

Supplementary Materials for

BioBits™ Explorer: A modular synthetic biology education kit

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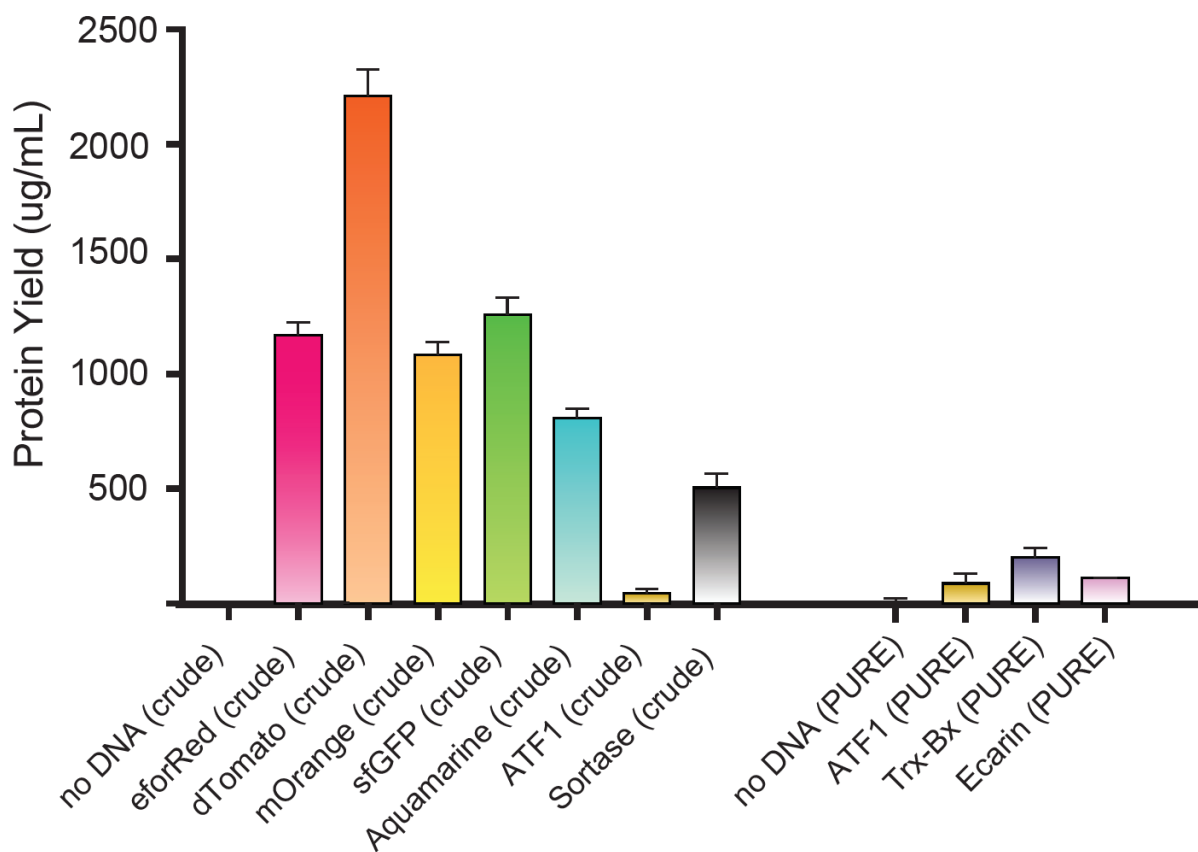


Fig. S1. Quantification of all proteins expressed in FD-CF. All of the FD-CF expressed proteins used in the demonstration experiments had high soluble yields (between 100 and >1000 $\mu\text{g/mL}$), as measured by ^{14}C -Leucine incorporation. Values represent averages and error bars represent standard deviations of $n=3$ biological replicates.

