLac-mRFP1 plasmid were constructed with varying proportions of unenriched extract and LacI-enriched extract. 5% LacI extract was selected for Modules 1-3 to have maximum signal and minimal leak.

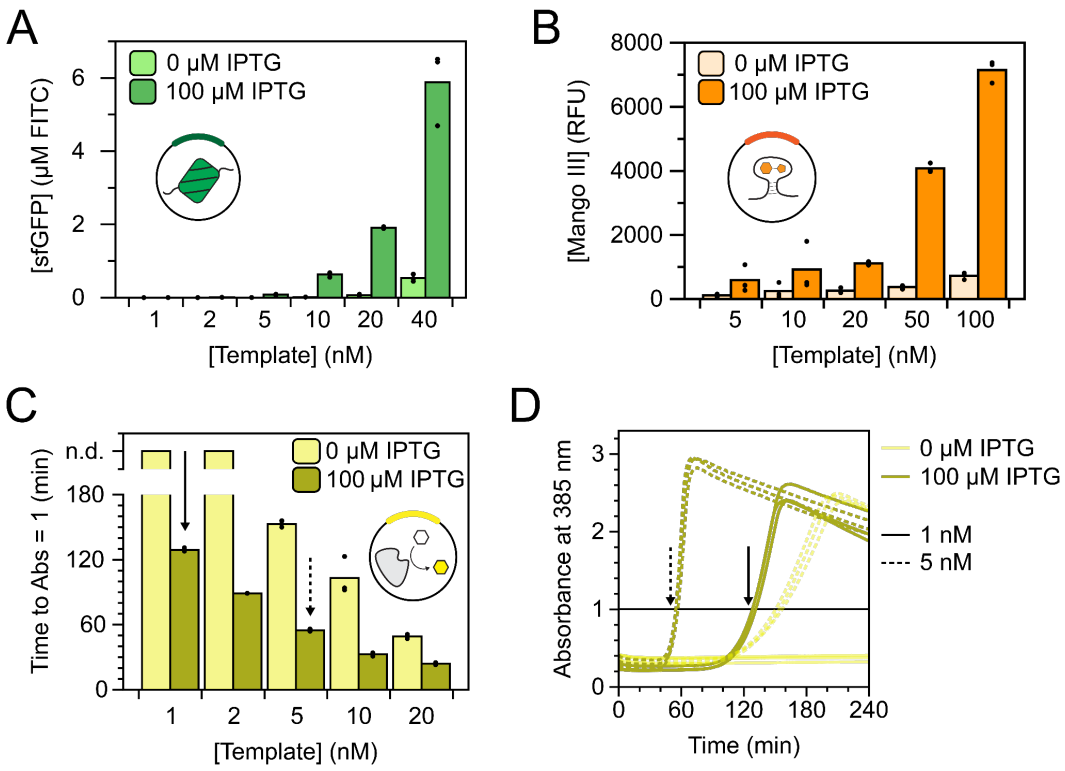

Figure S2. Reporter plasmid titrations. CFE reactions with 5% LacI-enriched extract were constructed with varying concentrations of expression templates for sfGFP (**A**), Mango III aptamer (**B**), and XylE (**C**) to maximize signal and minimize leak. The data in panel C represent time to a threshold absorbance of 1.0, linearly interpolated from absorbance measurements every two minutes over four hours, where the lower value indicates a faster time-to-result. The corresponding kinetic traces for two of the concentrations tested (1 nM and 5 nM) are shown in panel D, and the threshold absorbance of 1.0 is indicated with a solid black line.

