Don't be so specific:

exploiting diversity in synthesis to fast-track

synthetic biology

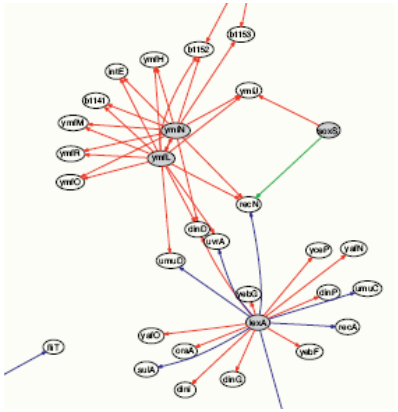
Tom Ellis

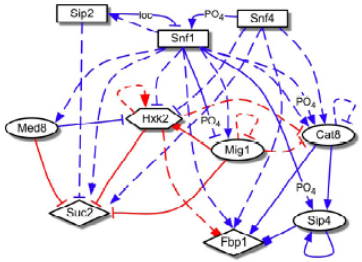
Jim Collins Group, Boston University

Collins Lab

Boston University

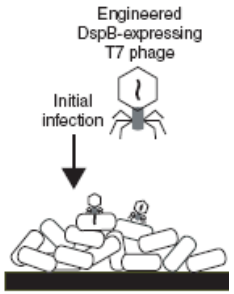
systems biology

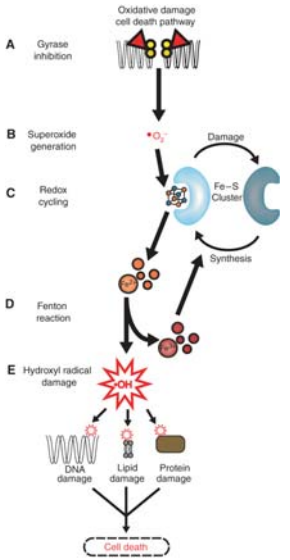




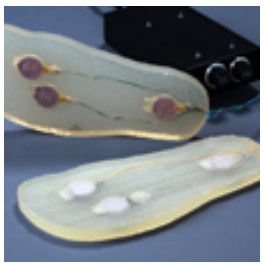
aging bioenergy mammalian disease

antibiotics

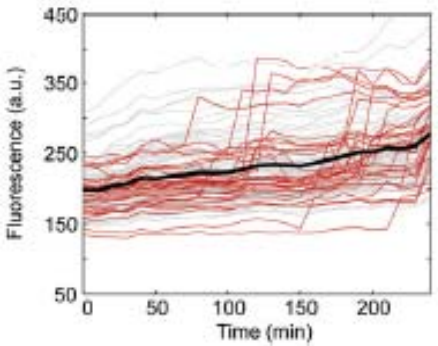




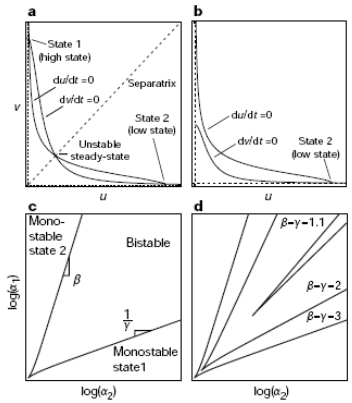
vibrating insoles



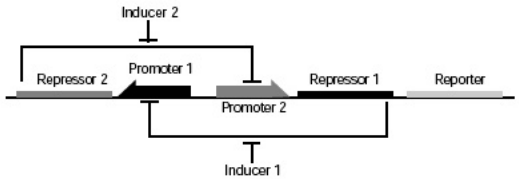
noise

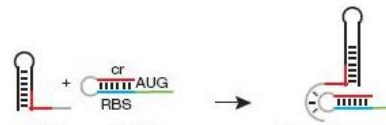


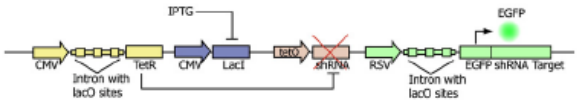
modeling



synthetic biology







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Tom Ellis 28-4-09

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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

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

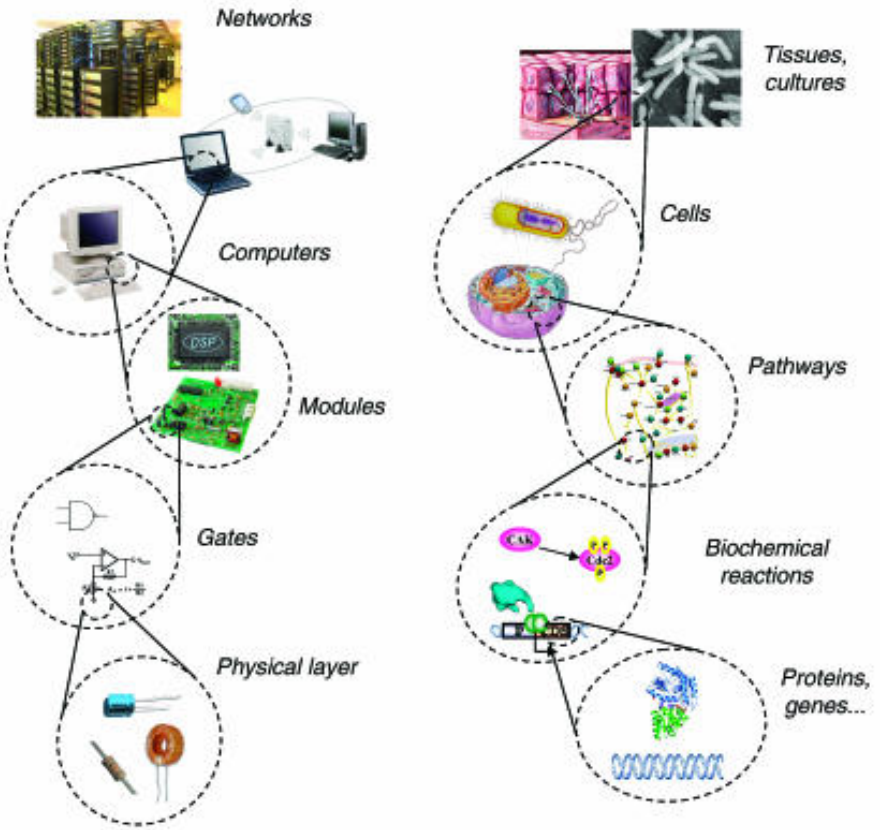
Constructing novel gene networks

Investigating biology by building
and modeling equivalent systems

Synthesizing entirely new biomolecules

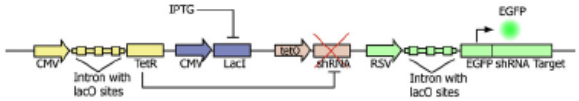
Rewriting genomes

Building new life



Andrianantoandro E et al, 2006

Building gene networks - everyone's favourite part of synthetic biology



Idea!

