Engineering synthetic gene networks

the IKEA way

Tom Ellis
Sept 2009

What is Synthetic Biology?

a new area of biological research that combines **science** and **engineering** in order to **design and build** ("synthesize") novel biological functions and systems
source: wikipedia

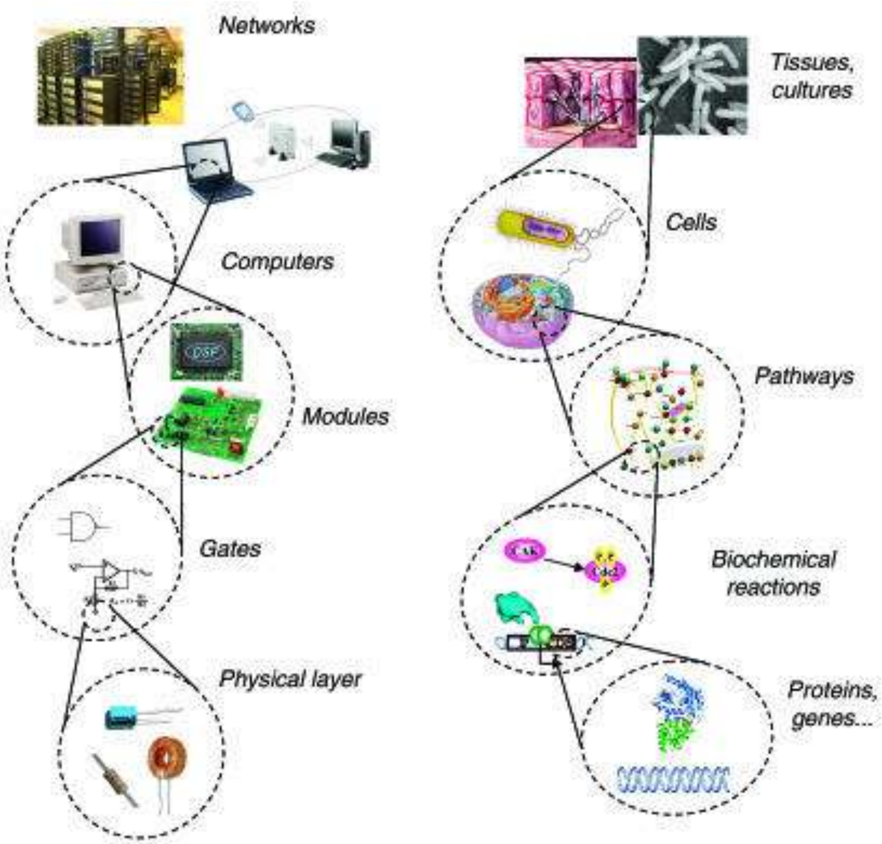
Constructing novel gene networks

Investigating biology by building
and modeling equivalent systems

Synthesizing entirely new biomolecules

Rewriting genomes

Building new life



Andrianantoandro E et al, 2006

The challenge of building gene networks

Andrianantoandro et al, 2006

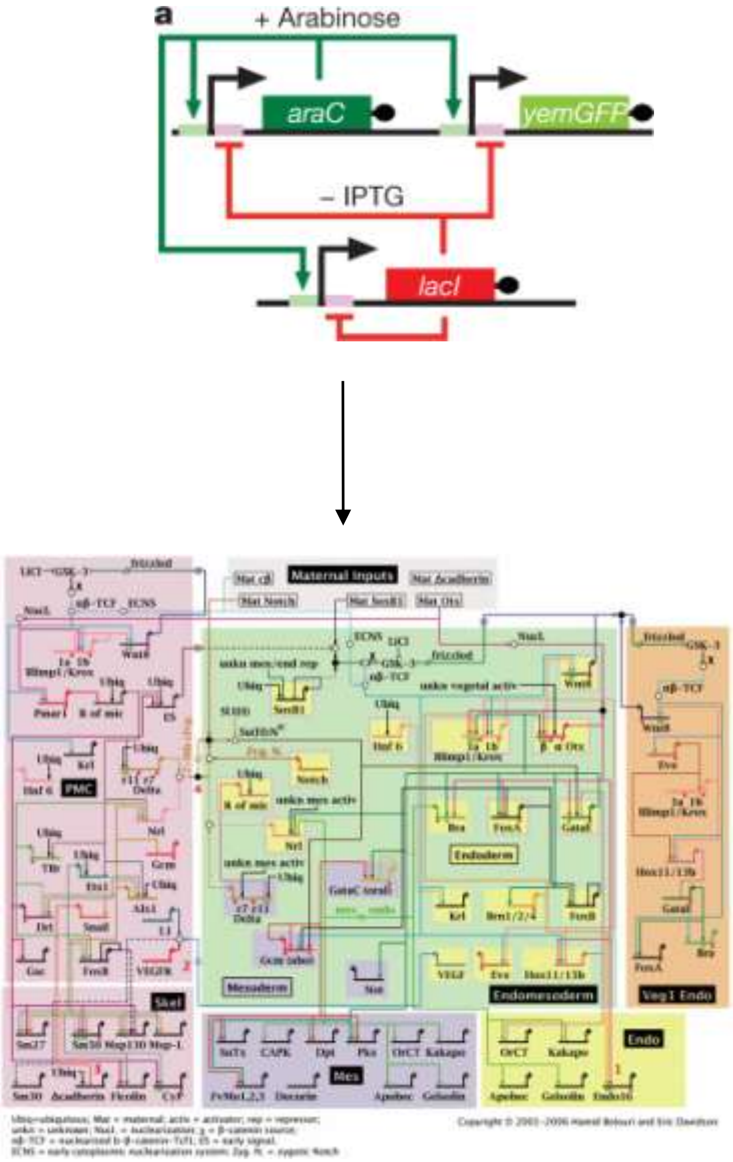
"…design of synthetic biological systems has become an **iterative** process of modeling, construction, and experimental testing that continues until a system achieves the desired behavior.

The process begins with the abstract design of devices, modules, or organisms, and is often guided by mathematical models. The synthetic biologist then tests the newly constructed systems experimentally. However, such initial attempts rarely yield fully functional implementations because of incomplete biological information.

Rational redesign based on mathematical models improves system behavior in such situations. Directed evolution is a complimentary approach, which can yield novel and unexpected beneficial changes to the system. These retooled systems are once again tested experimentally and **the process is repeated** as needed."

idea – x hours
model – x weeks
network – x years

Weiss R, 2006



The gene network engineering cycle

Idea!

↓

Design Your Network

↓

Find Suitable Parts

↓

Build Computer Model
and test out network

↓

Assemble Network DNA

↓

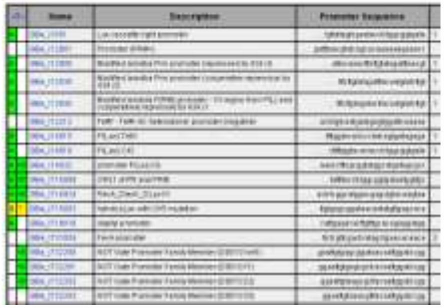
Test out Network in Cells

↓

It worked! :)

↓

put it to use



characterize
parts thoroughly

↑

more testing

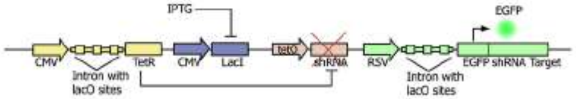
↑

Why?

↑

:(doesn't work

debugging



Tweak
Re-design
Retrofit

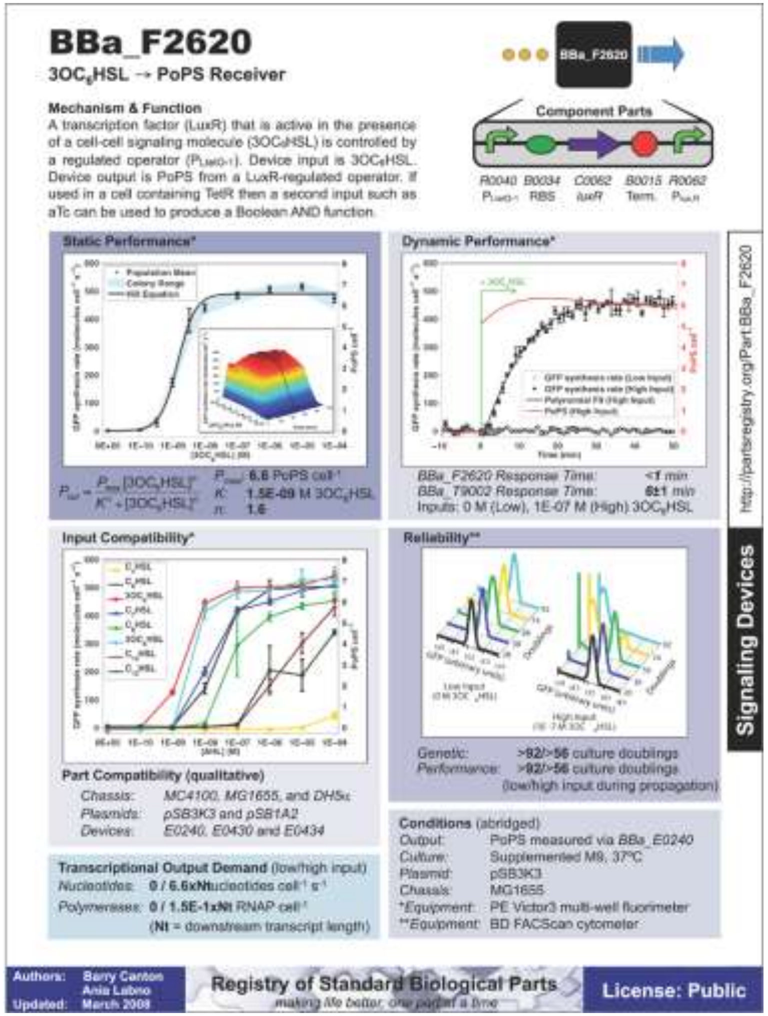
Tom Ellis Sept 09

Shortening the gene network engineering cycle

Better parts → Better Models
- on-going concern in Syn Bio standardisation
- impact on host cell compatibility

Directed Evolution
- use diversity to cheat the cycle
- requires selective pressures
- not always straightforward
- not rational design

More parts, more versions of each part
- square peg/round hole
- to optimize networks, need optimum parts
- tweak before failure



BBa_F2620

$3OC_6HSL \rightarrow$ PoPS Receiver

Mechanism & Function

A transcription factor (LuxR) that is active in the presence of a cell-cell signaling molecule ($3OC_6HSL$) is controlled by a regulated operator ($P_{LtetO-1}$). Device input is $3OC_6HSL$. 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*

$$P_{out} = \frac{P_{max}[3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

P_{max}: **6.6** PoPS cell^{-1}
K: **1.5E-09** 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) $3OC_6HSL$

Input Compatibility*

Reliability**

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

Part Compatibility (qualitative)

Chassis: MC4100, MG1655, and DH5α
Plasmids: pSB3K3 and pSB1A2
Devices: E0240, E0430 and E0434

Transcriptional Output Demand (low/high input)

Nucleotides: **0 / 6.6xNt**ucleotides cell^{-1} s^{-1}
Polymerases: **0 / 1.5E-1xNt** RNAP cell^{-1}
(**Nt** = downstream transcript length)

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

http://partsregistry.org/Part:BBa_F2620

Signaling Devices

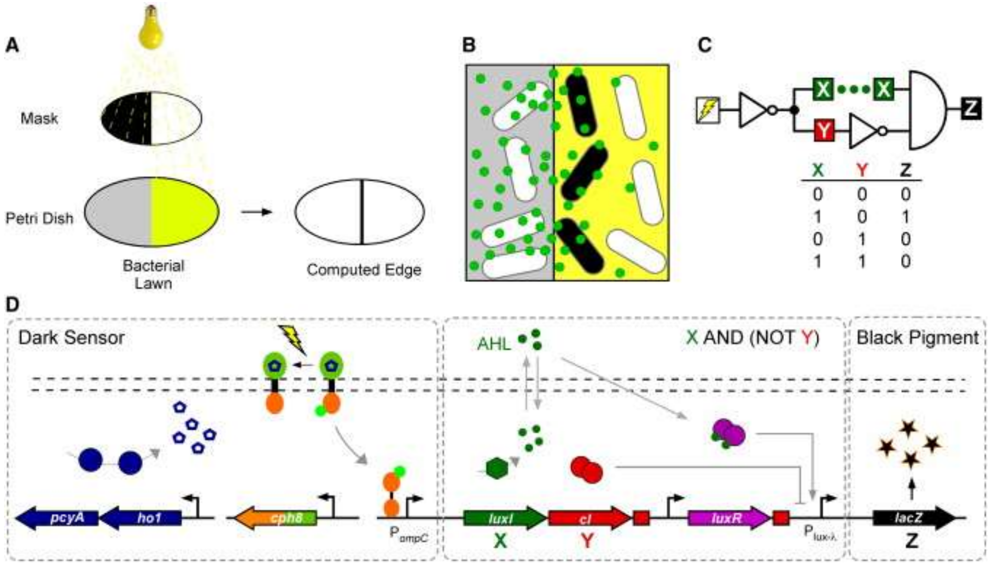
Authors: Barry Canton, Ania Labno
Updated: March 2008

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

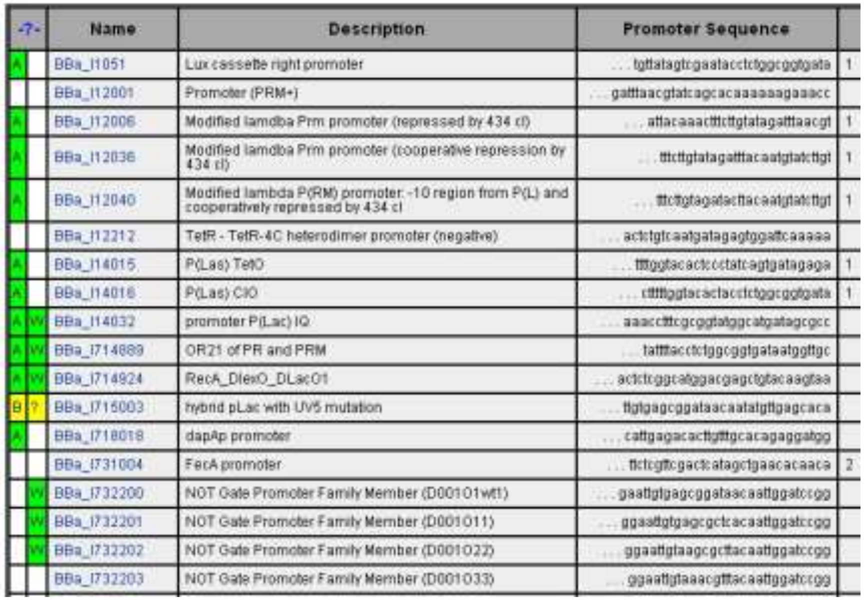
License: Public

Tom Ellis Sept 09

We’re playing with bricks we’ve found – ‘MacGyver’

