Standard Bio Parts Fab

Pilot Proposal

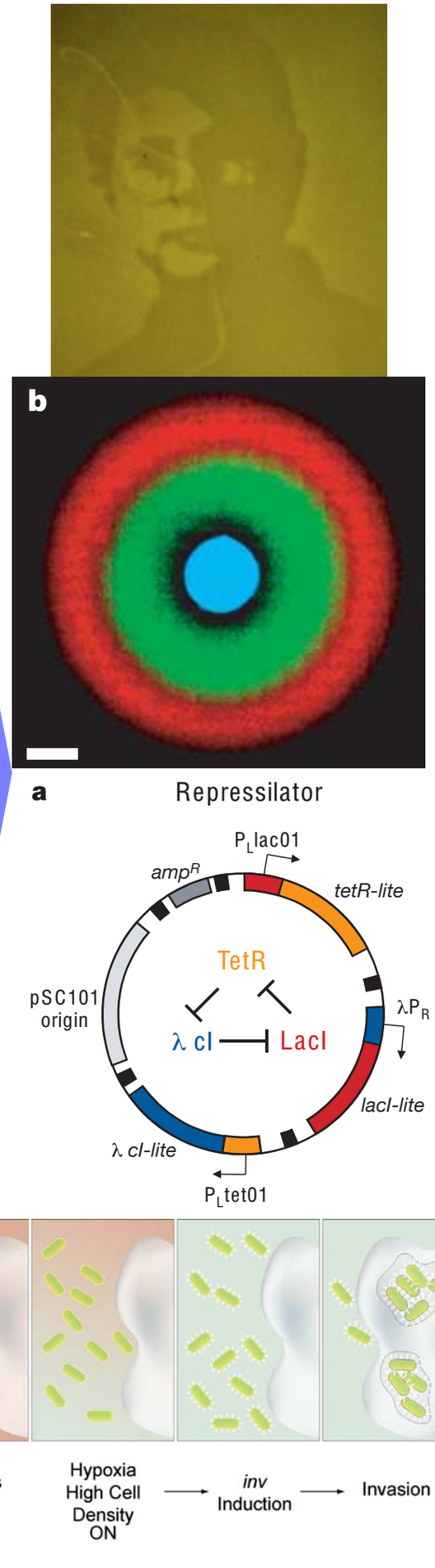
MIT SBWG Lunch
6 February 2007

Drew Endy
http://mit.edu/endy/

Design

Parts & Devices

Construction



Univeristy of Calgary, Canada
Missouri Western State Univeristy, USA
UCSF, USA
UC Berkeley, USA
Caltech, USA
UC Irvine, USA
University of Arizona, USA
University of Oklahoma, USA
University of Texas, USA
Prairie View A&M Univeristy, USA
Rice University, US
National Polytechnical Institute, Me
Team Latin A

a
pan
Japan

iGEM 2006

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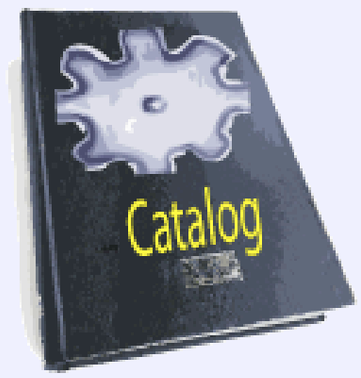


Registry of Standard Biological Parts

jump to part

BBa_

navigation

- Main Page
- Browse Part Types
- iGEM Wiki
- Community portal
- Recent changes
- Recent part changes

resources

- User Accounts
- Add a Part
- Part Searches
- DNA Repositories
- Sequence Analysis
- Assembly Tool
- Help

search

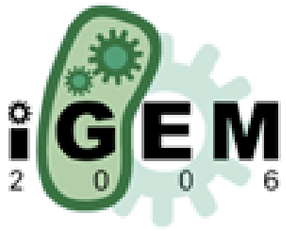
Go Search

toolbox

- What links here

Browse Parts by Type

Browse Parts by School



Featured Parts

Help & Documentation

Users & Groups

Registry Toolbox

Add a part

Search Parts

DNA Repositories

Sequence Analysis

Latest News

- [8/01/06] We have contact information for the creators of parts. You can access this information when you access "Hard Information" of a part.
- [8/01/06] A table made for yeast parts is now available on the Part Types page