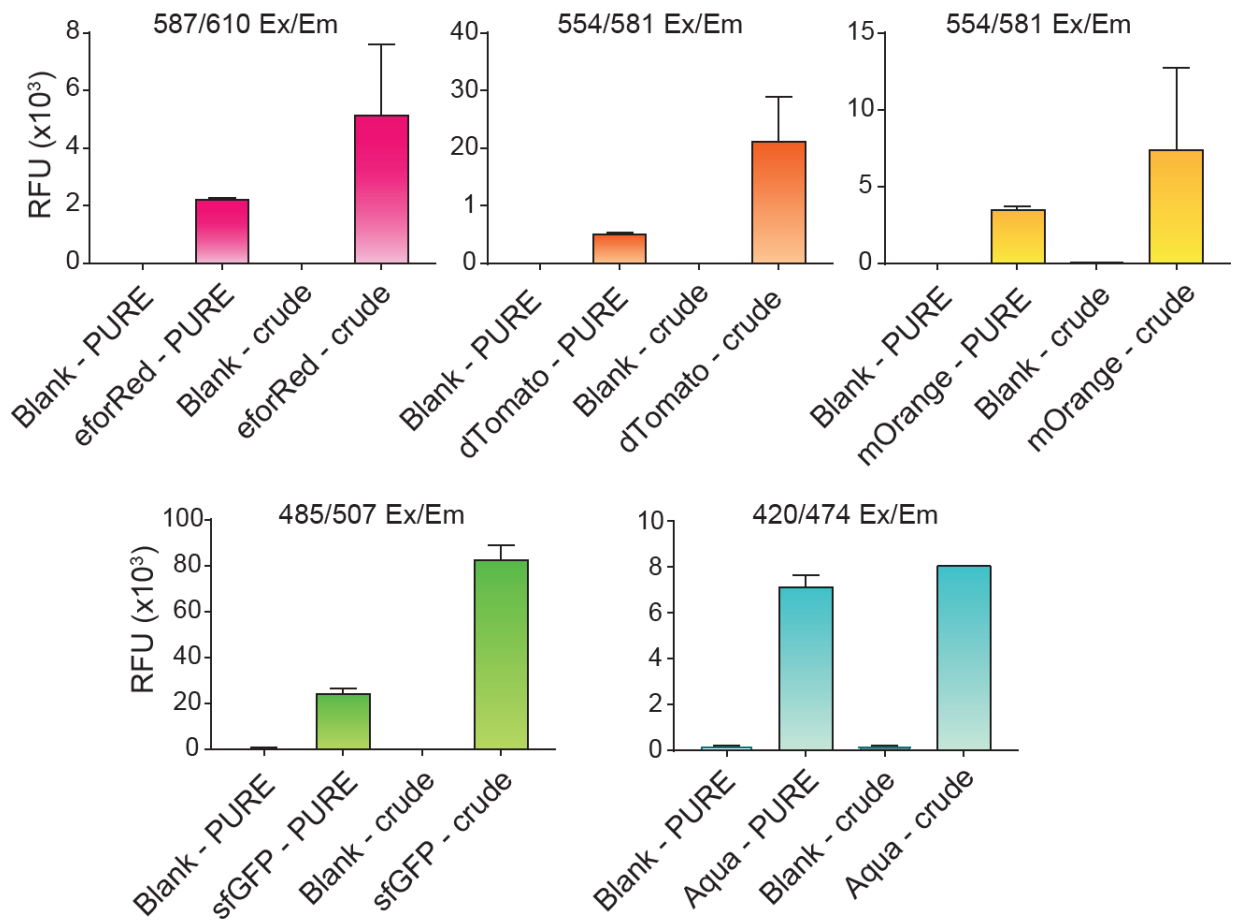


Fig. S2. Fluorescent proteins expressed in the PURE and crude extract systems. Endpoint fluorescent readouts of all expressed proteins in the commercial PURE or in-house crude system. Values represent averages and error bars represent average errors of $n \geq 2$ biological replicates.

