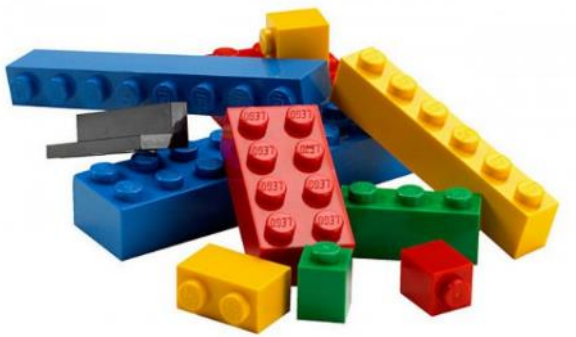
Synthetic Biology



www.brickartist.com
Nathan Sawaya

Philippe Bouloc
(philippe.bouloc@i2bc.paris-saclay.fr)

Institute for Integrative Biology of the Cell (I2BC)

CEA, CNRS, Univ. Paris-Sud, Univ. Paris-Saclay

Outline

- Definitions
- Origins of molecular biology
- Visionaries
- Why synthetic biology is possible?
- Synthetic biology standardization
- Simplistic vision?
- Examples of synthetic biology accomplishments

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Synthetic biology

Synthetic biology (*biologie synthétique = biologie de synthèse*) is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which **display functions that do not exist in nature**. This engineering perspective may be applied at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology **will enable the design of 'biological systems' in a rational and systematic way**.

Source: High-level Expert Group European Commission

As an emerging and **interdisciplinary field**, synthetic biology seeks to make the **design, construction and optimization of biological systems** easier, more predictable and reliable. This is achieved through the assembly of core biomolecules as engineered parts, which will either enhance the biological functions of existing systems or help with **creating novel biological functions and systems**.

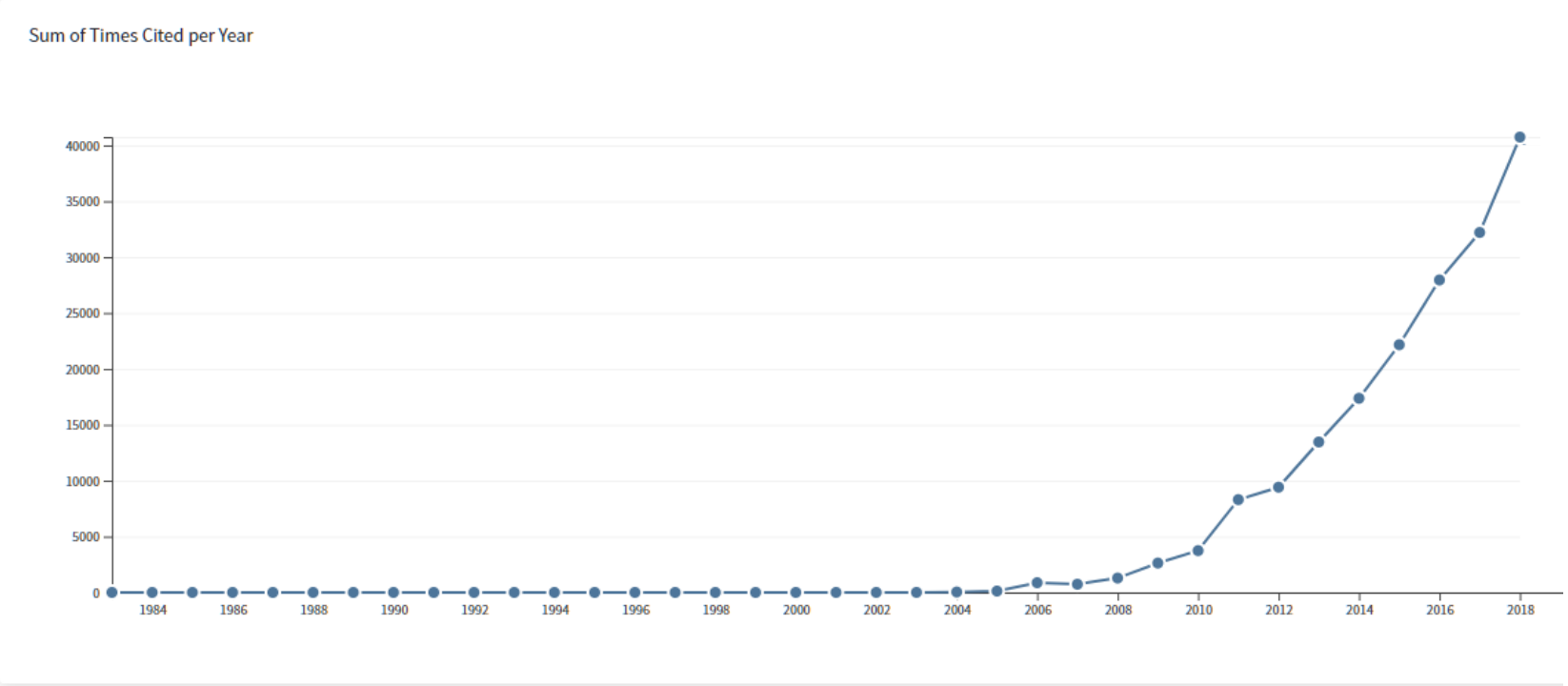
Source: GenScript

Systems biology (*biologie des systèmes*) is the computational and mathematical modeling of complex biological systems.

Source: https://en.wikipedia.org/wiki/Systems_biology

Synthetic biology, a new discipline

WEB OF SCIENCE™ THOMSON



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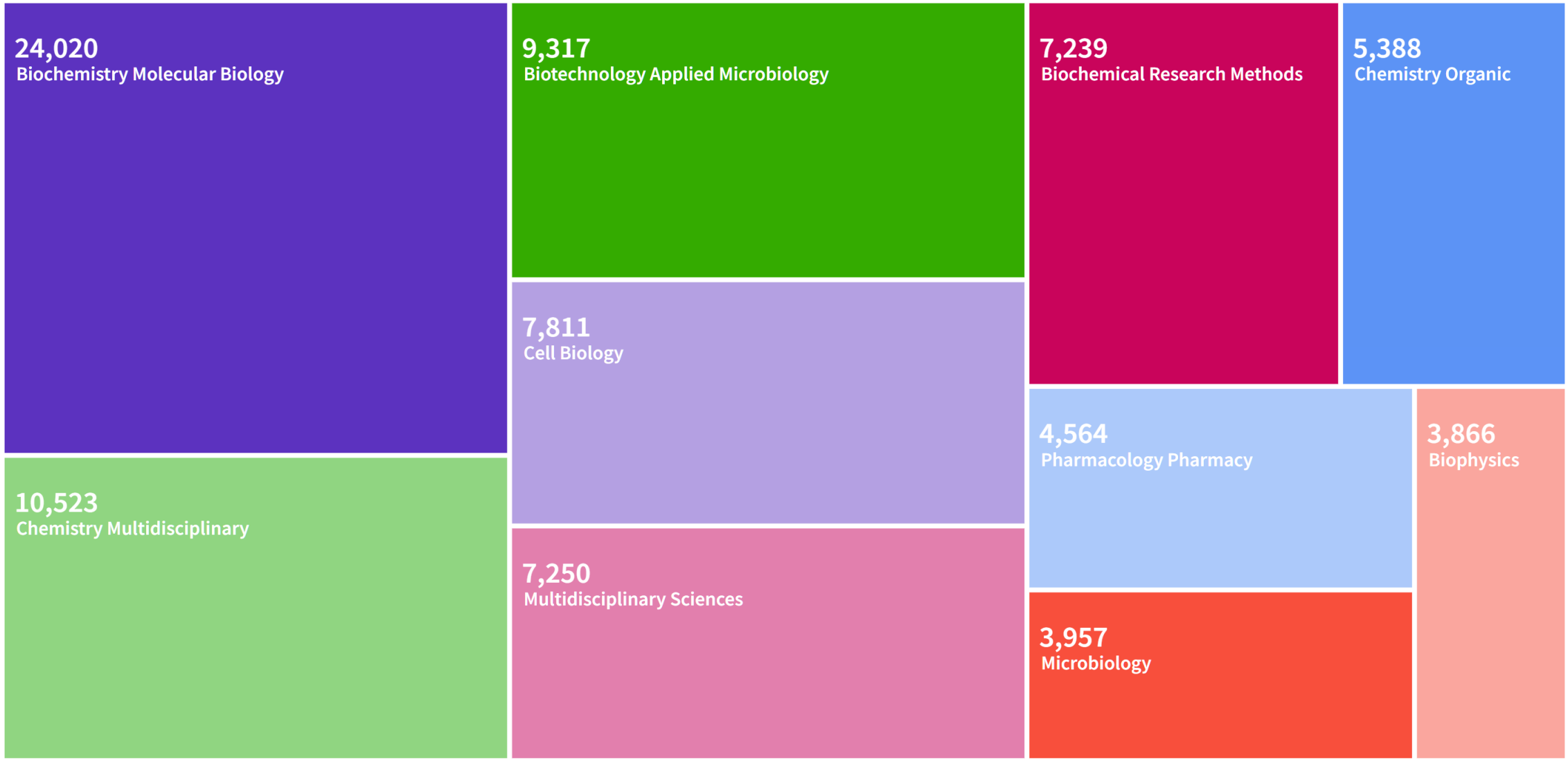
107,779 publications selected from Web of Science Core Collection



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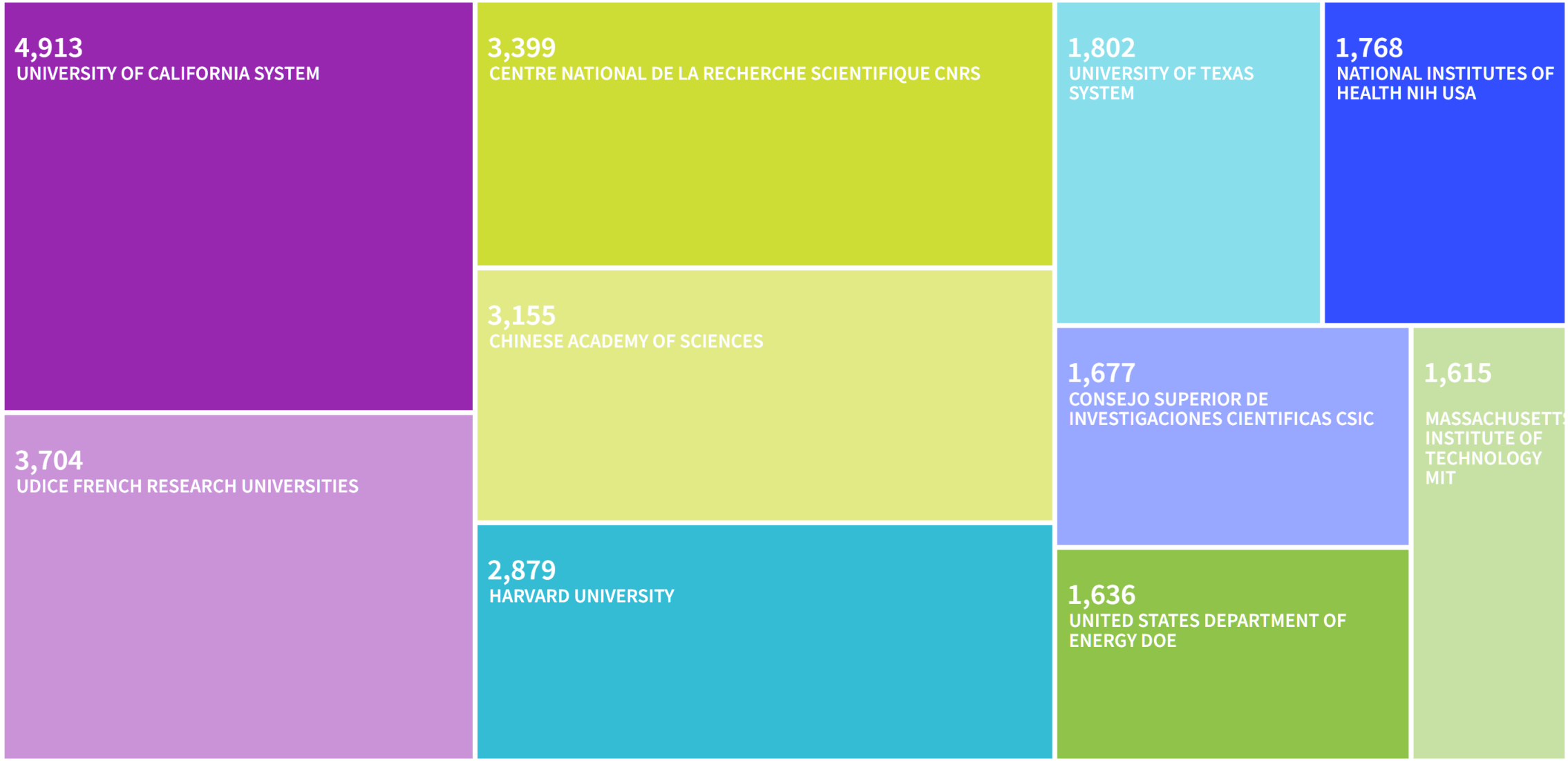

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Origins of modern biology

1859 - On the Origin of Species
Charles Darwin

1865 - Modern genetics
Gregor Mendel

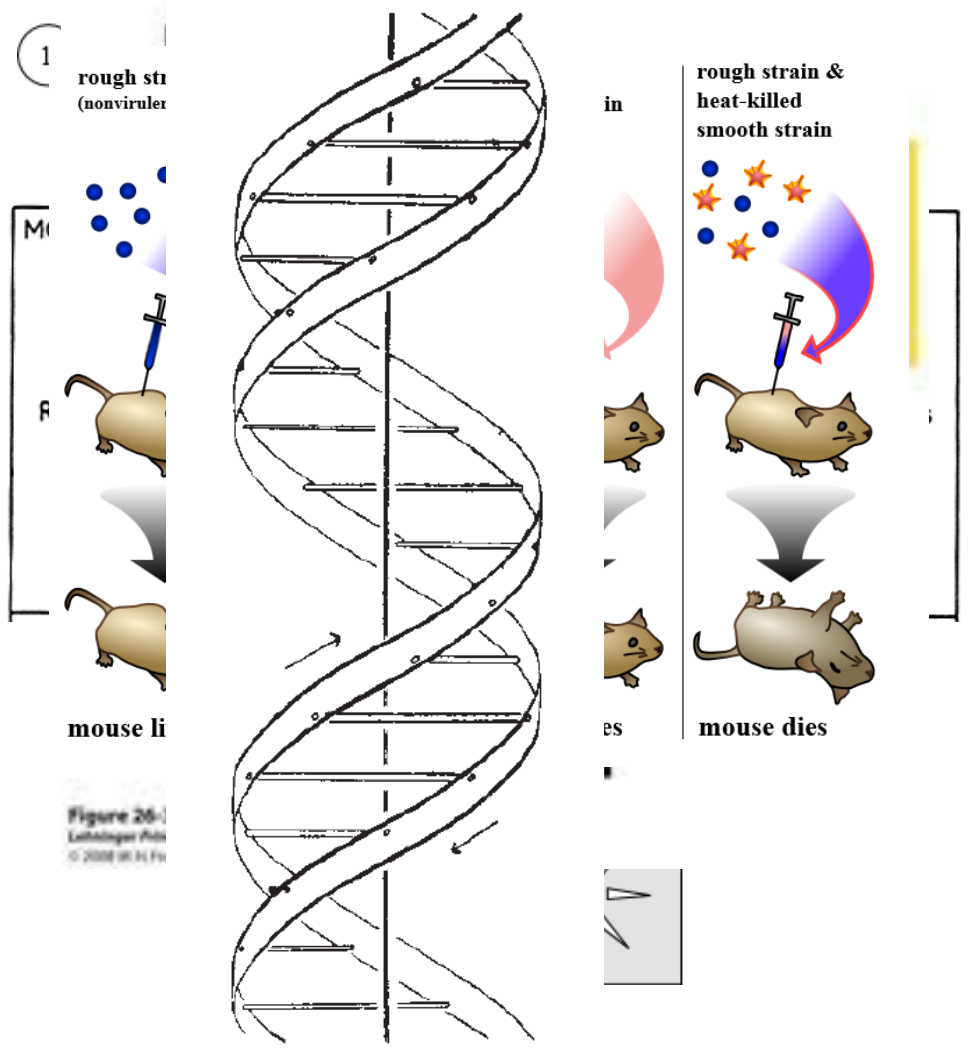
1928 - Transforming principle
Frederick Griffith

1944 - Genetic inheritance is due to DNA
Avery, MacLeod & McCarty

1953 - Structure of DNA
James Watson & Francis Crick

1956 - Central dogma of molecular biology
Francis Crick

1961 - Genetic regulatory mechanisms
François Jacob & Jacques Monod



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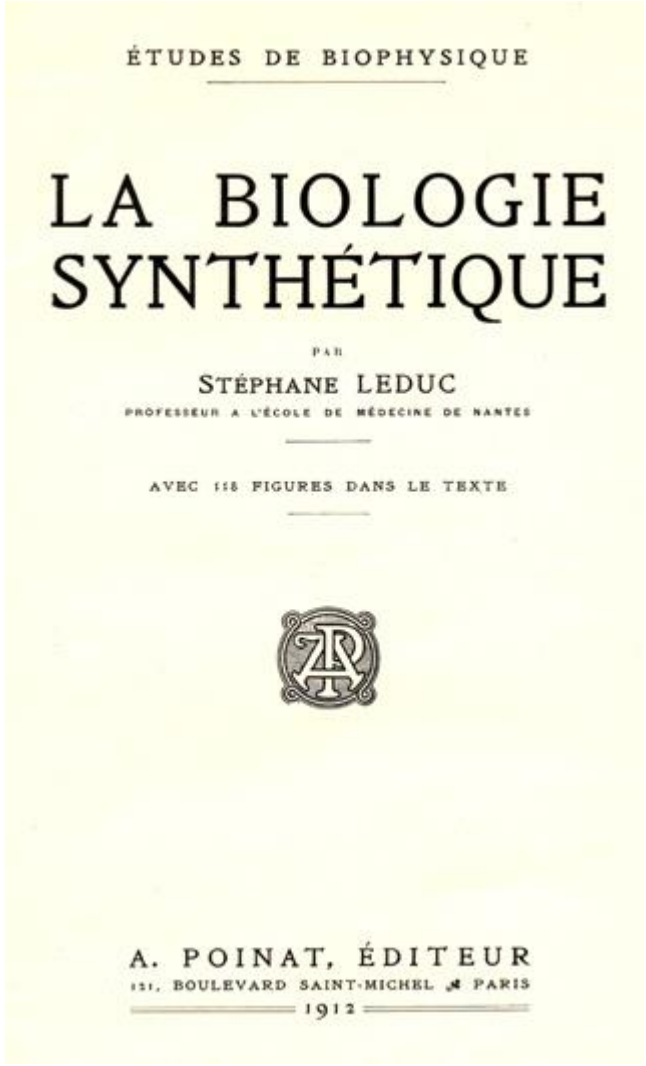
« La biologie synthétique »



ÉTUDES DE BIOPHYSIQUE

LA BIOLOGIE SYNTHÉTIQUE

PAR

STÉPHANE LEDUC

PROFESSEUR A L'ÉCOLE DE MÉDECINE DE NANTES

AVEC 118 FIGURES DANS LE TEXTE

A. POINAT, ÉDITEUR

121, BOULEVARD SAINT-MICHEL & PARIS

1912

Stéphane Leduc

Lorsqu'un phénomène, chez un être vivant, a été observé, et que l'on croit en connaître le mécanisme physique, on doit pouvoir reproduire ce phénomène isolément, en dehors de l'organisme vivant.