↓

Design Your Network

↓

Find Suitable Parts

↓

Build Computer Model and test out network

↓

Assemble Network DNA

↓

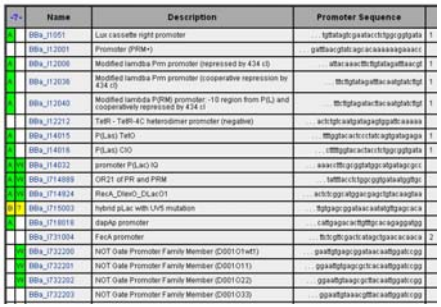
Test out Network in Cells

↓

It worked! :)

↓

put it to use



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BBa_I1051	Lux cassette right promoter	...tgttatagttcgaatacctctgtggtggtgata
BBa_I12001	Promoter (PRM+)	gatttaacgttatcagcaccaaaaaaagaaaccc
BBa_I12006	Modified lambda Prm promoter (repressed by 434 cI)	...attaccaaacttcttgtattagatttaacgt
BBa_I12036	Modified lambda Prm promoter (cooperative repression by 434 cI)	...ttcttgtatagatttaccaatgtatcttgt
BBa_I12040	Modified lambda P(RM) promoter: -10 region from P(L) and cooperatively repressed by 434 cI	...ttcttgtagatacttaccaatgtatcttgt
BBa_I12212	TetR - TetR-4C heterodimer promoter (negative)	...actctgtcaatgatagaggtggattcaaaaa
BBa_I14015	P(Lac) TetO	...tttggttaccactcctatcagtgatagaga
BBa_I14016	P(Lac) CIO	...ctttttggttaccactcctatcagtgatata
BBa_I14032	promoter P(Lac) IQ	...aaaccttttgcggtatggcatgatagcgccc
BBa_I714889	OR21 of PR and PRM	...tatttttaccttctggcggtgatgatatggttgc
BBa_I714924	RecA_DtetO_DLacO1	...actctcggcatggcaggctgttaccaagttaa
BBa_I715003	hybrid pLac with UV5 mutation	...ttgttgcaggcggattaaccaatatgttgtagcaccta
BBa_I718018	dapAp promoter	...cattgagaccacttgtttgtcaccagcaggcatggg
BBa_I731004	FecA promoter	...ttctcgttcgactcatagctgcaaccaccaacta
BBa_I732200	NOT Gate Promoter Family Member (D0010+wt1)	...gaattgttgagcggataaccaattggattccgg
BBa_I732201	NOT Gate Promoter Family Member (D001011)	...ggaattgttgagcggctcaccaattggattccgg
BBa_I732202	NOT Gate Promoter Family Member (D001022)	...ggaattgttaagcggttaccaattggattccgg
BBa_I732203	NOT Gate Promoter Family Member (D001033)	...ggaattgttaaaccgttaccaattggattccgg

:(doesn't work

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Systems often don't work first time

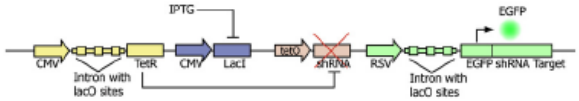
London Heathrow Terminal 5

What went wrong?

London Millennium Bridge

Retrofitted

Building gene networks - everyone's favourite part of synthetic biology



idea – hour

model – week

network – year

Idea!

↓

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↓

Find Suitable Parts

↓

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↓

Assemble Network DNA

↓

Test out Network in Cells

↓

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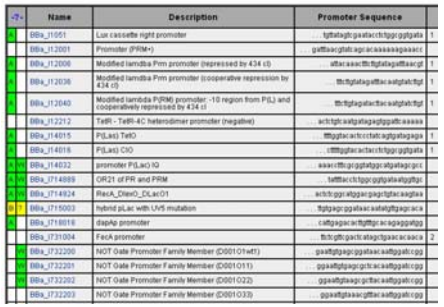
↓

put it to use



characterize parts thoroughly

↑

more testing

↑

Why?

↑

:(doesn't work

debugging

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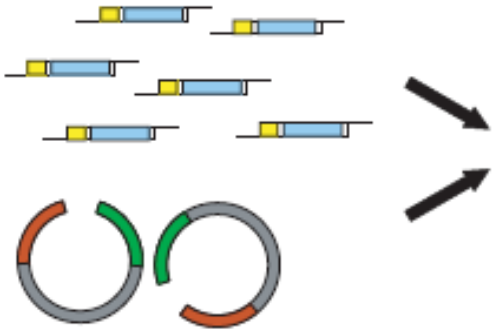
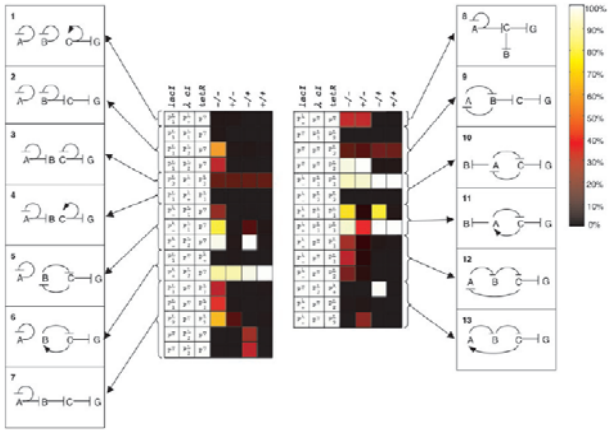
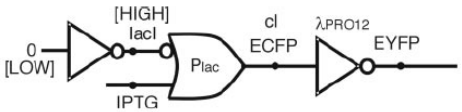
Alternative Approaches