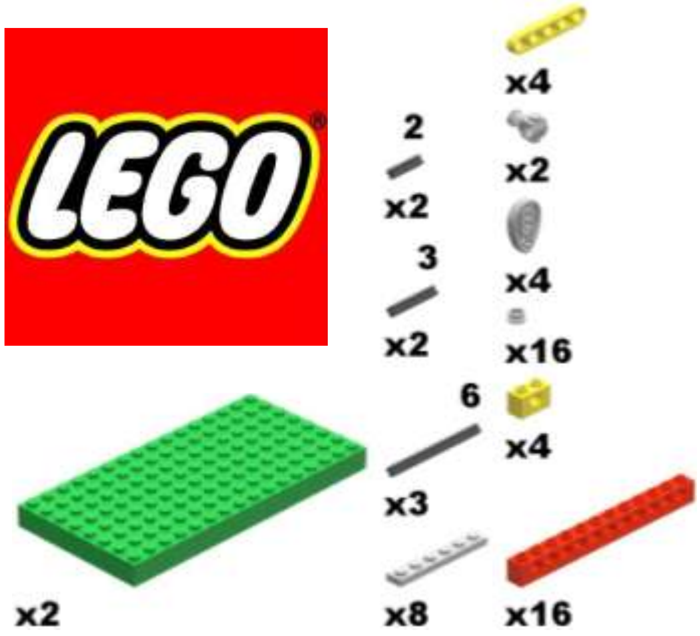


cyanobacter E.coli lambda phage V.fischeri E.coli

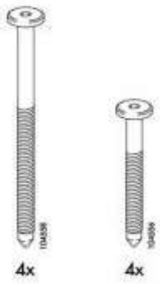


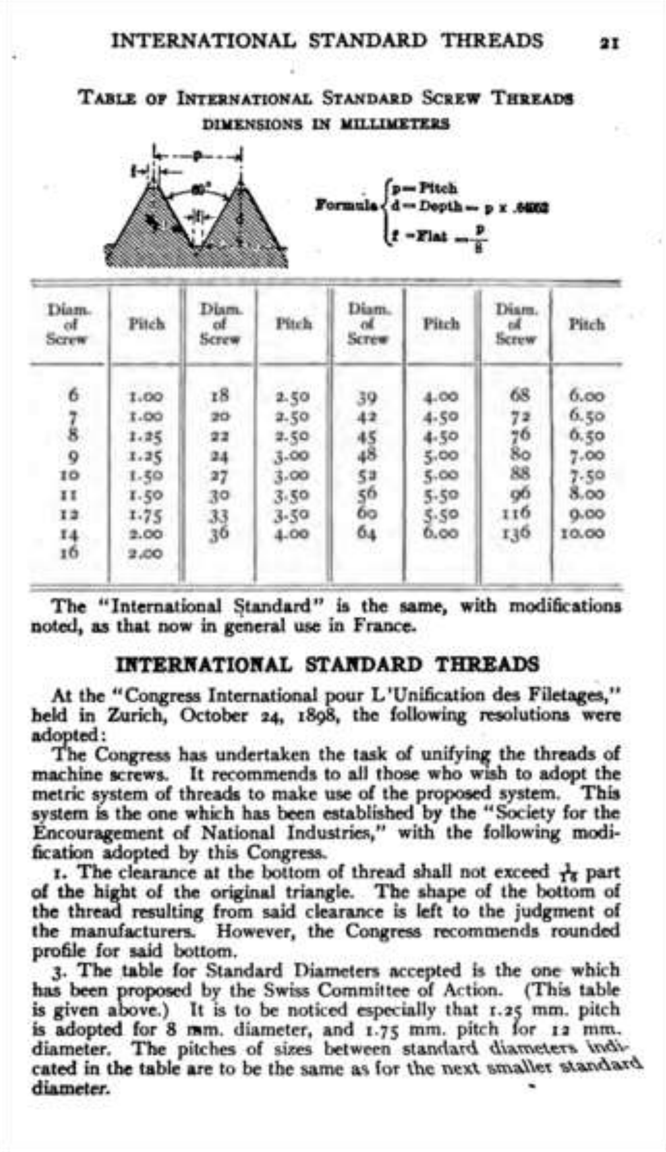
-?-		Name	Description	Promoter Sequence	
A		BBa_I1051	Lux cassette right promoter	. . . tgttatagtcgaatacctctggcggtgata	1
		BBa_I12001	Promoter (PRM+)	. . . gatttaacgtatcagcacaaaaaagaaacc	
A		BBa_I12006	Modified lamdba Prm promoter (repressed by 434 cI)	. . . attacaaactttcttgtatagatttaacgt	1
A		BBa_I12036	Modified lamdba Prm promoter (cooperative repression by 434 cI)	. . . tttcttgtatagatttacaatgtatcttgt	1
A		BBa_I12040	Modified lambda P(RM) promoter: -10 region from P(L) and cooperatively repressed by 434 cI	. . . tttcttgtagatacttacaatgtatcttgt	1
		BBa_I12212	TetR - TetR-4C heterodimer promoter (negative)	. . . actctgtcaatgatagagtggattcaaaaa	
A		BBa_I14015	P(Las) TetO	. . . ttttggtacactccctatcagtgatagaga	1
A		BBa_I14016	P(Las) CIO	. . . cttttggtacactacctctggcggtgata	1
A	W	BBa_I14032	promoter P(Lac) IQ	. . . aaacctttcgcggtatggcatgatagcgcc	
A	W	BBa_I714889	OR21 of PR and PRM	. . . tattttacctctggcggtgataatggttgc	
A	W	BBa_I714924	RecA_DlexO_DLacO1	. . . actctcggcatggacgagctgtacaagtaa	
B	?	BBa_I715003	hybrid pLac with UV5 mutation	. . . ttgtgagcggataacaatatgttgagcaca	
A		BBa_I718018	dapAp promoter	. . . cattgagacacttgtttgcacagaggatgg	
		BBa_I731004	FecA promoter	. . . ttctcgttcgactcatagctgaacacaaca	2
	W	BBa_I732200	NOT Gate Promoter Family Member (D00101wt1)	. . . gaattgtgagcggataacaattggatccgg	
	W	BBa_I732201	NOT Gate Promoter Family Member (D001O11)	. . . ggaattgtgagcgctcacaattggatccgg	
	W	BBa_I732202	NOT Gate Promoter Family Member (D001O22)	. . . ggaattgtaagcgcttacaattggatccgg	
		BBa_I732203	NOT Gate Promoter Family Member (D001O33)	. . . ggaattgtaaacgtttacaattggatccgg	

Toy models → Real world



More parts, more versions of each part





INTERNATIONAL STANDARD THREADS 21

TABLE OF INTERNATIONAL STANDARD SCREW THREADS
DIMENSIONS IN MILLIMETERS

Formula
p = Pitch
d = Depth = p x [illegible]
f = Flat = $\frac{p}{8}$

Diam. of Screw	Pitch	Diam. of Screw	Pitch	Diam. of Screw	Pitch	Diam. of Screw	Pitch
6	1.00	18	2.50	39	4.00	68	6.00
7	1.00	20	2.50	42	4.50	72	6.50
8	1.25	22	2.50	45	4.50	76	6.50
9	1.25	24	3.00	48	5.00	80	7.00
10	1.50	27	3.00	52	5.00	88	7.50
11	1.50	30	3.50	56	5.50	96	8.00
12	1.75	33	3.50	60	5.50	116	9.00
14	2.00	36	4.00	64	6.00	136	10.00
16	2.00						

The "International Standard" is the same, with modifications noted, as that now in general use in France.

INTERNATIONAL STANDARD THREADS

At the "Congress International pour L'Unification des Filetages," held in Zurich, October 24, 1898, the following resolutions were adopted:

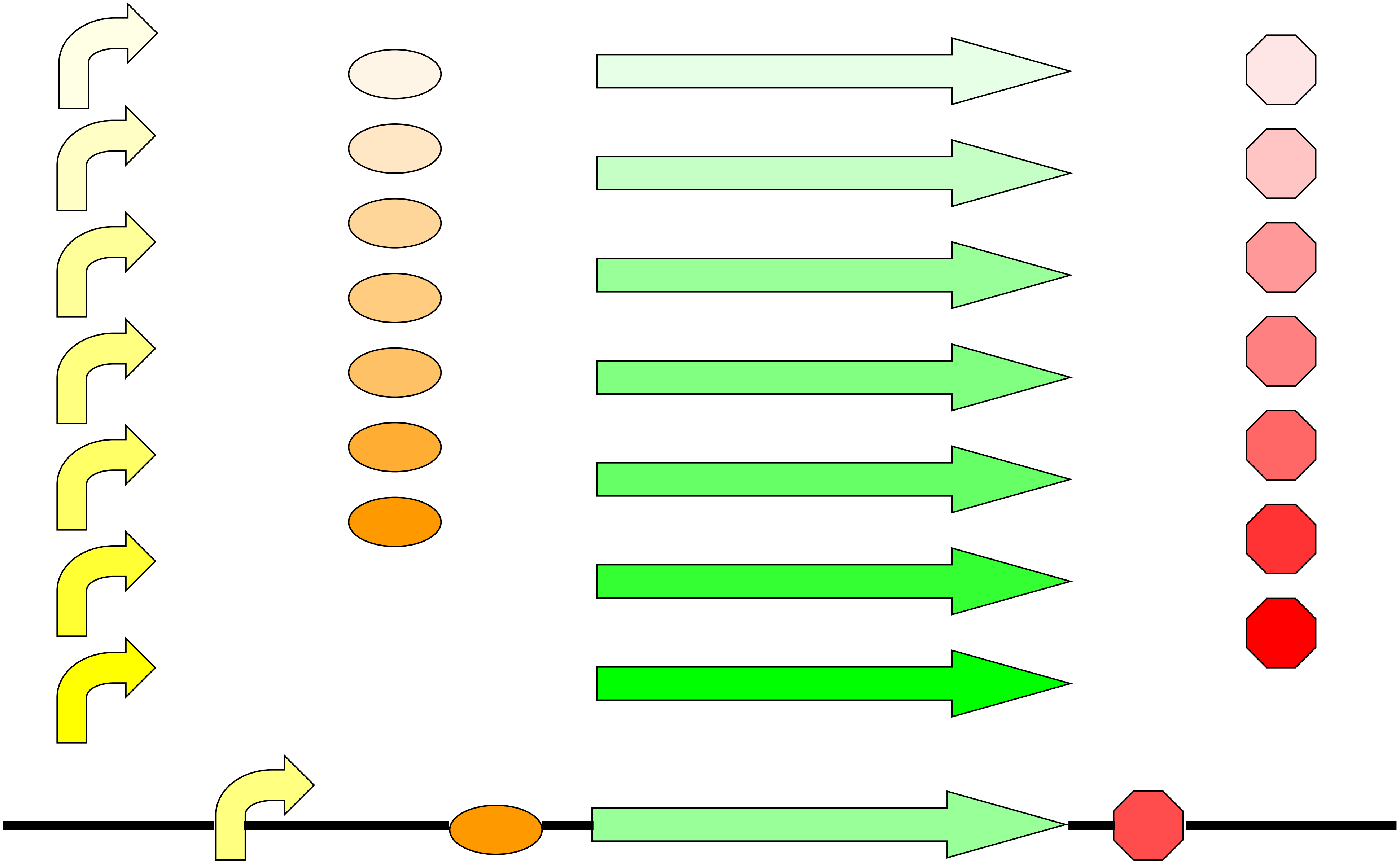
The Congress has undertaken the task of unifying the threads of machine screws. It recommends to all those who wish to adopt the metric system of threads to make use of the proposed system. This system is the one which has been established by the "Society for the Encouragement of National Industries," with the following modification adopted by this Congress.

1. The clearance at the bottom of thread shall not exceed $\frac{1}{16}$ part of the hight of the original triangle. The shape of the bottom of the thread resulting from said clearance is left to the judgment of the manufacturers. However, the Congress recommends rounded profile for said bottom.

3. The table for Standard Diameters accepted is the one which has been proposed by the Swiss Committee of Action. (This table is given above.) It is to be noticed especially that 1.25 mm. pitch is adopted for 8 mm. diameter, and 1.75 mm. pitch for 12 mm. diameter. The pitches of sizes between standard diameters indicated in the table are to be the same as for the next smaller standard diameter.