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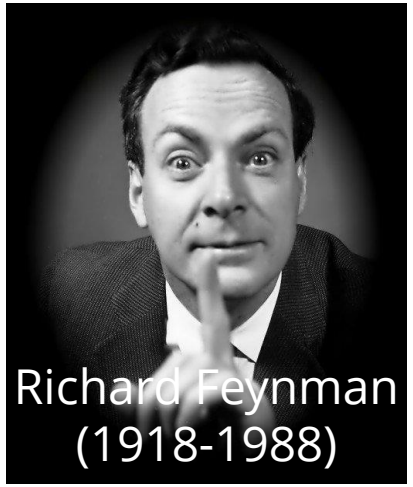
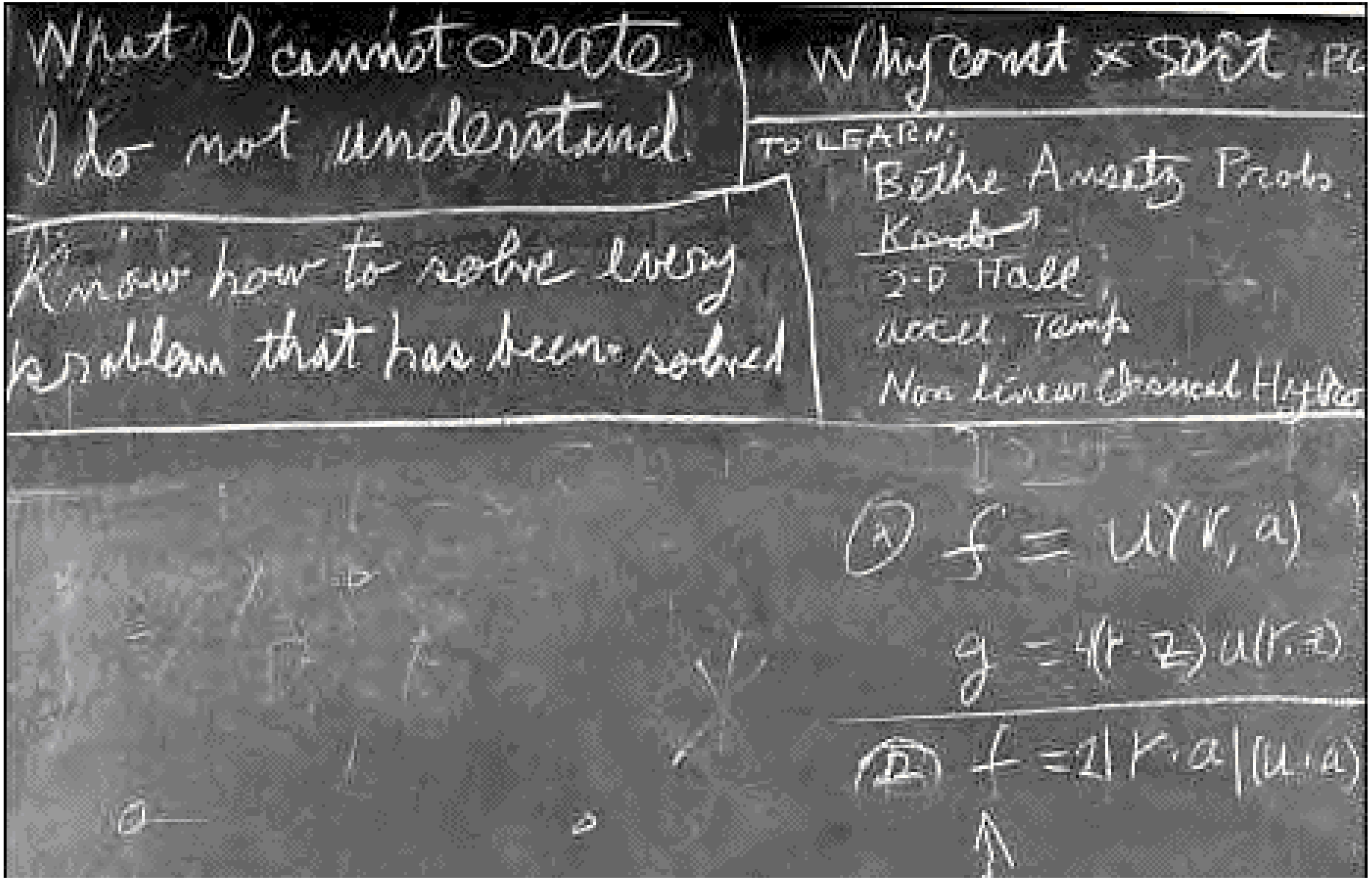
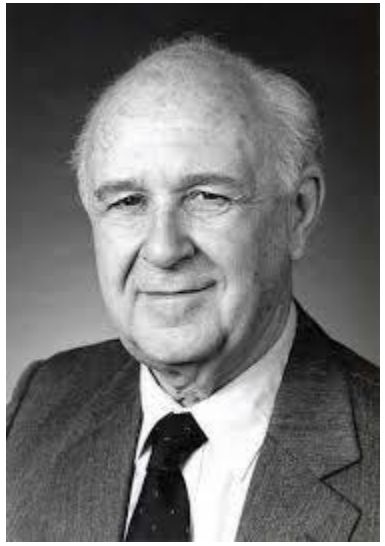
Concept


Richard Feynman
(1918-1988)



Unlimited expansion potential



"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

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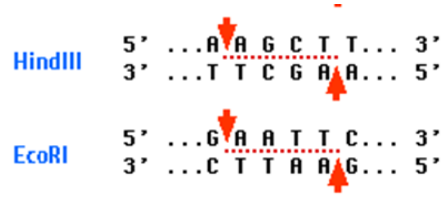
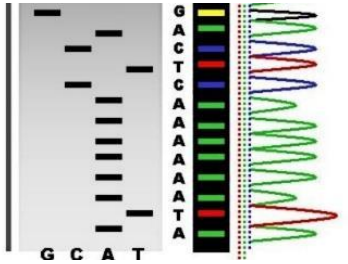
Essential steps

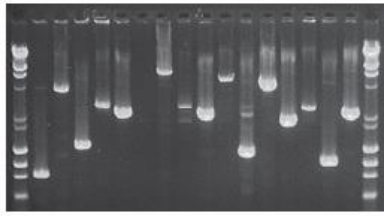
Restriction Enzymes
1970 Hamilton Smith (type II enzyme)
Arber, Nathans & Smith: 1978 Nobel Prize for physiology or medicine



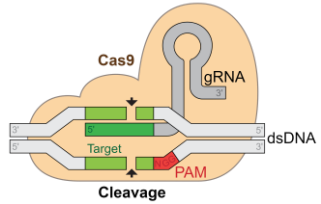
DNA sequencing
1977 Frederick Sanger
1977 Allan Maxam & Walter Gilbert
Gilbert & Sanger: 1980 Nobel Prize for chemistry



Polymerase chain reaction (PCR)
1983 Kary Mullis
Mullis & Smith: 1993 Nobel Prize for chemistry



CRISPR genome editing
2012 Emmanuelle Charpentier & Jennifer Doudna
Charpentier & Doudna: 2020 Nobel Prize for chemistry



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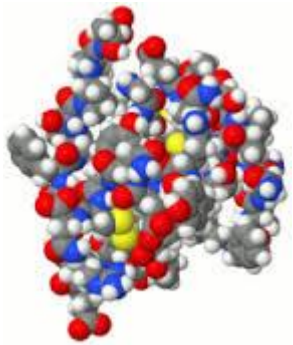
Essential steps

First GMO
1973 Stanley Cohen & Herbert Boyer: First GMO.
Patent on genetic recombination (UCSF et Stanford)

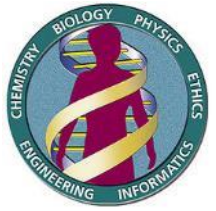
First biotech startup
1976 Genentech

First recombinant drug
1978 Human insulin cloned in *Escherichia coli*.
Arthur Riggs, Keiichi Itakura (Beckman Research Institute) and H. Boyer (Genentech)



First commercialized recombinant drug
1982 Biosynthetic human insulin

Human Genome Project (HGP)
2003 Sequencing completed
(13 years, 3 billion USD, 3.3 billion nts)



First human editing
2018 Removal of the CCR5 gene in an attempt to confer genetic resistance to HIV

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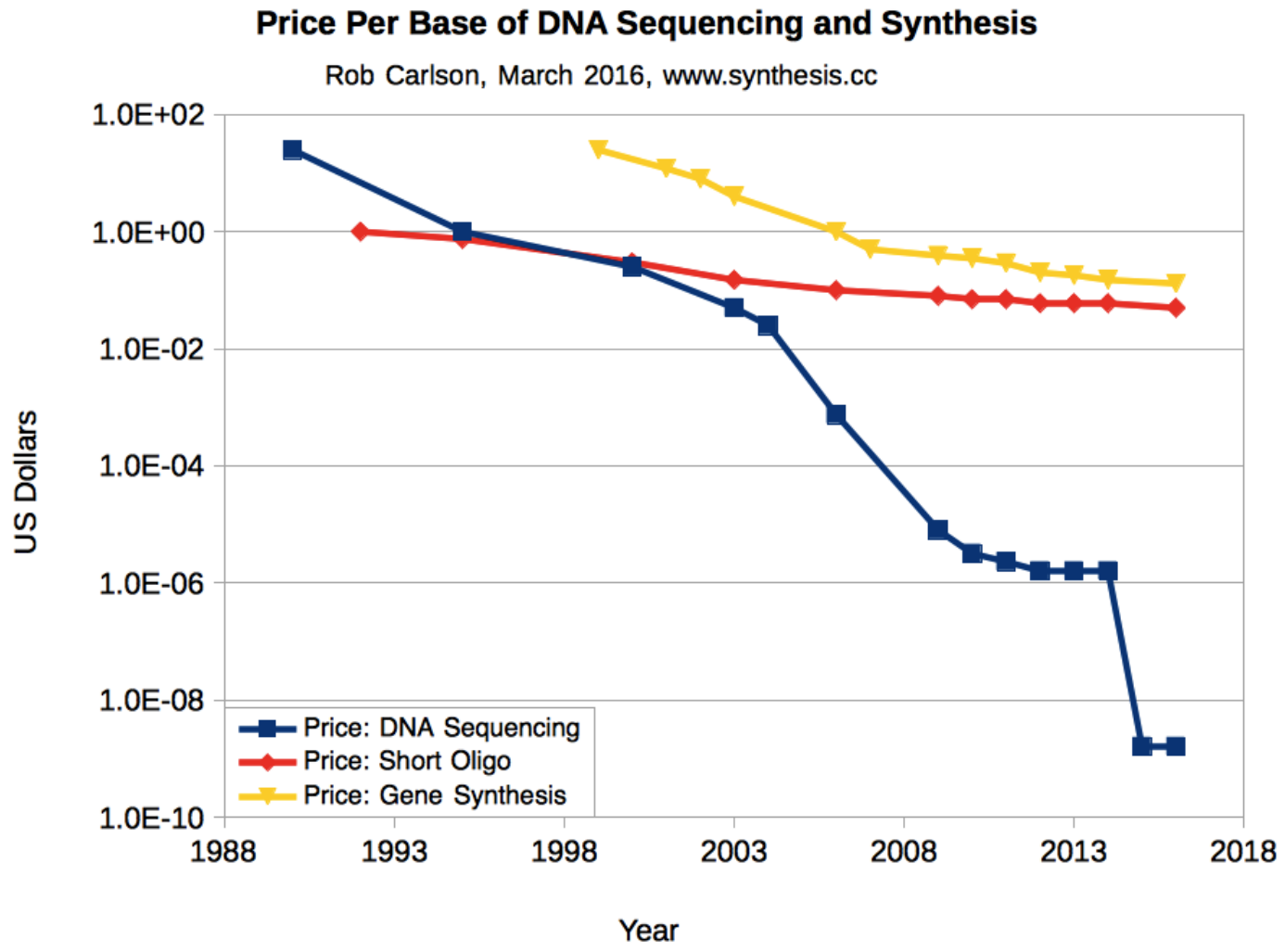
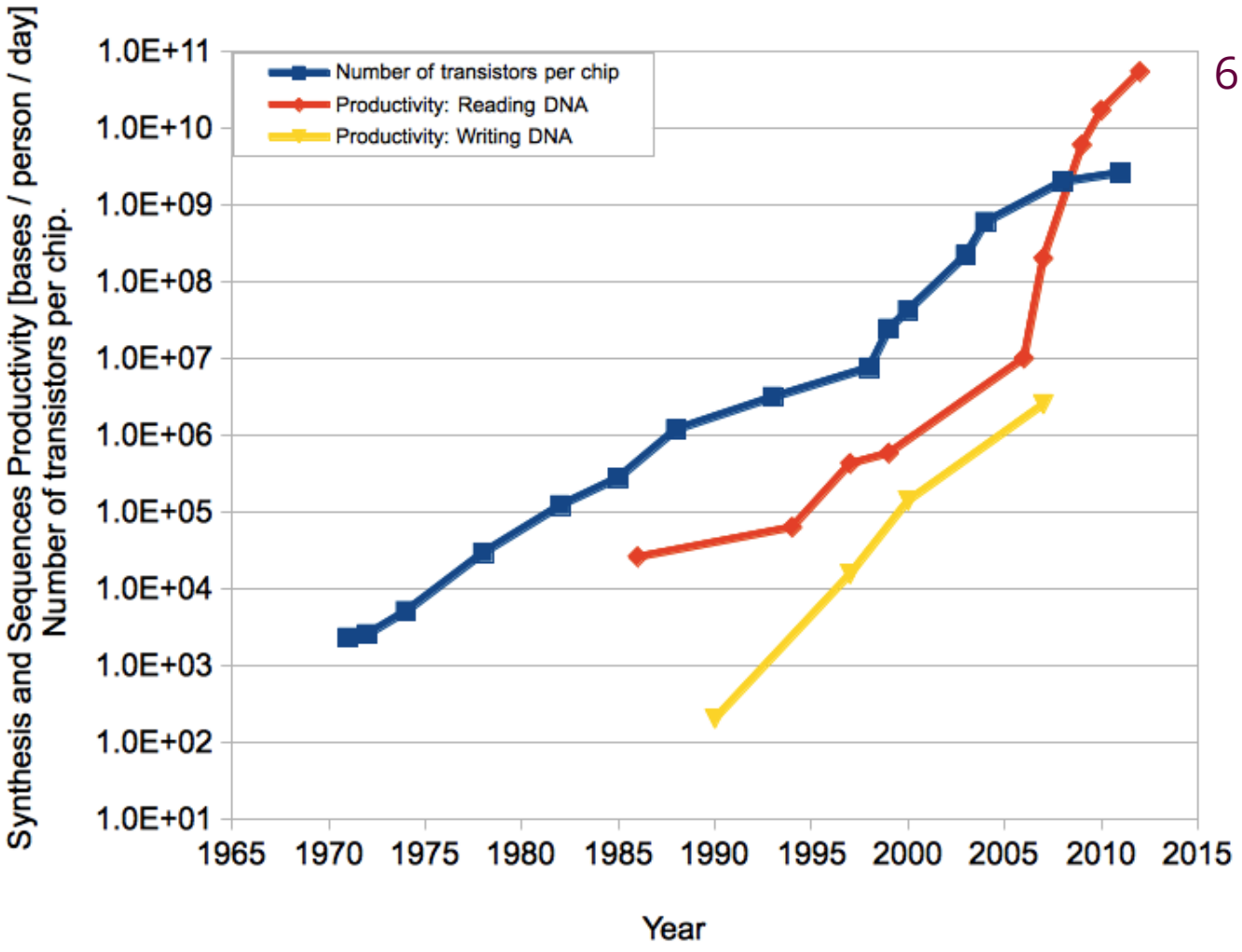
DNA sequencing and synthesis cost



10 logs

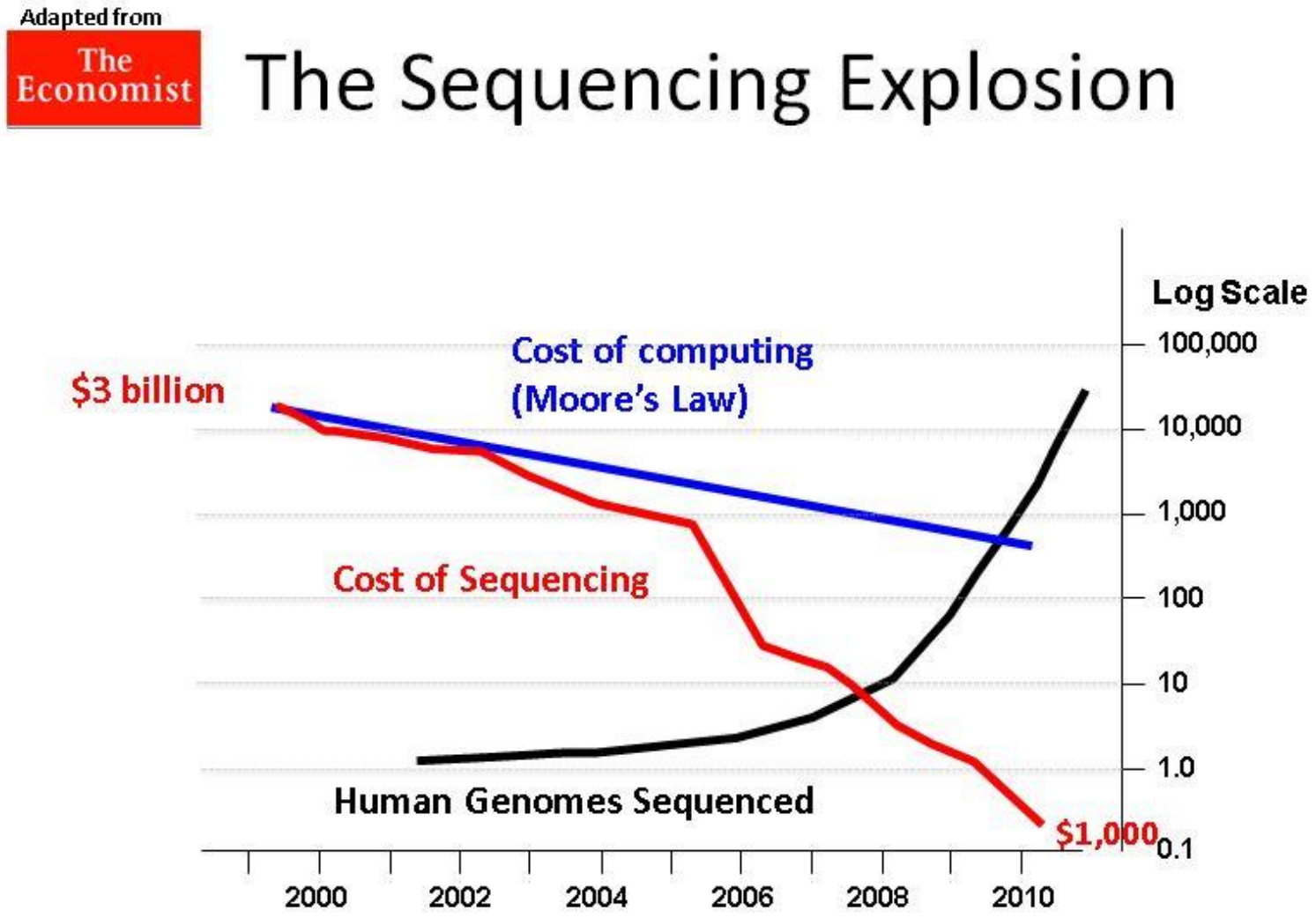
Productivity in DNA synthesis and sequencing



6 logs

Human genome sequencing price

Adapted from The Economist



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New revolution?

Industrial age → Digital age → Biological age

Oil-based feedstocks	→ Synthetic chemistry	→ Industrial process	→ Production
Bio-based feedstocks	→ Synthetic biology	→ Industrial process	→ Production

Adapted from Richard Kitney

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Expectation!

Top emerging technologies: World Economic Forum (WEF) 2012 Davos

1. Informatics for Adding Value to Information
2. Synthetic Biology and Metabolic Engineering

   The natural world is a testament to the vast potential inherent in the genetic code at the core of all living organisms. Rapid advances in synthetic biology and metabolic engineering are allowing biologists and engineers to tap into this potential in unprecedented ways, enabling the development of new biological processes and organisms that are designed to serve specific purposes – whether converting biomass to chemicals, fuels and materials, producing new therapeutic drugs or protecting the body against harm.
3. Green Revolution 2.0 – Technologies for Increased Food and Biomass
4. Nanoscale Design of Materials
4. Systems Biology and Computational Modeling / Simulation of Chemical and Biological Systems

Science News *from research organizations*

Scientists chart course toward a new world of synthetic biology

Engineering/synthetic biology can improve many aspects of life, but needs federal support

Date: June 19, 2019

Source: University of California - Berkeley

Summary: A team has compiled a roadmap for the future of synthetic or engineering biology, based on the input of 80 leaders in the field from more than 30 institutions. The report provides a strong case that the federal government should invest in this area, not only to improve public health, food crops and the environment, but also to fuel the economy and maintain the country's leadership in synthetic/engineering biology.