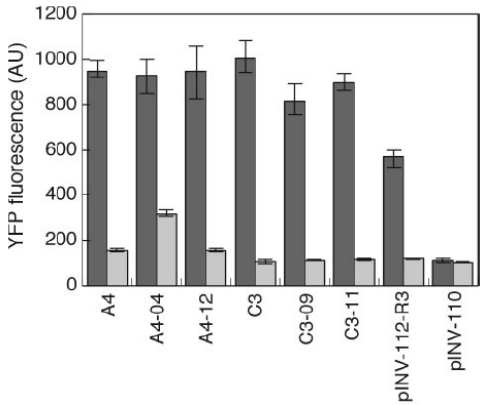


Module shuffling –
Guet at al, 2002

Directed evolution –
Yokobayashi et al, 2002



lacI	IPTG	cI	EYFP
0	0	1	0
0	1	1	0
1	0	0	1
1	1	1	0

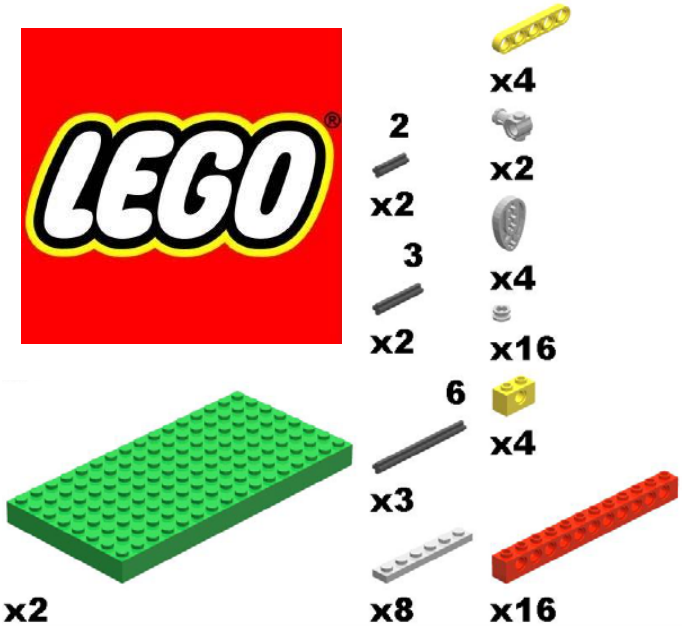


A role for **diversity** in synthetic biology

But…
These use diversity after model design

Can’t we introduce diversity before design?

Think of a set of screws


LEGO
x4
2
x2
x2
3
x2
x4
x16
6
x4
x3
x2
x8
x16


IKEA
IKEA
4x
4x
4x
2x
1x

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

Construct the intended networks (and use them)

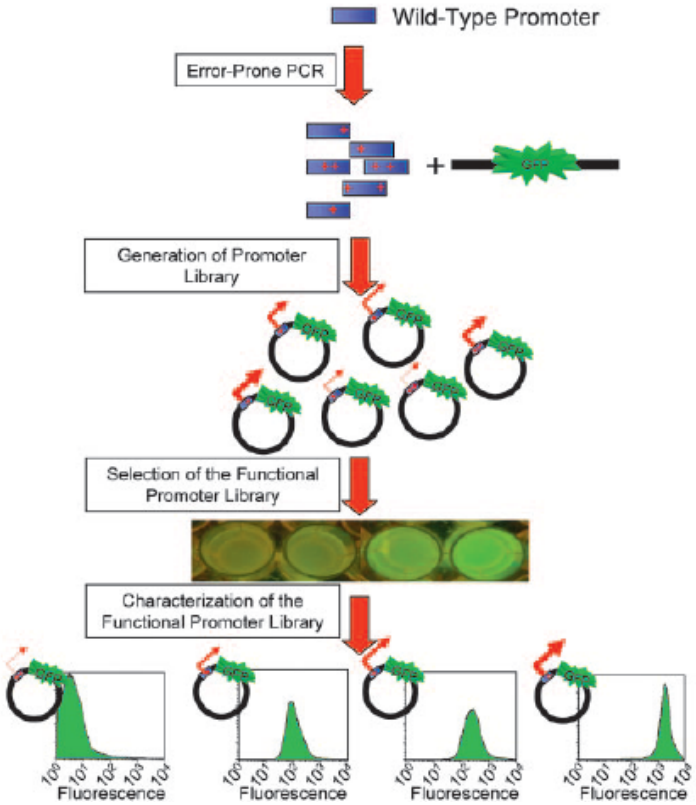
Bypass debugging

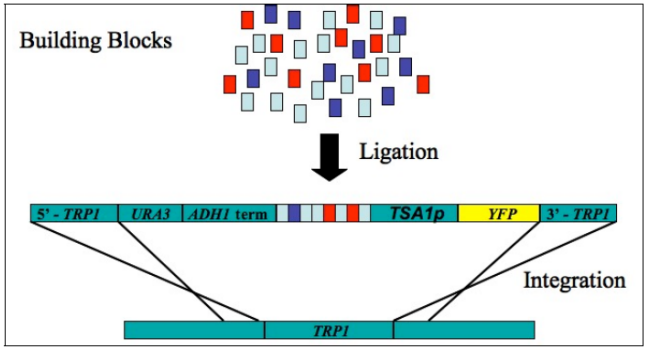
Promoter Library

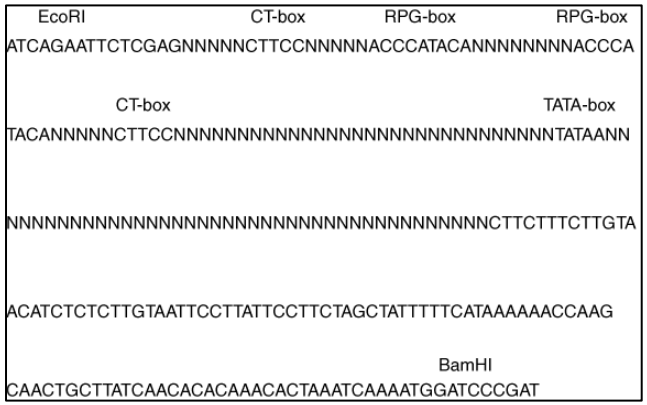
Synthesis techniques:
- By DNA shuffling
  Elowitz/Cohen
- By Mutation:
  Alper & Stephanopolous
- **By Synthesis:**
  Jensen & Hammer

