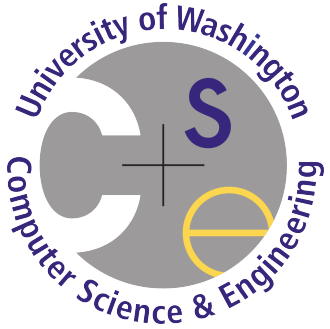
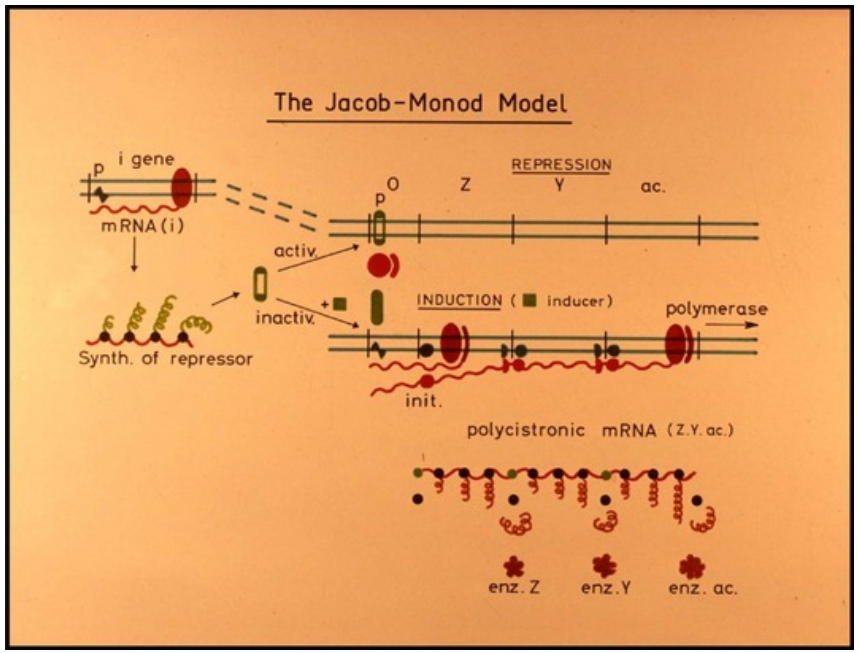


Module 8: Introduction to synthetic biology

CSE590: Molecular programming and neural computation.

Circuits

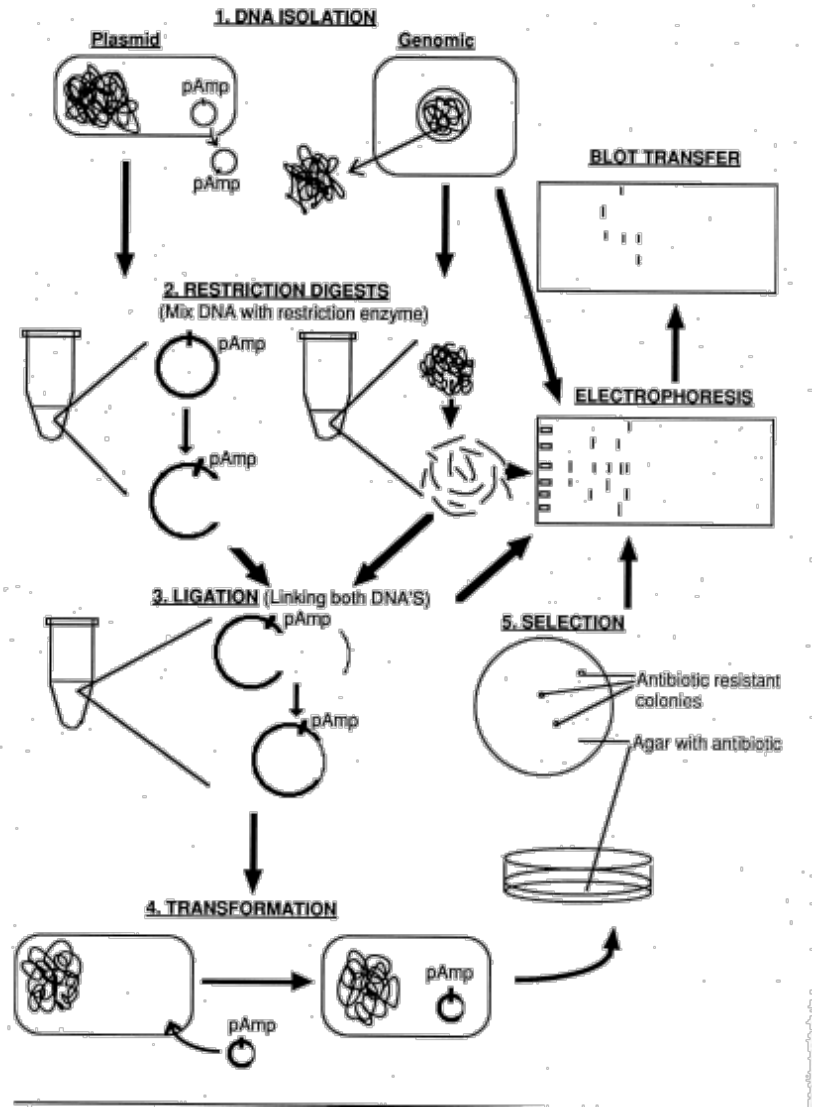
Jaques Monod (1910-1976): Elucidated regulation of gene expression by proteins to create feedback loops.



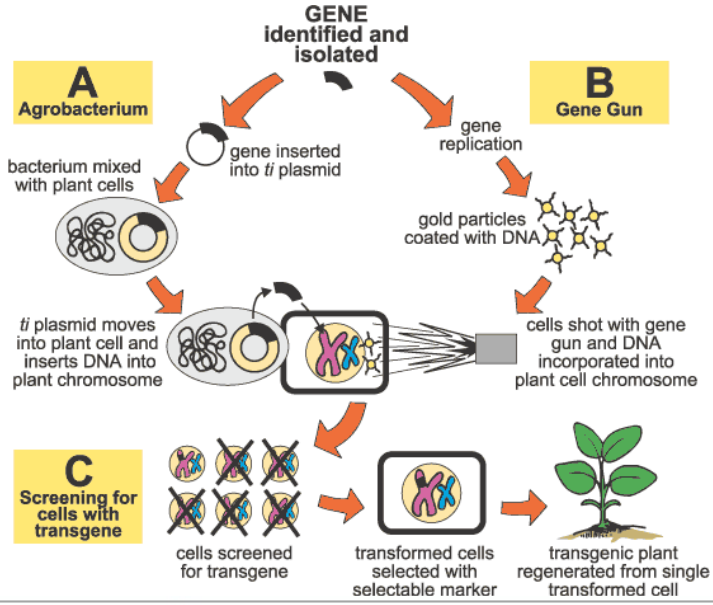
"Tout ce qui est vrai pour le Colibacille est vrai pour l'éléphant".

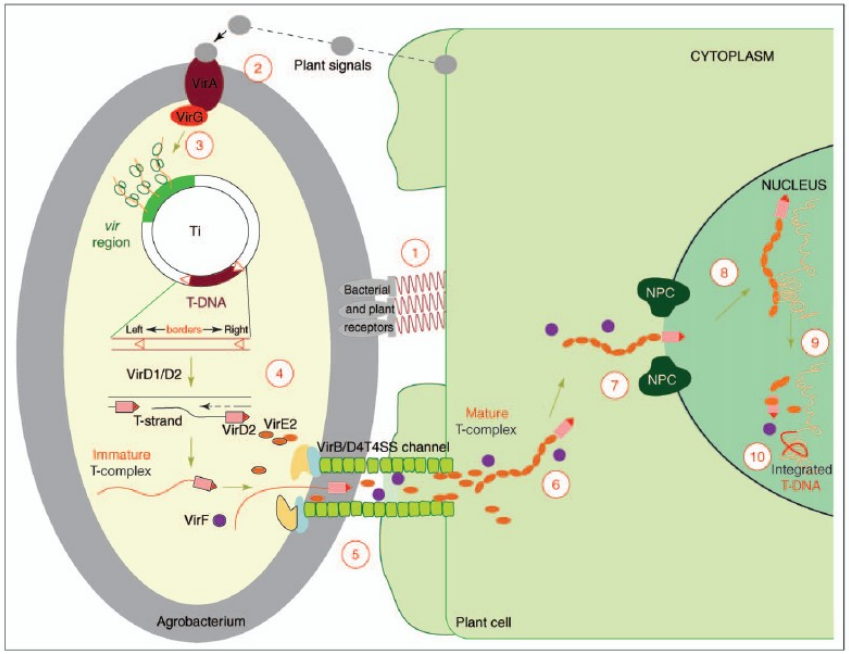
"All that is true for the Colon bacillus [e.g. e. coli] is true for the elephant"

Recombinant DNA

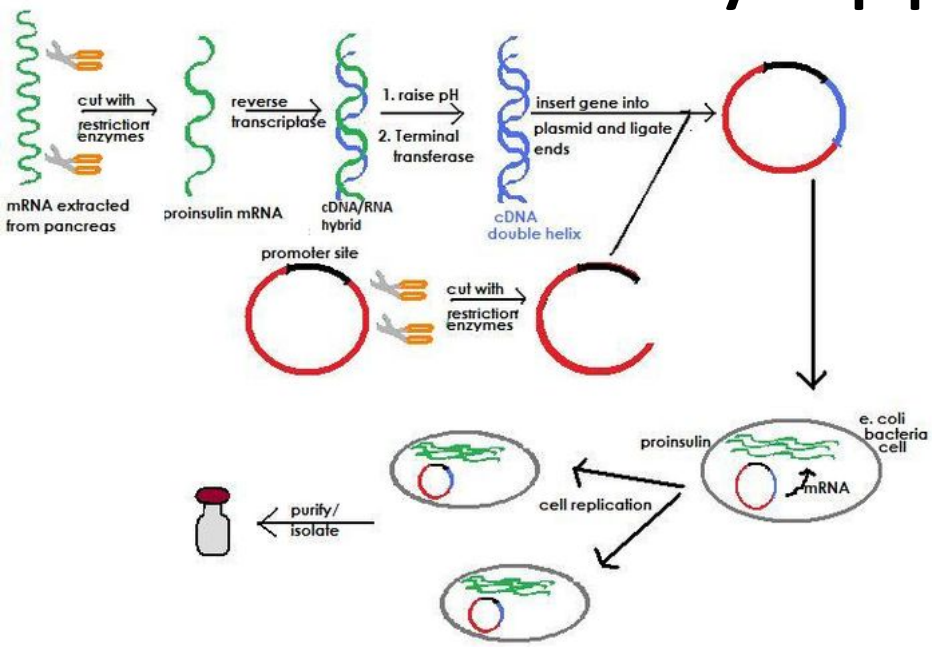


Chemical- or electroporation.





Early Applications



Insulin Production in E. coli in 1978.

Herbert Boyer (b. 1936) co-founded Genetech.