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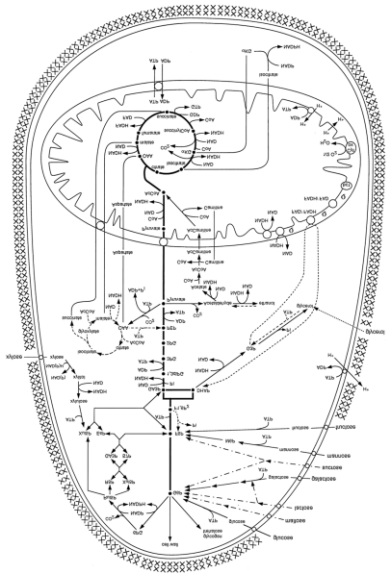
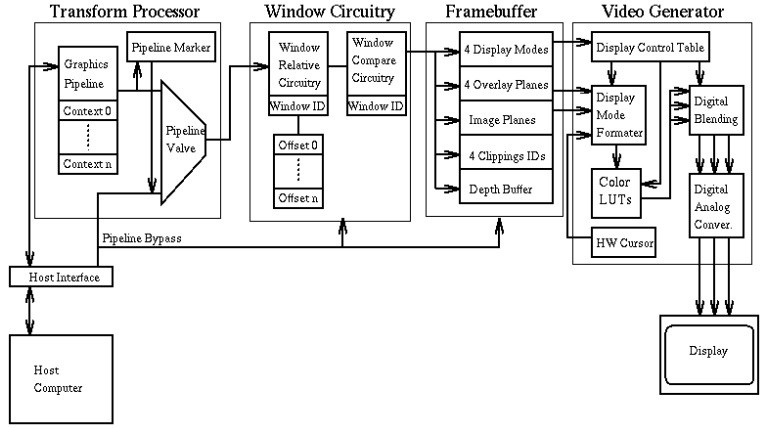
A computer analogy



Cell information	Processors (input-output)
DNA	programming information
Digital code	ATGC Open standard (e.g. codons)
Modular code	Genes
Error protection	DNA repair
Data compression	Overlapping ORFs Redundant backup (double helix, copy number)
Self-diagnostics	Apoptosis Firewalls (Species)
Operating system	Ribosomes



Adapted from Andrew Hessel

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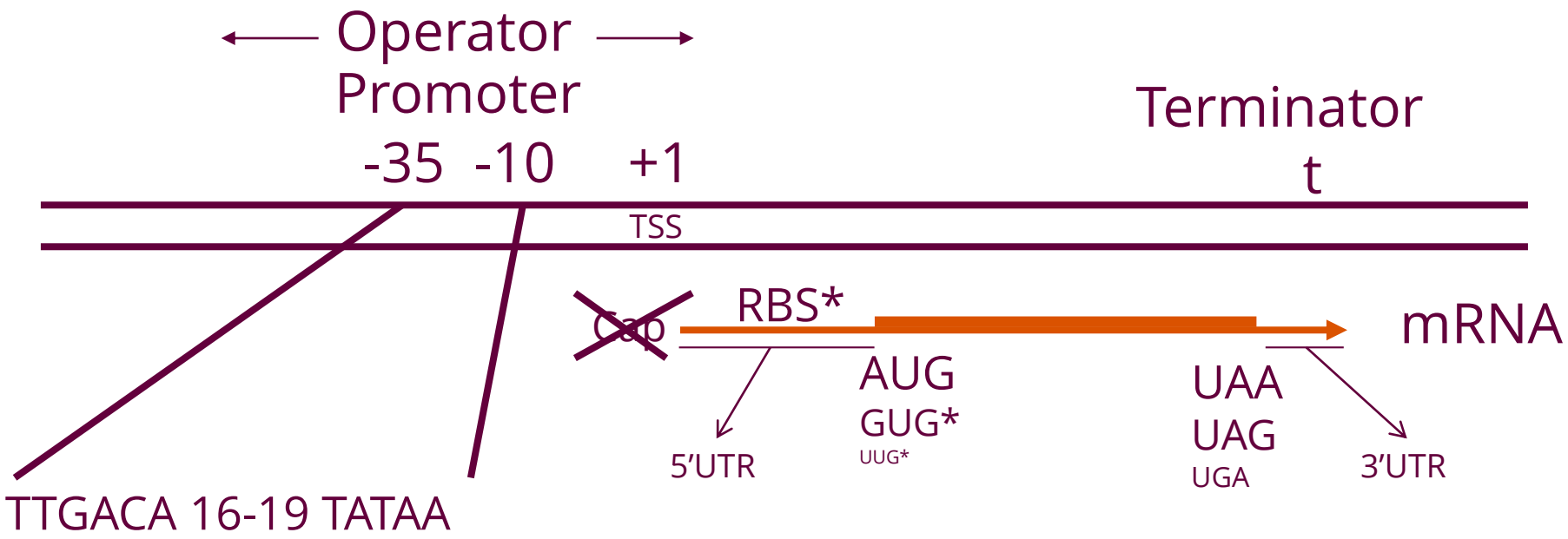
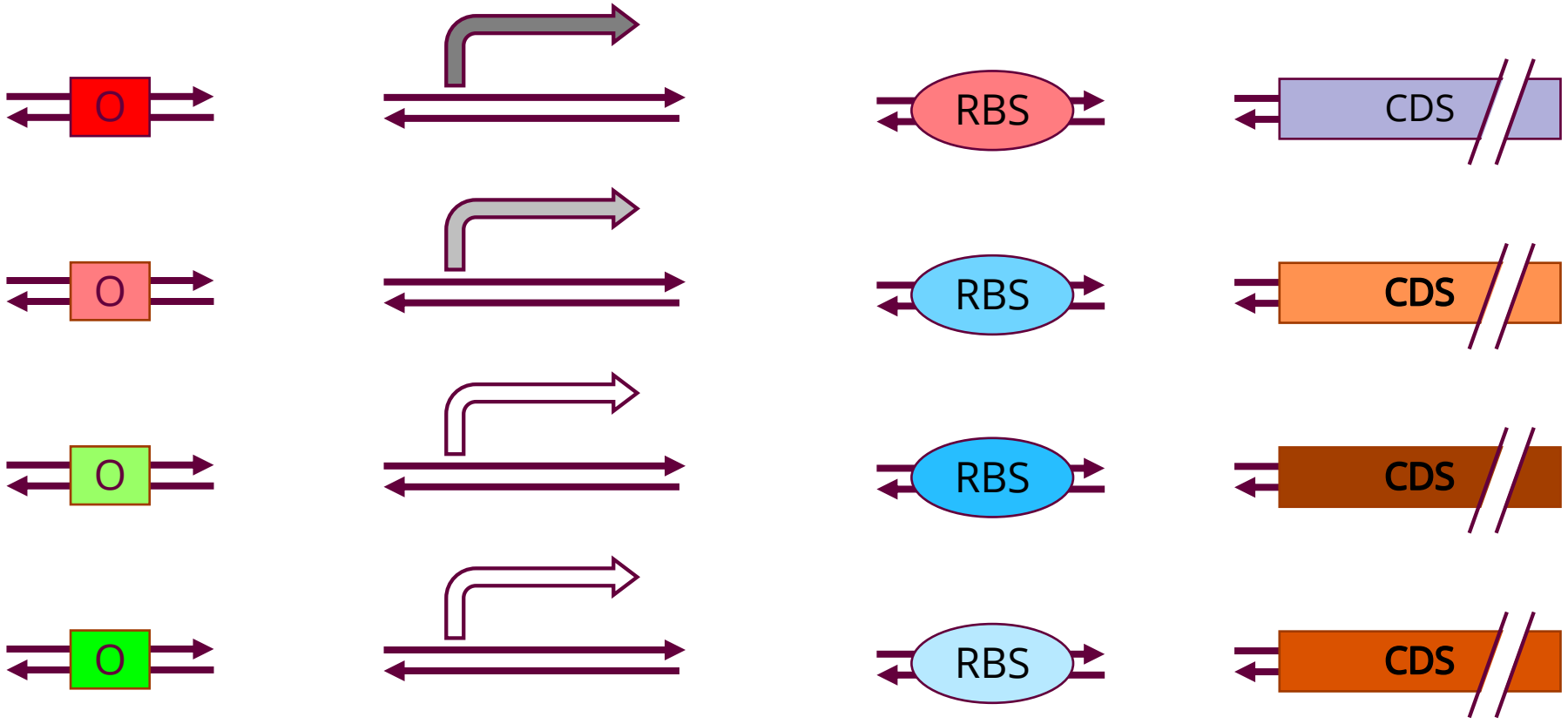
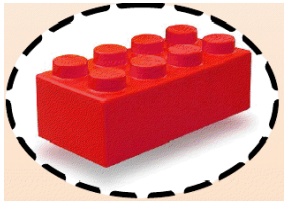
Transcription / translation



Modularity



Modularity



- **Parts:**

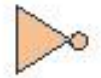
DNA sequence encoding some part of the genetic machinery

e.g.: promoters, operator, RBS, CDS, terminator)

- **Devices:**

Group of parts that work together for specific functions

e.g.: protein production, sensing-reporting, measurement, signal inversion, signaling, motility

Symbol	BioBrick parts
	Promoter
	Coding sequence
	RBS
	DNA
	Inverter
	Plasmid backbone
	Terminator

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Registry of standard biological parts
Biobricks Foundation (BBF)


Part assembly


System operation


Protein expression


Assembly of protein fusions


Part measurement


Screening of part libraries


Building BioBrick vectors

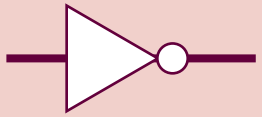
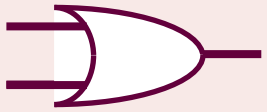
DNA synthesis


Other standards

Created by Drew Endy and Tom Knight:

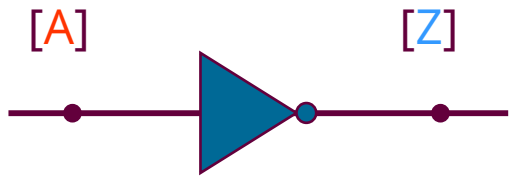
- Educate the public about biotechnology and synthetic biology
- Make biotech simple to do and available to the public
- Define ethical concerns and practices in synthetic biology

Logic gates

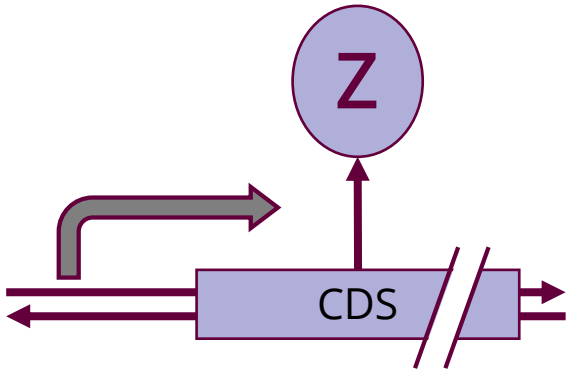
Function	Alg. Expres.	Symbol	Truth table
NOT	$\overline{A}$		A | | X 0 | | 1 1 | | 0
AND	AB		A | B | X 0 | 0 | 0 0 | 1 | 0 1 | 0 | 0 1 | 1 | 1
NAND	$\overline{AB}$		A | B | X 0 | 0 | 1 0 | 1 | 1 1 | 0 | 1 1 | 1 | 0
OR	$A+B$		A | B | X 0 | 0 | 0 0 | 1 | 1 1 | 0 | 1 1 | 1 | 1

Logic gate

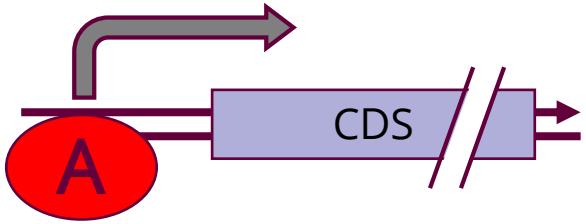
Proteins = Wires
Genes = Gates



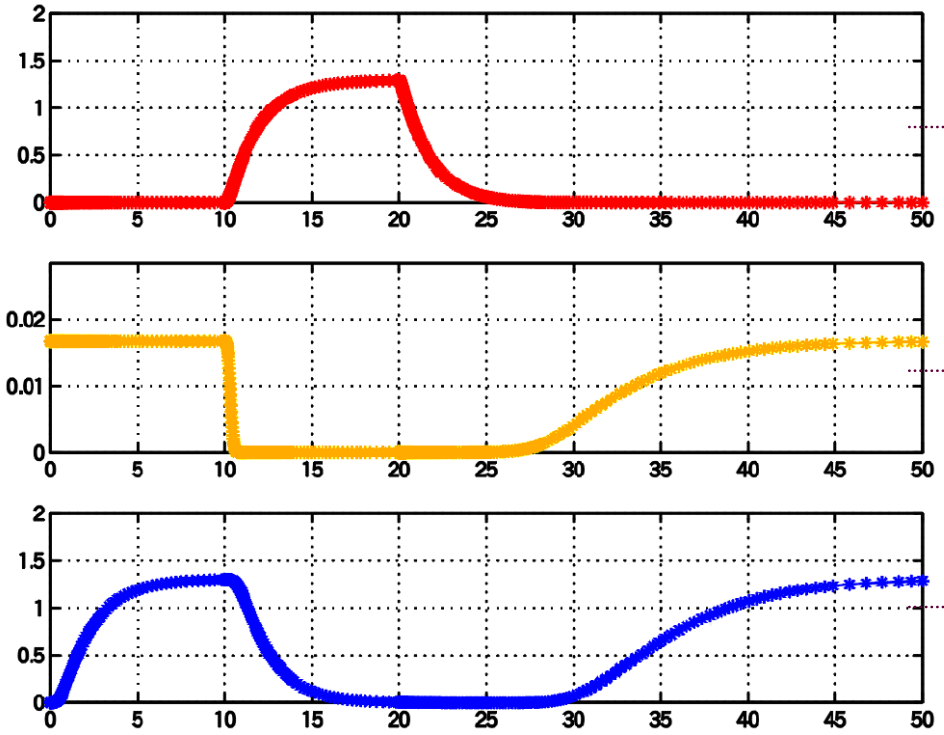
[A] = 0
[Z] = 1

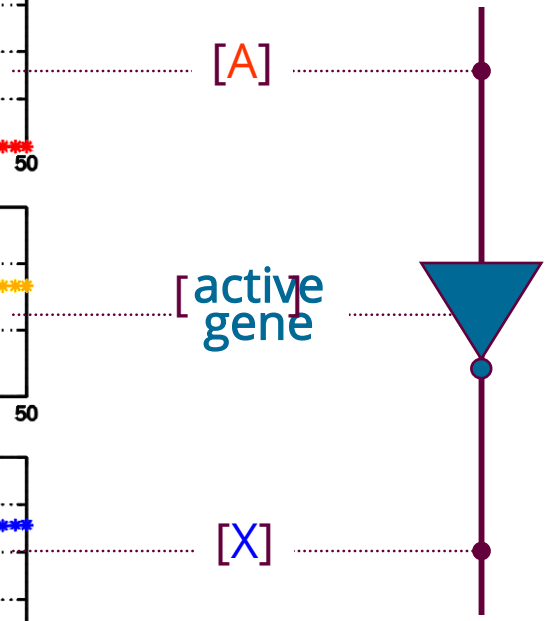


[A] = 1
[Z] = 0



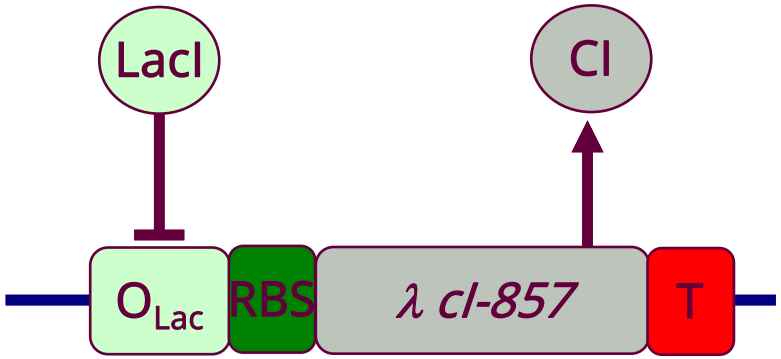
Inverter

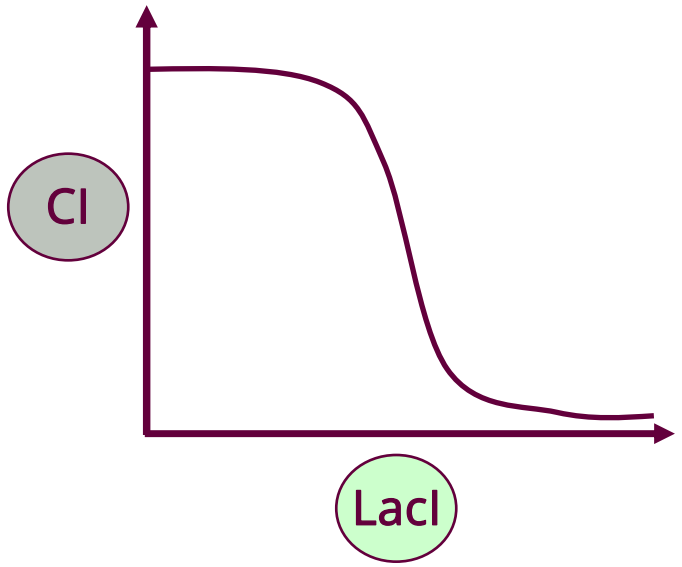




Adapted from Weiss et al, MIT

Inverter

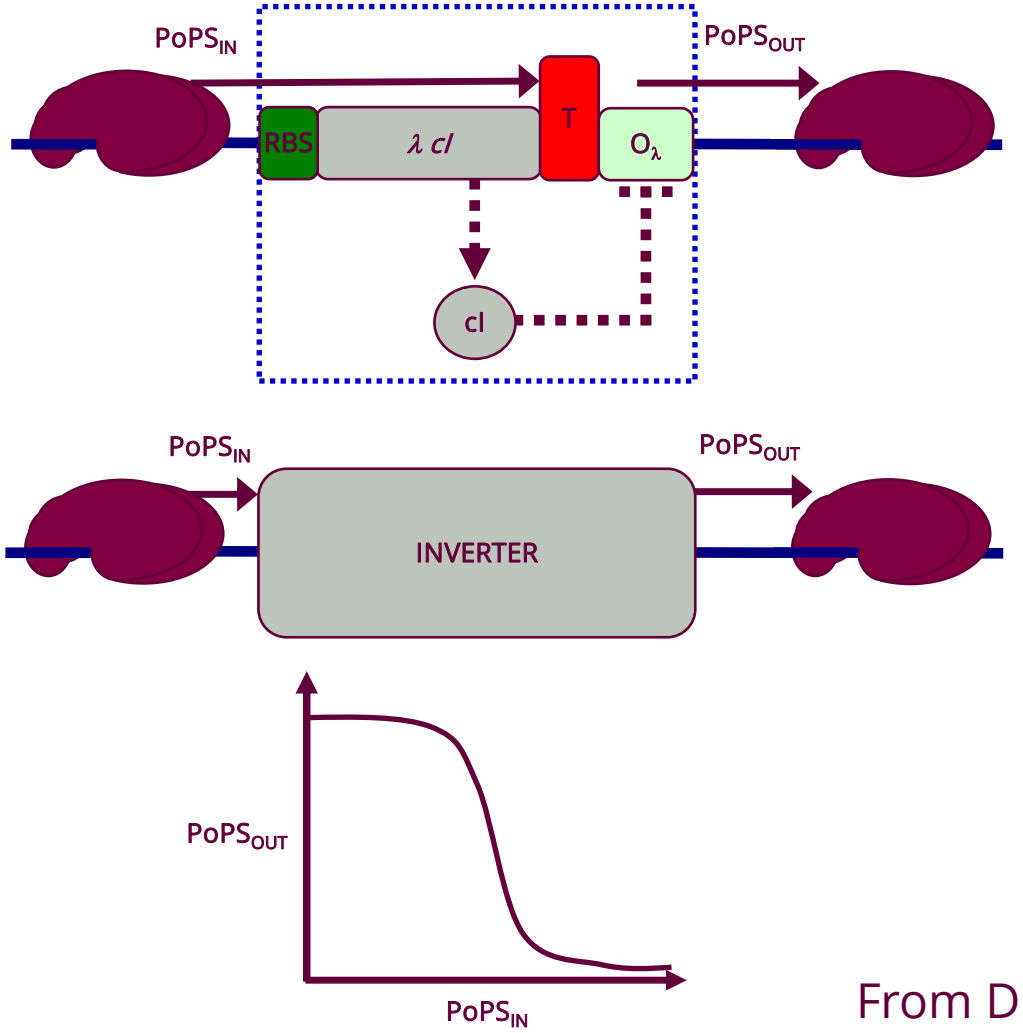




From Drew Endy , MIT

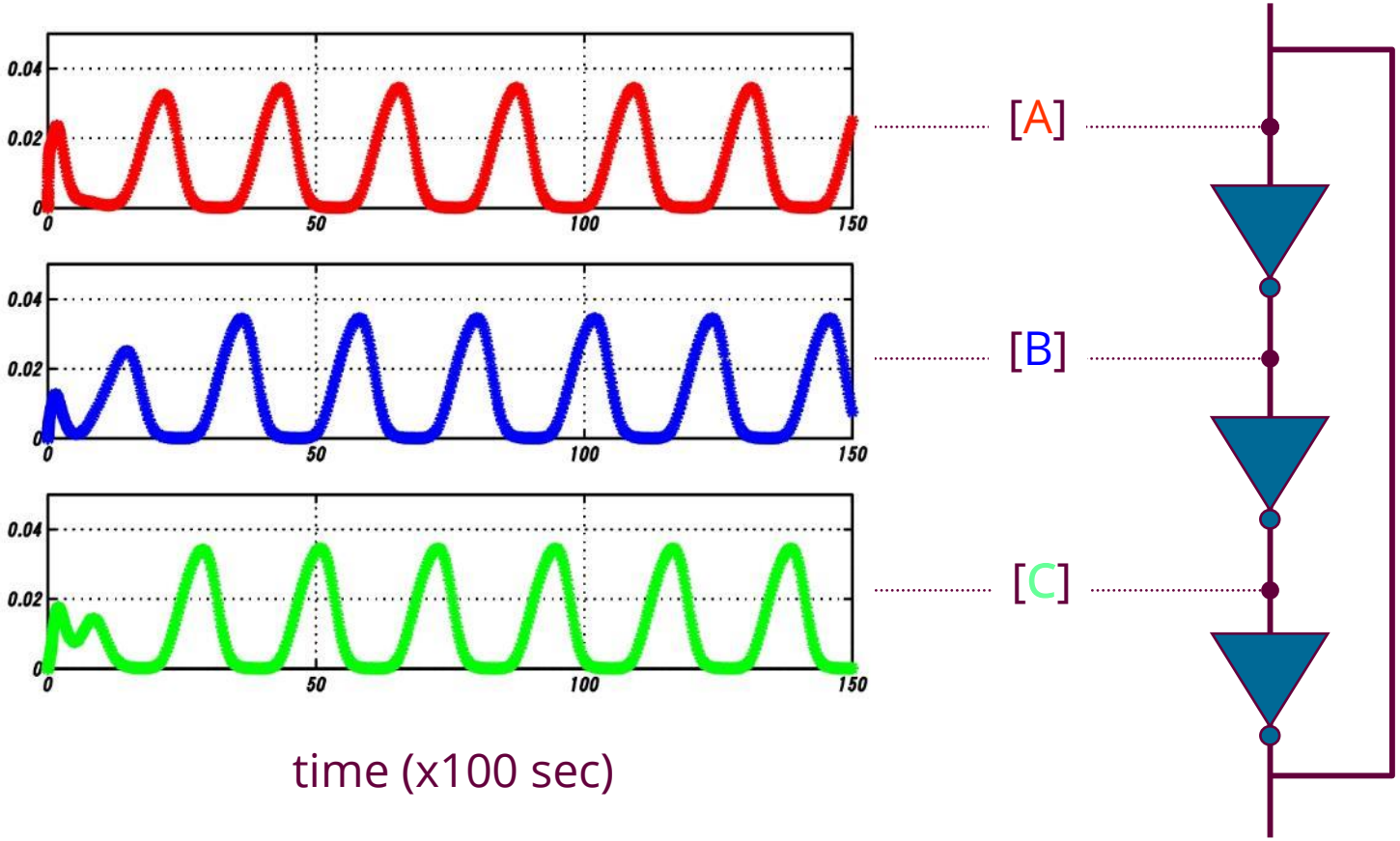
Polymerase Per Second = PoPS!