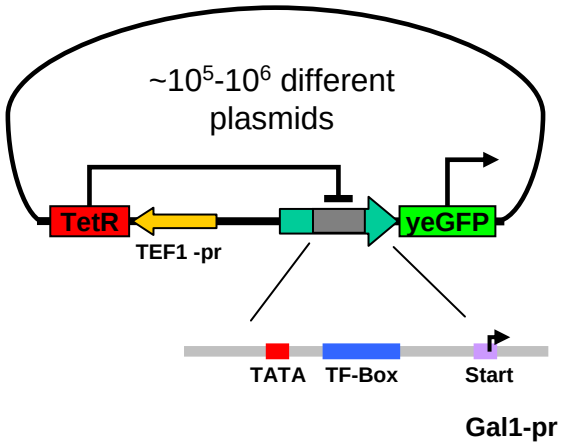
Made using oligos
Include regulation sites
Uses *de novo* design

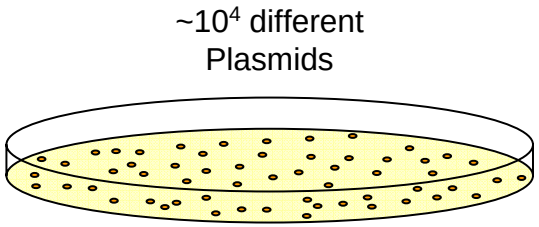
Characterizing in parallel

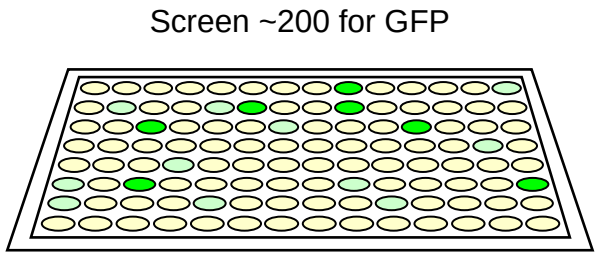






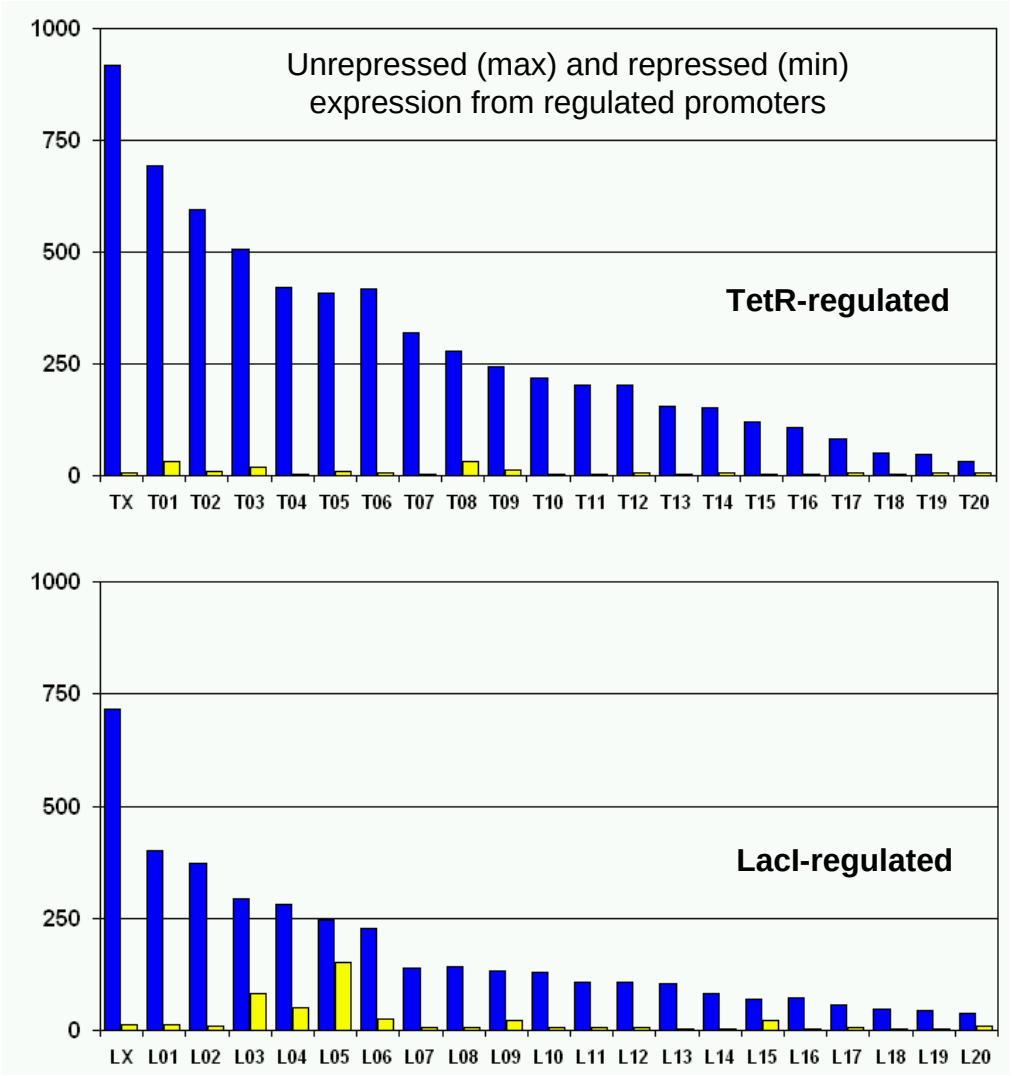






Select and characterize a library (20)

