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Supplementary Materials for

BioBits™ Bright: A fluorescent synthetic biology education kit

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/8/eaat5107/DC1)

Curriculum S3 (Microsoft Word format). Synthetic biology: Looking to nature to engineer new designs.
Curriculum S4 (Microsoft Word format). How fast is it really?
Curriculum S5 (Microsoft Word format). Super power protein!
Data S1 (Microsoft Excel format). This file contains example student-generated fluorescence data from the tunable protein expression laboratory activity (Fig. 3) and includes time-course data for modeling protein synthesis as an enzymatic reaction with varying amounts of substrate (DNA template).
Folder S1 (.zip format). This folder contains FreeCAD files and circuit diagrams to enable user construction of portable imagers and incubators.

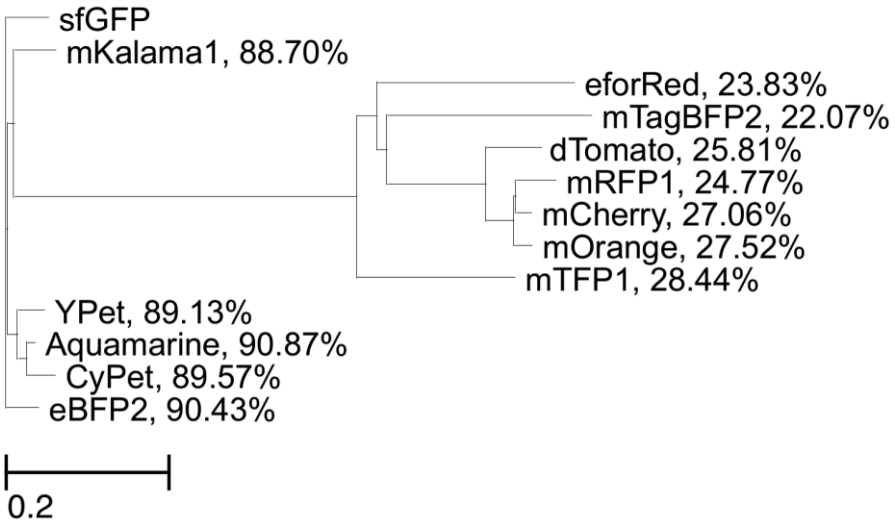

Fig. S1. Diversity of the fluorescent protein library facilitates evolution curriculum. Phylogenetic tree representing the amino acid sequences of the fluorescent protein library used in this study. The tree was constructed using the ETE toolkit in Python using the neighbor-joining method with results from a Clustal Omega multiple sequence alignment. Percentages represent the amino acid sequence homology to the standard CFPS reporter sfGFP.

