From Genetic Engineering to Synthetic Biology

Briefing for Ownership & Sharing Workshop

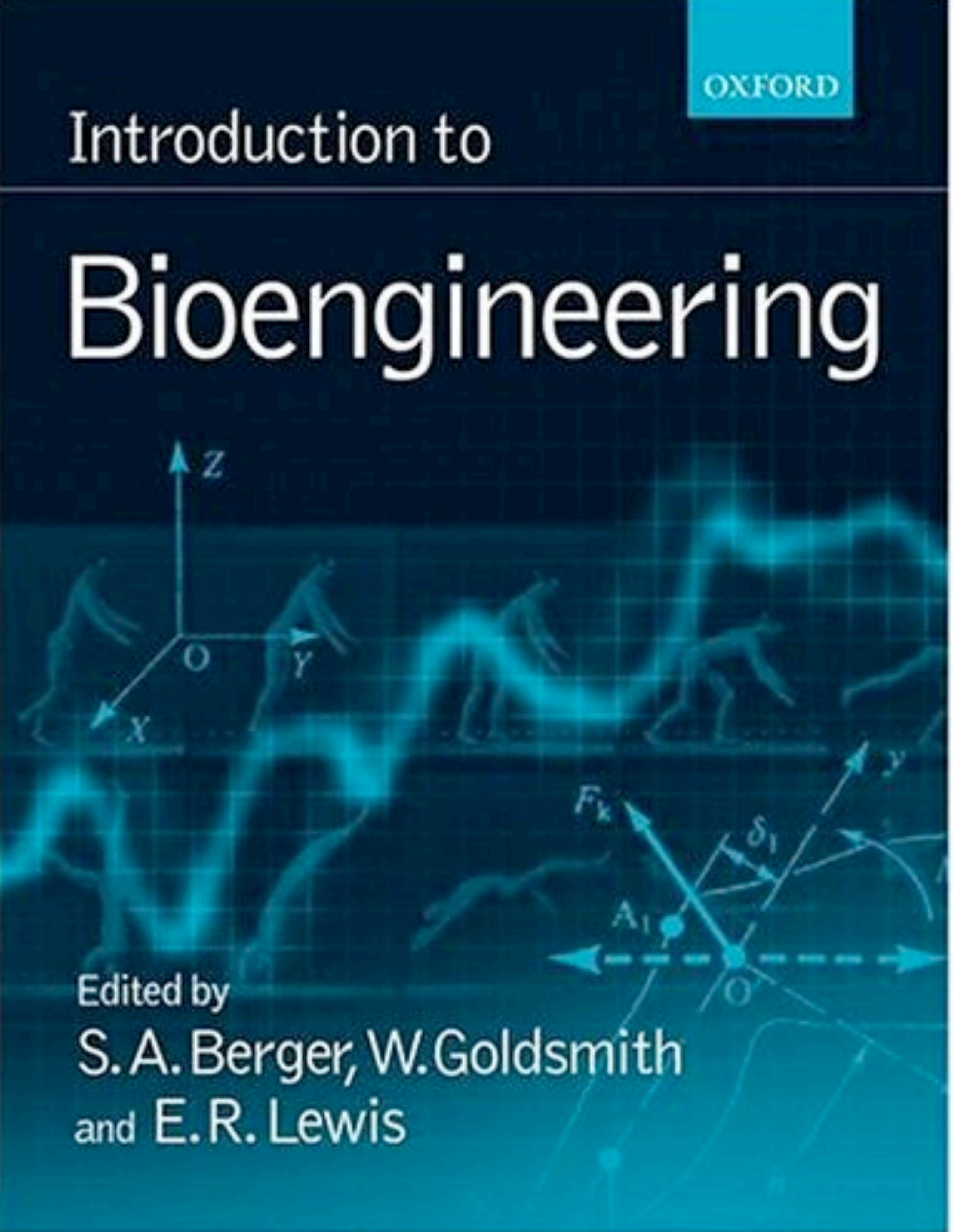
Drew Endy
Stanford Bioengineering
The BioBricks Foundation