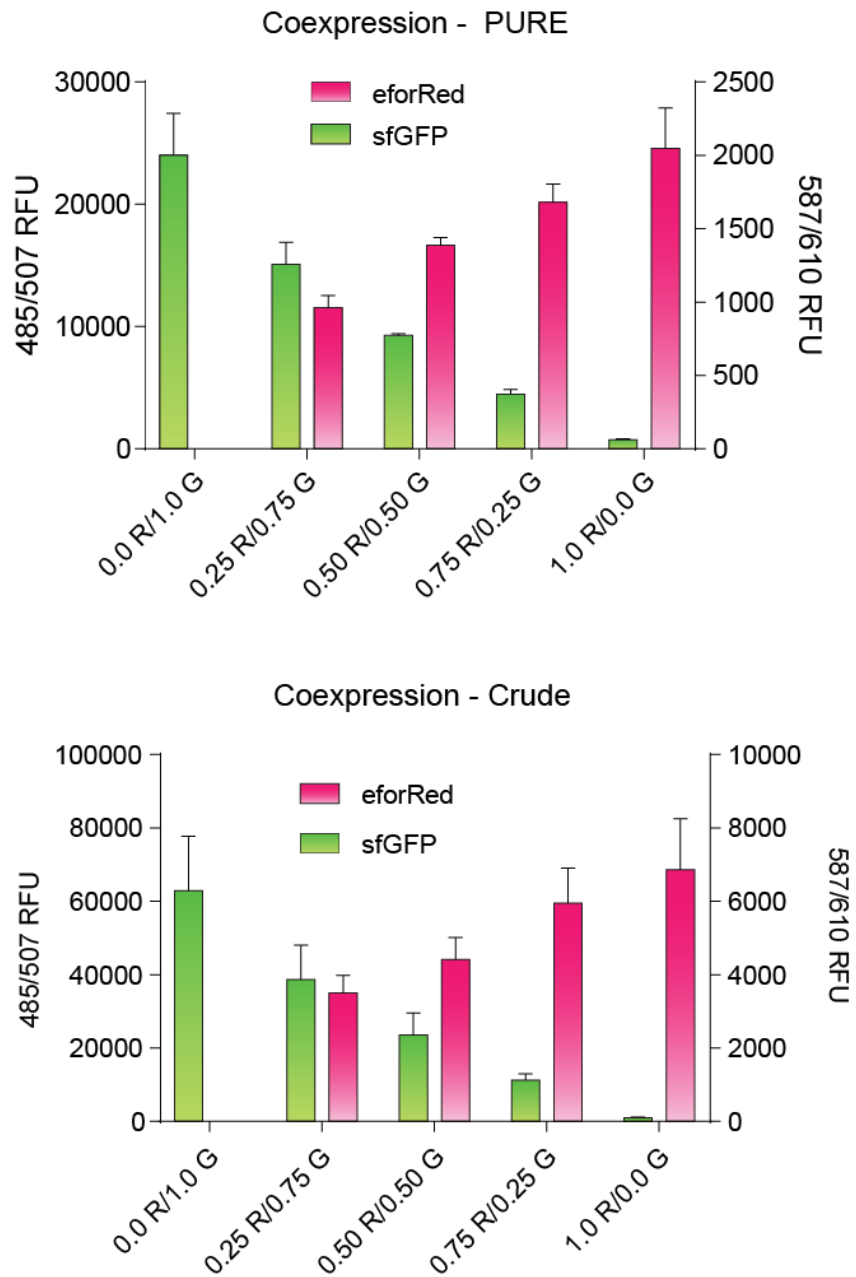


Fig. S3. Quantitative analysis of fluorescent proteins. Endpoint fluorescent readouts of co-expressed sfGFP and eforRed proteins in the PURE or crude extract system. Values represent averages and error bars represent standard deviations of n=3 biological replicates.