Report any bugs here | Request new features here | See new features here

ump to part

BBa_

avigation

- Main Page
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esources

- User Accounts
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earch

Go Search

oolbox

- What links here
- Related changes
- Upload file
- Special pages
- Printable version

Transcriptional Terminators

Available Transcriptional Terminators

Edit

-?-		Name	Description	Direction	Reversed Version	Biology	Efficiency * Fwd.	Rev.	Length
A	W	BBa_B0011	Terminator (luxICDABEG, +/-)	Bidirectional	BBa_B0021	LuxIA	0.419	0.636	46
A	W	BBa_B0014	Terminator (B0012, B0011)	Forward	BBa_B0024	B0012, B0011	0.604		95
A	W	BBa_B0015	Terminator (B0010, B0012)	Forward	BBa_B0025	(B0010, B0012)	0.984	0.295	129
A	W	BBa_B0021	Terminator (luxICDABEG, +/-)	Bidirectional	BBa_B0011	LuxIA (reversed)	0.639	0.419	46
A	W	BBa_B0025	Terminator (Reverse B0015)	Reverse	BBa_B0015	(B0010,B0012) reversed	0.295	0.984	129
A	W	BBa_J52016	Eukaryotic terminator						238
A		BBa_B0010	Terminator (T1)	Forward	BBa_B0020	T1			80
A	X	BBa_B0012	Terminator (T7 TE)	Forward	BBa_B0022	T7 TE	0.309	-0.368	41
A	X	BBa_B0013	Terminator (T7 TE, +/-)	Bidirectional	BBa_B0023	T7 TE	0.6	-1.09	47
A		BBa_B0017	Terminator (B0010, B0010)	Forward		B0010.B0010			168
A	X	BBa_B0022	Terminator (Reverse B0012)	Reverse	BBa_B0012	T7 TE (reversed)	-0.368	0.309	41
A	X	BBa_B0023	Terminator (Reverse B0013)	Bidirectional	BBa_B0013	T7 TE (reversed)	-1.09	0.6	47
A		BBa_B0024	Terminator (Reverse B0014)	Reverse	BBa_B0014	(B0012.B0011) reversed		0.604	95
A		BBa_B1004	Terminator (artificial, small, %T~=55)						34
A		BBa_J63002	yeast ADH1 terminator						225

* Click here for terminator measurement information.

Other Transcriptional Terminators

Edit

-?-		Name	Description	Direction	Reversed Version	Biology	Efficiency * Fwd.	Rev.	Length
M		BBa_B0016	Terminator (T7 RNAP specific, T_Phi)	Forward		T7 RNAP, T_Phi			48
		BBa_B0020	Terminator (Reverse B0010)	Reverse	BBa_B0010	T1 (reversed)			82
		BBa_B0050	Terminator (pBR322, +/-)	Bidirectional	BBa_B0060	pBR322			33
		BBa_B0051	Terminator (yciA/tonA, +/-)	Bidirectional	BBa_B0061	yciA/tonA			35
		BBa_B0052	Terminator (rrnC)	Forward	BBa_B0062	rrnC			41
		BBa_B0053	Terminator (His)	Forward	BBa_B0063	His			72
		BBa_B0054	-- No description --						69
		BBa_B0055	-- No description --						78
		BBa_B0060	Terminator (Reverse B0050)	Bidirectional	BBa_B0050	pBR322 (reversed)			33
		BBa_B0061	Terminator (Reverse B0051)	Bidirectional	BBa_B0051	yciA/tonA (reversed)			35
		BBa_B0062	Terminator (Reverse B0052)	Reverse	BBa_B0052	rrnC (reversed)			41
		BBa_B0063	Terminator (Reverse B0053)	Reverse	BBa_B0053	His (reversed)			72
		BBa_B1001	Terminator (artifical, small, %T~=90)	Bidirectional					34
		BBa_B1002	Terminator (artificial, small, %T~=85%)	Bidirectional					34
		BBa_B1003	Terminator (artificial, small, %T~=80)						34
		BBa_B1005	Terminator (artificial, small, %T~=25%						34
		BBa_B1006	Terminator (artificial, large, %T~>90)						39
		BBa_B1007	Terminator (artificial, large, %T~=80)						40
		BBa_B1008	Terminator (artificial, large, %T~=70)						40
		BBa_B1009	Terminator (artificial, large, %T~=40%)						40

For that reason, some scientists say, it might be difficult ever to make biological engineering as predictable as bridge construction.

