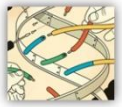
From parts-based synthetic biology to genome engineering

Imperial College London



Ellis Lab

CSynBI
Centre for Synthetic Biology and Innovation

Dr Tom Ellis

Centre for Synthetic Biology and Innovation
Department of Bioengineering
Imperial College London

Imperial College London

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Centre for Synthetic Biology and Innovation

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synthetic | biology

Synthetic Biology is the engineering of biology. It is an exciting new area of research combining science and engineering to design and build new biological functions and systems, and to understand existing biological life through its rational re-design

CSynBI
Centre for Synthetic Biology and Innovation

EPSRC

CSynBI is a partnership between Imperial College London and the BIOS Centre at

KING'S College LONDON BIOS

You are here - Welcome to LSE > BIOS

BIOS is an international centre for research and policy on social aspects of the life sciences and biomedicine.

Centre for Synthetic Biology and Innovation (CSynBI: April 2010 -)

- Co-Directors
  - Prof. Richard Kitney (Biologic, biosensors, CAD tools)
  - Prof. Paul Freemont (Biosensors, part characterisation)
- Academics in the Centre
  - Prof Nik Rose (Responsible Innovation and society)
  - Dr Geoff Baldwin (Directed evolution, DNA assembly)
  - Dr Travis Bayer (Metabolic engineering and biocatalysis)
  - Dr Guy-Bart Stan (Biomodelling analysis and control)
  - Dr Tom Ellis (DNA assembly, gene networks and genomes)
  - Dr Claire Marris (Responsible Innovation and society)
  - Dr Karen Polizzi (Biosensors for bioprocessing)



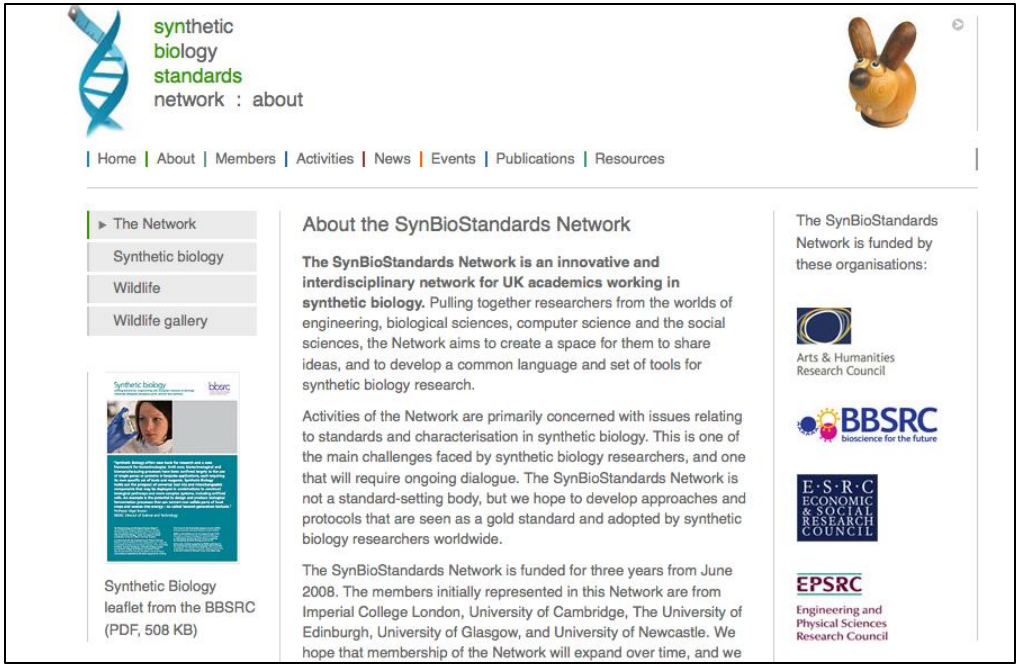






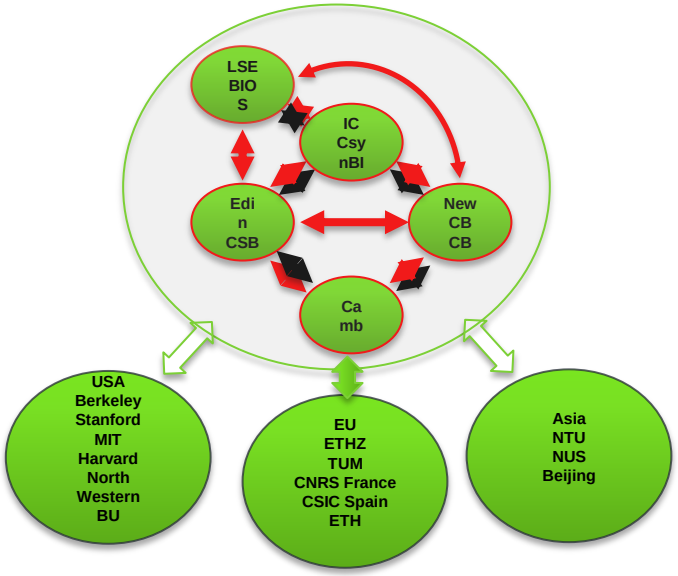
40+ researchers from interdiscplinary backgrounds

CSynBI collaborations and network



BBSRC SynBioStandards network

Member of the EU FP7 Standards

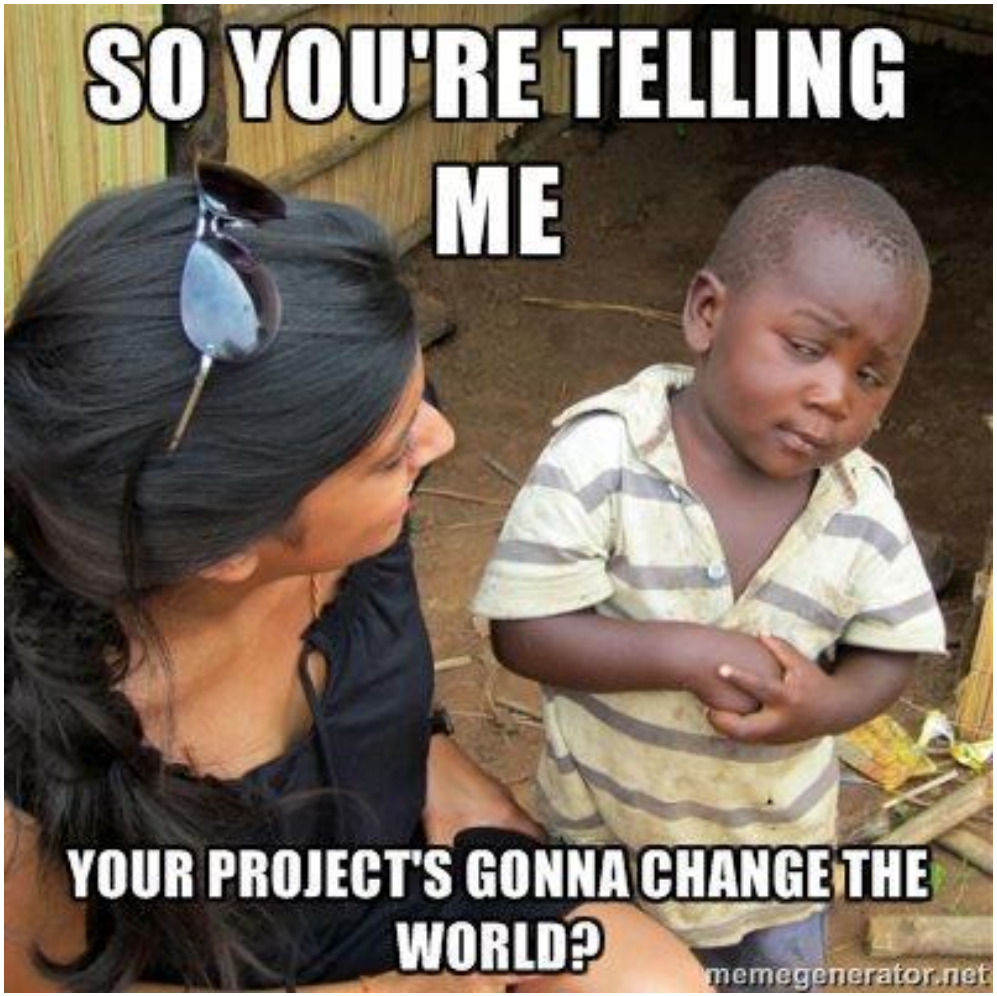


Flowers Consortium for Synthetic Biology

University of Edinburgh
University of Newcastle
University of Cambridge
Kings College London

Ellis Lab: **iGEM and** Synthetic Biology

iGEM shows us what Synthetic Biology could do



http://www.facebook.com/IgemMemes

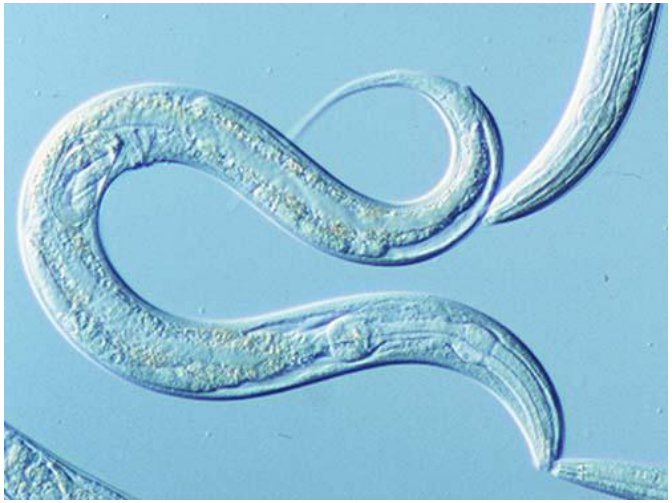
Ellis Lab: nuts and bolts for Synthetic Biology

- **Application-Scale synthetic biology (Apps)**
  - Promoter libraries for gene expression regulation
  - Rational synthesis of new biological parts
  - Accurate characterisation of regulatory parts

- **Genome Engineering Projects (OS)**
  - Synthetic Yeast (Sc2.0)
  - Designing and evolving genomes for applications