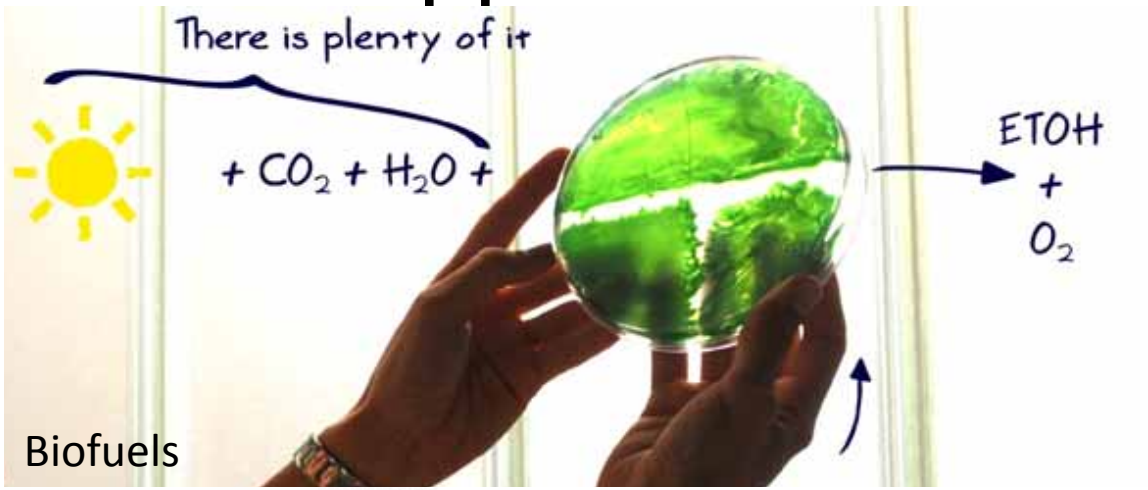
B. thuringiennsis + tobacco = insecticide resistant tobacco for cancer sticks!

Marc Van Montageau and Jozef Schell founded Plant Genetic Systems, now part of Bayer.

Bayer CropScience

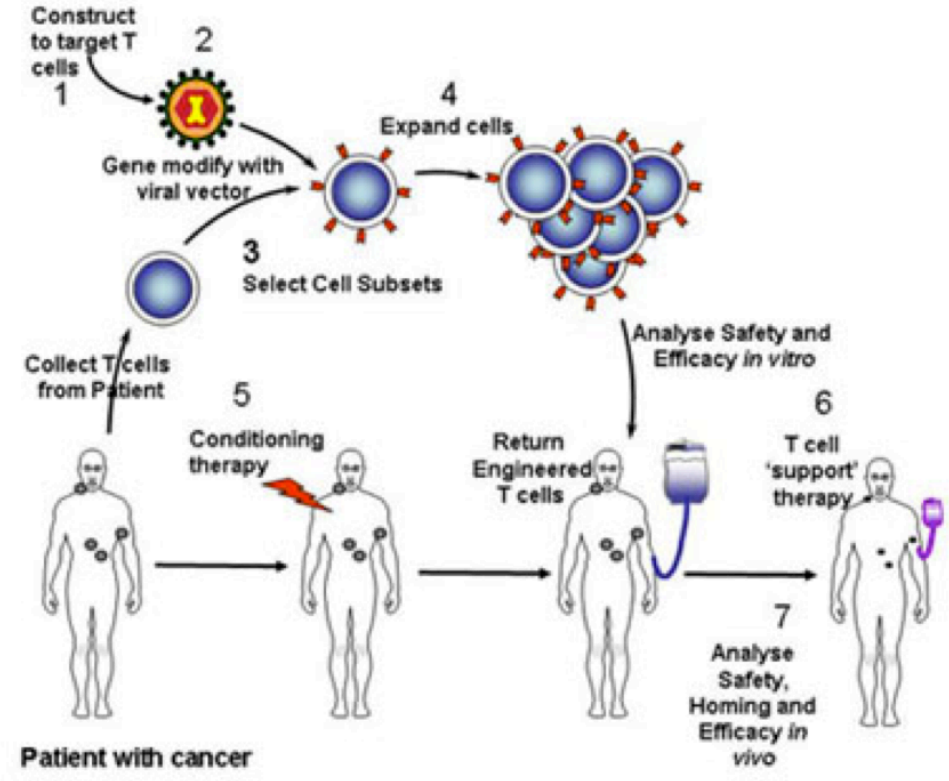
More Applications

Dolly, 1996



Biofuels

Cat with green fluorecent protein from jellyfish (2011).



T-cell therapy (in trials, 2007)

Genetic Engineering and Synthetic Biology

Jim Collins (BU)

Genetic Engineering is like replacing a light bulb in a 747 and saying you engineered a new kind of airplane.

-Jim Collins (2010, paraphrased)

E. coli engineered to respond to light, Voigt, Tabor, et al (2004).

Waclaw Szybalski (b. 1921)

Let me now comment on the question "what next". Up to now we are working on the descriptive phase of molecular biology. ... But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field with the unlimited expansion potential and hardly any limitations to building "new better control circuits" and ..... finally other "synthetic" organisms, like a "new better mouse". ... I am not concerned that we will run out of exciting and novel ideas, ... in the synthetic biology, in general.

- Waclaw Szybalski (1974)

NATURE | VOL 403 | 20 JANUARY 2000 | www.nature.com

The Birth of Synthetic Biology as a Research Field

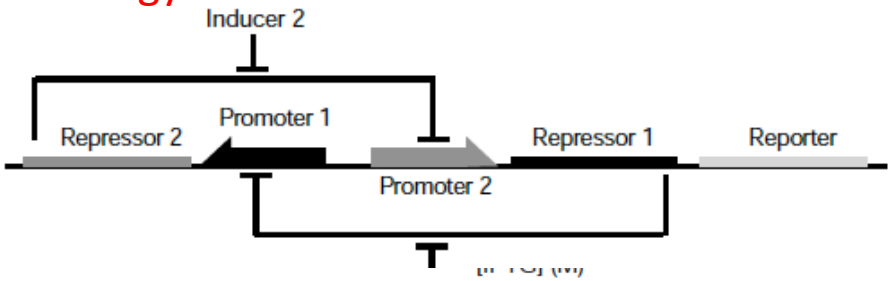
Construction of a genetic toggle switch in *Escherichia coli*

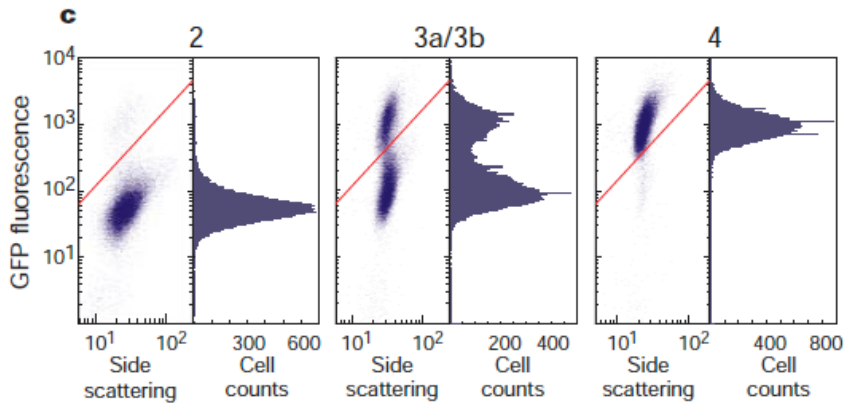
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

* *Department of Biomedical Engineering,* † *Center for BioDynamics and* ‡ *Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

It has been proposed[1] that gene-regulatory circuits with virtually any desired property can be constructed from networks of simple regulatory elements. These properties, which include multistability and oscillations, have been found in specialized gene circuits such as the bacteriophage λ switch[2] and the Cyanobacteria circadian oscillator[3]. However, these behaviours have not been demonstrated in networks of non-specialized regulatory components. Here we present the construction of a genetic toggle switch—a synthetic, bistable gene-regulatory network—in *Escherichia coli* and provide a simple theory that predicts the conditions necessary for bistability. The toggle is constructed from any two repressible promoters arranged in a mutually inhibitory network. It is flipped between stable states using transient chemical or thermal induction and exhibits a nearly ideal switching threshold. As a practical device, the toggle switch forms a synthetic, addressable cellular memory unit and has implications for biotechnology, biocomputing and gene therapy.

The design and construction of synthetic gene-regulatory net-





ring ampicillin resistance and containing the pBR322 ColE1 replication origin. The toggle switch genes are arranged as a type IV plasmid, as shown in Fig. 3. Although all genes and promoters are

Box 1

The toggle model

The behaviour of the toggle switch and the conditions for bistability can be understood using the following dimensionless model for the network:

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u \qquad (1a)$$

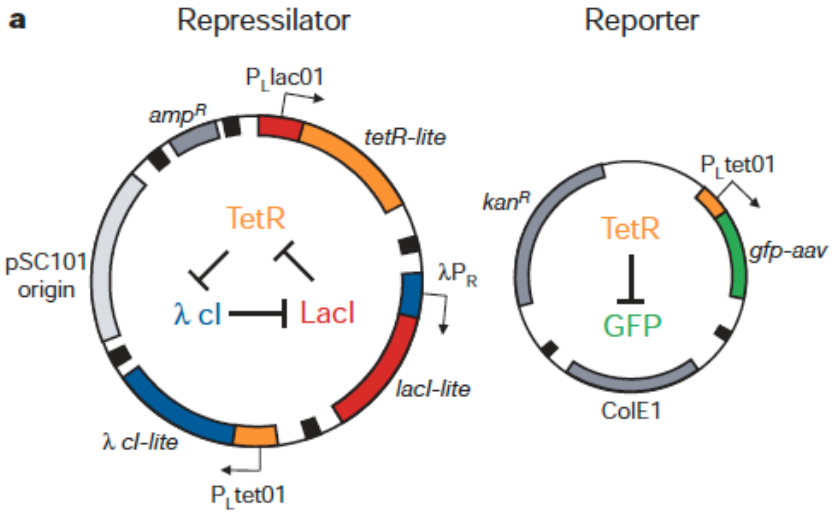
$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v \qquad (1b)$$

A synthetic oscillatory network of transcriptional regulators

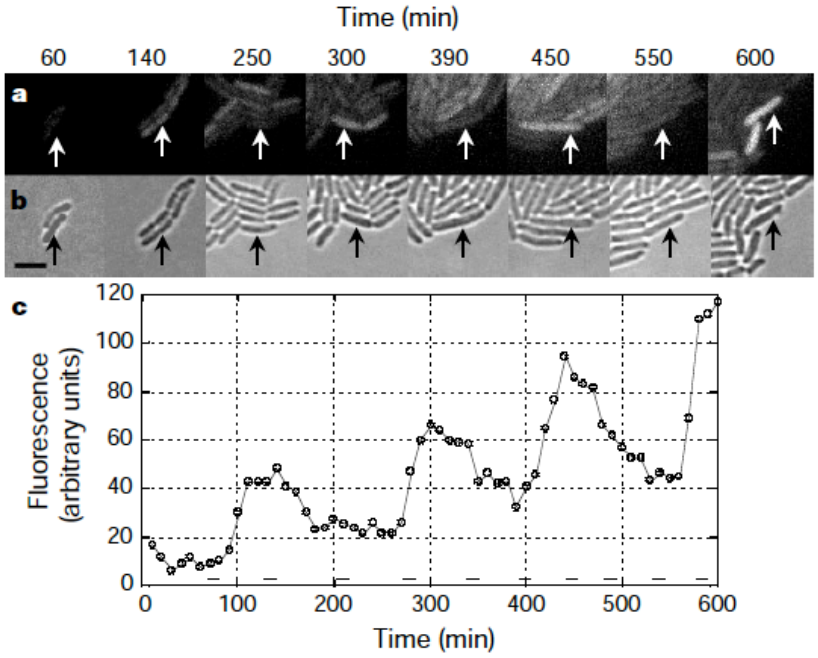
Michael B. Elowitz & Stanislas Leibler

Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA

Networks of interacting biomolecules carry out many essential functions in living cells[1], but the 'design principles' underlying the functioning of such intracellular networks remain poorly understood, despite intensive efforts including quantitative analysis of relatively simple systems[2]. Here we present a complementary approach to this problem: the design and construction of a synthetic network to implement a particular function. We used three transcriptional repressor systems that are not part of any natural biological clock[3–5] to build an oscillating network, termed