"There is no such thing as a standard component, because even a standard component works differently depending on the environment," Professor Arnold of Caltech said. "The expectation that you can type in a sequence and can predict what a circuit will do is far from reality and always will be."

Andrew Pollack
Custom-Made Microbes, at Your Service
New York Times
17 January 2006

BBa_F2620

3OC$_6$HSL → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 15 January 2007

Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC$_6$HSL is controlled by a TetR-regulated operator (BBa_R0040). Device input is 3OC$_6$HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

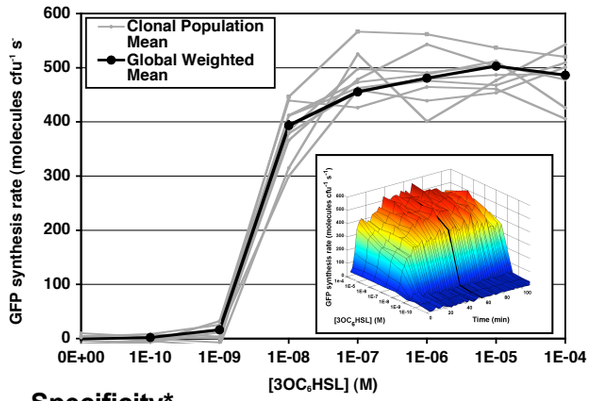
Characteristics

Input Swing: **1E-9 to 1E-6** M 3OC$_6$HSL, exogenous
Output Swing: **0±1 to 503±1** GFP molecules cfu^{-1} s^{-1}
Switch Point: **7±1 nM** 3OC$_6$HSL, exogenous
LH Response: **9 min** ($t_{50\%}$), **27 min** ($t_{90\%}$)

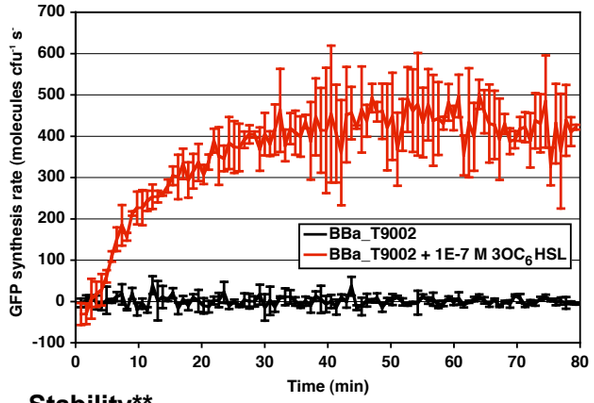
Key Parts

BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator

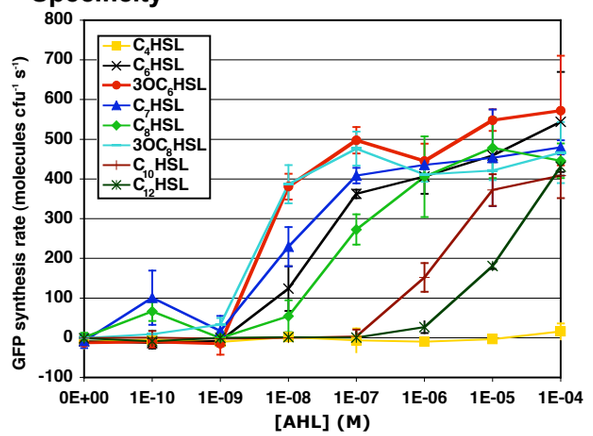
Transfer Function*



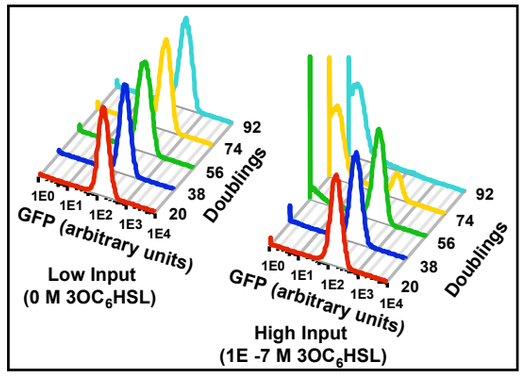
Response Time*



Specificity*



Stability**



Demand (low/high input)