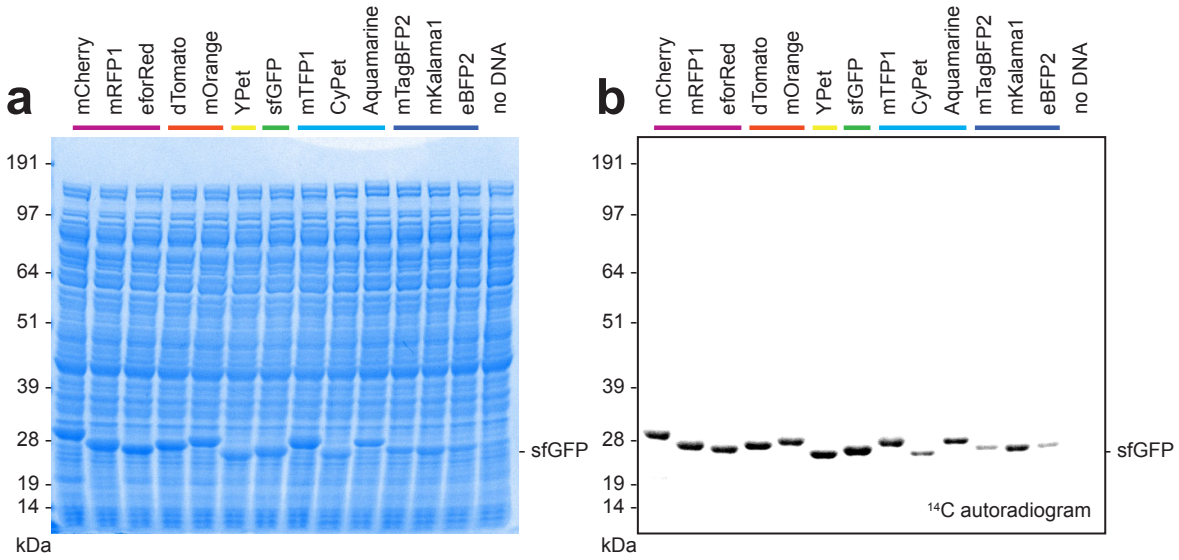

Fig. S2. Fluorescent protein library expresses with soluble, full-length products observed by SDS-PAGE and autoradiogram. Following cell-free protein synthesis for 20 hours at 30°C, reactions containing ^{14}C-leucine were centrifuged at 20,000xg for 10 min to remove insoluble or aggregated protein products. Soluble fractions were then analyzed by **(a)** SDS-PAGE and **(b)** ^{14}C-autoradiogram. Uncropped versions of both images are shown. All library members expressed with exclusively full-length product observable by both SDS-PAGE and autoradiogram.

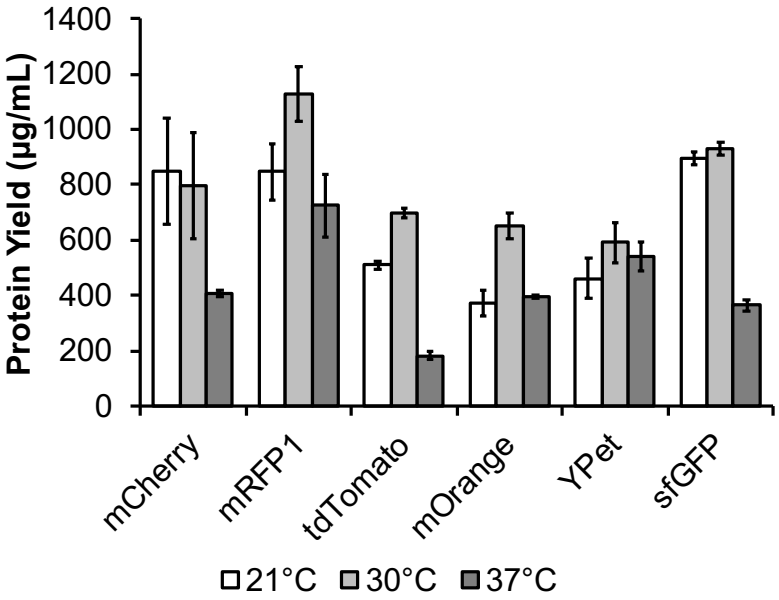

Fig. S3. FD-CF reactions tolerate a range of incubation temperatures. FD-CF reactions containing DNA template encoding mCherry, mRFP1, dTomato, mOrange, YPet, sfGFP were incubated at 37°C, 30°C, or 21°C. Reactions incubated at 37°C and 30°C were run for 20 hours, while reactions incubated at 21°C were run for 40 hours. Values represent averages and error bars represent standard deviations of N=3 biological replicates.

