Imperial College London

CSynBI
Centre for Synthetic Biology and Innovation

Synthetic Biology – Trends and Updates

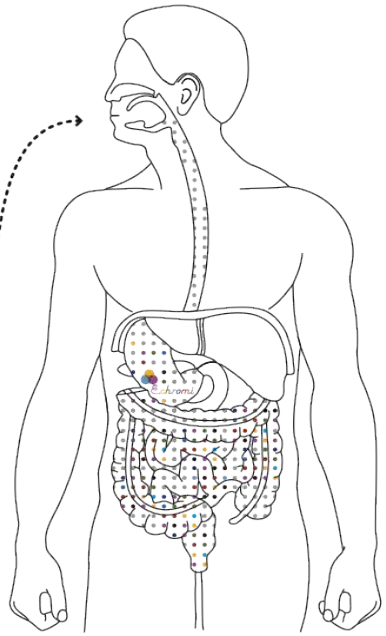
EC WORKSHOP ON SYNTHETIC BIOLOGY - FROM SCIENCE TO POLICY AND SOCIETAL CHALLENGES

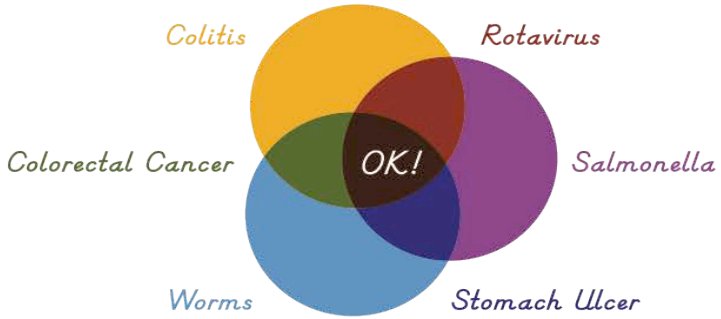
Luxembourg 9th Dec 2015

Professor Paul Freemont @paulfreemont
Co-director and Co-founder EPSRC Centre for Synthetic Biology and Innovation
Co-director and Co-founder of UK National Innovation and Knowledge Centre for Synthetic Biology Imperial College London, UK

SynBICITE
SYNTHETIC BIOLOGY INNOVATION COMMERCIAL AND INDUSTRIAL TRANSLATION ENGINE

WHAT IF ?





1. Drink
Synthetic E. chromi bacteria are ingested as a probiotic yoghurt.

2. Colonise
Colonising the gut, the E. chromi keep watch for the chemical markers of disease.

3. Monitor
If a disease is detected, the bacteria secrete an easily-read colour signal, visible in faeces.

E.chromi - cheap, personalised disease monitoring from the inside out.

E. chromi, 2009 University of Cambridge iGEM team

PNAS PNAS PNAS

Programmable bacteria detect and record an environmental signal in the mammalian gut

Jonathan W. Kotula[a,b,1], S. Jordan Kerns[a,b,1], Lev A. Shaket[b], Layla Siraj[b], James J. Collins[b,c,d], Jeffrey C. Way[b], and Pamela A. Silver[a,b,2]

[a]Department of Systems Biology, Harvard Medical School, Boston, MA 02115; [b]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115; [c]Departments of Biomedical Engineering and Medicine, and Center of Synthetic Biology, Boston University, Boston, MA 02215; and [d]Howard Hughes Medical Institute

Edited* by Richard D. Kolodner, Ludwig Institute for Cancer Research, La Jolla, CA, and approved February 19, 2014 (received for review November 25, 2013)

The mammalian gut is a dynamic community of symbiotic microbes that interact with the host to impact health, disease, and metabolism. We constructed engineered bacteria that survive in the mammalian gut and sense, remember, and report on their experiences. Based on previous genetic memory systems, we constructed a two-part system with a "trigger element" in which the lambda Cro gene is transcribed from a tetracycline-inducible promoter, and a "memory element" derived from the cI/Cro region of phage lambda. The memory element has an extremely stable cI state and a Cro state that is stable for many cell divisions. When *Escherichia coli* bearing the memory system are administered to mice treated with anhydrotetracycline, the recovered bacteria all have switched to the Cro state, whereas those administered to untreated mice remain in the cI state. The trigger and memory elements were transferred from *E. coli* K12 to a newly isolated murine *E. coli* strain; the stability and switching properties of the memory element were essentially identical in vitro and during passage through mice, but the engineered murine *E. coli* was more stably established in the mouse gut. This work lays a foundation for the use of synthetic genetic circuits as monitoring systems in complex, ill-defined environments, and may lead to the development of living diagnostics and therapeutics.

in which the lac repressor (*lacI*-) and *tetR*-encoded repressors inhibit the synthesis of the other protein, such that the system exists in two stable states that can be interchanged by environmental exposure to either isopropyl-β-D-thiogalactopyranoside or tetracycline. Ajo-Franklin et al. (21) developed a more general system in which a formally identified trigger element was separated from a bistable transcriptional memory element in yeast; in this way, a wide variety of input signals can be recorded using a single memory element with diverse trigger promoters. Burrill et al. (4) used this type of memory system to characterize gene-expression profiles in cells that responded differentially to a uniform exposure to DNA damaging agents. Thus, memory devices can be used in laboratory applications under controlled conditions.

Microbes carrying memory elements have potential for broad use as nondestructive environmental sensing systems. To realize this potential, such memory systems will need to be able to function in real-world environments beyond the controlled conditions of a laboratory. Thus, a memory device must be stable in either of two states for long periods of time, even in the presence of basal expression from a trigger element. DNA rearrangement systems may undergo an uninduced change of state resulting from leaky expression of a trigger element if the chances of s
ment increase linearly with expression levels. T

RESEARCH ARTICLE | CANCER

Programmable probiotics for detection of cancer in urine

Tal Danino[1,*], Arthur Prindle[2,*], Gabriel A. Kwong[1,†], Matthew Skalak[1], Howard Li[2], Kaitlin Allen[1], Jeff Hasty[2,3,4,‡] and Sangeeta N. Bhatia[1,5,6,7,8,‡,§]

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↵‡ Equally contributing senior authors.

Science Translational Medicine 27 May 2015:
Vol. 7, Issue 289, pp. 289ra84
DOI: 10.1126/scitranslmed.aaa3519

WHAT IF ?

Plasticity 2013 Imperial College iGEM team http://2013.igem.org/Team:Imperial_College

PLOS | ONE

RESEARCH ARTICLE

A Forward-Design Approach to Increase the Production of Poly-3-Hydroxybutyrate in Genetically Engineered *Escherichia coli*

Richard Kelwick[1,2]*‡, Margarita Kopniczky[1,2‡], Iain Bower[1,3], Wenqiang Chi[1,4], Matthew Ho Wai Chin[1,4], Sisi Fan[1,3], Jemma Pilcher[1,3], James Strutt[1,3], Alexander J. Webb[1,2], Kirsten Jensen[1,2], Guy-Bart Stan[1,4], Richard Kitney[1,4]*, Paul Freemont[1,2]*

1 Centre for Synthetic Biology and Innovation, South Kensington Campus, London, United Kingdom, **2** Department of Medicine, South Kensington Campus, London, United Kingdom, **3** Department of Life Sciences, South Kensington Campus, London, United Kingdom, **4** Department of Bioengineering, Imperial College London, South Kensington Campus, London, United Kingdom

‡ These authors are equal first authors on this work.
* p.freemont@imperial.ac.uk (PF); r.kitney@imperial.ac.uk (R. Kitney); r.kelwick@imperial.ac.uk (R. Kelwick)

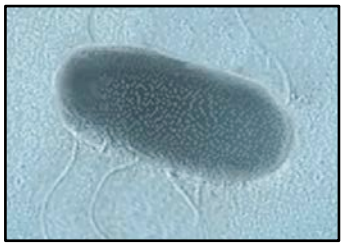
OPEN ACCESS

Citation: Kelwick R, Kopniczky M, Bower I, Chi W, Chin MHW, Fan S, et al. (2015) A Forward-Design Approach to Increase the Production of Poly-3-Hydroxybutyrate in Genetically Engineered

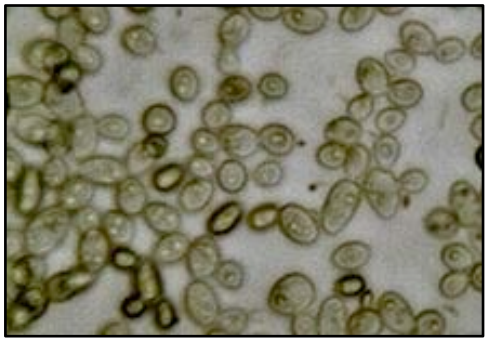
Abstract

Biopolymers, such as poly-3-hydroxybutyrate (P(3HB)) are produced as a carbon store in an array of organisms and exhibit characteristics which are similar to oil-derived plastics

WHAT IF ?



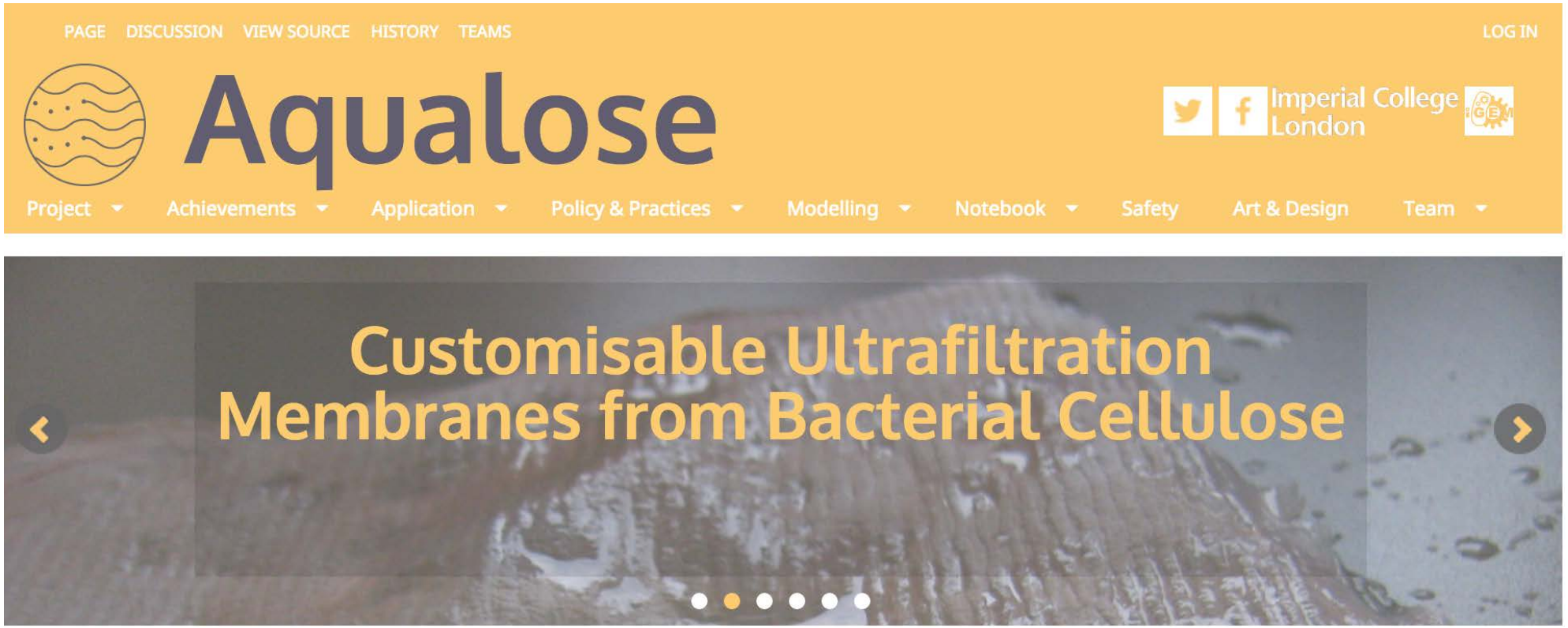
Gluconacetobacter xylinus



Gluconacetobacter xylinus + yeast

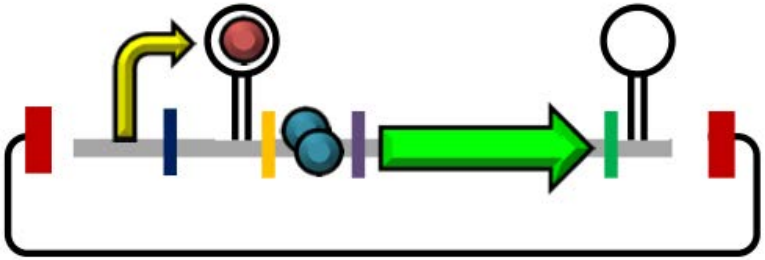
Suzanne Lee

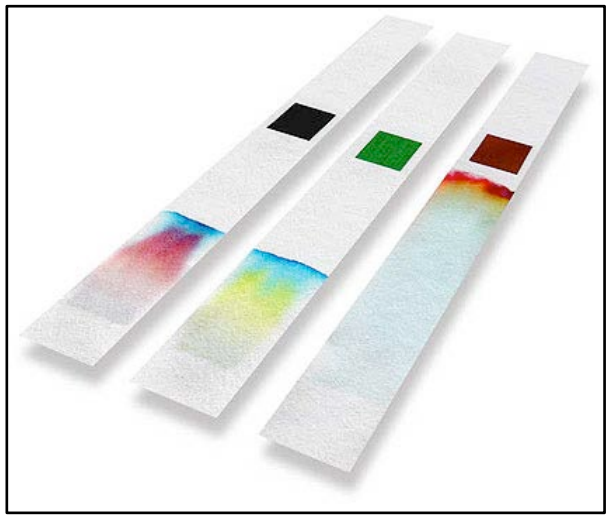
Imperial College IGEM 2014 Aqualose

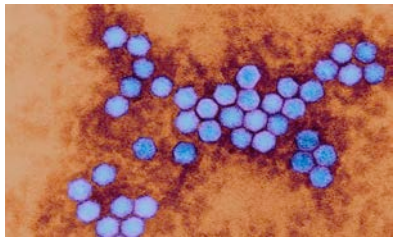


Genetic engineering of the cellulose-producing bacterium Komagataeibacter rhaeticus for production of novel biomaterials.
Florea et al 2015 in press

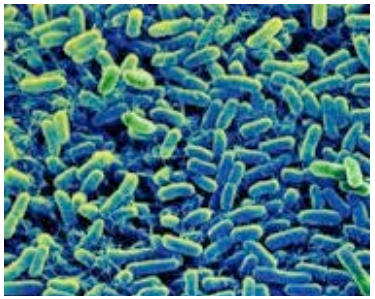
WHAT IF ?







viruses



bacteria

parasites

Cell

Resource

Paper-Based Synthetic Gene Networks

Keith Pardee,[1,2] Alexander A. Green,[1,2] Tom Ferrante,[1] D. Ewen Cameron,[2,3] Ajay DaleyKeyser,[1] Peng Yin,[1] and James J. Collins[1,2,3,*]

[1]Wyss Institute for Biological Inspired Engineering, Harvard University, Boston, MA 02115, USA

[2]Department of Biomedical Engineering and Center of Synthetic Biology, Boston University, Boston, MA 02215, USA

[3]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA

*Correspondence: jcollins@bu.edu

http://dx.doi.org/10.1016/j.cell.2014.10.004

SUMMARY

Synthetic gene networks have wide-ranging uses in reprogramming and rewiring organisms. To date, there has not been a way to harness the vast potential of these networks beyond the constraints of a laboratory or in vivo environment. Here, we present an in vitro paper-based platform that provides an alternate, versatile venue for synthetic biologists to operate and a much-needed medium for the safe deployment of engineered gene circuits beyond the lab. Commercially available cell-free systems are freeze

Earlier studies in the area of in vitro synthetic biology and cell-free systems have made important contributions to our understanding of fundamental molecular biology and biochemistry and, more recently, in the study of molecular switch dynamics and complex gene circuits (Hong et al., 2014; Karzbrun et al., 2014; Sun et al., 2014; Takahashi et al., 2014). These efforts, however, have focused on solution-phase reactions using fresh from frozen cell-free systems and often in liposomes with the goal of assembling artificial cells (Kuruma et al., 2009; Kobori et al., 2013). These solution-phase reactions are not stable or practical for handling outside of the lab and therefore miss the opportunity to leverage the abiotic and sterile nature of these systems.