Translational: **256/8048** ribosomes cfu^{-1}
3.8E3/1.2E5 charged tRNA cfu^{-1} s^{-1}

Compatibility

Chassis: Compatible with *MC4100*, *MG1655*, and *DH5α*
Plasmids: Compatible with *pSB3K3* and *pSB1A2*
Devices: Compatible with *E0240*, *E0430* and *E0434*
Crosstalk with systems containing *TetR (C0040)*
Signaling: Crosstalk with input molecules similar to 3OC$_6$HSL

Stability (low/high input)

Genetic: >**92/74** replication events**
Performance: >**92/74** replication events**

Conditions (abridged)

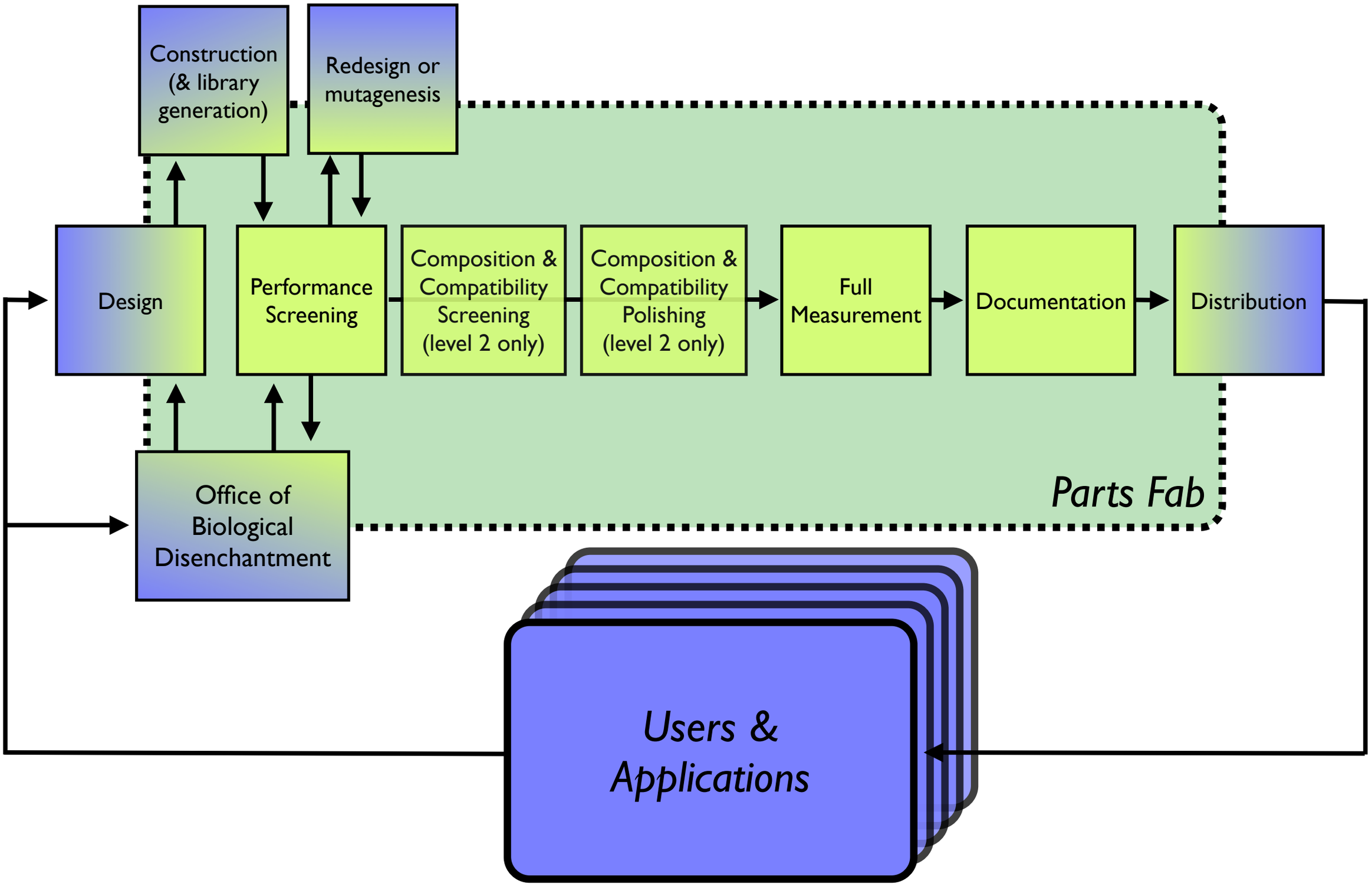
Output: Indirect via *BBa_E0240*
Vector: pSB3K3
Chassis: MG1655
Culture: Supplemented M9, 37ºC
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