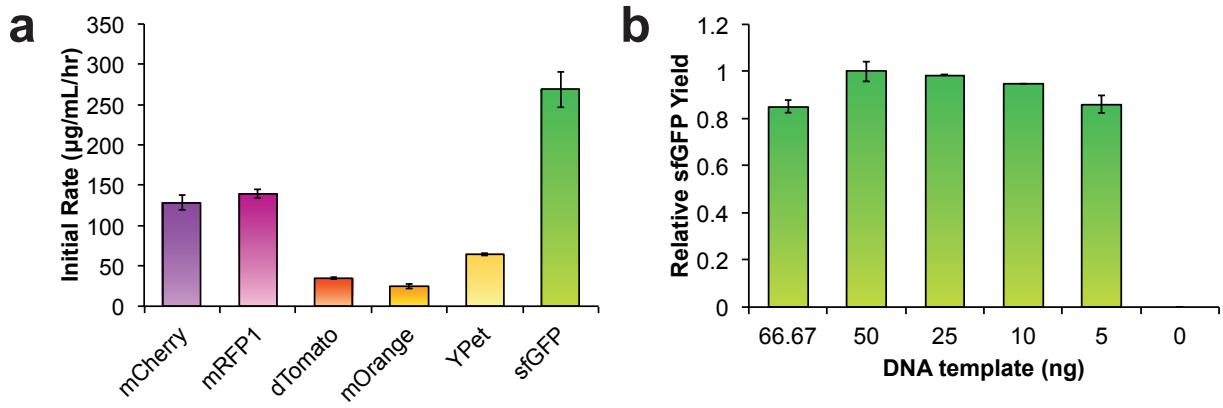

Fig. S4. DNA template is not limiting for in vitro sfGFP synthesis due to relatively high initial rates of protein synthesis. FD-CF reactions containing DNA template encoding mCherry, mRFP1, dTomato, mOrange, YPet, sfGFP were incubated at 30°C for 20 hours. **(a)** Initial rates of protein synthesis from reactions containing 66.67 ng DNA template were measured by fluorescence. **(b)** Endpoint yields for sfGFP synthesis measured *via* fluorescence at 20 hours show that protein synthesis is not limited by DNA template concentration. Values represent averages and error bars represent average errors of N≥2 biological replicates.

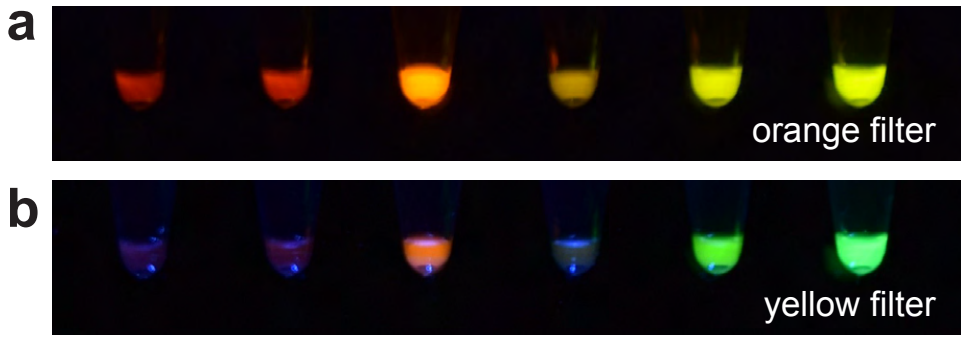

Fig. S5. Orange and yellow filters enable imaging of diverse fluorescent proteins in portable imagers. We constructed filters out of both translucent orange and yellow acrylic to visualize our fluorescent protein library. Red fluorescent proteins fluoresce more brightly through the orange filter **(a)** and yellow/green proteins appear brighter using the yellow filter **(b)**. Pictured are FD-CF reactions producing, from left to right, mCherry, mRFP1, dTomato, mOrange, YPet, and sfGFP.