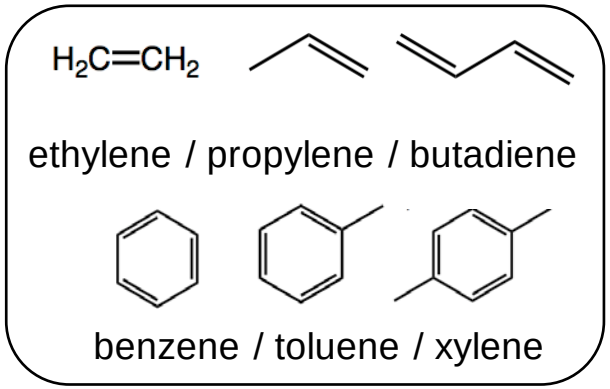
WHAT IF ?

petroleum

coal

natural gas

Carbon feedstocks



Building blocks

Value - added chemicals

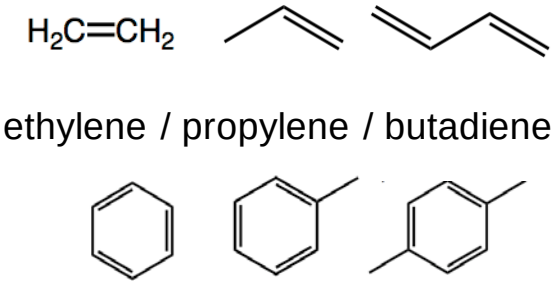
WHAT IF ?

CO_2

cellulosic crops

lignocellulosic waste

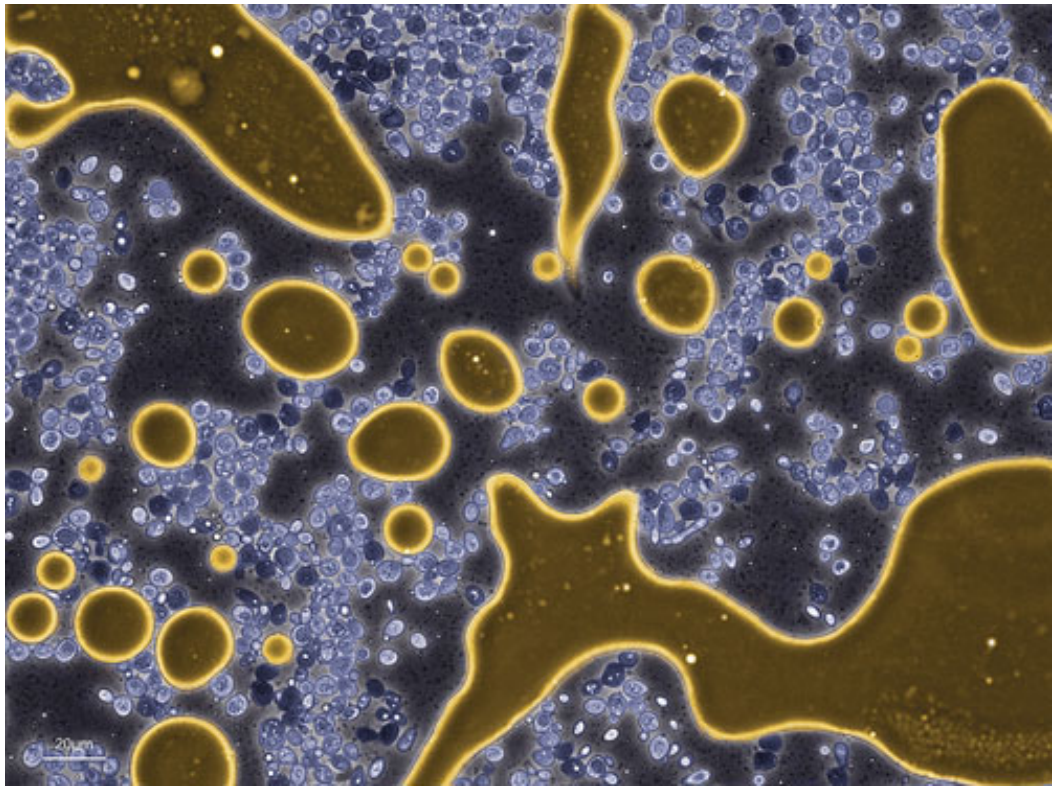
Biomass feedstocks



Building blocks

Value - added chemicals

S. cerevisiae secreting farnesene/biodiesel



~112K bases added
~41K bases removed
~450 single nucleotide changes

~1.25% of the genome!

AMYRIS.

DNA as a programmable material

WHAT IF ?


GenSpace'
ActiveDry
YEAST
All Natural Yeast


Eau de Yeast

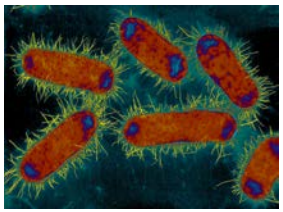

Almond
Coconut
Banana
Orange
Vanilla
Strawberry
Pineapple

DIY-Bio, Biohackers and the Growth of Community Labs

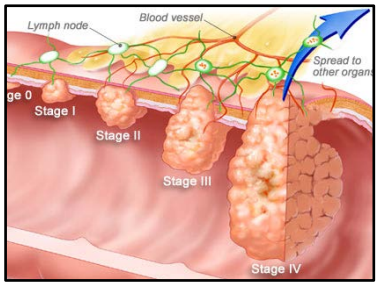
DIY BIO

WHAT IF ?

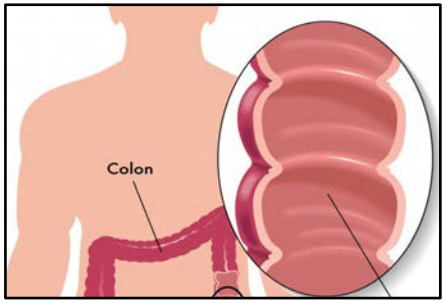
Engineered bacteria to detect and kill cancer cells



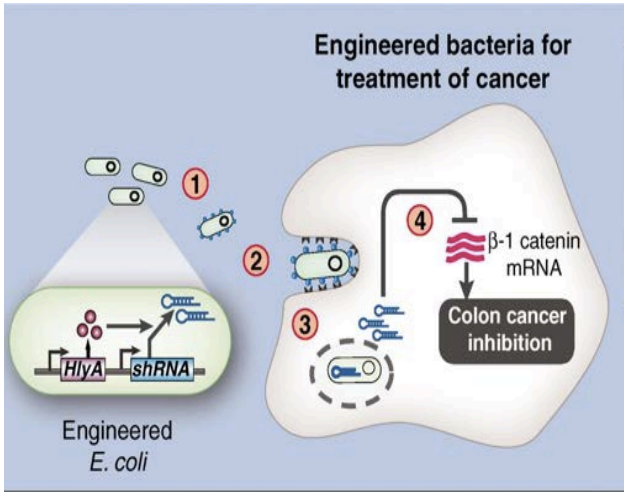
Engineered E. coli



Colorectal cancer



Healthy colon



(adapted *from Science 333: 6047 (2011); http://www.wisegeek.com/what-are-cold-forceps.htm); http://www.webmd.com/colorectal-cancer/ss/slideshow-colorectal-cancer-overview)*

Journal of Molecular Biology

Volume 355, Issue 4, 27 January 2006, Pages 619–627

Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria

J. Christopher Anderson[a, c], Elizabeth J. Clarke[c], Adam P. Arkin[a, b], Christopher A. Voigt[b, c]

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Letter

Nature Biotechnology **24**, 697 - 702 (2006)
Published online: 14 May 2006 | doi:10.1038/nbt1211

Short hairpin RNA–expressing bacteria elicit RNA interference in mammals

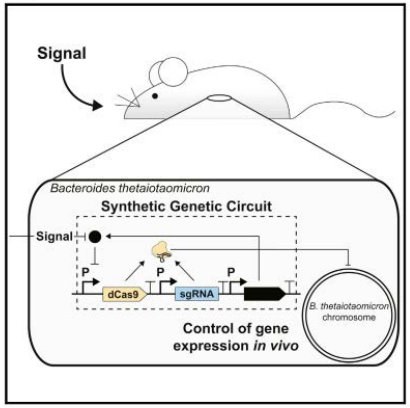
Shuanglin Xiang[1,2], Johannes Fruehauf[1,2] & Chiang J Li[1]

Article

Cell Systems

Programming a Human Commensal Bacterium, *Bacteroides thetaiotaomicron*, to Sense and Respond to Stimuli in the Murine Gut Microbiota

Graphical Abstract



Authors

Mark Mimee, Alex C. Tucker, Christopher A. Voigt, Timothy K. Lu

Correspondence

timlu@mit.edu

In Brief

The development of genetic parts to precisely program the human commensal gut bacterium *Bacteroides thetaiotaomicron* lays the foundation for microbiome engineering.

Highlights

- We develop sets of genetic parts for a human commensal bacterium
- Promoter and RBS libraries control gene expression over a 10,000-fold dynamic range
- Orthogonal, inducible sensors enable synthetic genetic memory and CRISPRi
- Genetic circuits respond to stimuli in a complex mouse gut microbiota

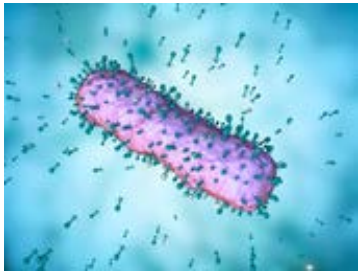
Mimee et al., 2015, Cell Systems 1, 62–71
July 29, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.cels.2015.06.001

CellPress

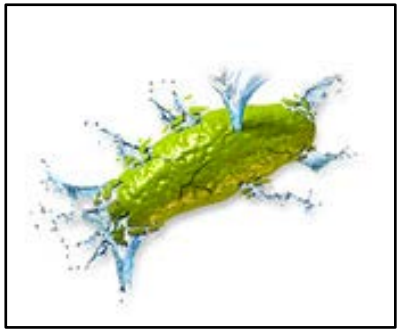
WHAT IF ?

Engineered phage and bacteria to target pathogens

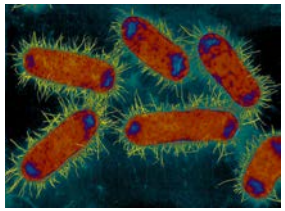
bacteriophage



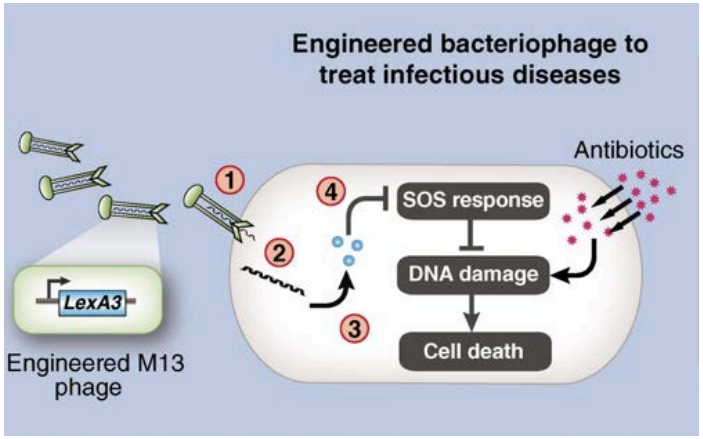
Attacking bacterium



Bacteria cell lyses and dies



Engineered E. coli



(adapted *from Science 333: 6047 (2011); http://www.sciencephoto.com/)*

PNAS PNAS PNAS

Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy

Timothy K. Lu[a,b] and James J. Collins[b,1]

[a]Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge, MA 02139; and [b]Howard Hughes Medical Institute, Center for BioDynamics and Department of Biomedical Engineering, Boston University, Boston, MA 02215

Edited by Arnold L. Demain, Drew University, Madison, NJ, and approved February 3, 2009 (received for review January 16, 2008)

Antimicrobial drug development is increasingly lagging behind the evolution of antibiotic resistance, and as a result, there is a pressing need for new antibacterial therapies that can be readily designed and implemented. In this work, we engineered bacteriophage to overexpress proteins and attack gene networks that are not directly targeted by antibiotics. We show that suppressing the SOS network in *Escherichia coli* with engineered bacteriophage enhances killing by quinolones by several orders of magnitude in vitro and significantly increases survival of infected mice in vivo. In addition, we demonstrate that engineered bacteriophage can enhance the killing of antibiotic-resistant bacteria, persister cells, and biofilm cells, reduce the number of antibiotic-resistant bacteria that arise from an antibiotic-treated population, and act as a strong adjuvant for other bactericidal antibiotics (e.g., aminoglycosides and β-lactams). Furthermore, we show that engineering bacteriophage to target non-SOS gene networks and to overexpress multiple factors also can produce effective antibiotic adjuvants. This work establishes a synthetic biology platform for the rapid translation and integration of identified targets into effective antibiotic adjuvants.

antibiotic adjuvants | antibiotic resistance | bacterial persistence | bacteriophage therapy | synthetic biology

ary pressures. Instead of overexpressing lethal genes, our desig
targets nonessential genes and the networks they regulate th
are not directly attacked by antibiotics. Combination therap
with different antibiotics, different bacteriophage, or antibioti
plus phage may reduce the incidence of phage resistance and/
antibiotic resistance (16–20). Therefore, by using a combinatio
of engineered antibiotic-enhancing phage and antibiotics, w
hoped to reduce the incidence of antibiotic resistance an
enhance bacterial killing.

Results

Targeting the SOS DNA Repair System. Bactericidal antibiotics (e.g
quinolones such as ofloxacin) induce hydroxyl radical formatio
that leads to DNA, protein, and lipid damage and ultimately t
cell death (8). DNA damage induces the SOS response (21, 22
which results in DNA repair (Fig. 1*A*). It has been shown th
bacterial killing by bactericidal antibiotics can be enhanced b
knocking out *recA* and disabling the SOS response (8). Here w
took an alternative approach and engineered M13mp18 phage t
overexpress *lexA3*, a repressor of the SOS response (23). Ove
expression of *lexA* to suppress the SOS system has been dem
onstrated to inhibit the emergence of antibiotic resistance (24
We used M13mp18, a modified version of M13 phage, as ou

Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

www.molecularsystemsbiology.com

molecular systems biology

Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

...min Saeidi[1], Choon Kit Wong[1], Tat-Ming Lo, Hung Xuan Nguyen[2], Hua Ling, Susanna Su Jan Leong, Chueh Loo Poh*
...atthew Wook Chang*

... of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore
...e authors contributed equally to this work
...ent address: Department of Biomedical Engineering, Duke University, Durham, NC, USA
...esponding author. CL Poh, Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, ...ore 637459, Singapore. Tel.: +65 6514 1088; Fax: +65 6794 7553; E-mail: cpoh@ntu.edu.sg or MW Chang, Division of Chemical and Biomolecular ...ering, School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore. ...+65 6513 8063; Fax: +65 6794 7553; E-mail: matthewchang@ntu.edu.sg

...ed 17.5.11; accepted 30.6.11

NANO LETTERS

Letter

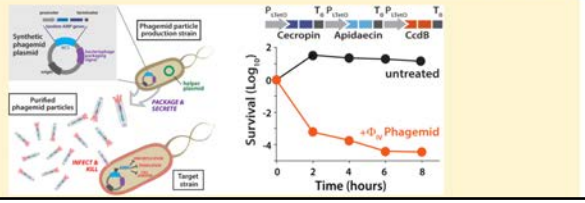
pubs.acs.org/NanoLett

Engineered Phagemids for Nonlytic, Targeted Antibacterial Therapies

Russell J. Krom,[†,‡,∥,⊥] Prerna Bhargava,[†,§,∥] Michael A. Lobritz,[†,§,∥,#] and James J. Collins*[,†,‡,§,∥]