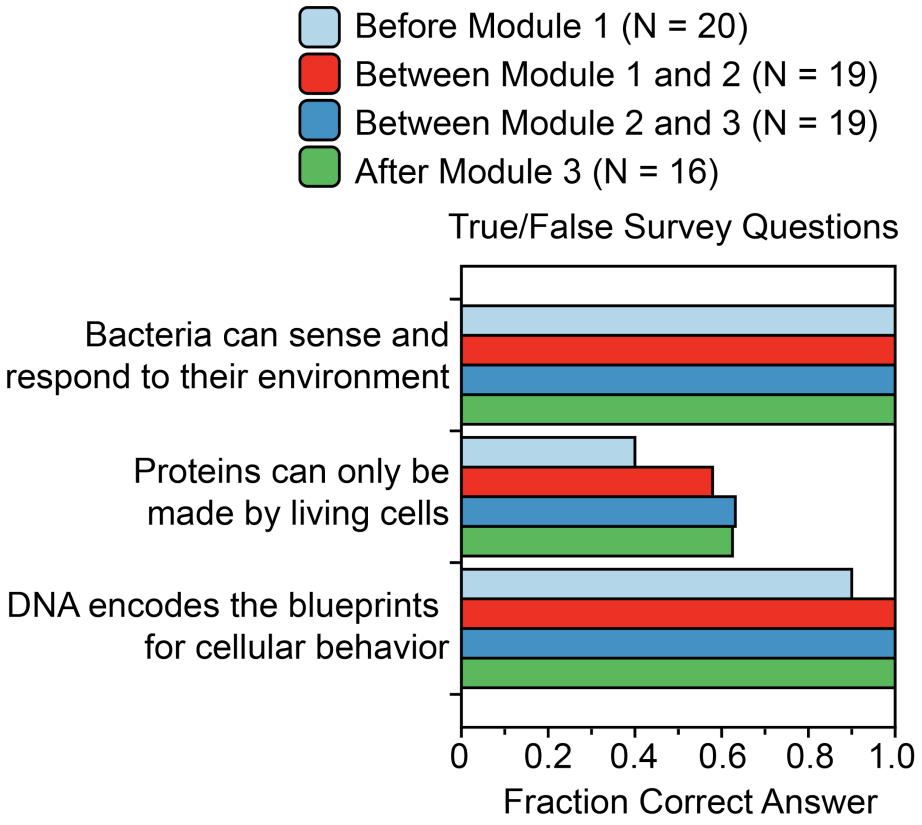

Figure S3. True/False survey questions. Students at Evanston Township High School completed true/false questions along with the perspective and comprehension questions in the survey. Most students correctly answered the first and third questions prior to completing the experiment modules, but the curricula did result in more students correctly answering the second question.

