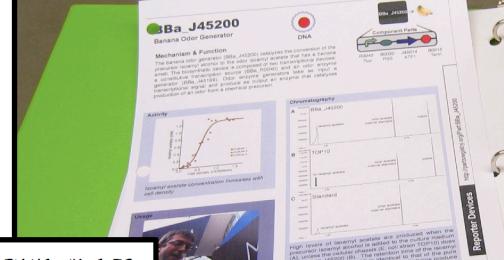


The Foo Camper's guide to biological engineering

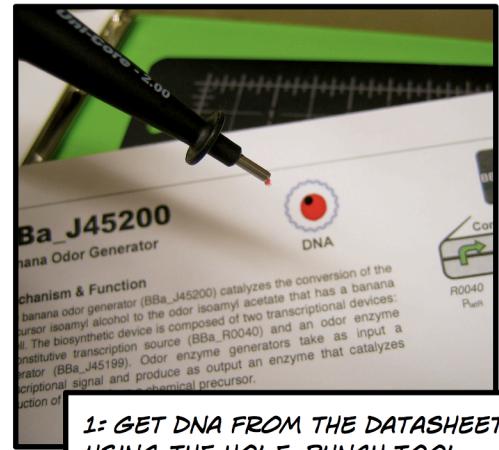
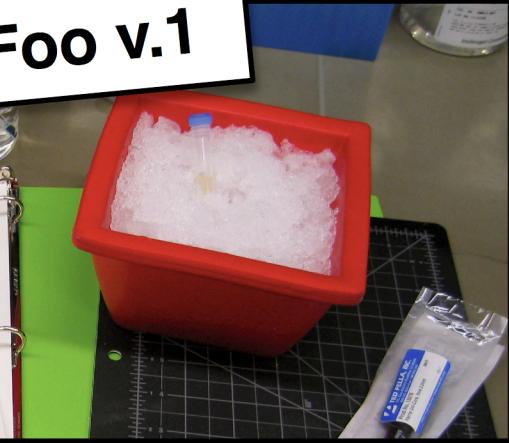
Ginkgo
BioWorks



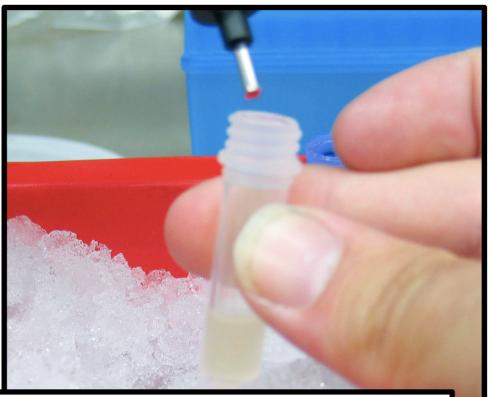
Transformation-Foo v.1



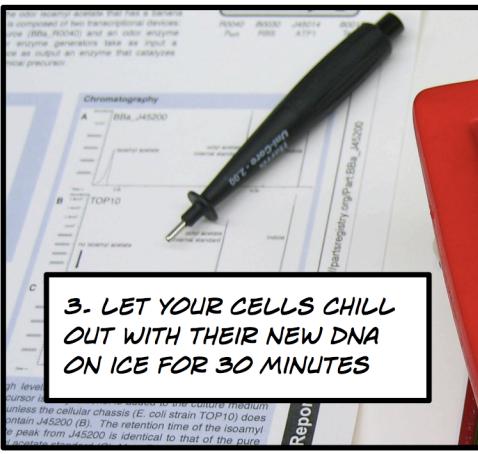
BY MC, JK, ERS



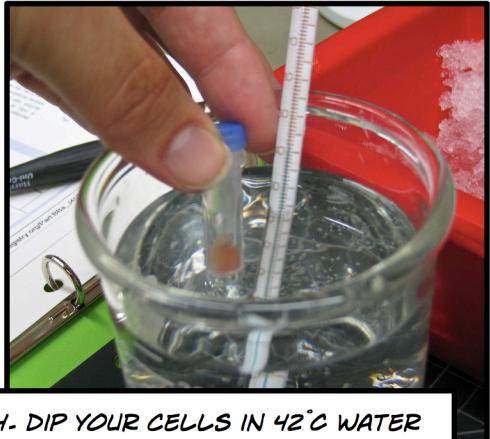
1: GET DNA FROM THE DATASHEET
USING THE HOLE-PUNCH TOOL



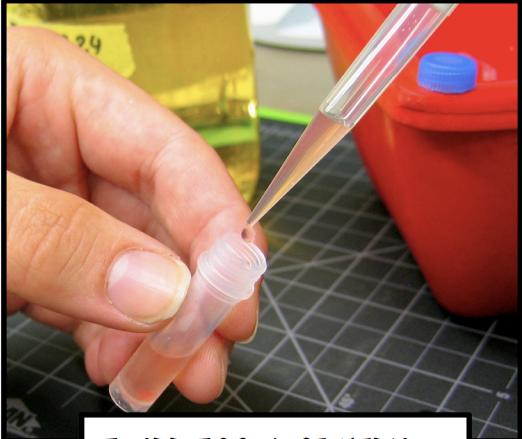
2. EJECT THE "DNA-CHAD" INTO
YOUR THAWED CELLS



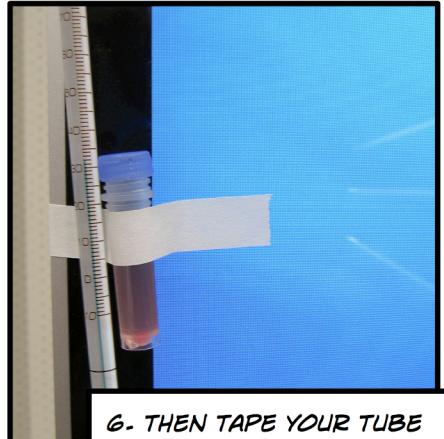
3. LET YOUR CELLS CHILL
OUT WITH THEIR NEW DNA
ON ICE FOR 30 MINUTES



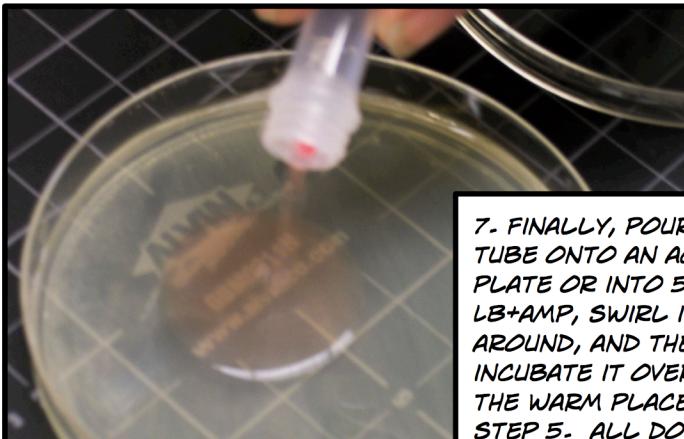
4. DIP YOUR CELLS IN 42°C WATER
FOR 1 MINUTE



5. ADD 500 µL OF MEDIA



6. THEN TAPE YOUR TUBE
TO SOMETHING WARM
(37°C) - A COMPUTER'S
COOLING FAN, OR TOP OF
MONITOR - FOR ONE HOUR



7. FINALLY, POUR YOUR
TUBE ONTO AN AGAR+AMP
PLATE OR INTO 5 mL OF
LB+AMP, SWIRL IT
AROUND, AND THEN
INCUBATE IT OVERNIGHT IN
THE WARM PLACE USED IN
STEP 5. ALL DONE!

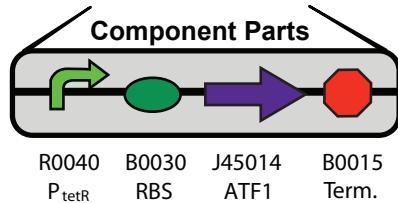
BBa_J45200

Banana Odor Generator

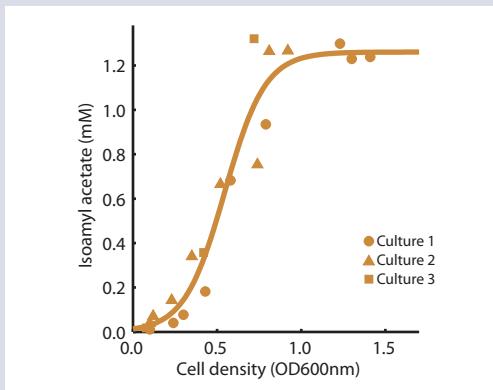


Mechanism & Function

The banana odor generator (BBa_J45200) catalyzes the conversion of the precursor isoamyl alcohol to the odor isoamyl acetate that has a banana smell. The biosynthetic device is composed of two transcriptional devices: a constitutive transcription source (BBa_R0040) and an odor enzyme generator (BBa_J45199). Odor enzyme generators produce as output an enzyme that catalyzes production of an odor from a chemical precursor.



Activity



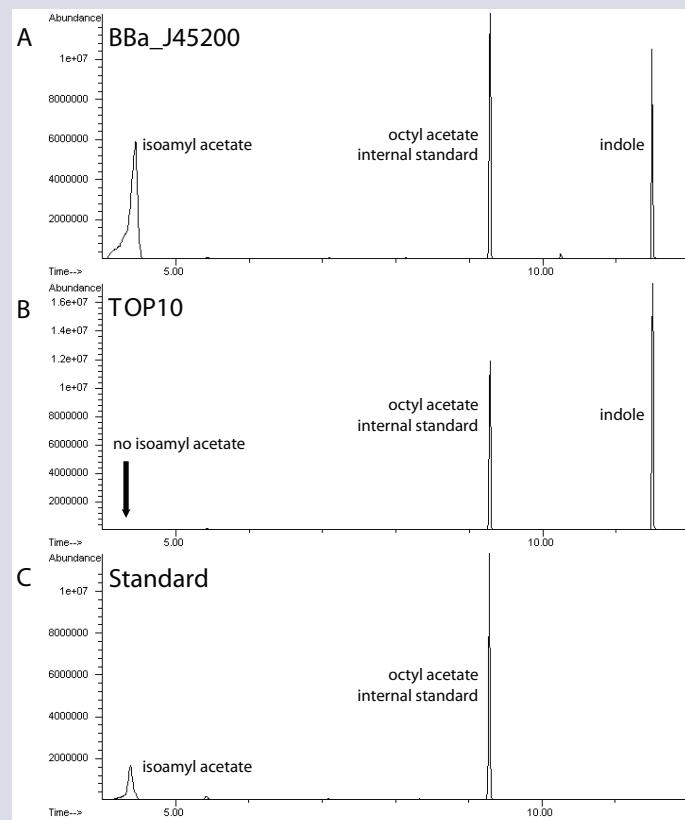
Isoamyl acetate concentration increases with cell density

Usage



Cells expressing ATF1 should be grown in the presence of isoamyl alcohol (5mM).

Chromatography



High levels of isoamyl acetate are produced when the precursor isoamyl alcohol is added to the culture medium (A), unless the cellular chassis (E. coli strain TOP10) does not contain J45200 (B). The retention time of the isoamyl acetate peak from J45200 is identical to that of the pure isoamyl acetate standard (C). Most E. coli strains produce indole. Octyl acetate was used as an internal standard for all samples containing isoamyl acetate

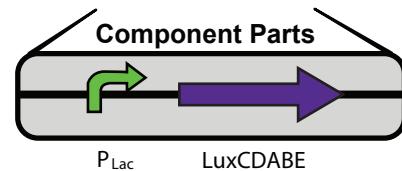
BBa_G10001

Visible Light Generator

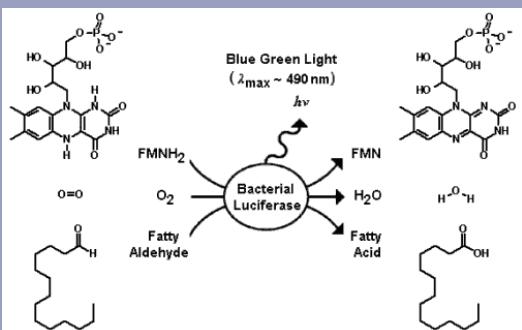


Mechanism & Function

The expression of the Lux operon is controlled by a regulated operator (P_{Lac}). If used in a cell containing lac repressor ($LacI$) then an input such as IPTG can be used to modulate the expression of the Lux operon. When expressed this operon produces the necessary enzymes to generate the fatty aldehyde substrates as well as the luciferase enzyme that converts luciferin to visible light.

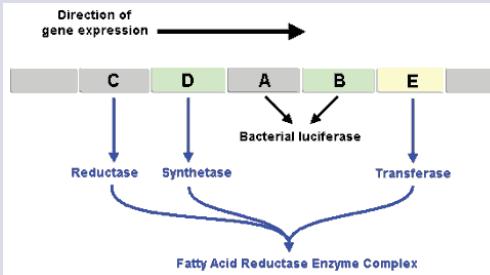


Reaction Mechanism



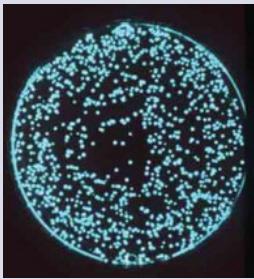
Bacterial luciferin is a reduced riboflavin phosphate (FMNH₂, above) which is oxidized in association with a long-chain aldehyde, oxygen, and a luciferase to produce visible light.

Lux Operon



The fatty acid reductase enzyme complex is needed to recycle the fatty aldehyde substrate in the reaction and luciferase is required to catalyze the reaction. No other exogenous enzymes are necessary since FMNH₂ is provided by the native electron transport chain in *E. coli*.

Usage



Cells expressing the lux operon are visible in low light in liquid culture or as colonies.

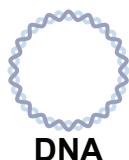
Origin



The Lux operon was isolated from *Vibrio fischeri* a bacteria found predominantly in symbiosis with marine animals such as the bobtail squid (above).

BBa_J04450

Red Fluorescent Protein Generator

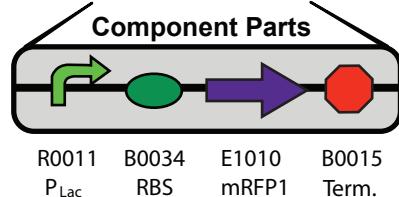


BBa_J04450 →

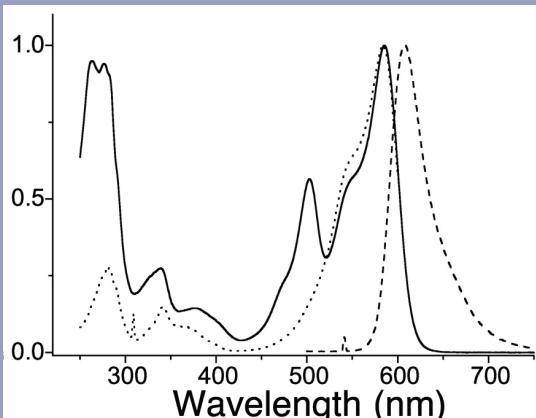


Mechanism & Function

The expression of the gene for mRFP1 is controlled by a regulated operator (PLac). If used in a cell containing lac repressor (LacI) then an input such as IPTG can be used to modulate the expression of mRFP1.

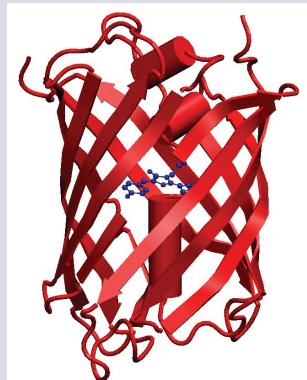


Spectra



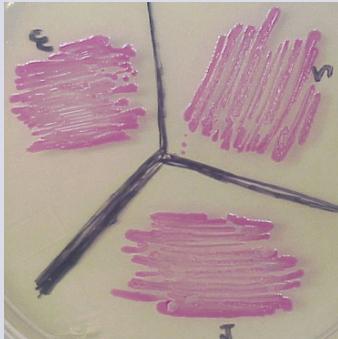
solid: absorptio[n, dotted: excitation,
dashed: emission

Structure



Protein structure for mRFP1 with the chromophore chemical structure shown in blue.