YEAST, E.COLI, BACILLUS AND THERMOPHILES

Gene regulation encodes complexity

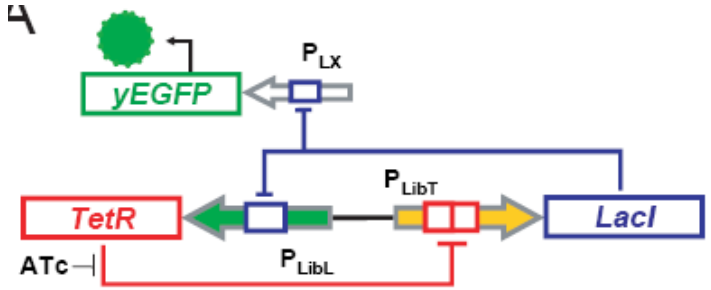


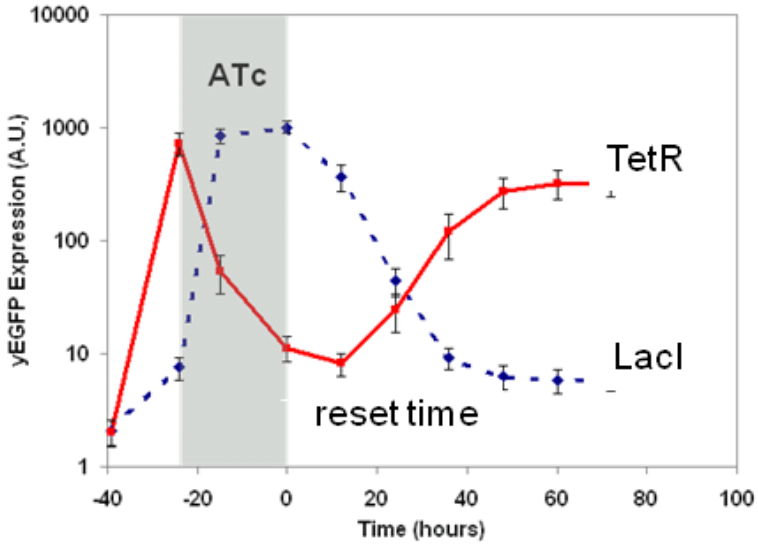
Nematodes – 19,000 genes

Humans – 20,000 genes

ENCODE Project 2012 – Human Genome contains “4 Million Switches”

Custom design of gene networks from parts

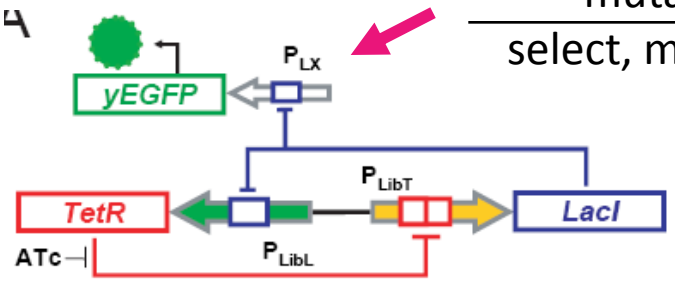




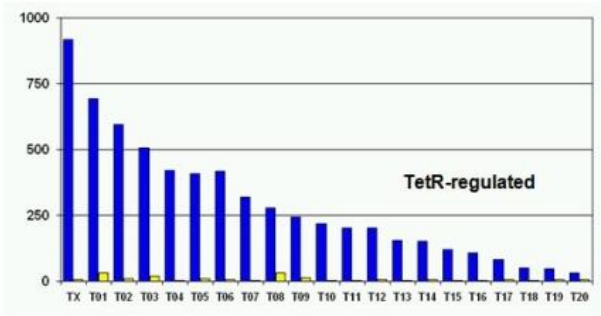
Timer network
Two competing genes + reporter gene
Constructed in yeast cells
Prototype characterised and modelled

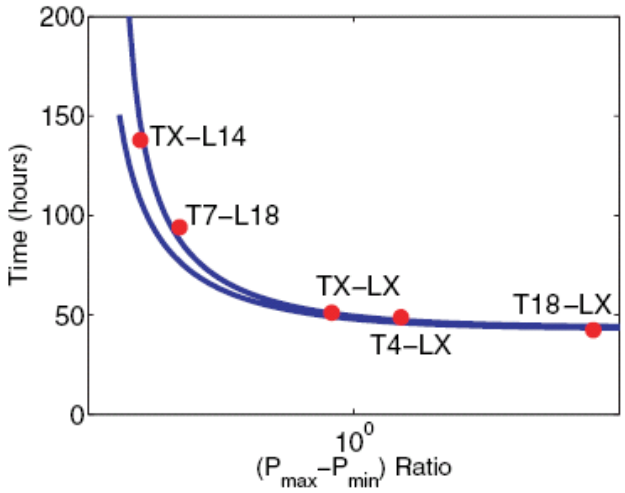
Nature Biotech - April 2009
Work with Jim Collins (BU) and Xiao Wang (now ASU faculty)

Gene networks - shaped by regulatory parts



mutate,
select, measure



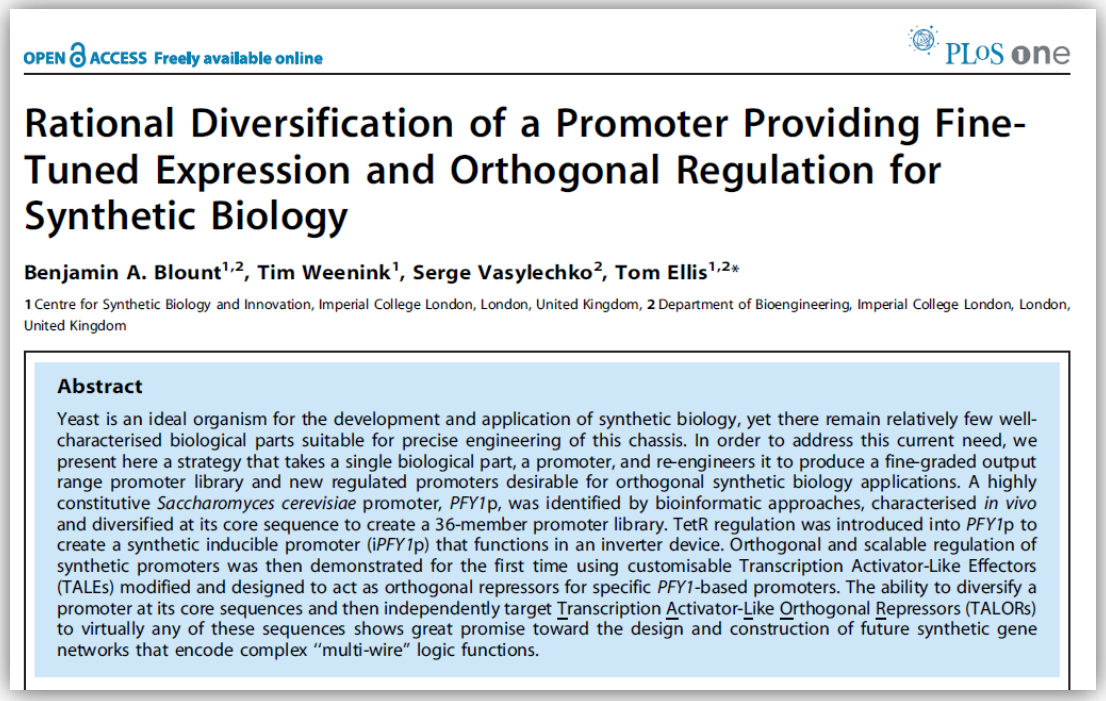


input data
predictive model

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

3 promoter ‘nodes’, 20 promoters per library = 8000 possible networks
Predictable custom gene networks with diverse reset times

Rational synthesis of new regulatory parts



OPEN ACCESS Freely available online

PLoS one

Rational Diversification of a Promoter Providing Fine-Tuned Expression and Orthogonal Regulation for Synthetic Biology

Benjamin A. Blount[1,2], Tim Weenink[1], Serge Vasylechko[2], Tom Ellis[1,2]*

1 Centre for Synthetic Biology and Innovation, Imperial College London, London, United Kingdom, **2** Department of Bioengineering, Imperial College London, London, United Kingdom

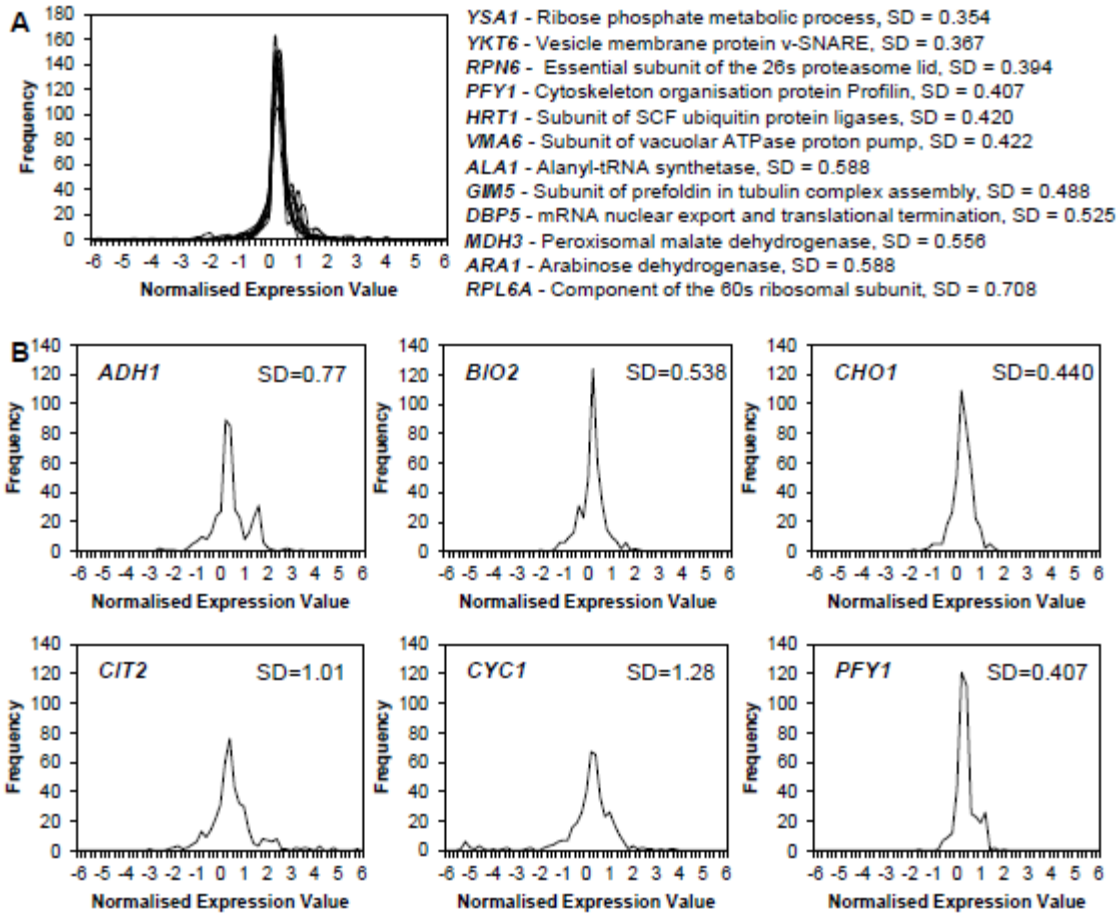
Abstract

Yeast is an ideal organism for the development and application of synthetic biology, yet there remain relatively few well-characterised biological parts suitable for precise engineering of this chassis. In order to address this current need, we present here a strategy that takes a single biological part, a promoter, and re-engineers it to produce a fine-graded output range promoter library and new regulated promoters desirable for orthogonal synthetic biology applications. A highly constitutive *Saccharomyces cerevisiae* promoter, *PFY1*p, was identified by bioinformatic approaches, characterised *in vivo* and diversified at its core sequence to create a 36-member promoter library. TetR regulation was introduced into *PFY1*p to create a synthetic inducible promoter (i*PFY1*p) that functions in an inverter device. Orthogonal and scalable regulation of synthetic promoters was then demonstrated for the first time using customisable Transcription Activator-Like Effectors (TALEs) modified and designed to act as orthogonal repressors for specific *PFY1*-based promoters. The ability to diversify a promoter at its core sequences and then independently target Transcription Activator-Like Orthogonal Repressors (TALORs) to virtually any of these sequences shows great promise toward the design and construction of future synthetic gene networks that encode complex "multi-wire" logic functions.