Michael Elowitz
(Caltech)



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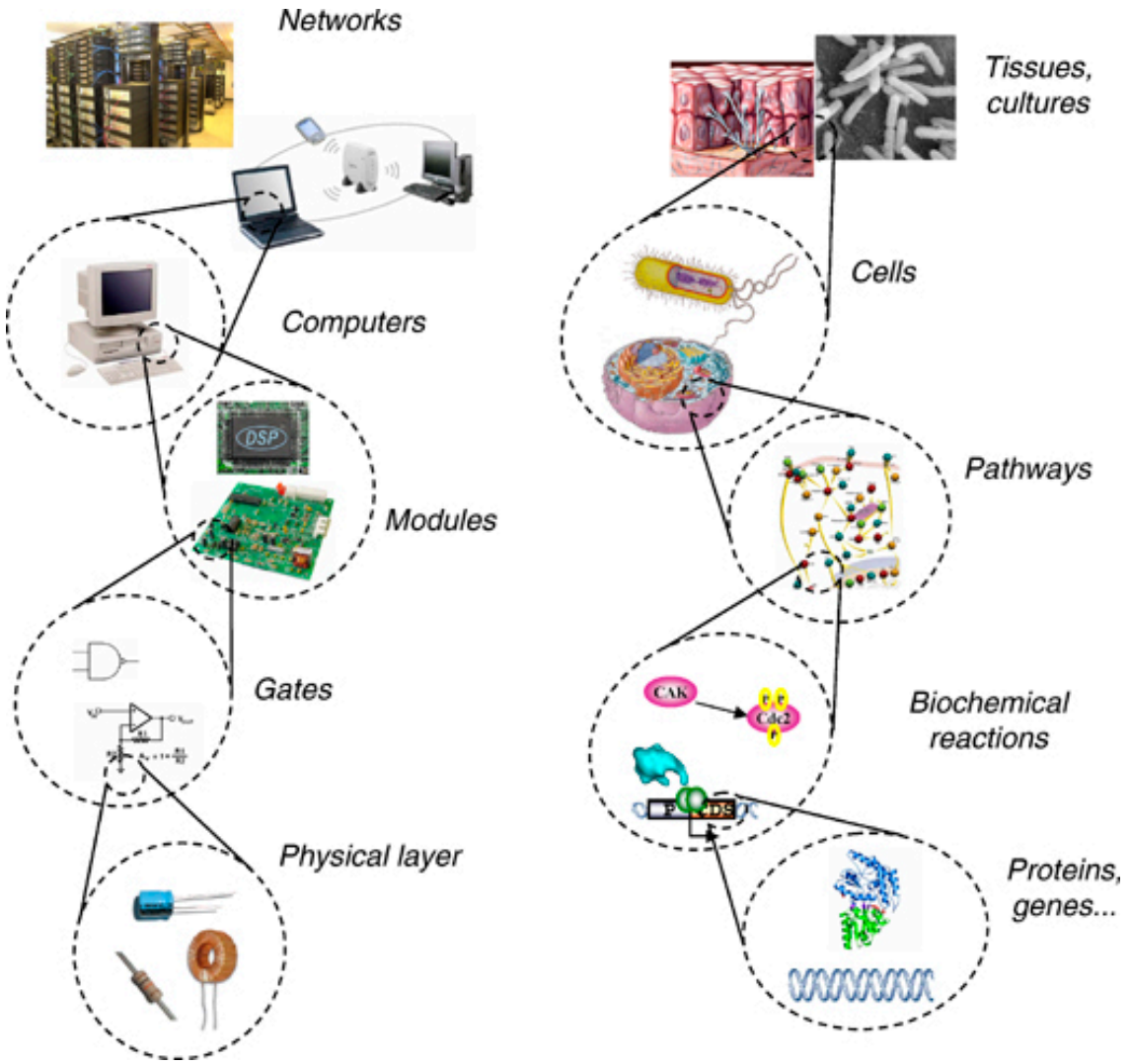
So What is Synthetic Biology?!?

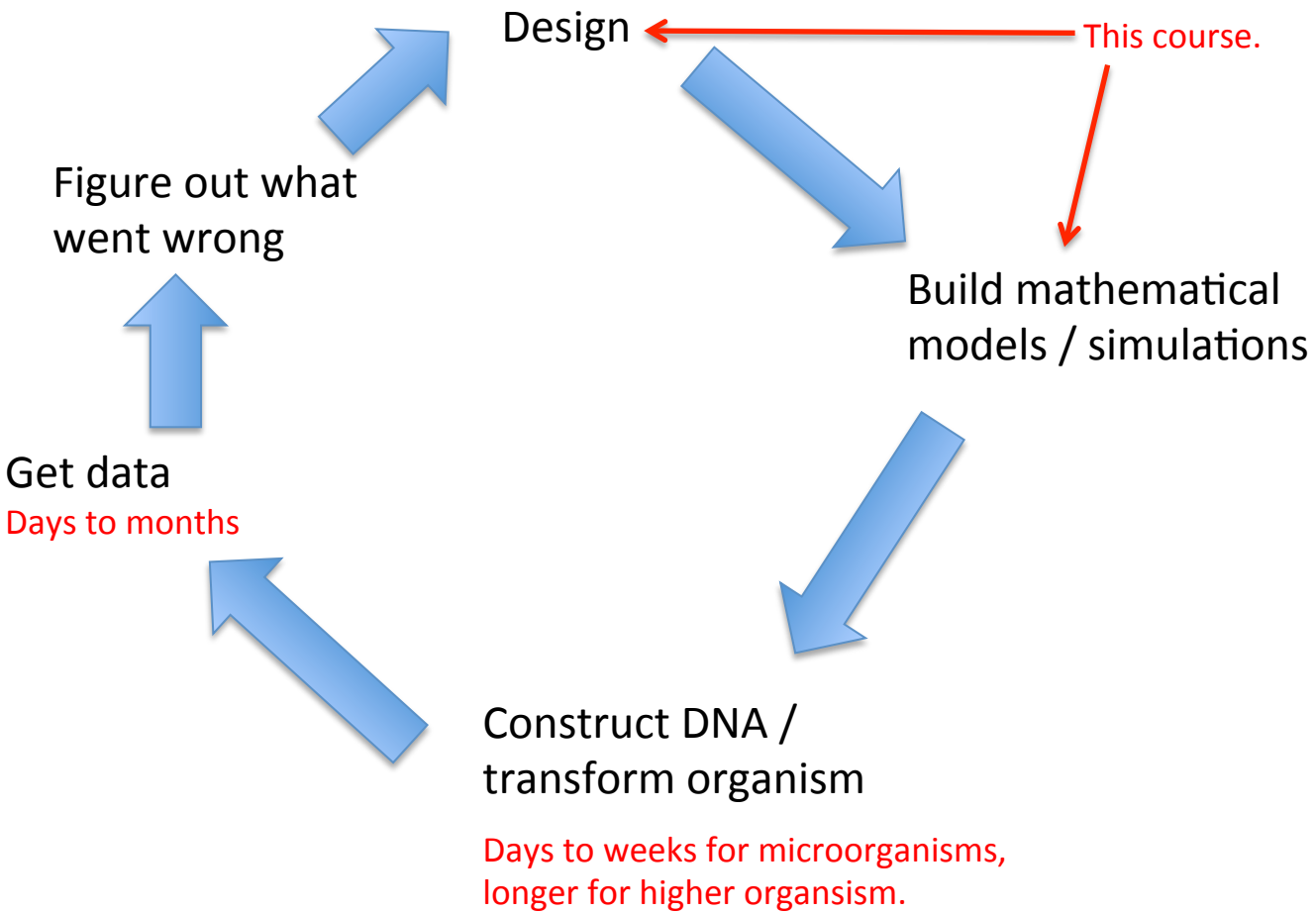


Engineering + Biology:

SB is what happens when computer scientists and electrical engineers learn genetic engineering: They make logic gates, amplifiers, oscillators, communication devices, and internets.

The goal is to engineer information processing systems for new living systems!

What Synthetic Biologists Do



Current Bottleneck: Cutting, pasting and integrating DNA is very laborious.

The changing economics of DNA synthesis

Robert Carlson

How are the economics of synthetic biology likely to develop in the coming years?

Biological technologies come in many different guises. For millennia, humans have used selection and breeding to direct the evolution of organisms in a sort of top-down approach, a powerful but unpredictable means to achieve a desired behavior. At the opposite extreme, genes and genomes can now be written from chemical precursors, a more precise but sometimes less effective means of producing a particular biological behavior—the design rules for bottom-up engineering of biology in the vast majority of cases are still poorly understood. In between, practicing metabolic engineers use any and all tools at hand to herd and cajole organisms into producing products with market value in the many hundreds of billions of dollars.

At the core of all these approaches to biological engineering is the creation of a particular genomic sequence that produces behaviors according to human desire or need.

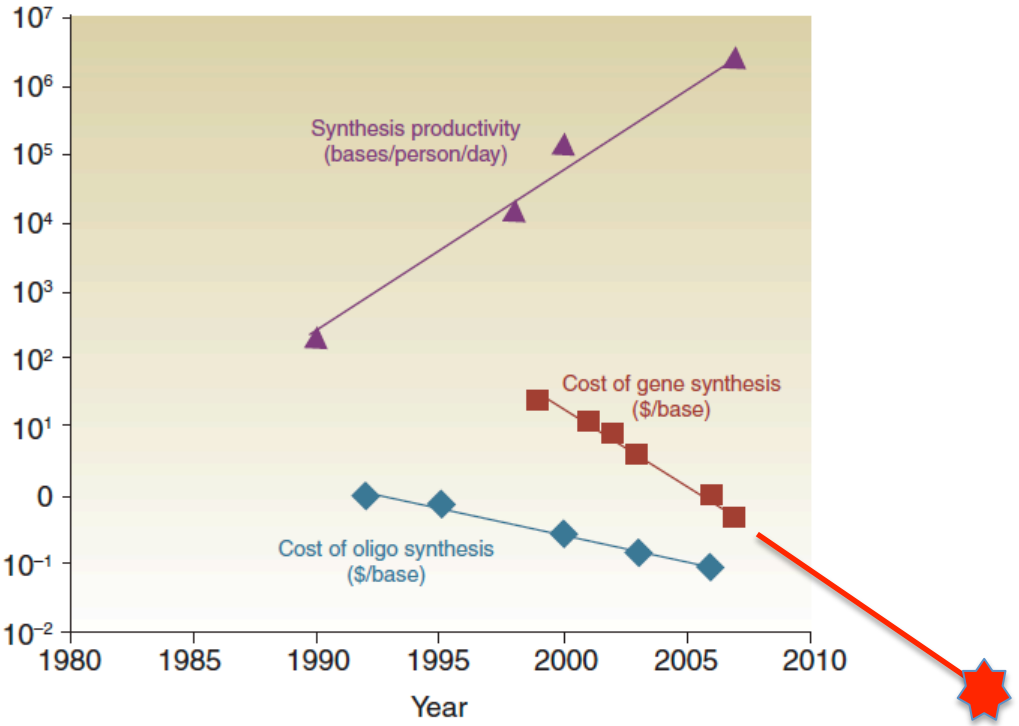


Figure 1 Productivity of oligo synthesis and cost of oligos and genes.

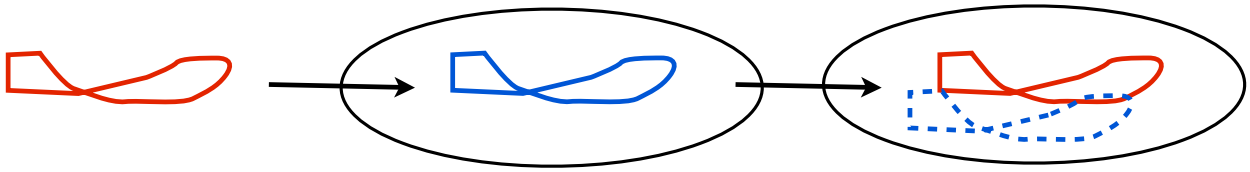
By 2015 or 2020:

Synthetic E. coli genome 4.6 Mbp x 10^{-4} dollars/bp = **$460** (less than an iPad)!