Signaling Devices

License: Public

Study	Source of Key Genetic Elements[a]	*E. coli* Chassis (genotype)	Plasmid (ori, resistance)	Conditions (medium, temperature)	Switch Point[b] (nM)	Response Time[c] (min)	Specificity[a,b] (summary)	Stability[a]	Demand[a]
BBa_F2620	*V. Fischeri* MJ1	MG1655 (F^- λ- ilvG- rfb-50 rph-1)	pSB3k3 (p15A, Kn^r)	Supplemented M9 medium, liquid culture, 37°C	7±1	9 (50%), 26 (90%)	Responds to 5 of 8 inputs (Figure 1)	>92/74	108,480 aa CFU^{-1} s^{-1}, 7232 ribosomes
Winson *et al.* , 1998[14]	*V. Fischeri* MJ1	JM109 (endA1 glnV44 thi-1 relA1 gyrA96 recA1 $mcrB^+$ Δ(lac-proAB) glnV44 e14- [F' traD36 $proAB^+$ $lacI^q$ lacZΔM15] hsdR17($r_K^- m_K^+$))	PSB401 (p15A, Tet^r)	LB, liquid culture, 30°C	~12	N/A	Responds to 7 of 11 inputs (Figure 2 (A, B)[14])	N/A	N/A
Lindsay *et al.* , 2005[15]	*V. Fischeri* MJ1	WM54 (MG1655 ΔlacX74)	pAL103 (p15A, Tet^r)	LB, liquid culture, 37°C	~5	N/A	N/A	N/A	N/A
Andersen *et al.* , 2001[16]	*V. Fischeri* MJ1	MC4100 (F^- araD139 Δ(argF-lac)U169* rspL150 relA1 flbB5301 fruA25‡ deoC1 ptsF25 e14-)	pJBA132 (p15A, Kn^r)	LB, liquid culture, 30°C	4-8	Figure 4[16]	Responds to 3 of 5 inputs (Figure 3 (C)[16])	N/A	N/A
Weiss, & Knight, 2000[17]	*V. Fischeri* MJ1	DH5α (F^- *end* A1 *gln* V44 *thi* -1 *rec* A1 *rel* A1 *gyr* A96 *deo* R *nup* G ϕ80d*lac* ZΔM15 Δ(*lac* ZYA-*arg* F)U169, *hsd* R17($r_K^- m_K^+$), λ–)	pRCV-3 (pMB1, Ap^r)	LB, liquid culture, 30°C	N/A	~15 (50%)	N/A	N/A	N/A
Weiss *et al.* , 2003[18]				Unspecified, liquid culture, 37°C	~30	N/A			
Collins *et al.* , 2005[19]	*V. Fischeri* MJ1	DH5α	pLuxR (p15A, Kn^r) pLuxGFPuv (ColE1, Cmr)	LB, liquid culture, 37°C	10	N/A	Figure 2 (A)[19] (unresponsive to C8HSL)	N/A	N/A
Collins *et al.* , 2006[20]							Figure 2[20] (unresponisve to C8HSL)		
Schaefer *et al.* , 1996[21]	*V. Fischeri* ES114	VJS533 ara Δ(lac-proAB)X111 rpsL ϕ80dlacZΔM15 recA56	pHV200I (ColE1, Ap^r)	LB, liquid culture, 30°C	>100	N/A	Responds to 3 of 18 inputs (Table 1 and text [21])	N/A	N/A
Boettcher & Ruby, 1995[22]	*V. Fischeri* ES114	VJS533	pHV200I	Bioassay medium, liquid culture, 23-25°C	>5000	~380 (50%)	N/A	N/A	N/A

Supplementary Table 2 - Comparison of previous genetic constructs similar to BBa_F2620. Genetic constructs based on the same DNA source can vary in genetic organization and regulatory elements. [a]Sequence difference exist between the two listed strains. For example, the MJ1 and ES114 LuxR proteins share 75% identity and 89% similarity at the amino acid level. [b]Please see main text for a description of each characteristic. [c]Input specificity is defined using a response switch point cutoff of two orders of magnitude from the cognate AHL switchpoint.