Forward engineering of diverse promoter libraries from a short, simple 'backbone promoter' (PFY1p)

2009 GAL1-based libraries: still dependent on Gal / Glu regulation
relatively long parts with homology

Rational synthesis of new regulatory parts



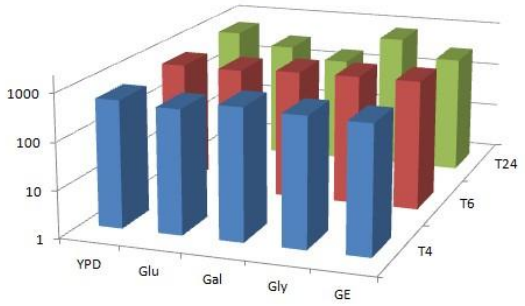
Scanned available bioinformatics data to identify very ‘stable’ yeast promoters

Candidates for natural constitutive promoters

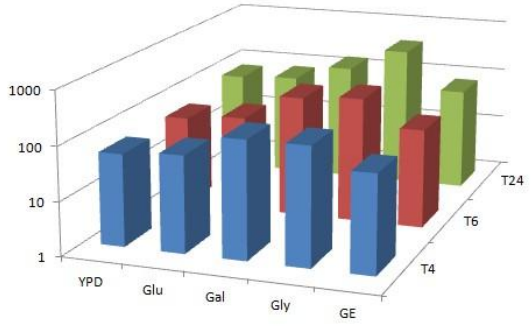
B Blount et al (2012) – PloS One

Rational synthesis of new regulatory parts

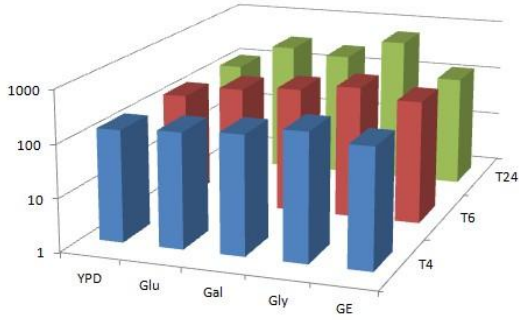
Expression of GFP from 6 selected promoters assessed by 96-well flow cytometry
Tested in different growth conditions (carbon source), single-copy integrations



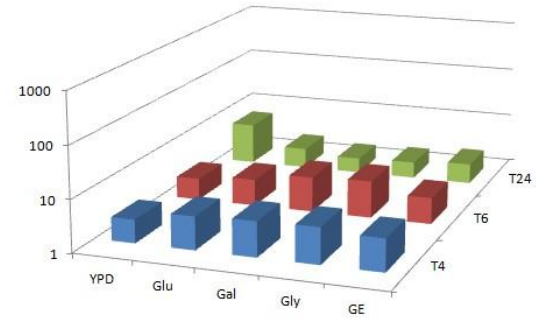
ADH1p **mCV = 0.293**
712 bp



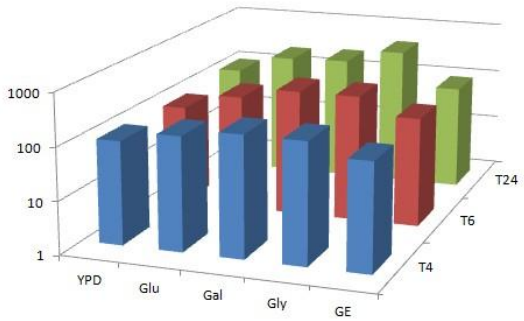
BIO2p mCV = 0.795
383 bp



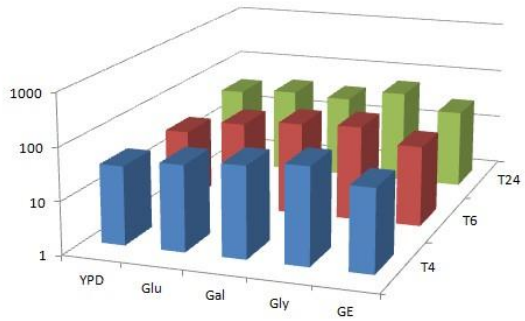
CHO1p mCV = 0.691
260 bp



CIT2p mCV = 0.507



CYC1p mCV = 0.671
416 bp

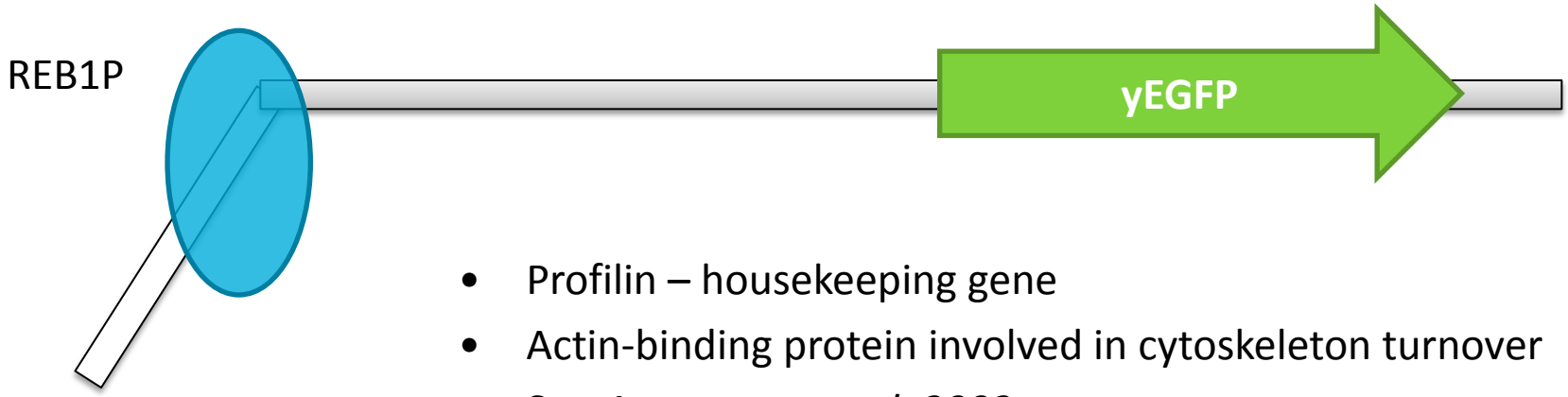


PFY1p **mCV = 0.326**
203 bp

B Blount et al (2012) – PloS One

Rational synthesis of new regulatory parts

Selected promoter - PFY1p - 150 to 200 bp long
No TATA box – instead just a long poly(dT) stretch (bend in DNA)
Only TF site is for REB1P, next to bend in DNA
Bend in DNA may mimic TFIID role giving constitutive expression

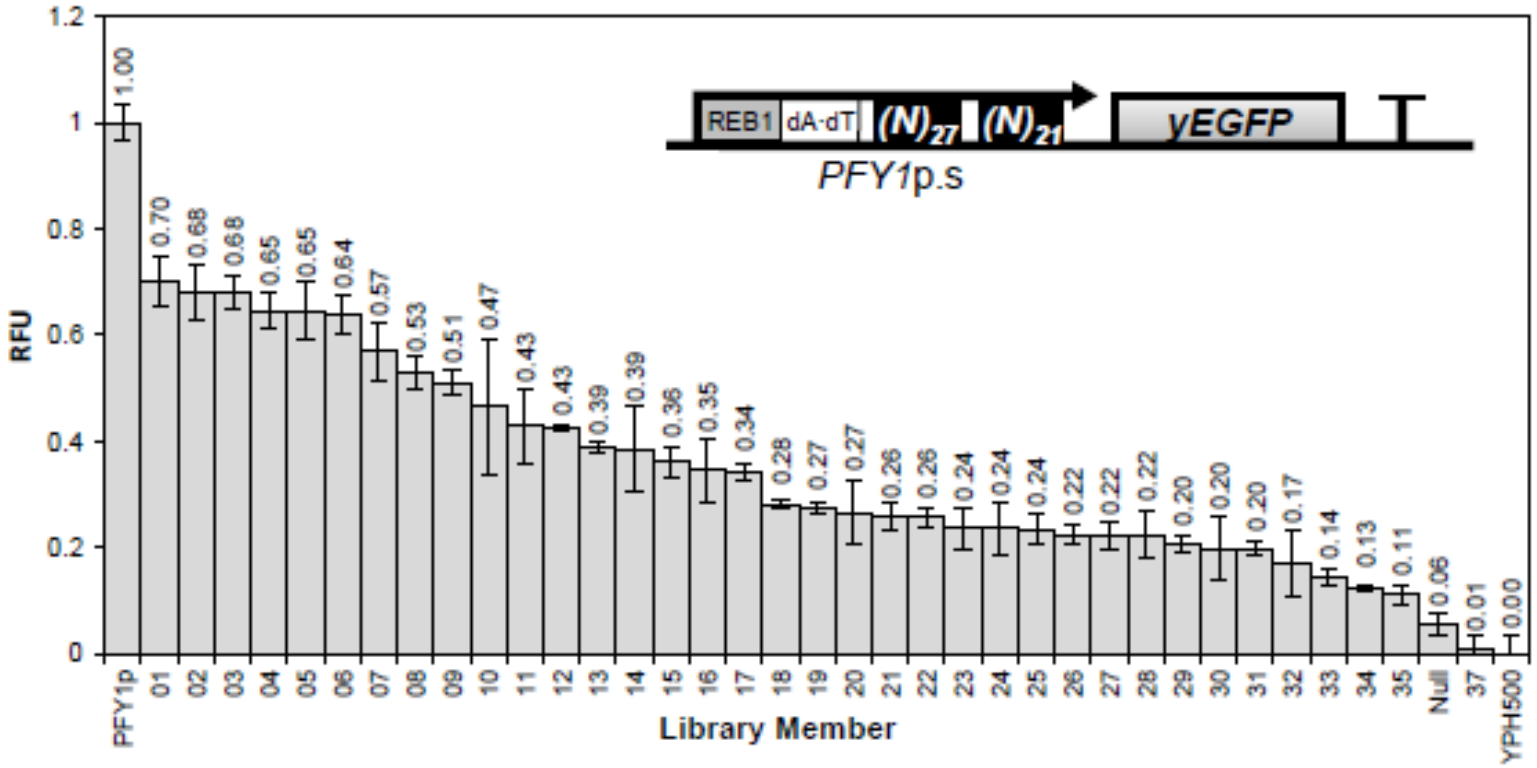


- Profilin – housekeeping gene
- Actin-binding protein involved in cytoskeleton turnover
- See *Angermayr et al. 2003*