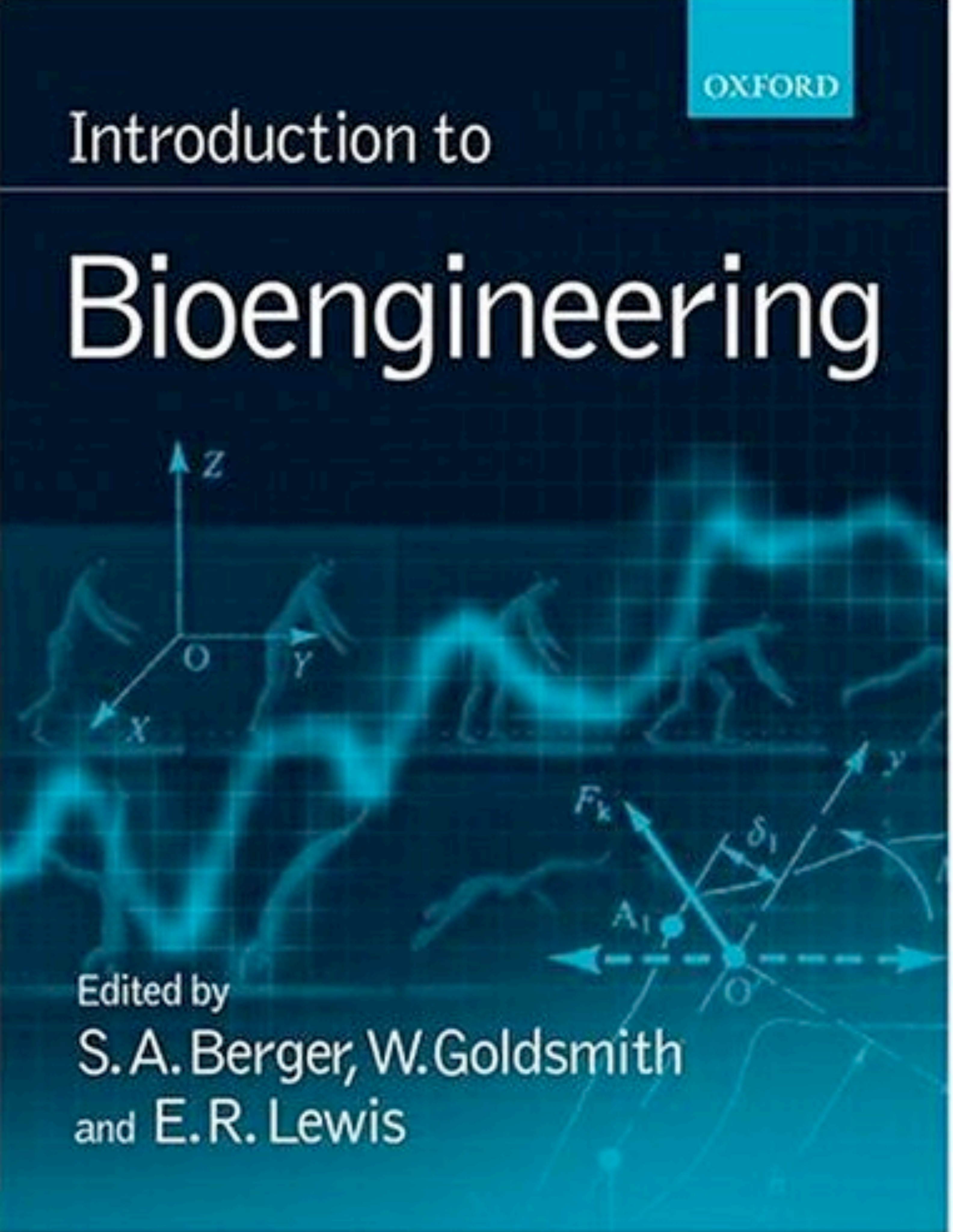
15 July 2013
London UK

Photo by Roger Lancaster (http://www.flickr.com/photos/rogeral/5813079061/); educational fair use

http://philross.org/#projects/mycotecture/

The living bridges of Cherrapunji, India are made from the roots of the Ficus elastica tree. (http://rootbridges.blogspot.c


OXFORD
Introduction to
Bioengineering
Edited by
S. A. Berger, W. Goldsmith
and E. R. Lewis



Third sentence:

“In the use of the term bioengineering in this book we exclude genetic engineering; that is, the systematic design of phenotypes by manipulation of genotypes.”