Construction (& library generation)
Redesign or mutagenesis
Design
Performance Screening
Composition & Compatibility Screening (level 2 only)
Composition & Compatibility Polishing (level 2 only)
Full Measurement
Documentation
Distribution
Office of Biological Disenchantment
Parts Fab
Users & Applications

Design Team (5):
- DNA engineer
- RNA engineer
- Protein engineer
- Device engineer
- Chassis engineer

Construction Team (3):
- Construction engineer (1)
- Construction techs (2)

Characterization Team (4):
- Characterization engineer (1)
- Characterization techs (3)

Users & Applications (internal)
- have to use the stuff that's being made

Community Team (4):
- Community engineer (1)
- Documentation techs (1)
- Registry developers (2)

Organization (2):
- Parts Fab lead (1)
- Parts Fab manager (1)

Also (#):
- Chemists (N)
  analytical, biochemical, synthetic
- Computing (N)
- Debuggers (N+1)

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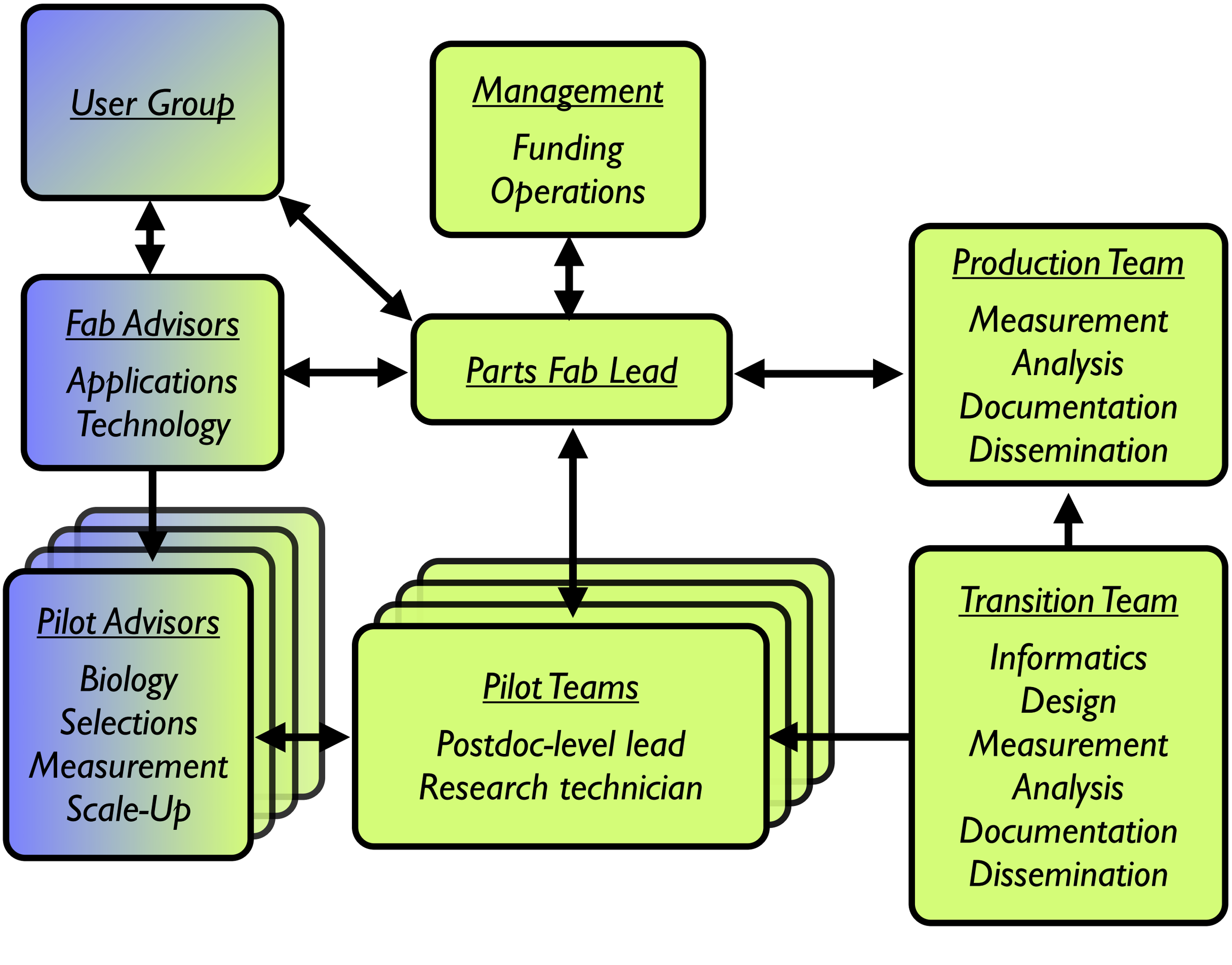
User Group
Management
Funding
Operations
Production Team
Measurement
Analysis
Documentation
Dissemination
Fab Advisors
Applications
Technology
Parts Fab Lead
Pilot Advisors
Biology
Selections
Measurement
Scale-Up
Pilot Teams
Postdoc-level lead
Research technician
Transition Team
Informatics
Design
Measurement
Analysis
Documentation
Dissemination