B Blount et al (2012) – PloS One

Rational synthesis of new regulatory parts

PFY1p is a medium-strength promoter

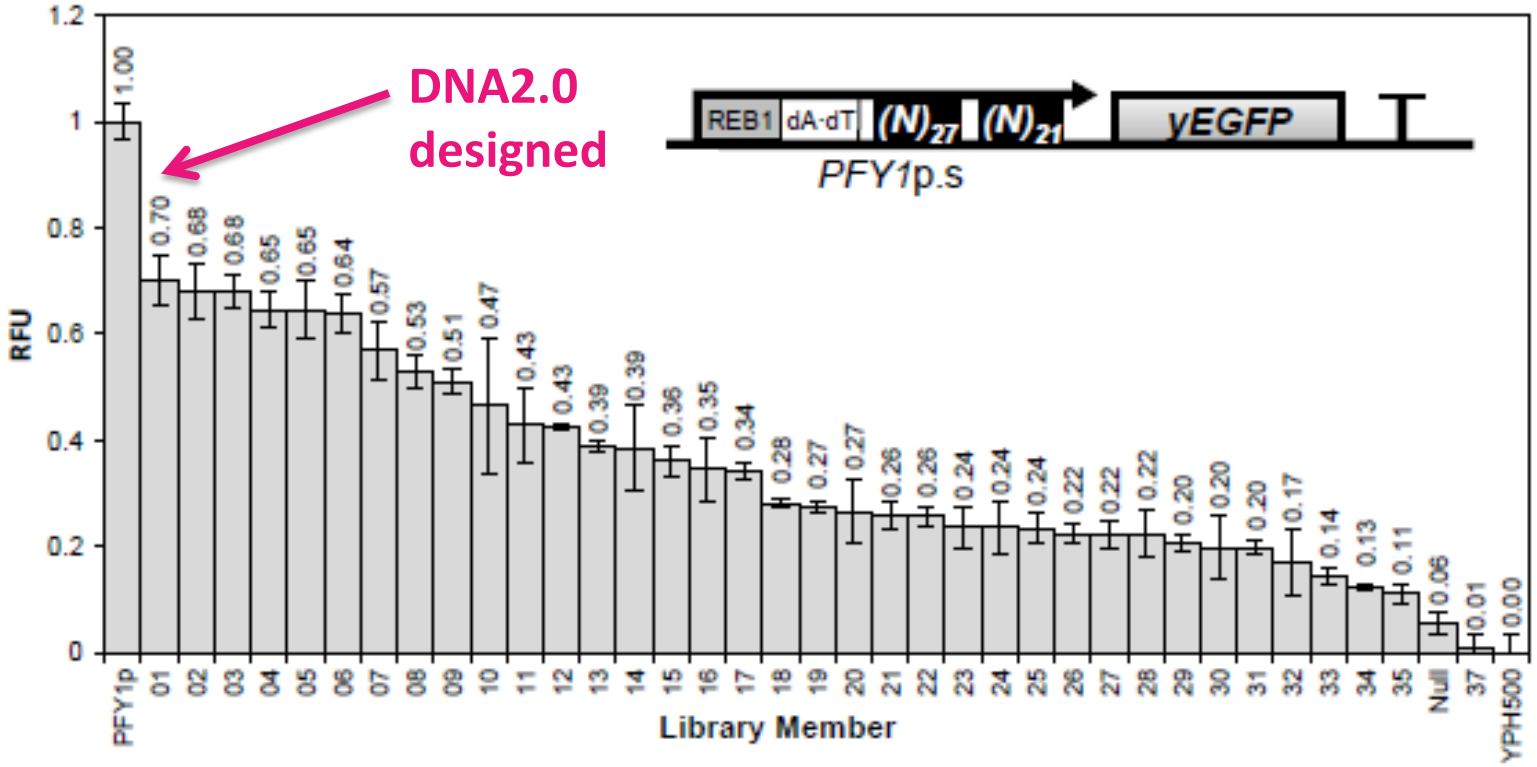


B Blount et al (2012) – PloS One

4^48 combinatorial space

Rational synthesis of new biological parts

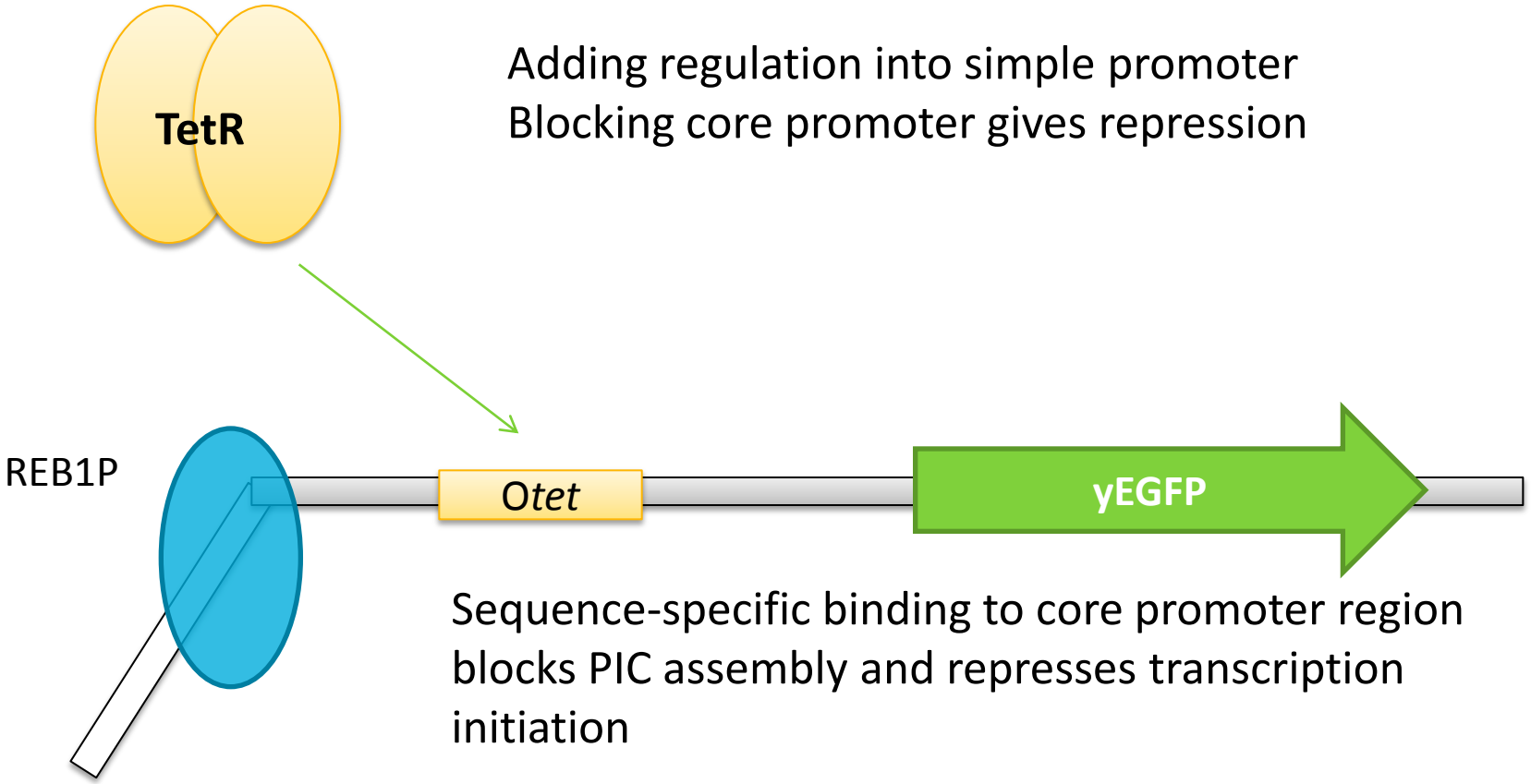
Rational design based output of 38 sample sequences: (4^48 space)
QSAM tools - J Jonsson *et al.* NAR 1993



Unpublished work - Anna Kress, Ben Blount and Tom Ellis with DNA2.0

DNA 2.0

Rational synthesis of new regulatory parts



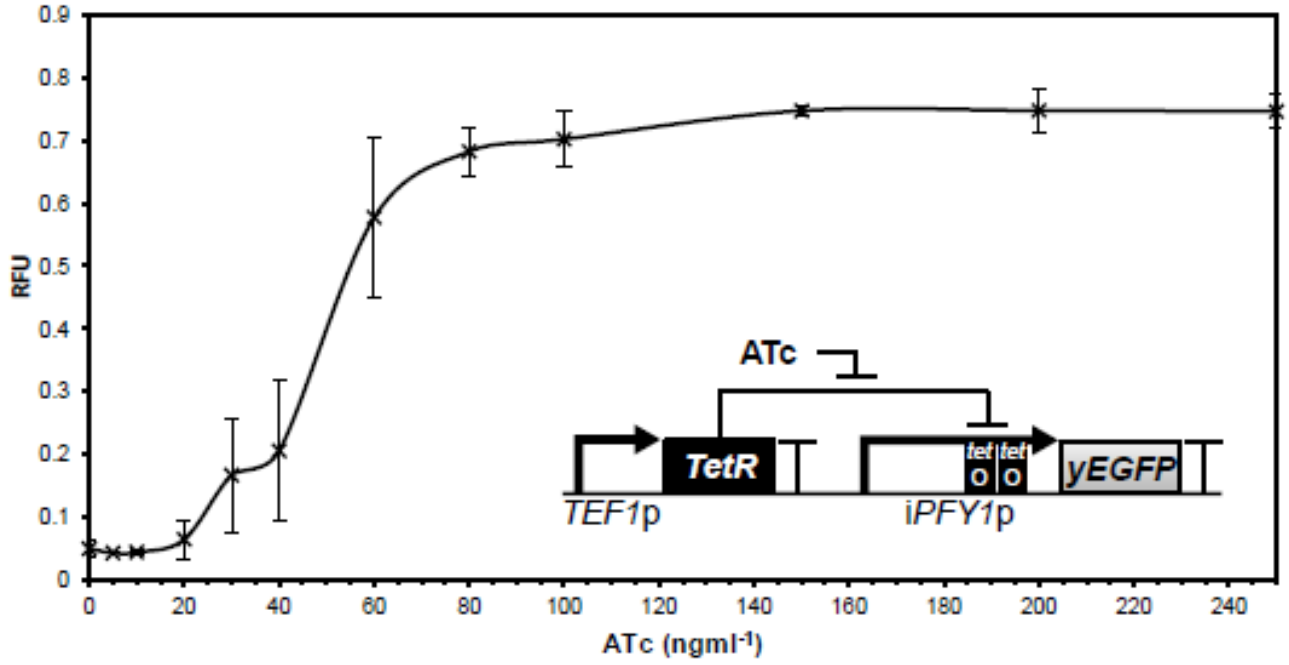
Adding regulation into simple promoter
Blocking core promoter gives repression

Sequence-specific binding to core promoter region blocks PIC assembly and represses transcription initiation

Can be done by adding operators (e.g. O*tet*)