Usage



Cells expressing mRFP1 are visibly red under white light and for optimal fluorescence detection should be excited at ~584nm and detected with a 600nm long pass filter.

Origin



mRFP1 is an engineered variant of dsRED fluorescent protein originally isolated in *Discosoma sp.* (Mushroom Coral)

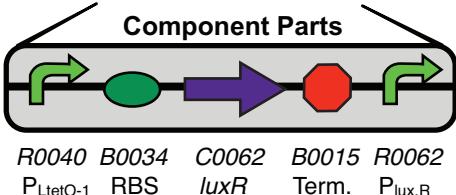
BBa_F2620

3OC₆HSL → PoPS Receiver

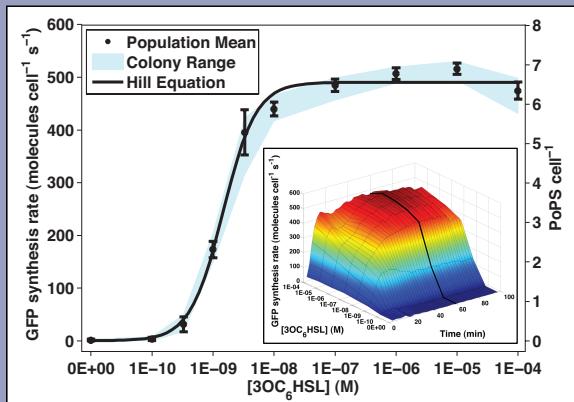


Mechanism & Function

A transcription factor (LuxR) that is active in the presence of a cell-cell signaling molecule (3OC₆HSL) is controlled by a regulated operator (P_{LtetO-1}). Device input is 3OC₆HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input such as aTc can be used to produce a Boolean AND function.



Static Performance*

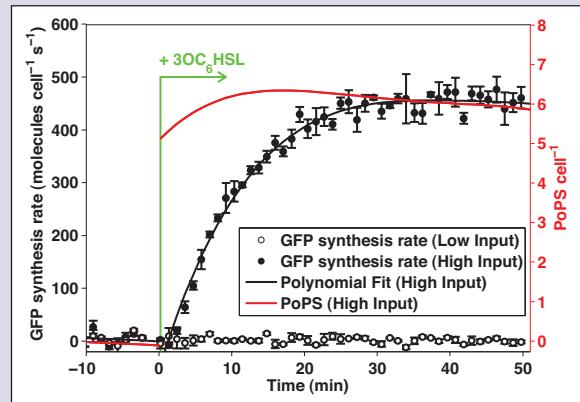


$$P_{out} = \frac{P_{max} [3OC_6HSL]^n}{K^n + [3OC_6HSL]^n} \quad P_{max}: 6.6 \text{ PoPS cell}^{-1}$$

$$K: 1.5E-09 \text{ M } 3OC_6HSL$$

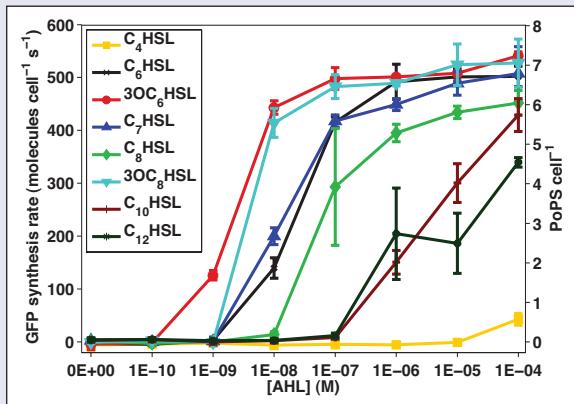
$$n: 1.6$$

Dynamic Performance*



BBa_F2620 Response Time: <1 min
BBa_T9002 Response Time: 6±1 min
 Inputs: 0 M (Low), 1E-07 M (High) 3OC₆HSL

Input Compatibility*



Part Compatibility (qualitative)

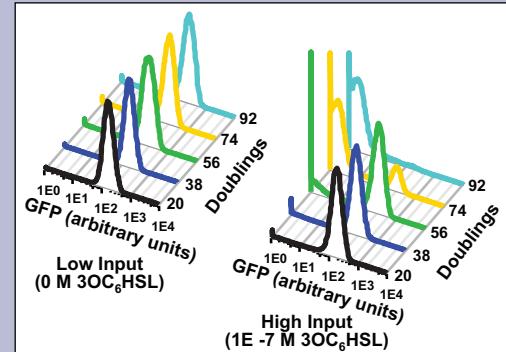
Chassis: MC4100, MG1655, and DH5 α
Plasmids: pSB3K3 and pSB1A2
Devices: E0240, E0430 and E0434

Transcriptional Output Demand (low/high input)

Nucleotides: 0 / 6xNt nucleotides cell $^{-1}$ s $^{-1}$

Polymerases: 0 / 1.5E-1xNt RNAP cell $^{-1}$
 (Nt = downstream transcript length)

Reliability**



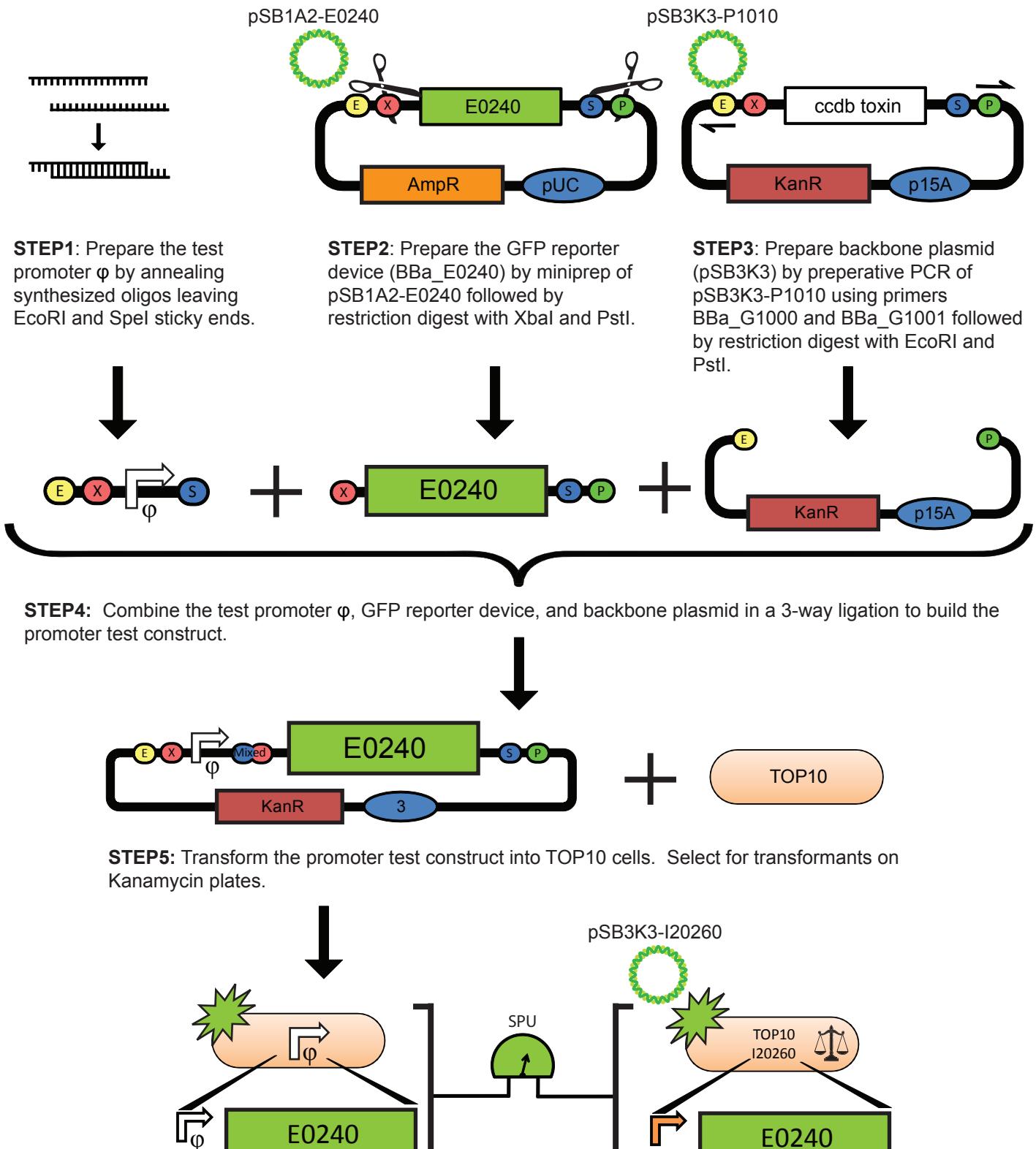
Genetic: >92/>56 culture doublings
Performance: >92/>56 culture doublings
 (low/high input during propagation)

Conditions (abridged)

Output: PoPS measured via BBa_E0240
Culture: Supplemented M9, 37°C
Plasmid: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 multi-well fluorimeter
***Equipment:* BD FACScan cytometer

BioBrick Promoter Measurement Kit

Assembly Instructions

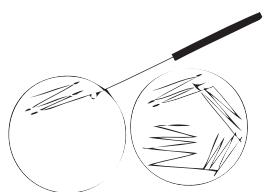


STEP6: Measure the activity of the test promoter ϕ relative to the activity of the reference standard promoter (BBa_J23101). Report promoter ϕ activity in relative units of Standard Promoter Units (SPUs).

BioBrick Promoter Measurement Kit

Measurement Instructions

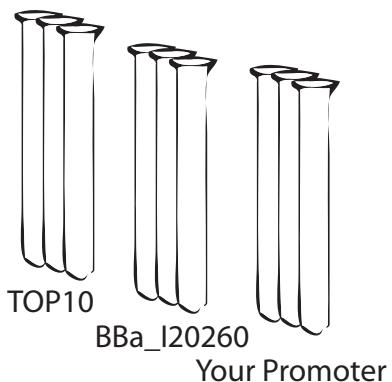
STEP1: Streak 3 plates



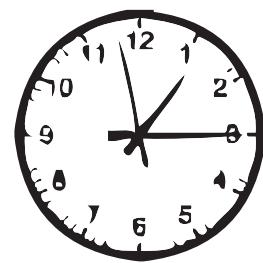
A: TOP10
B: BBa_I20260
C: Your promoter!



STEP 2: Pick 3 colonies from each plate to start overnight cultures in Supplemented M9 Media at 37 C (9 tubes)

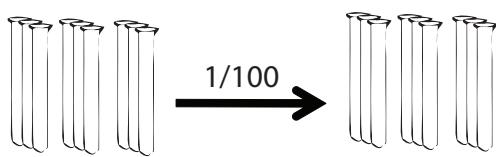


37C



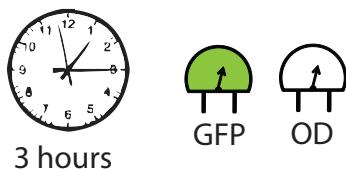
16 hours

STEP 3: Dilute 1/100 into fresh, pre-warmed media incubate at 37C (9 tubes)

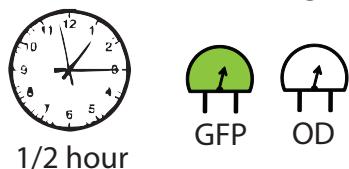


37C

STEP 4: After 3 hours measure GFP and OD



STEP 5: After another half hour measure GFP and OD again



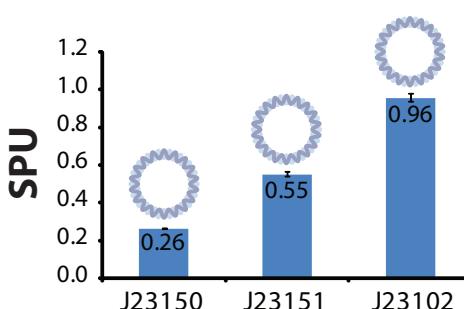
1/2 hour

STEP 6: Input your data into the SPU Calculator and report your results at: <http://partsregistry.org/measurement>

Practice Promoter Set

Weak (J23150), medium (J23151), and strong (J23102) promoters have already been assembled into promoter test constructs and are ready to measure! Transform the 3 test constructs into TOP10 and measure using the protocol above. Report your results at:

<http://partsregistry.org/measurement>



Refinement and standardization of synthetic biological parts and devices

Barry Canton^{1,4} Anna Labno^{2–4} & Drew Endy¹

The ability to quickly and reliably engineer many-component systems from libraries of standard interchangeable parts is one hallmark of modern technologies. Whether the apparent complexity of living systems will permit biological engineers to develop similar capabilities is a pressing research question. We propose to adapt existing frameworks for describing engineered devices to biological objects in order to (i) direct the refinement and use of biological ‘parts’ and ‘devices’, (ii) support research on enabling reliable composition of standard biological parts and (iii) facilitate the development of abstraction hierarchies that simplify biological engineering. We use the resulting framework to describe one engineered biological device, a genetically encoded cell-cell communication receiver named BBa_F2620. The description of the receiver is summarized via a ‘datasheet’ similar to those widely used in engineering. The process of refinement and characterization leading to the BBa_F2620 datasheet may serve as a starting template for producing many standardized genetically encoded objects.

Although many biotechnology applications have been developed¹, the scope and scale of imaginable applications exceed current abilities to implement them^{2,3}. In part this is because the design and construction of engineered biological systems remains an *ad hoc* process for which costs, times to completion and probabilities of success are difficult to estimate accurately⁴. Ideally, biological engineers might develop a design and construction framework that makes routine the incorporation of basic biological functions into many-component integrated genetic systems that behave as expected. Mature engineering disciplines have developed similar frameworks by using the concept of abstraction to define sets of standardized, functional objects that can be used in combination, together with composition rules⁵ that specify how such objects should be assembled.

Composition rules and abstraction are just beginning to be applied to the engineering of biology. For example, BioBrick standard biological parts (<http://partsregistry.org/>) are an early collection of genetically encoded functions that conform to simple rules supporting physical composition⁶ and guidelines for functional composition⁷ (Box 1 and Figs. 1 and 2). As a second example, in support

of functional composition, researchers recently developed a set of prokaryotic promoters that have reduced contextual dependencies in reported promoter activities⁸. We next need to produce quantitative descriptions⁹ that facilitate the reuse of first-generation parts and devices and enable the development of specifications prescribing the design of next generation parts and devices that are engineered to better support composition and abstraction.

Lessons from engineering experiences

Quantitative descriptions of devices in the form of standardized, comprehensive datasheets are widely used in the electrical¹⁰, mechanical, structural and other engineering disciplines (for examples see <http://www.mcmaster.com/>). A datasheet is intended to allow an engineer to quickly determine whether the behavior of a device will meet the requirements of a system in which the device might be used. Such a determination is based on a set of standard characteristics of device behavior, which are the product of engineering theory and experience^{10–13}. The characteristics typically reported on datasheets are common across a wide range of device types, such as sensors, logic elements and actuators: first, a definition of the function and interfaces of the device (inputs and outputs); second, the operating context of the device; third, measured characteristics describing the quantitative behavior of the device.