Synthetic Human Genome: 3.4 Bbp x 10^{-4} dollars/bp = **$340K** (less than a house)!

State of the art in genome synthesis

Installing a synthetic genome



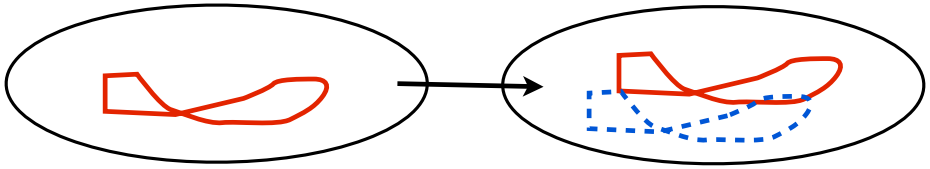
Synthetic genome
"new operating system"

Host cell

Install

Step 1: Complete synthesis of the genome from *Mycoplasma genitalium*, 580K basepairs

Step 2: Genome transfer from one cell to another (similar) cell



Mycoplasma mycoides capri

Mycoplasma capricolum

Craig Venter
Hamilton Smith
and others

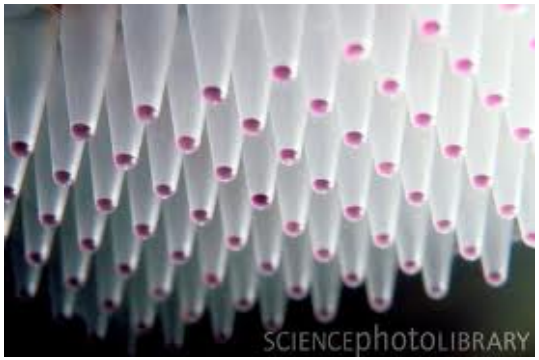
The Goal: Programming Life

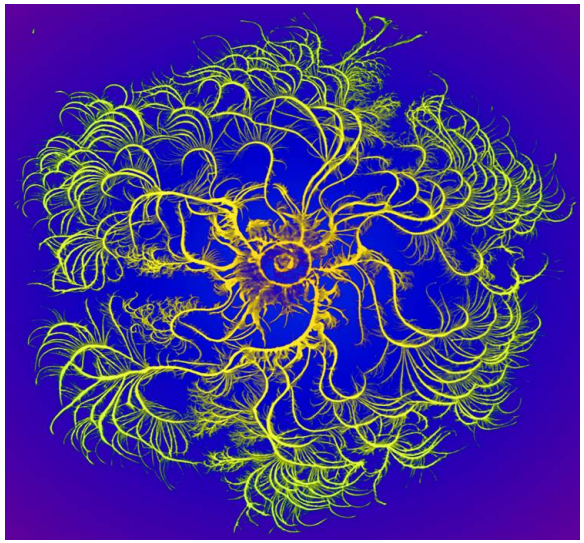
```
rate(k1) : {
  emit_signal ( ahl, se )
}

r.t > tr & get_signal ( ahl ) > sd : {
  emit_signal ( ahl, se ),
  r.t := 0
}
```

Compile

```
ATCTACGGATCGACTAGGACTACGGACTAGGCTACGGATCGGATGAGTACAGCTAC
AGCTAGCGACTTTTAVCACGCGGCGAGCTTAGCTAGCAGGAGCGATCGTAGGATCG
ACGTATCGATCGATGCTGTAGCGGCGCGAGGATGATGCATCGATCGACTACGCTAC
ACTTACACGACGACGATCGTAGGACGAGCGACGACTACGGAGAAAGAGCGCGAGCG
GGACTAGGCTACGGGACTAGGCTACGGGACTAGGCTACGGGACTAGGCTACGGGAC
GGACTAGGCTACGACATTACGGACTAGGCTACGCTTTTACTGGACTAGGCTACGTT
TCTACGGATCGACTAGGACTACGGACTAGGCTACGGATCGGATGAGTACAGCTACA
GCTAGCGACTTTTAVCACGCGGCGAGCTTAGCTAGCAGGAGCGATCGTAGGATCGT
CGTATCGATCGATGCTGTAGCGGCGCGAGGATGATGCATCGATCGACTACGCTACC
CTTACACGACGACGATCGTAGGACGAGCGACGACTACGGAGAAAGAGCGCGAGCGG
GACTAGGCTACGGGACTAGGCTACGGGACTAGGCTACGGGACTAGGCTACGGGACA
GACTAGGCTACGACATTACGGACTAGGCTACGCTTTTACTGGACTAGGCTACGTTT
```

Synthesize





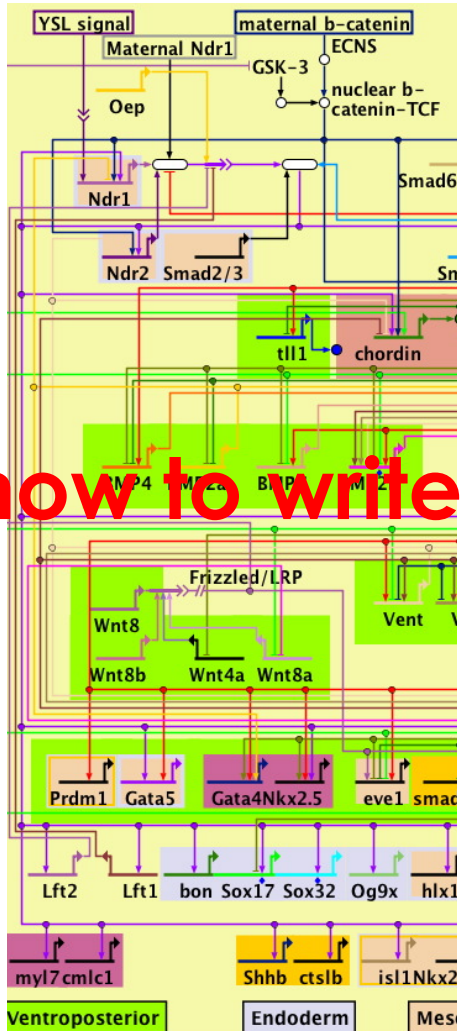
Transform,
boot up,
run

Biological inspiration

DNA Genome

...GTGGTACAGGTG
AATTTGGGTAGGCTA
AATTGTCCATAGTTT
ATGTGTGTGAATGAG
GGTGTATGGATGTTT
CTCAGAGATGGGTTG
CAGCTGGAAGGGCGT
CC[illegible]
TATGCTGGAGAAGTT
GCCGGTTCATTCTGC
TGTGGCGACCCCAGA
TTAATAAAAGGACTA
AGCCGAAAAGAAAAT
GAAACATATATATAT
ATATATATATATATA
TATATATATA...

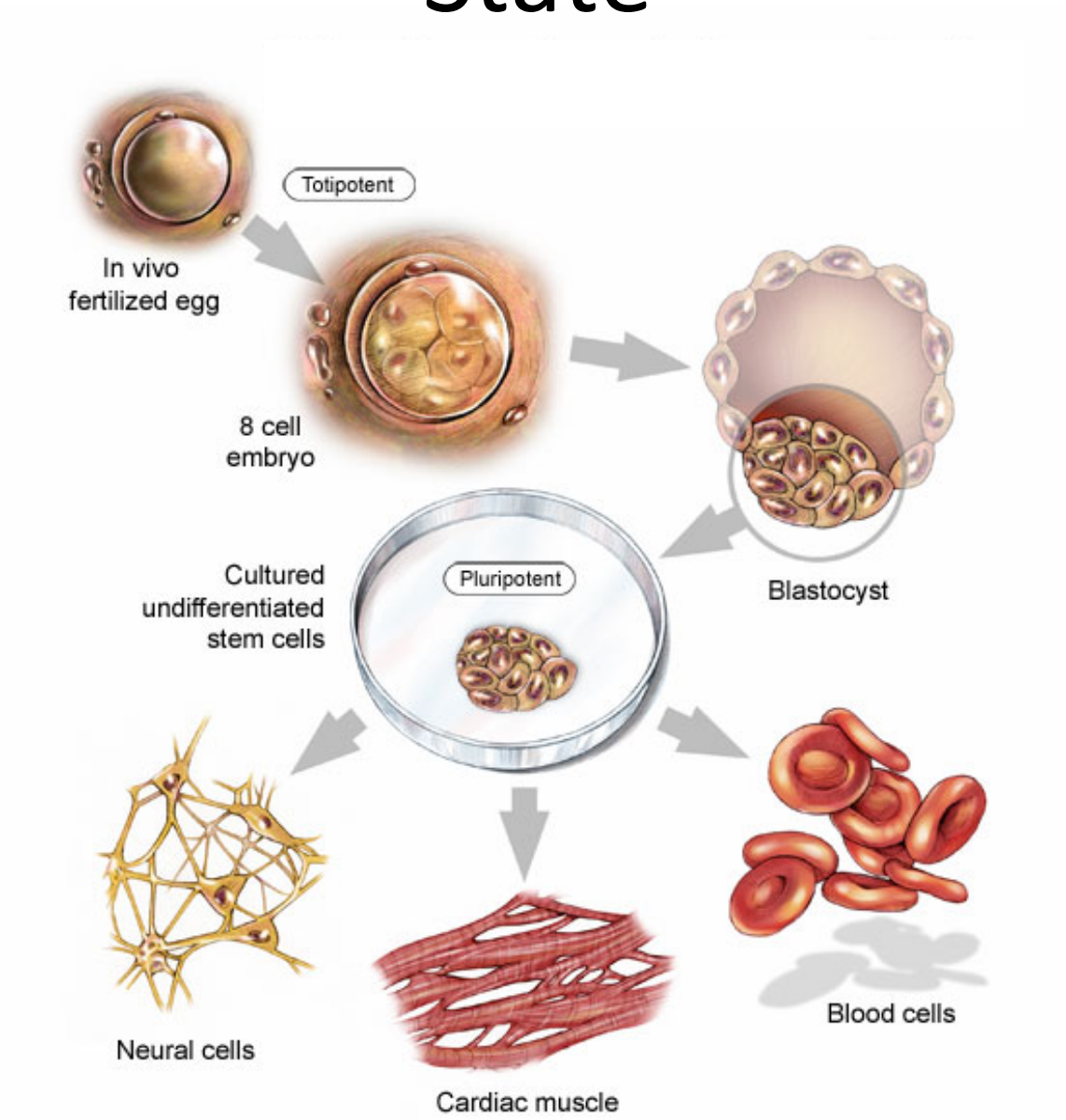
Regulatory Circuitry



Zebrafish Development

Can we learn how to write such a program?