Synthetic Biology

Drew Endy
Fellow of Biology & Biological Engineering, MIT
Patrick Lincoln
Director of Computer Science, SRI, Inc.
Richard Murray
Division Chair, Engineering & Applied Science, Caltech

Table of Contents

2003 *Synthetic Biology Study*

Free .PDF of full briefing via DOI 1721.1/38455

Synthetic Biology

Drew Endy
Fellow of Biology & Biological Engineering, MIT

Patrick Lincoln
Director of Computer Science, SRI, Inc.

Richard Murray
Division Chair, Engineering & Applied Science, Caltech

October 8, 2003

__Table of Contents__

Study Participants

- Drew Endy (chair)
- Patrick Lincoln (co-chair)
- Richard Murray (co-chair)
- Frances Arnold (Caltech)
- Ralph Baric (UNC)
- Roger Brent (TMSI)
- Rob Carlson (U.Washington)
- Jim Collins (BU)
- Lynn Conway (Michigan)
- Ron Davis (Stanford)
- Mita Desai (NSF)
- Eric Eisenstadt (DARPA)
- Stephanie Forrest (U.New Mexico)
- Seth Goldstein (CMU)
- Homme Hellinga (Duke)
- Tom Kalil (UC Berkeley)
- Jay Keasling (UC Berkeley)
- Doug Kirkpatrick (DARPA)
- Tom Knight (MIT)
- Bill Mark (SRI)
- John Mulligan (Blue Heron)
- Radhika Nagpal (MIT/Harvard)
- Carl Pabo (Sangamo)
- Randy Rettberg (MIT)
- Pam Silver (Harvard)
- Brad Smith (Johns Hopkins)
- Christina Smolke (Caltech)
- Gerry Sussman (MIT)
- Jack Thorpe (ISAT)
- Claire Tomlin (Stanford)
- Jeff Way (Lexigen)
- Chris Webb (Stanford)
- Ron Weiss (Princeton)
- Erik Winfree (Caltech)

October 8, 2003 4

Study participants included representatives from universities, industry, and government. Participants provided expertise in basic biological research, biological systems modeling, DNA synthesis, device analysis & design, self-assembly, systems analysis & design, computer science, electrical engineering, engineering theory, and biological security. Rich Entlich and other staff provided expert organizational support throughout the study.