Screws – standard threading but different length, width, input

More parts, more versions of each part

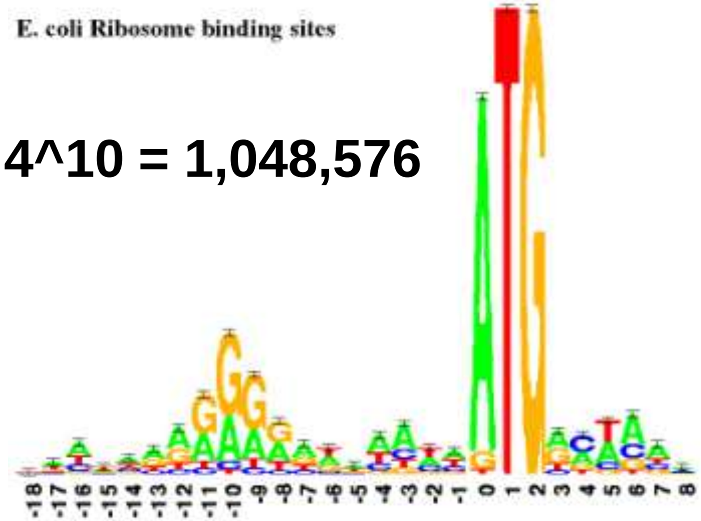


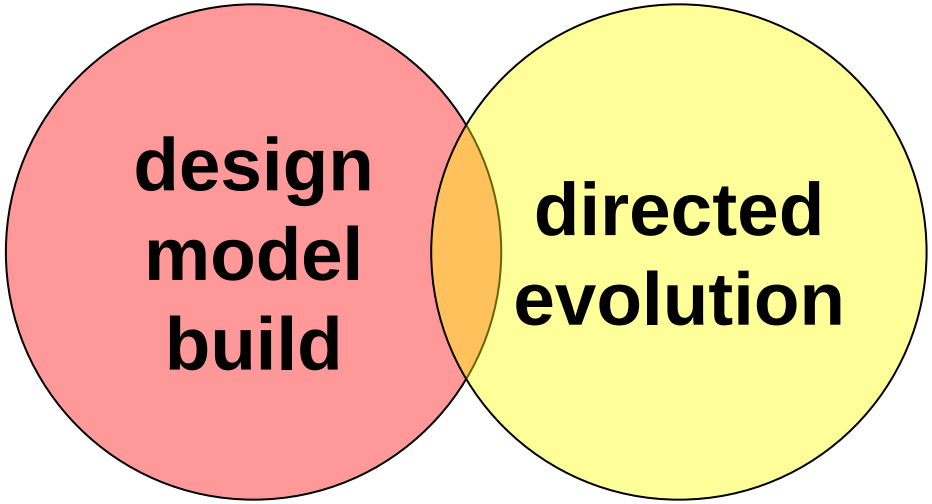
$7^4 = 2401$

Tom Ellis Sept 09

Rational use of diversity – Make libraries of new parts

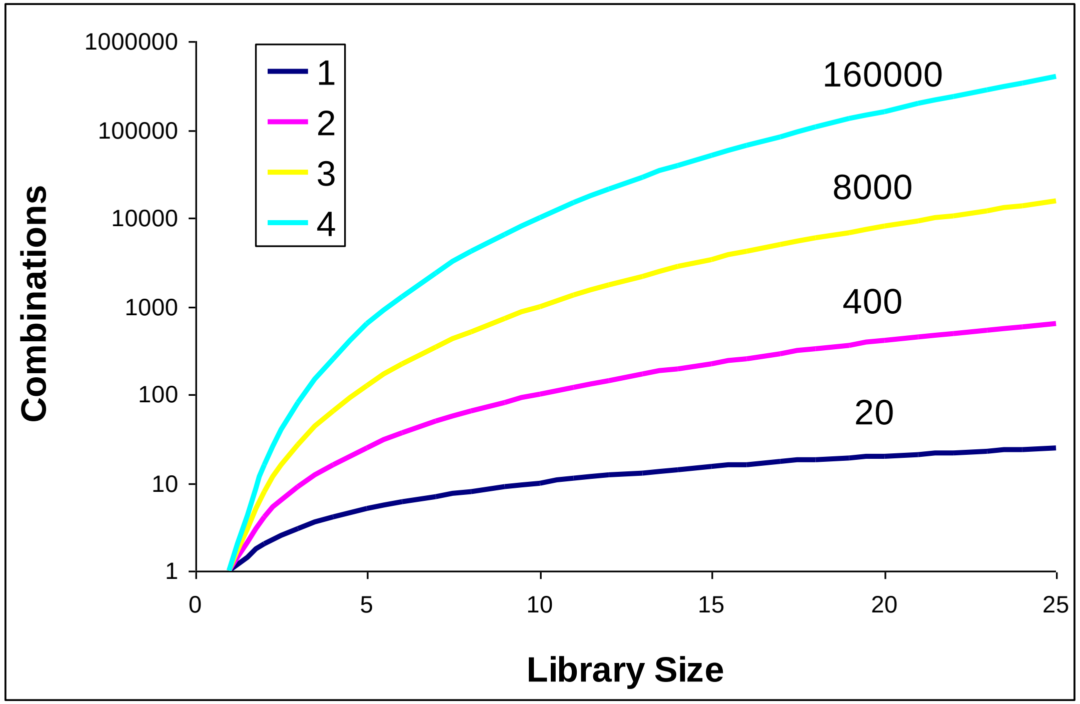
4^10 = 1,048,576



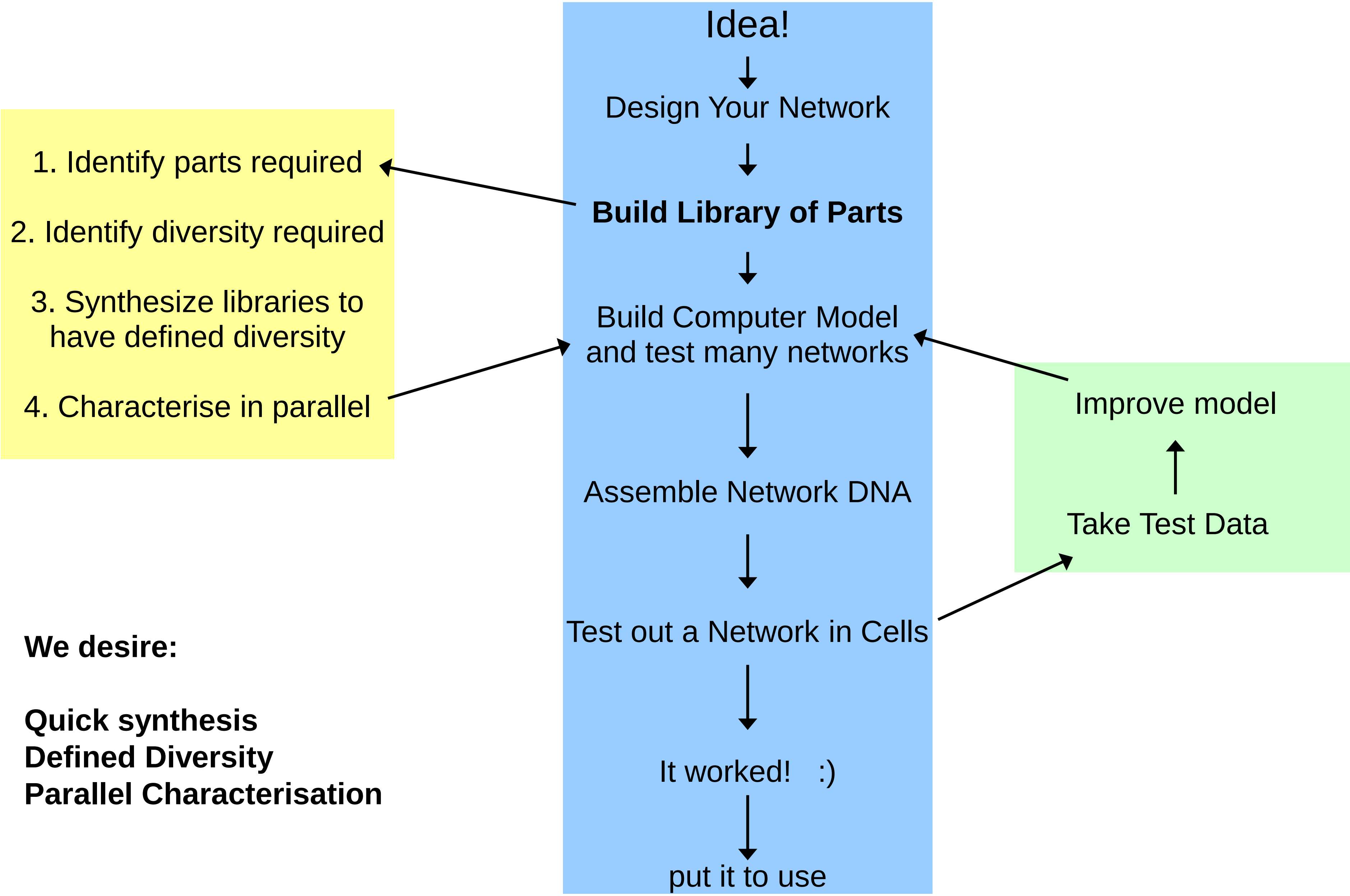


design
model
build

directed
evolution



A new gene network engineering cycle



Idea!

Design Your Network

Build Library of Parts

Build Computer Model
and test many networks

Assemble Network DNA

Test out a Network in Cells

It worked! :)

put it to use

1. Identify parts required
2. Identify diversity required
3. Synthesize libraries to have defined diversity
4. Characterise in parallel

Improve model

Take Test Data

We desire:

Quick synthesis
Defined Diversity
Parallel Characterisation

Tom Ellis Sept 09

The idea… to bypass debugging

1. Make libraries of parts using diversity *

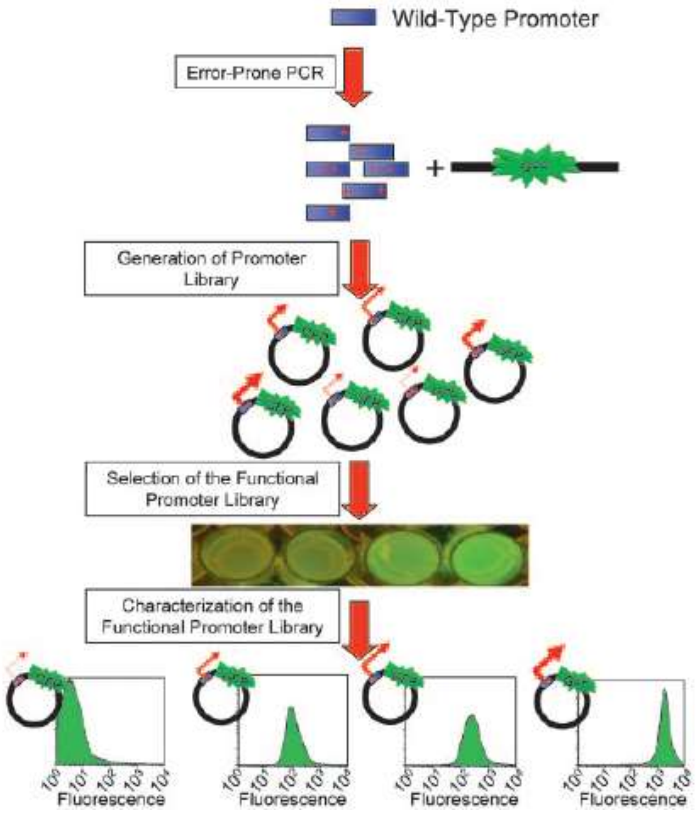
2. Make models of intended networks

3. Input library data into models

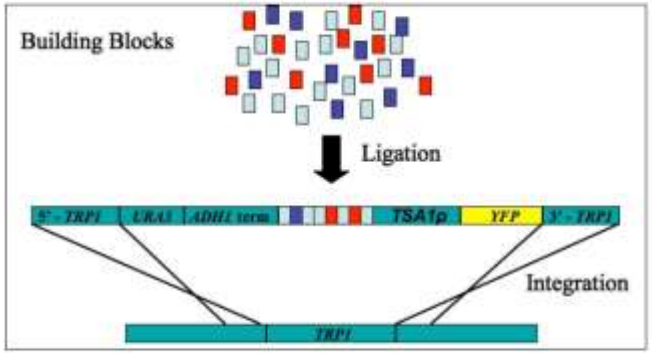
Models act as a guide - selecting the best library parts for the output function needed

* should be characterizing parts anyway, so no big deal

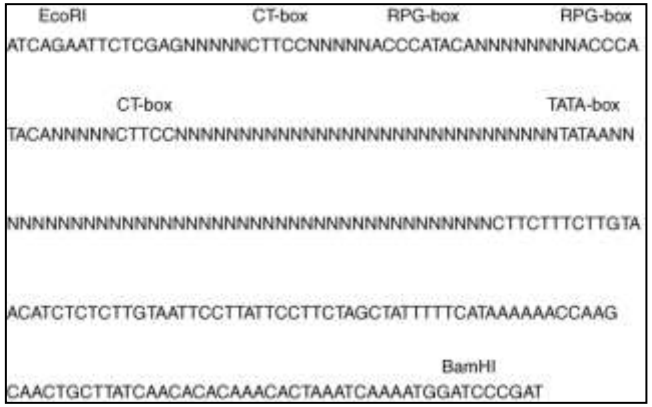
Library Synthesis Techniques - Promoters



By Mutation:
Alper & Stephanopolous



By DNA shuffling:
Elowitz/Cohen



EcoRI CT-box RPG-box RPG-box
ATCAGAATTCTCGAGNNNNNCTTCCNNNNNACCCATACANNNNNNNNNACCCA

CT-box TATA-box
TACANNNNNCTTCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTATAANN

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTTCTTTCTTGTA

ACATCTCTCTTGTAATTCCTTATTCCTTCTAGCTATTTTTCATAAAAAAACCAAG

BamHI
CAACTGCTTATCAACACACAAACACTAAATCAAAATGGATCCCGAT

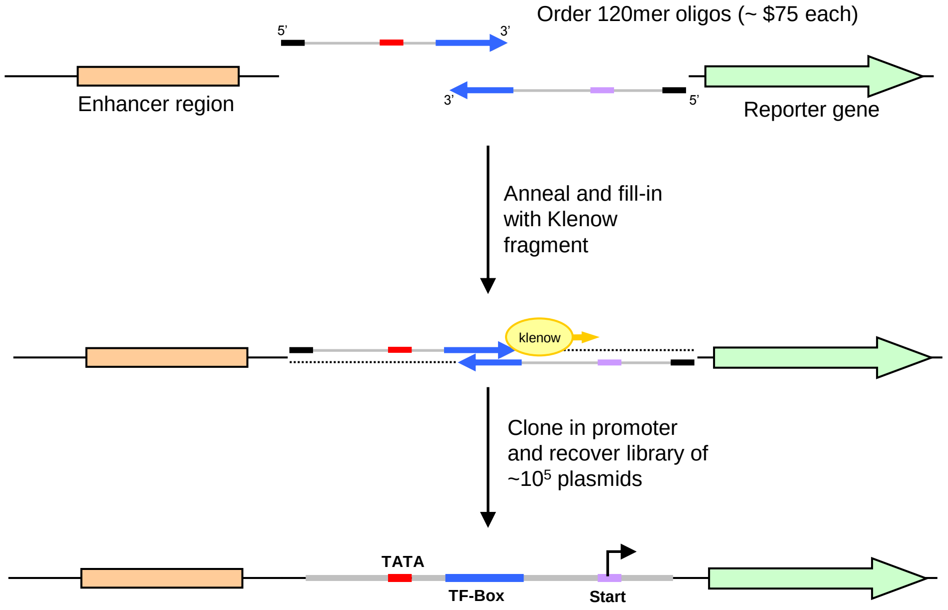
By Synthesis:
Jensen & Hammer

Tom Ellis Sept 09

Promoter Library Construction

Made using oligos
Include regulation sites
Uses *de novo* design

5’-PstI---$(N)_{35}$---TATA---$(N)_{11}$---*tet*O_2-$(N)_2$ -*tet*O_2- 3’