A crucial measured characteristic is the transfer function, which details the static relationship between device input(s) and output(s) and allows prediction of the equilibrium behavior of composed devices. The dynamic behavior of the device is often reported so that the response time of the device can be compared to the expected timing of the overall system. It is important to report compatibility of device function with other devices or different operating conditions whenever the context in which the device operates is expected to vary. The reliability, or expected time to failure of a device, is also relevant whenever correct device performance over longer timescales is required. Finally, a description of the power and material resources consumed by a device informs the choice of a suitable power supply and resource pools for the system.

We propose to adopt a similar framework for describing engineered biological devices. Despite the differences in materials and mechanisms, biological devices may often be defined with functions that are identical to the functions of electrical, mechanical and other types of existing engineered devices. Biological equivalents of sensors^{14–16}, logic gates^{17–19} and actuators²⁰ have all been demonstrated. Consequently, many of the characteristics found on existing device datasheets might also be useful for biological device datasheets. For example, the transfer function and dynamic behavior characteristics are directly applicable to any biological device with well-defined inputs and outputs. Compatibility of a biological device with genetic backgrounds, growth conditions or

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other devices would also be useful information to biological engineers. Describing the reliability of a biological device is likely to be important but may require the invention of novel metrics due to the self-replicating and evolving nature of biological systems. For example, device failure across many generations might be measured by the number of culture doublings before a nonfunctional mutant becomes fixed in the population. Resource consumption, in the form of a demand for nucleotides, aminoacylated transfer RNAs, polymerases, ribosomes, and so on, is

rarely reported for biological devices, yet such data would help biological engineers decide whether a cellular chassis is suitable to support a particular device or combination of devices.

Developing a prototypical device

We applied the generic framework outlined above to develop a genetically encoded receiver, BBa_F2620 (ref. 21). The receiver builds on work by biologists^{22–24} and early device engineers^{14,15,25–29} (Box 2);

Box 1 What is a standard biological part?

Each newly sequenced genome provides biotechnology researchers with additional natural genetic ‘parts’ to consider. These natural ‘parts lists’ include protein coding sequences, regulatory elements for gene expression and signaling and other functional genetic elements. However, such natural parts do not always behave as a would-be biological engineer might naively expect. For example, they cannot be reliably reused in combination with one another.

Most mature engineering fields depend on catalogs¹⁰ of synthetic parts. These engineered, synthetic parts are often easily distinguished from natural objects because they conform to standards for manufacturing and use. The production of synthetic parts almost invariably requires that raw materials taken from nature be refined and modified in order to produce constrained sets of synthetic objects that meet prescribed requirements—for example, silicon is purified and processed to form wafers that are used to produce microprocessors, whereas iron is refined, processed and machined to produce standardized steel nuts and bolts. If such examples are relevant to the engineering of biology, then an important next step is to attempt to refine and standardize natural biological parts.

What then is a standard biological part? We define a standard biological part to be a genetically encoded object that performs a biological function and that has been engineered to meet specified design or performance requirements. The requirements of greatest interest to us are those that enable reliable physical and functional composition (below). Practically, the engineering of biology now depends mostly on the design, construction and use of engineered DNA. Thus, the standardization of biological parts whose activities are directly encoded via DNA (e.g., a promoter) or via molecules whose primary structure is directly derived from DNA, such as RNA (e.g., a ribozyme) or proteins (e.g., a kinase), is of immediate importance. Other classes of biomolecules (e.g., oligosaccharides, metabolites, small molecules), although also important, are not yet as readily or widely engineered as DNA, RNA or protein, and thus are not yet considered within the engineering framework described here.

Physical composition is the process by which two or more parts or devices are materially connected (devices are combinations of one or more parts that together encode a well-defined, higher-order function). Standards supporting reliable physical composition underlie all other compositional standards. One early example of a

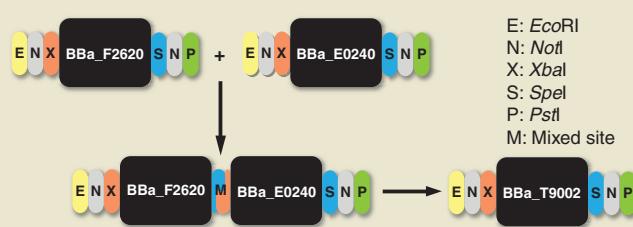


Figure 1 BioBrick assembly standard is a process that enables physical composition of BioBrick standard biological parts.

standard supporting physical composition of engineered biological parts and devices is the BioBrick assembly standard⁶, which allows standard objects to be assembled in an idempotent manner (Fig. 1). The BioBrick assembly standard requires the use of defined prefix and suffix sequences that contain specific restriction endonuclease sites (*Eco*RI, *Xba*I, *Spel*, *Pst*I and *Not*I). Consequently, to be compatible with the BioBrick assembly standard, a synthetic part must be engineered to remove any BioBrick restriction endonuclease sites found in the nucleotide sequence encoding the natural part.

Functional composition is the process and means of connecting the functional inputs and outputs of individual objects together such that the behavior of the composite object is as expected and not an emergent property of the connected parts or any expected interaction(s) with the environment. To support reliable functional composition, standardized objects must be designed to possess certain properties, only some of which are currently understood. For example, a standard signal carrier for device inputs and outputs supports the connection of engineered devices. One standard signal-carrier for transcription-based devices is the flow of RNA polymerases along DNA, measured in polymerases per second transcribing past a defined point on DNA (PoPS). The receiver BBa_F2620 is an engineered device that has been designed to produce a PoPS output signal and can therefore be connected to any other device that accepts a PoPS input signal (Fig. 2). The receiver itself was constructed from BioBrick standard parts via the BioBrick assembly standard.

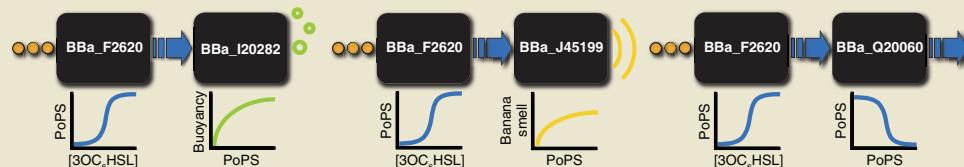


Figure 2 The use of PoPS, a standard signal carrier, enables functional composition of the receiver (BBa_F2620) with devices that accept a PoPS input.

ad hoc engineered constructs, similar in function to BBa_F2620, have been used to control programmed pattern formation, cell culture density and gene expression^{30,31}. BBa_F2620 is a composite device constructed by standard assembly⁶ from five BioBrick standard biological parts: a promoter (BBa_R0040), a ribosome binding site (BBa_B0034), the LuxR coding sequence (BBa_C0062), a transcription terminator (BBa_B0015) and the right lux promoter (BBa_R0062) (Supplementary Table 1 online). Detailed descriptions for each part are freely available online through the Registry of Standard Biological Parts (<http://partsregistry.org/>). We defined the input to the receiver to be the extracellular level of a chemical (3-oxohexanoyl-L-homoserine lactone, 3OC₆HSL) and the output to be a common gene expression signal, the flow of RNA polymerases along DNA (polymerases per second, or PoPS⁷). Hence, BBa_F2620 is a 3OC₆HSL-to-PoPS receiver. We choose to use a PoPS output for the receiver because PoPS possesses many characteristics likely to be necessary in a common signal carrier. First, it is a generic signal that can be used as the input to many other devices. Second, PoPS is a spatially directed signal that can only pass through the DNA molecule connecting the output of an upstream device to the input of a downstream device.

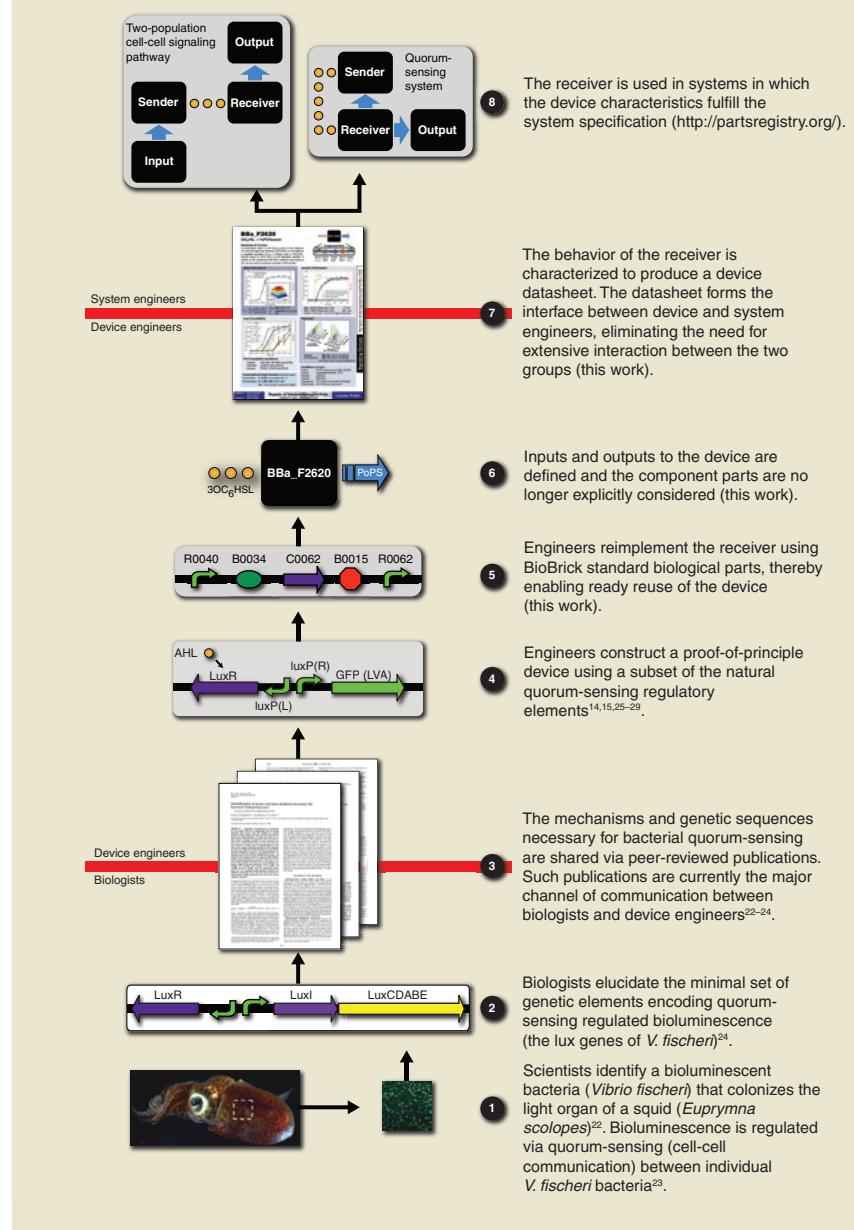
Characterizing the behavior of BBa_F2620

We used widely accessible technology to measure five characteristics that describe the behavior of the receiver under a particular set of operating conditions (described in Supplementary Notes and Supplementary Fig. 1 online). In all experiments, we measured the behavior of the receiver indirectly by measuring green fluorescent protein (GFP) expression from a downstream reporter device (BBa_E0240). The combination of the receiver device and the reporter device is a composite ‘system’ (BBa_T9002). We used independent experiments to parameterize a model of the behavior of the reporter device. This quantitative model allowed us to calculate the specific molecular output of the receiver from our observations of the dynamic behavior of the system (BBa_T9002). The detailed quantitative description of the receiver and its behavior are summarized on a device datasheet (Fig. 3 and Box 3).

We determined the transfer function of the receiver across a range of 3OC₆HSL input concentrations (see Supplementary Notes and Supplementary Fig. 2 online). A Hill equation model with three parameters described the data well (Supplementary Notes). The maximum, saturated output of the reporter was 490

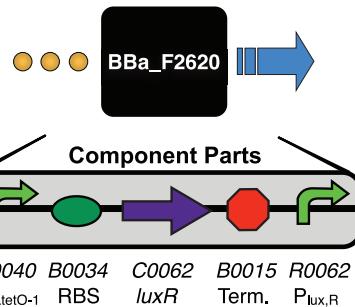
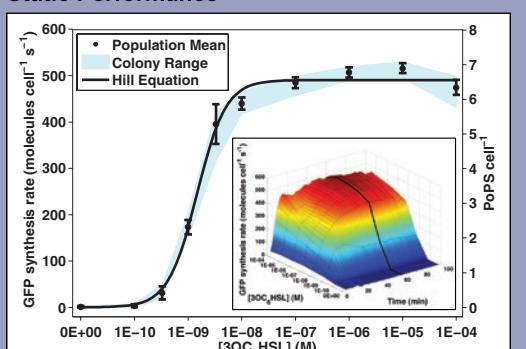
Box 2 From biological discovery to an engineered device

Very few synthetic biological parts are created from scratch (exceptions include RNA or peptide aptamers produced via multiple rounds of screening and selection, or a novel protein fold designed via modeling and simulation). Instead, most synthetic biological parts and devices are produced via a process that starts with the discovery and description of a natural biological function (Steps 1 and 2). Given the need for a particular biological function, engineers scour the scientific literature (Step 3) in hopes of finding suitable natural starting materials (if the necessary natural parts are unavailable or have not been discovered, engineers will often conduct or commission research to produce the needed parts). Once proof-of-principle engineered parts and devices have been demonstrated (Step 4), engineers can perform additional work (Steps 5 and 6) to improve the usability of the synthetic device by refining and standardizing the device in support of more reliable physical and functional composition (Box 1), as well as publishing a quantitative description of device behavior as a datasheet (Step 7; Fig. 3). Engineers working on higher-level systems, comprising many devices, can then readily make use of well-described synthetic biological devices (Step 8).



BBa_F2620 **$3\text{OC}_6\text{HSL} \rightarrow \text{PoPS Receiver}$** **Mechanism & Function**

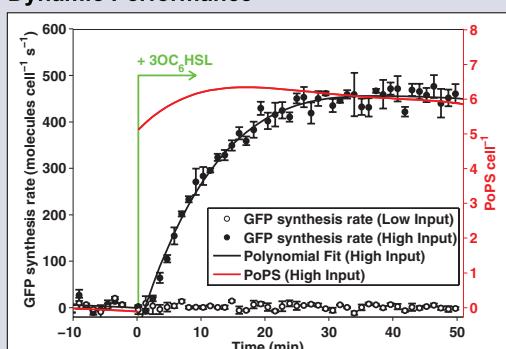
A transcription factor (LuxR) that is active in the presence of a cell-cell signaling molecule ($3\text{OC}_6\text{HSL}$) is controlled by a regulated operator ($P_{\text{LtetO-1}}$). Device input is $3\text{OC}_6\text{HSL}$. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input such as aTc can be used to produce a Boolean AND function.