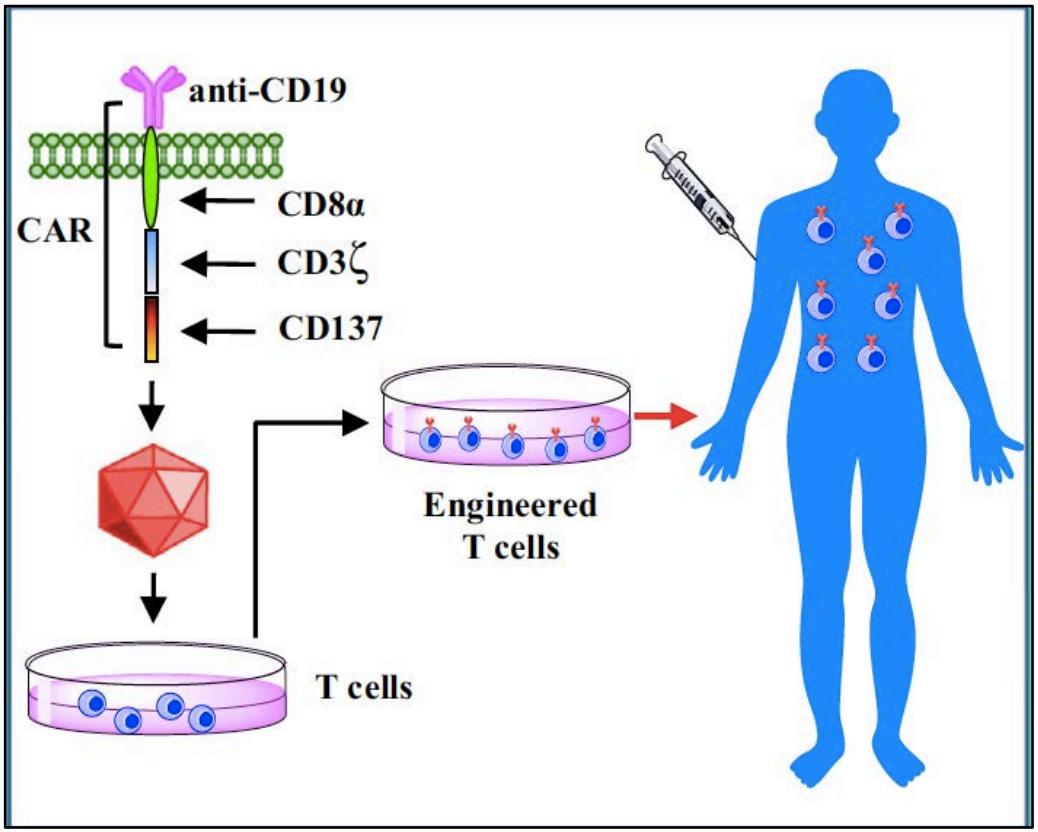
[†]Institute for Medical Engineering and Science, Department of Biological Engineering, and Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
[‡]Harvard-MIT Program in Health Sciences and Technology, Cambridge, Massachusetts 02139, United States
[§]Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, United States
[∥]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States
[⊥]Department of Molecular and Translational Medicine, Boston University, Boston, Massachusetts 02215, United States
[#]Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts 02114, United States

Supporting Information



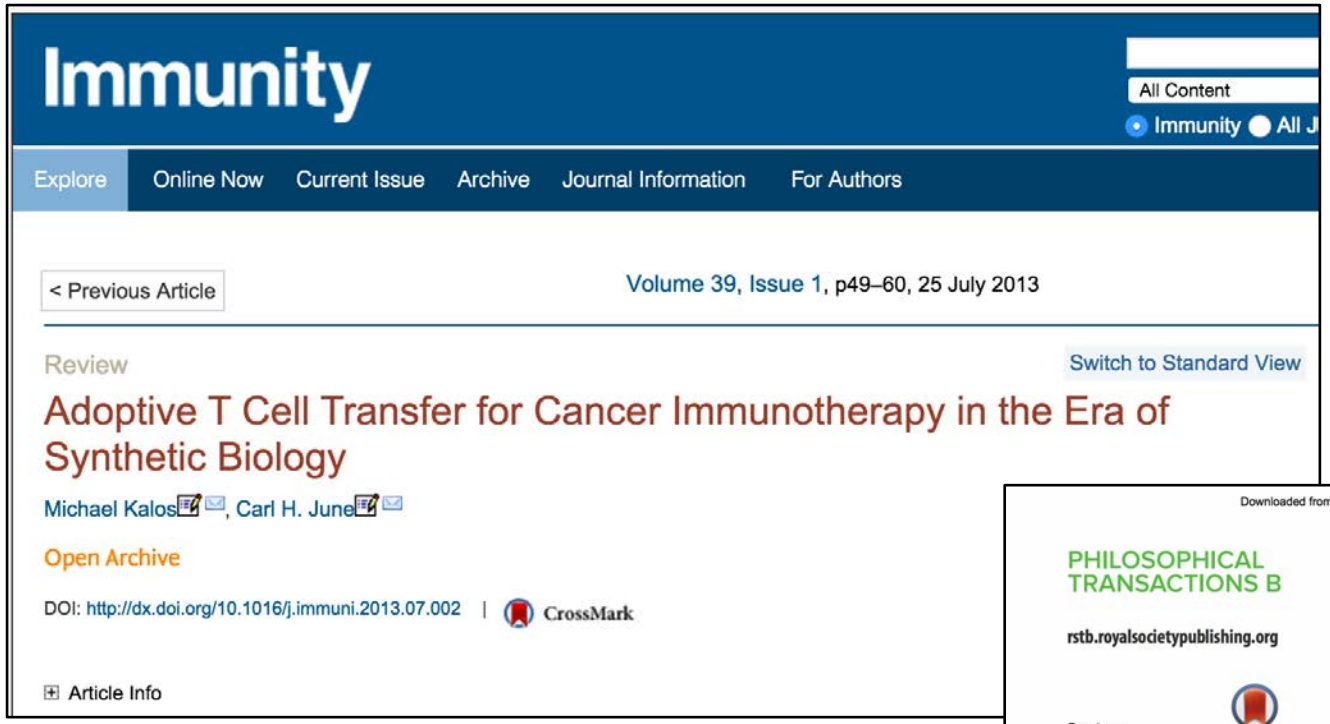
WHAT IF ?

Engineered T-cells to target cancer



T cell engineering

Porter et al. *N. Eng. J. Med.*, 365 (2011), pp. 725–733



Immunity

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Volume 39, Issue 1, p49–60, 25 July 2013

Review

Switch to Standard View

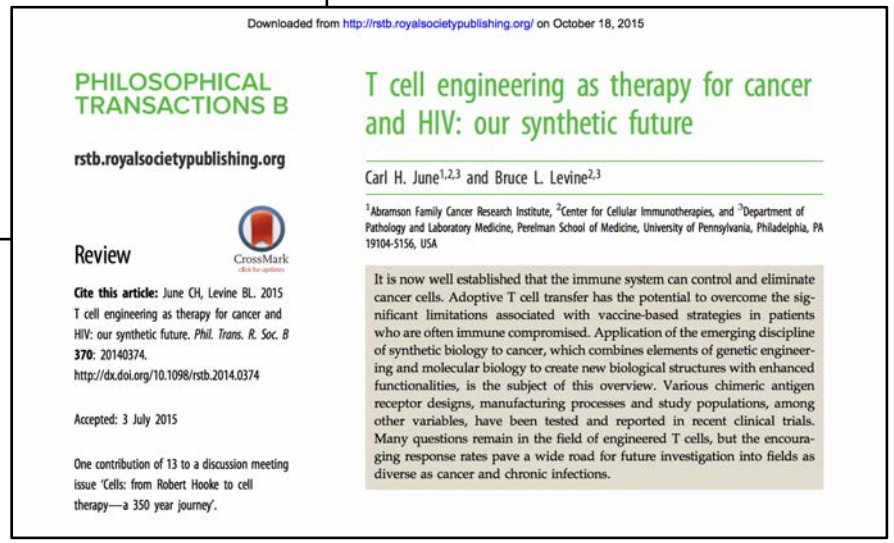
Adoptive T Cell Transfer for Cancer Immunotherapy in the Era of Synthetic Biology

Michael Kalos, Carl H. June

Open Archive

DOI: http://dx.doi.org/10.1016/j.immuni.2013.07.002 | CrossMark

Article Info



PHILOSOPHICAL TRANSACTIONS B

rstb.royalsocietypublishing.org

Review

Cite this article: June CH, Levine BL. 2015 T cell engineering as therapy for cancer and HIV: our synthetic future. *Phil. Trans. R. Soc. B* **370**: 20140374.
http://dx.doi.org/10.1098/rstb.2014.0374

Accepted: 3 July 2015

One contribution of 13 to a discussion meeting issue 'Cells: from Robert Hooke to cell therapy—a 350 year journey'.

T cell engineering as therapy for cancer and HIV: our synthetic future

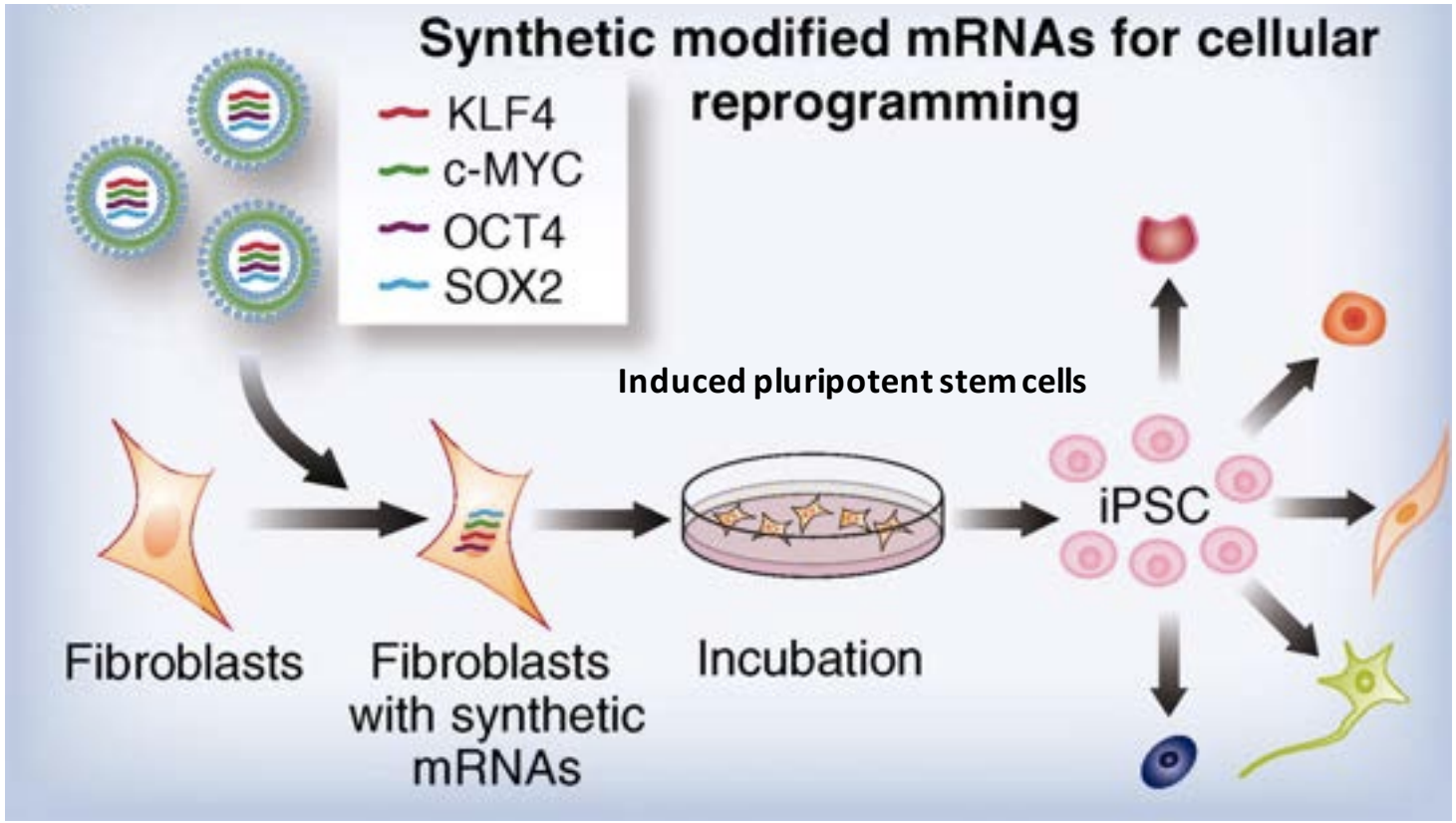
Carl H. June[1,2,3] and Bruce L. Levine[2,3]

[1]Abramson Family Cancer Research Institute, [2]Center for Cellular Immunotherapies, and [3]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-5156, USA

It is now well established that the immune system can control and eliminate cancer cells. Adoptive T cell transfer has the potential to overcome the significant limitations associated with vaccine-based strategies in patients who are often immune compromised. Application of the emerging discipline of synthetic biology to cancer, which combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities, is the subject of this overview. Various chimeric antigen receptor designs, manufacturing processes and study populations, among other variables, have been tested and reported in recent clinical trials. Many questions remain in the field of engineered T cells, but the encouraging response rates pave a wide road for future investigation into fields as diverse as cancer and chronic infections.

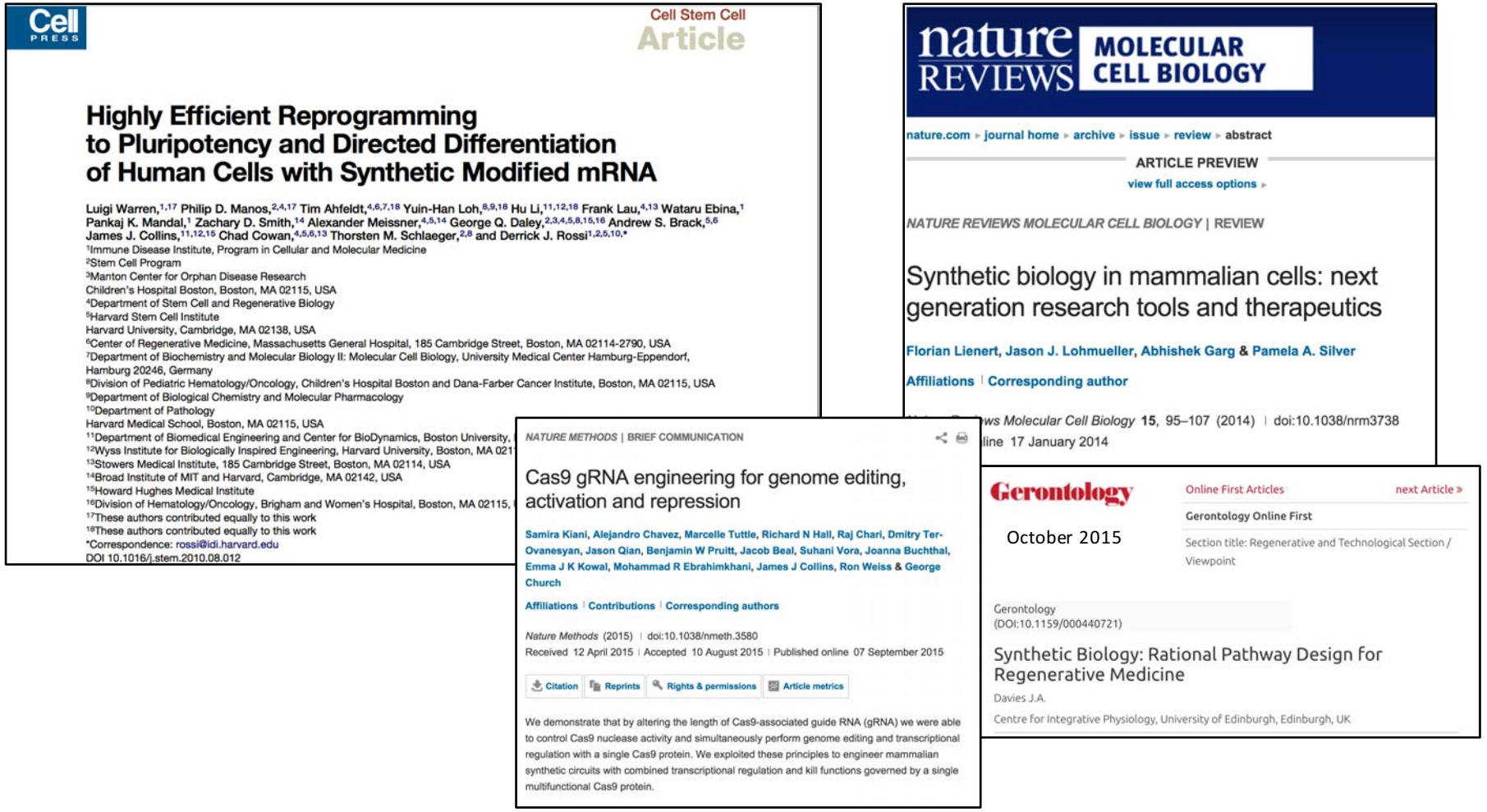
WHAT IF ?