Regulated Promoter Libraries

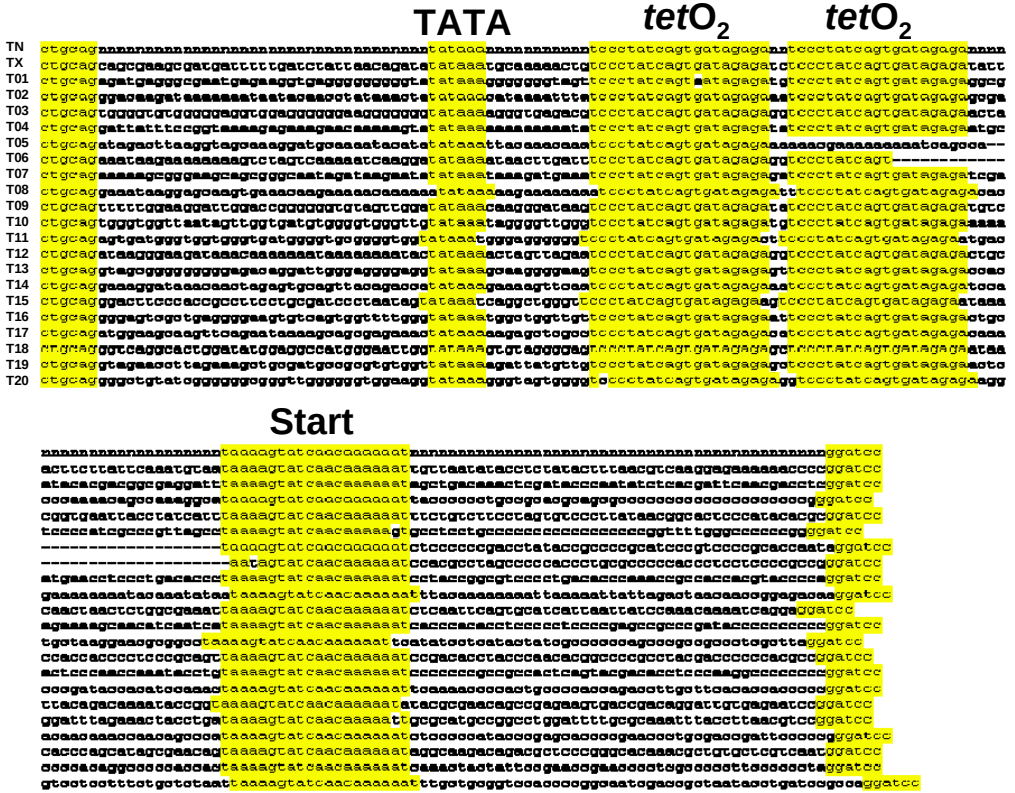


TetR-regulated promoters

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

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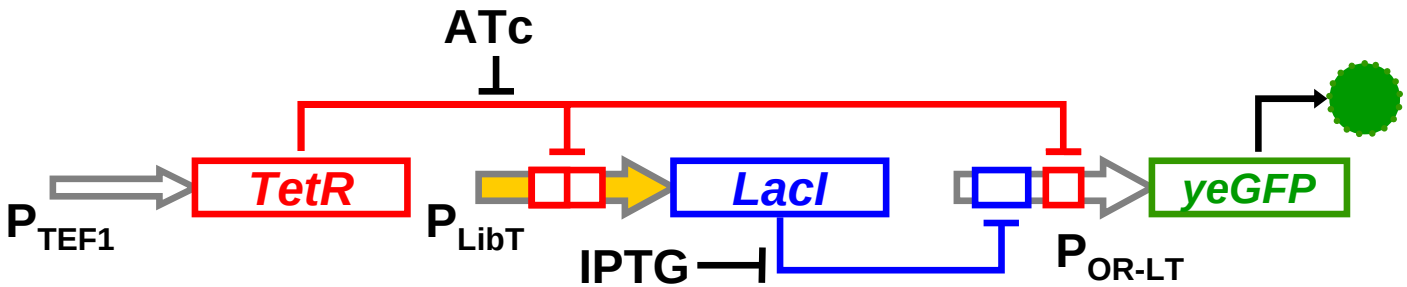
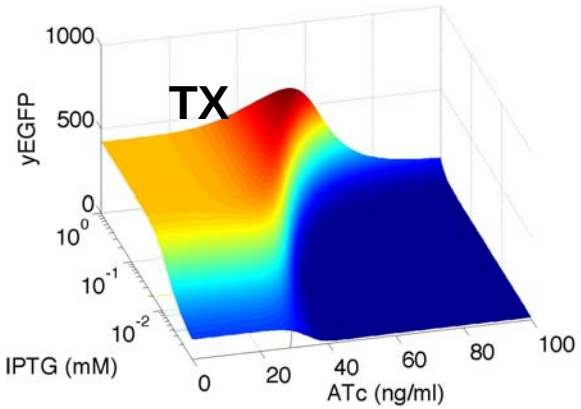
Giving it a go

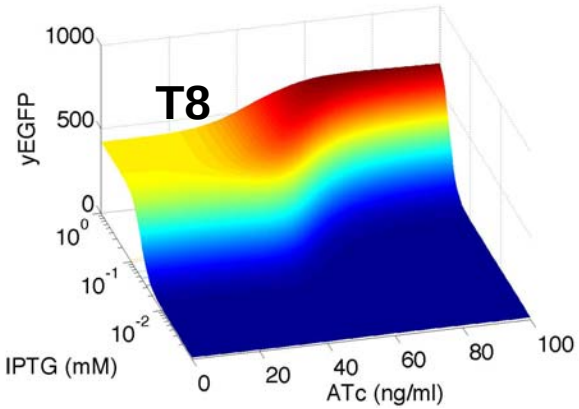
1 library = 21 networks

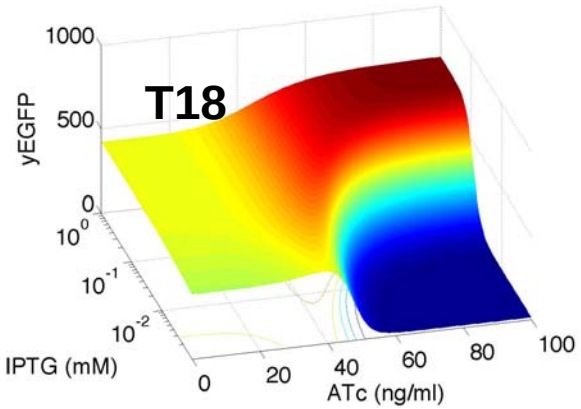
Feed forward loop motif - robust, non-linear

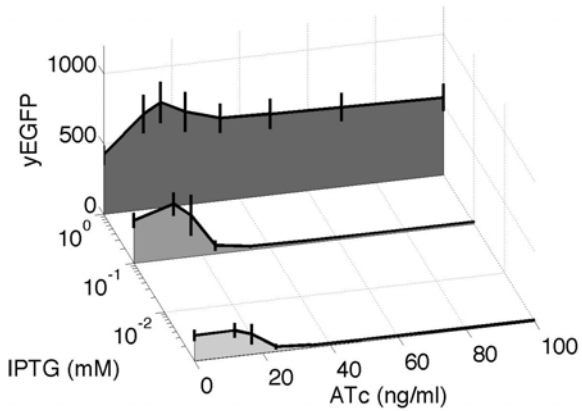
Modeling type:
prediction ahead
of assembly