State


Totipotent
In vivo
fertilized egg
8 cell
embryo
Blastocyst
Cultured
undifferentiated
stem cells
Pluripotent
Neural cells
Cardiac muscle
Blood cells

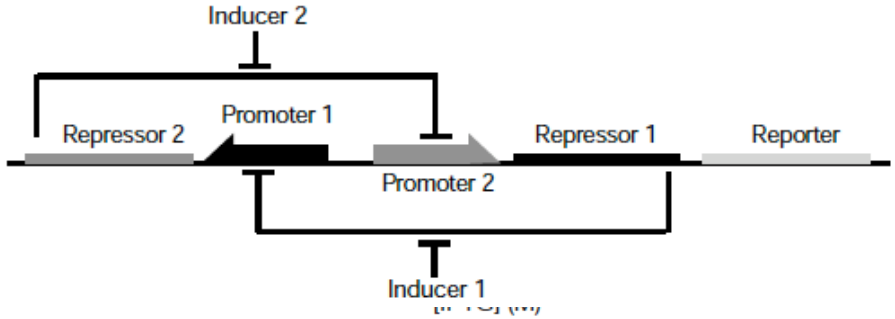
Construction of a genetic toggle switch in *Escherichia coli*

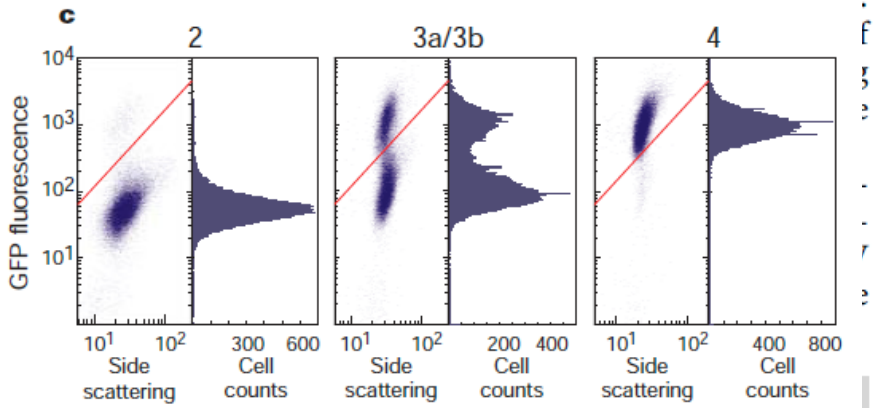
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

* *Department of Biomedical Engineering,* † *Center for BioDynamics and* ‡ *Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

It has been proposed[1] that gene-regulatory circuits with virtually any desired property can be constructed from networks of simple regulatory elements. These properties, which include multistability and oscillations, have been found in specialized gene circuits such as the bacteriophage λ switch[2] and the Cyanobacteria circadian oscillator[3]. However, these behaviours have not been demonstrated in networks of non-specialized regulatory components. Here we present the construction of a genetic toggle switch—a synthetic, bistable gene-regulatory network—in *Escherichia coli* and provide a simple theory that predicts the conditions necessary for bistability. The toggle is constructed from any two repressible promoters arranged in a mutually inhibitory network. It is flipped between stable states using transient chemical or thermal induction and exhibits a nearly ideal switching threshold. As a practical device, the toggle switch forms a synthetic, addressable cellular memory unit and has implications for biotechnology, biocomputing and gene therapy.

The design and construction of synthetic gene-regulatory net-





The toggle model

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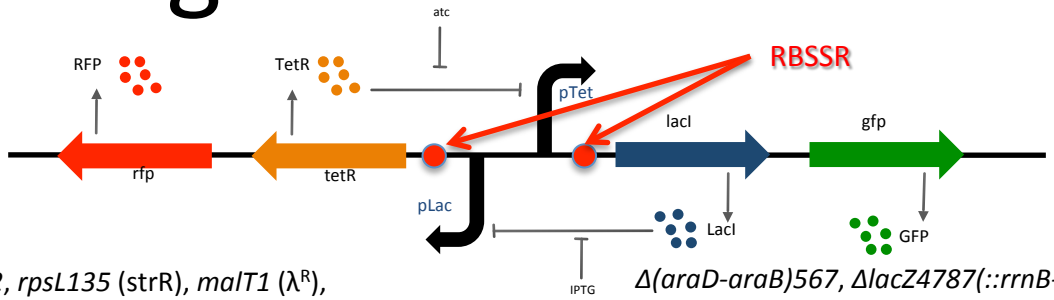
$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u \qquad (1a)$$

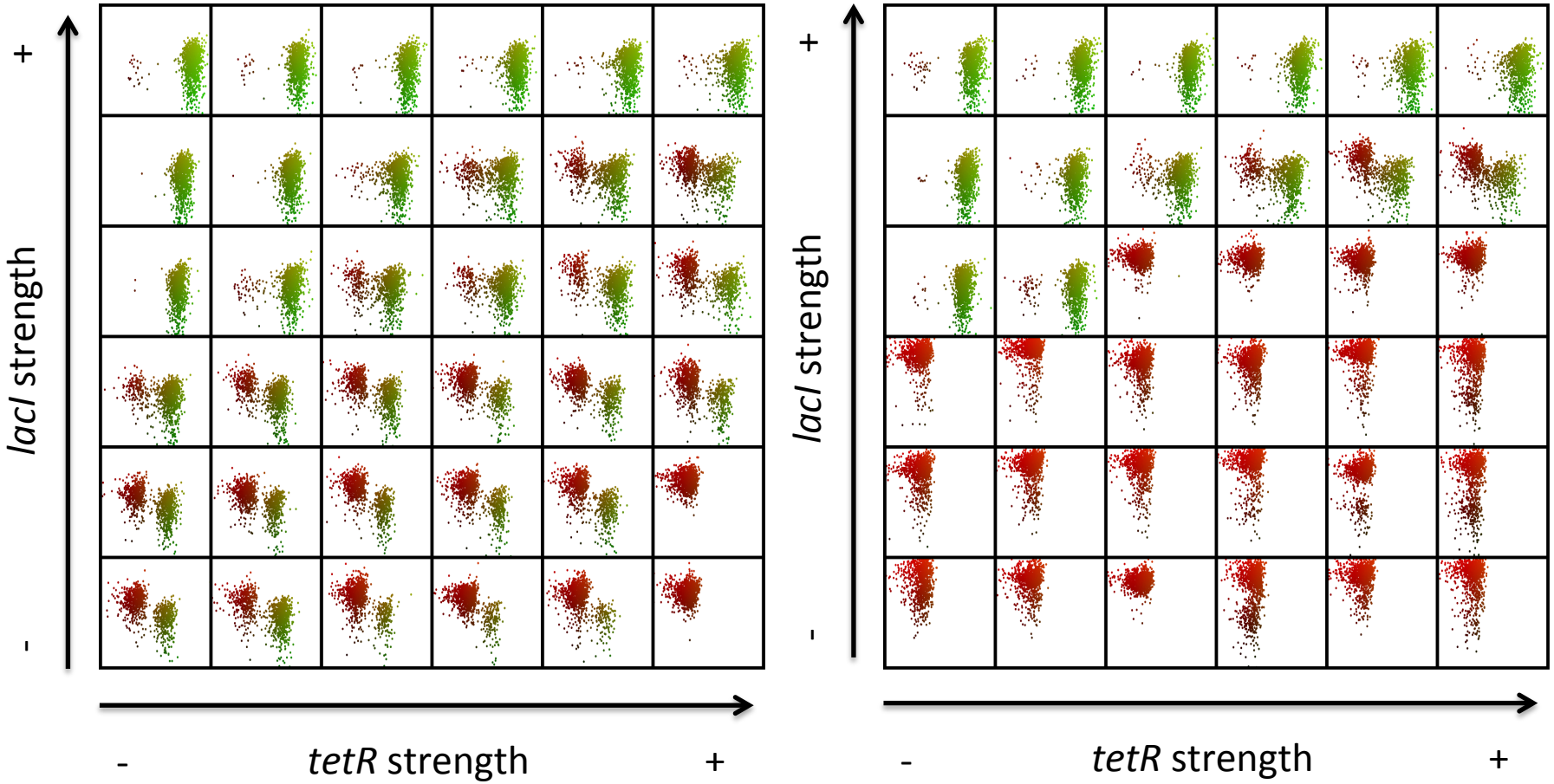
$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v \qquad (1b)$$

Tuning the Bistable Switch



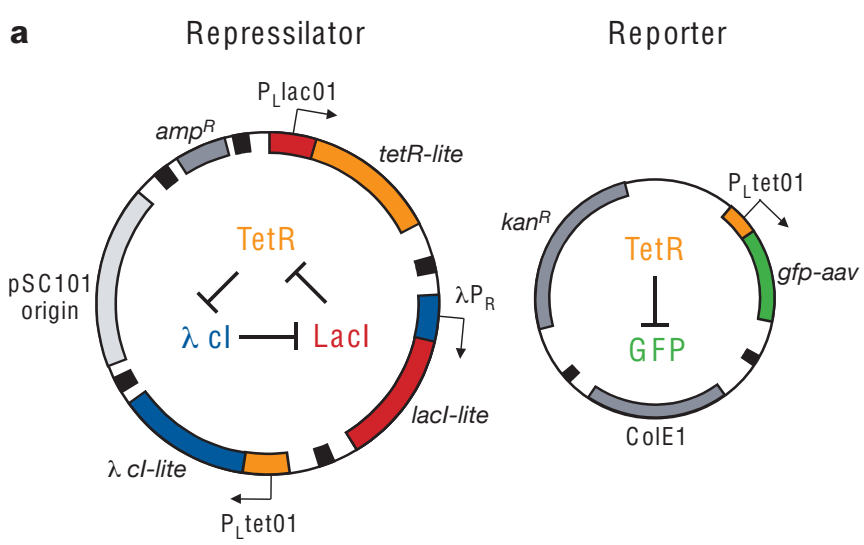
lacZ13(Oc), *lacI22*, *rpsL135* (strR), *malT1* (λ^R), *xyl-7*, *mtl-1*, *thi-1*

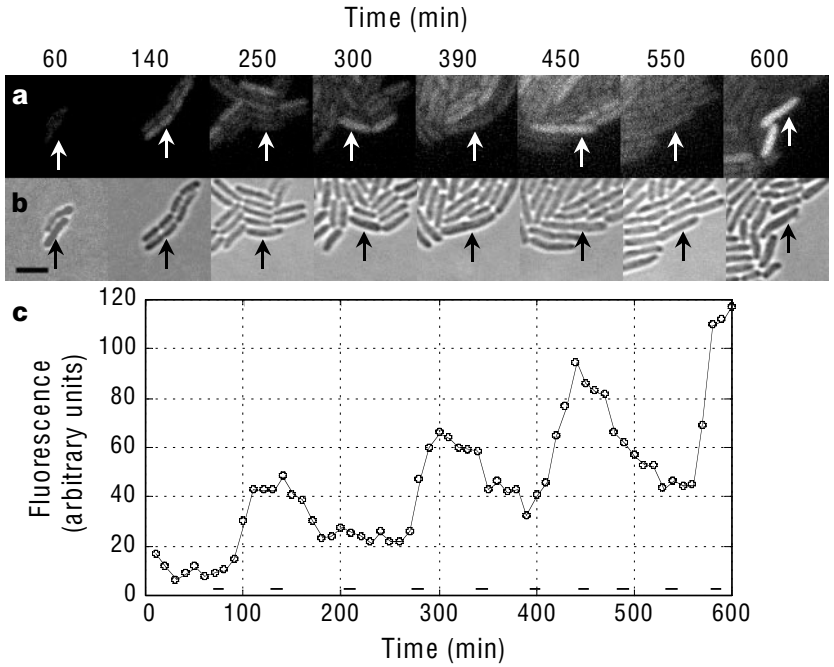
Δ(araD-araB)567, *ΔlacZ4787(::rrnB-3)*, *λ-*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*, *ΔlacI*



Egbert and Klavins, **Fine Tuning with Simple Sequence Repeats**, *Proceedings of the National Academy of Science*, Aug. 2012.

