Combining Engineering and Evolution in the Construction of Biological Systems

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(http://openwetware.org/wiki/Jason_Kelly)

Abstract

To date, engineered biological systems have been constructed via a variety of *ad hoc* approaches. The resulting systems should be thought of as pieces of art. We are interested in exploring how existing forward engineering approaches might be best combined with directed evolution to make routine the construction of engineered biological systems. We have specified a procedure for construction of biological systems via screening of subcomponent libraries and rational re-assembly. We have begun development of tools to enable this approach, including a FACS-based screening system to rapidly measure the input/output function of a genetic circuit. Additionally, we have designed a microfluidic system that enables more sophisticated screening and selection functions. Specifically, a microfluidic chemostat integrated with a cell sorter (i.e., a sort-o-stat). This microscope-based system will enable us to evaluate whether or not more complicated screens and selections will be of practical use in service of evolving engineered biological systems.

Framework for Engineering & Evolving Biological Systems

"There are only two ways we know of to make extremely complicated things. One is by engineering, and the other is evolution."
-Danny Hillis

Evolution	Engineering
•It works. Biology is good at generating large amounts of functional diversity	•It scales. No limit on system complexity.
•Slow & unpredictable, limited by the complexity of screens/selections.	•Requires functional composition of standard components

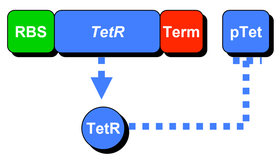
DNA is genetic material.

…AATGCGTAGCAA…

Parts are basic biological functions that can be encoded genetically.

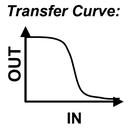
Type: Terminator
Efficiency: 97%
Temp: <Tm
Cell type: Any

Devices are combinations parts that encode human-defined functions.

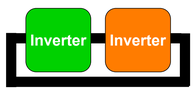


Type: Inverter
Protein: TetR
Latency: 0.5hr
Cell type: Any

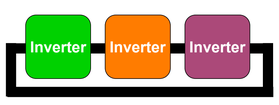
Transfer Curve:



Systems are combinations devices that encode human-defined functions.

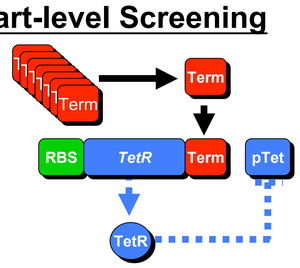


Type: Bi-stable Switch
Inverters: Q04400, Q04740
Latency: 0.5hr
Cell type: Any

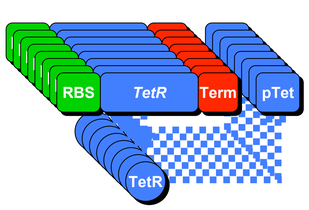


Type: Ring Oscillator
Inverters: Q04400, Q04740, Q04510
Latency: 0.5hr
Cell type: Any