Systematic cellular reprogramming



Cell therapy and regenerative medicine

Warren et al. *Cell Stem Cell* **7**, 618 (2010)



Cell PRESS

Cell Stem Cell
Article

Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA

Luigi Warren,[1,17] Philip D. Manos,[2,4,17] Tim Ahfeldt,[4,6,7,18] Yuin-Han Loh,[8,9,18] Hu Li,[11,12,18] Frank Lau,[4,13] Wataru Ebina,[1] Pankaj K. Mandal,[1] Zachary D. Smith,[14] Alexander Meissner,[4,5,14] George Q. Daley,[2,3,4,5,8,15,16] Andrew S. Brack,[5,6] James J. Collins,[11,12,15] Chad Cowan,[4,5,6,13] Thorsten M. Schlaeger,[2,8] and Derrick J. Rossi[1,2,5,10,*]
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Harvard Medical School, Boston, MA 02115, USA
[11]Department of Biomedical Engineering and Center for BioDynamics, Boston University,
[12]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 021
[13]Stowers Medical Institute, 185 Cambridge Street, Boston, MA 02114, USA
[14]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA
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DOI 10.1016/j.stem.2010.08.012

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NATURE REVIEWS MOLECULAR CELL BIOLOGY | REVIEW

Synthetic biology in mammalian cells: next generation research tools and therapeutics

Florian Lienert, Jason J. Lohmueller, Abhishek Garg & Pamela A. Silver

Affiliations | Corresponding author

ws Molecular Cell Biology 15, 95–107 (2014) | doi:10.1038/nrm3738
line 17 January 2014

NATURE METHODS | BRIEF COMMUNICATION

Cas9 gRNA engineering for genome editing, activation and repression

Samira Kiani, Alejandro Chavez, Marcelle Tuttle, Richard N Hall, Raj Chari, Dmitry Ter-Ovanesyan, Jason Qian, Benjamin W Pruitt, Jacob Beal, Suhani Vora, Joanna Buchthal, Emma J K Kowal, Mohammad R Ebrahimkhani, James J Collins, Ron Weiss & George Church

Affiliations | Contributions | Corresponding authors

Nature Methods (2015) | doi:10.1038/nmeth.3580
Received 12 April 2015 | Accepted 10 August 2015 | Published online 07 September 2015

Citation | Reprints | Rights & permissions | Article metrics

We demonstrate that by altering the length of Cas9-associated guide RNA (gRNA) we were able to control Cas9 nuclease activity and simultaneously perform genome editing and transcriptional regulation with a single Cas9 protein. We exploited these principles to engineer mammalian synthetic circuits with combined transcriptional regulation and kill functions governed by a single multifunctional Cas9 protein.

Gerontology

October 2015

Online First Articles next Article »

Gerontology Online First

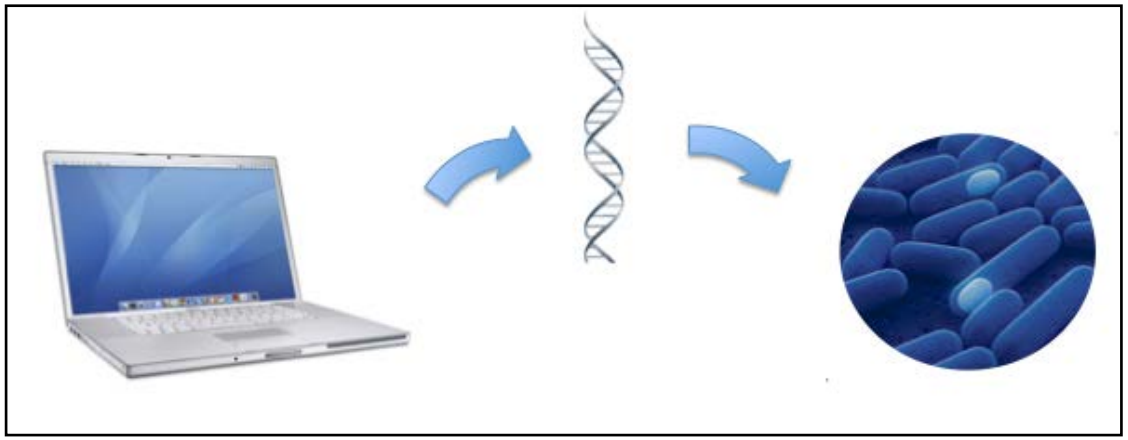
Section title: Regenerative and Technological Section / Viewpoint

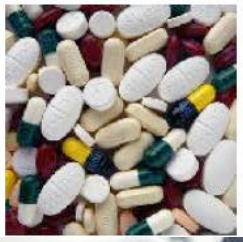
Gerontology
(DOI:10.1159/000440721)

Synthetic Biology: Rational Pathway Design for Regenerative Medicine

Davies J.A.
Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK

WHAT IF ?

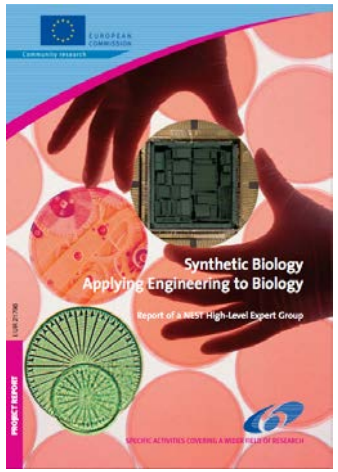


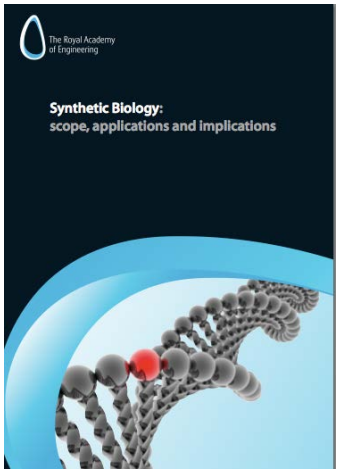


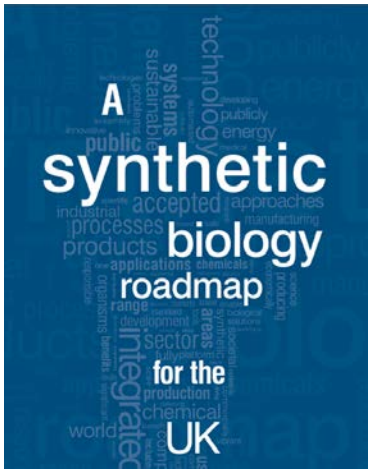
https://www.youtube.com/watch?v=ylSZN-1hhoY

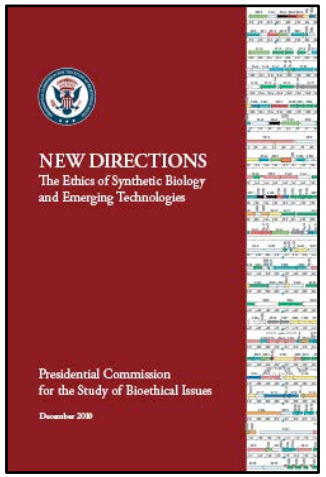
So why is synthetic biology causing such a big fuss?

"Synthetic biology aims to design and engineer biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems"





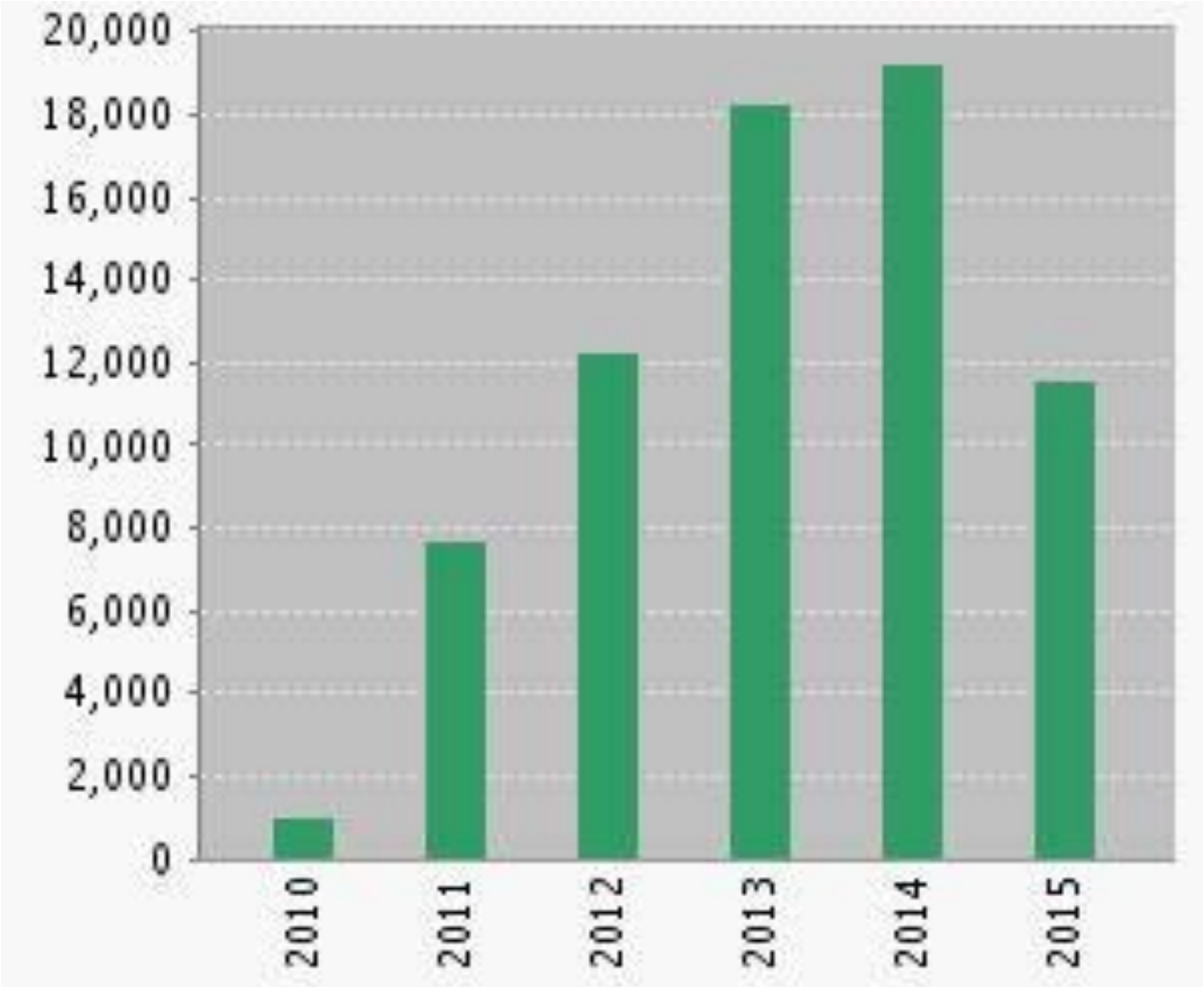




SCHER, SCENIHR, SCCS
operational definition for Synthetic Biology

"SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms."

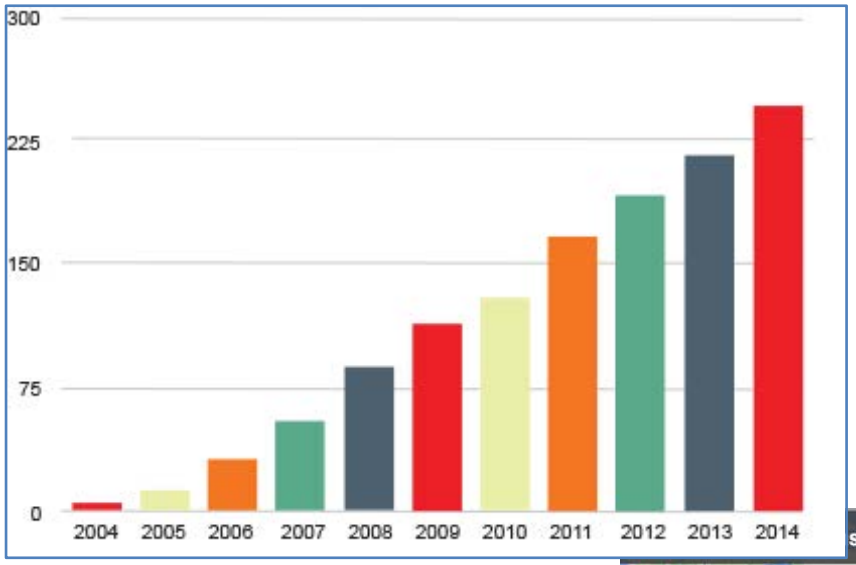
Synthetic Biology is a rapidly growing field



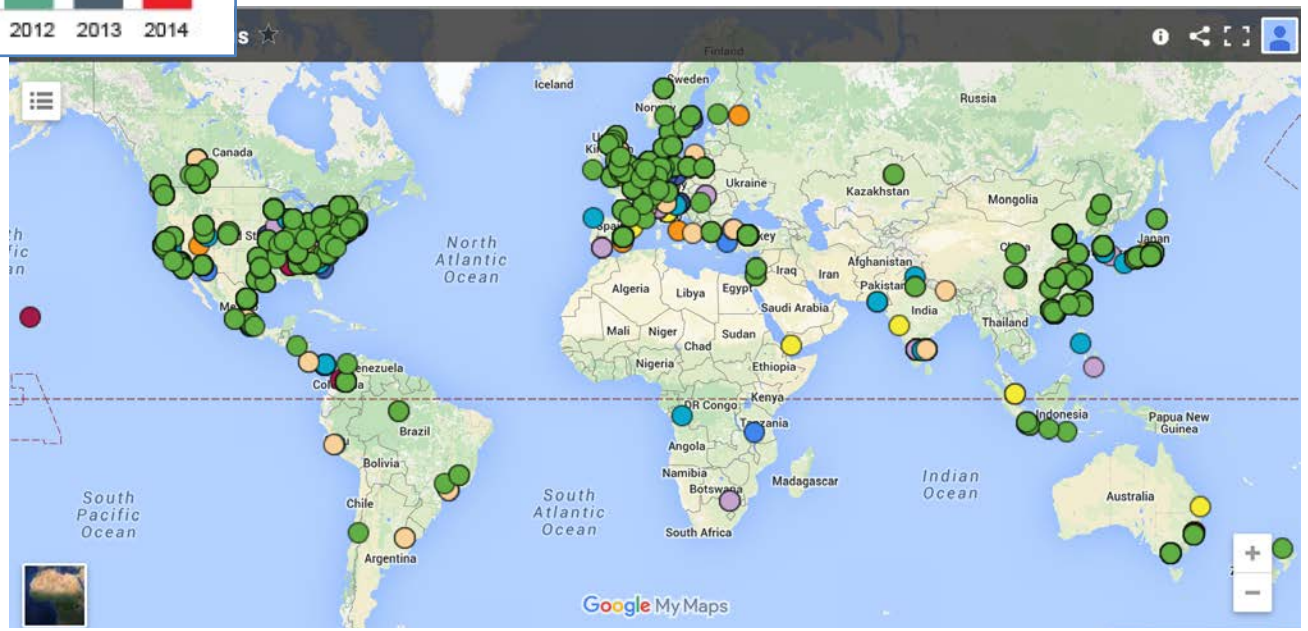
Citations of papers containing key works synthetic biology **70256 - 47,000 papers since 2001**

A growing community of student researchers – growth of iGEM

International Genetically Engineered Machine Competition



280 teams are registered for 2015
259 teams at the Jamboree
~15,000 iGEM alumni

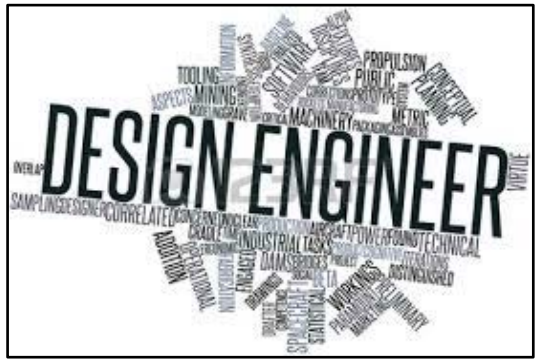


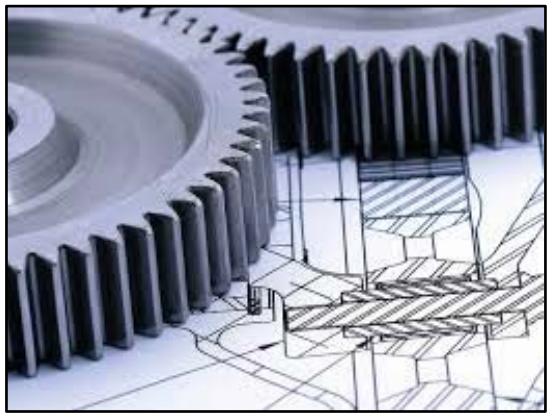
Synthetic Biology has a powerful vision for merging engineering design practice into the construction of biology systems and cells at the genetic level

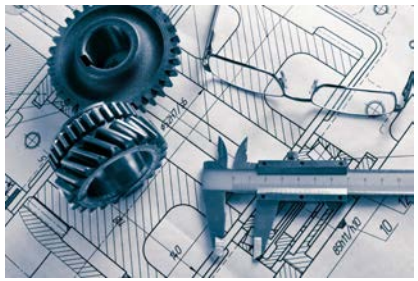
Basics of an engineering design framework

In engineering systems, robustness and stability are achieved by

(1) System control
(2) Redundancy
(3) Modular design
(4) Structural stability







An engineering design framework for Synthetic Biology

(1) System control (feed-back/ feed-forward biological control networks)
(2) Redundancy (gene duplication/ multiple regulatory pathways)
(3) Modular design (evolutionary robust / multi-functional / compartmental)
(4) Structural stability (homeostasis)

Hypothesis – Are these also intrinsic features of complex natural living systems?