Number of times that an RNA polymerase passes a specific point on DNA per unit time.



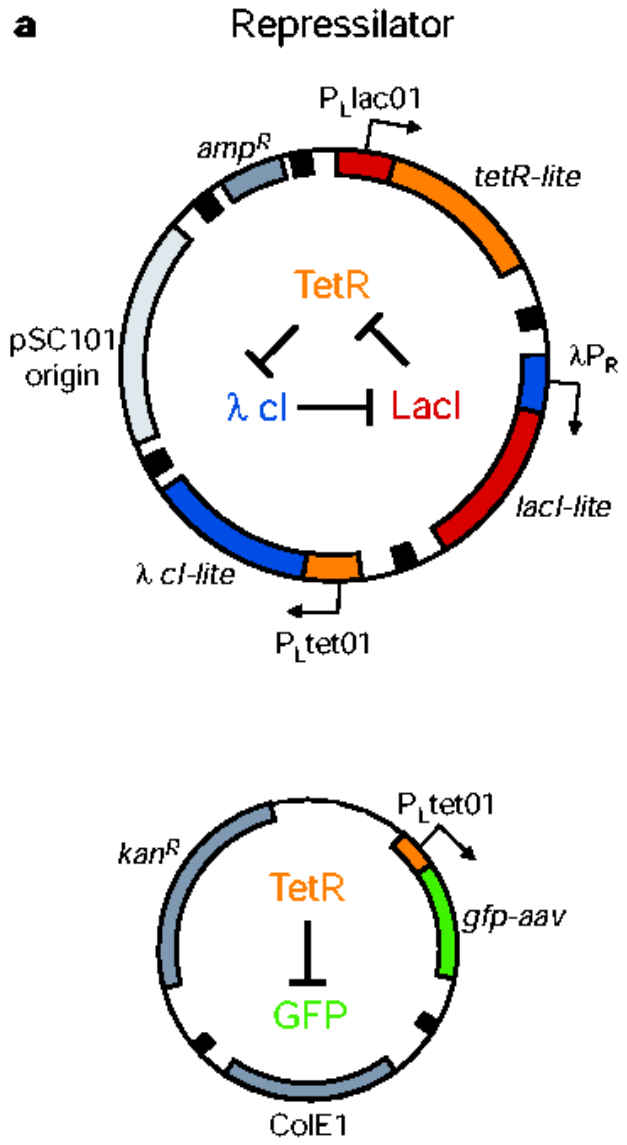
From Drew Endy , MIT

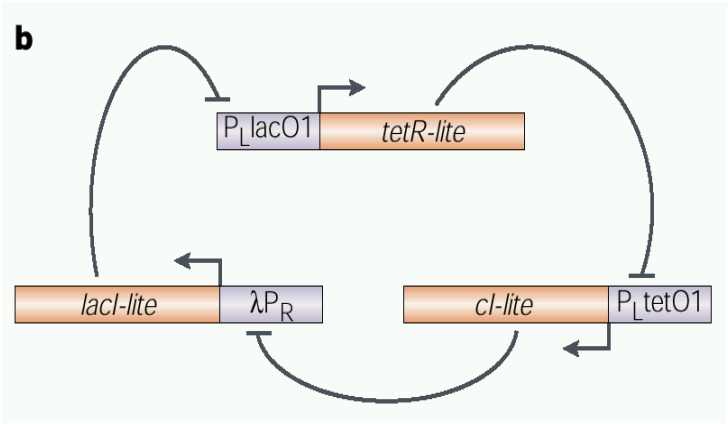
Oscillator



Adapted from Weiss et al, MIT

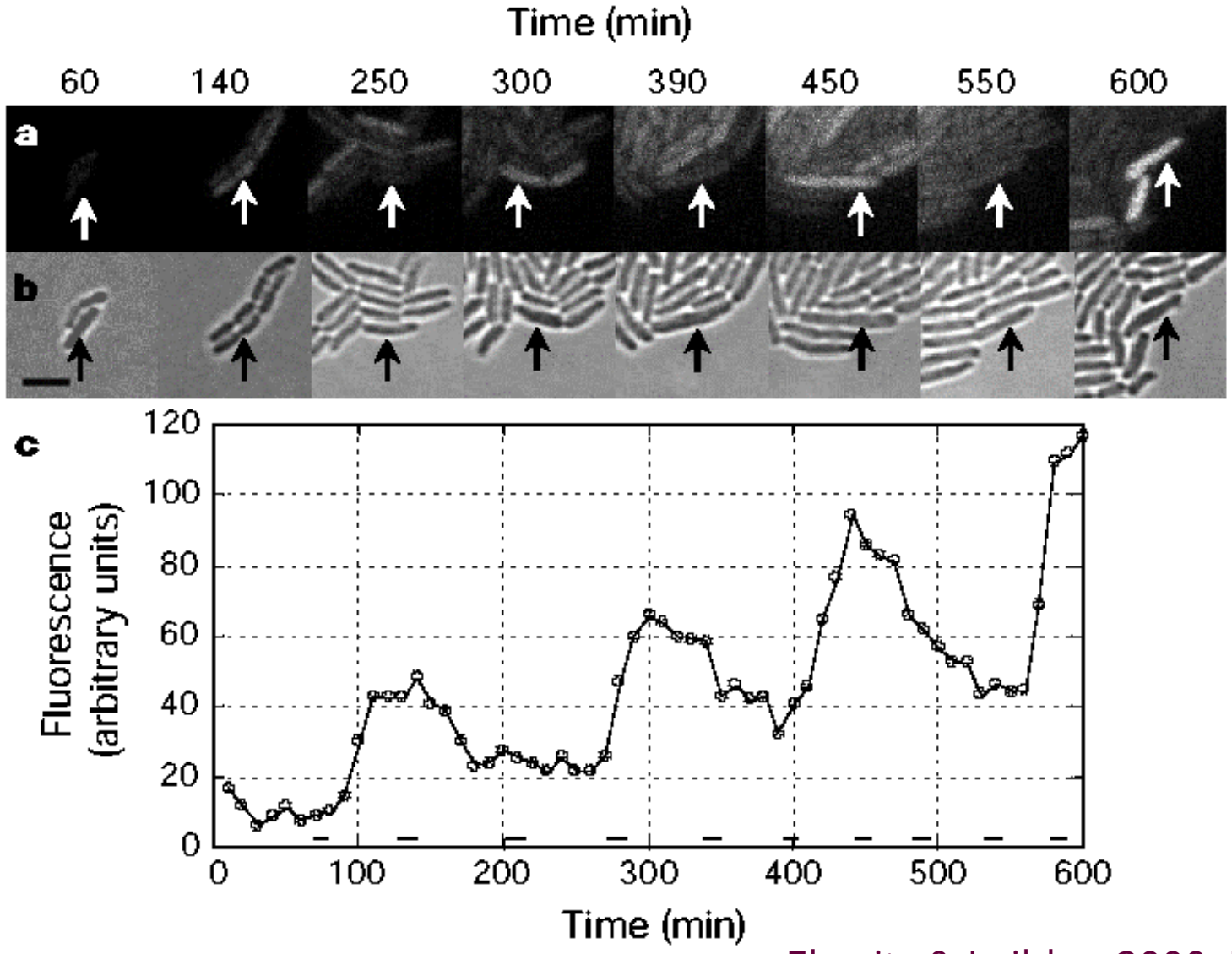
Oscillator





Elowitz & Leibler, 2000, Nature

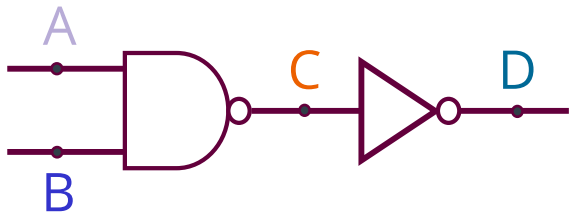
Oscillation



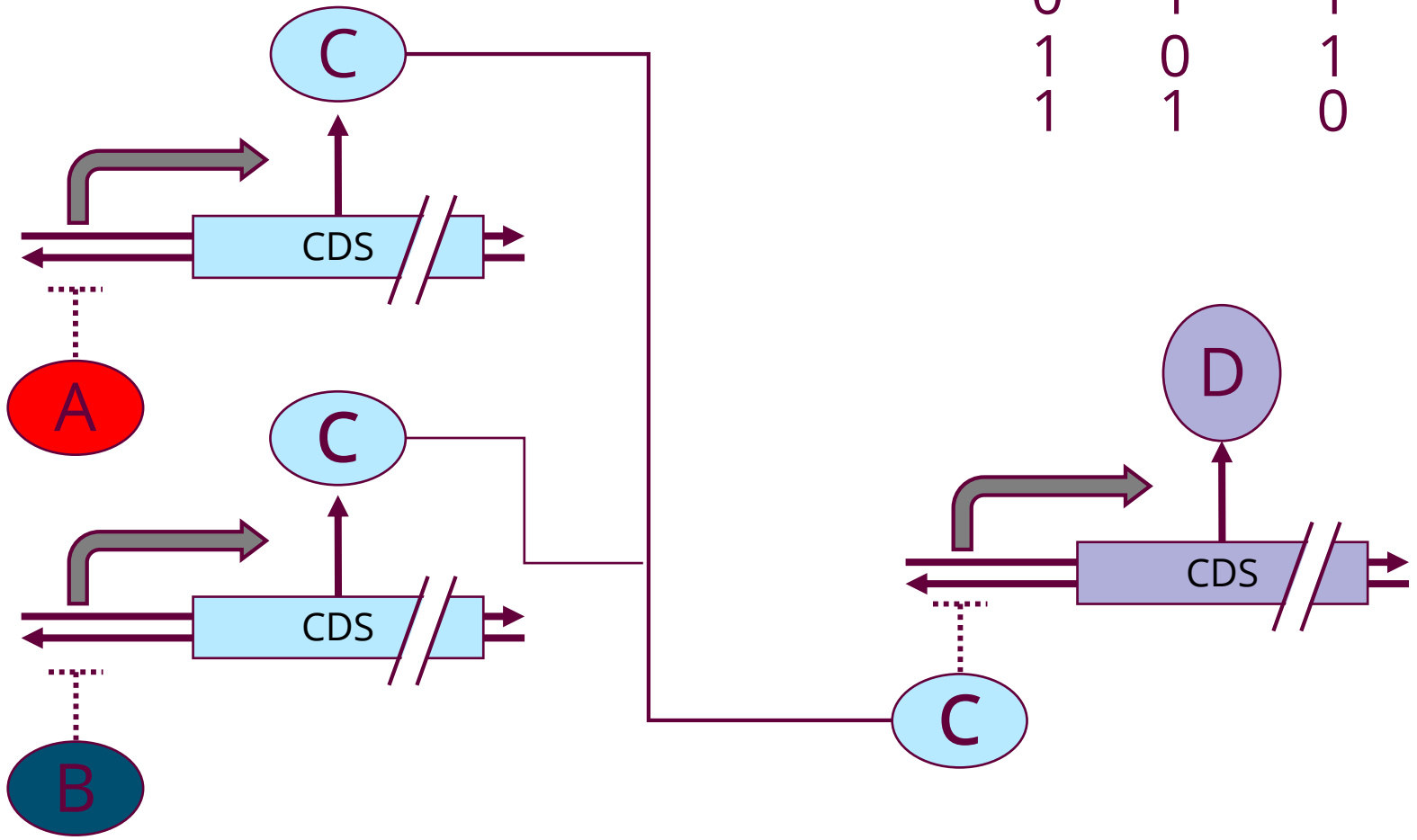
Elowitz & Leibler, 2000, Nature

Digital Circuit

NAND gate



In		Out	
B	A	C	D
0	0	1	0
0	1	1	0
1	0	1	0
1	1	0	1



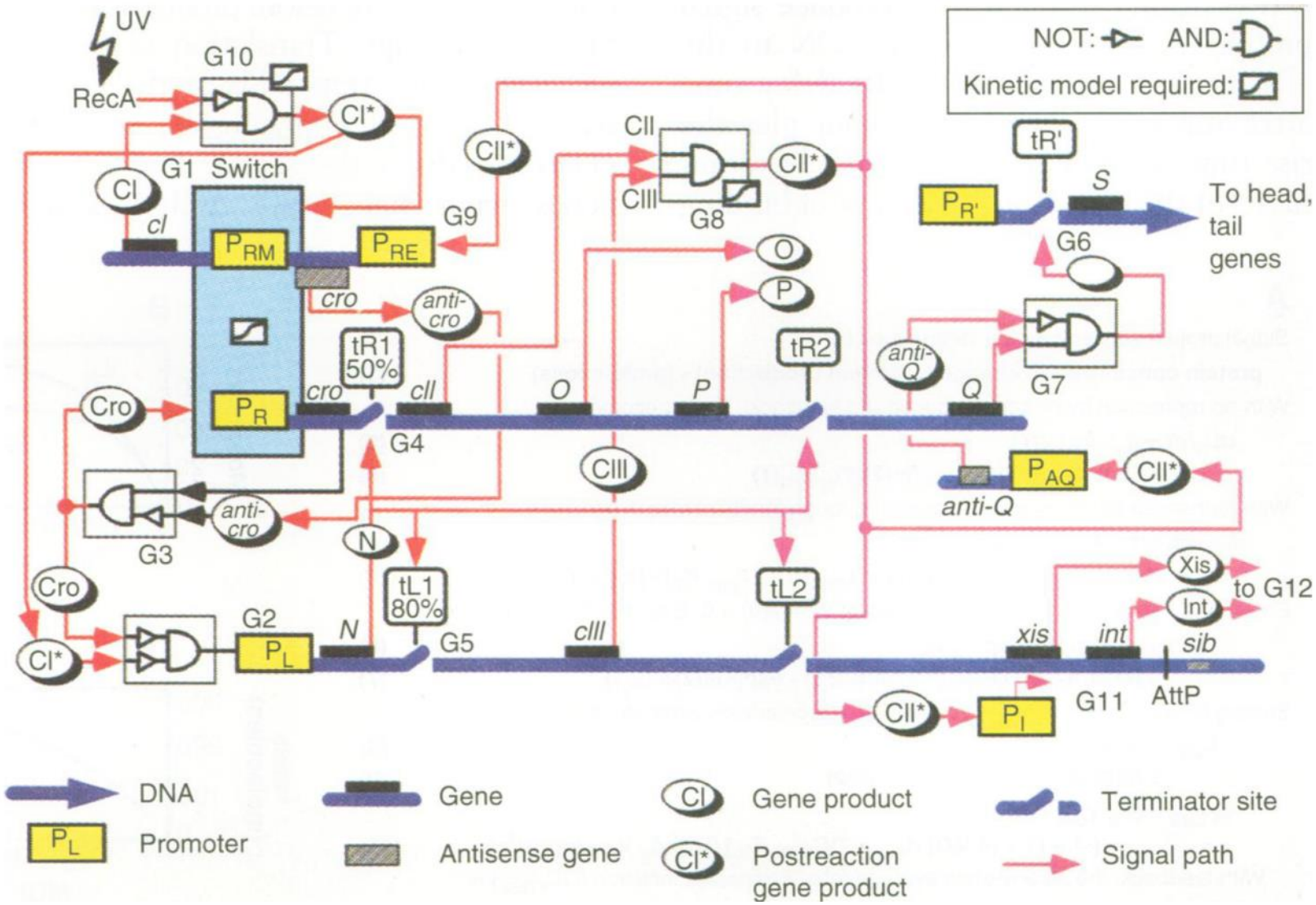
Organisms → Chassis

host containing devices:

- Often model bacteria (*e.g. Escherichia coli* or *Bacillus subtilis*)
- Unicellular eukaryotes (*e.g.*, *S. cerevisiae*)
- But also bacteriophage (virus), plants, mammals, anything, event synthetic organisms

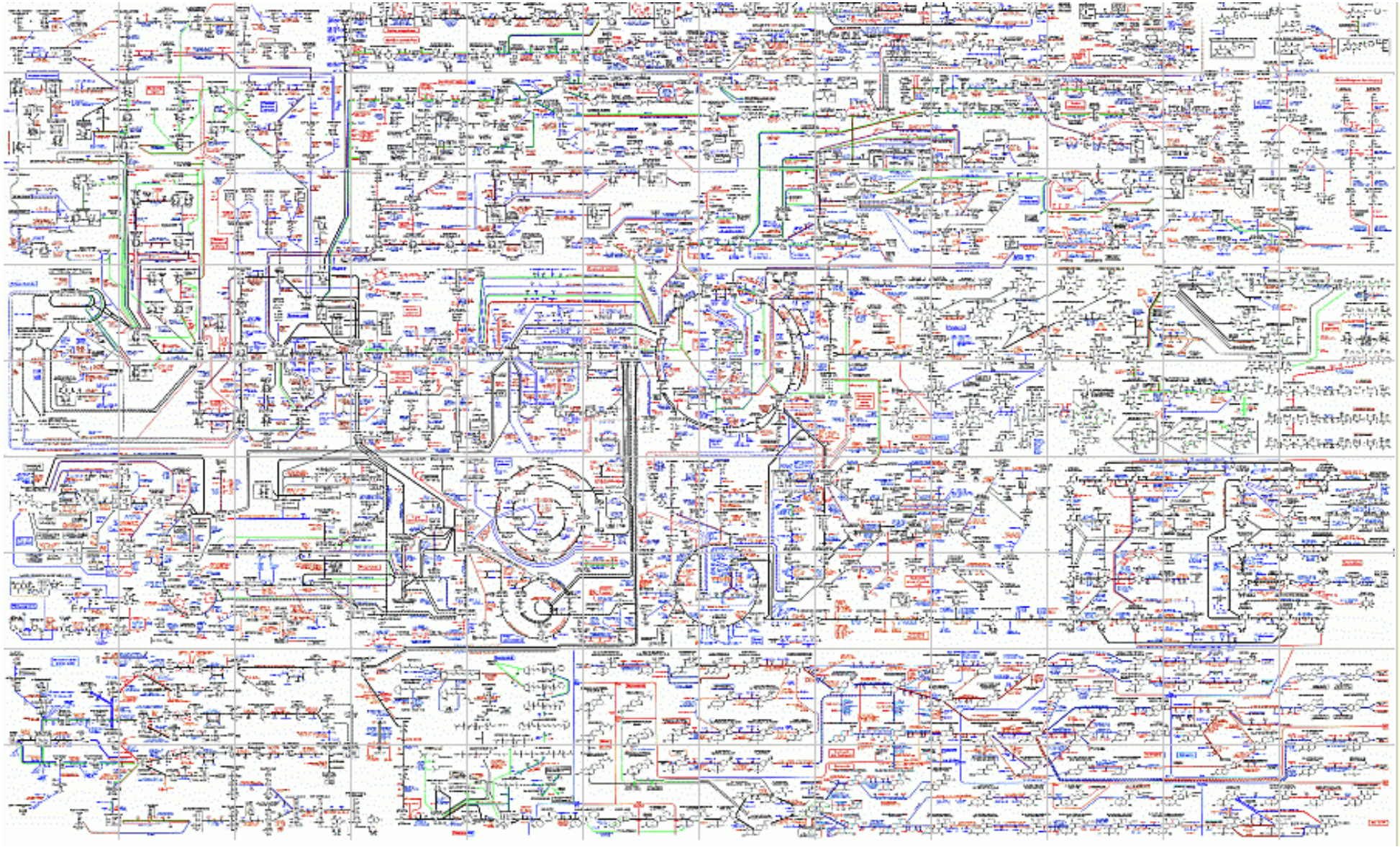
Simplistic vision?
Think big!