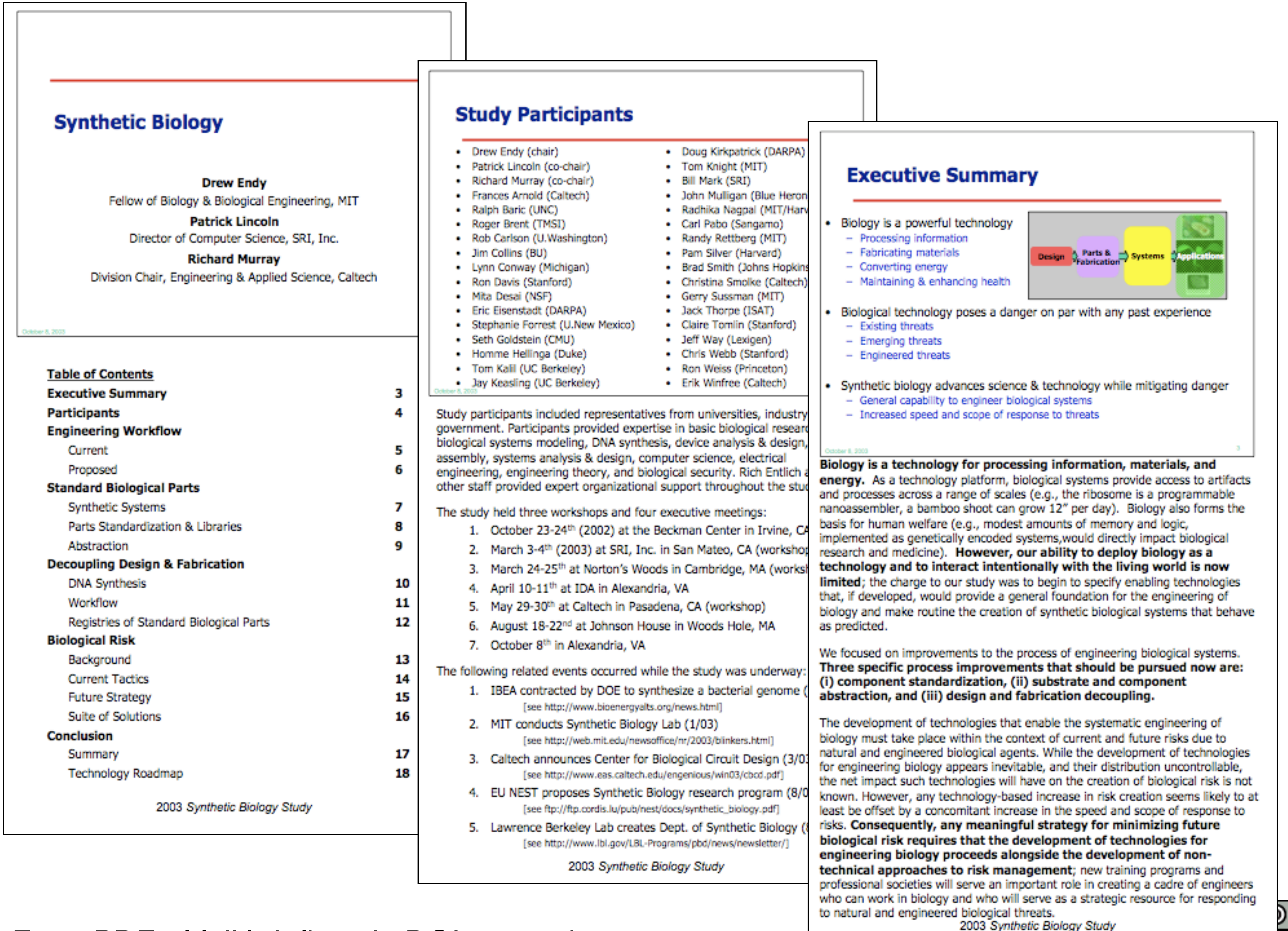
The study held three workshops and four executive meetings:

1. October 23-24th (2002) at the Beckman Center in Irvine, CA
2. March 3-4th (2003) at SRI, Inc. in San Mateo, CA (workshop)
3. March 24-25th at Norton's Woods in Cambridge, MA (workshop)
4. April 10-11th at IDA in Alexandria, VA
5. May 29-30th at Caltech in Pasadena, CA (workshop)
6. August 18-22nd at Johnson House in Woods Hole, MA
7. October 8th in Alexandria, VA

The following related events occurred while the study was underway:

1. IBEA contracted by DOE to synthesize a bacterial genome (11/02)
   [see http://www.bioenergyalts.org/news.html]
2. MIT conducts Synthetic Biology Lab (1/03)
   [see http://web.mit.edu/newsoffice/nr/2003/blinkers.html]
3. Caltech announces Center for Biological Circuit Design (3/03)
   [see http://www.eas.caltech.edu/engenious/win03/cbcd.pdf]
4. EU NEST proposes Synthetic Biology research program (8/03)
   [see ftp://ftp.cordis.lu/pub/nest/docs/synthetic_biology.pdf]
5. Lawrence Berkeley Lab creates Dept. of Synthetic Biology (8/03)
   [see http://www.lbl.gov/LBL-Programs/pbd/news/newsletter/]

Free .PDF of full briefing via DOI 1721.1/38455



Synthetic Biology

Drew Endy
Fellow of Biology & Biological Engineering, MIT
Patrick Lincoln
Director of Computer Science, SRI, Inc.
Richard Murray
Division Chair, Engineering & Applied Science, Caltech