B Blount et al (2012) – PloS One

Rational synthesis of new regulatory parts

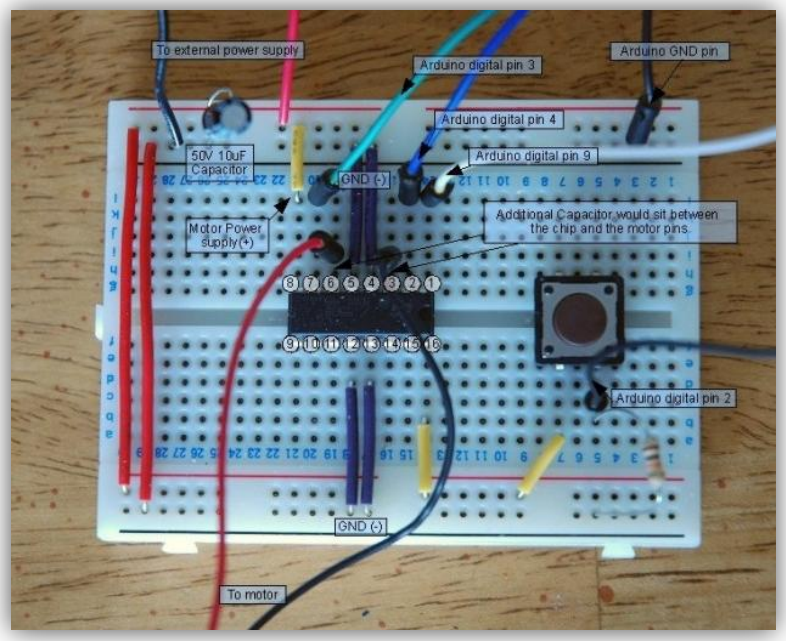


Synthetic inverter (NOT) gene network - tunable using ATc/Dox

B Blount et al (2012) – PloS One

Independent wires essential for synthetic regulation

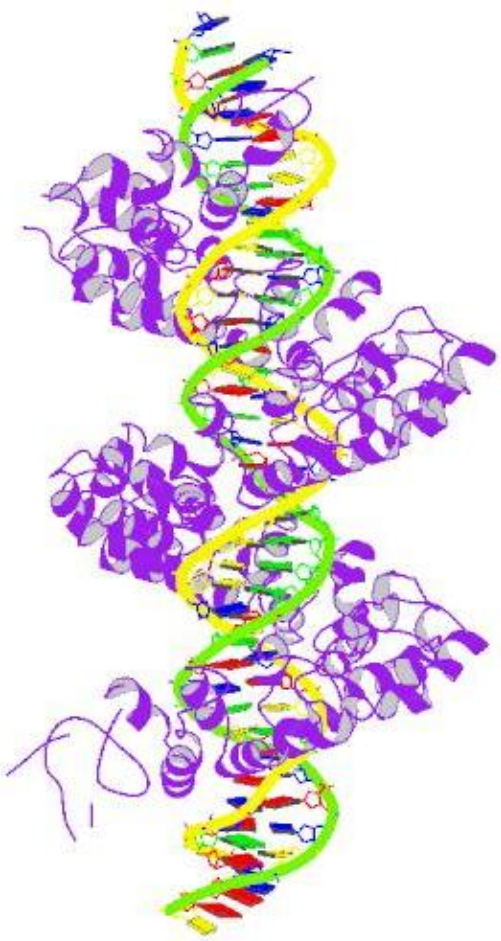
Genetic regulation uses transcription factors as wires, promoters as nodes
To avoid cross-talk (short circuiting) non-native ‘wires’ are required
e.g. LacI, TetR
Few of these are orthogonal TFs are available, limiting design complexity



Synthetic Biology

The Cell

Custom Repression with designer TALORS

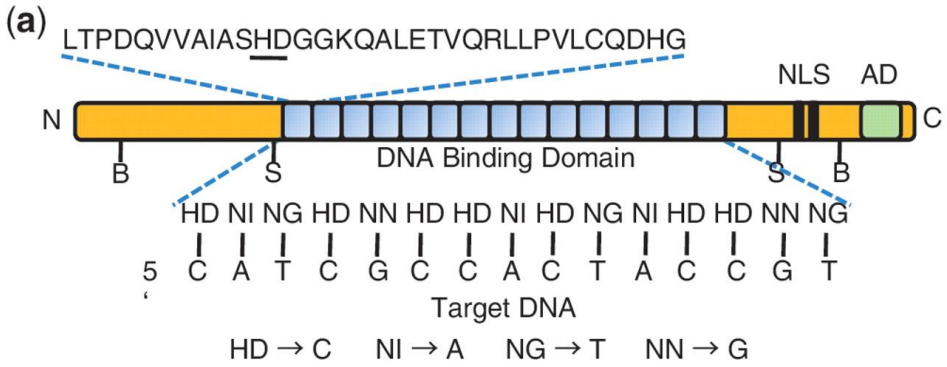


TAL-effectors are reconfigurable DNA-binders

TALEN – customisable nucleases

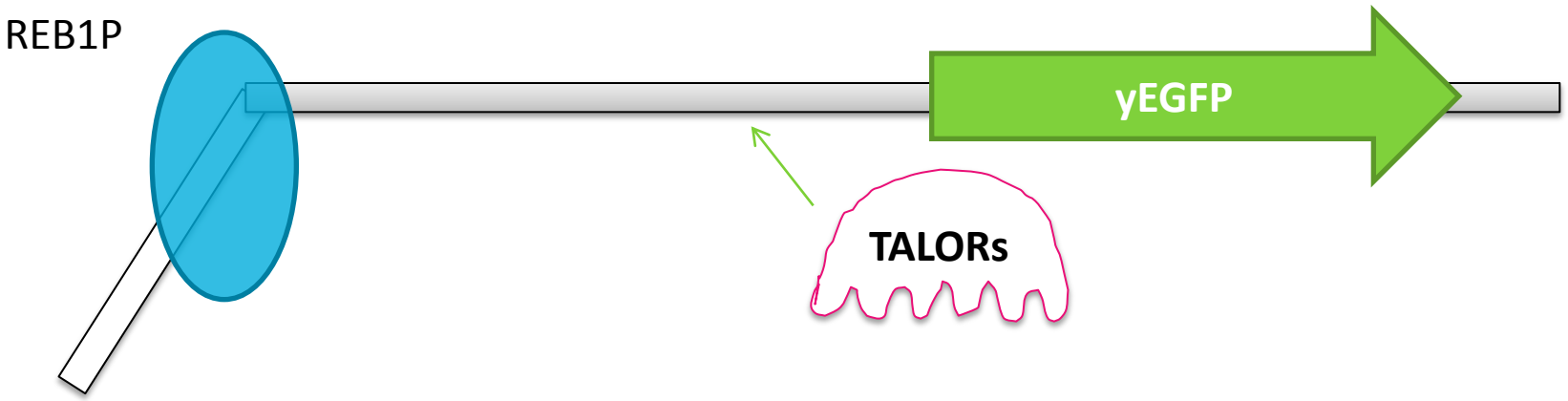
TALEs – customisable activators (plant, mammal)

TALOR = TAL Orthogonal Repressor



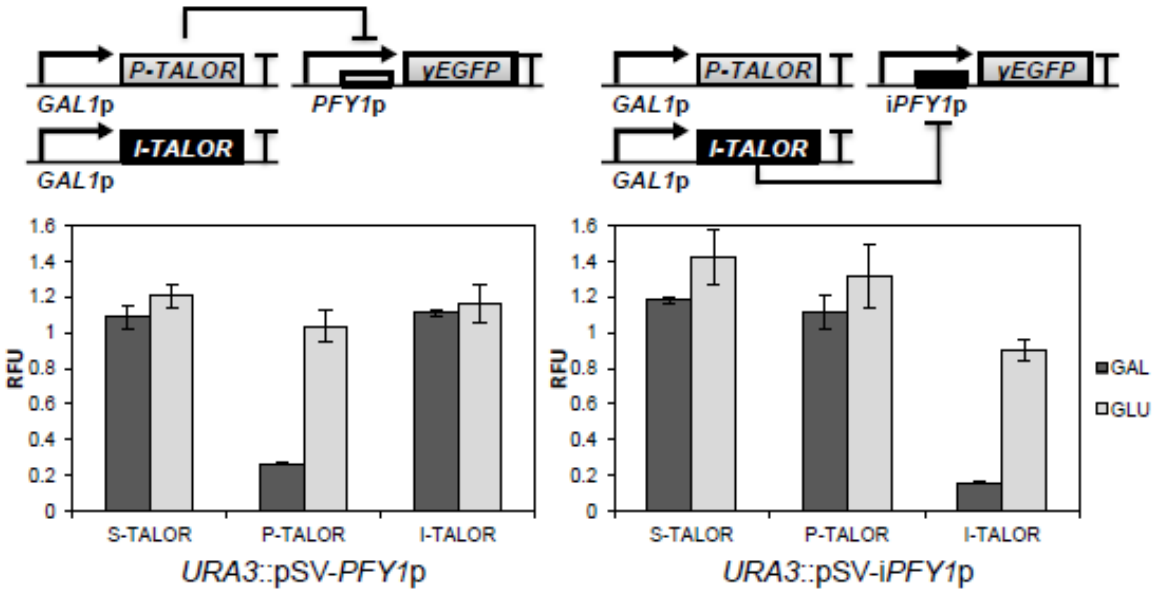
Cermak et al. 2011 NAR Methods Online

Rational synthesis of new regulatory parts



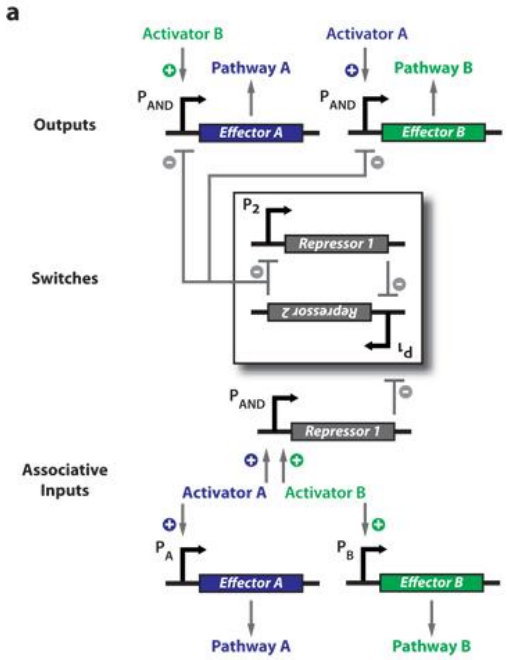
Scalable, orthogonal regulation

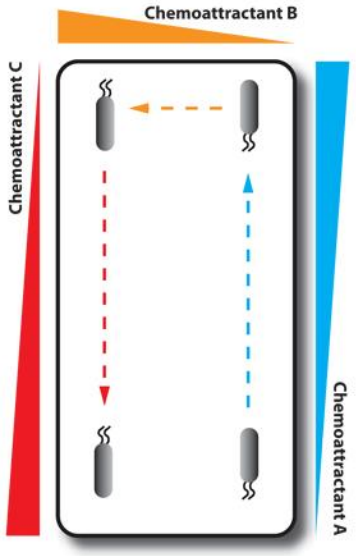
Almost unlimited new independent wires for gene regulatory networks