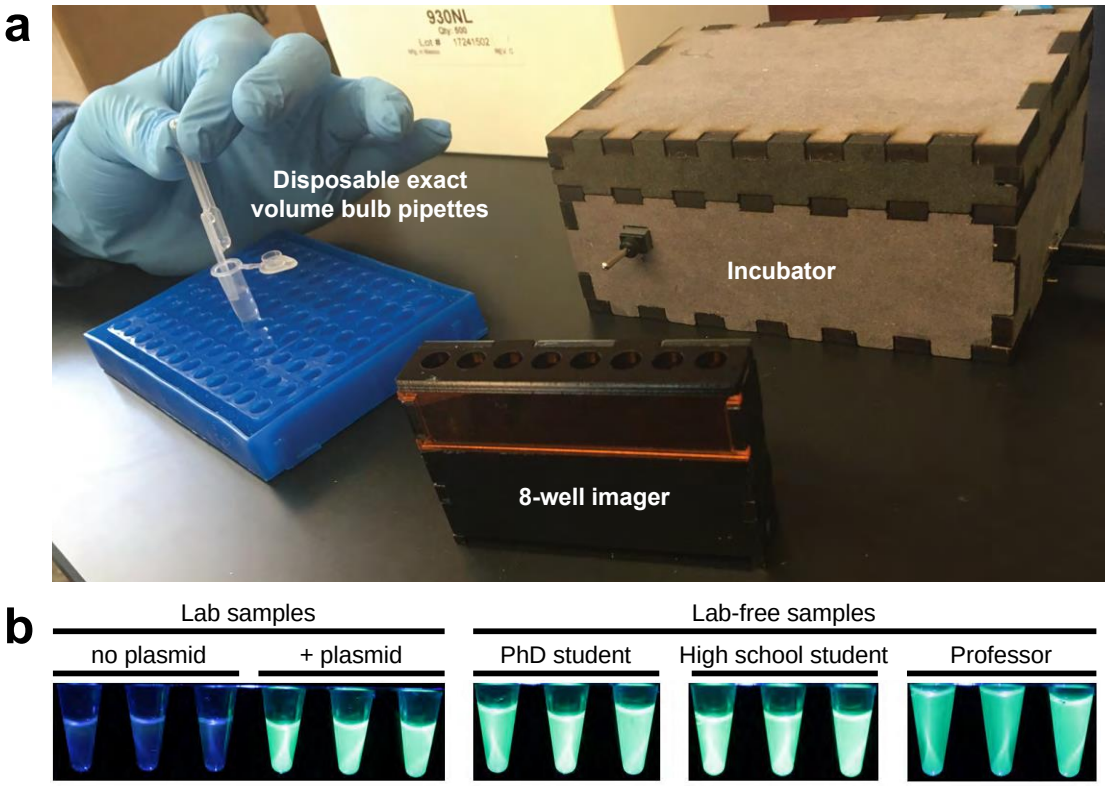

Fig. S6. FD-CF reactions can be run in a laboratory-free environment using low-cost, portable imagers and incubators. (a) Equipment used in “lab-free” experiments, including disposable 50 µL transfer pipettes, a portable imager, and a portable incubator. **(b)** sfGFP expression is visually consistent across different experiments and different operators. All images of reactions are scaled identically; variations in the volume of the reactions are due to pipetting differences across individual operators.