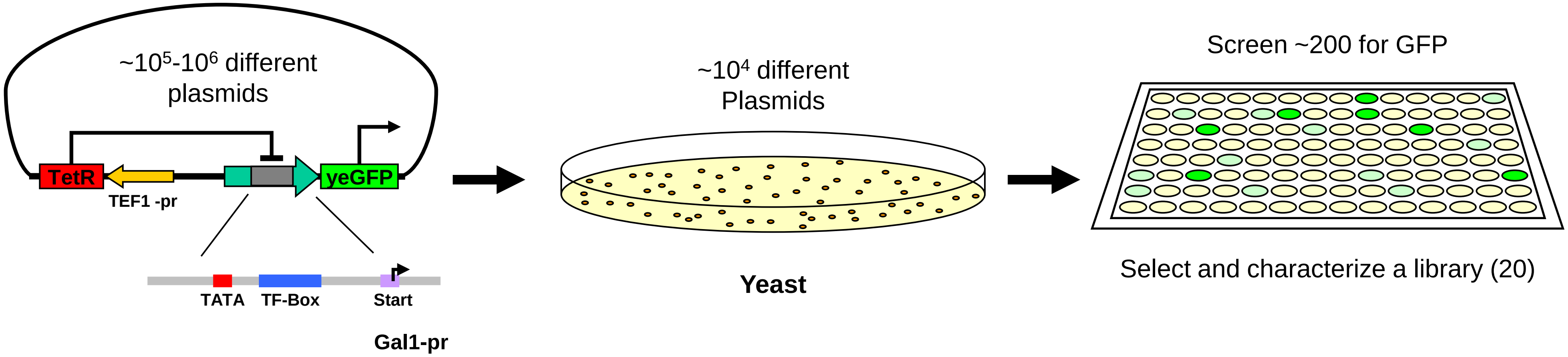


Order 120mer oligos (~ $75 each)

Anneal and fill-in with Klenow fragment

Clone in promoter and recover library of ~10^5 plasmids

Characterise in parallel



~10^5-10^6 different plasmids

~10^4 different Plasmids

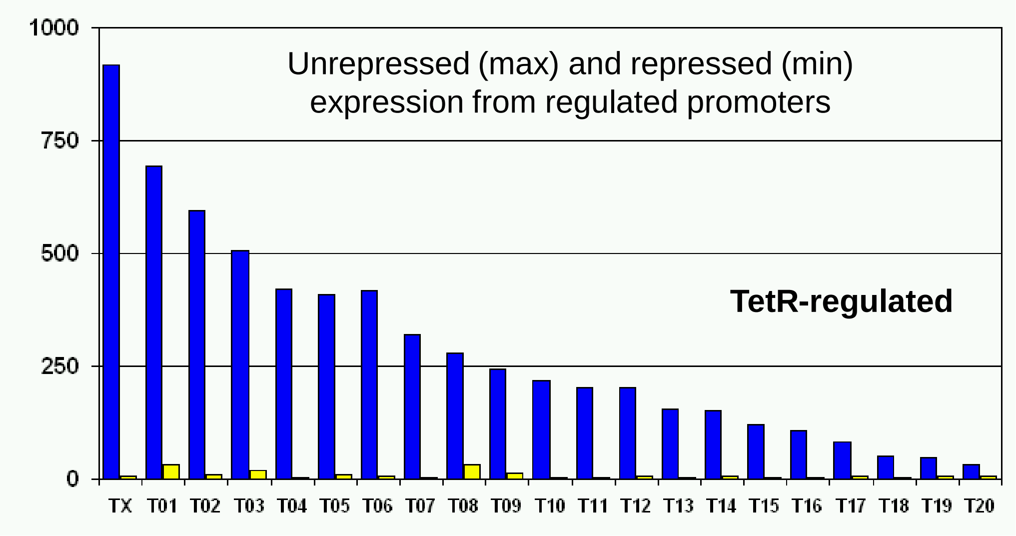
Yeast

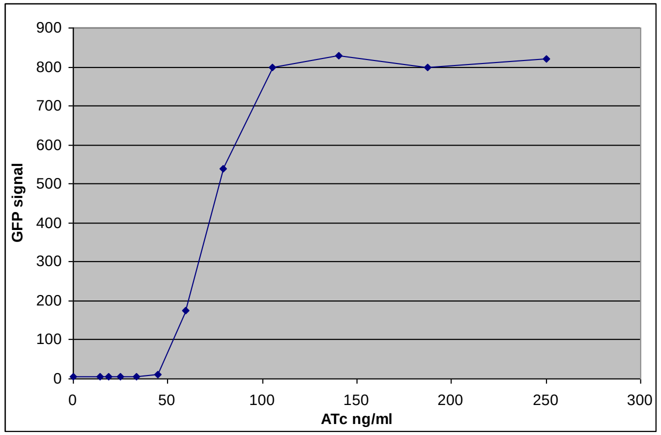
Screen ~200 for GFP

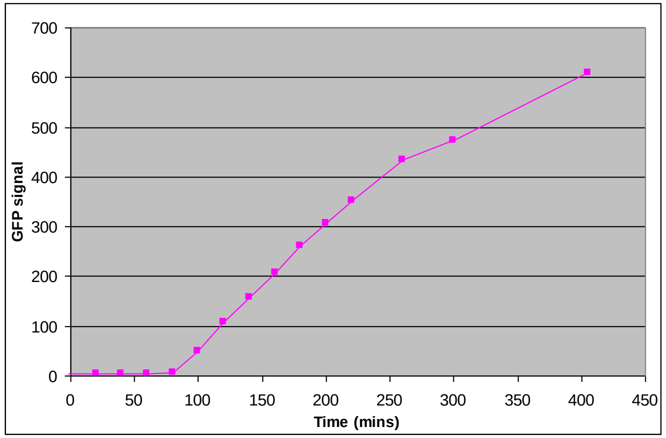
Select and characterize a library (20)

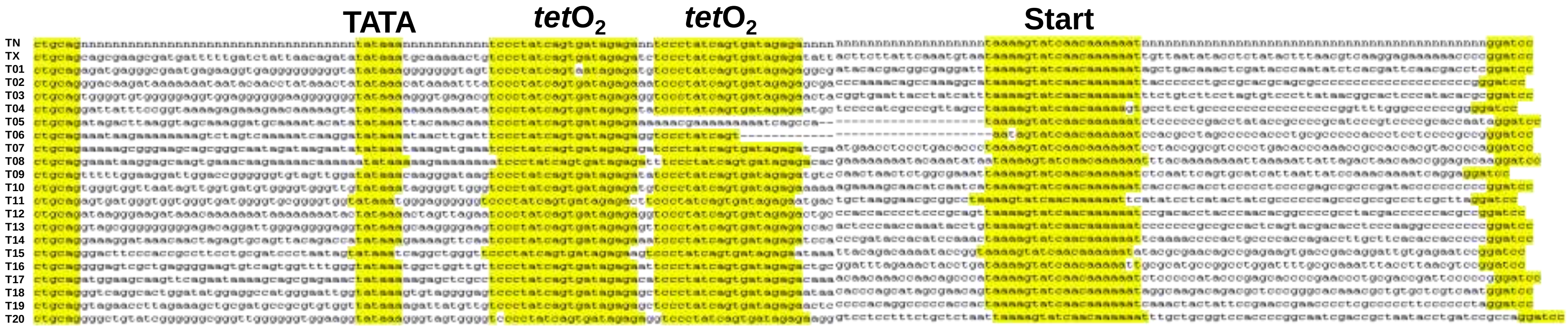
Tom Ellis Sept 09

Regulated Promoter Libraries



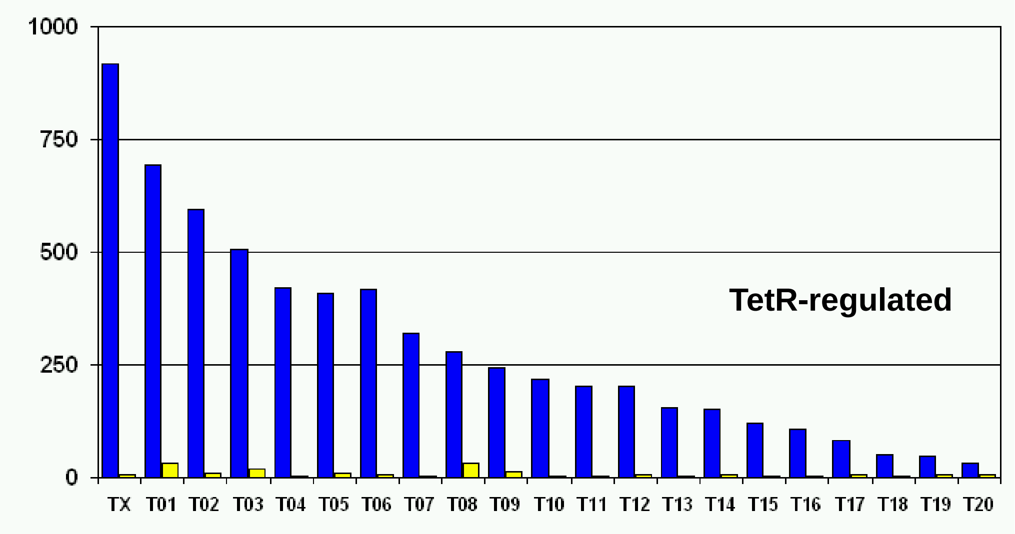


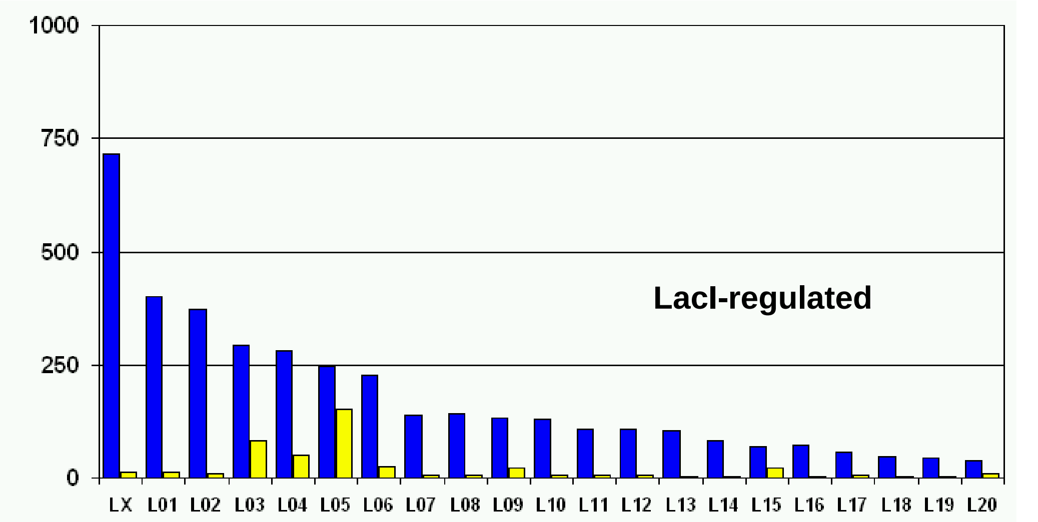




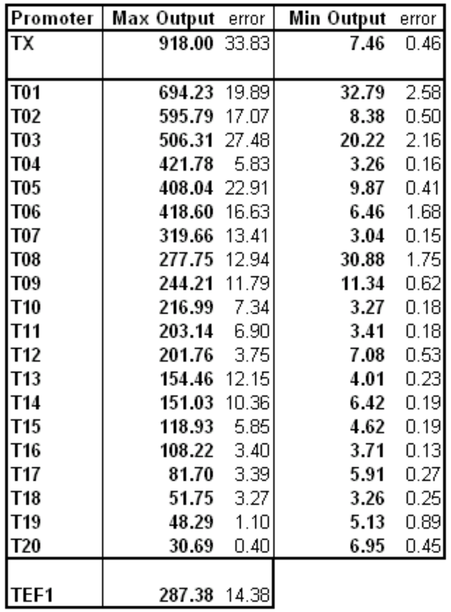
Tom Ellis Sept 09

Regulated Promoter Library Characterisation



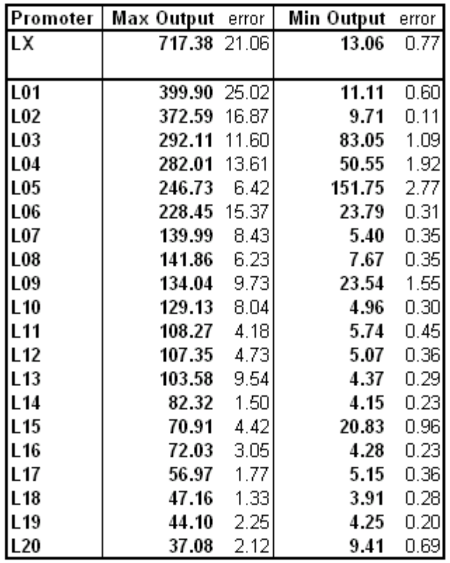


TetR-regulated promoters



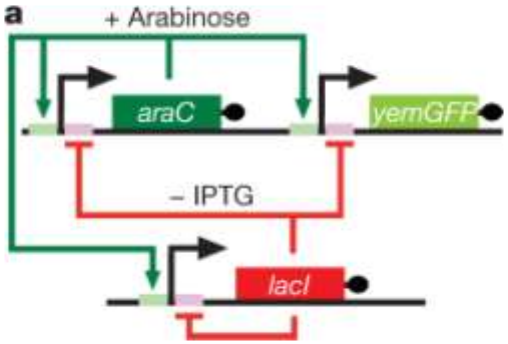
Promoter	Max Output	error	Min Output	error
TX	918.00	33.83	7.46	0.46
T01	694.23	19.89	32.79	2.58
T02	595.79	17.07	8.38	0.50
T03	506.31	27.48	20.22	2.16
T04	421.78	5.83	3.26	0.16
T05	408.04	22.91	9.87	0.41
T06	418.60	16.63	6.46	1.68
T07	319.66	13.41	3.04	0.15
T08	277.75	12.94	30.88	1.75
T09	244.21	11.79	11.34	0.62
T10	216.99	7.34	3.27	0.18
T11	203.14	6.90	3.41	0.18
T12	201.76	3.75	7.08	0.53
T13	154.46	12.15	4.01	0.23
T14	151.03	10.36	6.42	0.19
T15	118.93	5.85	4.62	0.19
T16	108.22	3.40	3.71	0.13
T17	81.70	3.39	5.91	0.27
T18	51.75	3.27	3.26	0.25
T19	48.29	1.10	5.13	0.89
T20	30.69	0.40	6.95	0.45
TEF1	287.38	14.38		