October 8, 2003

Table of Contents

2003 *Synthetic Biology Study*

Study Participants

- Drew Endy (chair)
- Patrick Lincoln (co-chair)
- Richard Murray (co-chair)
- Frances Arnold (Caltech)
- Ralph Baric (UNC)
- Roger Brent (TMSI)
- Rob Carlson (U.Washington)
- Jim Collins (BU)
- Lynn Conway (Michigan)
- Ron Davis (Stanford)
- Mita Desai (NSF)
- Eric Eisenstadt (DARPA)
- Stephanie Forrest (U.New Mexico)
- Seth Goldstein (CMU)
- Homme Hellinga (Duke)
- Tom Kalil (UC Berkeley)
- Jay Keasling (UC Berkeley)
- Doug Kirkpatrick (DARPA)
- Tom Knight (MIT)
- Bill Mark (SRI)
- John Mulligan (Blue Heron
- Radhika Nagpal (MIT/Harv
- Carl Pabo (Sangamo)
- Randy Rettberg (MIT)
- Pam Silver (Harvard)
- Brad Smith (Johns Hopkins
- Christina Smolke (Caltech)
- Gerry Sussman (MIT)
- Jack Thorpe (ISAT)
- Claire Tomlin (Stanford)
- Jeff Way (Lexigen)
- Chris Webb (Stanford)
- Ron Weiss (Princeton)
- Erik Winfree (Caltech)

October 8, 2003

Study participants included representatives from universities, industry government. Participants provided expertise in basic biological resear biological systems modeling, DNA synthesis, device analysis & design, assembly, systems analysis & design, computer science, electrical engineering, engineering theory, and biological security. Rich Entlich a other staff provided expert organizational support throughout the stu

The study held three workshops and four executive meetings:

1. October 23-24th (2002) at the Beckman Center in Irvine, CA
2. March 3-4th (2003) at SRI, Inc. in San Mateo, CA (worksho
3. March 24-25th at Norton's Woods in Cambridge, MA (works
4. April 10-11th at IDA in Alexandria, VA
5. May 29-30th at Caltech in Pasadena, CA (workshop)
6. August 18-22nd at Johnson House in Woods Hole, MA
7. October 8th in Alexandria, VA

The following related events occurred while the study was underway:

1. IBEA contracted by DOE to synthesize a bacterial genome (
   [see http://www.bioenergyalts.org/news.html]
2. MIT conducts Synthetic Biology Lab (1/03)
   [see http://web.mit.edu/newsoffice/nr/2003/blinkers.html]
3. Caltech announces Center for Biological Circuit Design (3/0
   [see http://www.eas.caltech.edu/engenious/win03/cbcd.pdf]
4. EU NEST proposes Synthetic Biology research program (8/0
   [see ftp://ftp.cordis.lu/pub/nest/docs/synthetic_biology.pdf]
5. Lawrence Berkeley Lab creates Dept. of Synthetic Biology (
   [see http://www.lbl.gov/LBL-Programs/pbd/news/newsletter/]

2003 *Synthetic Biology Study*

Executive Summary

- Biology is a powerful technology
  - Processing information
  - Fabricating materials
  - Converting energy
  - Maintaining & enhancing health
- Biological technology poses a danger on par with any past experience
  - Existing threats
  - Emerging threats
  - Engineered threats
- Synthetic biology advances science & technology while mitigating danger
  - General capability to engineer biological systems
  - Increased speed and scope of response to threats

Design → Parts & Fabrication → Systems → Applications

October 8, 2003 3

Biology is a technology for processing information, materials, and energy. As a technology platform, biological systems provide access to artifacts and processes across a range of scales (e.g., the ribosome is a programmable nanoassembler, a bamboo shoot can grow 12" per day). Biology also forms the basis for human welfare (e.g., modest amounts of memory and logic, implemented as genetically encoded systems,would directly impact biological research and medicine). **However, our ability to deploy biology as a technology and to interact intentionally with the living world is now limited**; the charge to our study was to begin to specify enabling technologies that, if developed, would provide a general foundation for the engineering of biology and make routine the creation of synthetic biological systems that behave as predicted.

We focused on improvements to the process of engineering biological systems. **Three specific process improvements that should be pursued now are: (i) component standardization, (ii) substrate and component abstraction, and (iii) design and fabrication decoupling.**

The development of technologies that enable the systematic engineering of biology must take place within the context of current and future risks due to natural and engineered biological agents. While the development of technologies for engineering biology appears inevitable, and their distribution uncontrollable, the net impact such technologies will have on the creation of biological risk is not known. However, any technology-based increase in risk creation seems likely to at least be offset by a concomitant increase in the speed and scope of response to risks. **Consequently, any meaningful strategy for minimizing future biological risk requires that the development of technologies for engineering biology proceeds alongside the development of non-technical approaches to risk management**; new training programs and professional societies will serve an important role in creating a cadre of engineers who can work in biology and who will serve as a strategic resource for responding to natural and engineered biological threats.