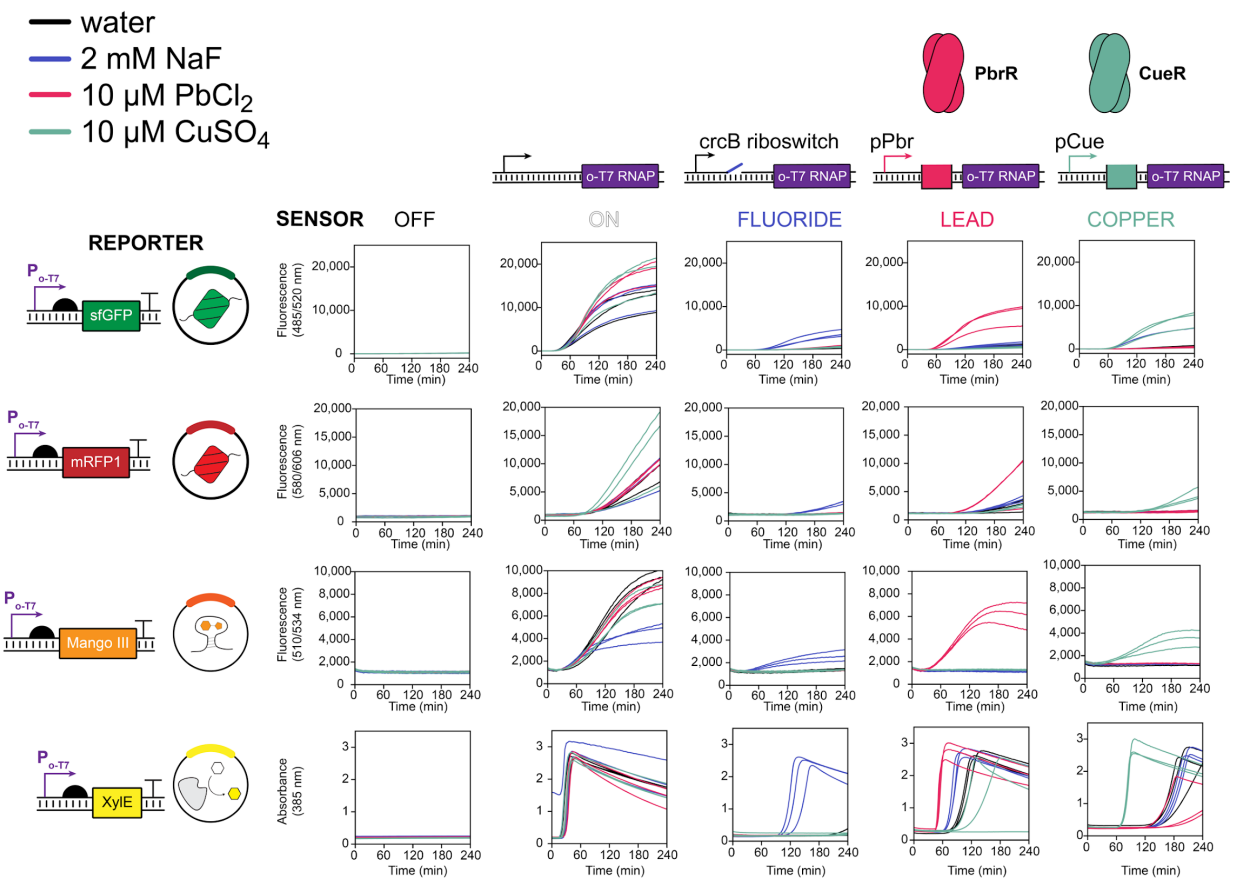

Figure S4. All cascade data pairs. Transcriptional cascades were run for all combinations of sensor and reporter plasmids available to students for Module 4 to verify modularity and effective construction of many unique sensing reactions. Plotted data represent the individual trajectories from three replicates of every possible lyophilized sensor reaction (5 sensor conditions rehydrated with each of 4 reporter plasmids and each of 4 inducer conditions).

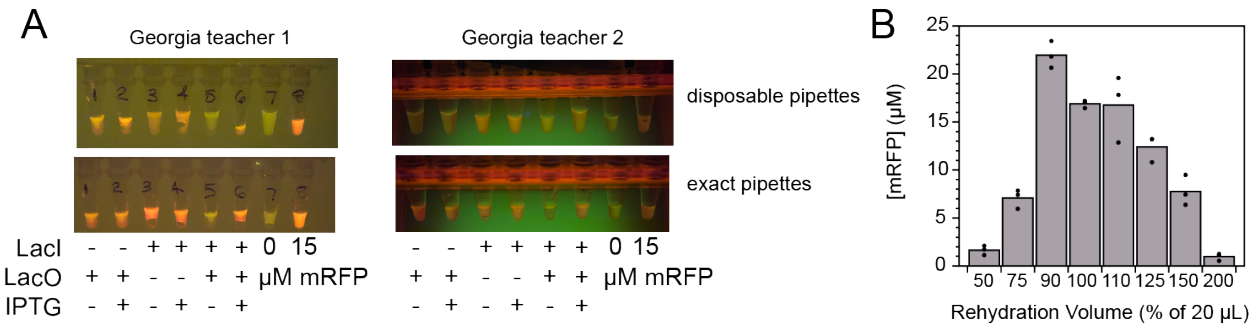

Figure S5. Troubleshooting large-scale Module 1 distribution. (**A**) Before the Georgia students performed Module 1, their teachers rehydrated the remaining reactions and observed results consistent with laboratory data (2 strip-tube replicates from each school). (**B**) The rehydration volume is a key consideration for CFE output. Plotted data represent the endpoint mRFP yield after four-hours when a 20 μL lyophilized IPTG-sensing reaction is rehydrated with varying volumes of a 100 μM IPTG stock. Significantly over- or under-diluting the CFE reactions can result in repressed protein synthesis. This is one of the main drawbacks of disposable pipettes that are less accurate and reliable than laboratory micropipettes.