**Static Performance***

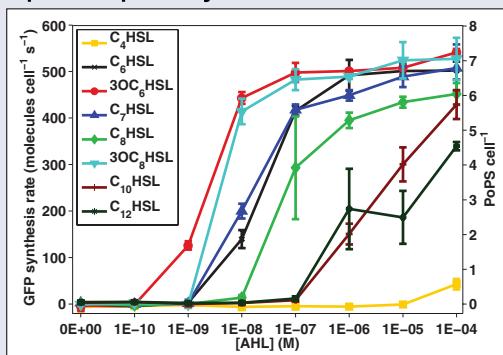
$$P_{\text{out}} = \frac{P_{\text{max}} [\text{3OC}_6\text{HSL}]^n}{K^n + [\text{3OC}_6\text{HSL}]^n} \quad P_{\text{max}}: 6.6 \text{ PoPS cell}^{-1}$$

$$K: 1.5\text{E}-09 \text{ M } 3\text{OC}_6\text{HSL}$$

$$n: 1.6$$

Dynamic Performance*

BBa_F2620 Response Time: <1 min
BBa_T9002 Response Time: 6 ± 1 min
 Inputs: 0 M (Low), $1\text{E}-07$ M (High) $3\text{OC}_6\text{HSL}$

Input Compatibility***Part Compatibility (qualitative)**

Chassis: MC4100, MG1655, and DH5 α

Plasmids: pSB3K3 and pSB1A2

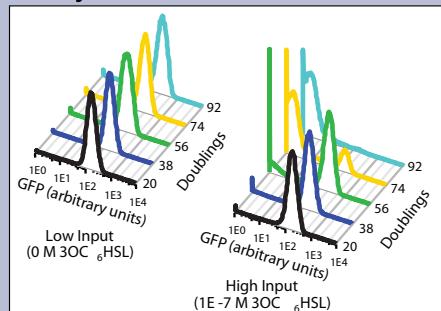
Devices: E0240, E0430 and E0434

Transcriptional Output Demand (low/high input)

Nucleotides: 0 / $6.6 \times \text{N}$ nucleotides $\text{cell}^{-1} \text{s}^{-1}$

Polymerases: 0 / $1.5\text{E}-1 \times \text{N}$ RNAP cell^{-1}

(Nt = downstream transcript length)

Reliability**

Genetic: >92/>56 culture doublings
Performance: >92/>56 culture doublings
 (low/high input during propagation)

Conditions (abridged)

Output: PoPS measured via BBa_E0240

Culture: Supplemented M9, 37°C

Plasmid: pSB3K3

Chassis: MG1655

***Equipment:** PE Victor3 multi-well fluorimeter

****Equipment:** BD FACScan cytometer

Authors: Barry Canton
Ania Labno
Updated: March 2008

Registry of Standard Biological Parts
making life better, one part at a time

License: Public

Figure 3 A prototypical 'datasheet' that summarizes current knowledge of the behavior of the receiver BBa_F2620. The datasheet, which includes a general description and a summary of relevant performance characteristics, is designed to support rapid reuse of the device. The description of the receiver is also available in electronic format²¹. A glossary for the datasheet is provided in **Box 3**.

± 10 GFP molecules/cell/s (uncertainties represent the 95% confidence interval for the parameter). From the measured GFP synthesis rate, we estimated a maximum output from the receiver of 6.6 ± 0.3 PoPS/cell. The minimum observed output was determined to lie between 0 and 3 GFP molecules/cell/s corresponding to a device output of ~ 0 PoPS/cell. Given such a low minimum observed output, we did not include a basal PoPS output in the model describing the output of the receiver. The receiver switch point, the input required for half-maximal output, is $1.5E-9 \pm 3E-10$ M $3OC_6HSL$. The Hill coefficient describing the steepness of the transition from low to high output is 1.6 ± 0.4 . The population distribution was monovariate at all input levels (data not shown). Given that the output of the receiver varies over two logs of input con-

centration, the receiver might be used either as an analog device with a graded output or as a digital device (with the high and low output levels still to be defined).

We determined the dynamic response of the receiver by quantifying the time-dependent increase in fluorescent protein synthesis rates after a step increase in input level from 0 to $1E-7$ M $3OC_6HSL$ (as described in **Supplementary Notes**). Assuming a first-order linear response with time delay, we calculated a response time constant of 6 ± 1 min and a delay of 1.5 ± 0.5 min. Independent experiments demonstrated that the observed dynamic response is largely due to the maturation rate of GFP (**Supplementary Notes** and **Supplementary Fig. 3** online). The model of the reporter device was used to calculate the time-dependent response

Box 3 Details of a datasheet

The following is a glossary of terminology and concepts in the datasheet of BBa_F2620 (**Fig. 3**).

BBa_F2620. The unique part number assigned to the device. The prefix, BBa, denotes a BioBrick part from the alpha release of BioBrick standard biological parts collection (<http://partsregistry.org/>). F denotes a cell-cell signaling device and the remaining numbers identify the specific device.

Static performance. This section contains data describing the steady-state relationship between the input and output of the device. The transfer function shows the input/output relationship 60 min after addition of input signal at which time the reporter device (BBa_E0240) is assumed to be at steady state. Hence, there is a linear relationship between the measured GFP synthesis rate and the PoPS output of the receiver. The inset shows the time and dose-dependent response of the receiver; the 60 min time point is indicated by a solid black line.

Population mean. The mean output level for either six or nine independent cultures at a given input level. Error bars represent the 95% confidence interval of the mean of the independent cultures.

Colony range. A range bounded by the lowest and highest outputs among the independent cultures at a given input level.

Hill equation. An equation relating the PoPS output per cell of the receiver (P_{out}) to the input concentration of $3OC_6HSL$. P_{max} represents the maximum output of the receiver, K is the device switch point and n is the Hill coefficient.

Dynamic response. This section describes the response of the receiver to a step increase in input level at 0 min. The mean GFP synthesis rates measured for three cultures of the composite part (BBa_T9002) are shown as filled (high input) or empty (low input) circles. Error bars represent s.d. across the independent cultures. The solid black lines are a linear fit to the data (**Supplementary Notes**). The time-dependent PoPS output from the receiver (shown as a solid red line) was calculated using a model of the dynamic behavior of the reporter device (**Supplementary Notes**).

Response time. The time for the output of the receiver to reach 67% of its final value was estimated from the calculated PoPS output of the receiver. The response time of the composite part (BBa_T9002) was calculated by fitting an exponential function to

the GFP synthesis rate data after the addition of $1E-7$ M $3OC_6HSL$ (**Supplementary Notes**).

Input compatibility. The dose response of the receiver to a variety of signaling compounds similar to $3OC_6HSL$ is presented. The data points represent the mean of three independent cultures, and the error bars represent the s.d. of the data for the three independent cultures.

Part compatibility. A list of other biological objects with which the receiver is known to be qualitatively functional.

Chassis. An organism, or genetic background, that can be used to support and power a particular engineered biological device. Details of specific genetic backgrounds can be found online (<http://partsregistry.org/>).

Reliability. The ability of the device to continue to function over many generations is reported. Here, fluorescence-activated cell sorting (FACS) data show the response of the device to a high input signal as a function of culture doublings. Two cases are shown, one in which the culture is propagated under low input conditions and one in which the culture is propagated under high input conditions.

Genetic reliability. The number of culture doublings before a mutant device represents at least 50% of the population. The reported figures are derived from the FACS data and confirmed by DNA sequencing analysis.

Performance reliability. The number of culture doublings before 50% of the population is unable to correctly respond to an input. The reported figures are derived from the FACS data.

Transcriptional output demand. The receiver requires resources from the cellular chassis in order to function. The demand for resources related to transcription is presented as a function of the length of the transcript produced by the output of the receiver.

Conditions. The growth conditions and measurement methods used to characterize the receiver are summarized on the datasheet (see **Supplementary Notes** for details).

License. The ownership, sharing and innovation terms by which the authors provide access to, and use of, the receiver together with the associated characterization data.

of the receiver given the observed response of the reporter. Using this method, we calculated a response time for the receiver of <1 min.

We measured receiver input specificity, which is the ability of the receiver to distinguish between its cognate input signal and similar chemical signals that might also be used in composite systems containing the receiver. Input specificity also describes the compatibility of the receiver within a particular set of related devices. We measured the response of the receiver to input signals carried by different acyl-homoserine lactones, both lacking the 3-oxo moiety and varying in side-chain length (**Supplementary Notes** and **Supplementary Table 2** online). The receiver responds to 3OC₆HSL and acyl-homoserine lactones with side chains of similar length. Any device that produces one of this subset of like acyl-homoserine lactones may be used to send a signal to the receiver. The compounds with the shortest and longest side chains produce very weak device responses, suggesting that the receiver could be used independently in parallel with other devices that respond to these compounds. The datasheet also lists the compatibility of the receiver with a range of genetic backgrounds, output devices and plasmids.

We measured the evolutionary reliability of the receiver coupled to the reporter device by following receiver performance as a function of culture doubling at low input levels (**Supplementary Notes**). Because evolutionary reliability is known to be dependent on levels of recombinant protein expression³², we measured the reliability of the receiver at low input levels so that GFP expression from the reporter device would be negligible. Receiver performance remained constant over 92 culture doublings. For comparison, we also measured the reliability of the composite system (BBa_T9002) at high input levels. Consistently, at high input levels, more than half the cells in the population were nonperforming within 74 culture doublings. Sequence analysis of non-performing mutants indicated that system failure results from a deletion between DNA sequences that are repeated in both the receiver and the reporter devices. Additional experiments confirmed that we were unable to isolate a population of cells that did not already carry the deletion (**Supplementary Notes** and **Supplementary Figs. 4** and **5** online). The failure observed here is an emergent behavior specific to the combination of the receiver and reporter devices. Emergent behavior might be avoided by the development of appropriate design rules. For example, when system operation across many culture doublings is required, repeat sequences sufficient in length and proximity to promote deletion events should be avoided.

We computed the output demand of the receiver using the observed rates of downstream protein synthesis (**Supplementary Notes**). The transcriptional output demand depends both on the output of the receiver and on the length of the transcript encoded by the downstream device (**Supplementary Notes**). At low inputs, the output of the receiver is ~0 and so places a negligible demand on the host cell. At high inputs, the output of the receiver requires $6.6 \times N_t$ nucleotides/cell/s and $0.15 \times N_t$ polymerases/cell, where N_t is the number of nucleotides in the transcript being produced from the output of the receiver. We did not measure the cellular resources required to produce the LuxR protein (BBa_C0062), an essential component of the receiver whose expression places an additional basal demand on the cell.

One function, many devices?

The natural biological system on which the design of the receiver is based has been used to produce other, functionally similar devices^{14,15,25–29}. We compared the behavior of our receiver to these earlier systems (none of which were constructed from BioBrick standard biological parts) to begin to evaluate whether or not the performance of the receiver might depend on external factors such as host cell genetic background, culture

conditions or laboratory environment (**Supplementary Table 3** online). None of the prior studies reported all the characteristics by which the receiver has been described here. What comparisons could be made suggested that the receiver switch point and response time are insensitive to host cell genotype or growth conditions but that the input compatibility is sensitive to host cell genotype or other variables. Notably, two studies reported device switch points that are 100-fold or more different from all other studies^{14,25}. This variation is likely explained by differences in sourcing genetic materials (**Supplementary Table 3**); the amino acid sequences of the LuxR proteins used in these two studies differ by 25% from those used in the other studies.

Summary and conclusions

Here, we developed a generic framework for defining and describing standard biological devices to support the reuse and refinement of many devices. To test the utility of our framework, we used relatively well-understood biological mechanisms to design a device that converts the extracellular level of 3OC₆HSL to PoPS, a common intracellular signal carrier that can be accepted as input by many standard biological devices. We constructed the receiver from five standard biological parts. We used a reporter device also encoded by standard biological parts to measure the quantitative and dynamic behavior of the receiver. Three aspects of our work enable easy reuse of the receiver: (i) our use of standards that support the reliable physical composition of genetic parts, (ii) a device design that produces an output signal that is a common signal carrier and (iii) our extensive and quantitative device description. As evidence, while this manuscript was in preparation, we made freely available the DNA encoding BBa_F2620 and its accompanying datasheet via the Registry of Standard Biological Parts (<http://partsregistry.org/>). Already, 18 higher-order systems incorporating the receiver have been successfully assembled and contributed back to the Registry by teams in the International Genetically Engineered Machines Competition (<http://igem.org/>).

The component parts of the receiver can be adapted to serve functions other than the one chosen here. For example, the behavior of the receiver could be modified in a predictable manner by choosing, as input, one of the acyl-homoserine lactones similar to 3OC₆HSL to which we have demonstrated that the receiver responds. As a second example, in a host cell that constitutively expresses Tet repressor, the receiver can perform a logical AND operation, producing a high output only in the presence of 3OC₆HSL and anhydrotetracycline (aTc). As a final example, removing the promoter regulating the transcription of the LuxR coding region would produce a device that has both a PoPS input and a 3OC₆HSL input. The resulting three-terminal device could be used to perform an AND operation, or as a 3OC₆HSL-dependent PoPS amplifier/attenuator. These examples highlight the value in considering the internal components, inputs and outputs of the receiver in detail to design novel devices. However, such value is gained at the expense of the convenience afforded by choosing a well-described ‘black-box’ device, such as the BBa_F2620 receiver.

Looking forward, much additional work is needed to make routine the engineering of many-component biological systems that behave as expected³³. For example, the framework for describing device behavior introduced here, or an improved framework, should be applied to describe many devices and device combinations. When characterizing combinations of devices, special attention should be paid to combinations that fail to produce the behavior predicted given descriptions of the individual devices. Careful characterization and analysis of such emergent behaviors is needed to support the development of design rules that prevent interactions between devices other than through the defined device inputs and outputs (such as the spontaneous selection for

a deletion within the composite system, BBa_T9002). As a second example, standard input and output signal levels might be defined so that any two devices, when connected, would be well matched. Understanding whether desired device behaviors (such as standard signal levels) can be best engineered via directed evolution, rational engineering or a combined approach^{29,34–36} will help researchers to produce well-behaved devices more quickly.

Finally, because the receiver can be used in many systems and because we hope to promote the collaborative development and unfettered use of open libraries of standard biological parts and devices, all of the information describing the receiver is freely available through the Registry of Standard Biological Parts, as mentioned above. We encourage researchers to contribute improvements to the design and description based on experiences with the operation of the receiver (or other parts and devices) directly to the registry. Ultimately, device descriptions such as that presented here should be available online in a machine-readable format that will enable the computer-aided design of many-component engineered biological systems.

Note: Supplementary information is available on the Nature Biotechnology website.

ACKNOWLEDGMENTS

We thank T. Knight; R. Rettberg; members of the Endy, Knight and Sauer labs and staff of the Registry of Standard Biological Parts for discussions, advice and materials throughout the work. We thank R. Brent, U. RajBhandary, C. Smolke, B. Studier and anonymous reviewers for comments on earlier versions of this manuscript. This research was supported by grants to D.E. from the US National Science Foundation, Defense Advanced Research Projects Agency and National Institutes of Health. B.C. was supported by a National University of Ireland training fellowship. Additional support was provided by the Massachusetts Institute of Technology.

AUTHOR CONTRIBUTIONS

B.C. and D.E. initiated the work. B.C., D.E. and A.L. designed the experiments. B.C. and A.L. performed the experiments. B.C., A.L. and D.E. analyzed the data and wrote the paper.

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Setting the standard in synthetic biology

Adam Arkin

Standards for characterization, manufacture and sharing of information about modular biological devices may lead to a more efficient, predictable and design-driven genetic engineering science.

Although genetic engineering—the technical ability to edit DNA—has led to impressive biotechnology applications, these generally require many years of work and trial-and-error experiments to implement¹. A concerted effort among synthetic biologists and allied fields might increase efficiency by developing rigorous characterization and manufacturing protocols linked to formal sharing of information and material through registries of biological parts and standard ‘datasheets’ (**Box 1**). In this issue, Endy and colleagues² present a case study demonstrating a possible datasheet for a biological part. Although they focus on a particular composite part—a genetically encoded cell-cell communication device—the authors’ broader assertion is that there is a science to be developed concerned with the proper packaging and characterization of ‘modular’ biological activities so that these may be efficiently assembled into applications. If successful, this science would yield the profound benefits seen in other engineering sciences but not yet realized in the biological engineering community.

Engineers are fond of standards. A good device standard defines sufficient information about discrete parts to allow the design of predictable complex composite systems. It also provides guidelines for the minimal characterization and manufacturing tolerances of new elements. If suitably designed, a standard can also lead to the abstraction of a composite element’s behavior into a few key functions and requirements, thereby greatly simplifying the design and analysis of the engineered system. If the abstractions are chosen just so, they may form a complete mathematical framework for design, as Boolean logic does in electronic engineering.