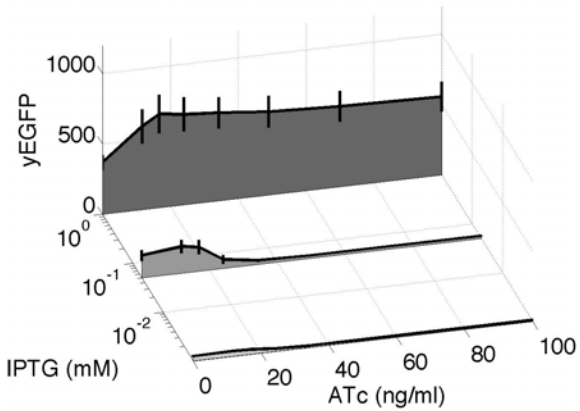


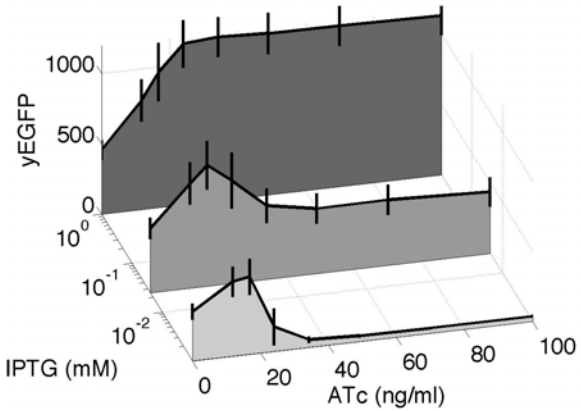










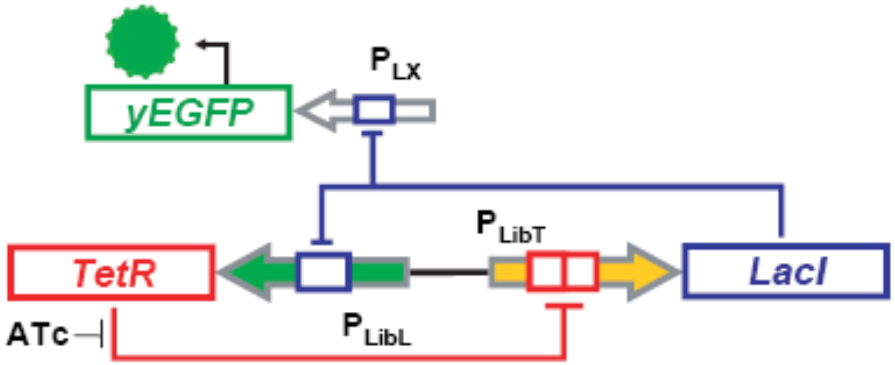


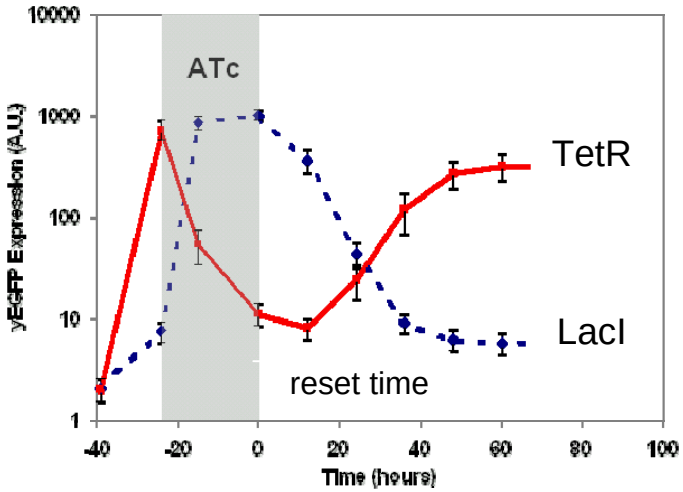
More complex case

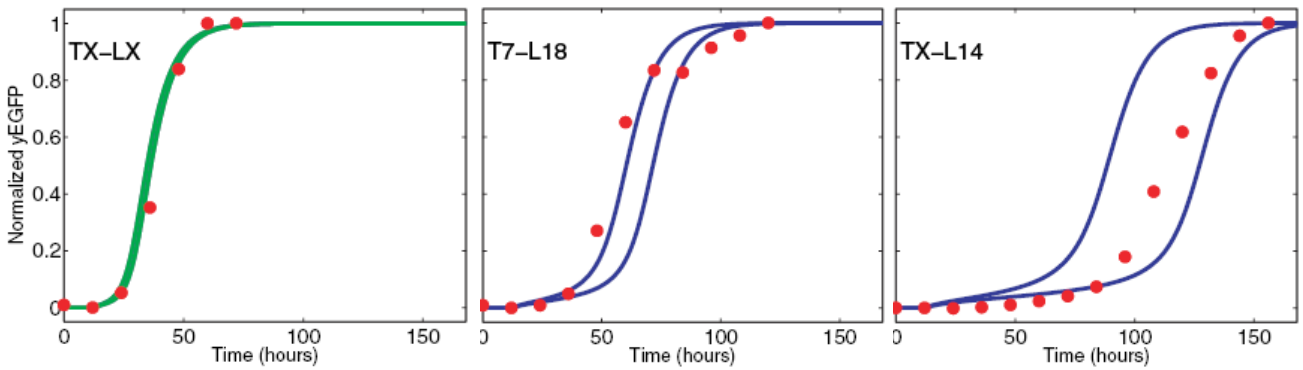
2 libraries = 441 networks

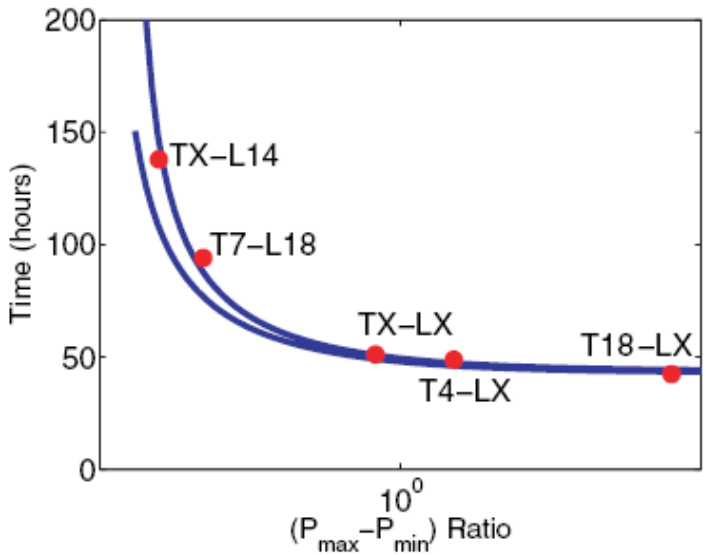
Monostable toggles that act as programmable ‘timers’
unbalanced mutual repression