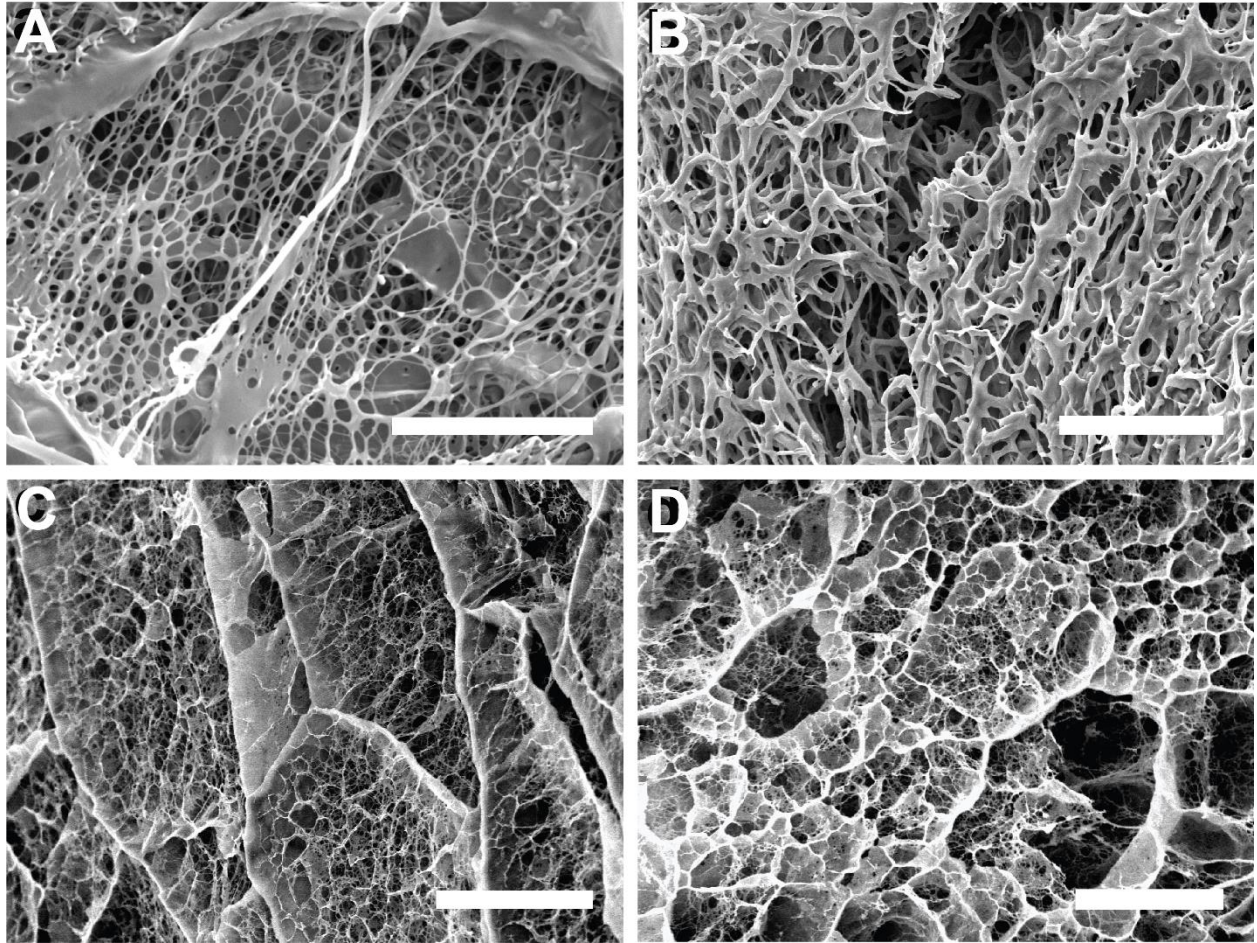


Fig. S4. Representative scanning electron microscopy images of hydrogel ultrastructures generated with FD-CF enzymes. PEG hydrogels crosslinked by FD-CF expressed sortase in (A) PURE and (B) crude extract. Fibrin hydrogels crosslinked by FD-CF expressed (C) ecarin in PURE and (D) batroxobin in PURE. All scale bars are 10 microns.