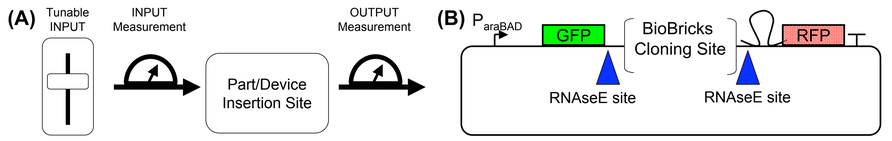
Part-level Screening



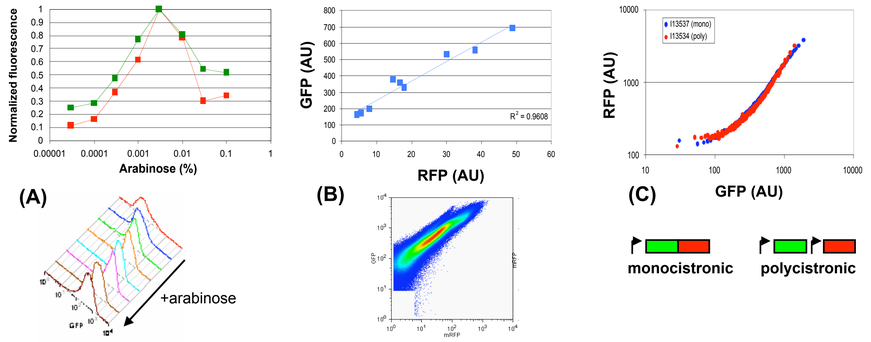
Device-level Screening



Screening Plasmid Design & Testing

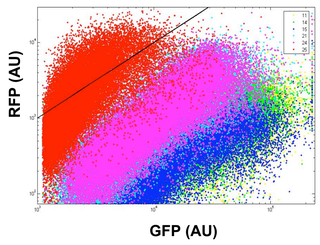


(A) Schematic of the components of the Screening Plasmid. The input and output of a genetic device can be measured in response to a range of inputs. **(B) Current implementation of the screening plasmid.** We are using the Pbad arabinose-inducible induction system [2] as a tunable input. GFP is a measure of input and RFP is a measure of output. A Biobricks cloning site enables easy insertion of any Biobricks part. RNase E sites create independence between the mRNA stability of the device being screened and the mRNA stability of the fluorescent proteins. In particular, we suspect mRFP1 contains internal RNaseE cut sites and have added a hairpin 5' of the coding region to slow degradation by RNase E. [3]

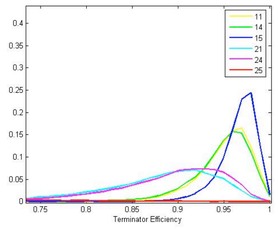


(A) Response of Screening Plasmid to varied arabinose concentrations. Cells were grown for 14 hours in the presence of 8 different arabinose concentrations. Data shown is the mean expression level of GFP (green) and RFP (red) based on measurement in a flow cytometer. Note the histogram shows a single population. **(B) Same data as in A, plotted RFP vs. GFP.** Note that the relationship between GFP and RFP remains constant across all arabinose concentrations. Dot plot is the concatenation of the 8 dot plots (e.g. all arabinose concentrations). **(C) Comparison of monocistronic and polycistronic constructs**. The similarity in the expression levels suggests that the RNAse E sites are working effectively.

Terminator Characterization



Characterization of 6 terminators from the Registry of Standard Biological Parts inserted into the Screening Plasmid. The black line is the best fit to the empty screening plasmid, and serves as a standard for 0% termination efficiency. Functional terminators should lie below the line, note that B0025 (red) is sometimes acting as a promoter.

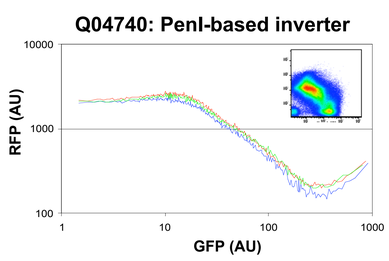


Histogram of calculated termination efficiencies for each terminator. Note that B0025 is mostly off scale.

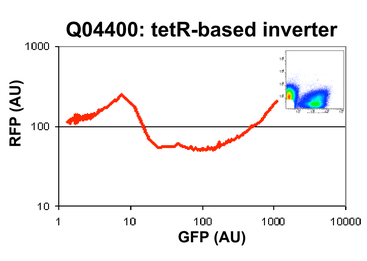
Terminator Registry Part #'s:

[B0011, B0014, B0015, B0021, B0024, B0025]

Inverter Characterization

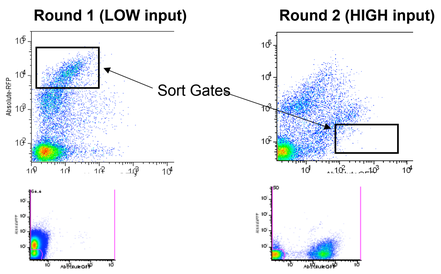


Characterization of Q04740. Dot plot of one replicate is shown in upper right. Mean RFP expression for 3 replicates is plotted against GFP showing characteristic inverter transfer curve.

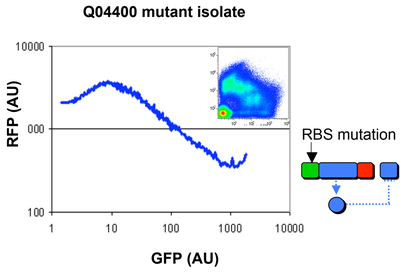


Characterization of Q04400. In this case the inverter appears to be "stuck" in the LOW output state, and as a result seemed to be a good candidate for library generation and device screening. (next section)

Inverter (Q04400) Library Screening



Conducted 2 rounds of screening – {LOW input, HIGH ouput} and {HIGH input, LOW output}. Upper dot plots are libraries, lower dot plots are original Q04400 under same arabinose conditions as the libraries.