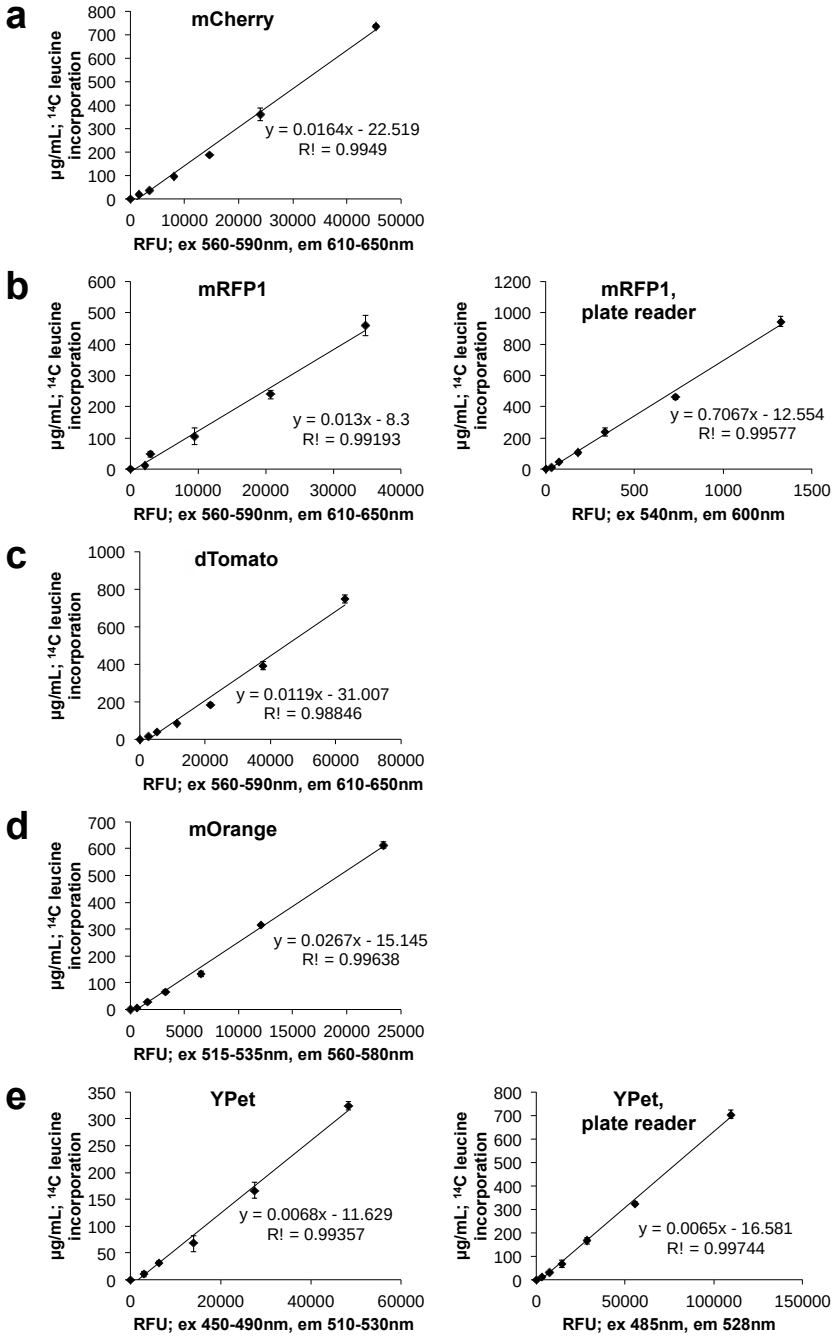

Fig. S7. Standard curves for converting fluorescence to protein concentrations. Standard curves were generated by correlating protein yields from serial dilutions of FD-CF reactions containing ^{14}C-leucine with measured fluorescence. Standard curves are shown for **(a)** mCherry, **(b)** mRFP1, **(c)** dTomato, **(d)** mOrange, and **(e)** YPet. Fluorescence was measured using a RT-PCR thermocycler unless fluorescence saturated the detector. If this was the case, fluorescence was measured using a plate reader after diluting reactions (4 µL FD-CF with 46 µL water for mRFP1 and 2 µL FD-CF with 48 µL water for YPet). Values represent means and error bars represent average errors of N=2 measurements.

Table S1. Cost analysis of portable imagers and incubators. The total cost to build working prototypes of our portable 8-well imager, 96-well imager, and incubator (switch and dial versions) are calculated below. Purchasing information for materials used to construct the prototypes are also included.