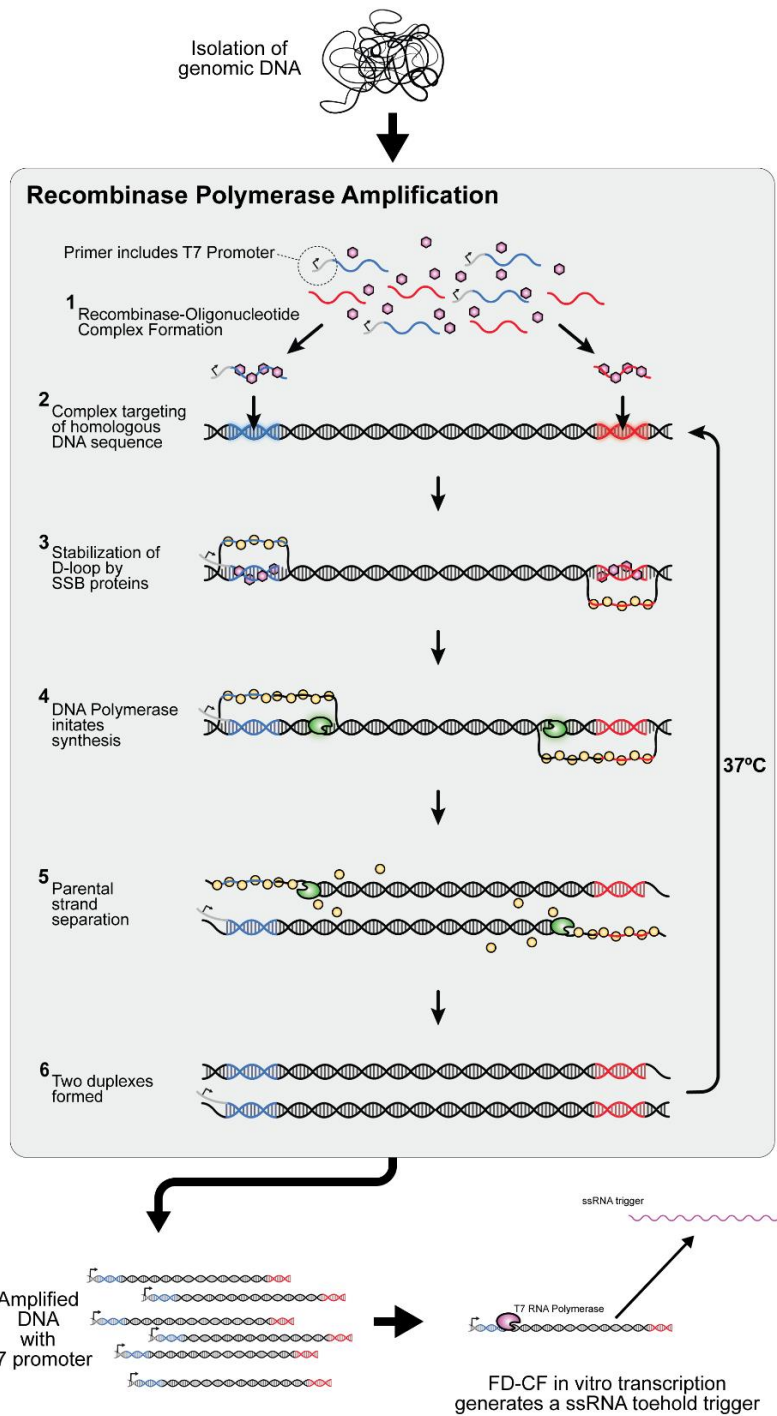


Fig. S5. Schematic of RPA reaction. From a genomic DNA sample, a specific region is isothermally amplified using Recombinase Polymerase Amplification. The primer includes a T7 promoter, such that the amplicons act as a template to generate a large amount of RNA trigger molecules when added to a FD-CF reaction. This results in signal amplification for toehold sensor activation.



Fig. S6. Detailed steps for isolating genomic DNA from fruits for environmental sensing activity. Photographs showing the DNA extraction process from fruit.