Table S1. Estimated cost of extract production at laboratory scale. Based on our previous analysis from the literature [1], we estimate a cost of $83 USD/mL extract. This assumes a labor cost of 20 $USD/hr and 12 person-hours/batch to yield $240 USD/batch of extract with 3 mL of extract yield per batch. Generating extract from higher cell density cultures (e.g., harvesting at 30 OD_{600} or more, instead of 3 OD_{600} as was done by Zawada et al. [2, 3]) would further reduce extract costs.

Growth media component	Vendor	Catalog Number	Cost ($USD/g)	g/L culture	Cost ($USD/L culture)
tryptone	Sigma	T9410	0.273	16	4.37
yeast extract	Sigma	9182	0.091	10	0.91
sodium chloride	Sigma	S9888	0.0789	5	0.39
potassium phosphate monobasic	Sigma	P0662	0.151	7	1.06
potassium phosphate dibasic	Sigma	P8281	0.268	3	0.80
dithiothreitol (DTT)	Sigma	D0632	14.7	0.154	2.26

Table S2. Estimated cost of module production at laboratory scale. Based on our previous analysis from the literature [1], we include a labor cost of $1.50 USD/module, noting that it required roughly four minutes for the preparation of a single set of reactions from the prepared reagents per scientist.

CFPS component	Vendor	Catalog Number	Cost ($USD/g)	g/L reaction	$USD/L reaction	$USD/module (120 µL)
magnesium glutamate	Sigma	49605	0.3	4.7	1.40	0.00
ammonium glutamate	MP Biomedicals	21805951	0.1	1.6	0.17	0.00
potassium glutamate	Sigma	G1501	0.4	26.3	9.41	0.00
ATP	Sigma	A2383	25.6	0.7	16.90	0.00
CTP	Sigma	C1506	503.0	0.5	226.4	0.03
UTP	Sigma	U6625	652.0	0.5	306.4	0.04
GTP	Sigma	G8877	713.0	0.4	313.7	0.04
folinic acid	Sigma	F7878	646.0	0.0	19.38	0.00
amino acids	Sigma	79248	551.0	0.2	110	0.01
PEP	Sigma	860077	233.0	6.2	1440	0.17
NAD	Sigma	N0632	38.3	0.2	8.81	0.00
CoA	Sigma	C3144	2360.0	0.2	496	0.06
oxalic acid	Sigma	241172	0.9	0.4	0.33	0.00
putrescine	Sigma	51799	707.0	0.1	63.6	0.01
spermidine	Sigma	S0266	31.0	0.2	6.82	0.00
HEPES	Sigma	H3375	0.8	13.6	10.27	0.00
plasmid DNA	Qiagen	12145X4	39750.0	0.1	3975	0.48
extract	N/A	N/A	83.26/mL	300 mL/L	24978	3.00
total					31982	3.84
total + labor						5.34

References

1. Silverman, A.D., A.S. Karim, and M.C. Jewett, *Cell-free gene expression: an expanded repertoire of applications.* Nature Reviews Genetics, 2020. **21**(3): p. 151-170.
2. Zawada, J. and J. Swartz, *Maintaining rapid growth in moderate-density Escherichia coli fermentations.* Biotechnology and Bioengineering, 2005. **89**(4): p. 407-415.
3. Zawada, J. and J. Swartz, *Effects of growth rate on cell extract performance in cell-free protein synthesis.* Biotechnology and Bioengineering, 2006. **94**(4): p. 618-624.