Modeling type: predictions based on single example





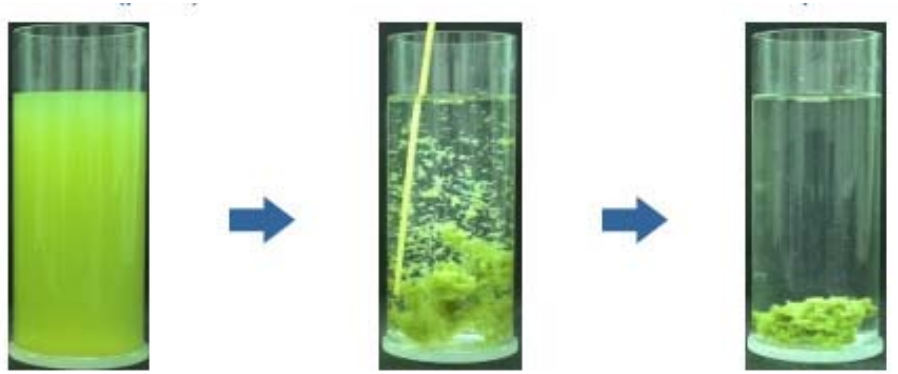




Predicted Relationship from
computational model + one experimental test case

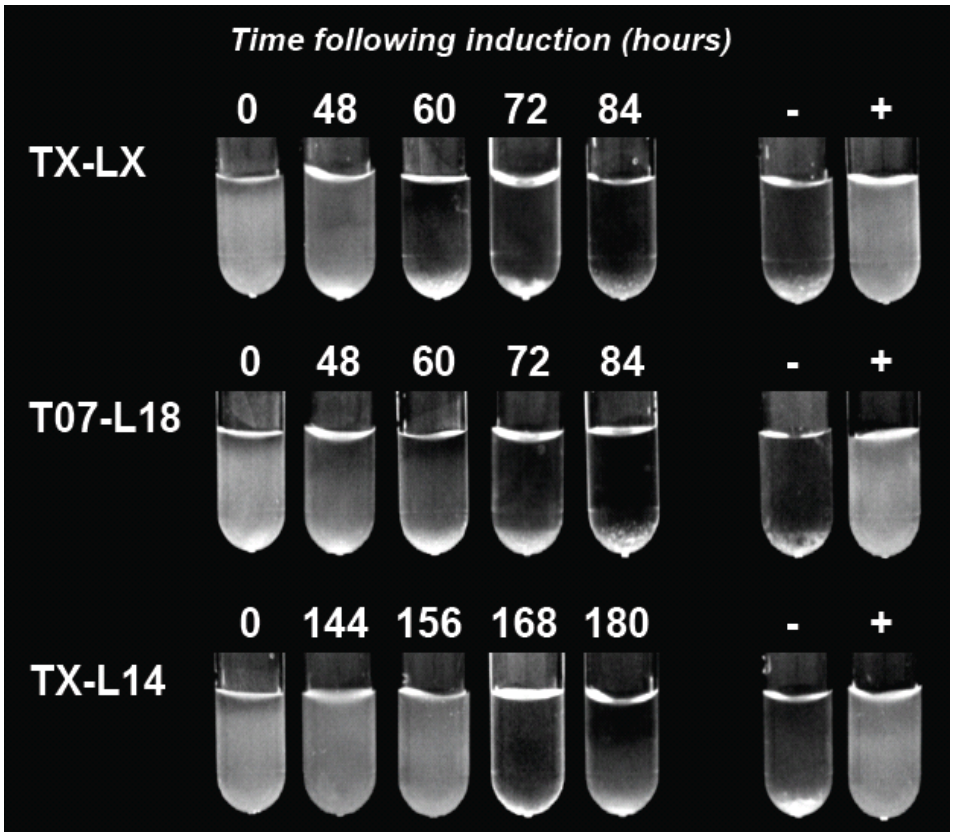
Applying the network

Yeast flocculation - sedimentation

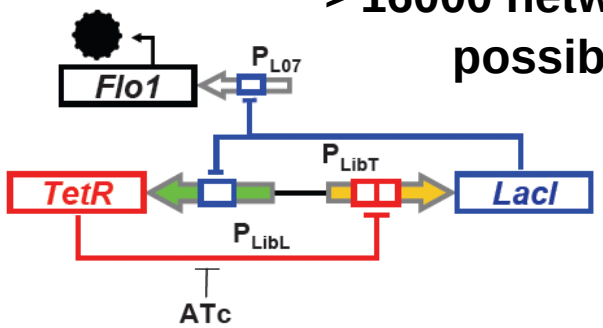


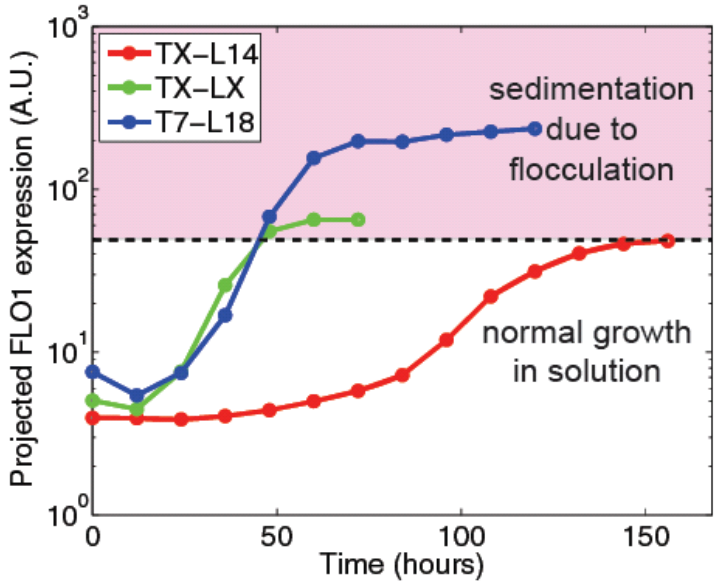
Why would this be of use?
Beer, wine… and now biofuels

Advantages of the system – controlled, predictable



>16000 networks possible





So what?