A systematic engineering framework for biological systems aims to test the hypothesis

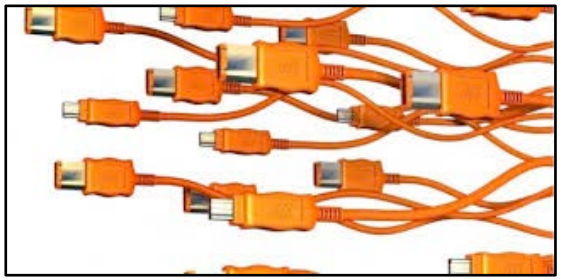
Can we use Biology to Build new Biology? Can we learn about biology through design and construction?

- Biological systems are modular
- Biological function is primarily encoded in DNA
- Large knowledge base of genome sequences
- Large diversity of biological parts (genes/regulatory)
- Increased understanding of molecular / cell biology
- New technologies to synthesize and assemble DNA

However……

Standardising biology poses challenges

- Biology is not fully ‘plug and play’
  - Context dependency
  - Evolution, adaptation and natural selection
  - Non-predictive stochastic behaviour
  - Self assembly and emergent properties
  - Non-linear dynamical processes
  - Multi-scale interactions



- Living cells have constrained volumes and high concentrations of biochemical components

One approach to overcome biological complexity in engineering biology is the use of **Systematic Design**

What is Systematic Design ?

Systematic design is founded on the following engineering principles

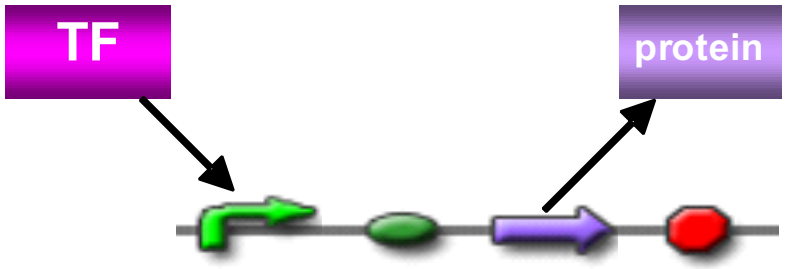
- Modularisation – interchangeable modules
- Standardisation – standard parts and processes
- Abstraction – reducing complexity

Systematic design aims to achieve
Robustness and Reproducibility

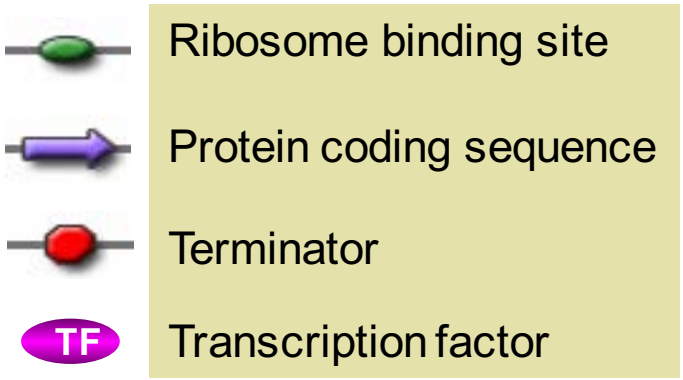
Key requirement is interoperability

A systematic design framework using genetic parts that encode biological function

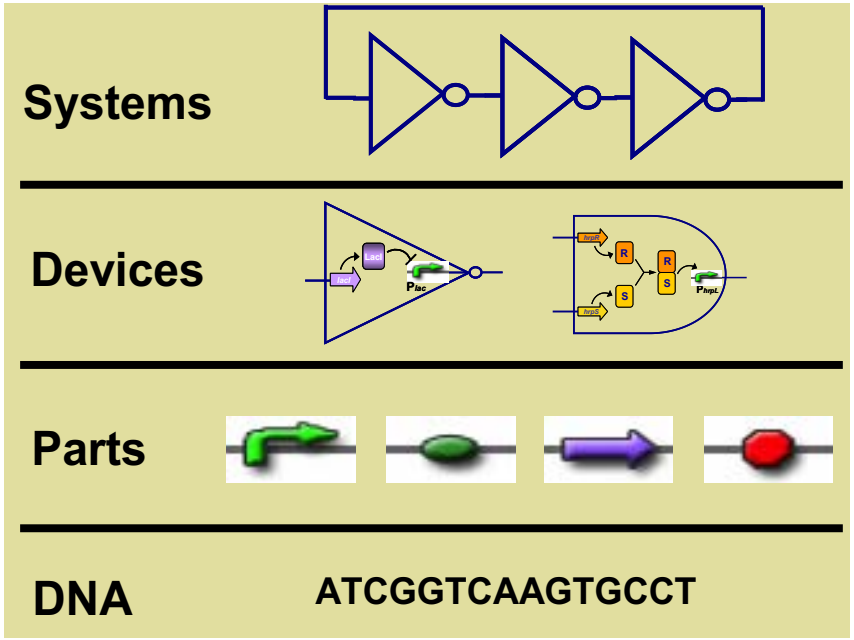
Modularity



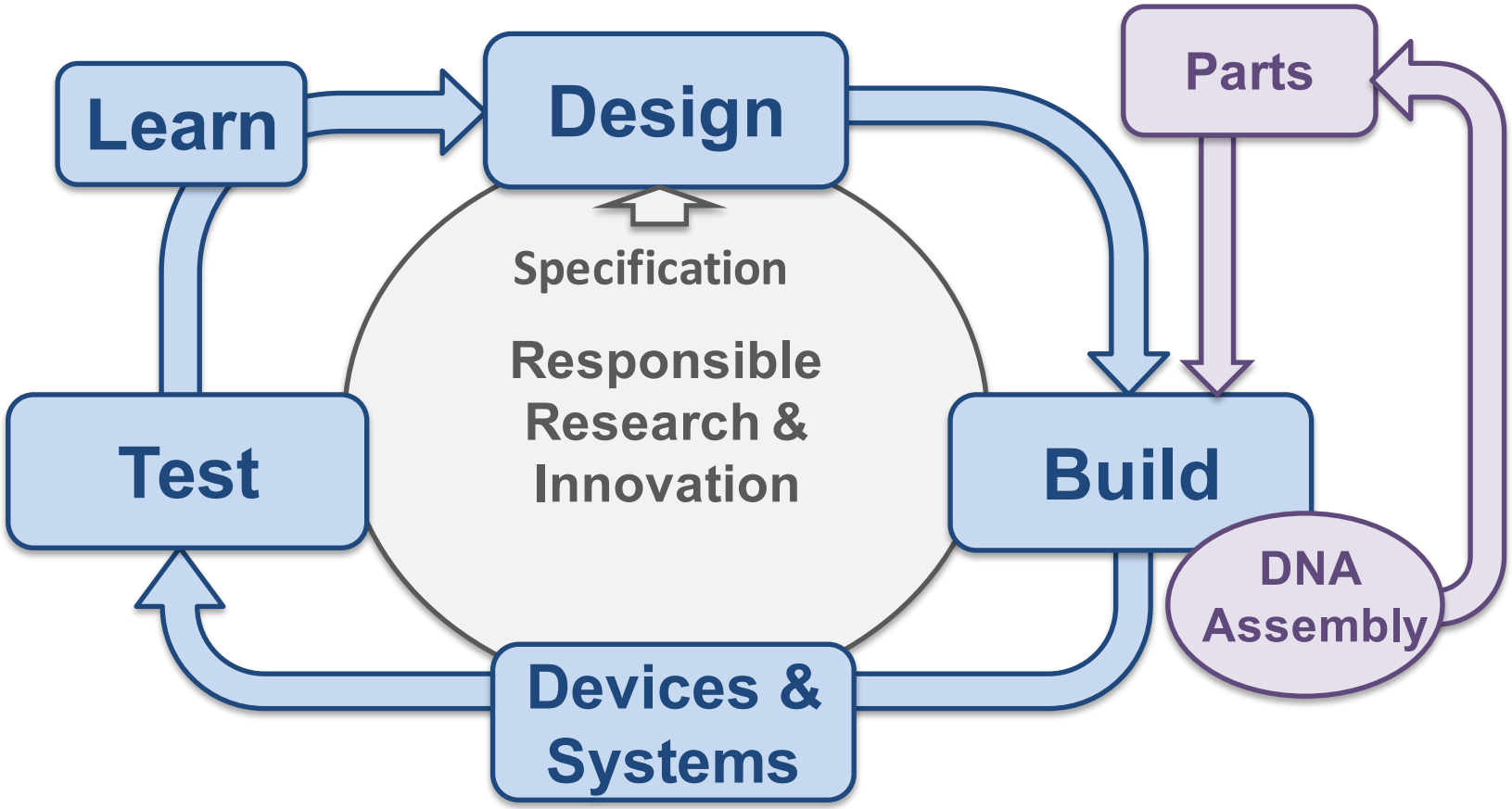
Typical gene transcription module



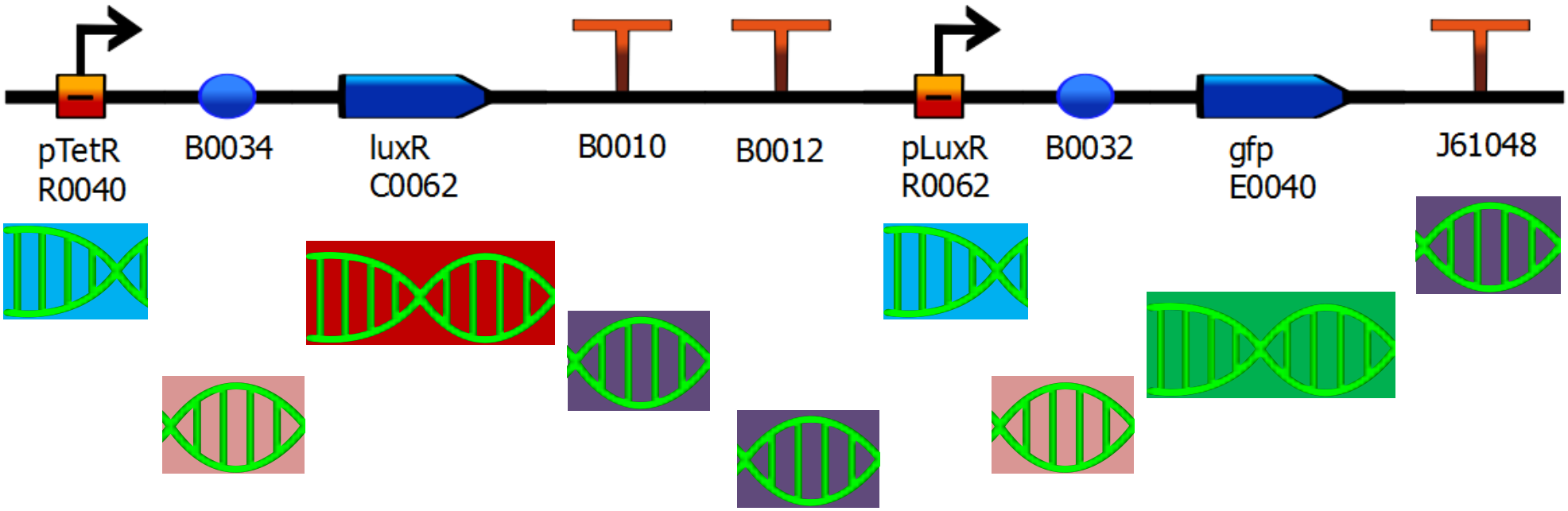
Abstraction hierarchy



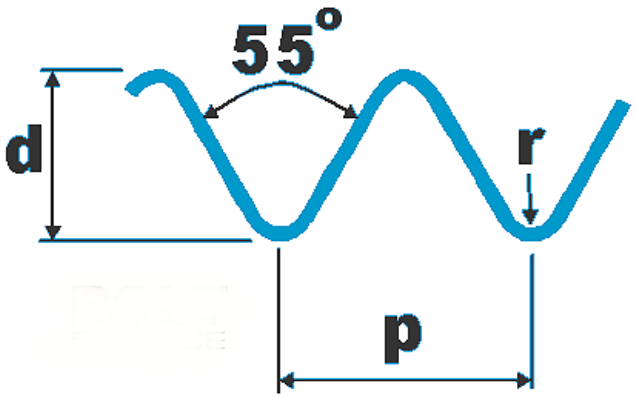
A systematic **DESIGN CYCLE** for Synthetic Biology



Can we build new biological systems with standardised DNA Parts?

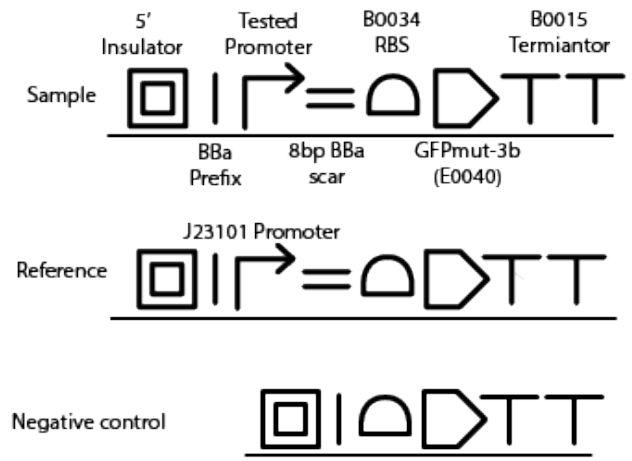


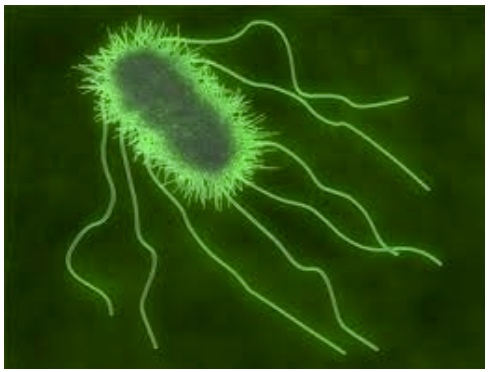
To enable forward engineering the synthetic biology field needs to develop standards



The first standard thread Sir Joseph Whitworth 1841

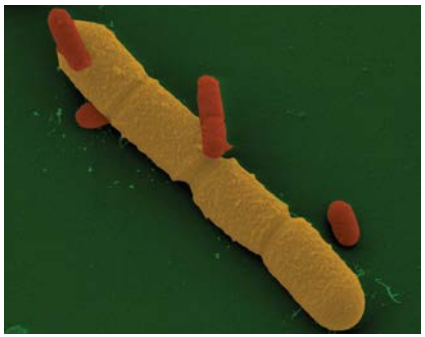
How do we standardise the construction of living matter?