Parts Fab Pilot:

- 3 year run
- Given success, ramping up

Operational Goals:

- Team recruited, trained & productive
- Vibrant designer & user community
- Pilot & user projects completed
- Leader insensitive (qualitative)

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Pilot Project:

- Team & process building
- Good chance of success
- Can start right away
- Widespread external utility
- Enable & improve subsequent work

Central Dogma Pilot:

- Enable forward engineering of DNA, RNA, GP levels in *E. coli* & *S. cerevisiae* for ~2 dozen components
- Open loop (largely)

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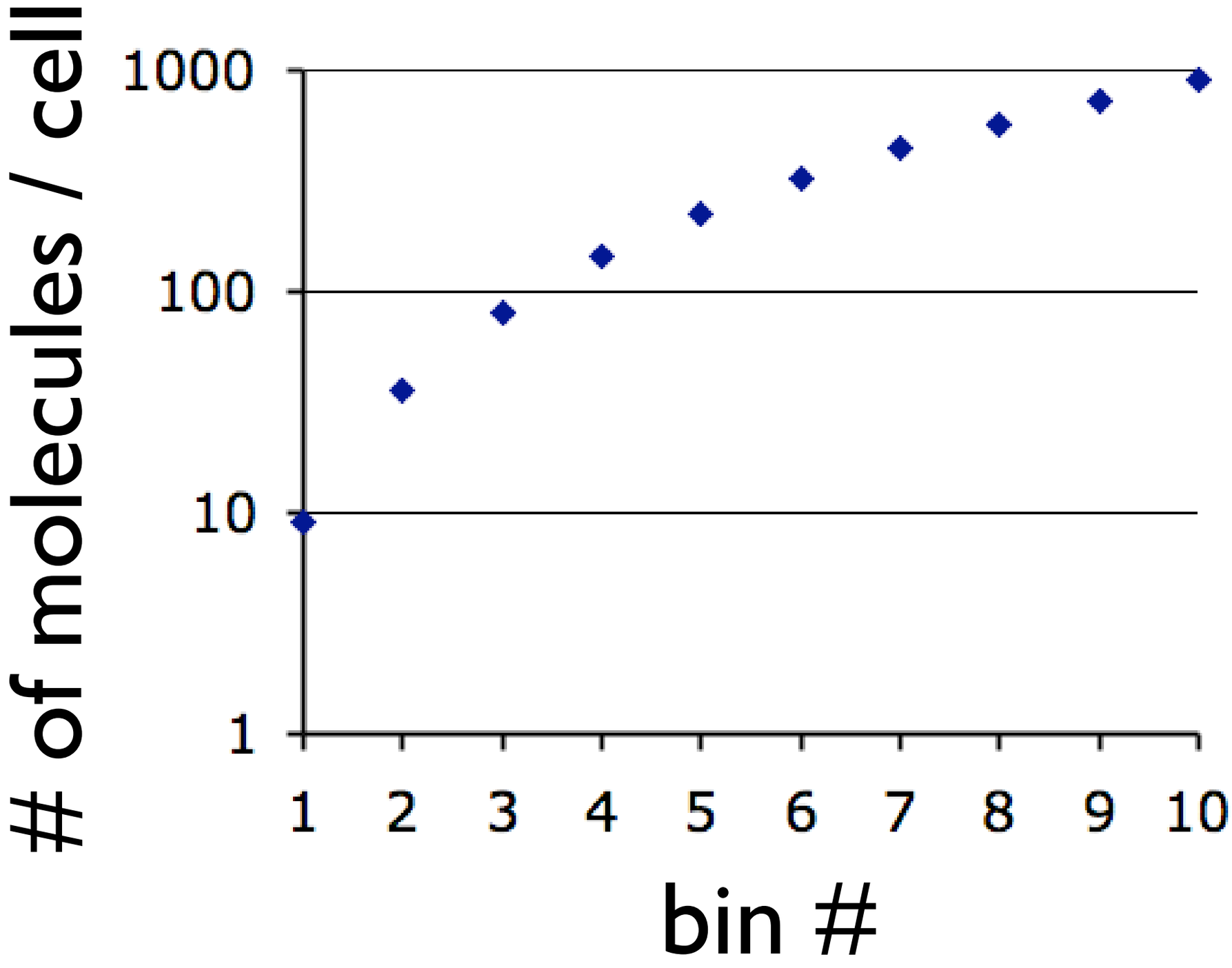
Naive Target Levels: Poisson Noise Bins