LacI-regulated promoters



Promoter	Max Output	error	Min Output	error
LX	717.38	21.06	13.06	0.77
L01	399.90	25.02	11.11	0.60
L02	372.59	16.87	9.71	0.11
L03	292.11	11.60	83.05	1.09
L04	282.01	13.61	50.55	1.92
L05	246.73	6.42	151.75	2.77
L06	228.45	15.37	23.79	0.31
L07	139.99	8.43	5.40	0.35
L08	141.86	6.23	7.67	0.35
L09	134.04	9.73	23.54	1.55
L10	129.13	8.04	4.96	0.30
L11	108.27	4.18	5.74	0.45
L12	107.35	4.73	5.07	0.36
L13	103.58	9.54	4.37	0.29
L14	82.32	1.50	4.15	0.23
L15	70.91	4.42	20.83	0.96
L16	72.03	3.05	4.28	0.23
L17	56.97	1.77	5.15	0.36
L18	47.16	1.33	3.91	0.28
L19	44.10	2.25	4.25	0.20
L20	37.08	2.12	9.41	0.69

Range of S_{min} and S_{max} = range of input and output

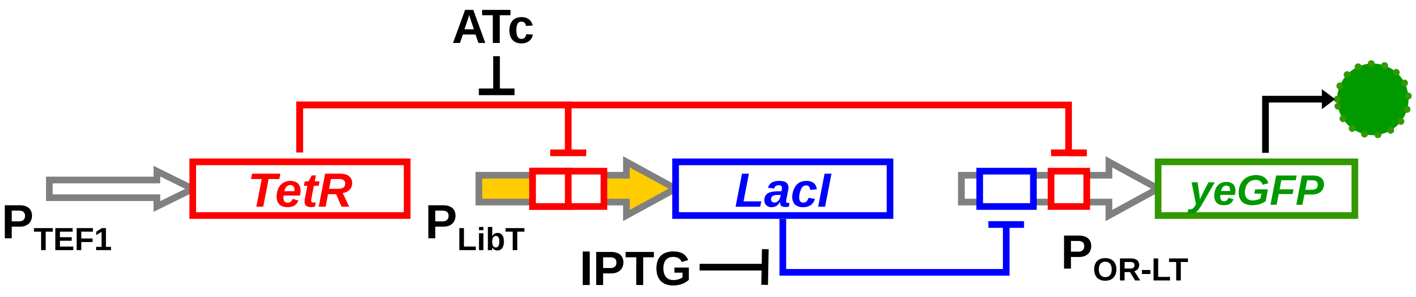


Tom Ellis Sept 09

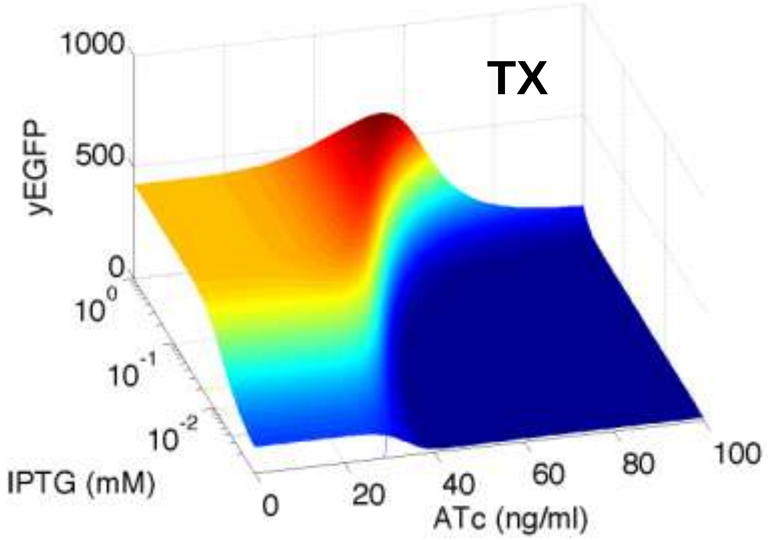
Giving it a go - What can we try that is interesting?

Negative feed-forward loop motif: robust, non-linear

Modeling type: prediction ahead of assembly

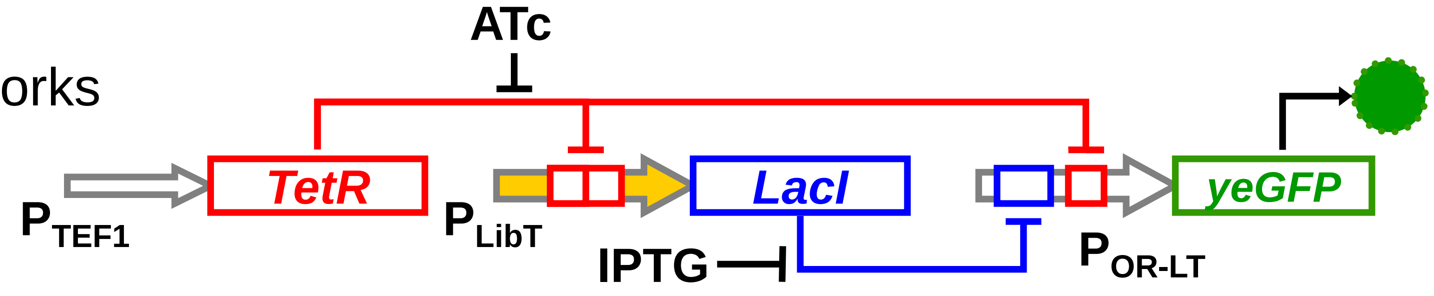


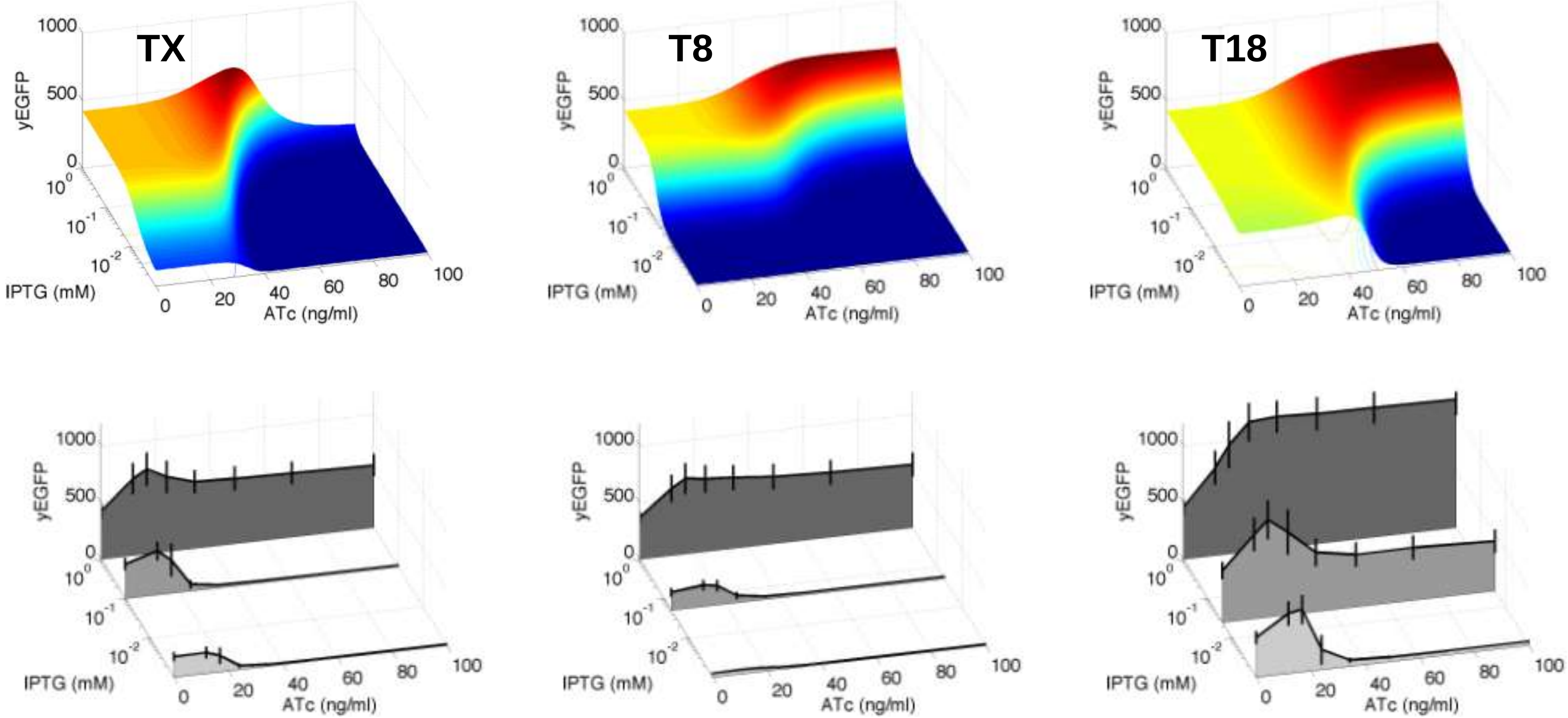
1 library = 21 networks



Feed-Forward Loop Network

Assemble example networks
single-copy
genome-integrated

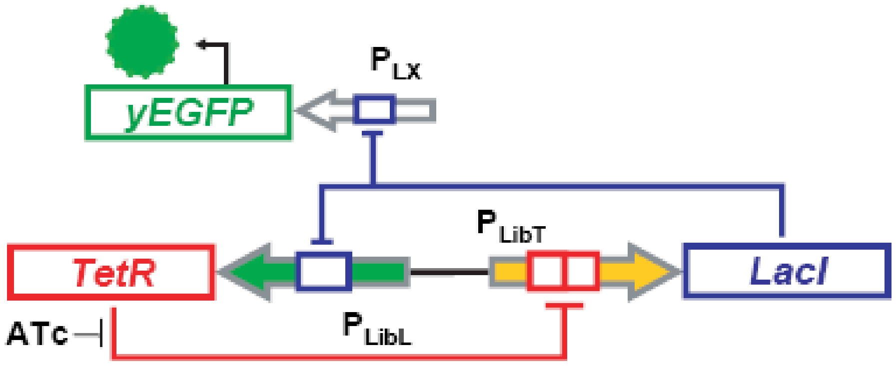


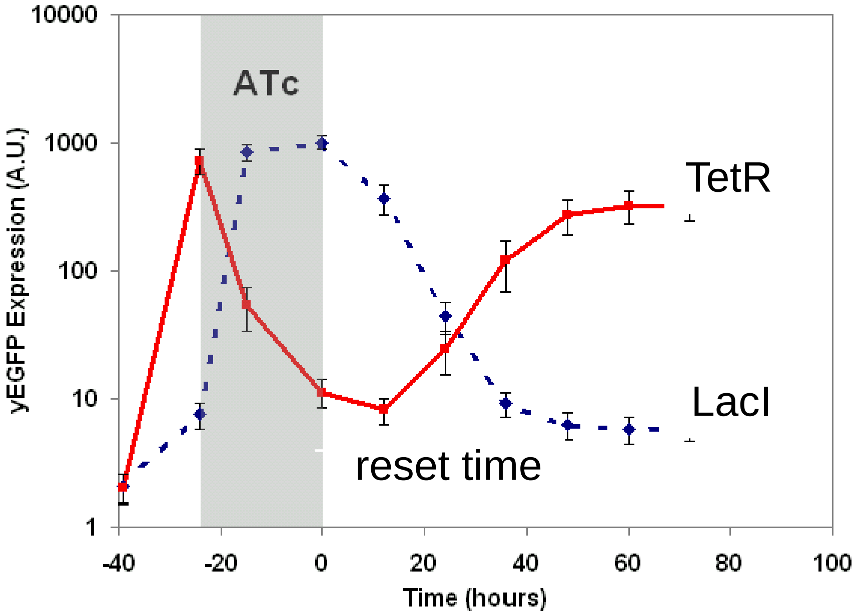


More complex case – Toggle ‘Timers’

Monostable toggles that act as programmable ‘timers’
 unbalanced mutual repression

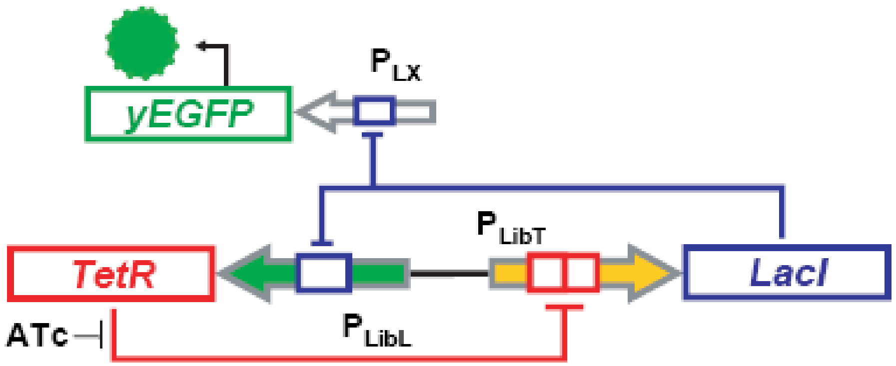
Modeling type: predictions based on single example