E. coli

B. subtilis



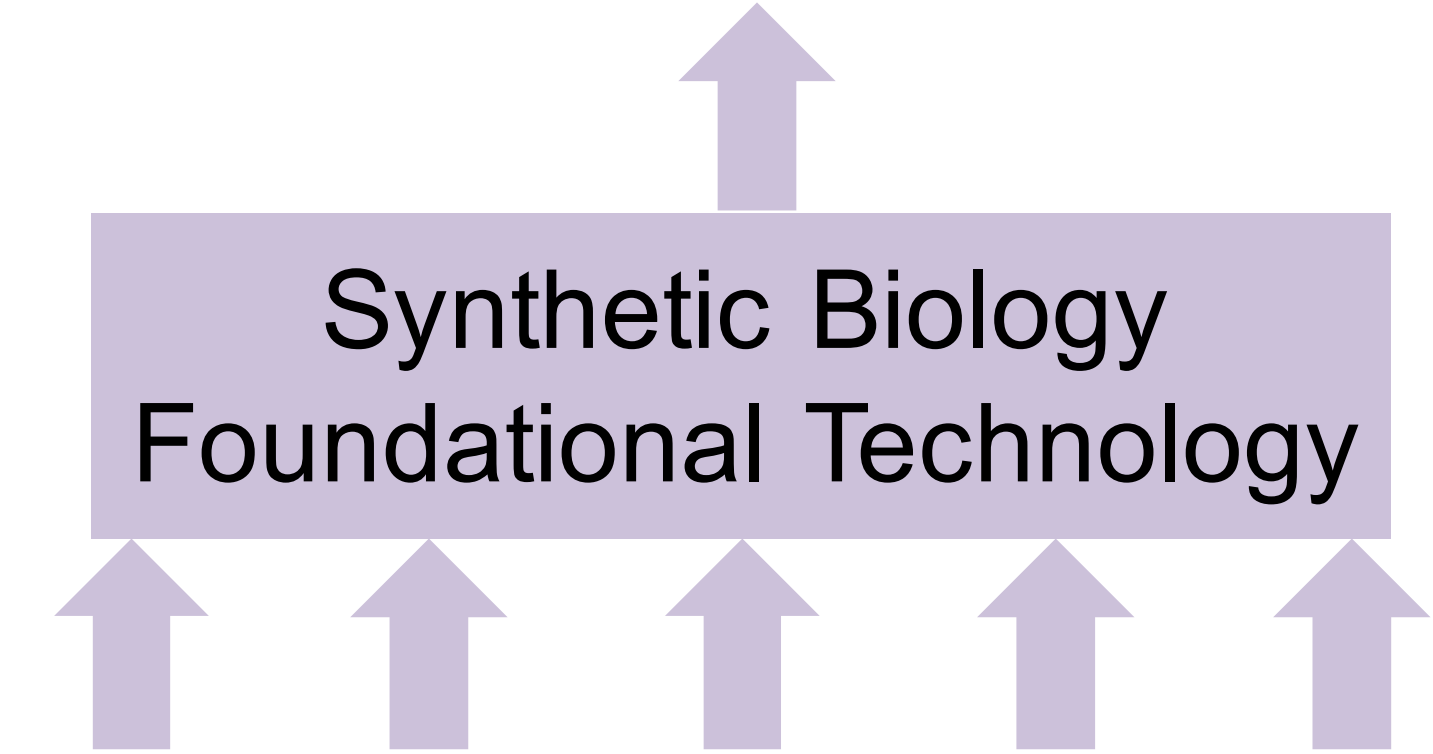
Bacillus megaterium

Standards development in synthetic biology

- Standard interchangeable biological parts

  - Physical standards (DNA)
    - Assembly standards (may not be needed with increasing DNA synthesis)
  - Functional standards
    - Standard culture conditions (media/temp/volume)
    - Standard measurements (e.g. Flow cytometry)
    - Standard strains of cell hosts or chassis
  - Digital Information standards
    - -SBOL
    - -SBML
    - -DICOM-SB

Different applications

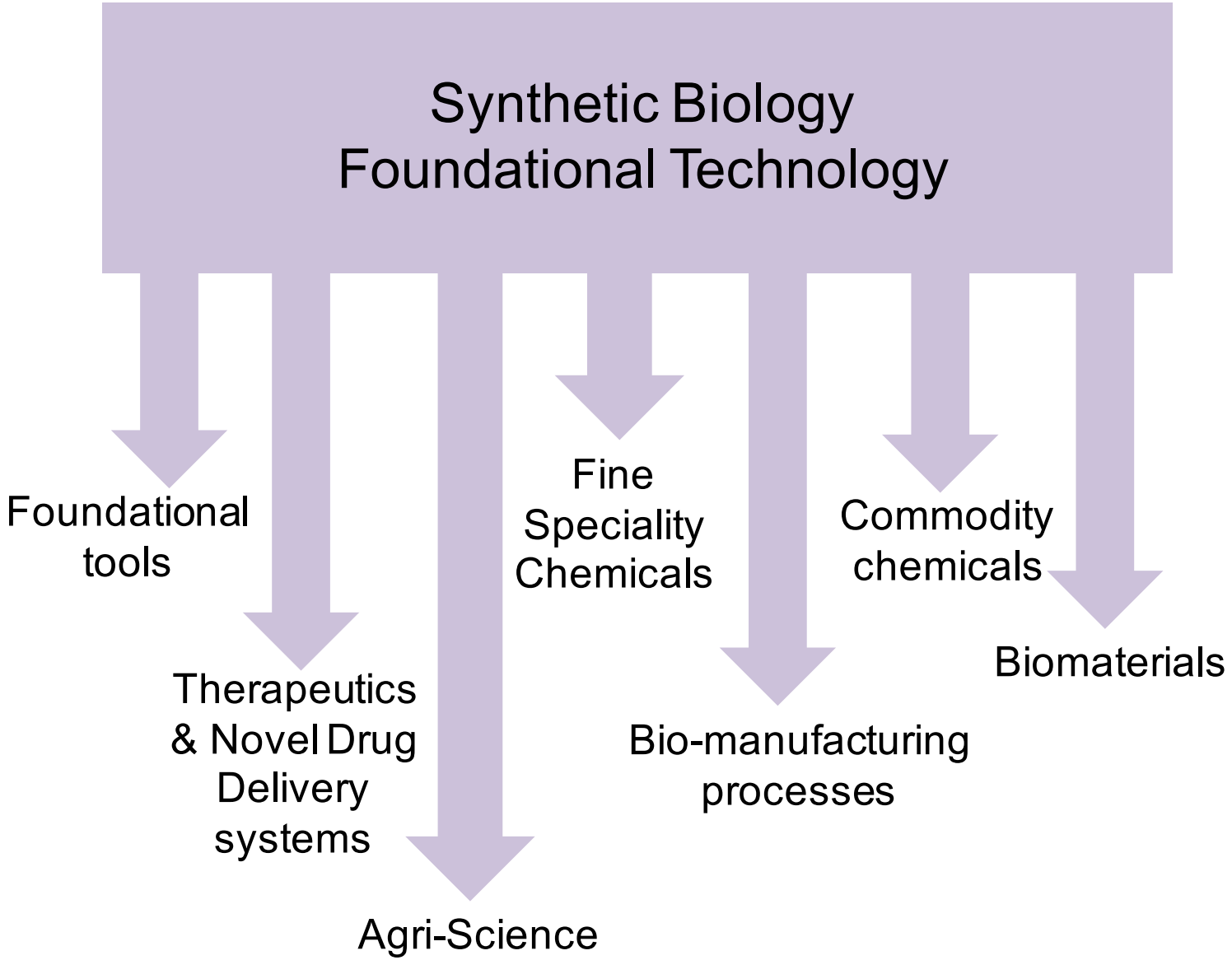


Current Synthetic Biology research trends

- Engineering of biological systems
  - Refactoring and Redesigning
  - Genome editing
  - Genome construction
  - Automation, standards and tools
  - Deskilling and open source

- Creating alternative biological systems
  - exobiology/XNA
  - Artificial cell and Cell free systems

Synthetic Biology application trends



DESIGN

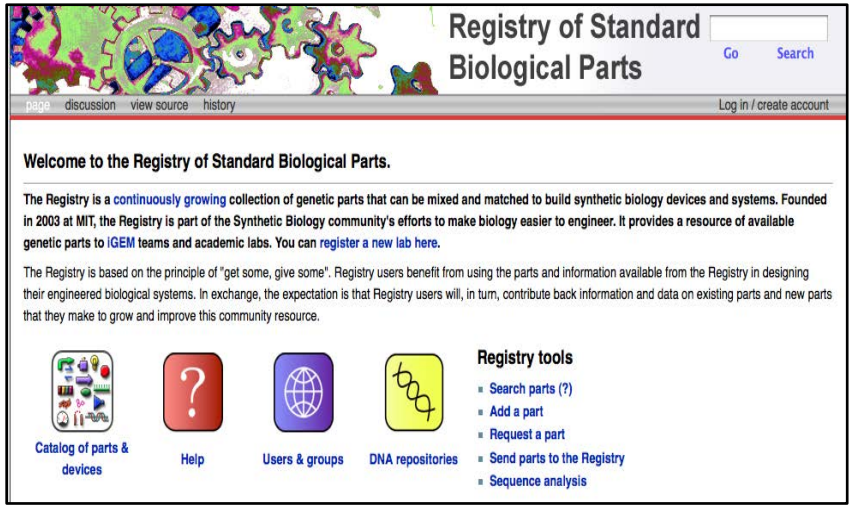
Available Bio-Design Tools

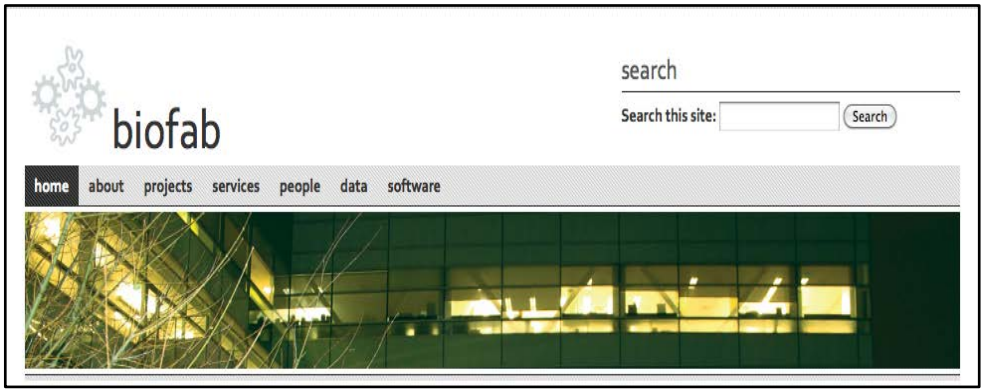
Pathway and circuit design
MATLAB: Simbiology http://www.mathworks.co.uk/products/simbiology/
OptCom http://maranas.che.psu.edu/software.htm
Genetic Engineering of Cells (GEC) http://research.microsoft.com/en-us/projects/gec/
Cell designer http://www.celldesigner.org/
ProMoT http://www.mpi-magdeburg.mpg.de/projects/promot/
GenoCAD http://www.genocad.org/
Operon calculator https://salis.psu.edu/software/OperonCalculator_EvaluateMode

Biopart design
Rosetta. http://maranas.che.psu.edu/software.htm
Cadnano. http://cadnano.org/
NUPAC http://www.nupack.org/
RNA Designer http://www.rnasoft.ca/cgi-bin/RNAsoft/RNAdesigner/rnadesign.pl
mfold/UNAfold http://mfold.rna.albany.edu/
RBS Calculator https://salis.psu.edu/software
RBS Designer http://rbs.kaist.ac.kr
UTR designer http://sbi.postech.ac.kr/utr_designer

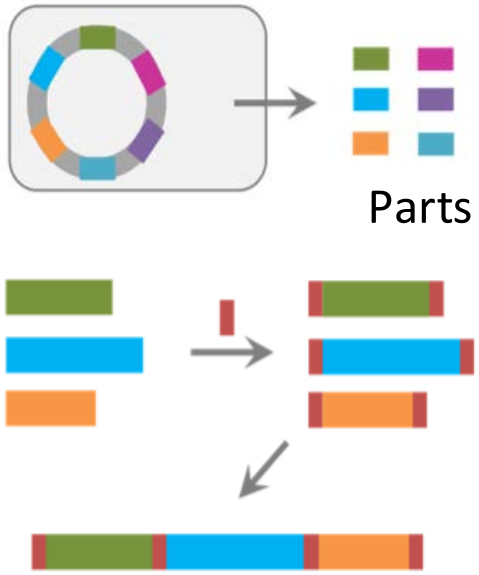
Miscellaneous
R2oDNA Designer http://r2odna.com/
SBOL http://www.sbolstandard.org/
SBOLv http://www.sbolstandard.org/visual

Part registries worldwide

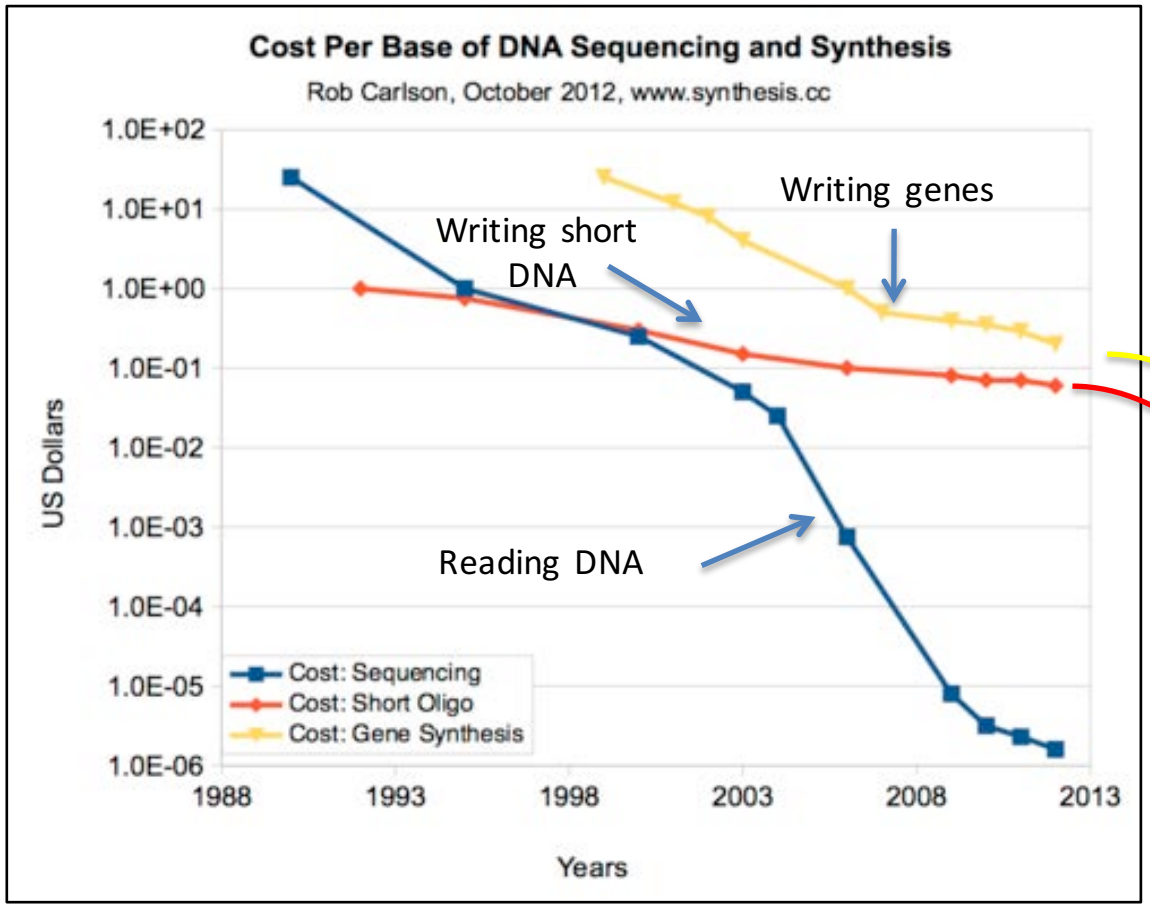




BUILD



Costs of DNA synthesis is driving the field



?