Table S1. Library of proteins and toehold switches that enable visual, olfactory, and tactile outputs for educational engagement.

| Protein | Output | Vector | Sequence Source | Addgene # |
|---------------------------------|---|---------------|--|------------------|
| eforRed | <i>Visual/fluorescent red</i> | pJL1 | iGEM part: BBa_K592012 | 106320 |
| dTomato | <i>Visual/fluorescent orange</i> | pJL1 | Addgene 54856 | 102631 |
| mOrange | <i>Visual/fluorescent orange/yellow</i> | pJL1 | PDB entry: 2H5O | 102632 |
| sfGFP | <i>Visual/fluorescent green</i> | pJL1 | PDB entry: 2B3P | 102634 |
| Aquamarine | <i>Fluorescent green/cyan</i> | pJL1 | Addgene 42889 | 106285 |
| ATF1 | Banana smell | pJL1 | IGEM part: BBa_J45014 | 106286 |
| Sortase | Tactile PEG-hydrogel | pJL1 | Gift from Linda Griffith | 106288 |
| Ecarin | Tactile fibrin hydrogel | pNP1 | US Patent #US20050164365 | 106289 |
| Thioredoxin-Batroxobin (Trx-Bx) | Tactile fibrin hydrogel | pJL1 | Thioredoxin (Trx): Uniprot: P0AA25, Batroxobin (Bx): UniProt: P04971.1 | 106290 |

| Toehold Switch | Corresponding RPA Primers for Trigger(s) | Addgene # |
|---------------------------|--|------------------|
| pCOLA banana sensor sfGFP | (Fwd)5'CATTCTAATACGACTCACTATAGGGATTATCTGCAAAA AACTACGG3' (Rev)5'TAAAAAGTGCTTCGGTGCAAAAATAAGAAACGAT3' | 107367 |
| pCOLA kiwi sensor sfGFP | (Fwd)5'ACATTCTAATACGACTCACTATAGCCCCGTTTTTGGGT GCTTGTGGT3' (Rev)5'CGGGATTCTGCAATTCACACCAAGTATCGCAT3' | 107368 |

Table S2. FD-CF reactions allow for inexpensive classroom synthetic biology education kits.

| BioBits™ Explorer – Core Kit | Cost/rxn (\$) | # Rxns | Cost in kit (\$; w/ 10) repeats) |
|---|----------------------|------------------|---|
| <i>Activity: Co-expressed fluorescent</i> | | | |
| Plasmids | 0.04 | 16 | 6.40 |
| CF reagents | 0.10 | 16 | 16.00 |
| | | Sub-total | 22.40 |
| <i>Activity: Odor-generating enzymes</i> | | | |
| Plasmids | 0.04 | 3 | 1.20 |
| CF reagents | 0.10 | 3 | 3.00 |
| Odor substrates | 2.29 | 3 | 68.70 |
| | | Sub-total | 72.90 |
| <i>Activity: Sortase-generated hydrogels</i> | | | |
| Plasmids | 0.07 | 3 | 2.10 |
| CF reagents | 0.21 | 3 | 6.30 |
| Hydrogel substrates | 2.34 | 3 | 70.20 |
| | | Sub-total | 78.60 |
| <i>Reusable components</i> | | | |
| 96-well incubator | | | 20.00 |
| 8-well illuminator | | | 15.00 |
| Nuclease-free water | | | 1.00 |
| | | Sub-total | 36.00 |
| Total: | | | 209.90 |

| BioBits™ Explorer – Add-on Kit | Cost/rxn (\$) | # Rxns | Cost in kit (\$; w/ 10) repeats) |
|---|----------------------|------------------|---|
| DNA Extraction Supplies | 0.21 | 4 | 8.40 |
| RPA reagents (1 rxn supplies whole class) | 4.27 | 4 | 17.08 |
| CF reagents | 4.41 | 4 | 176.40 |
| | | Sub-total | 201.88 |
| Total: | | | 201.88 |