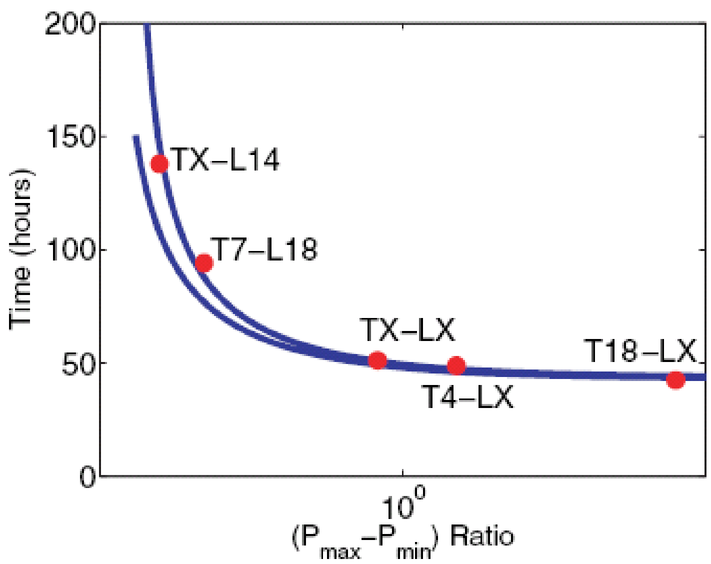


2 libraries = 441 networks

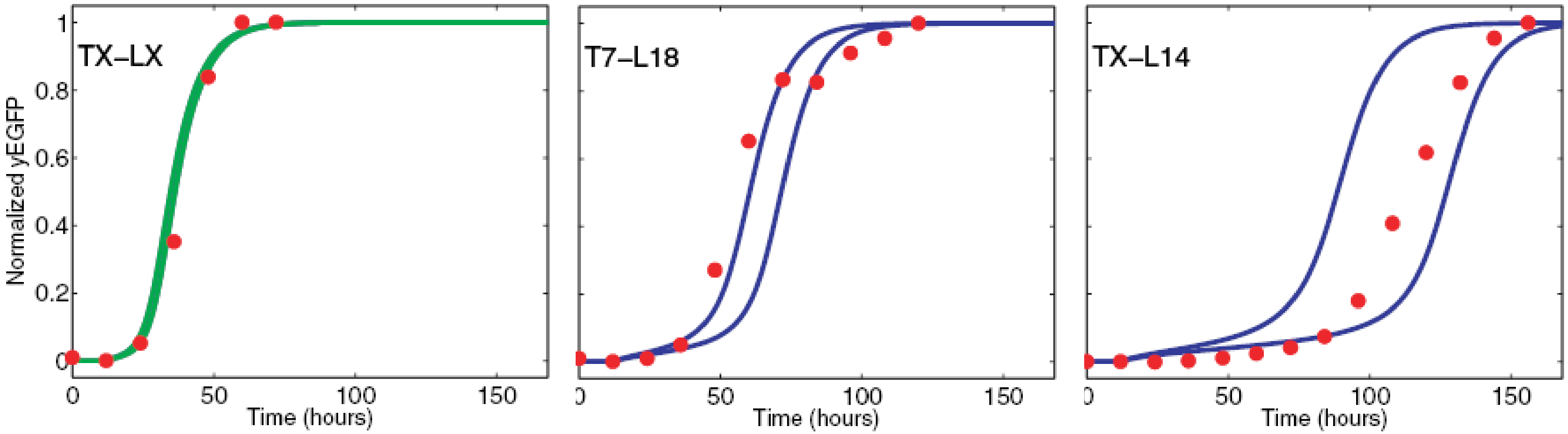
Toggle 'Timers'



Toggle reset times can be extrapolated from promoter properties



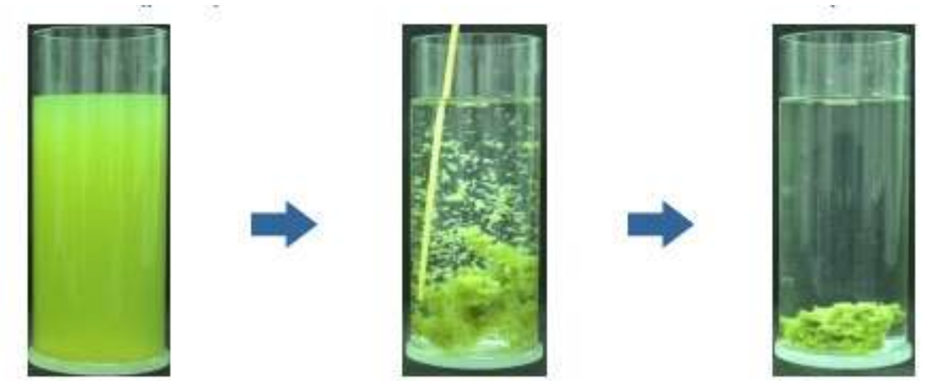
Predicted relationship from computational model + one experimental test



Tom Ellis Sept 09

Applying the network – a phenotype more interesting than GFP

Yeast flocculation: high expression of Flo1 = cell sedimentation

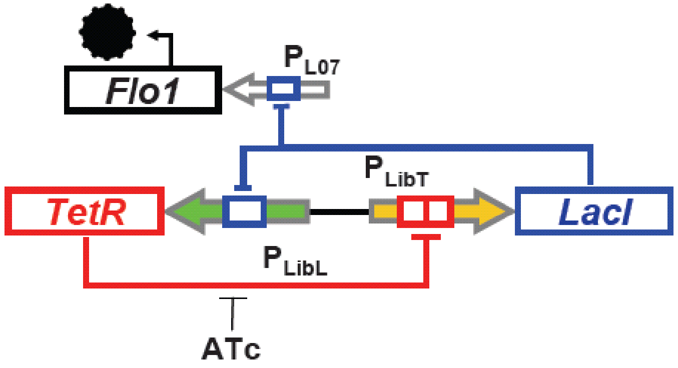


Why would this be of use?
Beer, wine, waste… and now biofuels

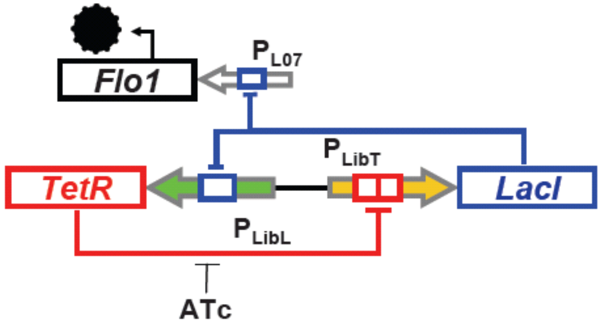
Advantages of the system – controlled, predictable

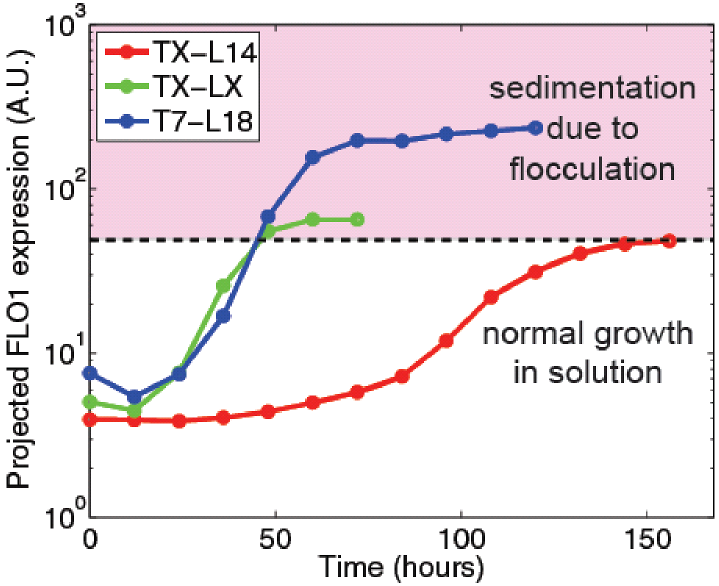
Flo1 can be direct replacement for GFP

Sedimentation is a threshold event

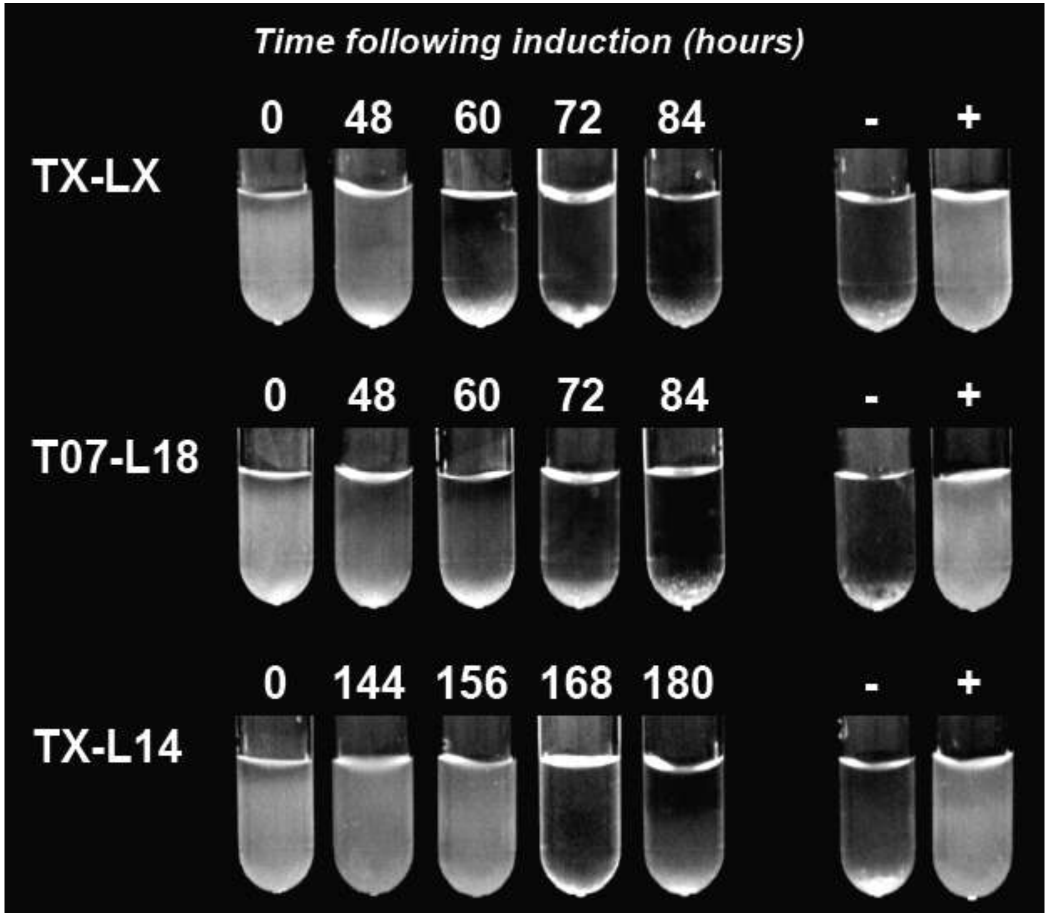


Yeast Flocculation Control

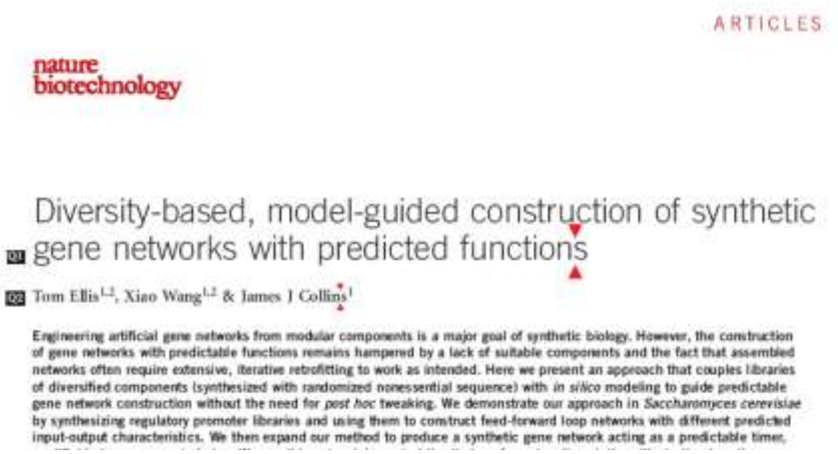




>16000 networks possible



Tom Ellis Sept 09



ARTICLES

nature biotechnology

Diversity-based, model-guided construction of synthetic gene networks with predicted functions

Tom Ellis[1,2], Xiao Wang[1,2] & James J Collins[1]

Engineering artificial gene networks from modular components is a major goal of synthetic biology. However, the construction of gene networks with predictable functions remains hampered by a lack of suitable components and the fact that assembled networks often require extensive, iterative retrofitting to work as intended. Here we present an approach that couples libraries of diversified components (synthesized with randomized nonessential sequence) with *in silico* modeling to guide predictable gene network construction without the need for *post hoc* tweaking. We demonstrate our approach in *Saccharomyces cerevisiae* by synthesizing regulatory promoter libraries and using them to construct feed-forward loop networks with different predicted input-output characteristics. We then expand our method to produce a synthetic gene network acting as a predictable timer,

nature.com

The world's best science and medicine on your desktop

21 April 2009

Biotechnology: A better engineered beer

Diversity-based, model-guided construction of synthetic gene networks with predicted functions.