Cost per base
– sequencing ~0.000001 $
– synthesis ~0.10 – 0.28 $

Rob Carlson http://www.biodesic.com/

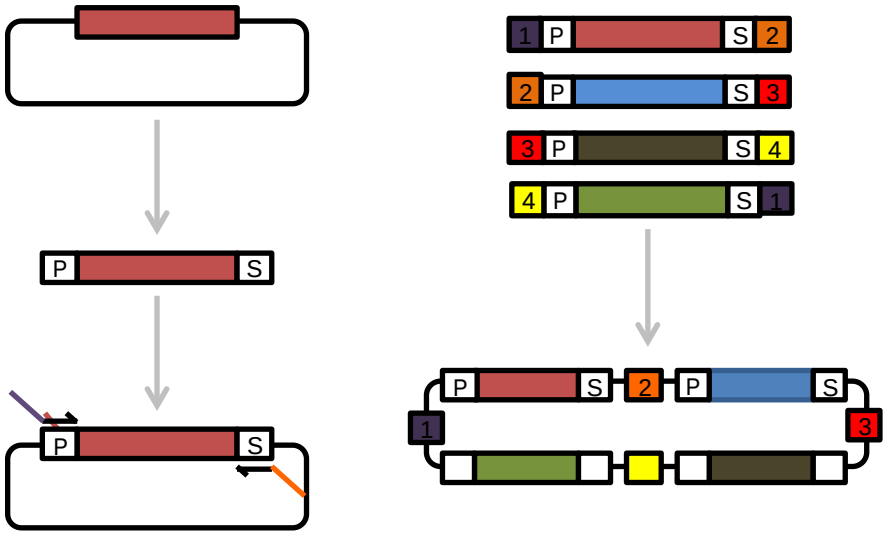
DNA assembly Standards

Tom Ellis, Geoff Baldwin

Interoperability

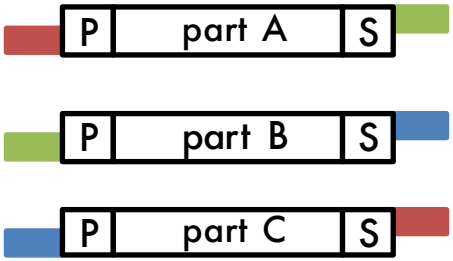
MODAL – Modular Overlap Directed Assembly with Linkers

(A. Casini et al NAR 2014a and 2014b)



BASIC - Biopart Assembly Standard for Idempotent Cloning

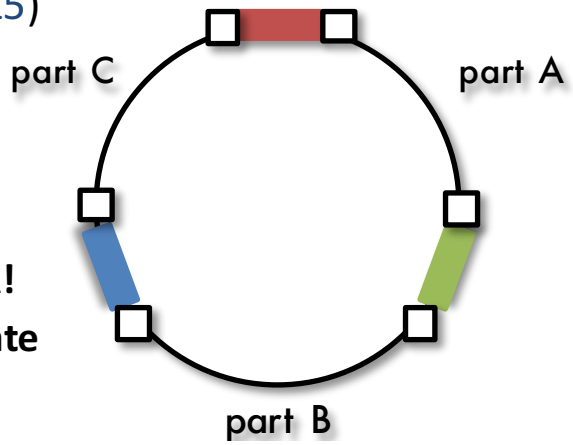
M. Storch et al ACS Synbio 4 :791 (2015)



parts ligation

Long-overhang assembly No PCR!

Robust reactions easy to automate



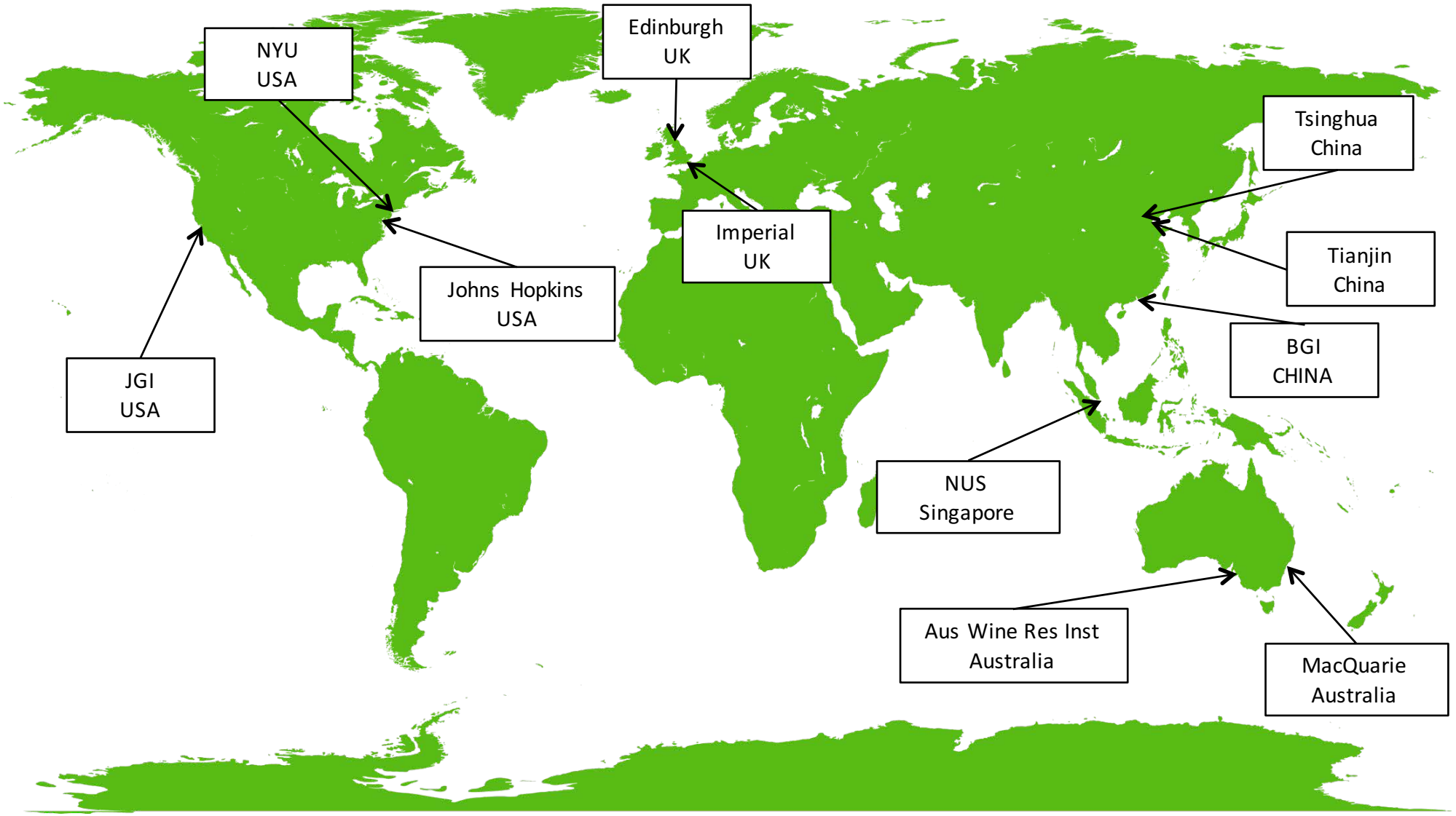
Constructing Synthetic Yeast: **Sc2.0**

Design, Synthesise & Assemble a modified version of the
S. cerevisiae genome 12 million bp and 16 chromosomes

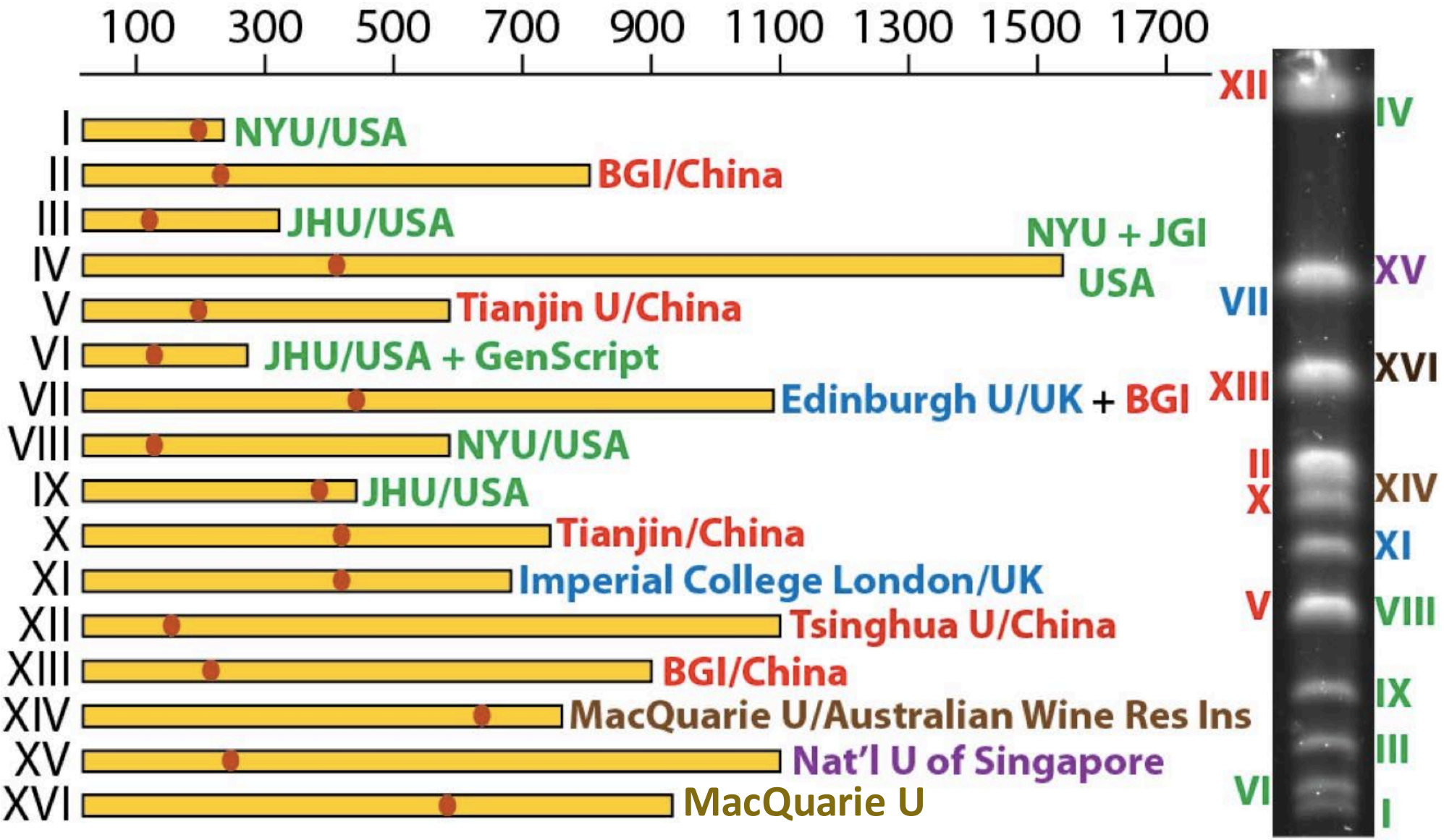
www.syntheticyeast.org
www.syntheticyeastresource.com

Jef Boeke (NY Medical School)

A global synthetic biology project



Sc2.0: 16 chromosomes, 12 million bp



2014 – Completed Syn Chromosome III

Sciencexpress Resea

EMBARGOED UNTIL 2:00 PM US ET THURSDAY, 27 MARC

Total Synthesis of a Functional Designer Eukaryotic Chromosome

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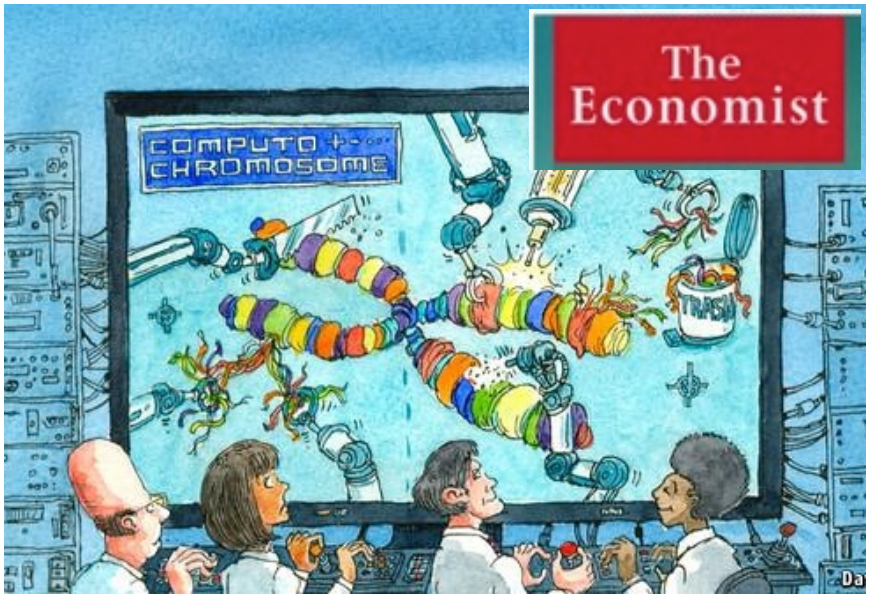
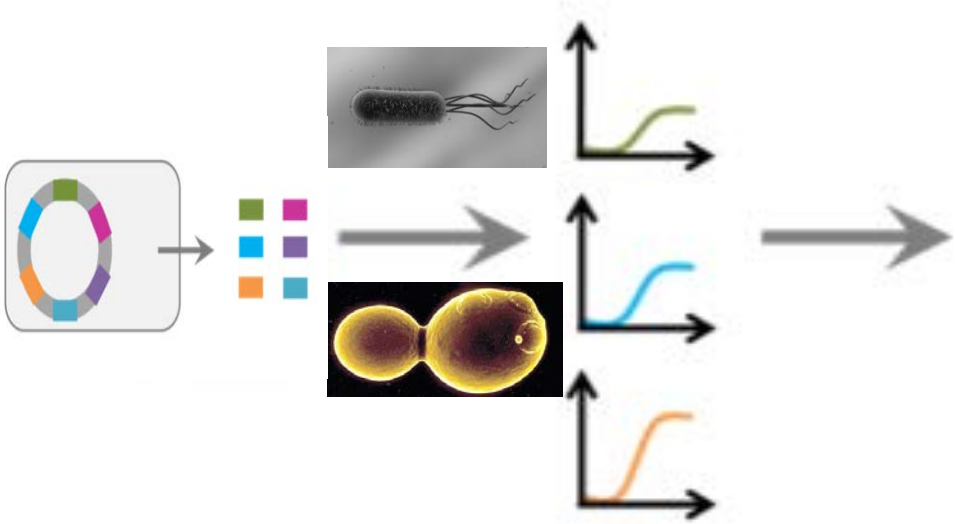
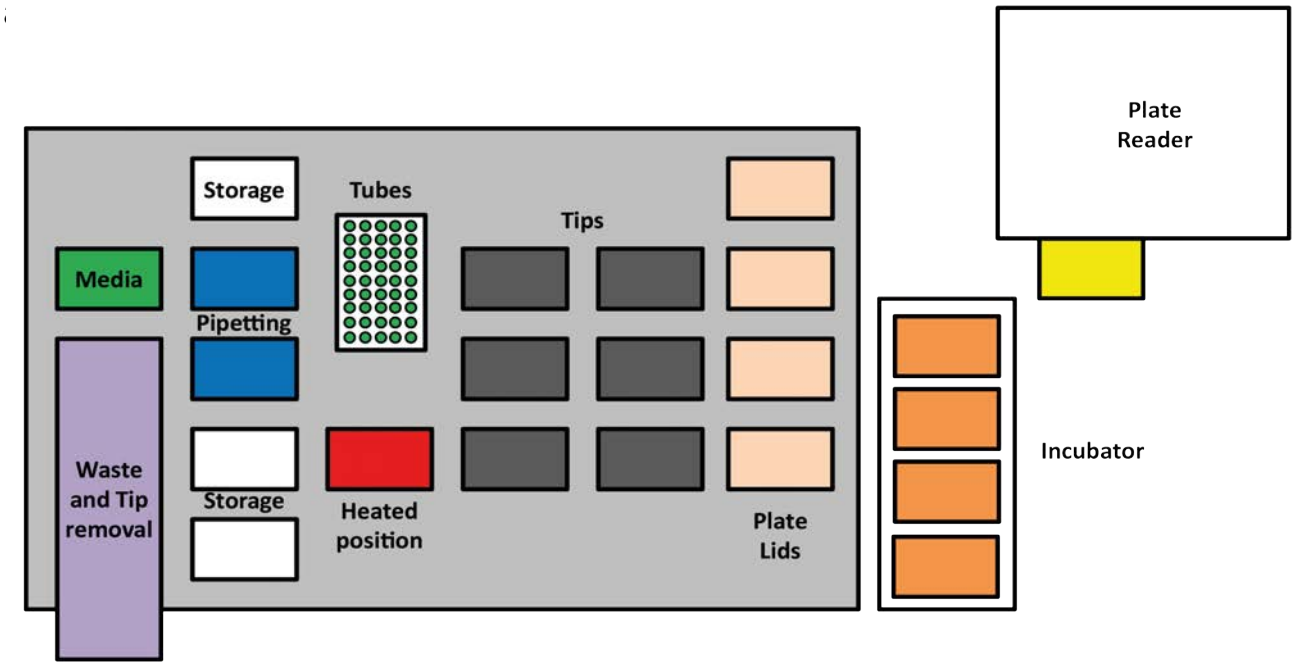
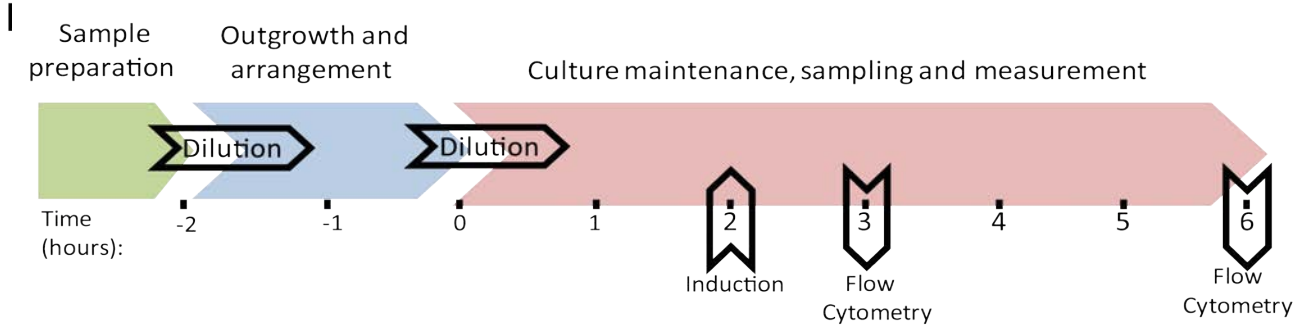