Datasheets are an embodiment of such engineering standards. They contain a formal set of context-dependent, input-output behaviors, tolerances, requirements, physical interconnect ‘form factors’ (the mechanical

requirement for physical incorporation of the device into a system) and other details about a particular part or subsystem. This compact form enables engineers to rapidly select from a vast list the parts that will meet their design requirements. Adherence to the set of standards ensures that each device and

the systems made from them will work as advertised.

Endy and colleagues² characterize a cell-cell communication receiver to demonstrate what it might take to create such standards. First, the form factor of the device is stated to be compliant with the BioBrick standard with which

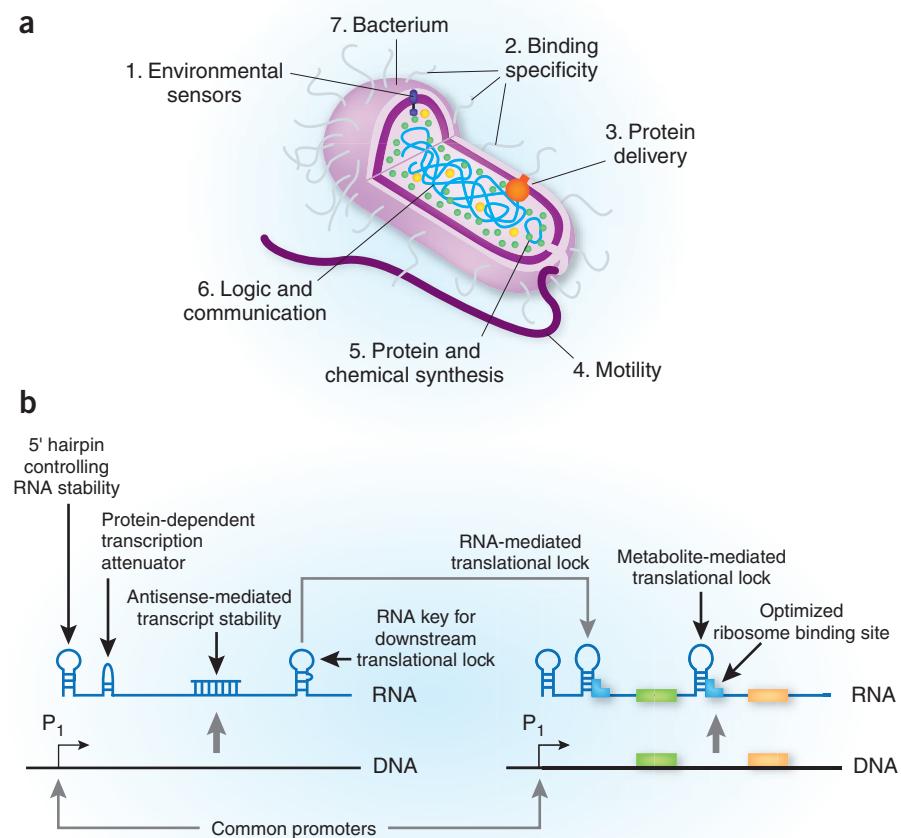


Figure 1 Examples of synthetic biological devices. (a) Different classes of a biological device. Tunable devices: 1 and 2. These devices perform functions whose particular features, such as specificity or affinity, may be changed with difficulty and only modestly. Once a datasheet is made for one member of a class, it can be applied to other members. Specific and/or complex devices: 3,4,5,7. These devices perform complex specialized functions used in one or a few variant forms in different applications. Their datasheets are necessarily non-standard and must be defined for each new device. Designable/scalable devices: 6. These devices are structured such that many new variants with new specificity or activity are easily designed (see b). Datasheets are reusable and become stable and mature quickly. (b) Gaining control over the central dogma with designable/scalable RNA devices. The diversity of known RNA structure-based mechanisms for regulating all aspects of gene expression and the emerging principles for altering their specificities suggest that it may be possible to develop designable, predictable and large families of parts to homogenize gene regulatory network design. The development of datasheet formats for each of the classes illustrated here would greatly aid the exchange of information about these parts and the assessment of their quality.

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Endy is associated. BioBricks is a protocol for the relatively easy cloning and physical linking of biological parts together on a DNA strand. Parts adhering to this standard in the MIT Registry of Standard Biological Parts (<http://parts.mit.edu/>) follow a formal nomenclature. From its 'name', it may be inferred that the authors' part, BBa_F2620, is (i) compliant with the alpha release of the BioBricks repository (BBa); (ii) is of the 'F' or signaling class of function (there are thirteen such classes); and (iii) has an accession number of 2620.

Second, the authors define their device's input and output. The input is the chemical concentration of a particular homoserine lactone ($3\text{OC}_6\text{HSL}$), a type of small organic molecule produced in certain bacterial quorum-sensing systems³. The output is defined as PoPS (polymerases per second), or the number of RNA polymerases crossing a particular point in DNA per unit time. In this case, the output is from a specific promoter in the construct. The PoPS unit is also a standard of a sort, defining a 'common carrier' of transcriptional information (like current or voltage in electrical devices).

BBa_F2620 is a composite part made up of five other BioBrick components. These, in their order on the DNA strand, are: a TetR promoter, a particular ribosome binding site, the *luxR* gene (whose product is a transcription factor activated by the presence of certain HSLs), a transcriptional terminator and the LuxR-sensitive promoter. In cells lacking TetR, LuxR is constitutively expressed and will activate its promoter when the cell is exposed to certain HSLs. It is consistent with synthetic biology philosophy that one could characterize each of the subcomponents and from these predict the composite behavior of the overall device. It is equally consistent, as done by Endy and colleagues², to encapsulate all this internal function into a 'gray box' in which only the properly characterized input-output behavior is necessary for an engineer to know how to use this 'abstracted' composite component in a larger design. The authors cite many other groups that used this encapsulated device, BBa_F2620.

This philosophy (suitably hedged with caveats by the authors) raises the question of what proper characterization entails. Clearly, one should vary the inputs and measure the outputs. But should these be steady-state or dynamic measurements? What range of concentrations of input should be assayed? What methods will best quantify PoPS, and what precision is necessary? Should the response be measured during an exponential or stationary phase of growth? At 37 °C or 25 °C? In minimal media or rich? In *Escherichia coli* DH5α or MG1655? In a multicopy plasmid or integrated in the genome? Even for the obvious input-output behavior, the list

of considerations goes on and is highly dependent on the final application. Synthetic biological devices reported in the past make somewhat arbitrary, if reasonable, choices about which of these to consider (see, e.g., ref. 4).

Endy and colleagues² also show that it might be important to measure device properties beyond those of the designed inputs and outputs. How responsive is the device to other members of the HSL family of signaling molecules? To what extent does operation of the device drain cellular resources and affect growth? What mechanisms of mutation inactivate the device, and how quickly do such mutants take over? The authors do an admirable job of implementing methods for measuring device performance for a wide array of these conditions, even using blunt instruments for measurement of expression such as green fluorescent protein. Their compact summary of results in their datasheet is impressive in its communication of these complex results, although there is much to be found in their supplementary information as well.

It is easy to throw stones. For example, the BBa_F2620 data sheet states that the device is "qualitatively" compatible in different hosts. This seems strangely noncommittal for a datasheet. How would an engineer use this beyond knowing there was work to be done in strains of *E. coli* other than MG1655? Why weren't key device properties, such as turn-off time, measured? How constrained were the parameters of the model by the data for the primary input $3\text{OC}_6\text{HSL}$? Was the mathematical form of the model appropriate for the other HSLs tested? Did each bacterium in the population show identical behavior, or was there single-cell heterogeneity? Should a stochastic model have been used to describe the device? There are challenges here in model selection and parameter estimation known to plague every engineering science. Whereas aspects of this problem in synthetic biology have been considered by other laboratories^{5–8}, the characterization of BBa_F2620 sets up perhaps the first nearly complete standard to which future attempts can be compared. However, unlike many other engineering disciplines, biology does not yet possess a theory of what the minimal information about a biological part should be. But, in the words of Voltaire, "The perfect is the enemy of the good."

Endy and colleagues' results² also underscore conceptual issues with defining datasheets for biological parts. There is much uncertainty about what affects the behavior of biological circuitry and systems. For example, what precisely differs between the *E. coli* strains MG1655 and DH5α that causes differences in BBa_F2620 function? What untested cellular functions might this device perturb? Synthetic biologists might control for such issues by agreeing

to use and characterize devices in a number of common 'chassis' organisms, but what happens when we try to put different devices into the same strain?

Even carefully designed device interfaces can yield unpredictable interactions. Imagine a PoPS-out device whose polymerases exit an open reading frame and a PoPS-in device that starts with a ribosome binding site followed by an open reading frame. The resulting multicistronic transcript formed by the composition could, for example, yield new RNA structures that affect both the expression of the upstream gene and the rate of polymerase read-through to the downstream gene. There are also likely to be parasitic and unpredictable interactions among components as well as with the host. It is possible, for example, that introduction of a particular device in a design will drain necessary common resources (such as ribosomes or transcription factors) from another device. There also may be unpredicted interactions among component and/or host molecules. Or a new device might place the cell in a stressed state that affects both growth and mutation rates of other devices.

In addition to the challenges posed by unpredictability, the other key challenge to standardization is the sheer heterogeneity of biological device types. There are elementary types of parts, such as DNA-binding protein domains⁹, and extremely complex 'composite' parts, such as type III protein secretion systems¹⁰. Clearly, these represent very different categories of function whose properties require distinct types of experiments to characterize their behaviors, tolerances and compatibilities (Fig. 1a and Box 1). Even DNA-binding domains may belong to different protein families, each with a different set of key properties to measure (e.g., the ability to be fused with transcriptional activation domains).

It may seem as if there is such an infinite number of functions arrived at by evolution that there is no hope for standardizing their characterization. However, though large, the space of different types of elementary functions is finite and has been assembled in a limited number of ways to create the variety of organisms we see today. There are modules of function and evolvable structures of proteins and circuits that are shared, tuned and rewired across and within organisms to create new behaviors. This suggests that evolution has perhaps arrived at a tunable basis set of parts from which new complex organismal function can be rapidly evolved^{11,12}. It seems we can exploit this for our own designs.

Some of these tunable functions, such as ribosome binding sites¹³, riboswitches¹⁴, eukaryotic protein interaction scaffolds¹⁵ and zinc-finger

Box 1 What is in a datasheet?

Synthetic biology aims to create the standards, abstractions and protocols to make design and manufacturing of new biological function inexpensive, efficient, predictable and reliable. The standards seek to define modules of biological function both with regard to how parts are physically linked and which of their behaviors must be characterized to enable a designer to predict how they will function as a group. Datasheets are compact, prescribed formats for formally communicating this information. For every biological device, there will be certain information common to all devices (top) and data and protocols specific to the particular device in question (examples below).

Generic datasheet format for a biological device

Part-UID: an accession number for a registry

Name: a name compliant with a standard nomenclature

Brief description of function: a few paragraphs describing the device's key behaviors

Description of use and significance: a short narrative on the uses conceived for the device

Notes on usage: context dependencies, compatibilities, growth phase and media requirements, etc.

References: publications on the device and its use in larger systems

Authorship: information about the creators of the device

Declaration of intellectual property: information on patents and licenses associated with the device

Safety class: what sort of lab can use the device

Sequence: FASTA sequence

Packaging type: protocol for physical linkage (e.g., BioBrick version alpha)

Annotated sequence: the functions and other part-UIDs that make up the device

Data: any measurements on the device (see examples below)

Property measured: one sentence or less

Chassis ID: the host used for testing the system

Vector ID: the location of the device in the host's genetic material

Property description: what is measured and why (1–2 paragraphs)

Protocol used: all information needed to understand the measurement

Measurement data: data file including author information (may be different from above), the data format and the data

Possible cell-cell communication measurements (after Canton *et al*²)

- Steady-state and dynamic induction curves of the output promoter by different homoserine lactone inputs in different cells
- Reliability over time (mutational inactivation rate)
- Homogeneity of induction in members of the population
- Effect of induction on cellular growth rate
- Effect of growth phase on above function of the device
- Reference to DNA-Binding Protein Domain datasheet for element of current device, LuxR

Possible DNA-binding protein domain measurements

- Crystal structure
- Binding constants for different DNA sequences
- Toxicity of overexpression in different cells
- Stability in different cells
- Composability with different transcriptional activation domains

Possible therapeutic bacterium measurements

- Survival in different hosts
- Tissue localization in different hosts
- Cell-type targeting efficiency in different hosts
- Immune response of host to therapeutic organism
- Dose-efficacy curves of therapeutic organism
- Efficacy of safety measures
- Mutation rate

proteins¹⁶ (whose functionality can be changed by engineering a few key sites), suggest that a careful choice of parts 'families' developed to support synthetic biological application would be very powerful. A family of parts is a set of devices derived from the same basic core structure in which each member has been slightly modified to vary a particular key property. Since members of the family are closely related they are likely to share physical mechanisms and therefore characterization protocols.

For example, it may be possible to find a small set of parts families that allow us to construct transcriptional-translational control circuits

of any complexity at will. Designable RNA elements that control mRNA stability, transcription and translation have all been reported; there are examples of each that are sensitive to metabolites, proteins or other RNA in the cell¹⁴. Designing and characterizing large families of such RNA parts responsive to different inputs could be a large step in gaining control over the central dogma (**Fig. 1b**).

Such a basis set would allow each RNA logic gate to be transcribed from a separate copy of the same promoter, thus providing to each gate a homogeneous 'transcriptional power'. The physical basis for the functioning of these

parts is largely governed by RNA-folding physics and in many cases may be designable by Watson-Crick base pairing. This part set may be as close as we can get to a scalable, physically homogeneous, computationally designable basis set of biological parts. The network effect gained by having many labs working simultaneously to standardize characterization and design of these parts is greatly facilitated by the use of datasheets and repositories like those proposed by Endy and colleagues². Assessment of both data quality as well as efficacy of design and prediction tools are greatly enhanced by such central resources, as users of the main

biological databases and competitors in CASP (Critical Assessment of Techniques for Protein Structure Prediction) can attest.

Unavoidably, some devices will be nearly one-off characterizations. Complex multi-factor systems such as type III secretion, flagellar biosynthesis or photosynthetic systems will require very specialized measurements for their characterization¹⁰. The existence of specialized parts is prevalent in other engineering systems. But the work of Endy and colleagues² and others in the community gives hope that there will be basis sets of parts that make scalable, predictable, reliable design of certain functions a reality for biological systems.

No standard, however mature, is set in stone. It must evolve with the development of a field and its technology. Some engineering fields have more formal and less mutable standards than others owing to the nature of their substrate and the uncertainties that plague their manufacture and deployment. Standards can be quite contentious things, especially when the principles of design and the predictability of manufacture are still in their infancy. Synthetic biology is in early gestation, although it is developing quickly. BBa_F2620 is built, for example, to comply with BioBricks version alpha, in which cutting and pasting together of parts is accomplished by particular restriction enzymes and ligation protocols. New protocols for efficient and automated cloning and assembly of synthetic biological parts are being continually developed. The ability to simply synthesize very large pieces of DNA quickly, cheaply and without error is rapidly improving, as are methods for integrating these large constructs into organisms. Whole viral and bacterial genomes have been constructed in one or a few lengths of synthetic DNA^{17,18}. Further, our ability to measure the circuit behavior in cells, even at single-molecule resolution, is rapidly advancing. Thus, what constitutes satisfying standards for manufacturing and characterization is changing quickly as well. In the words attributed to Ken Olsen, the founder of Digital Equipment Corporation: "The nicest thing about standards is that there are so many of them to choose from."

Those of us with pressing practical or commercial applications of synthetic biology will certainly use whatever means necessary to create and optimize our systems and may feel that it is too early and burdensome to develop standards. But it is in our interests to contribute to this mission both because we are familiar with the practical need for and limitations of different proposed approaches and because we have the most to gain if the effort is successful. With their work, Endy and colleagues² have enunciated a challenge. However difficult and imperfect our stan-

dards may be, let's push this idea to its limits and see where it will take us.