Image: Punchstock

Latest news

- US environment agency declares greenhouse gases a threat
- NIH announces draft stem-cell guidelines
- Australia launches carbon capture institute

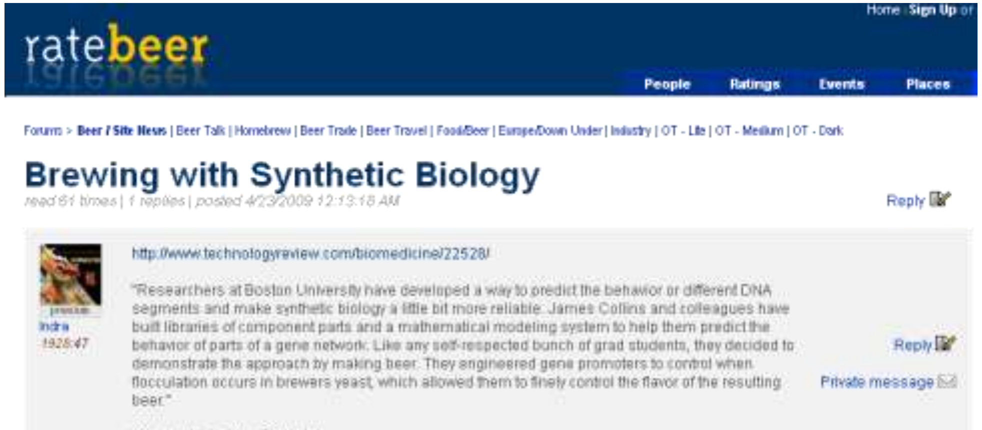
Wednesday, April 22, 2009

Brewing with Synthetic Biology

A new approach offers a more efficient way to design biological "circuits."

By Courtney Humphries

Synthetic biology rests on the hope that biological "parts" like DNA and proteins can be engineered and assembled just like a machine or computer circuit, but the field still



Brewing with Synthetic Biology

read 61 times | 1 replies | posted 4/23/2009 12:13:18 AM

Indra
1928:47

http://www.technologyreview.com/biomedicine/22528/

"Researchers at Boston University have developed a way to predict the behavior or different DNA segments and make synthetic biology a little bit more reliable. James Collins and colleagues have built libraries of component parts and a mathematical modeling system to help them predict the behavior of parts of a gene network. Like any self-respected bunch of grad students, they decided to demonstrate the approach by making beer. They engineered gene promoters to control when flocculation occurs in brewers yeast, which allowed them to finely control the flavor of the resulting beer."

Yes, I got this from Slashdot.

Brewing with Synthetic Biology

April 23rd, 2009 by Jabba

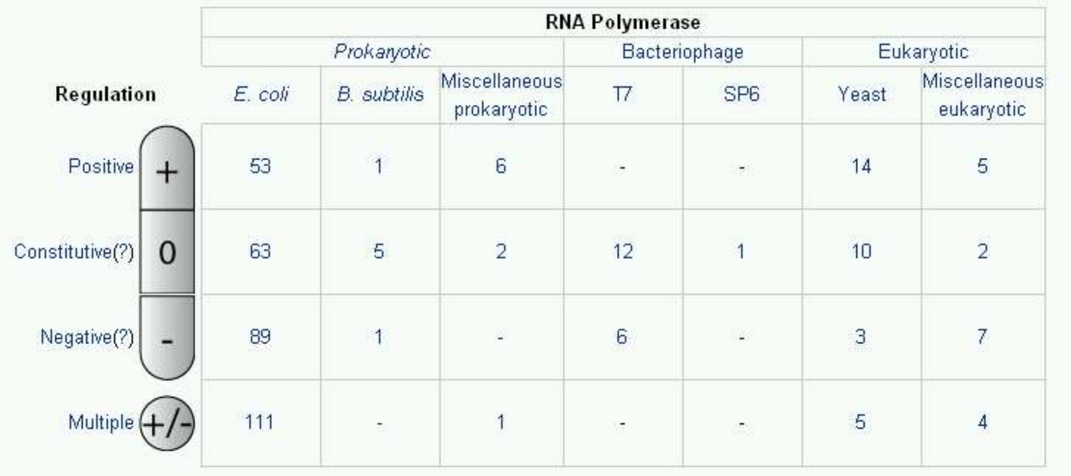
Synthetic biology rests on the hope that biological "parts" like DNA and proteins can be engineered and assembled just like a machine or computer circuit, but the field still

"While we may not fully understand the terminology and the processes involved, we do know that Collins has used the technology to brew beer. Really good beer."
"We love the idea of this RoboBeer, but they'd better not start toying around with PBR."
Sunrise Post, 26-4-09

Advantages

1. Fast
2. Predictive / Rational
3. Desired output levels
4. Fine-tuning of response
5. Parallel characterisation
6. Exponential
7. Provides parts for community

If every iGEM team made 1 new biobrick by this method…
>2000 new parts?



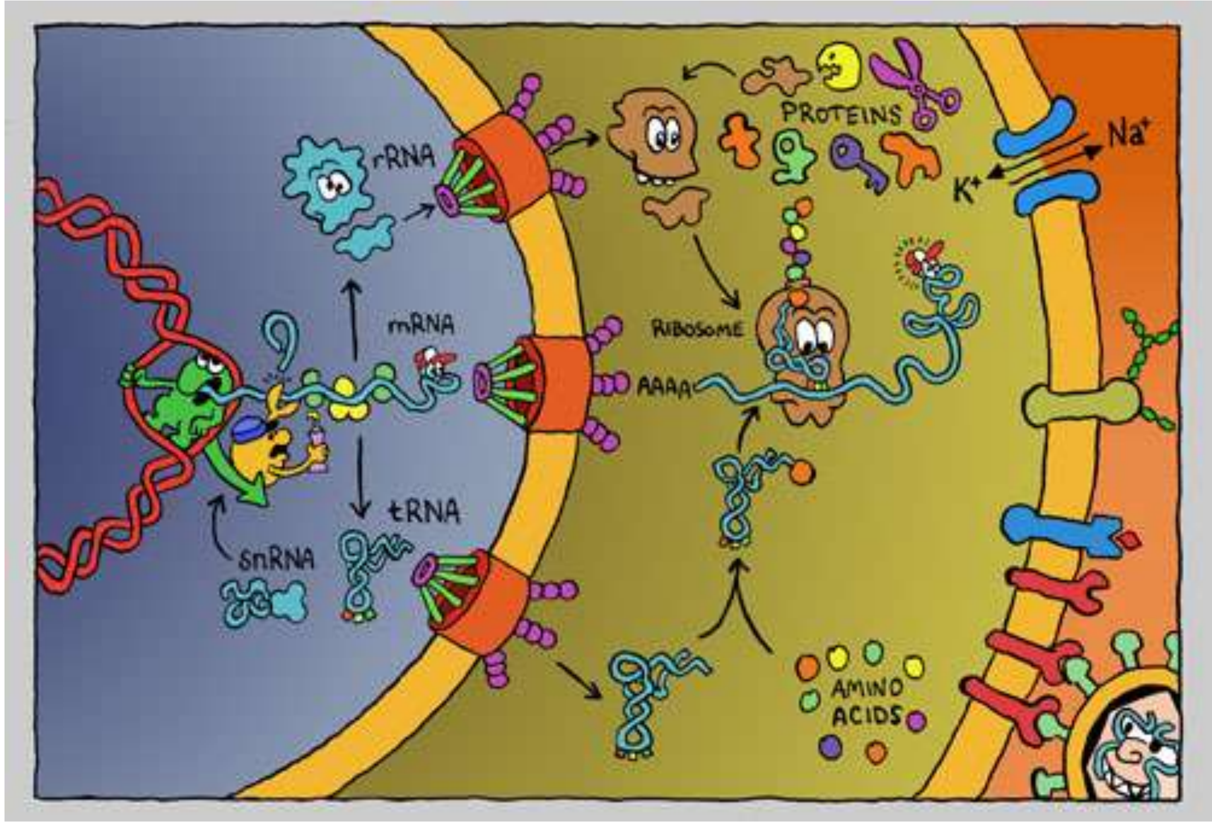
	RNA Polymerase						
	Prokaryotic			Bacteriophage		Eukaryotic	
Regulation	*E. coli*	*B. subtilis*	Miscellaneous prokaryotic	T7	SP6	Yeast	Miscellaneous eukaryotic
Positive +	53	1	6	-	-	14	5
Constitutive(?) 0	63	5	2	12	1	10	2
Negative(?) -	89	1	-	6	-	3	7
Multiple +/-	111	-	1	-	-	5	4

Beyond Yeast Regulatory Promoters…

Bacterial, mammalian regulatory promoters

Libraries of other parts:

… RBS, Terminators, RNA UTRs, Codon usage, Peptide tags, Modular Proteins

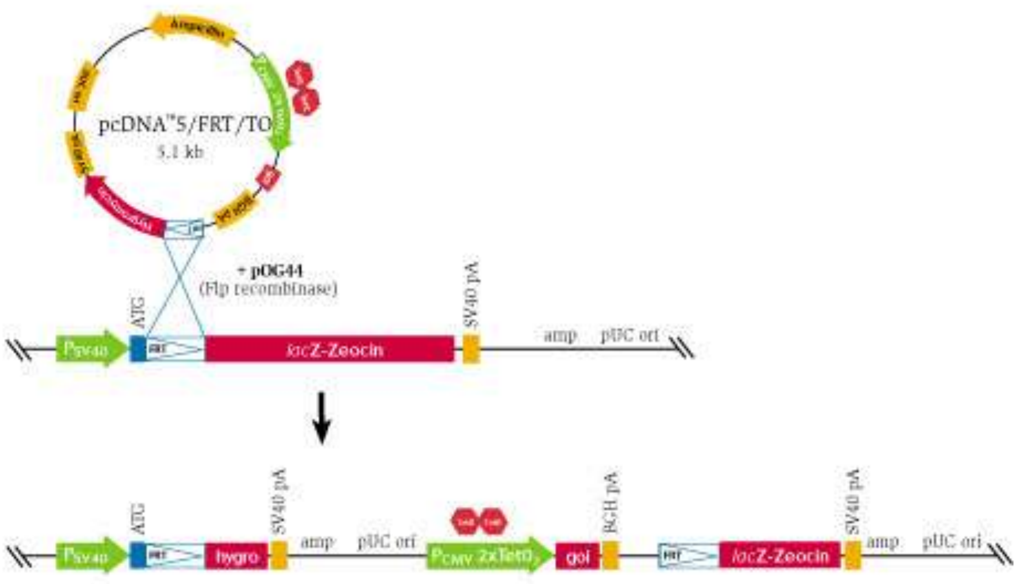


Working on now…

Mammalian promoter library

Single site integration
Flow cytometric sorting

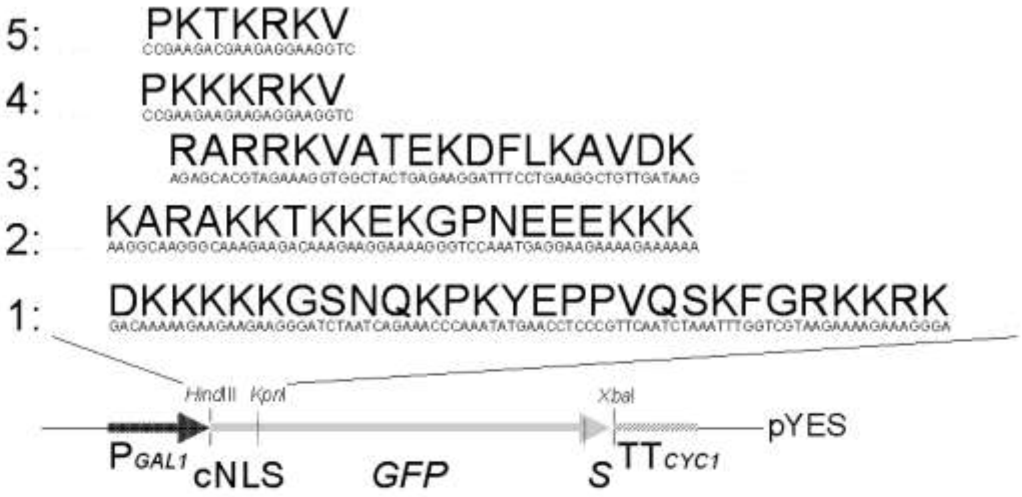
Figure 1 - Integration of the Flp-In™ T-REx™ vector



Peptide tags in mammalian systems

Nuclear Localization Sequence

Splicing regulation



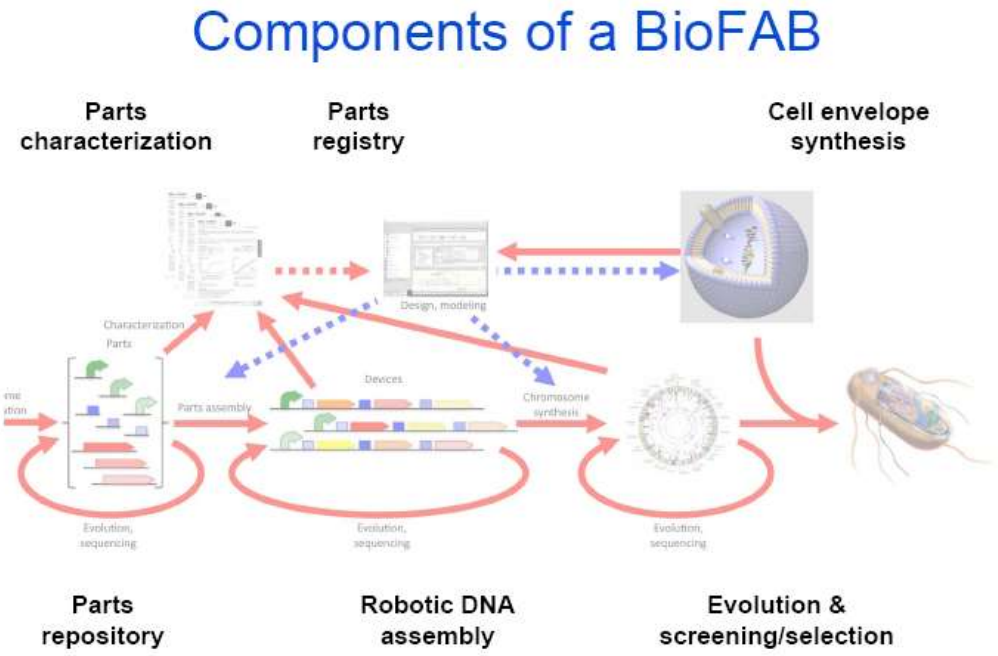
Where could this lead?