“Diversity? Isn’t this just degenerate synthesis?”

“Libraries aren’t new”

“Predictive models aren’t new”

“I’m too busy to make multiple parts”

“Can I just use yours?”

Advantages

Fast
Predictive
Desired output levels
Fine-tuning of response

Provides parts for community

What to apply it to?

Regulatory networks
Modular bioparts
RNA – eg. RBS/polyA
Protein binding sites

Investigate motifs/modules

Follow-ups

Promoters with activation
Mammalian cells, *E.coli*
More complex networks

Sequence/output relation
Digital understanding

Future Vision

Implementable in a BioFAB
Scaled-up libraries

All parts made this way?
Diversify from start chassis

Tom Ellis – Techniques, Construction and Implementation

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…

Xiao Wang – Modeling and Predictions

doing even more amazing work with Matlab – e.g. cells that count

Done with help from:

Jim Collins, Boston University
Henry H Lee, Boston University
Peter R Jensen, Biocentrum DTU
Kevin Verstrepen, KU Leuven

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, modifiable by component choice. We use this network to control the timing of yeast sedimentation, illustrating how the plug-and-play nature of our design can be readily applied to biotechnology.

Synthetic biology promises to transform biotechnology by applying engineering principles to biological systems[1]. In less than a decade this field has already yielded technological applications, providing new avenues for drug manufacture[2,3], biofabrication[4] and therapeutics[5,6], while also showing promise in alternative energy[7]. A major focus of the field is the synthesis of gene networks with predictable behavior[8–10], either to endow cells with novel functions[11–15] or to study analogous natural systems[8,16–19]. Despite a booming community and notable successes, the basic task of assembling a predictable gene network from biomolecular parts remains a considerable challenge and often takes many months before a desired network is realized[20]. If Directed evolution has been shown to provide a short-cut through this phase[21] but is complicated by the additional work needed to couple networks to selective pressures.

This time-consuming *post hoc* tweaking phase stems in part from having to work with a limited set of imperfect components. Although this lack of reliable parts is being addressed by community efforts[26], it remains an acute problem because most of the available components are inadequately characterized. For example, many promoters are simply characterized as being 'weak' or 'strong'. What is needed to resolve this problem and fast-track synthetic biology is an approach that creates libraries of components ahead of any assembly. Then, by

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Wednesday, April 22, 2009

Brewing with Synthetic Biology

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

By Courtney Humphries

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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 has some way to go before this is the case. As much as biologists know about the structure and function of biological molecules, their behavior when interacting with one another is still unpredictable.

A new approach detailed in this week's issue of the journal *Nature Biotechnology* offers a more systematic approach

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Brewing with Synthetic Biology

read 61 times 1 replies | posted 4/23/2009 12:15:18 AM

Reply

Indra
1928147

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

"Researchers at Boston University have developed a way to predict the behavior of 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.

Reply

Private message

JoeMcPhee
4331239

I think this stuff could be interesting, but controlling flocculation is hardly critical when most breweries filter anyway. I remember reading about this synthetic biology briefly that they were trying to assemble, but I think that they'll need to demonstrate something much more novel than this before people really jump onto the bandwagon.

4/23/2009 8:30:30 AM

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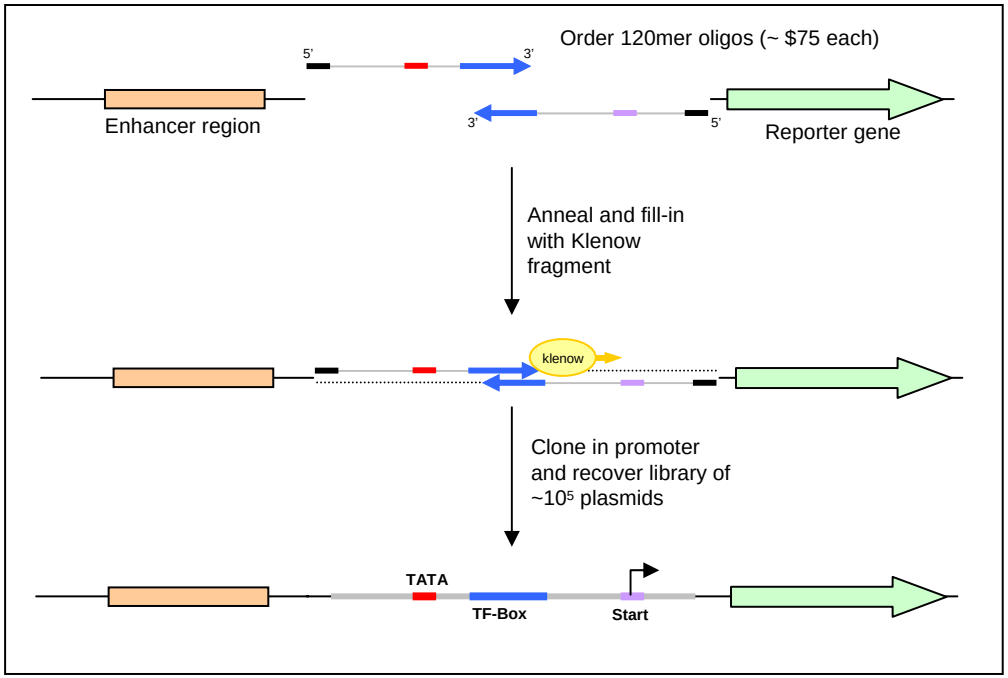
Promoter Construction

Cloning to get set-up and get appropriate controls

Make everything modular!

Work large scale (pooling colonies from plates), use plate-reader and then flow cytometer to pick 20 clones

Take repeatable measurements of each library member



5'-PstI---$(N)_{35}$---TATA---$(N)_{11}$---*tetO$_2$*-$(N)_2$-*tetO$_2$*- 3' ⋯▸ ***Klenow pol extension***

◂⋯ **3'-*tetO$_2$* ---$(N)_{23}$---Start---$(N)_{44}$---BamHI-5'**