Genetic circuit of λ lysis-lysogeny decision

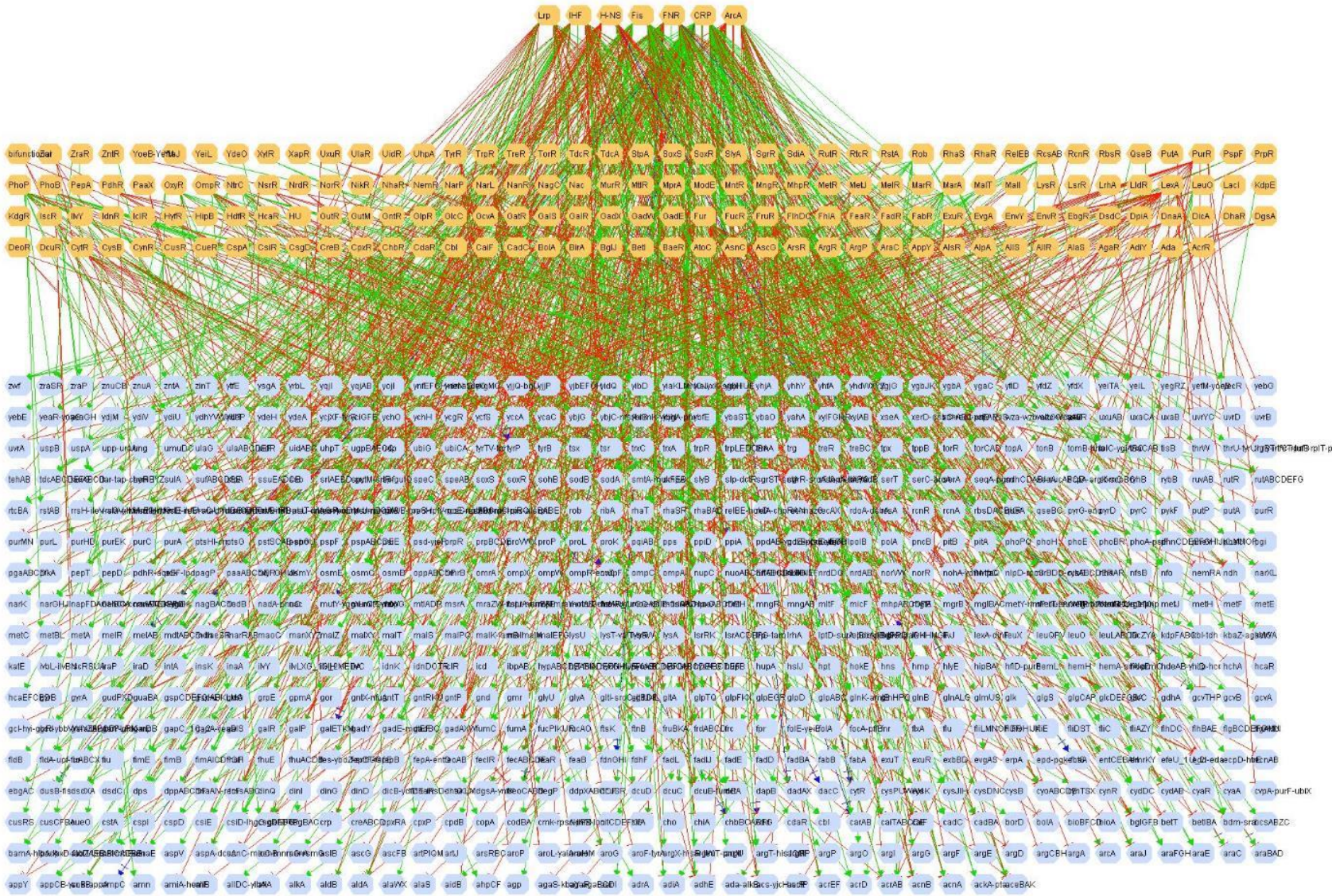


McAdams and Shapiro 1995

Metabolic pathways



Régulateurs et opérons chez *E. coli*



iBioFAB at University of Illinois

https://www.youtube.com/watch?v=Hwb735qZ-IQ

The Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB) is a fully integrated computational and physical infrastructure that supports rapid design, fabrication, validation/quality control, and analysis of genetic constructs and organisms. As the first "living foundry" in the world, the iBioFAB provides a new manufacturing paradigm for chemicals, materials, and biologics.

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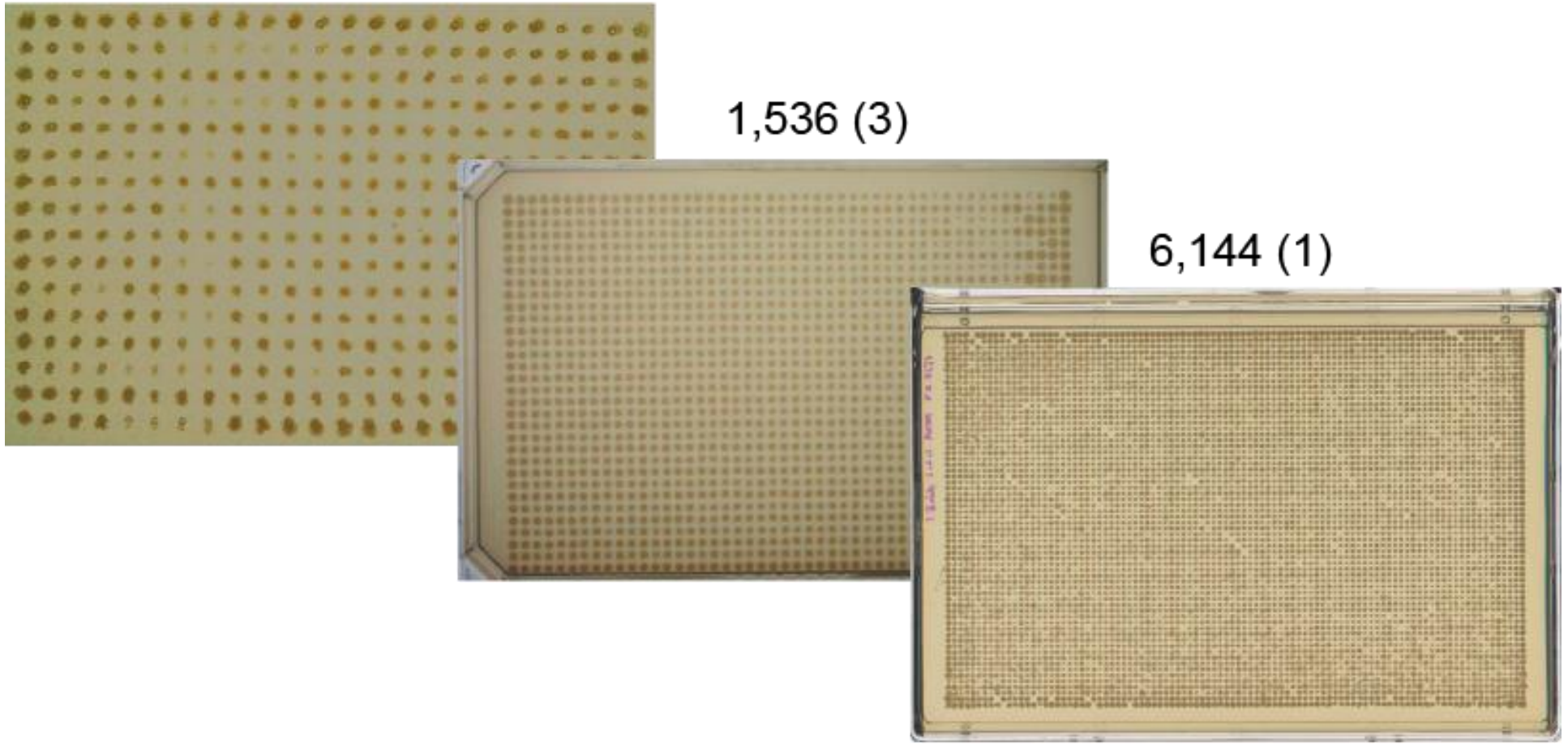
Increasing the density of colony on plate

384 / plate (12 plates / genome)

1,536 (3)

6,144 (1)



Scanning system.

Max 74,000 colonies / day

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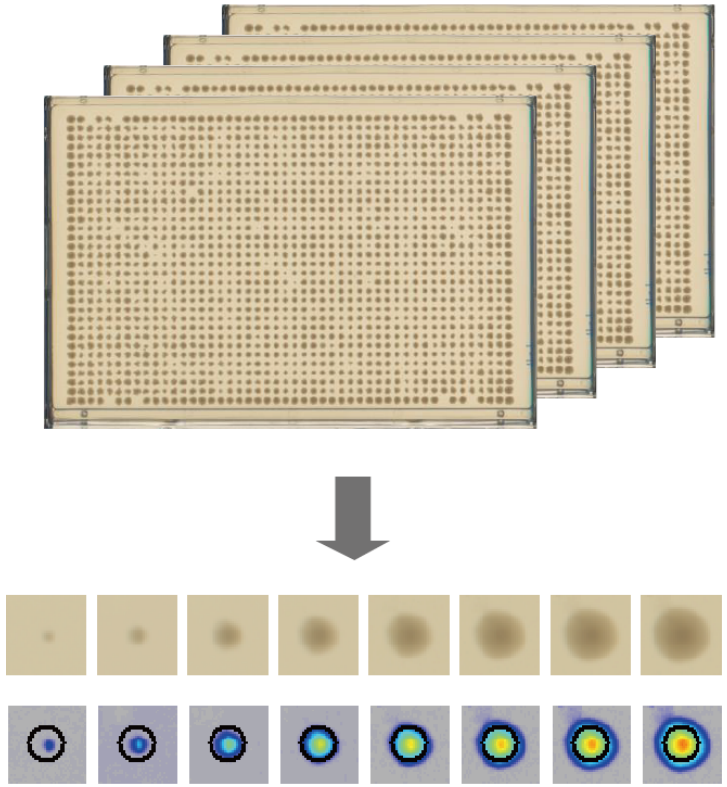
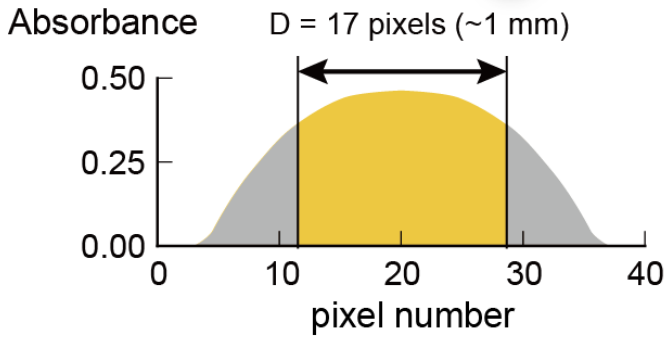
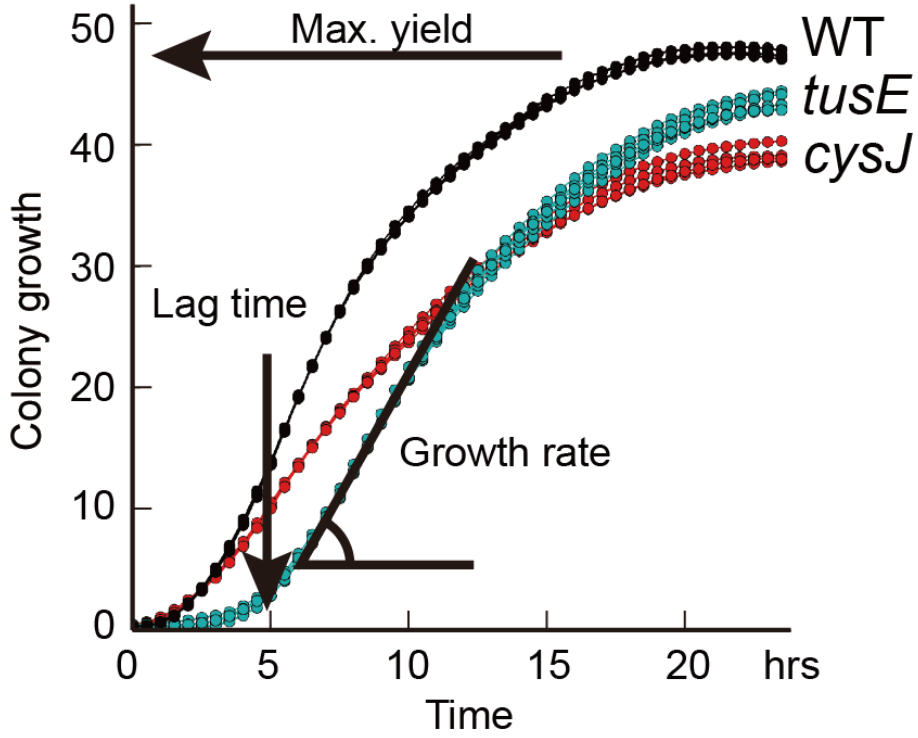
Accurate measurement of cell growth

Time series quantification







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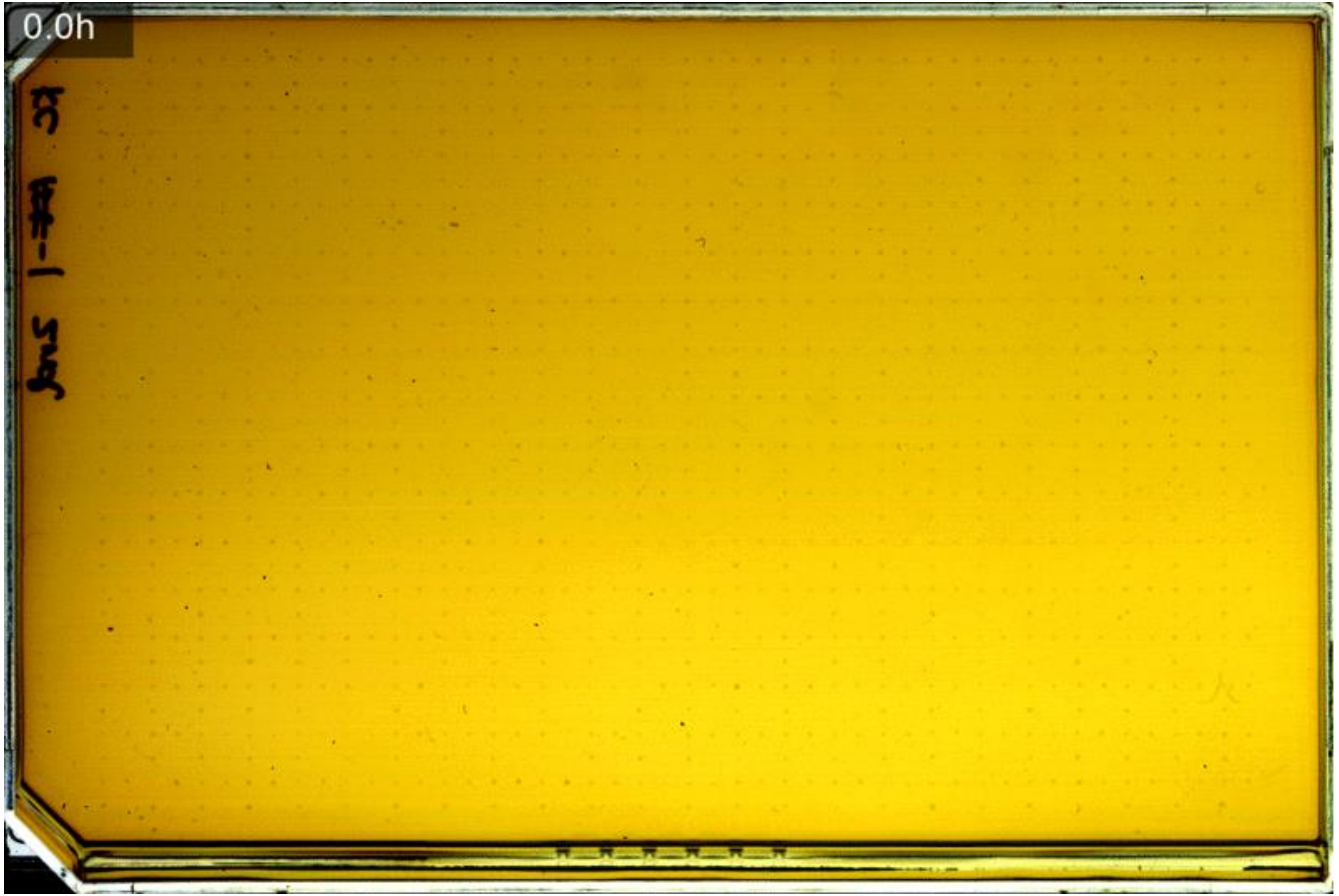

0.0h

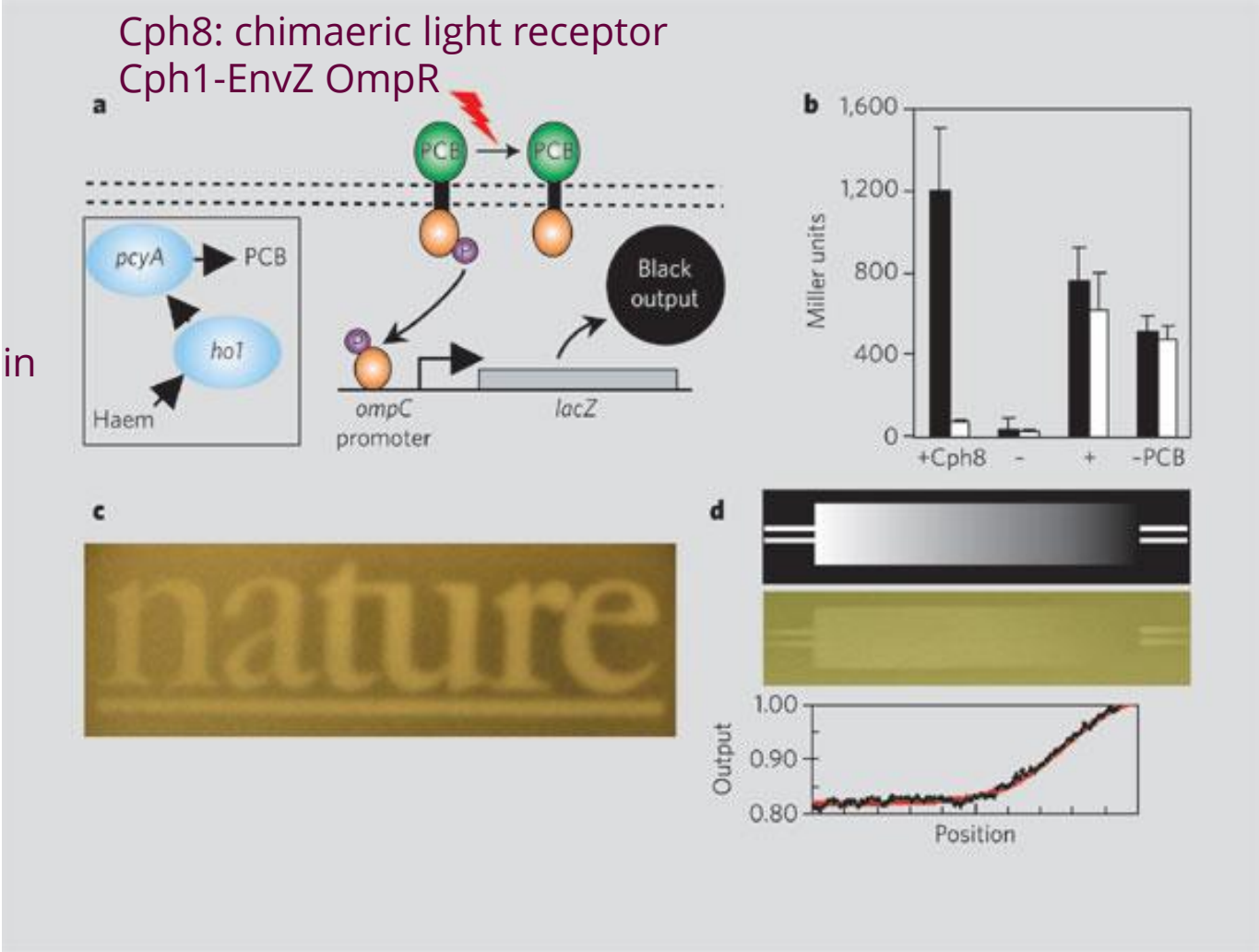
Outline

- Definitions
- Origins of molecular biology
- Visionaries
- Why synthetic biology is possible?
- Synthetic biology standardization
- Simplistic vision?
- Examples of synthetic biology accomplishments

Light imaging by engineered *Escherichia coli*

Cph8: chimaeric light receptor
Cph1-EnvZ OmpR

PCB:
phycocyanobilin



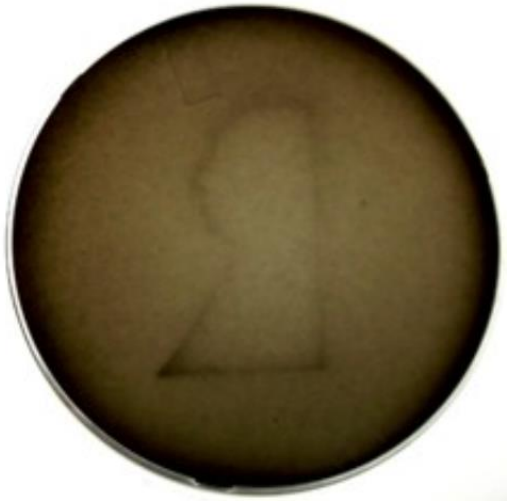
Levskaya et al. Nature. 2005; 438(7067):441-2.