TEST



Automation characterisation platform v1.0





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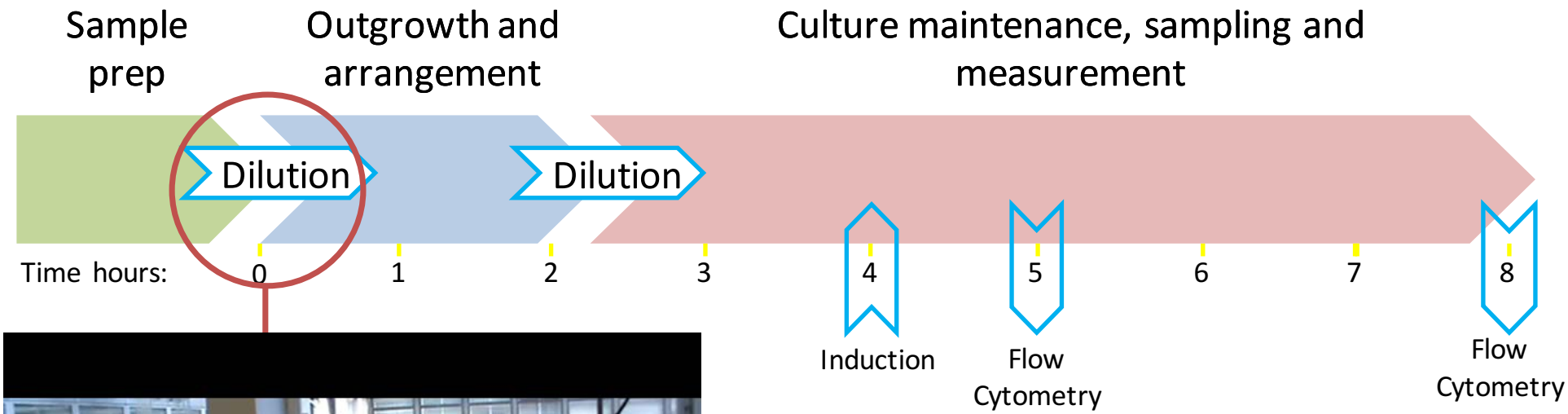
Automation characterisation platform v1.0



- Cells kept at similar phase of growth
  - Ensure high quality data
  - Ensures cells are at an appropriate population for assay
- Growth and measurement separated
  - Reduces noise in data
  - Greatly reduces evaporation

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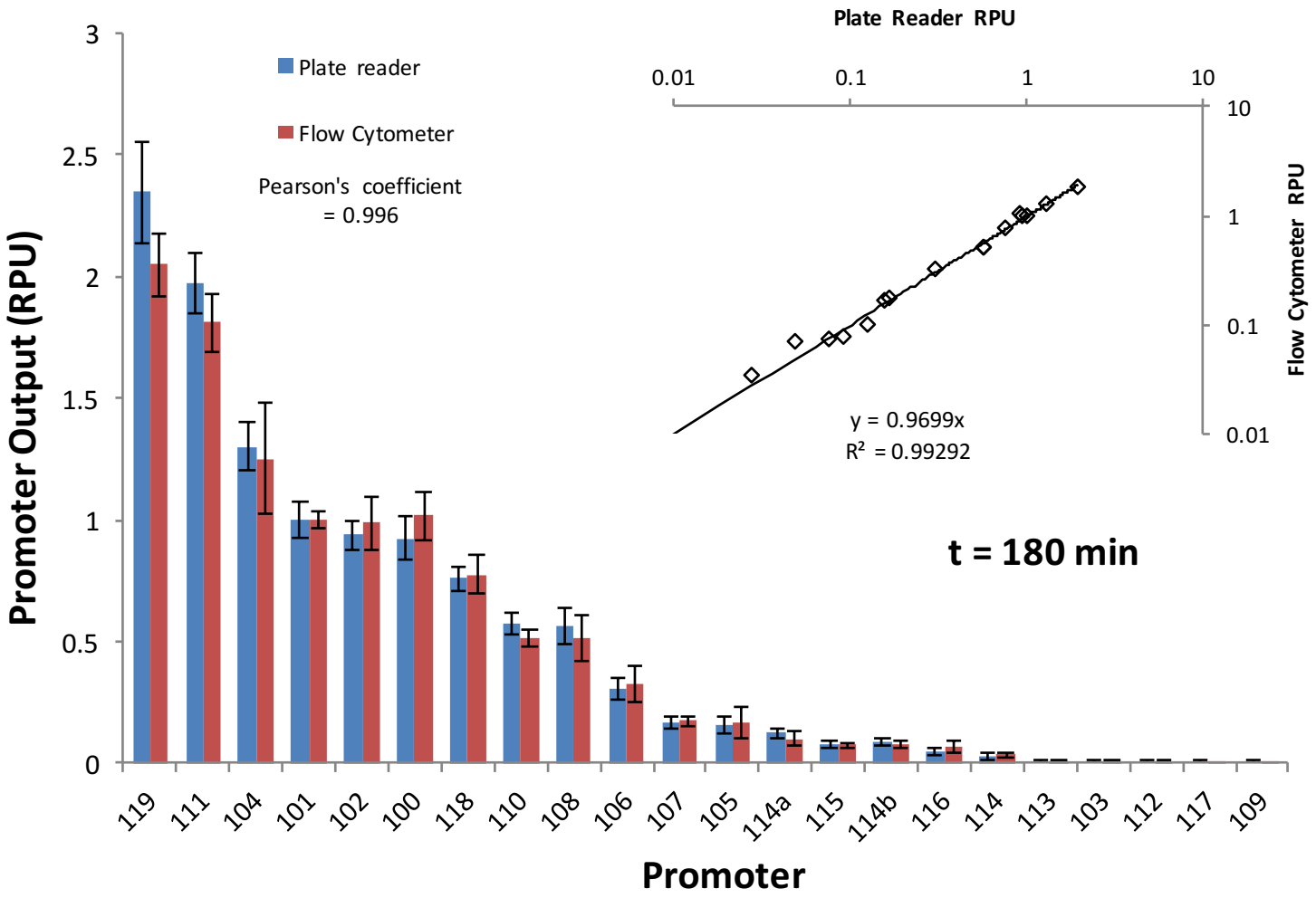
Anderson 22x promoter characterisation



Single cell versus population measurements show high degree of correlation

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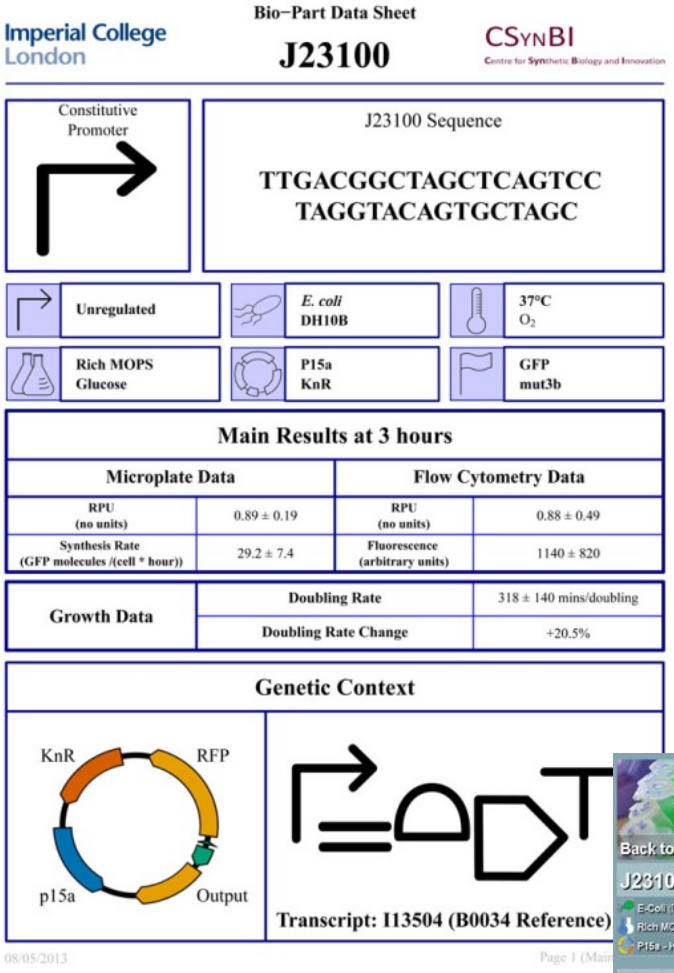
Data Sheets for Parts and SynBIS



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Bio–Part Data Sheet

J23100

CSynBI Centre for Synthetic Biology and Innovation

Constitutive Promoter

J23100 Sequence

TTGACGGCTAGCTCAGTCC
TAGGTACAGTGCTAGC

Unregulated	*E. coli* DH10B	37°C O_2
Rich MOPS Glucose	P15a KnR	GFP mut3b

Main Results at 3 hours

Microplate Data		Flow Cytometry Data	
RPU (no units)	0.89 ± 0.19	RPU (no units)	0.88 ± 0.49
Synthesis Rate (GFP molecules /(cell * hour))	29.2 ± 7.4	Fluorescence (arbitrary units)	1140 ± 820

Growth Data		
	Doubling Rate	318 ± 140 mins/doubling
	Doubling Rate Change	+20.5%

Genetic Context

Transcript: I13504 (B0034 Reference)

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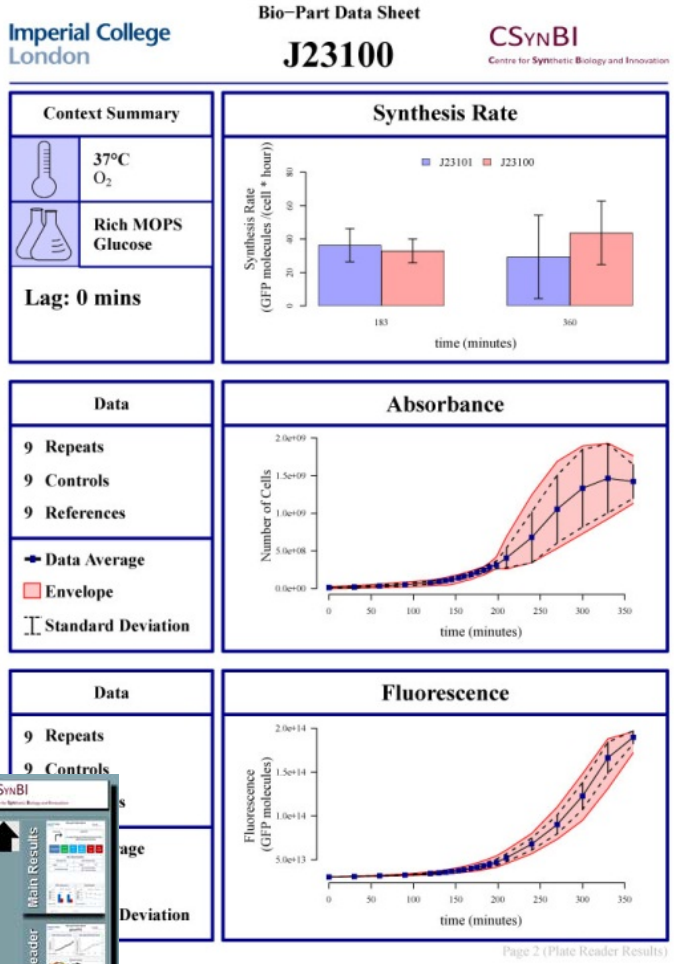

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Bio–Part Data Sheet

J23100

CSynBI Centre for Synthetic Biology and Innovation

Context Summary

37°C O_2

Rich MOPS Glucose

Lag: 0 mins

Synthesis Rate

Data

9 Repeats
9 Controls
9 References

Data Average
Envelope
Standard Deviation

Absorbance

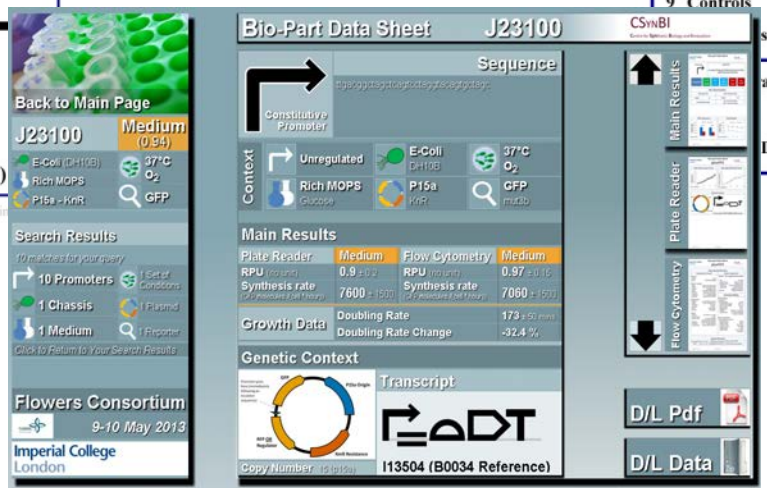
Data

9 Repeats
9 Controls

Deviation

Fluorescence

Page 2 (Plate Reader Results)



Automation characterisation platform v2.0 @Imperial College

Summary

- Automation and standardised metrology is accelerating the application of synthetic biology
- Data for part / device characterisation is being shared openly
- New chassis are being constructed e.g. Sc2.0
- Standards are being developed and shared
- Huge growth and interest by younger researchers
- Non-biologists are now doing synthetic biology e.g. Engineers
- Significant growth of community labs worldwide- see (www.biobuilder.org)