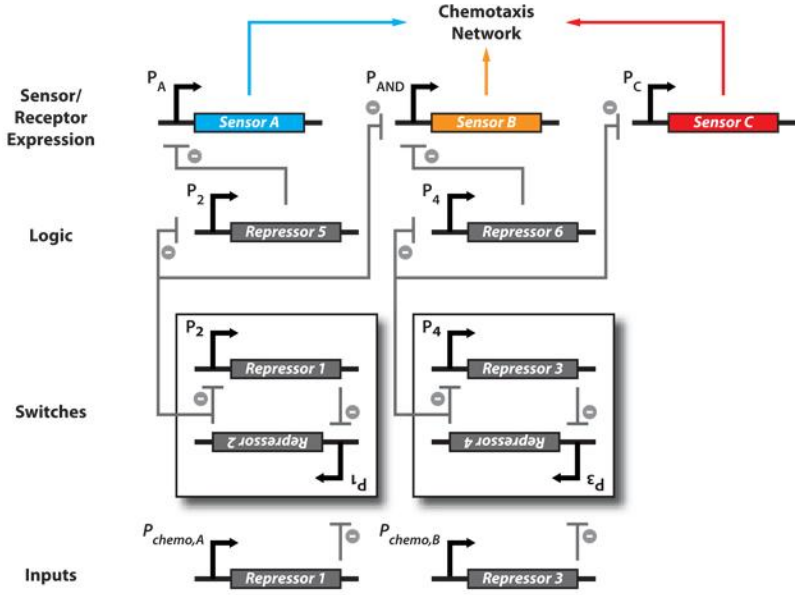


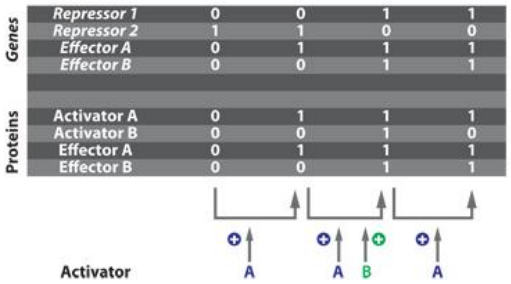
B Blount et al (2012) – PloS One

Part libraries for advanced biological Apps









Adaptive Learning Networks: *e.g.* associated memory

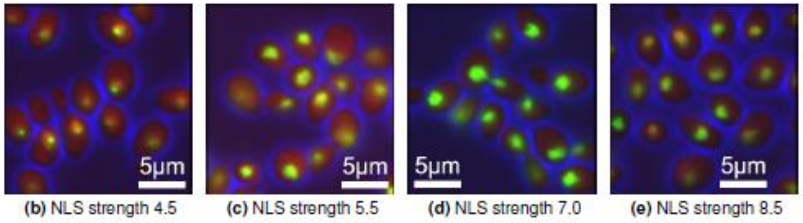
Intelligent Biosensors: *e.g.* navigating bacteria

Next-generation synthetic gene networks
Timothy K Lu, Ahmad S Khalil & James J Collins
Nature Biotechnology 27, 1139 - 1150 (2009)

Engineering and characterisation of essential regulatory parts for Syn Bio

***S. cerevisiae* Yeast**

- Constitutive Promoters
- Programmable Transcription Factors
- Protein Degradation Tags
- Nuclear Localisation Sequences



(b) NLS strength 4.5 (c) NLS strength 5.5 (d) NLS strength 7.0 (e) NLS strength 8.5

Thermophile Bacteria

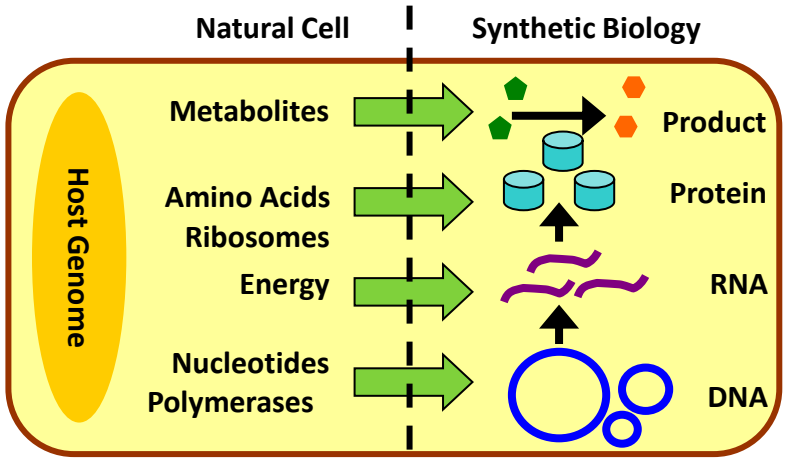
- Constitutive Promoters
- Sugar-responsive Transcription Factors
- New plasmid systems

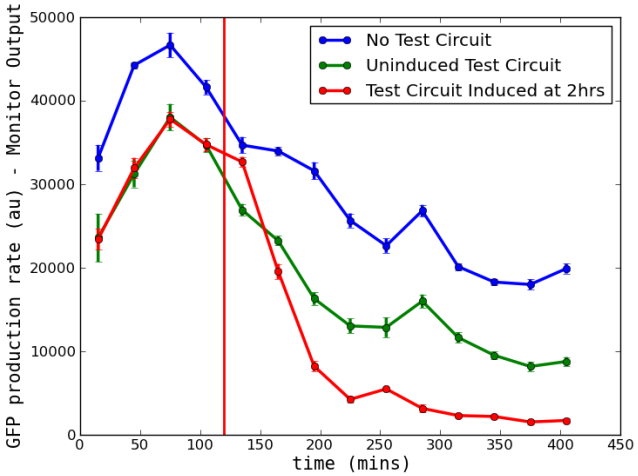
***E. coli* Bacteria**

- Measuring and predicting cellular cost
- Live measurement of transcription
- Custom designed plasmid vectors

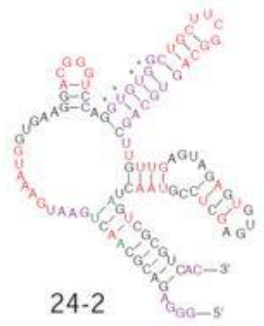
New characterisation technologies

1. Live measurement of expression cost

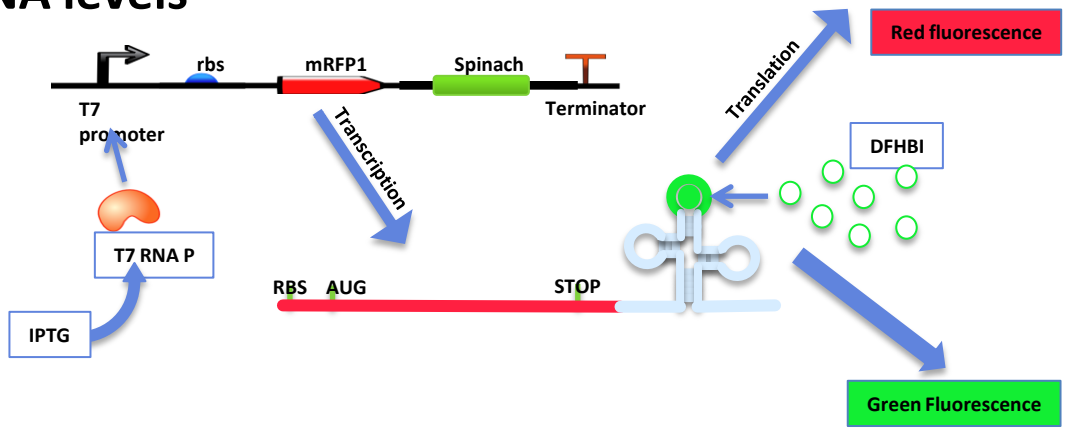




2. Live measurement of mRNA levels

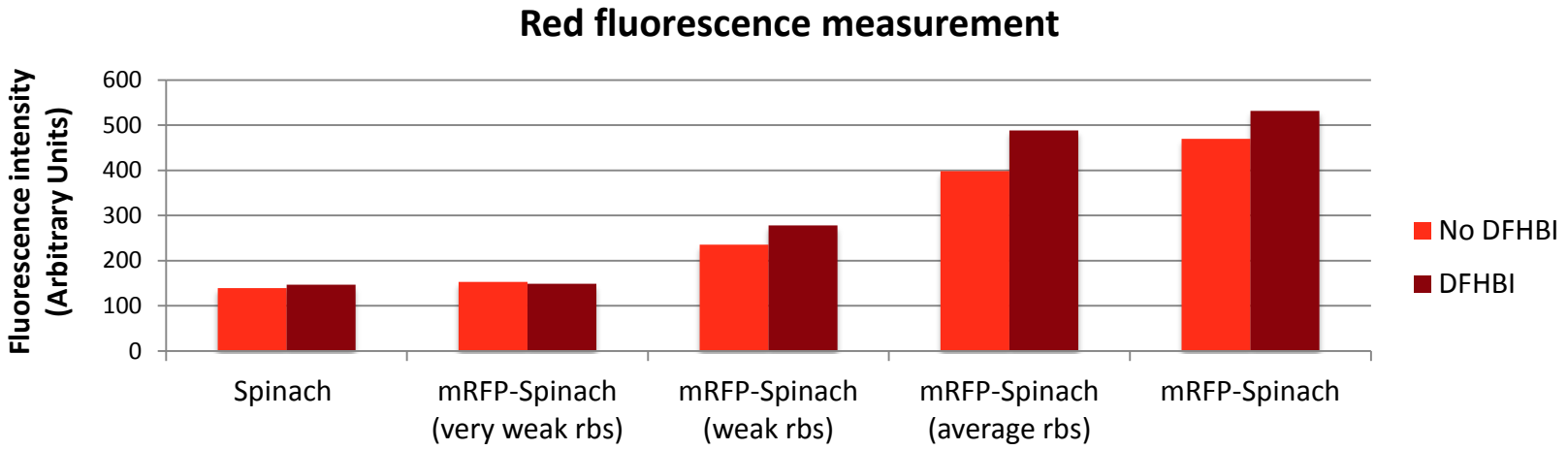


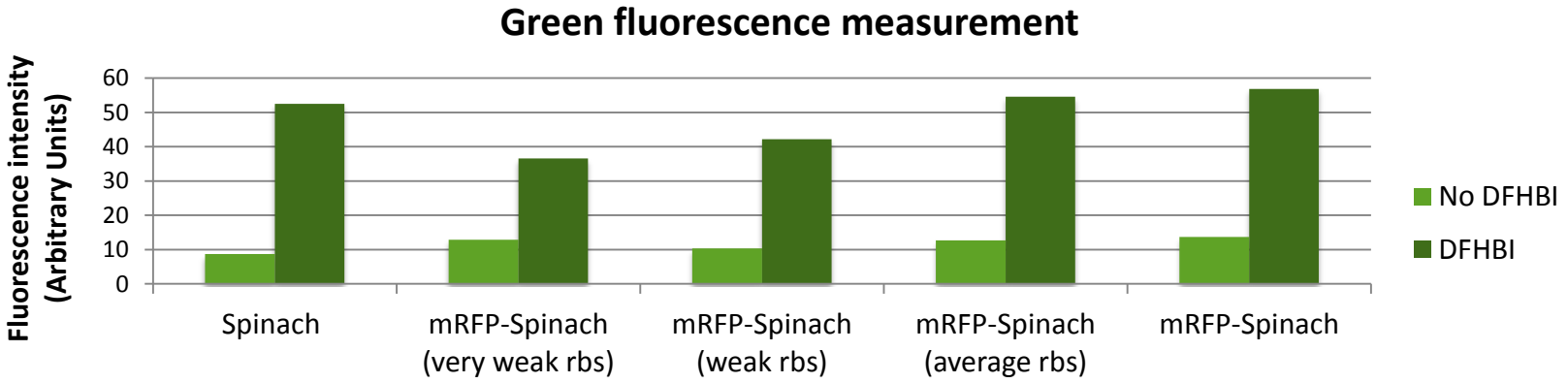
Jaffrey Lab
Spinach RNA



Rhys Algar, Georgious Pothoulakis, Guy-Bart Stan and Tom Ellis - unpublished

New characterisation technologies





Beyond ‘Apps’ to ‘OS’ Synthetic Biology: Genome Engineering

How do we link genome synthesis work with parts-scale synthetic biology?