Photo strain

Projected Mask

Photo strain

Edge detector strain



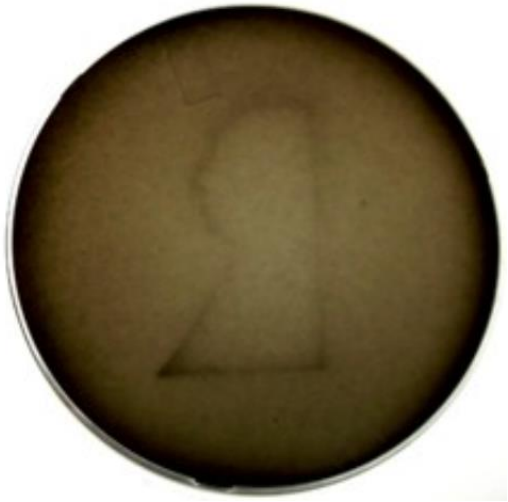
Tabor et al. Cell. 2009; 137(7):1272-81.

Edge detector ?

Projected Mask

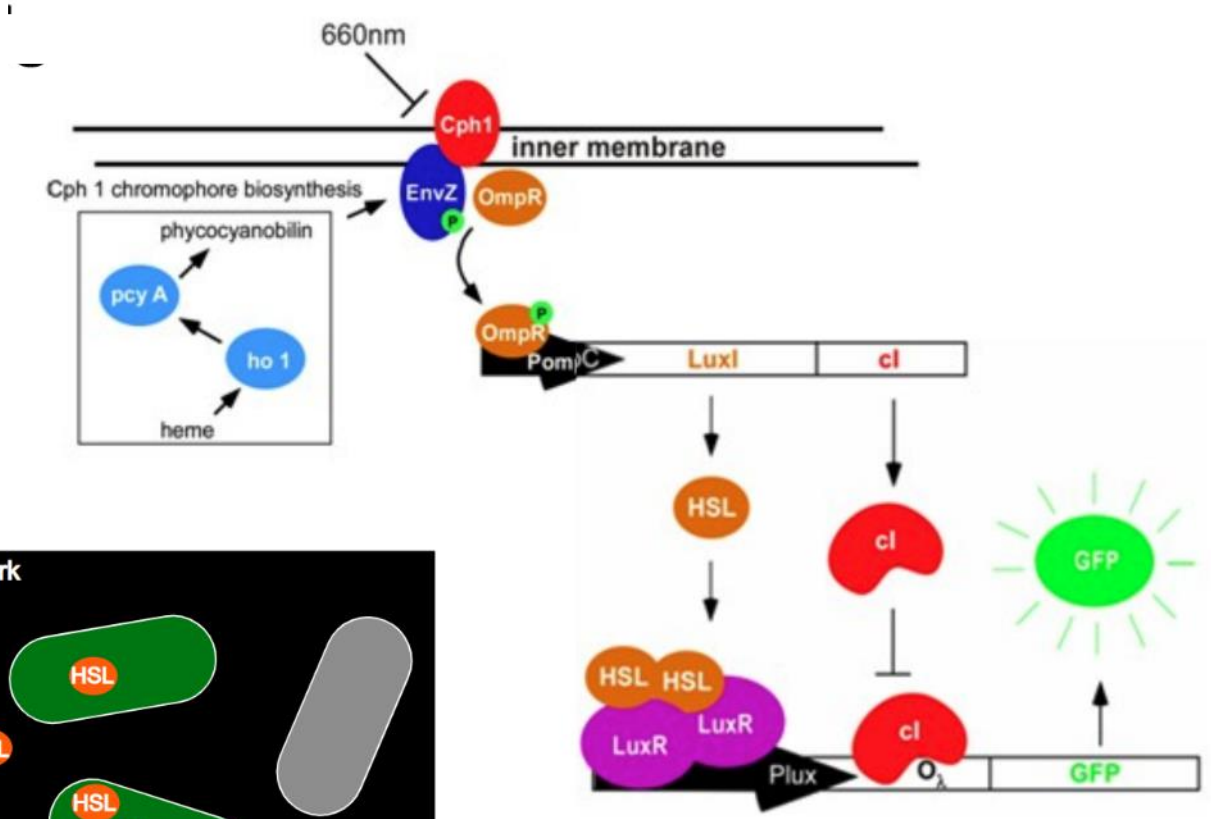
Photo strain

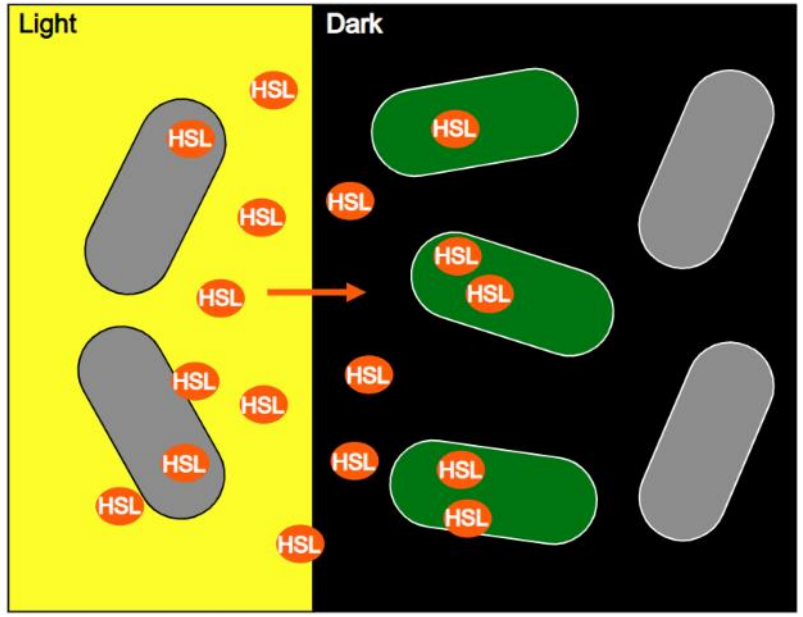
Edge detector strain



Tabor et al. Cell. 2009; 137(7):1272-81.

Edge detector





Tabor et al. Cell. 2009; 137(7):1272-81.

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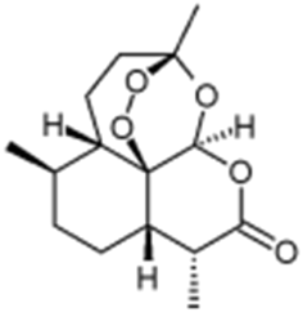
Engineering an existing pathway.



Malaria: about 200 millions human infected (plasmodium)

The artimisinine is an antimalarial, extracted from the plant *Artemisia annua* (sweet wormwood) (herbal medicine ginghaosu for more than 2000 years)

Youyou Tu (prix Nobel 2015)

- Quantity produced insufficient, fluctuating costs

- Jay Keasling (UC Berkeley) used a synthetic biological approach: transplanted genes from yeast and from the sweet wormwood tree into the bacterium *Escherichia coli* to produce the artemisinin precursor, amorphadiene.

- Artemisinin produced by Sanofi

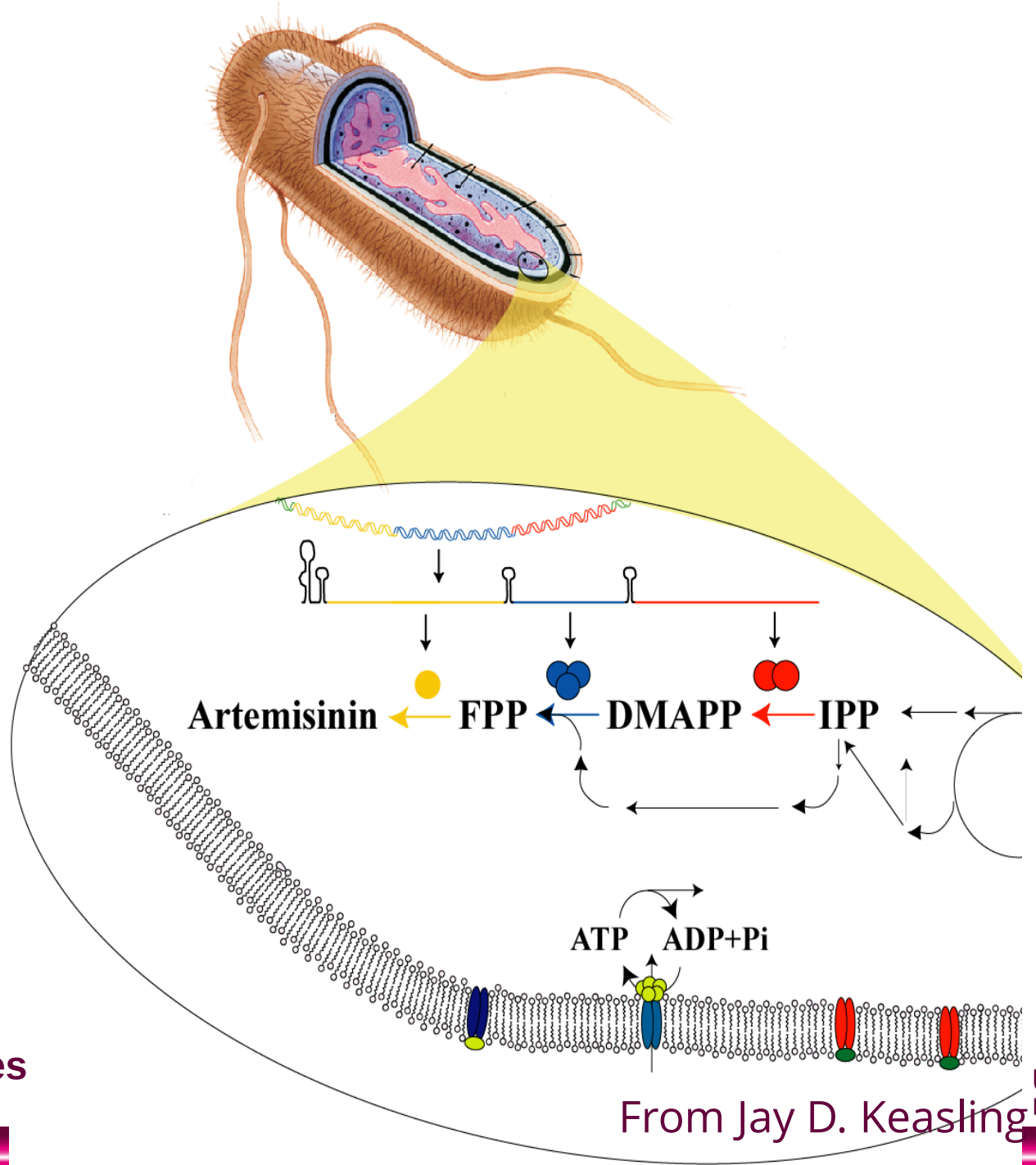
Microbial production of artemisinin

- **Advantages**
  - Microbial fermentations are relatively simple to scale up
  - Inexpensive starting materials can be used
- **Challenges**
  - Need the genes for all of the enzymes in the pathway
  - Not always simple to express in microbes the genes from very different organisms
  - Need to balance metabolic pathways to optimize production
  - Need a good “platform organism” with appropriate gene expression tools