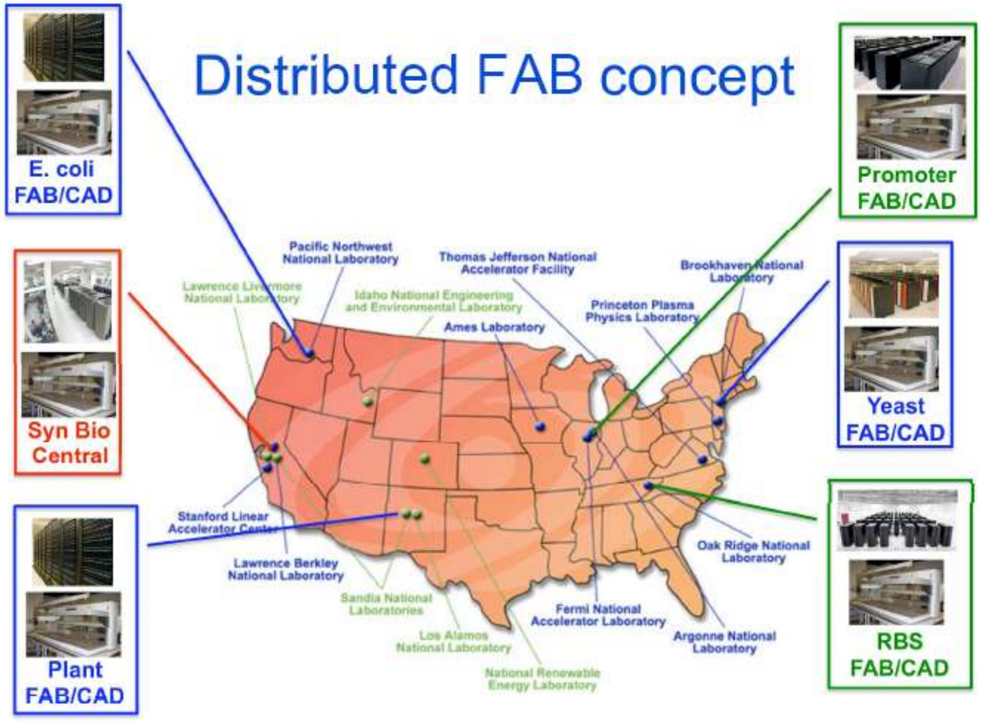
Scaled-up libraries

Degenerate DNA synthesis

High-throughput screening

Implementable in a BioFAB





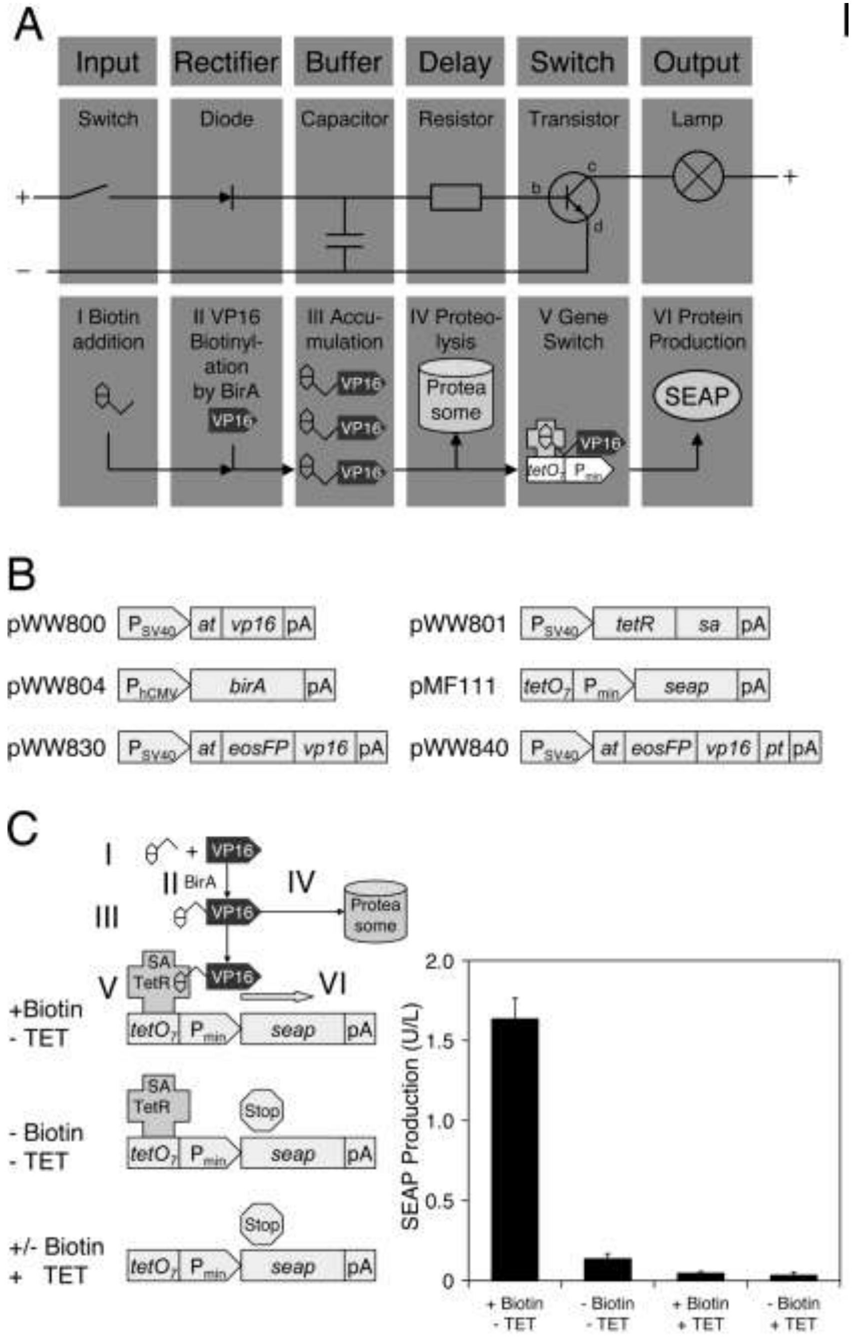
Tom Ellis Sept 09

Synthesis of new parts

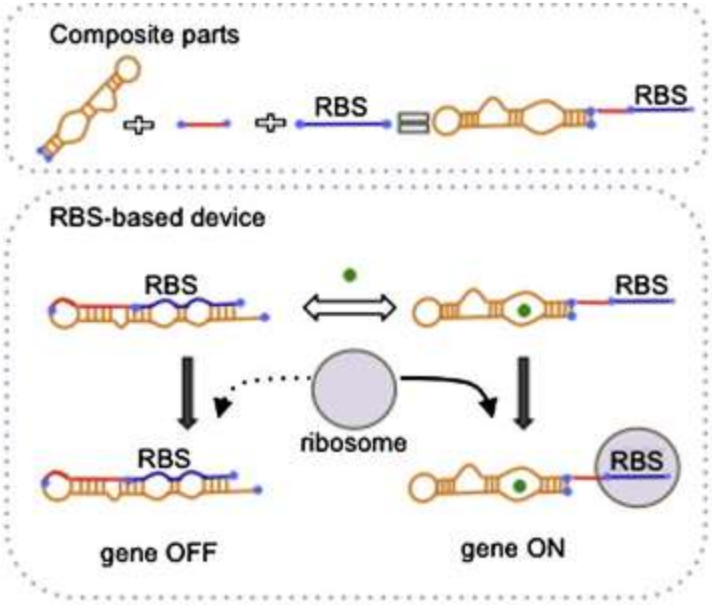
Hybrid / *de novo* part design

Modular motifs
Stepwise changes

Start from chassis part



Fussenegger lab



Smolke lab

Tom Ellis

Techniques, Construction and Implementation of Gene Networks

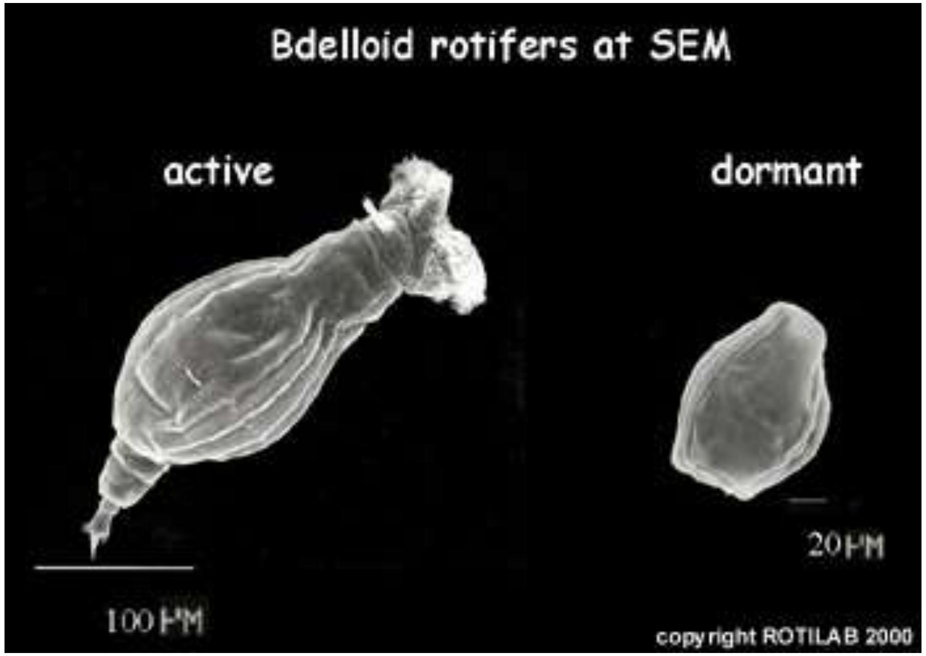
now at **University of Cambridge**, Dept of Biotechnology and Chemical Eng.

Mammalian cell synthetic biology

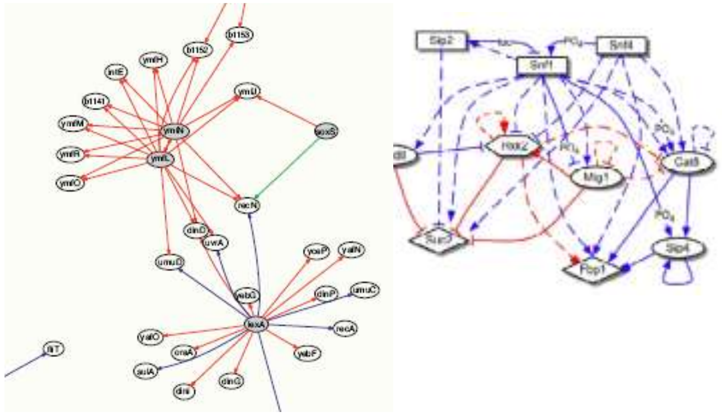
Engineer dry-life tolerance into cells

genetic, metabolic and protein engineering

And other ideas…



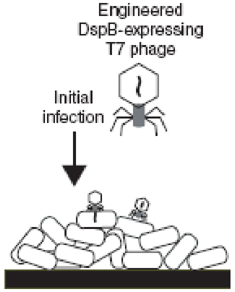
systems biology

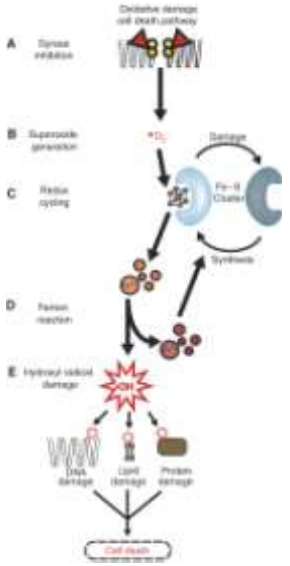


beer

aging bioenergy mammalian disease

antibiotics





Collins Lab

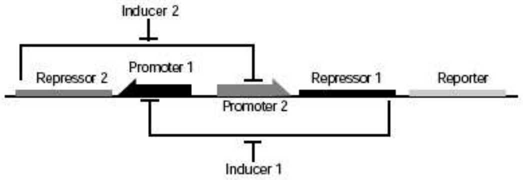
Boston University

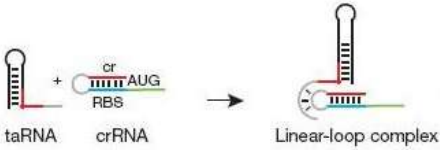
Xiao Wang – Matlab
Henry Lee – Ideas

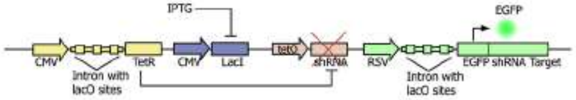
Peter R Jensen, Biocentrum DTU
Kevin Verstrepen, KU Leuven

vibrating insoles

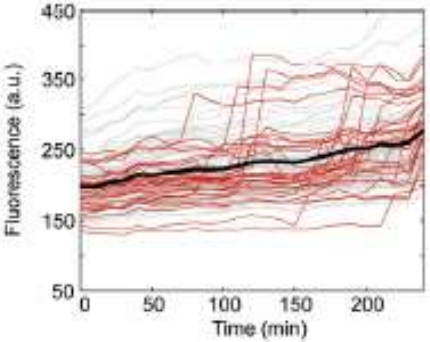
synthetic biology



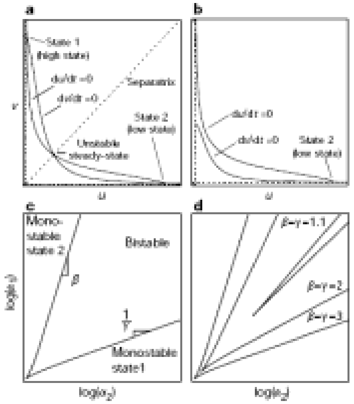




noise



modeling



BOSTON UNIVERSITY

Tom Ellis 2009