DNA:

- Replication origins
- Markers

RNA:

- Promoters
- Terminators
- RNase recognition sites
- Hairpins
- (Di)codon usage

Protein:

- RBS, 5'UTR, IRES
- Processing tags

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Terminators:

- 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% η
- At least 10 sequence-distinct instances at each η level
- Leverage informatics resources to support design?

How?

- Dual FP test device (colony, culture & microscope)
- Re-sequencing technology?

Quality Levels:

- Level 1. Works in isolation
- Level 2. Works in combination

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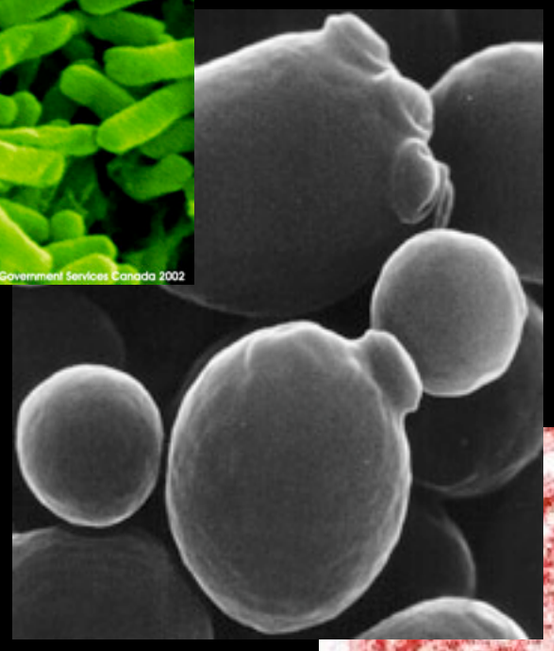
Enzymatic saccharification of biomass (to be secreted from engineered fermentation chassis):
Endocellulase
Exocellulase
Beta-glucosidase
Ligninase
Xylanase

Cell wall synthesis:
Glycosyltransferase
Methyltransferase
Acetyltransferase
Class III peroxidase
Laccase
Ascorbate peroxidase
NADPH oxidase
Copper amine oxidase
Oxalate oxidase

Transporters:
ABC transporters
Aquaglycerolporins

Fuel synthesis pathways:
Acyl-ACP thioesterase
Acetyl-CoA carboxylase
Fatty aldehyde reductase
Fatty alcohol reductase
Fatty acid decarbonylase

Lipids:
Cyanobacterial desaturase
Thioesterase
Keto-acyl ACP synthase



Prelim. list c/o
Blake A. Simmons, Ph.D.
Manager, Energy Systems Department
Sandia National Laboratories

Known Challenges (Opportunities):

- Potential for data type complexity
- Data type may vary with part type
- Ownership & sharing framework
- Safety & security strategy
- Parallel efforts (eventually)

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Future ability to usefully engineer information as genetic material will be at least as important as our current prowess manipulating information via silicon.

Parts framework seems likely to work. But, collecting quality data on failure will require a high quality production-scale resource.

Fostering an open community of parts designers and users around a common resource will float all (biotechnology) boats, from energy to carbon to environment to materials to health.

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