From Jay D. Keasling

Synthesis of artemisinin in *E. coli*

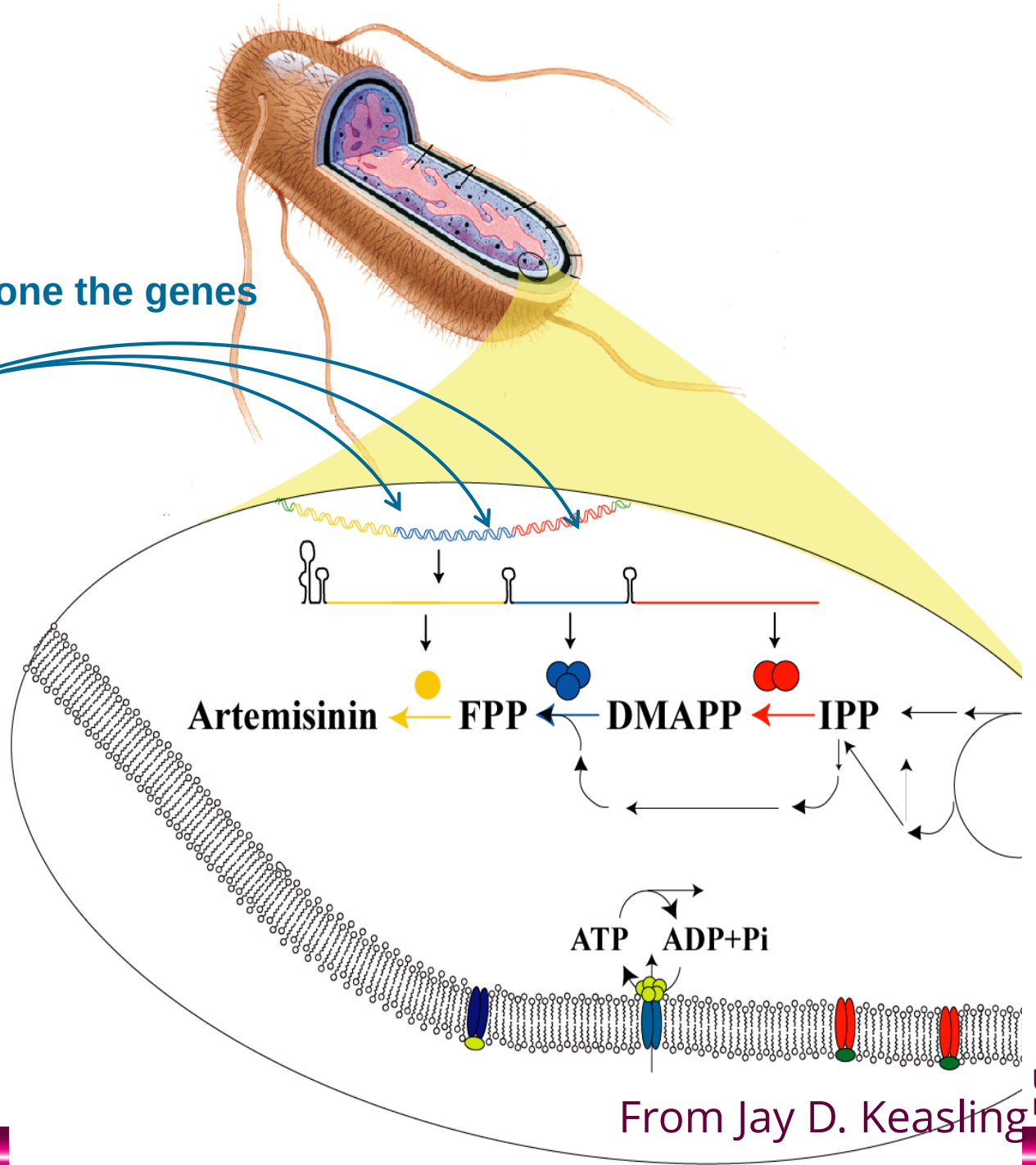
Identify the enzymes



From Jay D. Keasling

Synthesis of artemisinin in *E. coli*

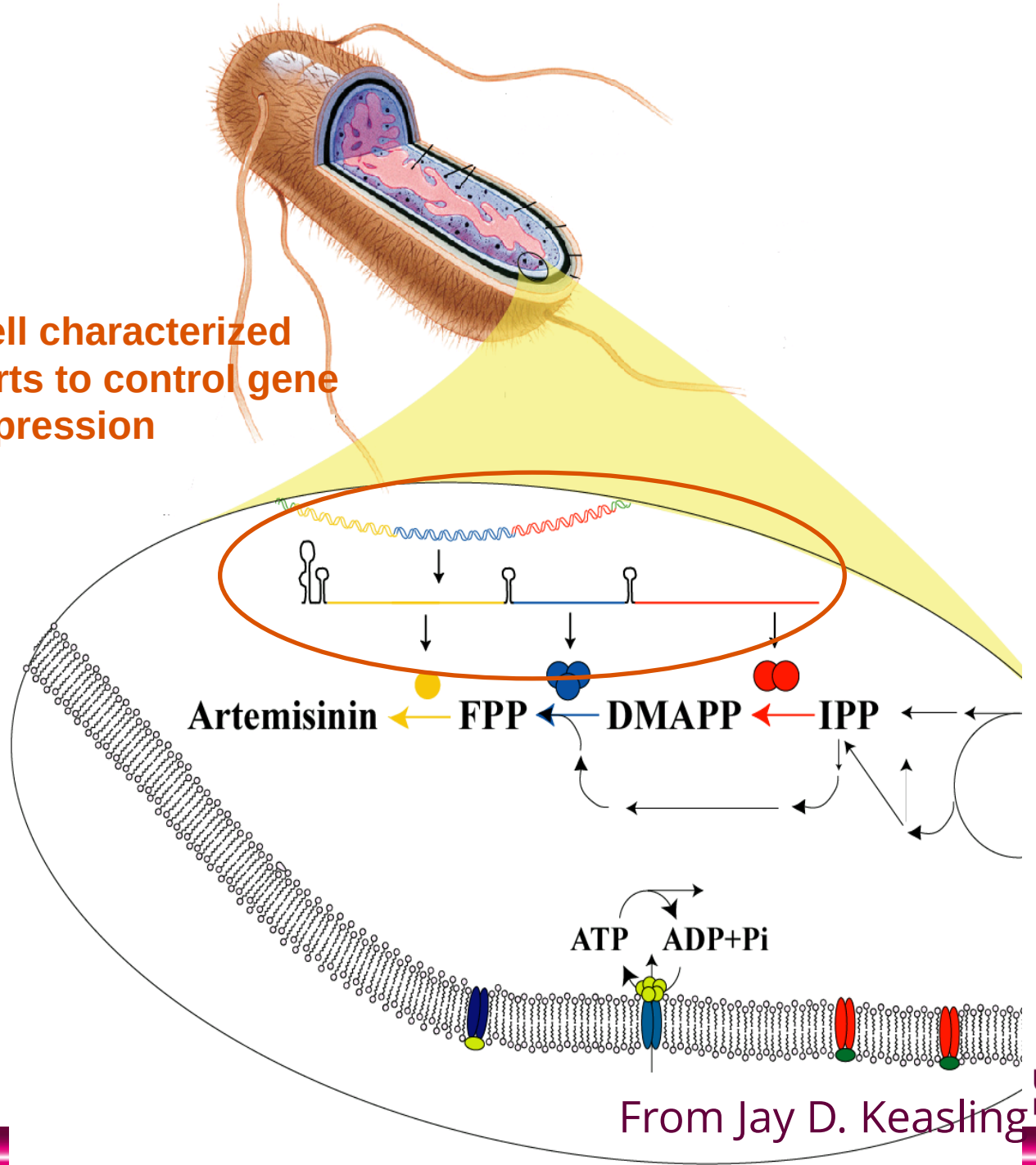
Clone the genes



From Jay D. Keasling

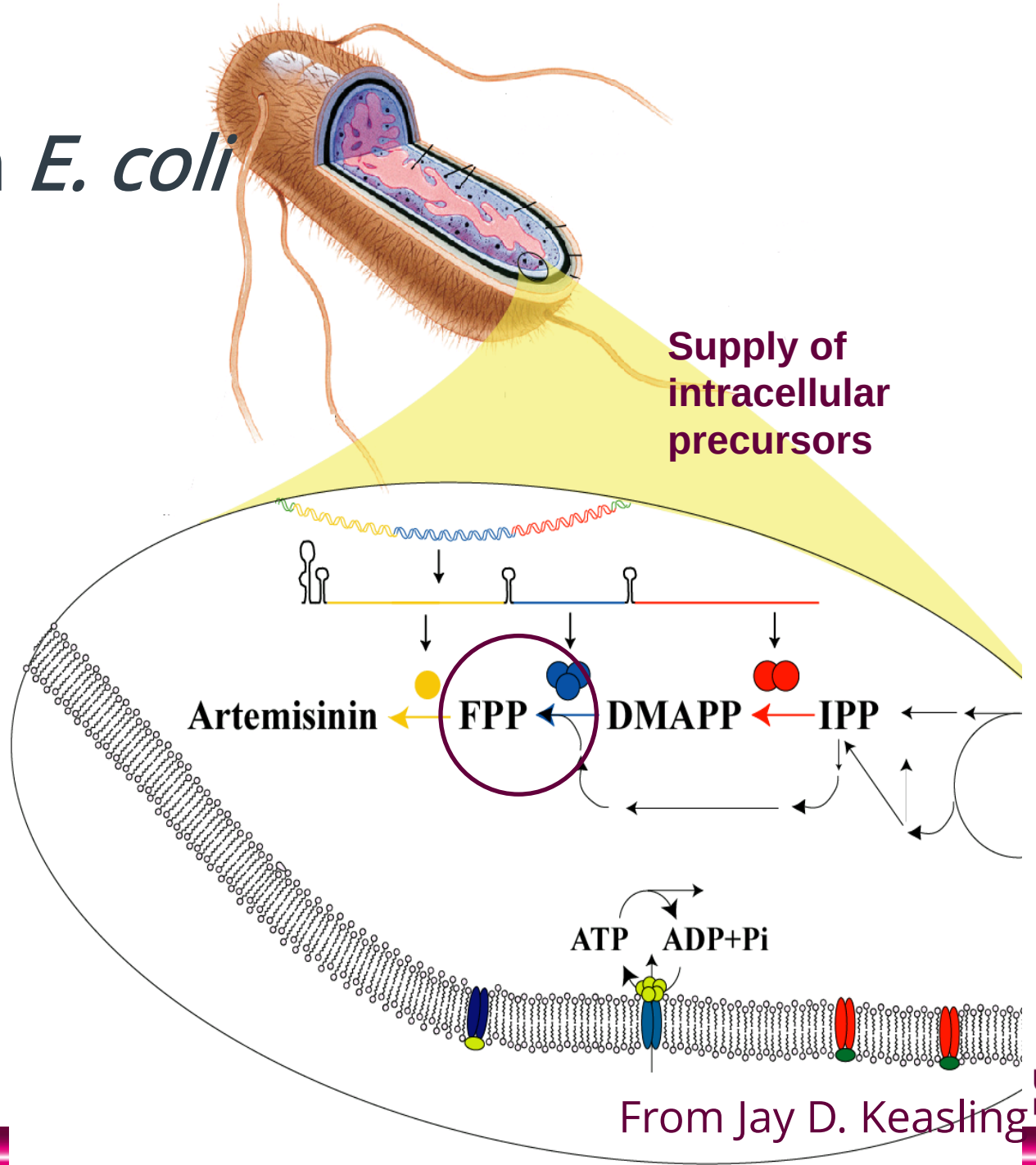
Synthesis of artemisinin in *E. coli*

Well characterized parts to control gene expression



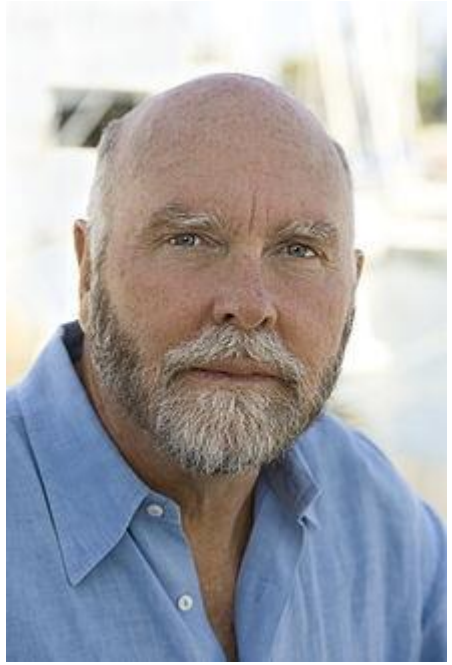
From Jay D. Keasling

Synthesis of artemisinin in *E. coli*



From Jay D. Keasling

Creating life



John Craig Venter (born October 14, 1946)
American biotechnologist, biochemist, geneticist, and businessman.

1995	first complete genome sequence
2003	ΦX174 completely assembled *in vitro* from synthesized oligonucleotides
2003	first draft sequence of the human genome
2010	Built the genome of a bacterium from scratch First Minimal Synthetic Bacterial Cell

Science August 2007

RESEARCH ARTICLE

Genome Transplantation in Bacteria: Changing One Species to Another

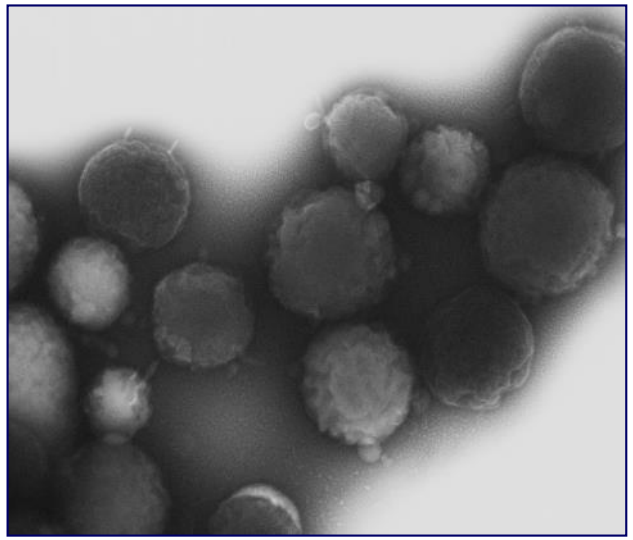
Carole Lartigue, John I. Glass,* Nina Alperovich, Rembert Pieper, Prashanth P. Parmar, Clyde A. Hutchison III, Hamilton O. Smith, J. Craig Venter

As a step toward propagation of synthetic genomes, we completely replaced the genome of a bacterial cell with one from another species by transplanting a whole genome as naked DNA. Intact genomic DNA from *Mycoplasma mycoides* large colony (LC), virtually free of protein, was transplanted into *Mycoplasma capricolum* cells by polyethylene glycol–mediated transformation. Cells selected for

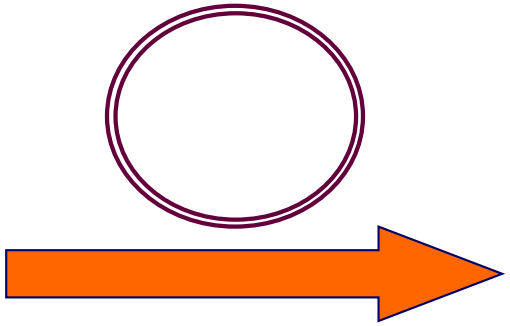
from incompatibility between the two genomes (*6*). Transplantation of nuclei as intact organelles into enucleated eggs is a well-established procedure in vertebrates (*7*–*9*). Our choice of the term "genome transplantation" comes from the similarity to eukaryotic nuclear transplantation in which one genome is cleanly replaced by another.

Genome transplantation is a requirement for the establishment of the new field of synthetic genomics. It may facilitate construction of useful microorganisms with the potential to solve pressing societal problems in energy production, environmental stewardship, and medicine. Chemically synthesized chromosomes must

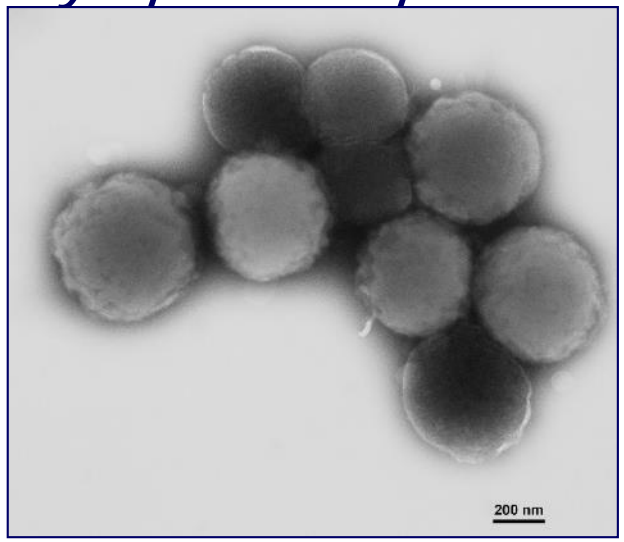
Mycoplasma mycoides



gDNA DONOR



Mycoplasma capricolum



RECIPIENT CELLS

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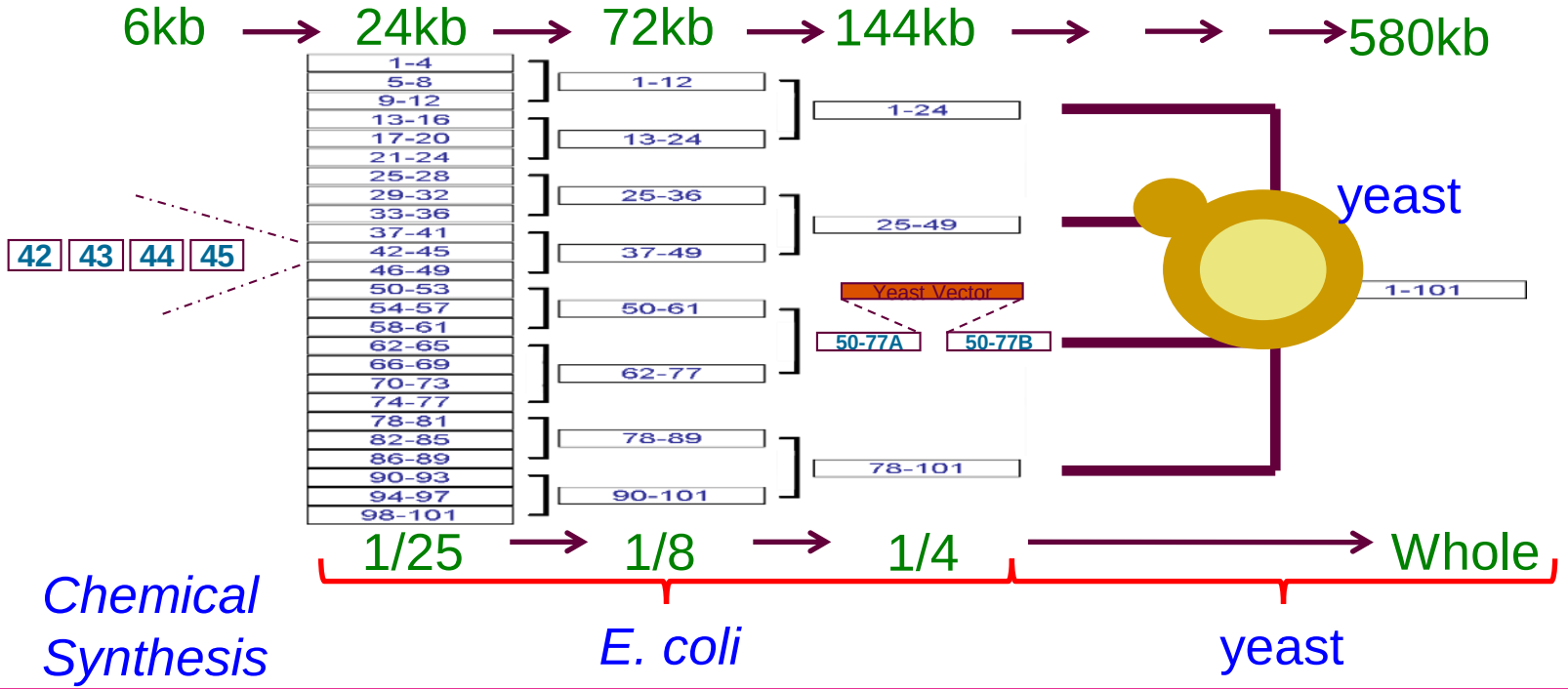
Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stockwell, Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison III, Hamilton O. Smith*

genome, we needed to establish convenient and reliable methods for the assembly and cloning of much larger synthetic DNA molecules.

Strategy for synthesis and assembly. The native 580,076-bp *M. genitalium* genome sequence (*Mycoplasma genitalium* G37 ATCC 33530 genomic sequence; accession no. L43967) (*3*) was partitioned into 101 cassettes of approximately 5 to 7 kb in length (Fig. 1) that were individually synthesized, verified by sequencing, and then joined together in stages. In general, cassette boundaries were placed between genes so that each cassette contained one or several complete genes. This will simplify the future



Science August 2009

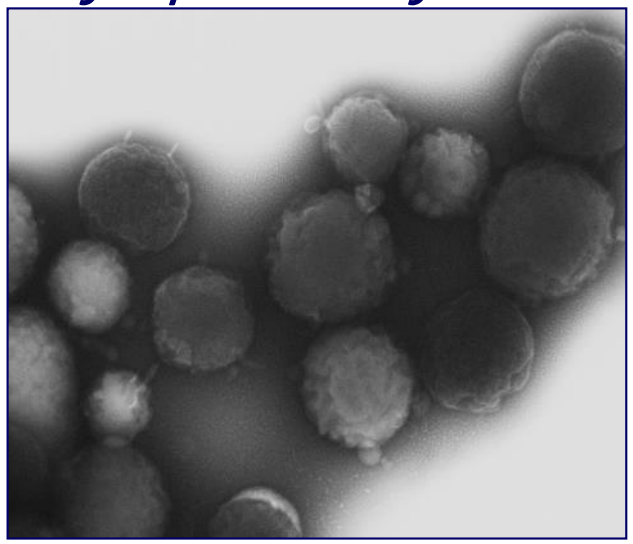
Creating Bacterial Strains from Genomes That Have Been Cloned and Engineered in Yeast

Carole Lartigue,[1] Sanjay Vashee,[1]† Mikkel A. Algire,[1] Ray-Yuan Chuang,[1] Gwynedd A. Benders,[2] Li Ma,[1] Vladimir N. Noskov,[1] Evgeniya A. Denisova,[1] Daniel G. Gibson,[1] Nacyra Assad-Garcia,[1] Nina Alperovich,[1] David W. Thomas,[1]* Chuck Merryman,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter,[1,2] John I. Glass[1]

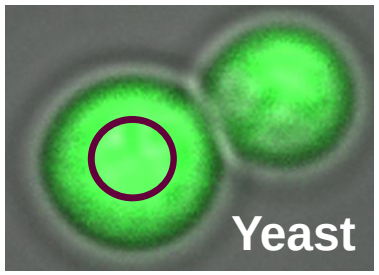
We recently reported the chemical synthesis, assembly, and cloning of a bacterial genome in yeast.

omously replicating sequence, for selection and propagation in yeast as a yeast centromeric plasmid (YCp). Direct genomic sequencing (*8*) of one clone (YCpMmyc1.1) showed that the entire vector integrated into the genome. This clone grew robustly and transplanted efficiently into *M. capricolum* (*9*), so it was chosen for cloning into yeast. The genome of this clone will be called YCpMmyc1.1 throughout this paper, regardless of the cellular source. YCpMmyc1.1 can refer to: (i) the original *M. mycoides* strain (the "native" *M. mycoides* YCpMmyc1.1 genome), (ii) the same genome cloned in yeast, (iii) the genome transplanted from *M. mycoides* or from yeast, or (iv) this

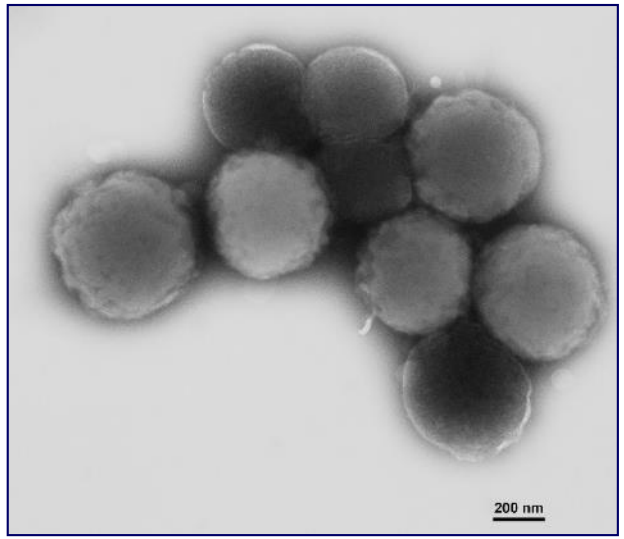
Mycoplasma mycoides



gDNA DONOR



Mycoplasma capricolum



RECIPIENT CELLS

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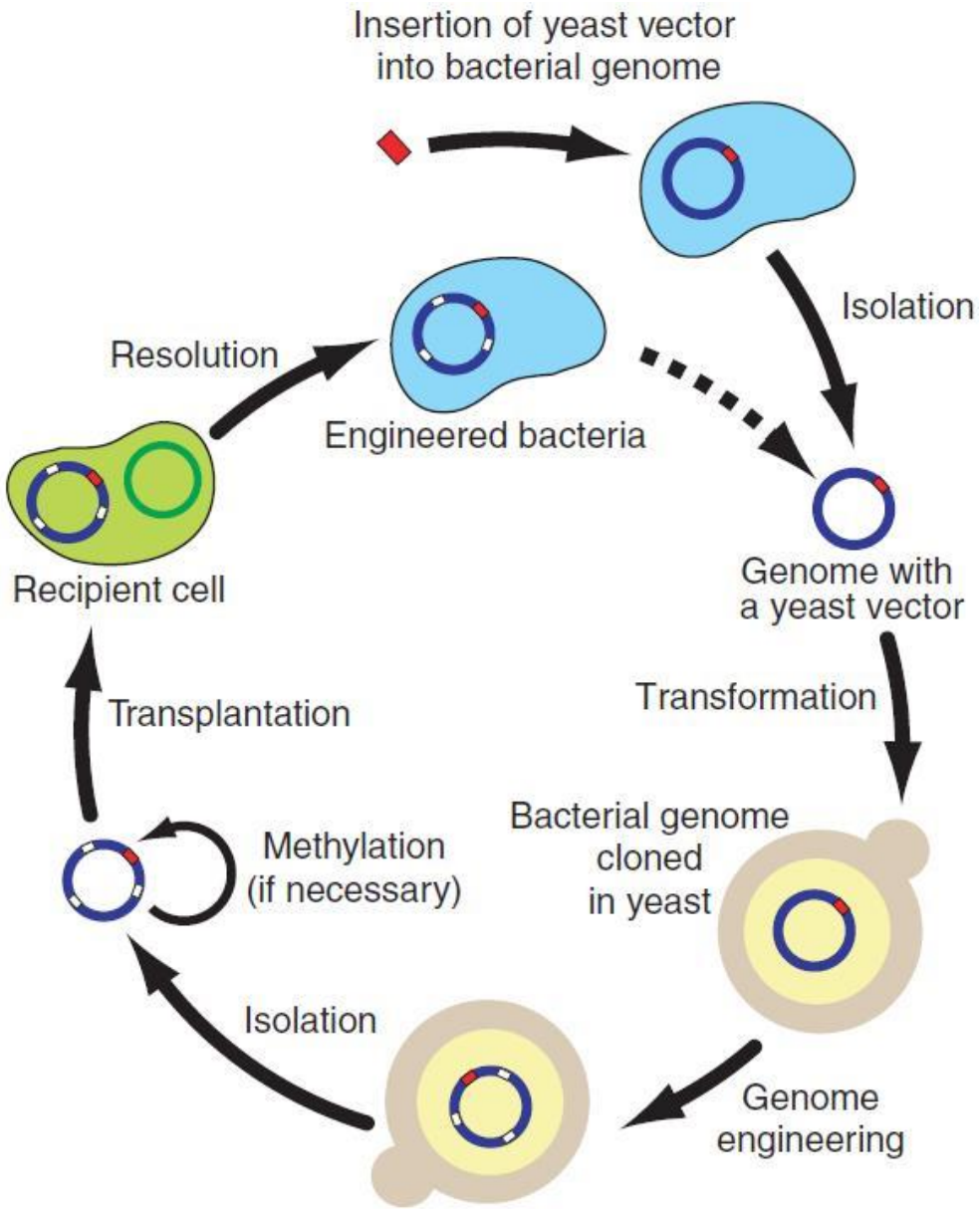

Insertion of yeast vector
into bacterial genome
Isolation
Resolution
Engineered bacteria
Recipient cell
Genome with
a yeast vector
Transformation
Transplantation
Bacterial genome
cloned
in yeast
Methylation
(if necessary)
Isolation
Genome
engineering

Science May 2010

RESEARCH ARTICLE

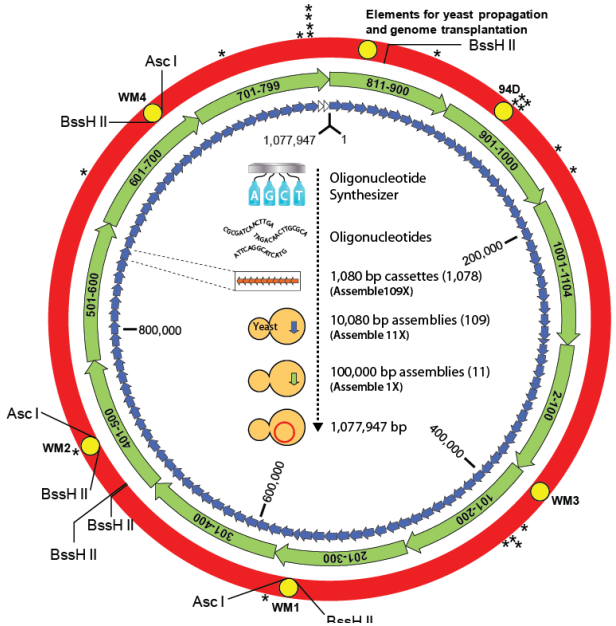
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