This view is shared by many in the field and is a central thrust within the Synthetic Biology Engineering Research Center (SynBERC, <http://www.synberc.org/>), to which the authors of this paper, and I, belong. There may also be an opportunity for journals to foster this activity during a period when only a few specialize in the field. For example, a newly launched journal, *Synthetic Biology*, will be accepting datasheets in the spirit of Figure 1 of the paper² (and the examples in Box 1 above). Authors will be requested to store experimental constructs in a public repository. (In the spirit of full disclosure, I am Editor-in-Chief of this journal.)

Such community repositories will yield the most benefit when synthetic-biology designs scale to systems requiring many interacting parts, thereby limiting the utility of even inspired tinkering to optimize function. Our planes and computer processors are made possible by sophisticated engineering programs that model characterized parts that are designed and manufactured to work together predictably. Although we cannot quite yet imagine what synthetic biological applications might require the numbers and quality of elements on which these advanced technological systems rely, it is economically and socially important that we improve the efficiency, reliability and predictability of our biological designs. Engineering cells for production of chemicals in a fermentor

remains a key technical and economic challenge¹. But there also exist critical applications beyond the bioreactor—in the environment, in agriculture and in medicine—for which it would be at least soothing to know that they could be engineered for dependable and safe function. Setting the standards—high standards—is a clear prerequisite.

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The long and short of carbon nanotube toxicity

Kostas Kostarelos

Toxicological and pharmacological studies suggest guidelines for the safe use of carbon nanotubes in medicine.

The unique physical, chemical and electronic properties of carbon nanotubes (CNTs) have generated much interest in their potential medical applications. Although most studies have assessed the pharmacological efficacy, stability and toxicity of CNTs *in vitro*¹, two recent reports, in the *Journal of Toxicological Sciences*² and *Nature Nanotechnology*³, explore

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their carcinogenic risk *in vivo*. Notably, these studies reveal that CNTs delivered to the abdominal cavity of mice can induce a response resembling that associated with exposure to certain asbestos fibers. What is the significance of these findings for efforts to develop CNTs as delivery vehicles for therapeutic and diagnostic agents?

Carbon nanotubes are seamless cylindrical structures comprising single or multiple concentric graphene sheets. Applications of both single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs) have long been haunted by fears of toxicity because of their

Methodology

Open Access

Engineering BioBrick vectors from BioBrick parts

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Published: 14 April 2008

Received: 14 February 2008

Journal of Biological Engineering 2008, **2**:5 doi:10.1186/1754-1611-2-5

Accepted: 14 April 2008

This article is available from: <http://www.jbioleng.org/content/2/1/5>

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Abstract

Background: The underlying goal of synthetic biology is to make the process of engineering biological systems easier. Recent work has focused on defining and developing standard biological parts. The technical standard that has gained the most traction in the synthetic biology community is the BioBrick standard for physical composition of genetic parts. Parts that conform to the BioBrick assembly standard are BioBrick standard biological parts. To date, over 2,000 BioBrick parts have been contributed to, and are available from, the Registry of Standard Biological Parts.

Results: Here we extended the same advantages of BioBrick standard biological parts to the plasmid-based vectors that are used to provide and propagate BioBrick parts. We developed a process for engineering BioBrick vectors from BioBrick parts. We designed a new set of BioBrick parts that encode many useful vector functions. We combined the new parts to make a BioBrick base vector that facilitates BioBrick vector construction. We demonstrated the utility of the process by constructing seven new BioBrick vectors. We also successfully used the resulting vectors to assemble and propagate other BioBrick standard biological parts.

Conclusion: We extended the principles of part reuse and standardization to BioBrick vectors. As a result, myriad new BioBrick vectors can be readily produced from all existing and newly designed BioBrick parts. We invite the synthetic biology community to (1) use the process to make and share new BioBrick vectors; (2) expand the current collection of BioBrick vector parts; and (3) characterize and improve the available collection of BioBrick vector parts.

Background

The fundamental goal of synthetic biology is to make the process of engineering biology easier. Drawing upon lessons from the invention and development of other fields of engineering, we have been working to produce methods and tools that support the design and construction of genetic systems from standardized biological parts. As developed, collections of standard biological parts will

allow biological engineers to assemble many engineered organisms rapidly [1]. For example, individual parts or combinations of parts that encode defined functions (devices) can be independently tested and characterized in order to improve the likelihood that higher-order systems constructed from such devices work as intended (Canton, Labno, and Endy, submitted) [2,3]. As a second

example, parts or devices that do not function as expected can be identified, repaired, or replaced readily [4,5].

We define a biological part to be a natural nucleic acid sequence that encodes a definable biological function, and a standard biological part to be a biological part that has been refined in order to conform to one or more defined technical standards. Very little work has been done to standardize the components or processes underlying genetic engineering [6]. For example, in 1996, Rebatchouk *et al.* developed and implemented a general cloning strategy for assembly of nucleic acid fragments [7]. However, the Rebatchouk *et al.* standard for physical composition of biological parts failed to gain widespread acceptance by the biological research community. As a second example, in 1999, Arkin and Endy proposed an initial list of useful standard biological parts but such a collection has not yet been fully realized [8]. In 2003, Knight proposed the BioBrick standard for physical composition of biological parts [9]. Parts that conform to the BioBrick assembly standard are BioBrick standard biological parts. In contrast to the previous two examples, the BioBrick physical composition standard has been used by multiple groups (Canton, Labno, and Endy, submitted) [10-12], and adoption of the standard is growing. For example, each summer, hundreds of students develop and use BioBrick standard biological parts to engineer biological systems of their own design as a part of the International Genetically Engineered Machines competition [13]. Additional technical standards defining BioBrick parts are set via an open standards setting process led by The BioBricks Foundation [14].

The key innovation of the BioBrick assembly standard is that a biological engineer can assemble any two BioBrick parts, and the resulting composite object is itself a BioBrick part that can be combined with any other BioBrick parts. The idempotent physical composition standard underlying BioBrick parts has two fundamental advantages. First, the BioBrick assembly standard enables the distributed production of a collection of compatible biological parts [15]. Two engineers in different parts of the world who have never interacted can each design a part that conforms to the BioBrick assembly standard, and those two parts will be physically composable via the standard. Second, since engineers carry out the exact same operation every time that they want to combine two BioBrick parts, the assembly process is amenable to optimization and automation, in contrast to more traditional *ad hoc* molecular cloning approaches.

The Registry of Standard Biological Parts (Registry) exemplifies the advantage offered by a physical composition standard such as the BioBrick assembly standard [15]. The Registry currently maintains a collection of over 2,000

BioBrick standard biological parts. Every part in the Registry has a BioBrick part number that serves as the unique identifier of the part (for example, BBa_I51020). The Registry maintains information about each part including its sequence, function, and, if available, user experiences. DNA encoding each BioBrick standard biological part is stored and propagated in *Escherichia coli* plasmid-based vectors [16-19]. Biological engineers can obtain parts from the Registry and assemble them using the BioBrick assembly standard in order to construct many-component synthetic biological systems.

All BioBrick parts are currently maintained on a set of plasmids that includes pSB1A3-P1010, pSB3K3-P1010, pSB4A3-P1010 (see Naming of BioBrick vectors in Methods). However, these BioBrick vectors are *ad hoc* designs that were cobbled together from common cloning plasmids such as pUC19 [20-22]. As a result, whenever a new vector is needed for use with BioBrick parts, a biological engineer must design and assemble the new BioBrick vector from scratch.

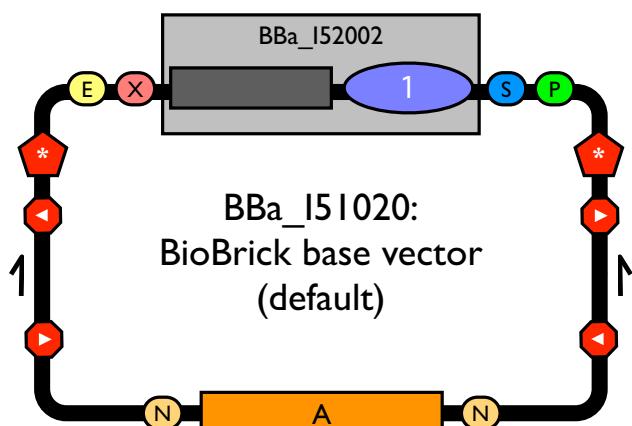
Several plasmid-based cloning systems that support the manipulation, propagation, and expression of DNA fragments have been developed [20-29]. The Gateway® recombinational cloning system and associated vectors are arguably the closest example of a vector standard in biological research [30,31]. For example, several genome-wide collections of open reading frames (ORFeomes) have been compiled using the Gateway® cloning system [32-34]. The Gateway® system has even been extended to allow assembly of multiple DNA fragments [35,36]. However, the Gateway® system generally requires customized assembly strategies for each new system and therefore does not provide the advantages afforded by the BioBrick standard (above).

Thus, we sought to extend the advantages of BioBrick standard biological parts to the vectors that propagate BioBrick parts. To do this, we developed a new process for engineering BioBrick vectors. The process leverages existing and newly designed BioBrick parts for the ready construction of many BioBrick vectors. To demonstrate the utility of the new process, we constructed seven new BioBrick vectors from the base vector. We also successfully used the new vectors to assemble BioBrick standard biological parts.

Results

The BioBrick base vector (BBa_I51020)

The process for engineering BioBrick vectors from BioBrick parts is primarily based upon a newly designed BioBrick part: BBa_I51020 [Genbank:[EU496089](#)]. The new part is a BioBrick base vector that serves as a scaffold for construction of new BioBrick vectors (Figure 1). Starting

**Figure 1**

The BioBrick base vector (BBa_I51020). Schematic diagram of BBa_I51020: a BioBrick base vector designed to facilitate construction of new BioBrick vectors. Parts from the collection listed in Figure 5 were used to construct BBa_I51020.

from the base vector, new vectors can be built using plasmid replication origins and antibiotic resistance markers that conform to the BioBrick standard for physical composition. Thus, the base vector enables the ready reuse of vector parts available from the Registry of Standard Biological Parts. Use of the base vector to construct BioBrick vectors ensures standardization and uniformity in any resulting BioBrick vectors. For convenience, the base vector includes both a high copy replication origin and ampicillin resistance marker, so the base vector itself is capable of autonomous plasmid replication for easy DNA propagation and purification [37].

All BioBrick vectors derived from the BioBrick base vector have five key features. First, BioBrick vectors include a complete BioBrick cloning site to support the propagation and assembly of BioBrick standard biological parts [9]. Second, BioBrick vectors contain a positive selection marker in the cloning site to ameliorate one of the most common problems during assembly of BioBrick parts: contamination of the ligation reaction with uncut plasmid DNA [38]. Any cells transformed with the BioBrick vector produce the toxic protein CcdB and do not grow [39-41]. Cloning a BioBrick part into the cloning site of the vector removes the toxic *ccdB* gene. Third, BioBrick vectors contain a high copy origin in the cloning site to facilitate increased yields from plasmid DNA purification [42,43]. Again, cloning a BioBrick part into the cloning site removes the high copy origin in the cloning site thereby restoring replication control to the vector origin. Fourth, BioBrick vectors include transcriptional terminators and translational stop codons flanking the cloning

site to insulate the proper maintenance and propagation of the vector from any possibly disruptive function encoded by inserted BioBrick parts [44-47]. Fifth, BioBrick vectors include verification primer annealing sites sufficiently distant from the cloning site to check the length and sequence of the cloned BioBrick part. The primer annealing sites are identical to those found in commonly used BioBrick vectors, such as pSB1A3-P1010, to support backwards compatibility.

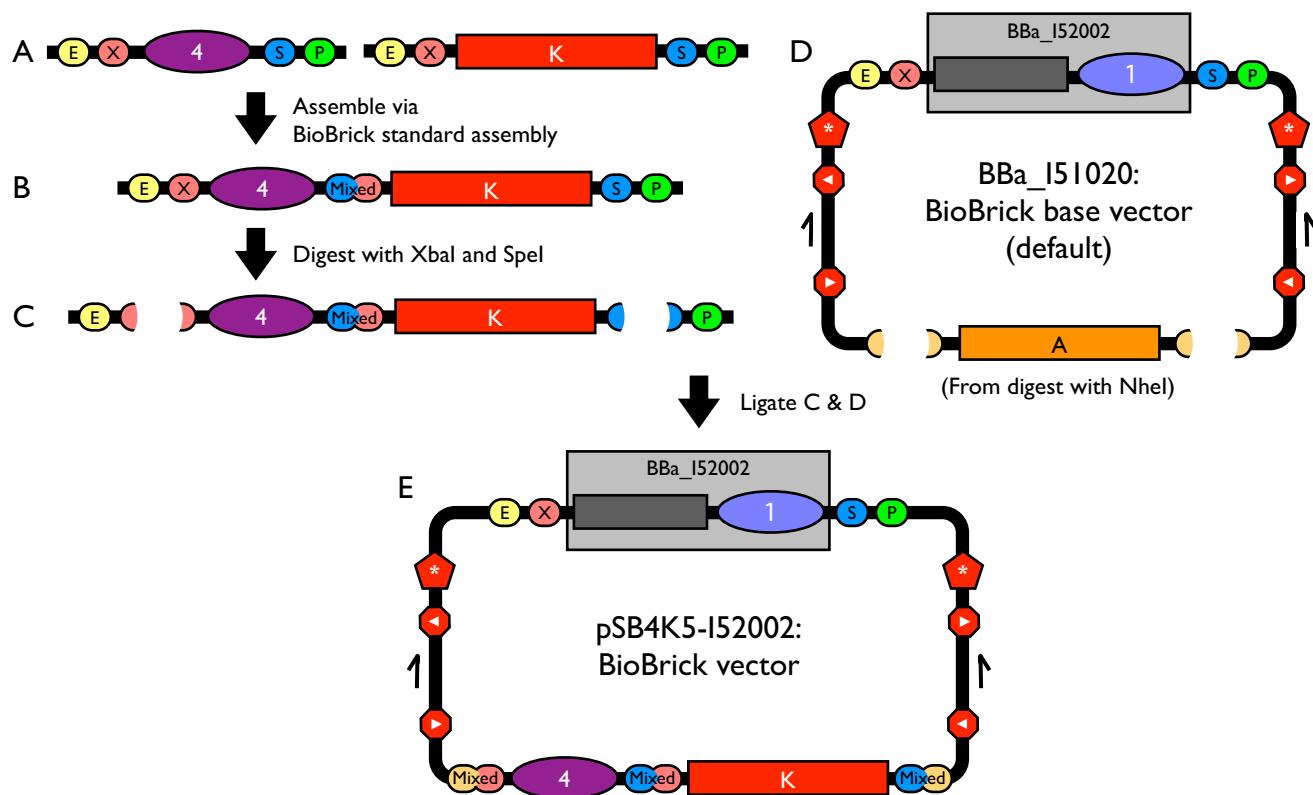
Constructing new BioBrick vectors using the BioBrick base vector

Constructing new BioBrick vectors starting from the BioBrick base vector requires just two assembly steps (Figure 2). The replication origin and antibiotic resistance marker should each be BioBrick standard parts. To construct a BioBrick vector, assemble the origin and antibiotic resistance marker via BioBrick standard assembly (first assembly step). Then, digest the resulting composite part with restriction enzymes XbaI and SpeI, and digest the BioBrick base vector with NheI to excise the ampicillin resistance marker. Next, ligate the composite origin and resistance marker to the linearized base vector (second assembly step). XbaI, SpeI, and NheI all generate compatible DNA ends that, when ligated with a DNA end from one of the other enzymes, produce a non-palindromic sequence that cannot be cut by any of the three enzymes. Thus, proper assembly of the vector eliminates any BioBrick enzyme sites and ensures that the resulting vector adheres to the BioBrick physical composition standard. Finally, transform the ligation product into a strain tolerant of *ccdB* expression, such as *E. coli* strain DB3.1 [48,49].