The Repressilator



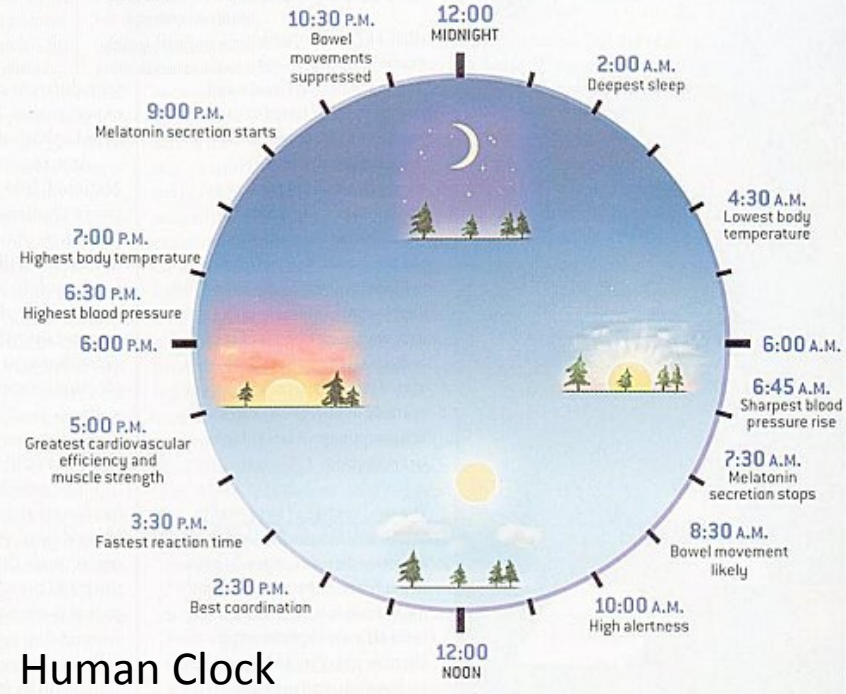


A biological oscillator
(Elowitz, 2001)

The Repressilator

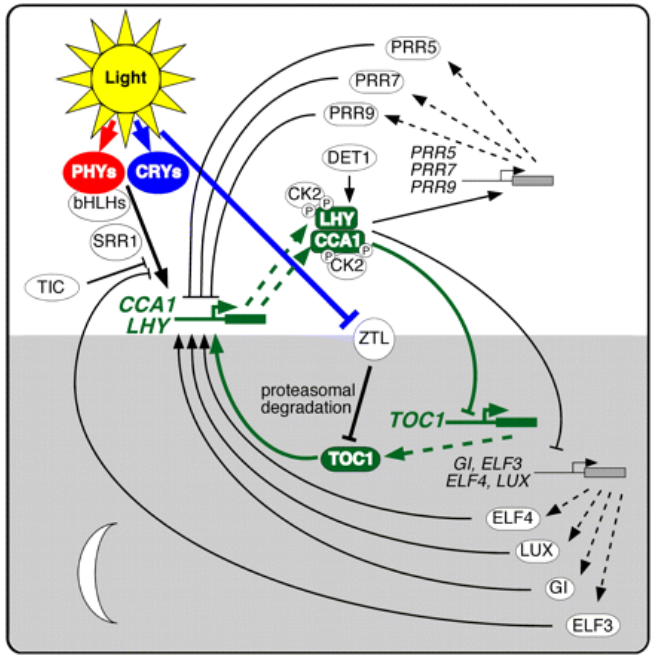
A biological oscillator
(Elowitz, 2001)