Component	Cost ($/unit)	Manufacturer	Product No
8-well Imager Total	**14.13**		
Black acrylic	0.95	Inventables	24112-03
Transparent yellow acrylic	0.12	Inventables	24165-03
Transparent orange acrylic	0.12	Inventables	24164-03
SCIGRIP Weld-On 16 Cement	0.12	TAP Plastics	10886
448 nm blue LED	6.97	Luxeon Star LEDs	SP-05-V4
Thermal adhesive tape for LEDs	0.94	Luxeon Star LEDs	LXT-R-10
Aluminum	0.35	McMaster-Carr	9146T38
Battery pack (3 AAA) with switch	1.56	Digi-Key	SBH431-1AS-ND
AAA rechargeable batteries	3.00	Amazon	RFQ420
96-well Imager Total	**32.01**		
Black acrylic	7.05	Inventables	24112-01
Transparent yellow acrylic	0.85	Inventables	24165-03
Transparent orange acrylic	0.85	Inventables	24164-03
SCIGRIP Weld-On 16 Cement	0.29	TAP Plastics	10886
448 nm blue LEDs (2)	13.94	Luxeon Star LEDs	SP-05-V4
Thermal adhesive tape for LEDs	3.77	Luxeon Star LEDs	LXT-R-10
Aluminum	0.70	McMaster-Carr	9146T38
Battery pack (3 AAA) with switch	1.56	Digi-Key	SBH431-1AS-ND
AAA rechargeable batteries	3.00	Amazon	RFQ420
Switch Incubator Total	**19.79**		
Black medium-density fiberboard	5.67	Inventables	30463-04
Screw, 0.25 in	1.20	Inventables	25297-01
Gorilla® wood glue	0.20	Inventables	26032-01
Mini solder-able breadboard	2.95	SparkFun	PRT-12702
N-channel MOSFET	0.95	SparkFun	COM-10213
Heating pad	3.95	SparkFun	COM-11288
MicroB USB breakout	1.95	SparkFun	BOB-12035
10K resistor	0.10	Digi-Key	10.0KXBK-ND
1-20K resistor (2x)	0.20	Digi-Key	variable
110K resistor (2x)	0.20	Digi-Key	110KXBK-ND
Temperature sensor	1.84	Digi-Key	TC622VAT-ND
Switch	0.58	Digi-Key	EG1903-ND
Dial Incubator Total	**19.67**		
Black medium-density fiberboard	5.67	Inventables	30463-04
Screw, 0.25 in	1.20	Inventables	25297-01
Gorilla® wood glue	0.20	Inventables	26032-01
Mini solder-able breadboard	2.95	SparkFun	PRT-12702
N-channel MOSFET	0.95	SparkFun	COM-10213
Heating pad	3.95	SparkFun	COM-11288
MicroB USB breakout	1.95	SparkFun	BOB-12035
10K resistor	0.10	Digi-Key	10.0KXBK-ND
110K resistor	0.10	Digi-Key	110KXBK-ND
Temperature sensor	1.84	Digi-Key	TC622VAT-ND
Potentiometer	0.76	Digi-Key	987-1726-ND

Table S2. Cost analysis for BioBits™ Bright. An estimate of the total cost to assemble BioBits™ Bright is calculated below. BioBits™ Bright will include enough reagents to run lab modules I and II for a 30-person classroom with groups of 2 students. The kit also includes our low-cost, portable imagers and incubators to enable use outside of a laboratory setting or in resource-limited classrooms. See **Table S3** for a detailed cost analysis of FD-CF reactions.

Component	Cost ($/unit)	Quantity	Cost in Kit ($)	Module
5 µL FD-CF rxns	0.05	150	7.50	I
96-well FD-CF rxns	1.00	15	15.00	II
Incubator	19.79	1	19.79	I & II
8-well imager	14.13	1	14.13	I & II
96-well imager	32.01	1	32.01	I & II
Freeze-dried plasmid (1200+ rxns)	1.60	6	9.60	I & II
Nuclease-free water (mL)	0.04	25	1.07	I & II
	BioBits™ Bright Total		**99.10**	**$/kit**

Table S3. Cost analysis of FD-CF reactions. The total cost to assemble FD-CF reactions is ~$0.01 per µL. This comes out to ~$0.05 per 5 µL reaction used in the DNA titration module, or ~$1.00 per 96 well plate used in the design-build-test module. In the table, amino acid cost accounts for 2 mM each of the 20 canonical amino acids purchased individually from Sigma. Extract cost is based on a single employee making 50 mL lysate from a 10 L fermentation, assuming 30 extract batches per year and a 5-year equipment lifetime. Component source is also included in the table if it is available to purchase directly from a supplier. Homemade or user-supplied components cannot be purchased directly and must be prepared by the end user according to procedures described in the Methods section.