To support the construction of new BioBrick vectors, we built four new antibiotic resistance markers and two replication origins all as BioBrick standard biological parts. The four antibiotic resistance markers express proteins that confer resistance to ampicillin (BBa_P1002 [Genbank:[EU496092](#)]), kanamycin (BBa_P1003 [Genbank:[EU496093](#)]), chloramphenicol (BBa_P1004 [Genbank:[EU496094](#)]), and tetracycline (BBa_P1005 [Genbank:[EU496095](#)]), respectively [50-53]. The two replication origins were derived from the pSC101 (BBa_I50042 [Genbank:[EU496096](#)]) and p15A (BBa_I50032 [Genbank:[EU496097](#)]) replicons, respectively [54,55]. We used the described procedure, base vector, and new vector parts to construct seven new BioBrick vectors: pSB4A5-I52002, pSB4K5-I52002, pSB4C5-I52002, pSB4T5-I52001, pSB3K5-I52002, pSB3C5-I52001, and pSB3T5-I52001 [Genbank:[EU496098](#)-[EU496104](#)].

Assembling BioBrick parts using a new BioBrick vector

BioBrick vectors support assembly of new BioBrick standard parts. The new vectors are compatible with prefix or

**Figure 2**

How to build new BioBrick vectors. Assembly strategy for a new BioBrick vector using the BioBrick base vector BBa_I51020. (A) The replication origin and antibiotic resistance cassette should each be BioBrick standard biological parts. (B) Assemble the desired replication origin and antibiotic resistance cassette via BioBrick standard assembly to construct a composite origin and antibiotic resistance cassette. (C) Digest the resulting BioBrick composite part with XbaI and SpeI. (D) To excise the ampicillin resistance marker, digest the base vector with NheI. XbaI, SpeI, and NheI all generate compatible cohesive DNA ends that, when ligated with a DNA end from one of the other enzymes, produce a non-palindromic sequence that cannot be cut by any of the three enzymes. Finally, ligate the digested composite origin and resistance marker to the digested base vector. (E) The result is the new BioBrick vector pSB4K5-I52002.

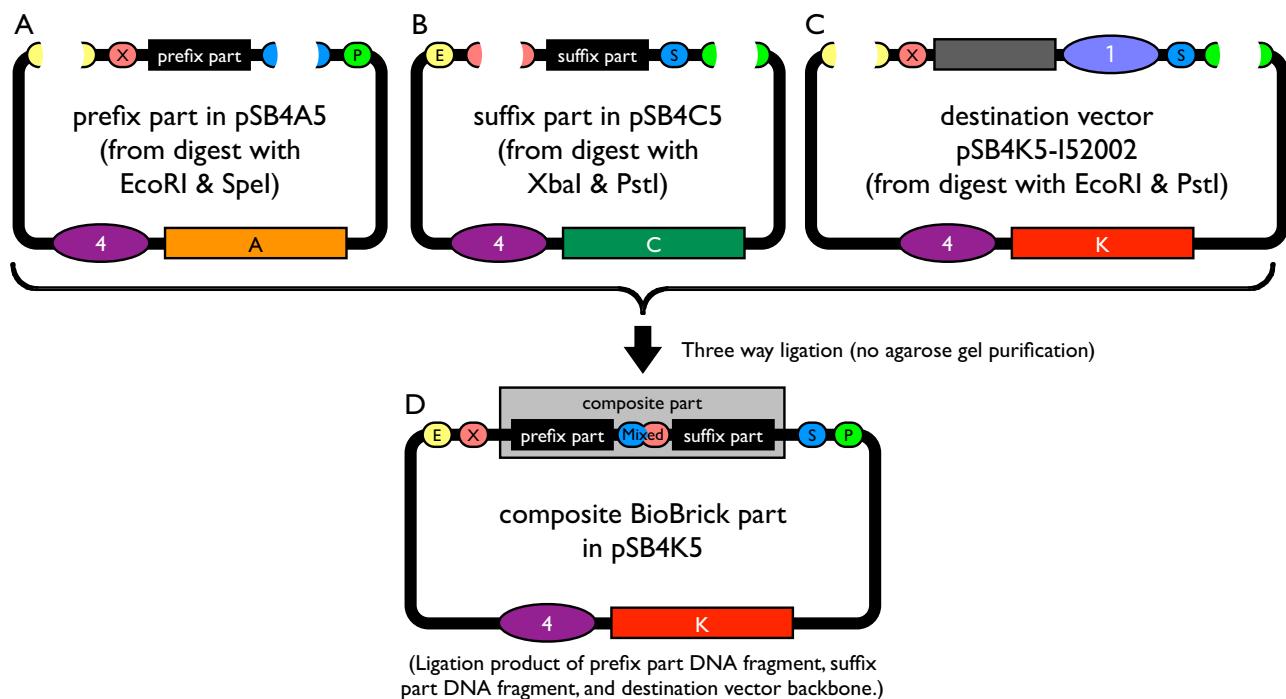
postfix insertions of BioBrick parts as originally described [9]. Alternatively, the new vectors also support three antibiotic based assembly (3A assembly; Figure 3; Shetty, Rettberg, and Knight, in preparation) [56]. 3A assembly is a method for assembling one part (the prefix part) upstream or 5' to a second part (the suffix part) in the BioBrick cloning site of a BioBrick vector (the destination vector). 3A assembly favors correct assembly of the prefix and suffix BioBrick parts in the destination vector through a combination of positive and negative selection. Briefly, 3A assembly works as follows: Digest the prefix part with EcoRI and SpeI, the suffix part with XbaI and PstI, and the destination vector with EcoRI and PstI. Then, ligate the two parts and destination vector and transform into competent *E. coli*. Plate the transformed cells on LB agar plates supplemented with antibiotic corresponding to the destination vector resistance marker. Most of the resulting col-

onies should contain the composite BioBrick part cloned into the destination vector.

To confirm that our new BioBrick vectors function as expected, we assembled new BioBrick standard biological parts using four of the vectors that we constructed. To demonstrate that the composite BioBrick parts were correctly assembled using our new vectors, we performed a colony PCR amplification of the assembled parts and determined that the PCR product length was correct (Figure 4). Each part was also verified to be correct via sequencing with primers that anneal to the verification primer binding sites (BBa_G00100 and BBa_G00102).

Discussion

We developed a new process for engineering BioBrick vectors from BioBrick parts. The process now makes possible the ready construction of many, new BioBrick vectors

**Figure 3**

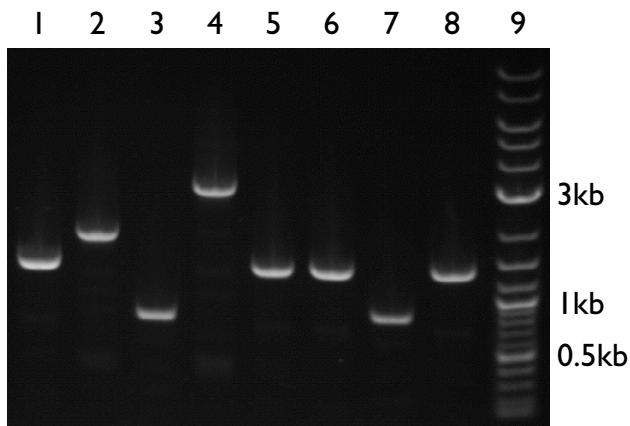
How to use a new BioBrick vector for standard assembly. Assembly strategy for two BioBrick standard biological parts using a new BioBrick vector. (A) Digest the prefix part with enzymes EcoRI and SphI. (B) Digest the suffix part with restriction enzymes XbaI and PstI. (C) Digest the destination vector (*pSB4K5-I52002*) into which the two parts will be assembled with restriction enzymes EcoRI and PstI. Without agarose gel purification of the linearized DNA, ligate the three fragments, transform into *E. coli* and plate on LB agar plates supplemented with the antibiotic corresponding to the destination vector resistance marker. (D) Most of the resulting colonies contain the composite BioBrick part cloned into the destination vector.

using the growing collection of BioBrick parts available from the Registry of Standard Biological Parts. Moreover, new BioBrick vectors can be constructed from the BioBrick base vector in just two assembly steps. Finally, any BioBrick vectors derived from the BioBrick base vector have five key features designed to facilitate the cloning, assembly, and propagation of BioBrick parts. We used the process to construct seven new BioBrick vectors and used the vectors to assemble new BioBrick parts.

Design of new BioBrick vectors parts

To adhere to the BioBrick standard for physical composition, BioBrick vector parts need only be free of the BioBrick restriction enzyme sites. However, we chose to design anew all BioBrick vector parts (Figure 5), so that we could completely specify their DNA sequences. We compiled a list of potentially useful endonuclease sites for removal from all new BioBrick vector parts (Table 1). We targeted each group of endonuclease sites for removal for a different reason. We targeted recognition sites of enzymes that produce compatible cohesive ends to the BioBrick enzymes because such enzymes often prove useful in constructing new variants of BioBrick vectors. We

targeted offset cutter sites because they may be useful in alternative restriction enzyme-based assembly methods [57]. We targeted homing endonuclease sites because they are commonly used in genome engineering [58]. We targeted some nicking endonuclease sites because they can be useful for specialized cloning applications [59]. Finally, we targeted several additional restriction endonuclease sites to keep them available for use by new standards for physical composition. Our list of endonuclease sites constitutes a set of target sequences that should be considered for removal from any newly synthesized BioBrick part, if possible. The target sequence set will change as the synthetic biology community develops new standards for physical composition of BioBrick parts. Some of the targeted endonuclease sites were naturally absent from the DNA sequences encoding our new vector parts. For any remaining sites, we removed the recognition sequences from the BioBrick vector parts by introducing point mutations. However, the functions of the *pSC101* and *pUC19*-derived plasmid replication origins were sensitive to introduced mutations, so the replication origins used in this work are not free of all targeted endonuclease sites (see Methods). Similarly, issues during synthesis led

**Figure 4**

Using the new BioBrick vectors. To verify the function of the new BioBrick vectors, we performed a colony PCR using primers that anneal to the verification primer binding sites. To check the length of the resulting PCR products, we electrophoresed the reactions through an 0.8% agarose gel. Lanes 1–8 are the PCR products resulting from the amplification of the following BioBrick parts cloned into new BioBrick vectors. The desired PCR product lengths are in parentheses. Lane 1 is pSB4A5-I52001 (1370 bp), lane 2 is pSB4K5-T9003 (1883 bp), lane 3 is pSB4C5-E0435 (814 bp), lane 4 is pSB4T5-P20061 (2988 bp), lane 5 is pSB3K5-I52002 (1370 bp), lane 6 is pSB3C5-I52001 (1370 bp), lane 7 is pSB3T5-I6413 (867 bp), and lane 8 is BBa_I51020 (1370 bp). Lane 9 is 1 μ g of 2-log DNA ladder (New England Biolabs, Inc.). The 0.5 kb, 1 kb, and 3 kb DNA fragments in the DNA ladder are annotated.

to an unnecessary redesign of the *ccdB* positive selection marker, so it too is not free of all targeted endonuclease sites.

Construction of BioBrick base vector

To realize our designs for new BioBrick vectors, we contracted for DNA synthesis of the four antibiotic resistance markers, pSC101 replication origin and the entire BioBrick base vector. However, synthesis of the BioBrick base vector was problematic (see Methods). The issues that arose during synthesis are briefly discussed here, because they are relevant to anyone interested in synthesizing new BioBrick parts. Difficulties during synthesis stemmed from the inclusion of both a *ccdB* positive selection marker that is toxic to most *E. coli* strains and a synthetic replication origin that proved incapable of supporting replication of the BioBrick base vector. Commercial DNA synthesis processes currently rely on cloning, assembly, and propagation of synthesized DNA in *E. coli*. In general, for parts whose function are incompatible with growth and replication of *E. coli*, the processes of DNA design and DNA synthesis cannot be easily decoupled. Improve-

ments in commercial DNA synthesis are needed that free the process from dependence on *in vivo* DNA propagation and replication.

Conclusion

The goal of synthetic biology is to make the process of design and construction of many-component, engineered biological systems easier. In support of this goal, a technical standard for the physical composition of biological parts was developed [9]. Here, we extended the same principles of part reusability and standardization of physical composition to the vectors that are used to assemble and propagate BioBrick parts. Using the process described here, new BioBrick vectors can be produced from existing and newly designed BioBrick parts. As a result, myriad new vectors with diverse functions can be built readily to support the engineering of many-component systems. We invite the community to build on this work in several ways. First, we invite the community to use the process described here to construct more BioBrick vectors and share them via the Registry of Standard Biological Parts. Second, we invite the community to expand the collection of parts for making BioBrick vectors. For example, shuttle vector parts, compatible replication origins, and additional antibiotic resistance markers would all be useful contributions to the Registry. Third, we invite the community to further characterize and improve the BioBrick parts that make up BioBrick vectors. For example, important parameters to measure include plasmid copy number, and transcriptional and translational read-through into and out of the BioBrick cloning site.

Methods

Design of BioBrick vector parts and the BioBrick base vector

We designed all BioBrick vector parts and the BioBrick base vector using Vector NTI® Suite 7 for Mac OS X by Invitrogen Life Science Software in Carlsbad, CA. We removed endonuclease recognition sites from the designed parts either manually or using GeneDesign vβ;2.1 Rev 5/26/06 [60].

Construction of BioBrick vector parts

We contracted for DNA synthesis of the four antibiotic resistance markers and the pSC101 replication origin to the DNA synthesis company Codon Devices, Inc. in Cambridge, MA. The four antibiotic resistance markers (BBa_P1002-P1005) were easily synthesized as designed. Testing confirmed that the four markers conferred resistance to the corresponding antibiotics. Synthesis of the pSC101 origin was also straightforward. However, testing revealed that our design for the pSC101 origin (BBa_I50040) was nonfunctional as a replication origin. We successfully reconstructed a functional pSC101 replication origin (BBa_I50042) via PCR of an existing plas-

Part Number	Function	Notation
BBa_G00000	BioBrick cloning site prefix	
BBa_G00001	BioBrick cloning site suffix	
BBa_P1016	<i>ccdB</i> positive selection marker	
BBa_I50022	pUC19-derived high copy replication origin	
BBa_B0042	translational stop sequence	
BBa_B0053 & BBa_B0054	forward transcriptional terminator	
BBa_B0055 & BBa_B0062	reverse transcriptional terminator	
BBa_G00100	forward verification primer annealing site (VF2)	
BBa_G00102	reverse verification primer annealing site (VR)	
BBa_B0045	NheI restriction site	
BBa_P1006	ampicillin resistance marker (reverse orientation)	
BBa_P1002	ampicillin resistance marker	
BBa_P1003	kanamycin resistance marker	
BBa_P1004	chloramphenicol resistance marker	
BBa_P1005	tetracycline resistance marker	
BBa_I50042	pSC101 replication origin	
BBa_I50032	p15A replication origin	

Figure 5

New BioBrick vector parts. The Registry part number, function, and graphical notation of each constructed BioBrick vector part are listed. The part collection includes (1) BBa_G00000: BioBrick cloning site prefix including the EcoRI (E) and XbaI (X) restriction enzyme sites, (2) BBa_G00001: BioBrick cloning site suffix including the SphI (S) and PstI (P) restriction enzyme sites which, together with the BioBrick prefix, forms a BioBrick cloning site for compatibility with all BioBrick standard biological parts, (3) BBa_P1016: positive selection marker *ccdB* to improve yield of insert-containing clones during part assemblies, (4) BBa_I50022: pUC19-derived high copy replication origin within the BioBrick cloning site that allows for easy plasmid DNA purification of the base vector and any derived vectors, (5) BBa_B0042: a short DNA sequence that has translational stop codons in all six reading frames to prevent translation into or out of the BioBrick cloning site, (6) BBa_B0053-B0055 and BBa_B0062: forward and reverse transcriptional terminators flanking the BioBrick cloning site to prevent transcription into or out of the BioBrick cloning site, (7) BBa_G00100 and BBa_G00102: sequence verification primer annealing sites for primers VF2 and VR, (8) BBa_B0045: NheI (N) restriction site for insertion of desired replication origin and resistance marker to construct vector of interest, (9) BBa_P1006: ampicillin resistance selection marker to facilitate propagation of the base vector, (10) BBa_P1002-P1005: four antibiotic resistance markers, and (11) BBa_I50042 and BBa_I50032: pSC101 and p15A replication origins. Each part is used either as a component of the BioBrick base vector BBa_I51020 (1–9) or to construct new BioBrick vectors (10–11).