Rhythm

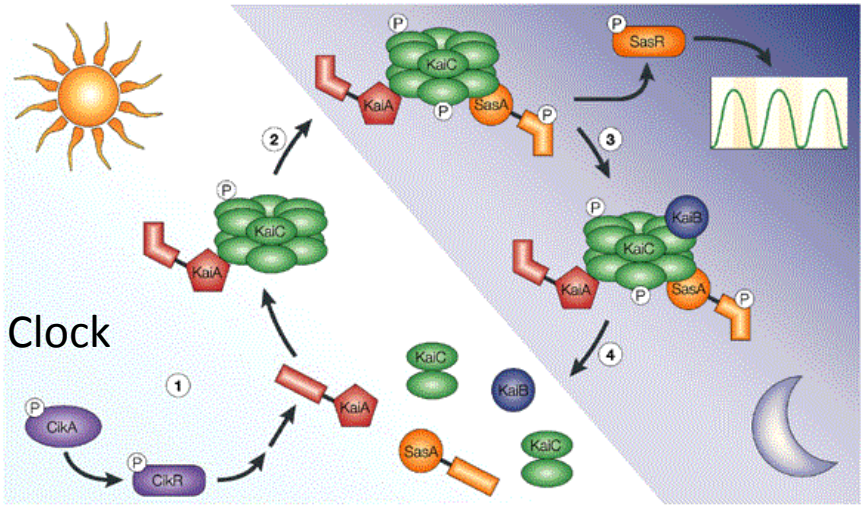


Human Clock

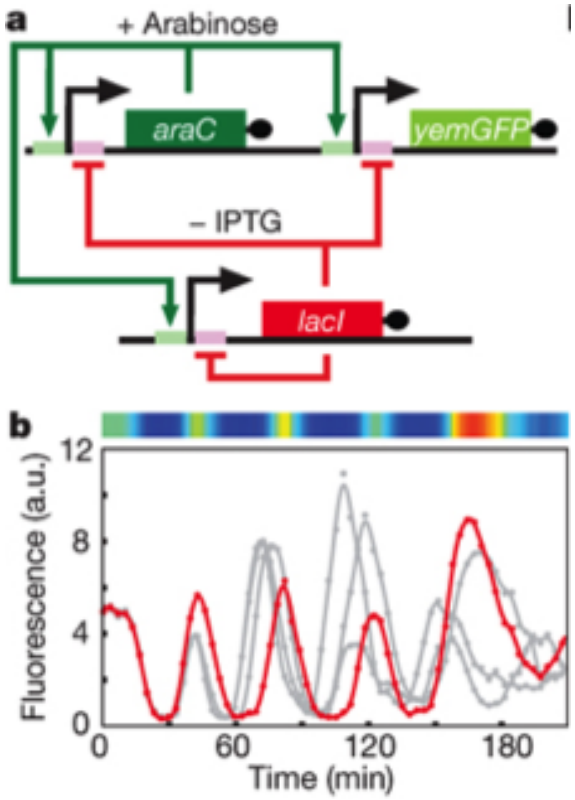
Plant Clock

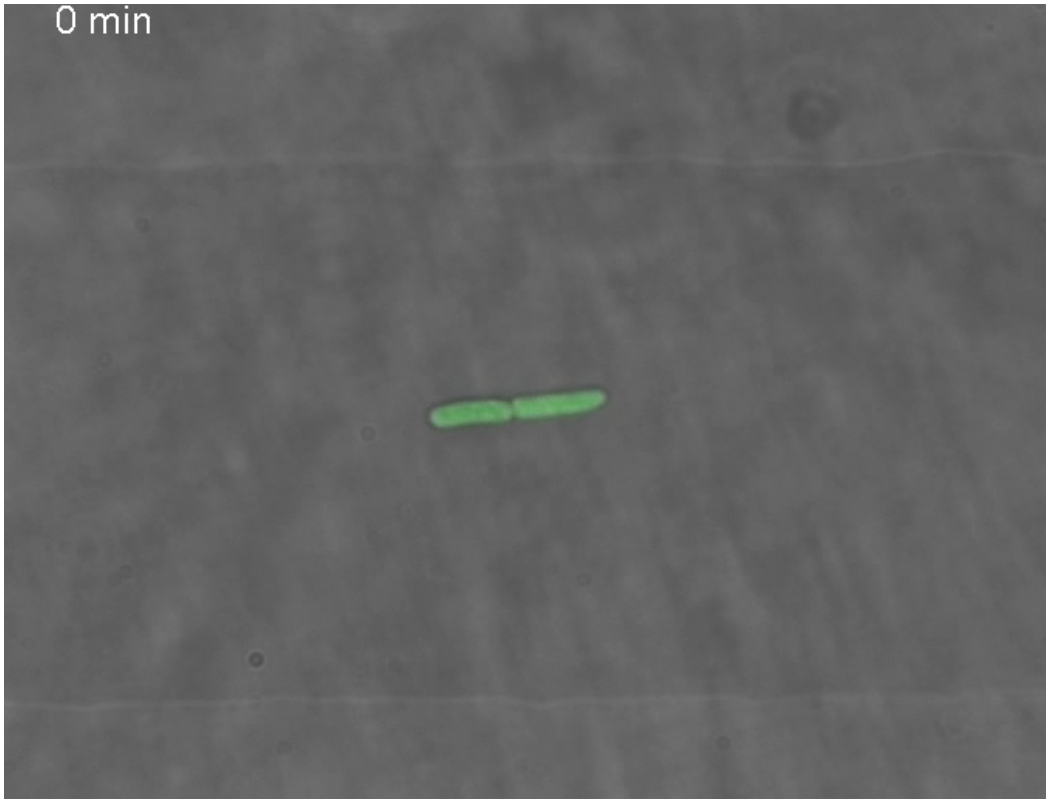


Cyanobacterial Clock



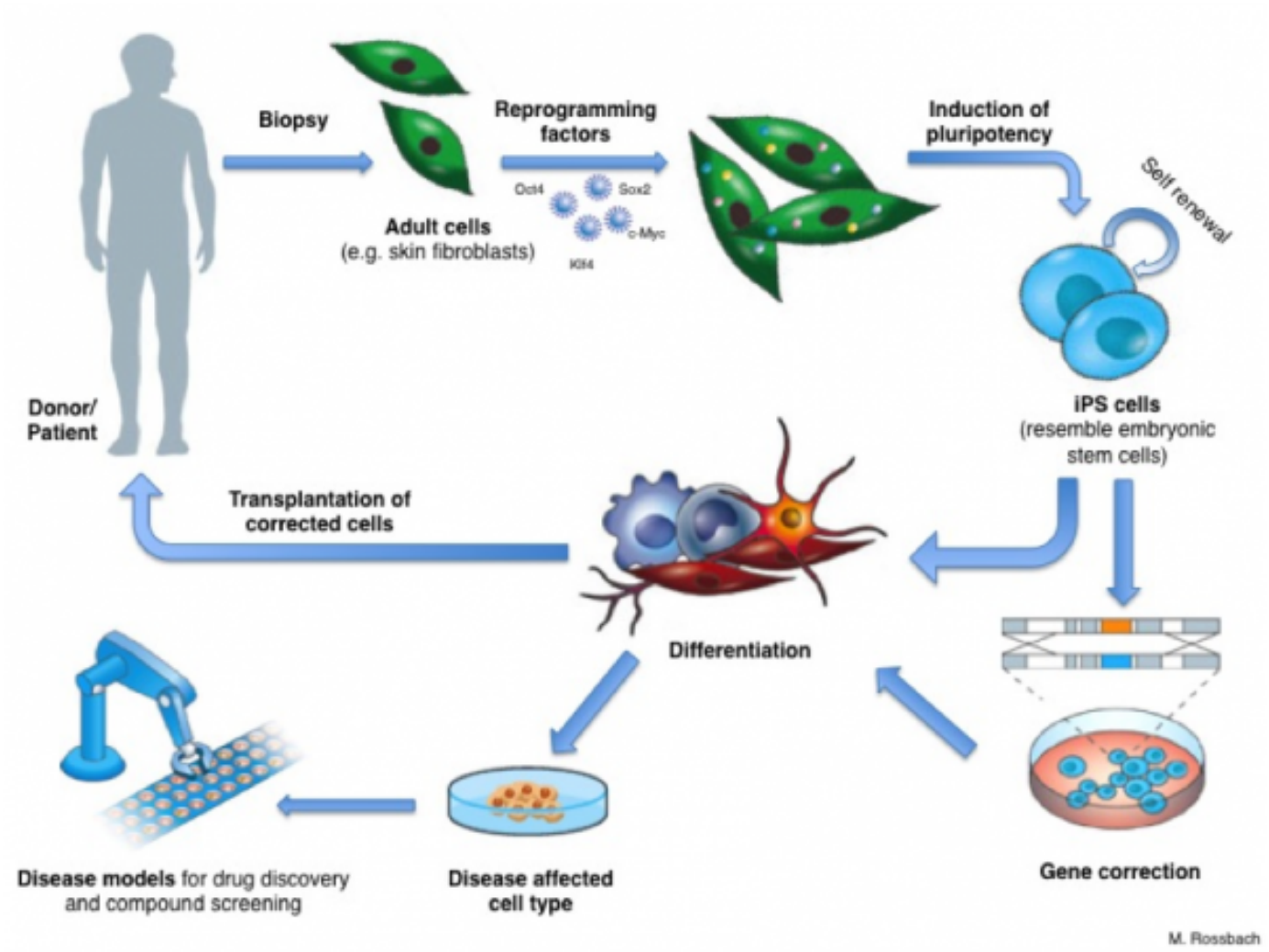
An improved synthetic oscillator





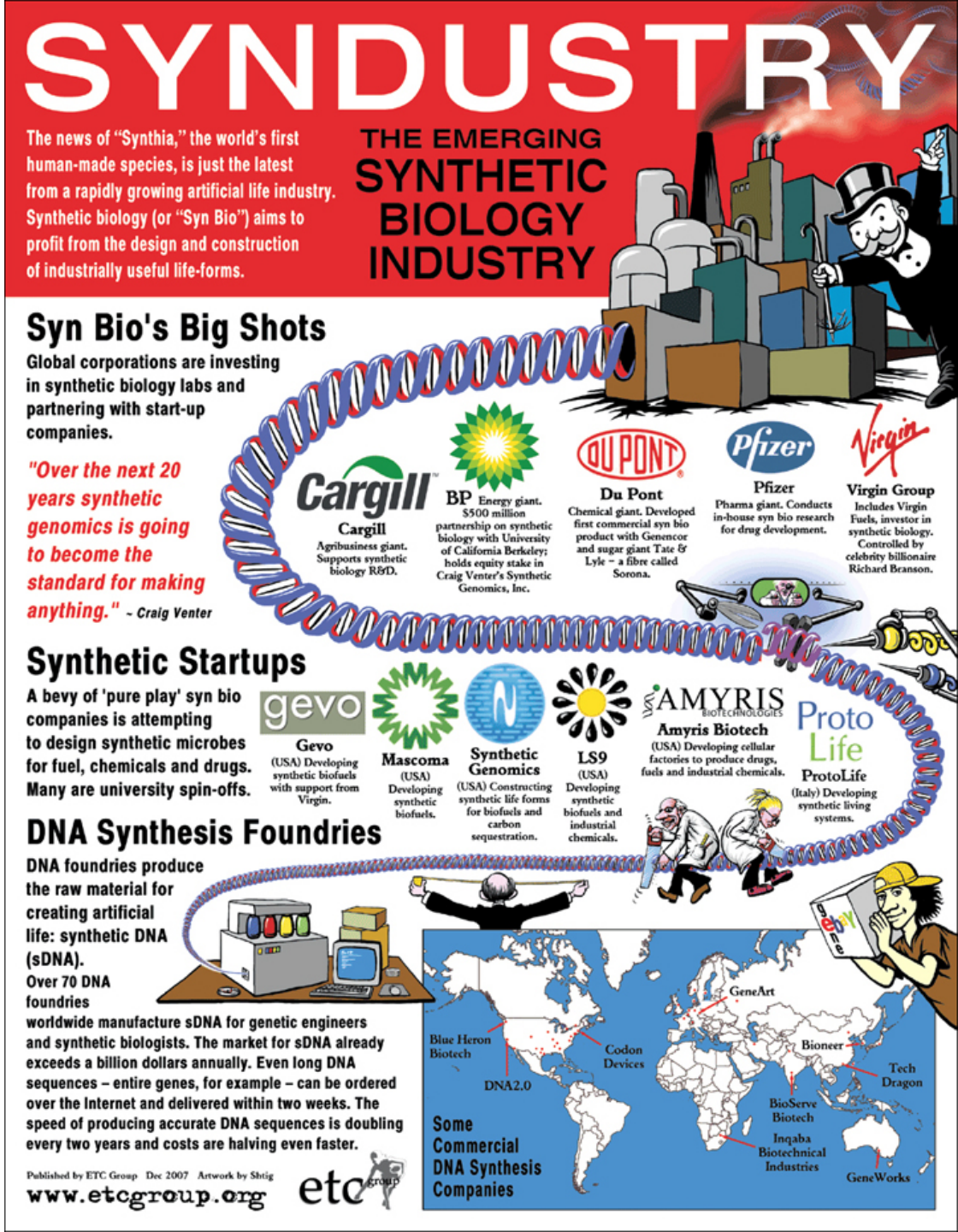
A biological oscillator
(Hasty lab)

Cellular reprogramming



Applications

- Tissue Engineering
- Diagnostics
- Therapeutics
- Chemical Synthesis
- Materials



Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]

Malaria is a global health problem that threatens 300–500 million people and kills more than one million people annually[1]. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*[2,3]. Synthetic antimalarial drugs and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing[4,5]. Artemisinin, a sesquiterpene lactone endoperoxide extracted from *Artemisia annua* L (family Asteraceae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant *Plasmodium* spp., but is in short supply and unaffordable to most malaria sufferers[6]. Although total synthesis of artemisinin is difficult and costly[7], the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of artemisinin[8,9]. Here we report the engineering of *Saccharomyces cerevisiae* to produce high titres (up to 100 mg l^{-1}) of artemisinic acid using an engineered mevalonate pathway, amorphadiene synthase, and a novel cytochrome P450 monooxygenase (*CYP71AV1*) from *A. annua* that performs a three-step oxidation of amorpha-4,11-diene to artemisinic acid. The synthesized arte-

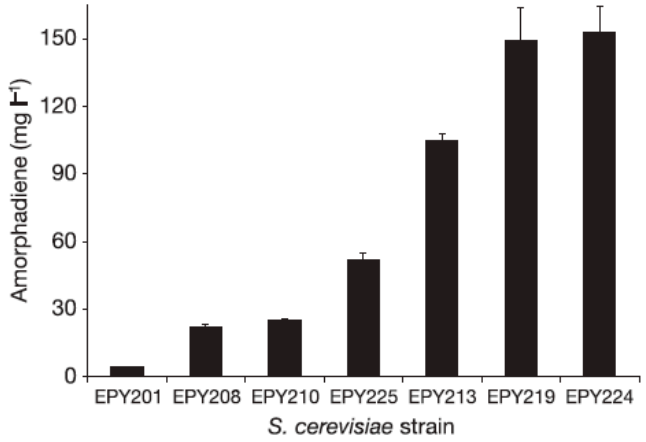
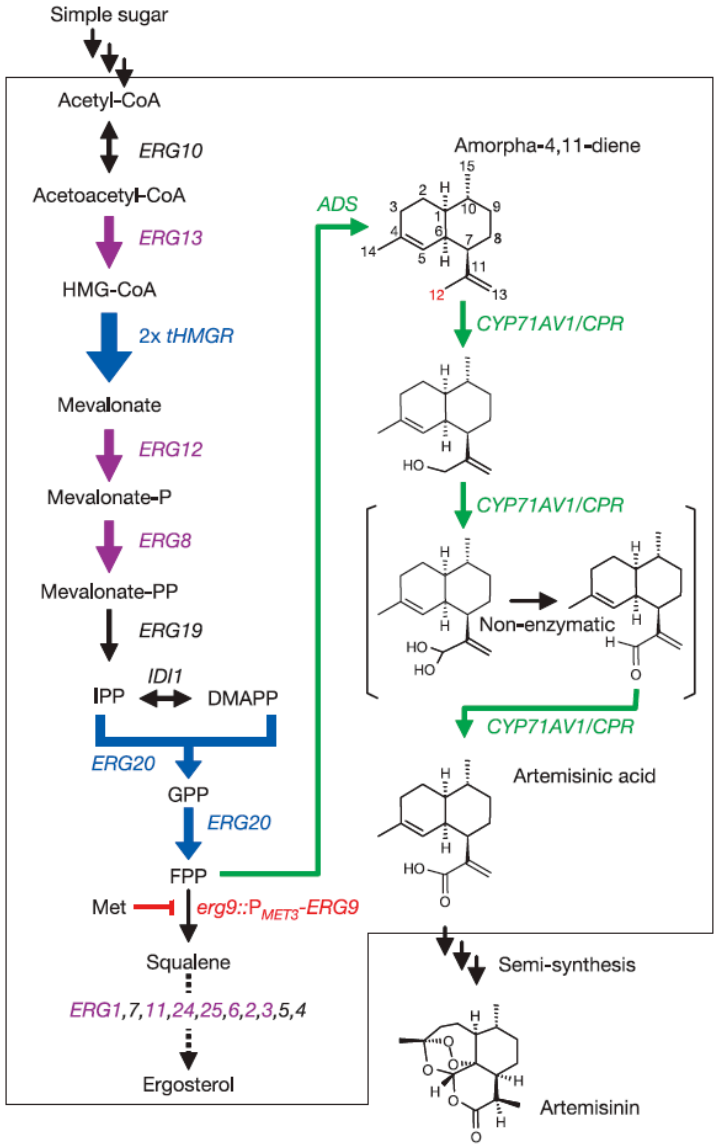


Figure 2 | Production of amorphadiene by *S. cerevisiae* strains. The various *S. cerevisiae* strains are described in the text. Cultures were sampled after 144 h of growth, and amorphadiene levels were quantified. Data, shown as total production, are mean ± s.d. ($n = 3$).



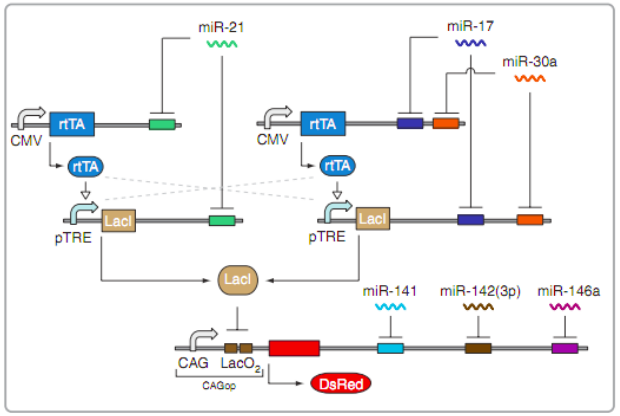
Not much about metabolic engineering in this course.