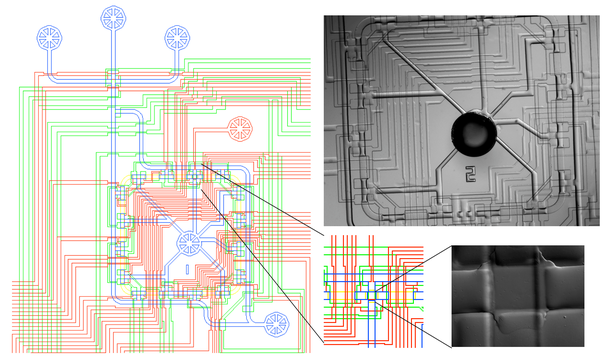


Characterization of Q04400 mutant. We were able to isolate a properly functioning inverter following 2 rounds of screening.

RBS mutation: AAAGAGG<A_G>GAAA

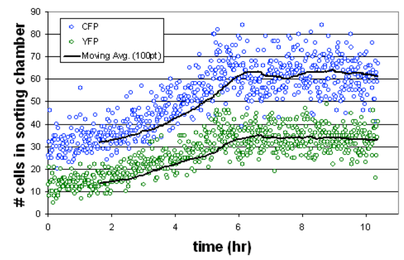
Sortostat

A microfluidic chemostat integrated with a cell sorter, which we call a "sort-o-stat", will enable more complicated selections to be applied to a population of cells in continuous culture. In particular, time varying selective pressures as well as very specific selection strengths can be applied. We will evaluate whether or not these more sophisticated selective pressures will be of practical use in service of evolving engineered biological systems. Selection can be based on any characteristic that can be reliably measured via microscopy.

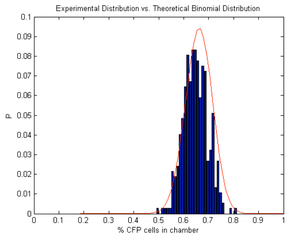


- Total Reactor Volume = 16nL
- Sorting chamber = 1/50th of total reactor volume
- Modification and extension of design by Balagadde et al. [4]

Normal Chemostat Operation

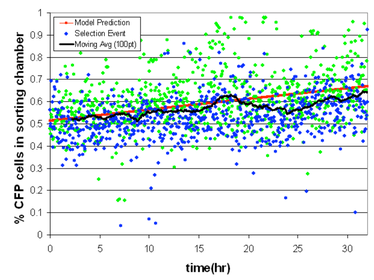


Sortostat was run with no selective pressure after being inoculated with cells growing in log phase from a batch culture.

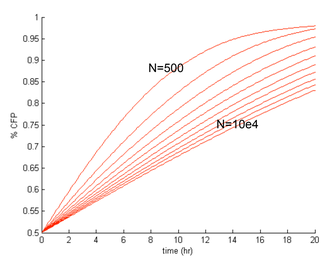


Analysis of steady state region (>6hrs) suggests that the %CFP cells found in the sorting chamber is binomially distributed (0.01 significance level)

Selective Pressure Turned ON



Sortostat was run with selective pressure, sorting against cells expressing YFP. Based on the rate of sorting events (1/3 min^{-1}) and initial cell counts, the mathematical model predicted the effect of sorting on the population relatively well.



Graph depicts the performance limits of the device based on a mathematical model at the maximum screening rate for populations 500-10e4 cells / reactor. Smaller populations have wider distribution and thus will face a greater selective pressure.

Future Work

- Further characterization and specification of device performance
- Tuning of oscillation frequency by selective pressure
- Selection for reduction in noise in gene expression across population
- Selection for a specific expression level of a fluorescent protein.
- Other ideas?

Acknowledgements

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- OpenWetWare community
- NSF Graduate Research Fellowship

References

[1] Yokobayashi et al., Directed evolution of a genetic circuit. Proc Natl Acad Sci U S A. 2002 Dec 24;99(26):16587-91. Epub 2002 Nov 25.

[2] Khlebnikov et al,Modulation of gene expression from the arabinose-inducible araBAD promoter. J Ind Microbiol Biotechnol. 2002 Jul;29(1):34-7.

[3] Effect of gene location, mRNA secondary structures, and RNase sites on expression of two genes in an engineered operon. Biotechnol Bioeng. 2002 Dec 30;80(7):762-76.

[4] Balagadde et al., Long Term Monitoring of Bacteria Undergoing Programmed Population Control in a Microchemostat. Science. 2005 Jul 1;309(5731):137-40.