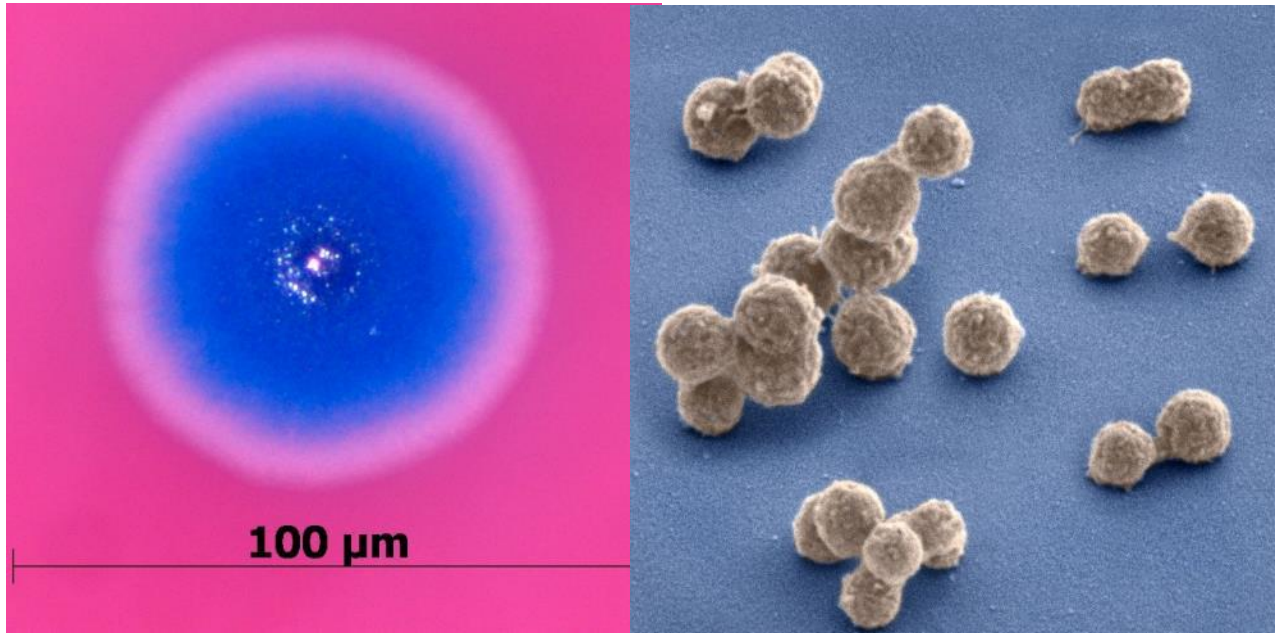
Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (*8*, *9*, *11*). One was the genome donor used





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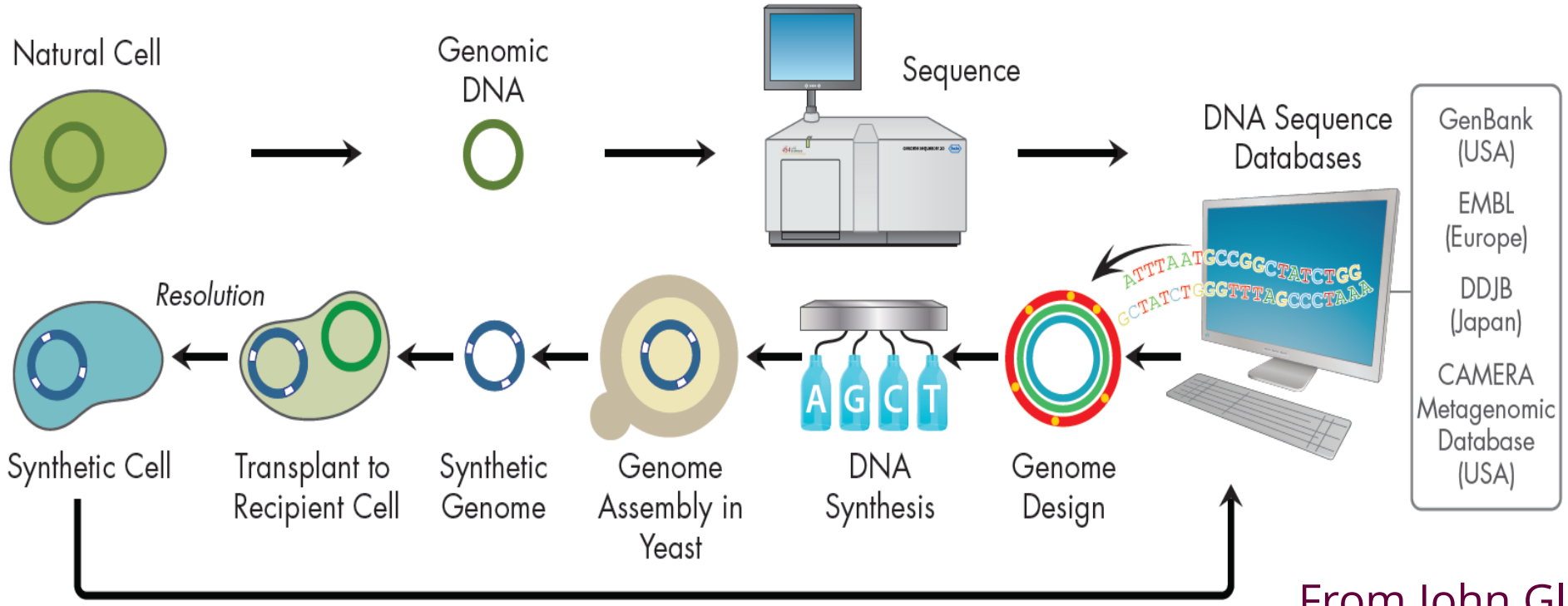
Moving life into the digital world and back



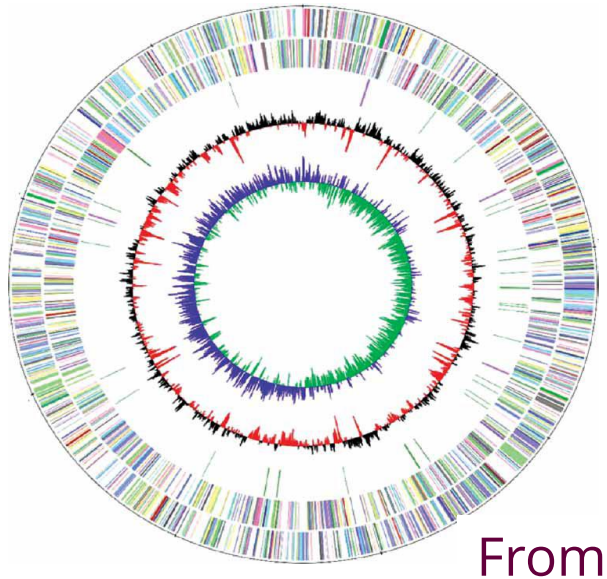
From John Glass
The J. Craig Venter Institute

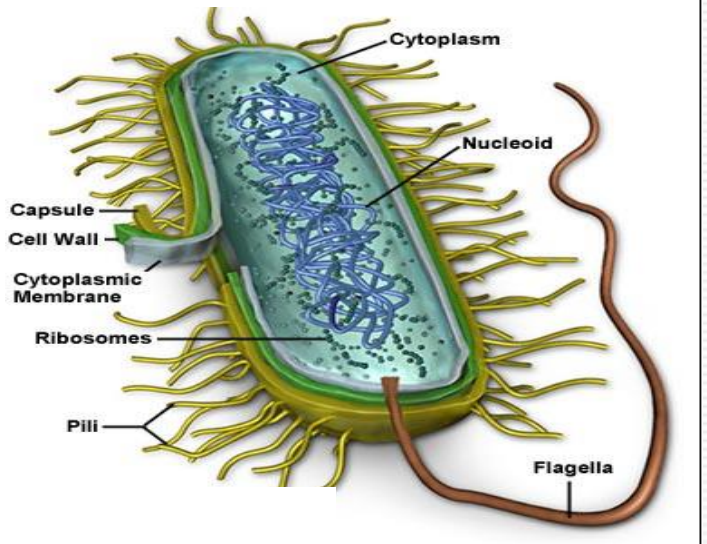
Our capacity to build microbes capable of solving human problems is limited only by our imagination

Synthetic Organism Designer 1.0

Design | Codon Opt. | Oligo Synthesis

Organism	Cell Membrane	Division Type	Form of Metabolism	Structural Genes	Control & Safety
Archaea	Gram +	Meiosis	Photosynthesis	Rubisco (30,000)	Auxotrophic Marker
Bacteria	Gram -	Mitosis	Methanogenesis	Ferredoxin (82,000)	Suicide Gene
Single Cell Euk	Archaeal	Cellular Septation	Glycolysis	Plastocyanin (9,000)	
Virus	Euk		Calvin Cycle	ATP Synthase (100,000)	
Multicellular Euk			Pentose Phosphate		





From John Glass
The J. Craig Venter Institute

Total synthesis of *E. coli* with a recoded genome

Jason Chin
MRC
Medical Research Council
Cambridge

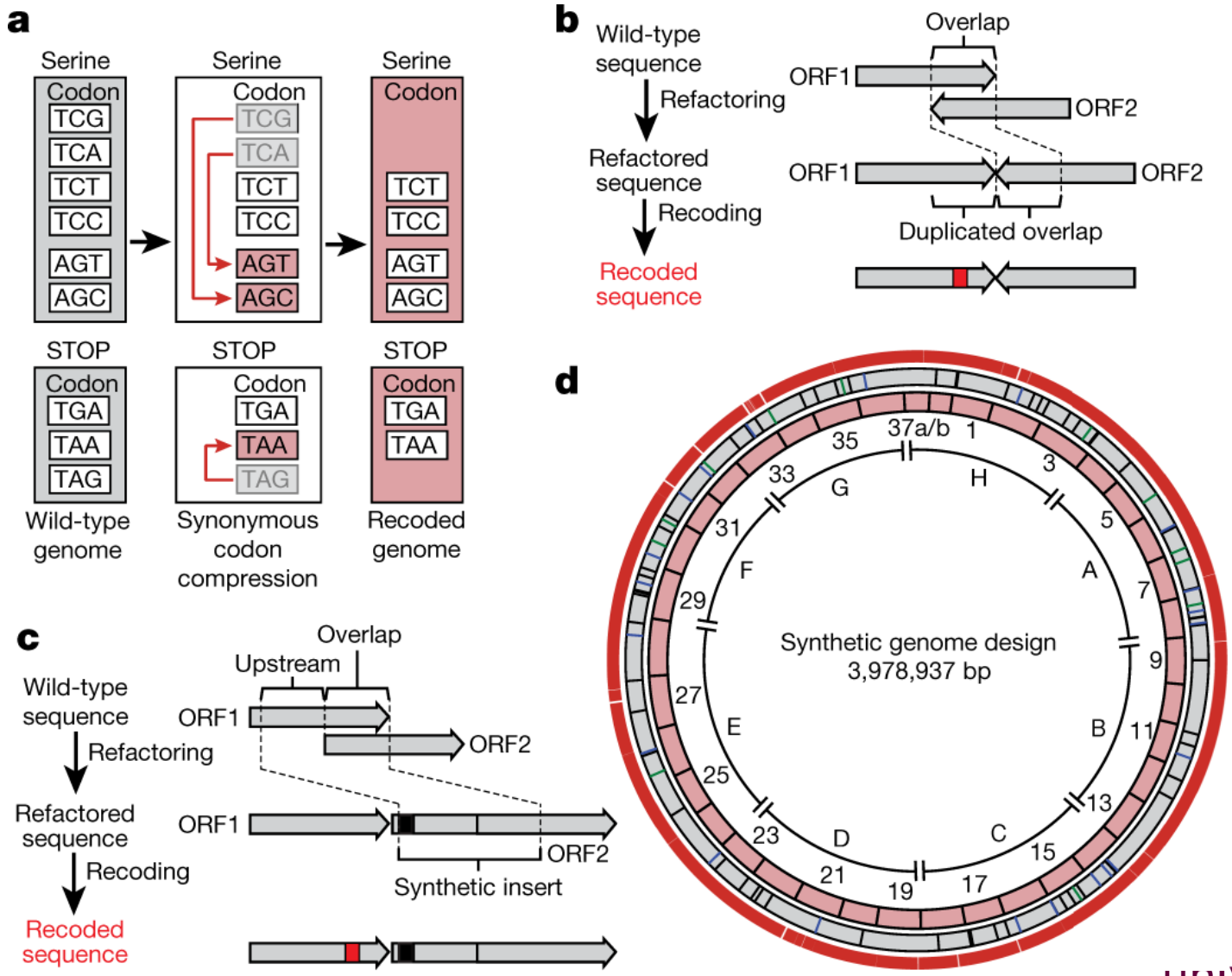
Synthesized the entire genome of *E. coli*.

61 codons

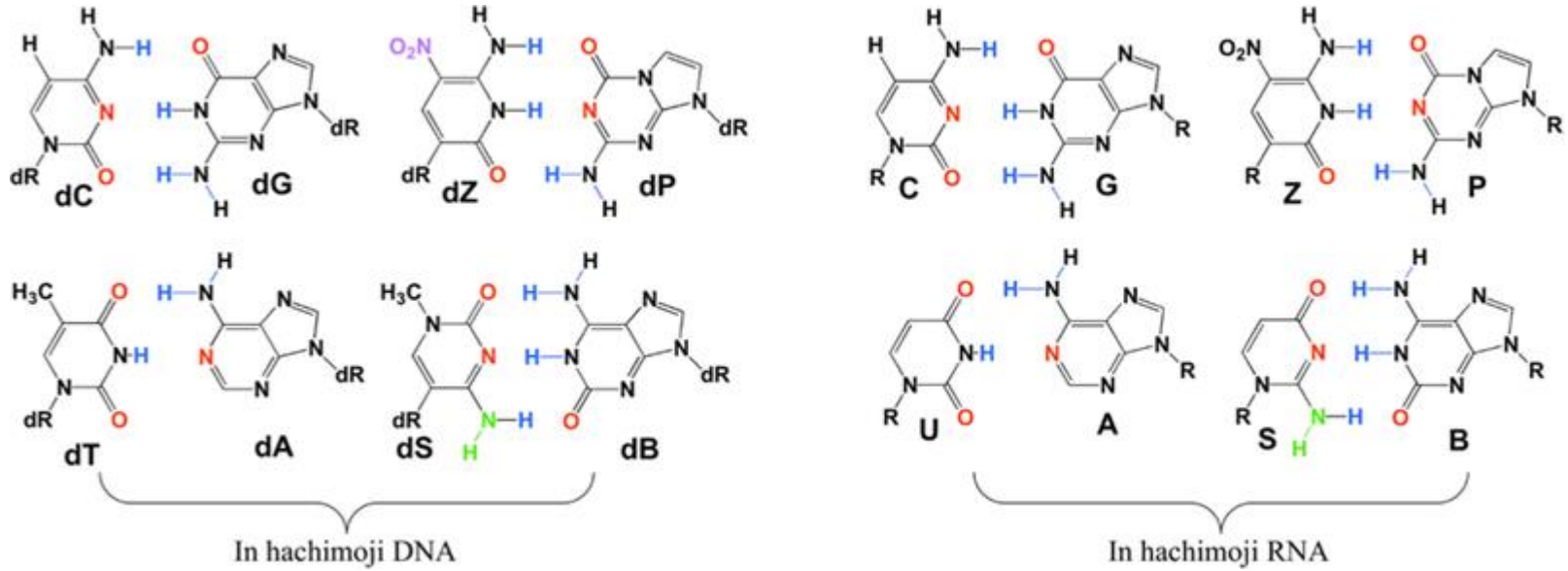
Replacement of natural DNA with its corresponding synthetic version

Not be able to decode DNA from any other organism

Nature. 2019
569:514-518

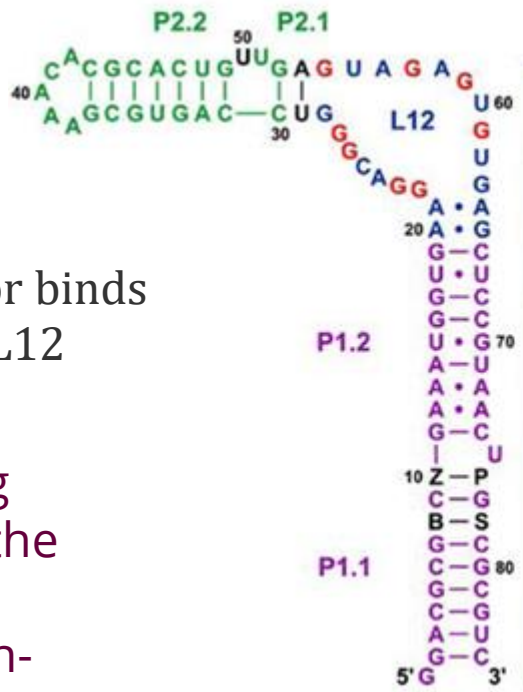


Hachimoji DNA

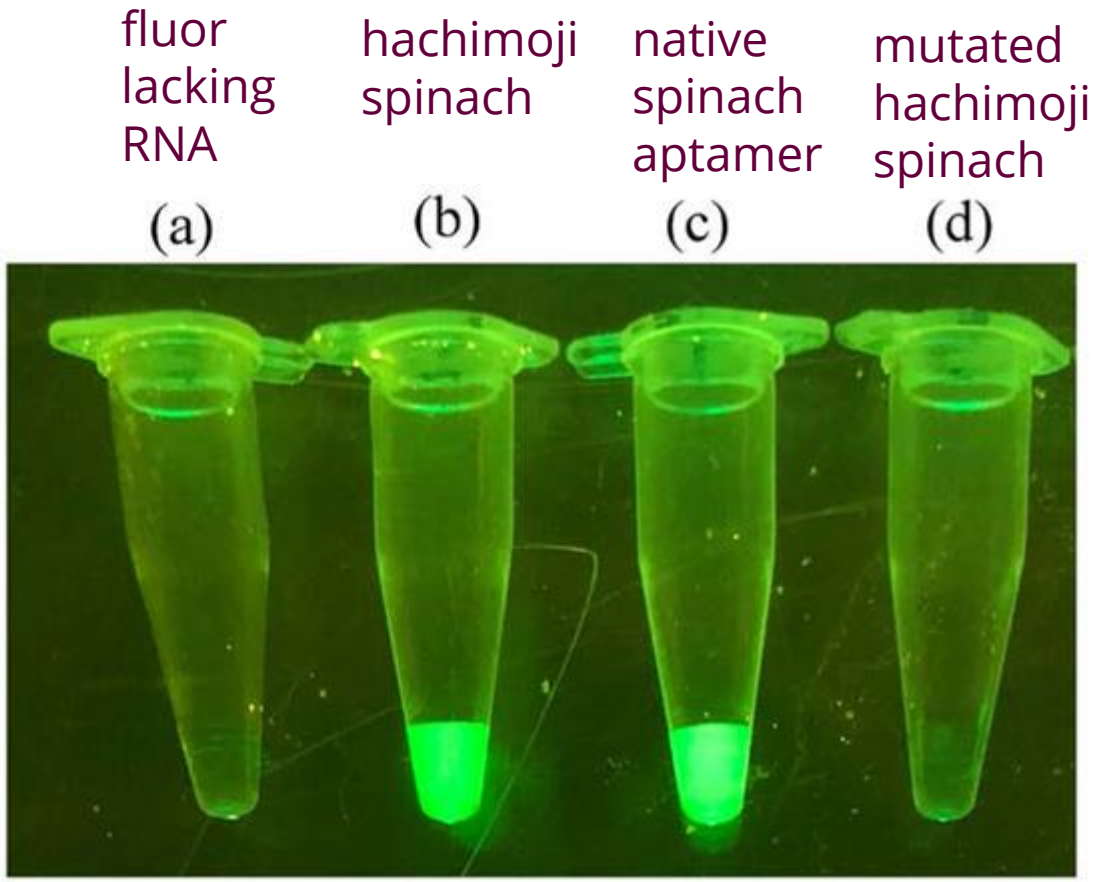


Science. 2019 Feb 22; 363(6429): 884–887.
doi: 10.1126/science.aat0971

Hachimoji spinach variant aptamer



The fluor binds in loop L12



Spinach is a short RNA tag that can be inserted into the target RNA sequence and turns on an otherwise non-fluorescent dye.

Science. 2019 Feb 22; 363(6429): 884–887.
doi: 10.1126/science.aat0971

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Possible uses of synthetic & engineered species

- Increase basic understanding of life
- Increase the predictability of synthetic biological circuits
- Become a major source of energy
- Replace the petrol-chemical industry
- Enhance bioremediation
- Clean up water
- Drive antibiotic and vaccine discovery & production
- Gene therapy