Diagnostics and Imaging

Multi-Input RNAi-Based Logic Circuit for Identification of Specific Cancer Cells

Zhen Xie,[1,2]* Liliana Wroblewska,[2] Laura Prochazka,[3] Ron Weiss,[2,4]† Yaakov Benenson[1,3]†‡

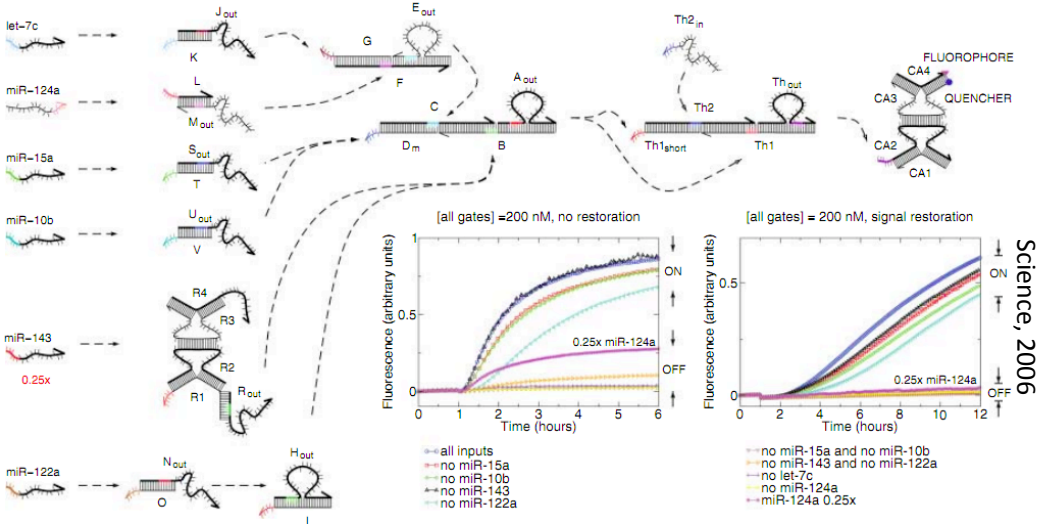
miR-21 AND miR-17-30a AND NOT(miR-141) AND NOT(miR-142(3p)) AND NOT(miR-146a)



Science, 2010

Enzyme-Free Nucleic Acid Logic Circuits

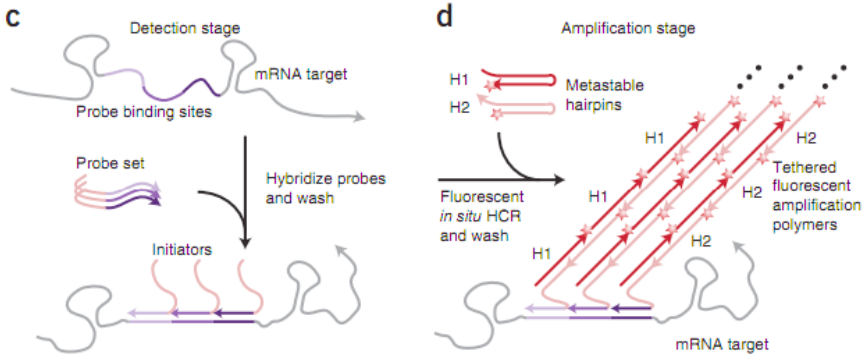
Georg Seelig,[1] David Soloveichik,[2] David Yu Zhang,[2] Erik Winfree[2,3]*

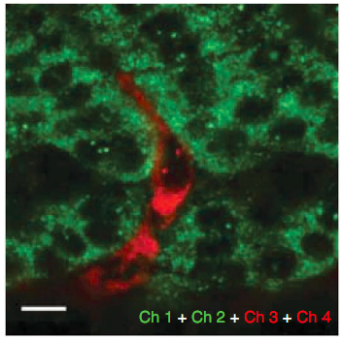


Science, 2006

Programmable *in situ* amplification for multiplexed imaging of mRNA expression

Harry M T Choi[1], Joann Y Chang[1], Le A Trinh[2], Jennifer E Padilla[1], Scott E Fraser[1,2] & Niles A Pierce[1,3]





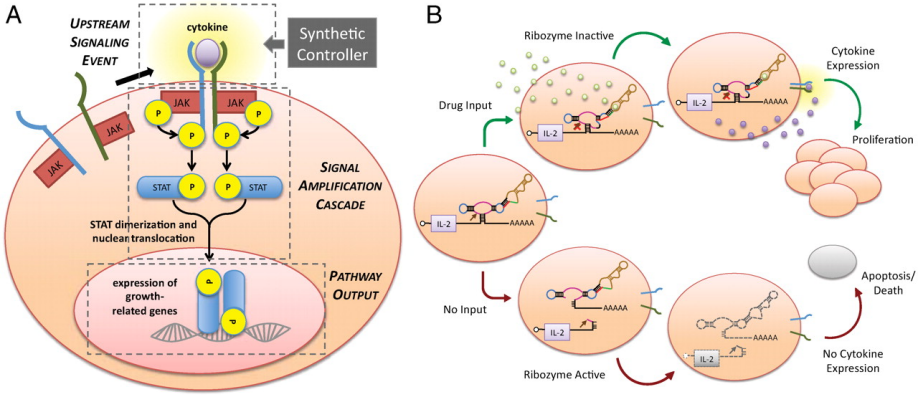
Nature BioTech, 2010

Not much about human cells in this course.

Therapeutics

Genetic control of mammalian T-cell proliferation with synthetic RNA regulatory systems

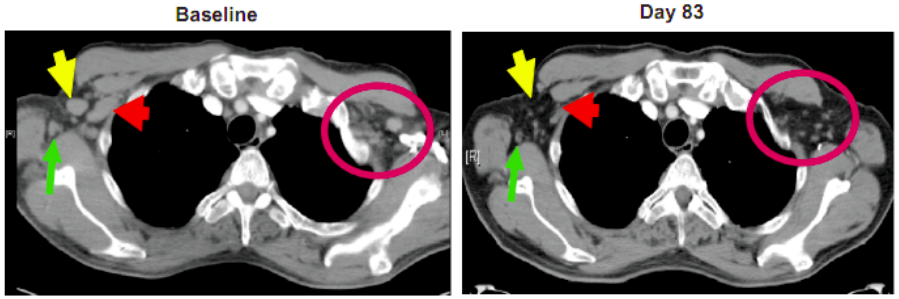
Yvonne Y. Chen[a], Michael C. Jensen[b,1], and Christina D. Smolke[c,1,2]



PNAS, 2010

T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia

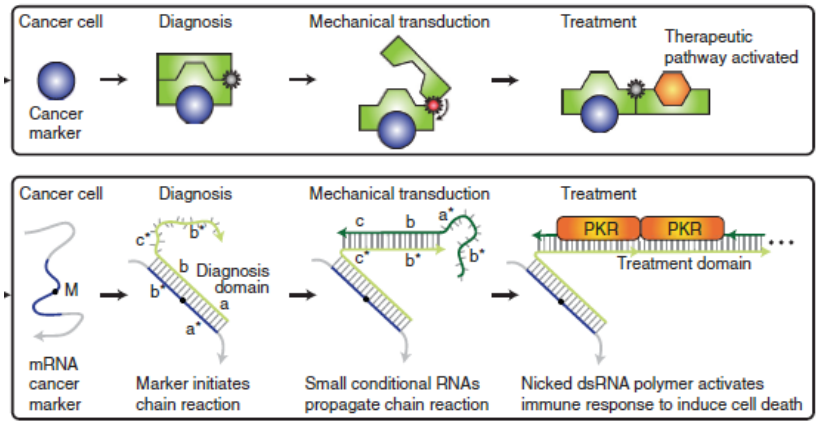
Michael Kalos[1,2,*], Bruce L. Levine[1,2,*], David L. Porter[1,3], Sharyn Katz[4], Stephan A. Grupp[5,6], Adam Bagg[1,2] and Carl H. June[1,2,†]

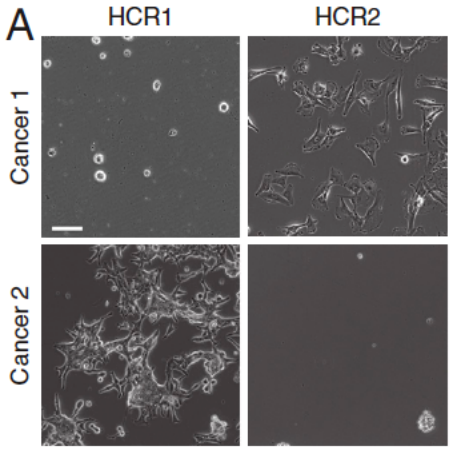


Science Trans Med, 2011

Selective cell death mediated by small conditional RNAs

Suvir Venkataraman[a], Robert M. Dirks[a,b], Christine T. Ueda[b], and Niles A. Pierce[a,c,1]





PNAS 2010

Table 1 Selected advanced biofuel companies

Company	Process	Producing Organism
Ethanol		
Algenol Biofuels, Naples, Florida	Photosynthesis in contained bioreactor	Cyanobacteria
BioGasol, Ballerup, Denmark	Glucose & xylose fermentation	Yeast, anaerobic, thermophilic bacteria
Codexis[1], Redwood City, California	High performance enzymes via gene shuffling	Not available
Coskata, Warrenville, Illinois	High temperature biomass gasification and fermentation using carbon monoxide and hydrogen	Not disclosed
Dupont Danisco Cellulosic Ethanol, Itaca, New York	Combined cellulosic conversion and fermentation	*Zymomonas mobilis*
Gevo, Englewood, Colorado	Production of higher alcohols via amino acid biosynthetic pathway	Yeast
Green Biologics, Abingdon, UK	Modified classical acetone, butanol ethanol (ABE) fermentation	Clostridium species, Geobacillus species
Joule Biotechnologies, Cambridge, Massachusetts	Helioculture modified photosynthetic process in closed bioreactor	Modified photosynthetic organisms
Lanza Tech, Auckland, New Zealand	Fermentation process using carbon monoxide and hydrogen from syngas and fluegas	Not disclosed
LS9, S San Francisco	Biodiesel fermentation via fatty acid metabolism	*E. coli*
Mascoma, Lebanon, New Hampshire	Combined lignocelulose conversion with fermentation	Yeast, *Clostridium thermocellum*
Qteros, Marlborough, Massachusetts	One-step bacterial lignocellulose conversion and fermentation	*Clostridim phytofermentans*
TMO Renewables, Guildford, UK	Combined cellulosic conversion and fermentation	Geobacillus TM242
Verdezyne, Carlsbad, California	Improved yeast fermentation based on microbial glycolytic pathway and xylose isomerase	Yeast
Verenium, Cambridge, Mass	Combined cellulosic conversion and fermentation	Ethanologenic bacteria
Zeachem, Lakewood, Colorado	Hybrid biochemical and thermochemical process involving acetic acid fermentation	Naturally occurring acetate producing bacteria
Diesel		
Amyris Biotechnologies, Emeryville, California	Isoprenoid biosynthesis via mevalonate pathway	Yeast
Aurora Biofuels, Alameda, California	Photosynthesis in open pond system	Naturally occurring algae
OPX Biotechnologies, Boulder, Colorado	Undisclosed	*E. coli*
Algal oils		
Sapphire Energy, San Diego, California	Photosynthesis	Photosynthetic algae
Solazyme, S. San Francisco	Photosynthesis	Photosynthetic algae
Solix Biofuels, Fort Collins, Colorado	Photosynthesis in close system photobioreactor	Photosynthetic algae
Synthetic Genomics, La Jolla, California	Combined phtosynthetic production and secretion	Photosynthetic algae

[1]Codexis is a technology provider to cellulosic ethanol producer Iogen, of Ottawa, Canada.

FIVE HARD TRUTHS FOR SYNTHETIC BIOLOGY

Can engineering approaches tame the complexity of living systems? **Roberta Kwok** explores five challenges for the field and how they might be resolved.

Many of the parts are undefined

A biological part can be anything from a DNA sequence that encodes a specific protein to a promoter, a sequence that facilitates the expression of a gene. The problem is

The circuitry is unpredictable

Even if the function of each part is known, the parts may not work as expected when put together, says Keasling.

The complexity is unwieldy

As circuits get larger, the process of constructing and testing them becomes more daunting. A system developed by Keas-

Many parts are incompatible

Once constructed and placed into cells, synthetic genetic circuits can have unintended effects on their host. Chris

Variability crashes the system

Synthetic biologists must also ensure that circuits function reliably. Molecular activities inside cells are prone to random fluctuations, or noise. Variation in growth con-

"The field has had its hype phase. Now it needs to deliver."
— Martin Fussenegger

http://www.nature.com/nature/focus/syntheticsytemsbiology/index.html