Table I: Endonuclease sites targeted for removal from BioBrick vector parts.

Endonuclease	Description
EcoRI, XbaI, SpeI, PstI	BioBrick restriction site
Apol, MfeI	Produces compatible ends to EcoRI
AvrII, NheI	Produces compatible ends to XbaI and SpeI
NsiI SbfI	Produces compatible ends to PstI
AarI, Acul, BbsI, BciVI, BfuAI, BmrI, BsaI, BsgI, BsmBI, BsmI, BspMI, BsrDI, BtgZI, Earl, EcoP15I, FokI, SapI, TspRI	Offset cutter
I-CeuI, I-SceI, PI-PspI, PI-SceI, I-PpoI	Homing endonuclease
Nt.BbvCI, Nt.BstNBI, Nt.AlwI	Nicking endonuclease
AgeI, Ascl, BamHI, BbvCI, FseI, HindIII, KsaI, Ncol, NdeI, NgoMIV, PaaI, PmeI (MssI), RsrII, SacI, SalI, SfiI, SgfI, SgrAI, SrfI, Swal (Smil), XcmI, XbaI, XmaI, XmnI, ZraI	Restriction endonuclease

A list of endonuclease sites targeted for removal from BioBrick vectors parts. The endonuclease sites were targeted for removal to enable various end-user DNA cloning and manipulation applications.

mid. Thus, we presume that one or more of the introduced point mutations to eliminate endonuclease sites were deleterious to the plasmid replication function of the designed origin. We did not attempt to synthesize the p15A replication origin (BBa_I50032). Instead, like the pSC101 origin, we constructed p15A origin by PCR of an existing plasmid.

We constructed the functional pSC101 replication origin by PCR using pSB4A3-P1010 as a template and amplification primers I50042-f (5'-GTT TCT TCG AAT TCG CGG CCG CTT CTA GAG CTG TCA GAC CAA GTT TAC GAG-3') and I50042-r (5'-GTT TCT TCC TGC AGC GGC CGC TAC TAG TAG TTA CAT TGT CGA TCT GTT C-3'). We constructed the p15A replication origin by PCR using pSB3K3-P1010 as a template and amplification primers I50032-f (5'-GTT TCT TCG AAT TCG CGG CCG CTT CTA GAG ATG GAA TAG ACT GGA TGG AG-3') and I50032-r (5'-GTT TCT TCC TGC AGC GGC CGC TACT TAG TAA ACA CCC CTT GTA TTA CTG-3'). Each reaction was a mix of 45 μ L PCR SuperMix High Fidelity, 31.25 pmoles each of forward and reverse primer, and 1 ng template DNA in a 50 μ L total volume. The PCR conditions were an initial denaturation step of 95°C for 15 mins followed by 40 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 68°C for 2.5 minutes. Finally, the reactions were incubated at 68°C for 20 minutes. We then added 20 units DpnI restriction enzyme to each reaction to digest the template DNA. The reactions were incubated for 2 hours at 37°C and then heat-inactivated for 20 minutes at 80°C. We purified both reactions using a MinElute PCR Purification kit according to the manufacturer's directions (QIAGEN, Germany). The pSC101 and p15A origin PCR products were used directly for assembly of the BioBrick vectors.

Construction of BioBrick base vector

We also contracted for synthesis of the entire BioBrick base vector. However, we encountered two issues during synthesis of the base vector. First, troubleshooting efforts

during synthesis compromised the design of the base vector: failed attempts to clone the base vector into an *E. coli* strain intolerant of expression of the toxic protein CcdB led to an unnecessary redesign of the *ccdB* positive selection marker in the BioBrick base vector (from BBa_P1011 to BBa_P1016 [Genbank:[EU496090](#)]). Second, faulty part design adversely impacted the synthesis process: our pUC19-based replication origin design was similarly non-functional, so the base vector could not be propagated as specified. Yet, synthesized DNA for the BioBrick base vector was nevertheless provided. We eventually determined that the provided DNA was actually a fusion of two slightly different copies of the base vector: one with the designed, nonfunctional version of the pUC19 origin (BBa_I50020) and one with a functional version of the pUC19 origin (BBa_I50022 [Genbank:[EU496091](#)]). To obtain a single, corrected version of the BioBrick base vector, we performed a restriction digest of the provided base vector DNA with EcoRI. We then re-ligated 1 μ L of a ten-fold dilution of the linearized base vector DNA. For detailed reaction conditions, see Assembly of BioBrick parts using the new BioBrick vectors. We transformed the religated BioBrick base vector into *E. coli* strain DB3.1 via electroporation and plated the transformed cells on LB agar plates supplemented with 100 μ g/mL ampicillin to obtain the corrected BioBrick base vector BBa_I51020 [48,61,62]. Correct construction of the BioBrick base vector was verified by DNA sequencing by the MIT Biopolymers Laboratory.

Assembly of BioBrick vectors

We assembled the new BioBrick vectors as described (Figure 2). For detailed reaction conditions, see Assembly of BioBrick parts using the new BioBrick vectors. However, we used the synthesized BioBrick base vector BBa_I51019 instead of the corrected BioBrick base vector BBa_I51020, since, at the time, we had not yet identified the issue with the provided synthesized DNA. As a result, we obtained a mixture of new vectors. Four of the constructed vectors

have a functional version of the pUC19 origin (BBa_I50022) in the BioBrick cloning site and propagate at high copy (vectors with BBa_I52002: pSB4A5, pSB4K5, pSB4C5, and pSB3K5). The other three vectors have a nonfunctional version of the pUC19 origin (BBa_I50020) in the BioBrick cloning site and propagate at low copy (vectors with BBa_I52001: pSB4T5, pSB3C5, and pSB3T5). We chose to describe all seven vectors here for two reasons. First, all seven new BioBrick vectors can be used for the propagation and assembly of BioBrick parts; the vectors pSB4T5, pSB3C5, and pSB3T5 are just slightly less convenient for plasmid DNA purification. Second, the difficulties that we encountered during construction of the BioBrick base vector are illustrative of the current interdependence of DNA design and DNA synthesis (see Discussion).

Assembly of BioBrick parts using the new BioBrick vectors

We assembled BioBrick composite parts as described (Figure 3). We performed all restriction digests by mixing 0.5–1 μg DNA, 1X NEBuffer 2, 100 $\mu\text{g}/\text{mL}$ Bovine Serum Albumin, and 1 μL each needed restriction enzyme in a 50 μL total volume. Restriction digest reactions were incubated for at least 2 hours at 37°C and then heat-inactivated for 20 minutes at 80°C. We then dephosphorylated the destination vector into which the parts were assembled. (When assembling BioBrick vectors, we dephosphorylated the composite origin and resistance marker to prevent circularization of this DNA fragment.) We performed dephosphorylation reactions by adding 5 units Antarctic Phosphatase and 1X Antarctic Phosphatase Reaction Buffer in a total volume of 60 μL to the heat-inactivated restriction digest reaction. We incubated dephosphorylation reactions for 1 hour at 37°C and inactivated the phosphatase by heating to 65°C for 5 minutes. We purified all reactions using a MinElute PCR Purification kit according to the manufacturer's directions (QIAGEN). We performed all ligation steps by mixing 2–4 μL of each purified, linearized DNA, 1X T4 DNA Ligase Reaction Buffer, and 200 units T4 DNA Ligase in a 10 μL total volume. We incubated the ligation reactions for 20 minutes at room temperature. We transformed all assembled Bio-

Brick parts into *E. coli* strain TOP10 via chemical transformation [63–65]. (We transformed the assembled BioBrick vectors into *E. coli* strain DB3.1 via electroporation [48,61,62].) Transformed cells were plated on LB agar plates supplemented with 100 $\mu\text{g}/\text{mL}$ ampicillin, 50 $\mu\text{g}/\text{mL}$ kanamycin, 35 $\mu\text{g}/\text{mL}$ chloramphenicol, or 15 $\mu\text{g}/\text{mL}$ tetracycline as appropriate. We identified clones with correct construction of BioBrick parts by growth on the plates supplemented with the correct antibiotic, lack of growth on plates supplemented with other antibiotics, length verification by colony PCR (see next section), and DNA sequencing by the MIT Biopolymers Laboratory.

Verification of correct BioBrick part assembly via colony PCR

To demonstrate the correct assembly of BioBrick parts using the new BioBrick vectors, we performed a colony PCR using primers that anneal to the verification primer binding sites. We picked one colony and diluted it into 100 μL water. Then we mixed 9 μL PCR SuperMix High Fidelity, 6.25 pmoles VF2 primer (5'-TGC CAC CTG ACG TCT AAG AA-3'), 6.25 pmole VR primer (5'-ATT ACC GCC TTT GAG TGA GC-3'), and 1 μL colony suspension. The PCR conditions were as described previously but using an annealing temperature of 62°C and an elongation time of 3.5 minutes. We diluted the reactions four-fold with water and then performed an agarose gel electrophoresis of 20 μL of each diluted reaction using a 0.8% E-Gel®. We also electrophoresed 1 μg of 2-log DNA ladder (New England Biolabs, Inc., Ipswich, MA) to verify the length of each PCR product. The gel was imaged with 302 nm transilluminating ultraviolet light using an ethidium bromide emission filter and an exposure time of 614 milliseconds.

Materials for all PCR and agarose gel electrophoresis steps in this work were purchased from the Invitrogen Corporation in Carlsbad, CA unless otherwise specified. Reagents for all restriction digest, dephosphorylation, and ligation reactions were purchased from New England Biolabs, Inc., Ipswich, MA. All PCR and temperature-controlled incubation steps were done in a DNA Engine Peltier Thermal

Table 2: Numeric abbreviations for plasmid replication origins in BioBrick vector nomenclature.

Number	Replication origin	Copy number	Purpose
1	modified pMB1 derived from pUC19	500–700	Easy plasmid DNA purification
2	F and PI lytic derived from pSCANS-I-BNL [67]	1–2 inducible to high copy	Inducible copy number
3	p15A derived from pMR101	10–12	Multi-plasmid engineered systems
4	rep101, repA derived from pSC101	5	Small cell to cell copy number variation
5	derived from F plasmid	1–2	Improved plasmid stability
6	pMB1 derived from pBR322	15–20	Multi-plasmid engineered systems

BioBrick vector names take the form pSB#X#. The first number indicates the identity of the origin of replication. The number, corresponding replication origin, expected plasmid copy number and typical purpose of that origin are listed [38]. To expand the list to include additional replication origins, document additions at the Registry of Standard Biological Parts [66].

Table 3: Letter abbreviations for antibiotic resistance markers in BioBrick vector nomenclature.

Code	Antibiotic
A	ampicillin
C	chloramphenicol
E	erythromycin
G	gentamycin
K	kanamycin
N	neomycin
Na	nalidixic acid
R	rifampicin
S	spectinomycin
St	streptomycin
T	tetracycline
Tm	trimethoprim
Z	zeocin

BioBrick vector names take the form pSB#X#. The letter X indicates the antibiotic to which the vector confers resistance. The letter code and corresponding antibiotic resistance marker are listed. The absence of a letter indicates that no antibiotic is present. Multiple resistance markers in a vector are indicated by successive codes in alphabetical order e.g., AK, StT, AC and AKT. To expand the list to include additional antibiotic resistance markers, document additions at the Registry of Standard Biological Parts [66].

Cycler (PTC-200) or DNA Engine OPTICON™ from MJ Research, Inc. (now Bio-Rad Laboratories, Inc., Hercules, CA).

Naming of BioBrick vectors

BioBrick vector names take the form pSB#X#. The letters pSB are an acronym for plasmid Synthetic Biology. The first number denotes the origin of replication (Table 2). The letter X identifies the antibiotic resistance marker(s) present in the vector (Table 3). Vectors with multiple

resistance markers have multiple, successive letters. Finally, the last number in the vector name is a version number to differentiate between the various implementations of the pSB series of vectors (Table 4). When referring to both a BioBrick standard biological part and the vector in which it is cloned, the convention is to use the form [vector name]- [part number] such as pSB4K5-T9003. To refer to BioBrick vectors to be used for construction of BioBrick parts, use the full vector name and default cloned part. For example, pSB4A3-P1010, pSB1A10-P1010, pSB4K5-I52002, and pSB3T5-I52001 are all available vectors from the Registry of Standard Biological Parts. However, for convenience, vector names are often abbreviated to pSB4A3, pSB1A10, pSB4K5, and pSB3T5, respectively. New plasmid-based vectors constructed from the BioBrick base vector BBa_I51020 should be named pSB#X5-I52002 where the # is determined by the identity of the replication origin and the letter X is determined by the antibiotic resistance marker(s) present. To expand the BioBrick vector nomenclature, submit new vectors or vector parts to the Registry of Standard Biological Parts and document any new annotation needed [66]. The BioBricks Foundation is leading an open standards setting process should any revisions to the BioBrick vector nomenclature beyond addition of new replication origins, antibiotic resistance markers and version numbers be needed.

Abbreviations

PCR – polymerase chain reaction. bp – base pairs. kb – kilobase (1000 base pairs).

Table 4: Numeric abbreviations for vector version number in BioBrick vector nomenclature.

Number	Key features	Purpose	Example	Designer
0	absent or incomplete BioBrick cloning site		pSB2K0	Brookhaven National Lab
1	complete BioBrick cloning site (BCS)	assembly of BioBrick parts	pSB4A1	Reshma Shetty
2	5' terminator and BCS	transcriptional insulation of vector upstream of cloned BioBrick part	pSB1A2	Tom Knight
3	5' terminator and BCS and 3' terminator	transcriptional insulation of vector downstream of cloned BioBrick part	pSB1AC3	Reshma Shetty & Tom Knight
4	pSB2K3-derived vector free of many restriction sites	Genome refactoring [68]	pSB2K4	Leon Chan
5	constructed from BioBrick base vector	standardized BioBrick vector design	pSB4K5	Reshma Shetty
6	Reserved	-	-	-
7	BCS flanked by terminator BBa_B0015	transcriptional insulation of cloned BioBrick part	pSB1A7	Karmella Haynes
8	Unassigned	-	-	-
9	Unassigned	-	-	-
10	Screening plasmid v1.0 [69]	characterization of single input, single output transcriptional devices	pSB1A10	Josh Michener & Jason Kelly

BioBrick vector names take the form pSB#X#. The second number indicates the BioBrick vector version number. The version number, key features, purpose for which that version was designed, example vector, and vector designer(s) are listed. To expand the list to include new vector version numbers, document additions at the Registry of Standard Biological Parts [66].

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

RS and TK designed the BioBrick standard biological parts and vectors described in this work. RS carried out all construction and testing. RS and DE wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Austin Che for useful discussions regarding the design of the BioBrick base vector and the new BioBrick vector parts. Funding for this work was provided by the NSF Synthetic Biology Engineering Research Center. RS was supported by an NSF Graduate Research Fellowship.

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