Component	Cost ($/µL rxn)	Supplier	Product No
$Mg(Glu)_2$	negligible	Sigma	49605
NH_4Glu	negligible	MP	02180595
KGlu	negligible	Sigma	G1501
ATP	negligible	Sigma	A2383
GTP	0.00026	Sigma	G8877
UTP	0.00022	Sigma	U6625
CTP	0.00019	Sigma	C1506
Folinic acid	0.00002	Sigma	47612
tRNA	0.00021	Roche	10109541001
Amino acids	negligible	Sigma	
PEP	0.00178	Roche	10108294001
NAD	negligible	Sigma	N8535-15VL
CoA	0.00033	Sigma	C3144
Oxalic acid	negligible	Sigma	P0963
Putrescine	negligible	Sigma	P5780
Spermidine	negligible	Sigma	S2626
HEPES	negligible	Sigma	H3375
Extract	0.00737	homemade	
Total	**0.01**	**$/µL rxn**	
	0.05	**$/5 µL rxn**	
	1.00	**$/96 well plate**	

Table S4. Plasmids used in this study. The source of the gene sequence and/or DNA used to construct each member of the fluorescent protein library is listed.

Plasmid	Sequence Source	Plasmid Source	Addgene #
pJL1 mCherry	Clontech 632524	This work	102629
pJL1 mRFP1	BBa_E1010	This work	102630
pJL1 eforRed	BBa_K592012	This work	106320
pJL1 dTomato	Addgene 54856	This work	102631
pJL1 mOrange	Addgene 54751	This work	102632
pJL1 YPet	Addgene 54860	This work	102633
pJL1 sfGFP	Addgene 69496	Jewett lab unpublished	102634
pJL1 mTFP1	Addgene 54553	This work	102635
pJL1 CyPet	Addgene 14030	This work	102636
pJL1 Aquamarine	Addgene 42889	This work	106285
pJL1 mTagBFP2	Addgene 54572	This work	102638
pJL1 mKalama1	Addgene 14892	This work	102639
pJL1 eBFP2	Addgene 14891	This work	102640

Other Supplementary Materials for this manuscript include the following:

Curriculum S1. Let it glow! This pre-lab assignment and lab protocol is designed to support the BioBits™ Bright tunable protein expression module with an optional inquiry-based extension activity.

Curriculum S2. What factors affect CFPS yields? This curriculum piece outlines an inquiry-based lab activity modeled on the BioBits™ Bright tunable protein expression lab. In this activity, students are guided through an independent investigation of biochemical factor(s) that influence *in vitro* protein synthesis yields.

Curriculum S3. Synthetic biology: Looking to nature to engineer new designs. This curriculum piece outlines a research project in which students are challenged to design and present a solution to a societal challenge of their choice using synthetic biology.

Curriculum S4. How fast is it really? This assignment, designed for high school math classrooms, asks students to calculate rates of transcription and translation.

Curriculum S5. Super power protein! This curriculum piece outlines a research project in which students have the opportunity to design their own super power after learning about some of the "super powers" (e.g., fluorescence) of proteins in biology. Students will present their super power and design an *in vitro* program that illustrates the super power *via* expression of fluorescent proteins in a 96-well plate.

Data S1. This file contains example student-generated fluorescence data from the tunable protein expression laboratory activity (Fig. 3) and includes time-course data for modeling protein synthesis as an enzymatic reaction with varying amounts of substrate (DNA template).

Folder S1. This folder contains FreeCAD files and circuit diagrams to enable user construction of portable imagers and incubators.