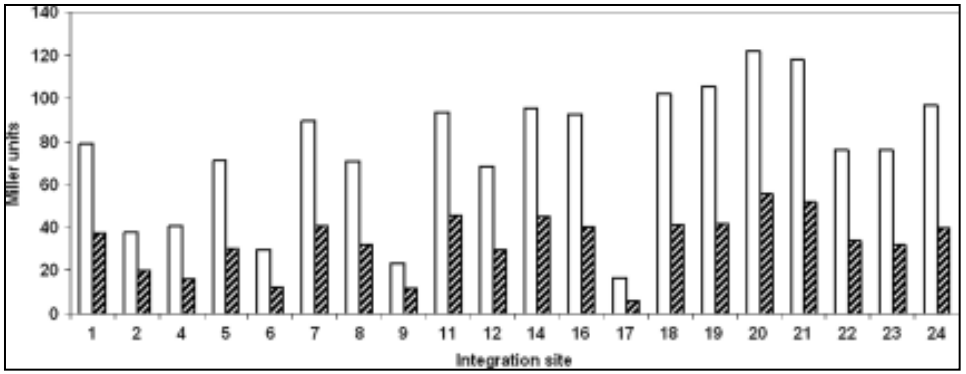
Synthetic Biology’s grand challenge

A designed parts-based genome

How can we understand genome writing rules?

YEAST

Gene expression has been shown to be significantly affected by genomic location



Flagfeldt et al. Yeast 2009

E. COLI

Promoter behaviour is dependent on the location of the promoter within the genome

	mArray	RNAseq	g-YFP	Plasmid
mArray		0.431	0.220	0.149
RNAseq	0.431		0.292	0.203
g-YFP	0.220	0.292		0.042
Plasmid	0.149	0.203	0.042	

Zhu and Ellis. Unpublished

Sc2.0 - Yeast Genome Engineering

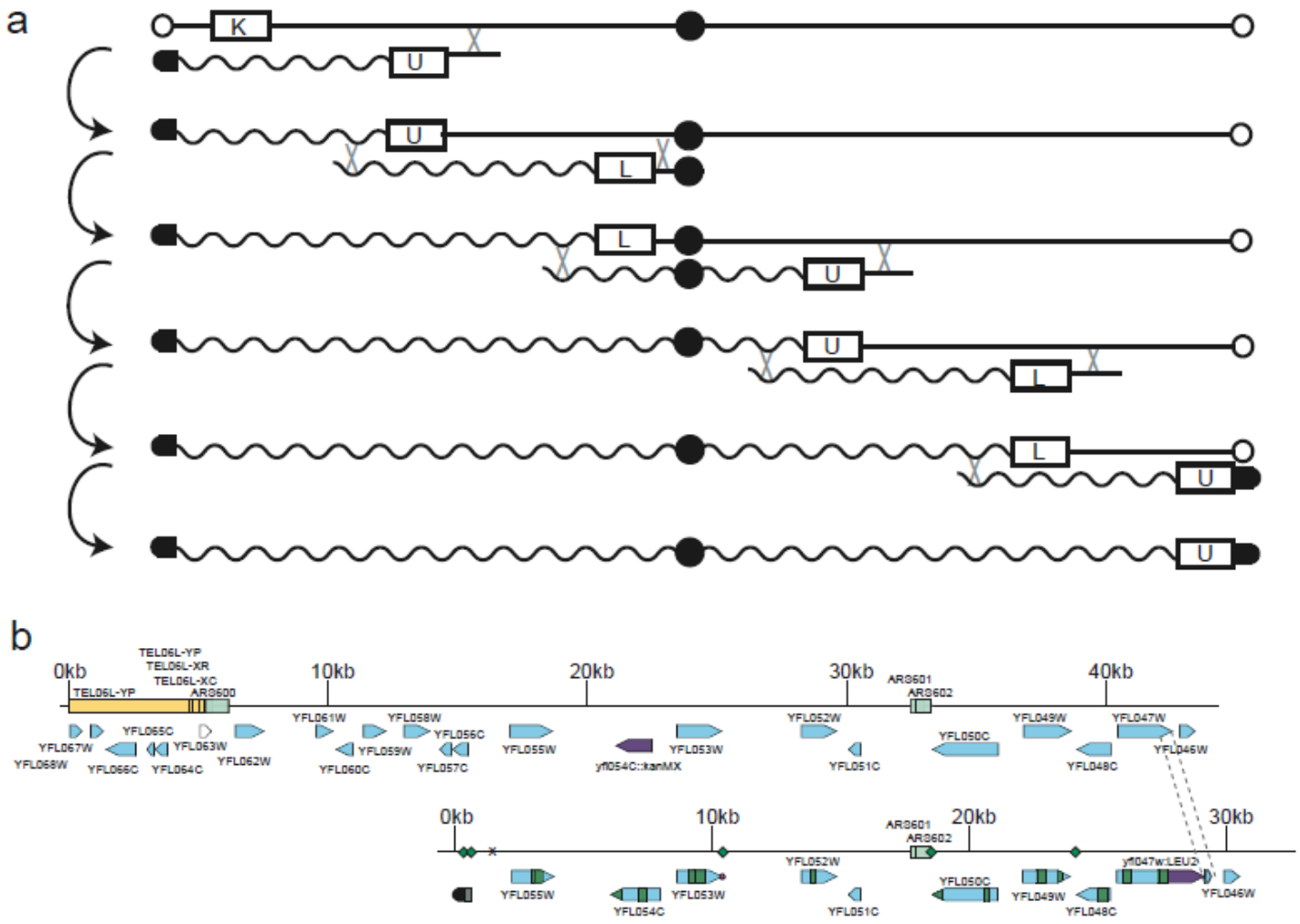
Prof. Jef Boeke, Johns Hopkins University, USA

Project = Synthetic Yeast 2.0 http://biostudio.bme.jhu.edu/sc2/

A major international project now in 4 countries: USA, China, UK and India

Jessica S. Dymond *et al.* **Synthetic chromosome arms function in yeast and generate phenotypic diversity by design**. *Nature*, 2011; DOI: 10.1038/nature10403

Replacing native sequence with synthetic in yeast



Reiterative Recombination Method

Requires two selectable markers

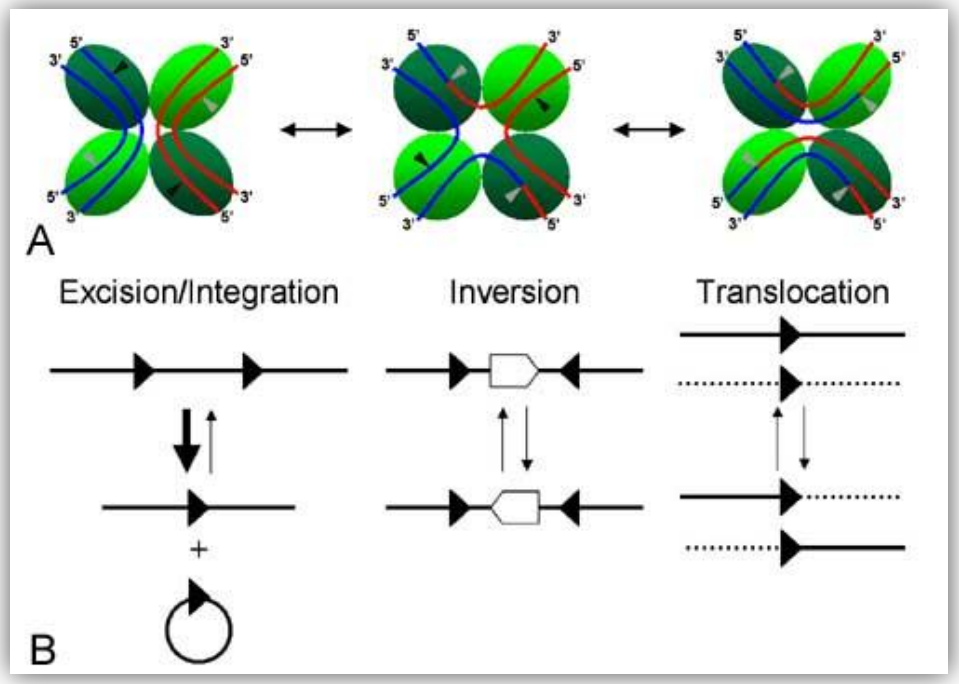
Makes use of yeast's ability to recombine matching sequences

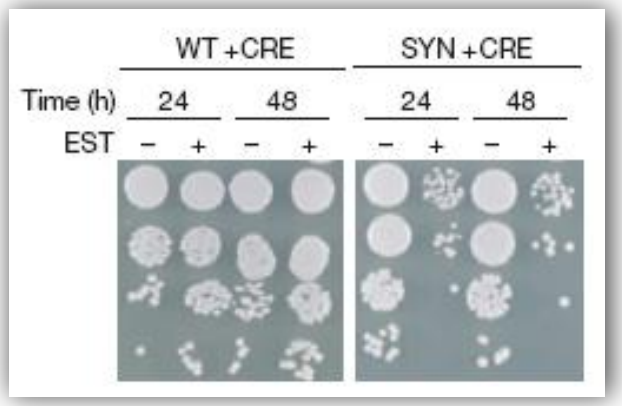
SERIAL process

Sc2.0 - Yeast Genome Engineering

Automated refactoring of genomes

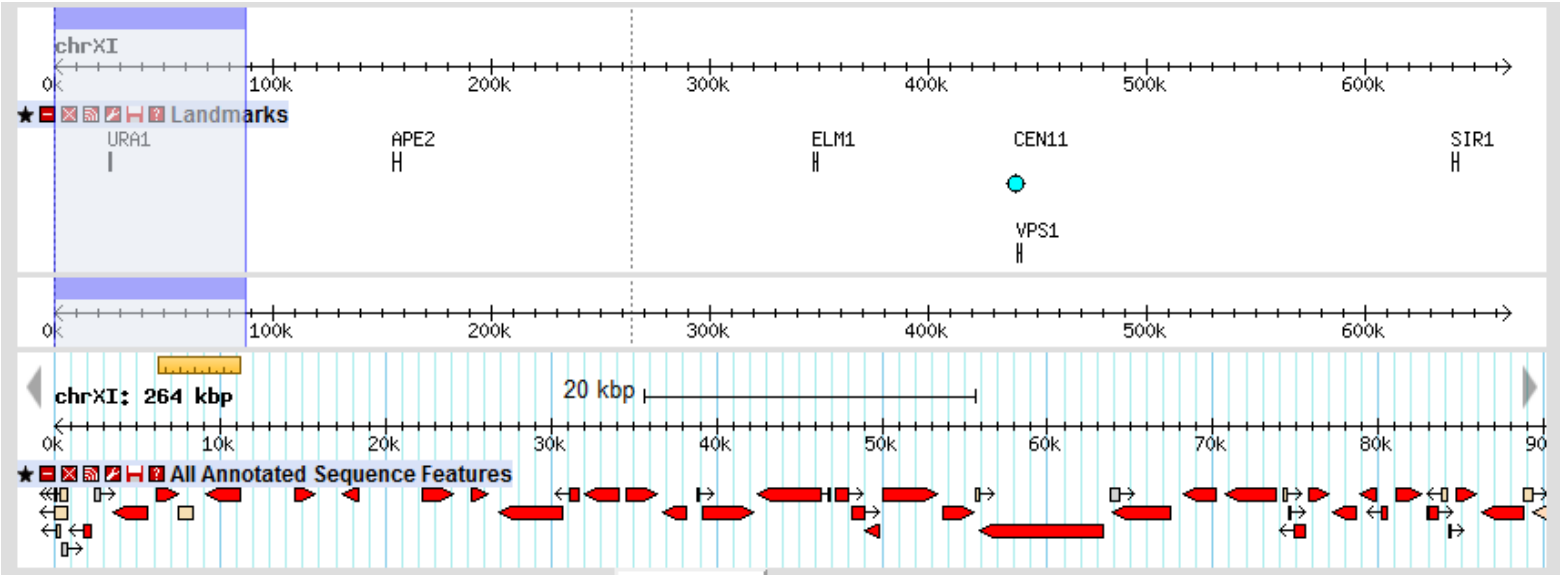
- Symmetrical loxP sites inserted in the 3'UTR of all non-essential genes, and at synthetic landmarks. This generates the SCRaMbLE toolkit.
- LoxPsym sites are cut and moved around by Cre Recombinase
- SC2.0 has inducible Cre expression. Add oestradiol = whole genome shuffle





Automatic refactoring of genome – un-needed genes will be lost

Sc2.0 - Yeast Genome Engineering



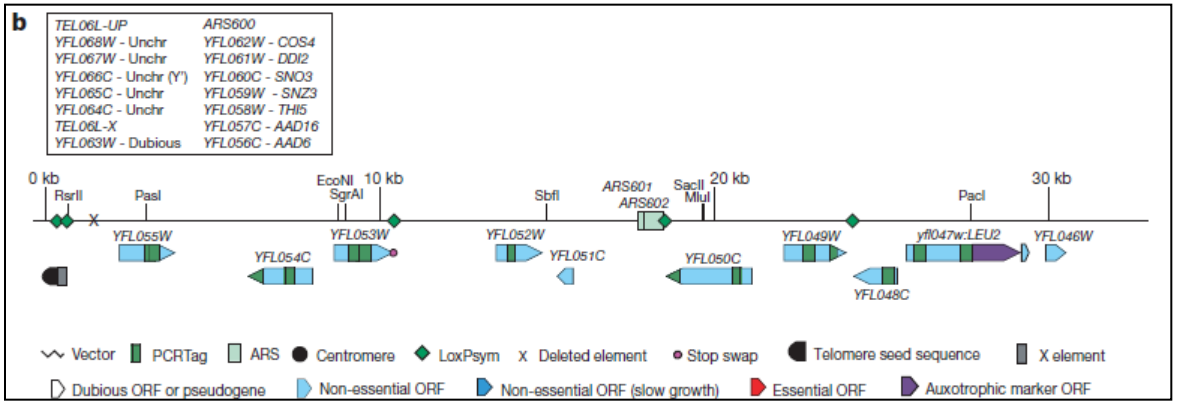
UK Chromosome
SynChr XI – 0.67 Mbp

Imperial College London

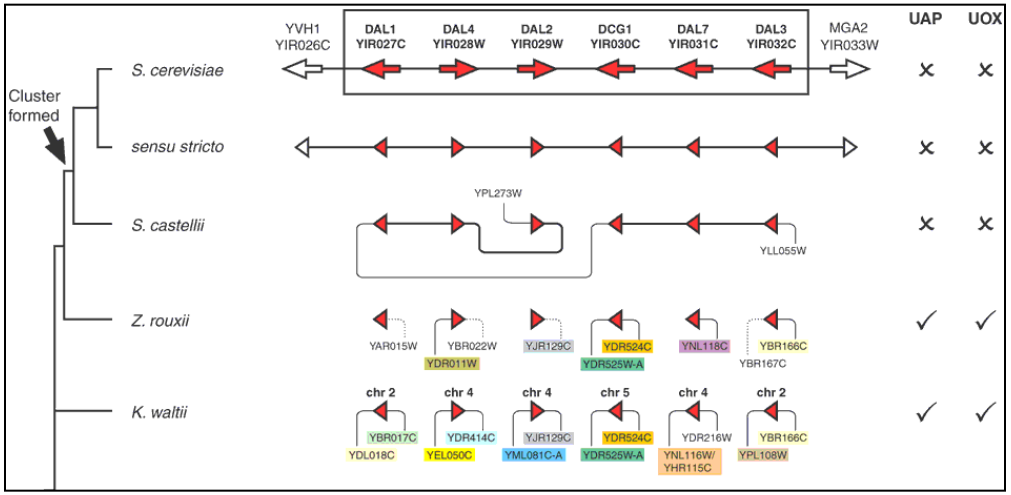
CSynBI
Centre for Synthetic Biology and Innovation

Genome writing rules for applications

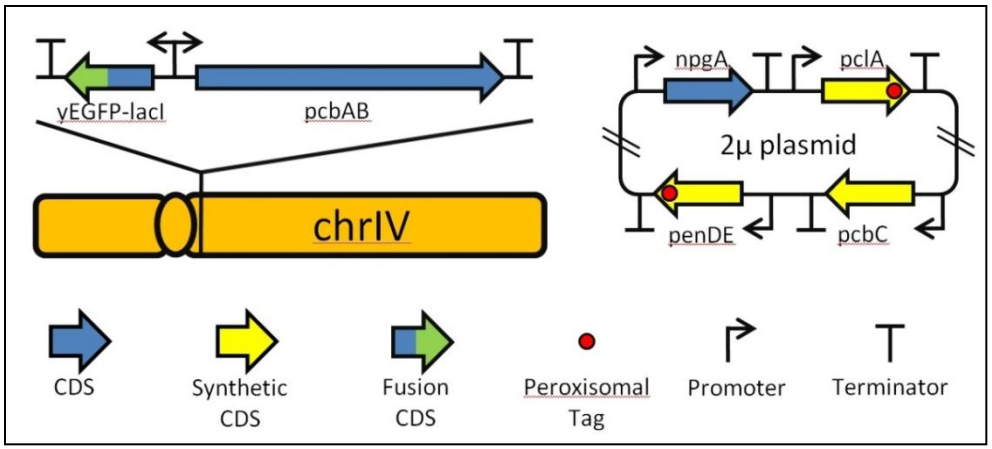
How does location matter for construction of gene networks and pathways?



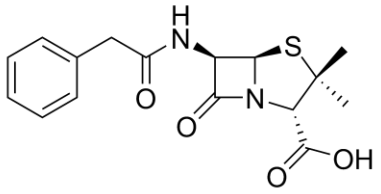
Evolution of DAL cluster in yeasts

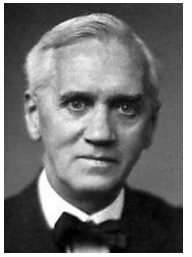


Gene layouts for novel Secondary Metabolism

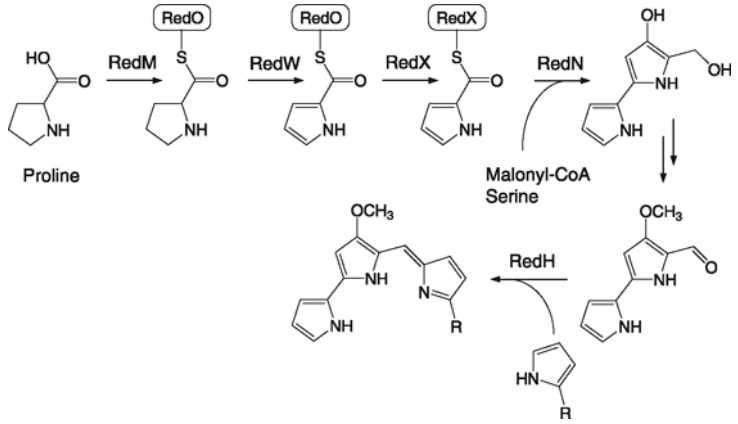


Antibiotic Production
+ Sc2.0 Genome





1. Penicillin Biosynthesis encoded into synthetic yeast chromosomes



2. Modular re-factoring of Prodigiosins pathways with combinatorial assembly

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Anna Kress
Yaksha Novicic
Ben Reeve
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Elena Martinez-Klimova
Georgios Pothoulakis

Alumni

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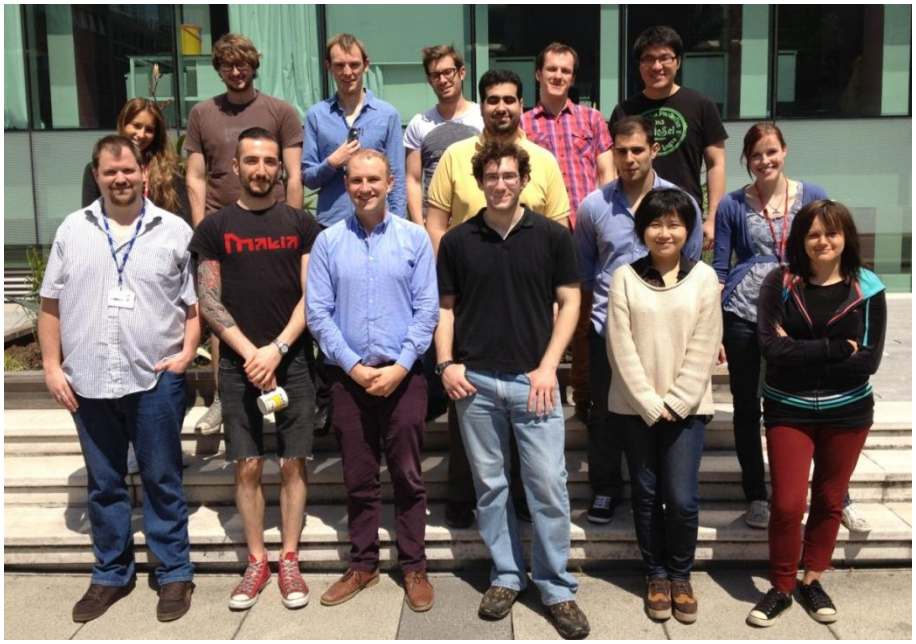
Charles Fracchia
Serge Vasylechko



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BioBricks Foundation

Biotechnology in the public interest

BioBricks FOUNDATION

BioBricks Foundation SB6.0: The Sixth International Meeting on Synthetic Biology

July 9-11, 2013 | Imperial College, London, UK

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Imperial College, London

Lodging during the conference will be available at Imperial College and at hotels close by. Details and rates will be announced when available. **Please check back for registration information, and be sure to sign up for the BioBricks mailing list below.**

For more information, contact us at sb6-info@biobricks.org.