2003 *Synthetic Biology Study*

Free .PDF of full briefing via DOI 1721.1/38455

Our charge was, "*to specify enabling technologies that, if developed, would provide a general foundation for the engineering of biology and make routine the creation of synthetic biological systems that behave as predicted.*"

Free .PDF of full briefing via DOI 1721.1/38455

Our charge was, "*to specify enabling technologies that, if developed, would provide a general foundation for the engineering of biology and make routine the creation of synthetic biological systems that behave as predicted.*"

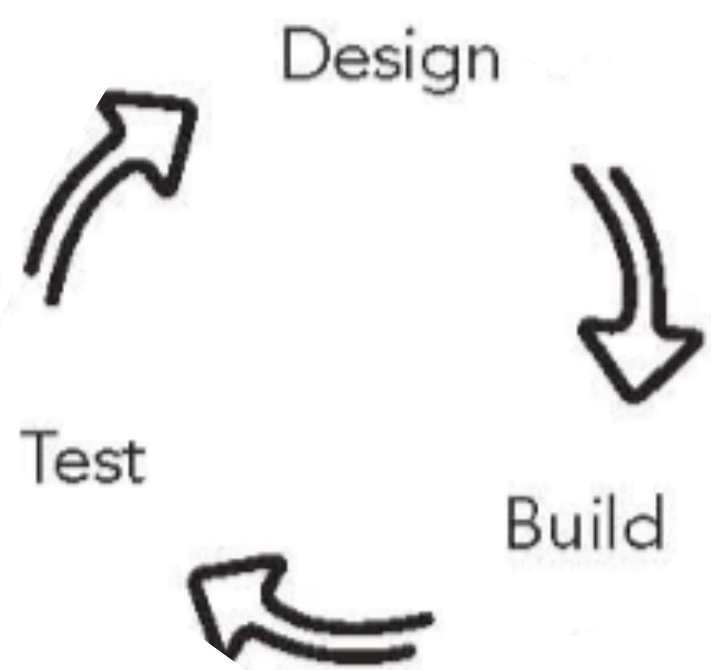
Our stated findings were, "*Three specific process improvements that should be pursued now are: (i) component standardization, (ii) substrate and component abstraction, and (iii) design and fabrication decoupling.*"

Free .PDF of full briefing via DOI 1721.1/38455

By *process* we meant the **core** of the *engineering cycle*.

Graphic from "Synthetic Aesthetics" MIT Press (2014)

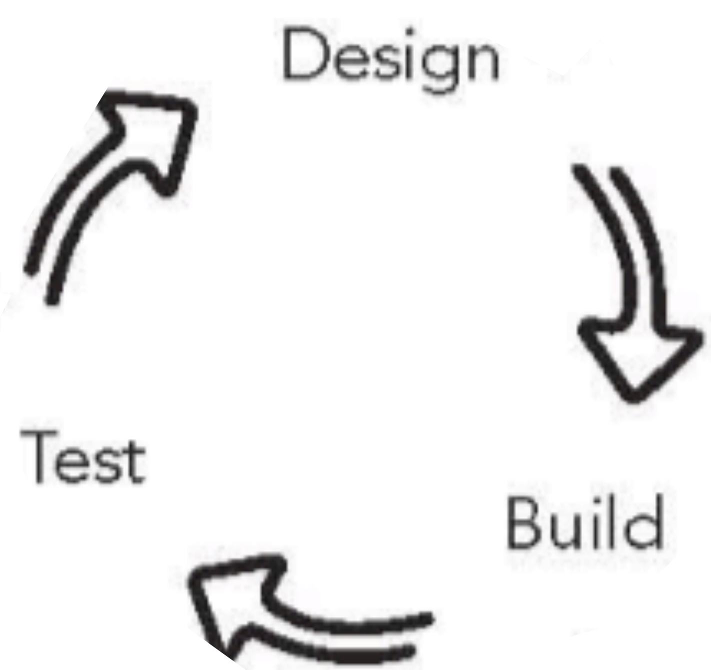
By *process* we meant the **core** of the *engineering cycle*.



Graphic from "Synthetic Aesthetics" MIT Press (2014)

By *process* we meant the **core** of the *engineering cycle*.

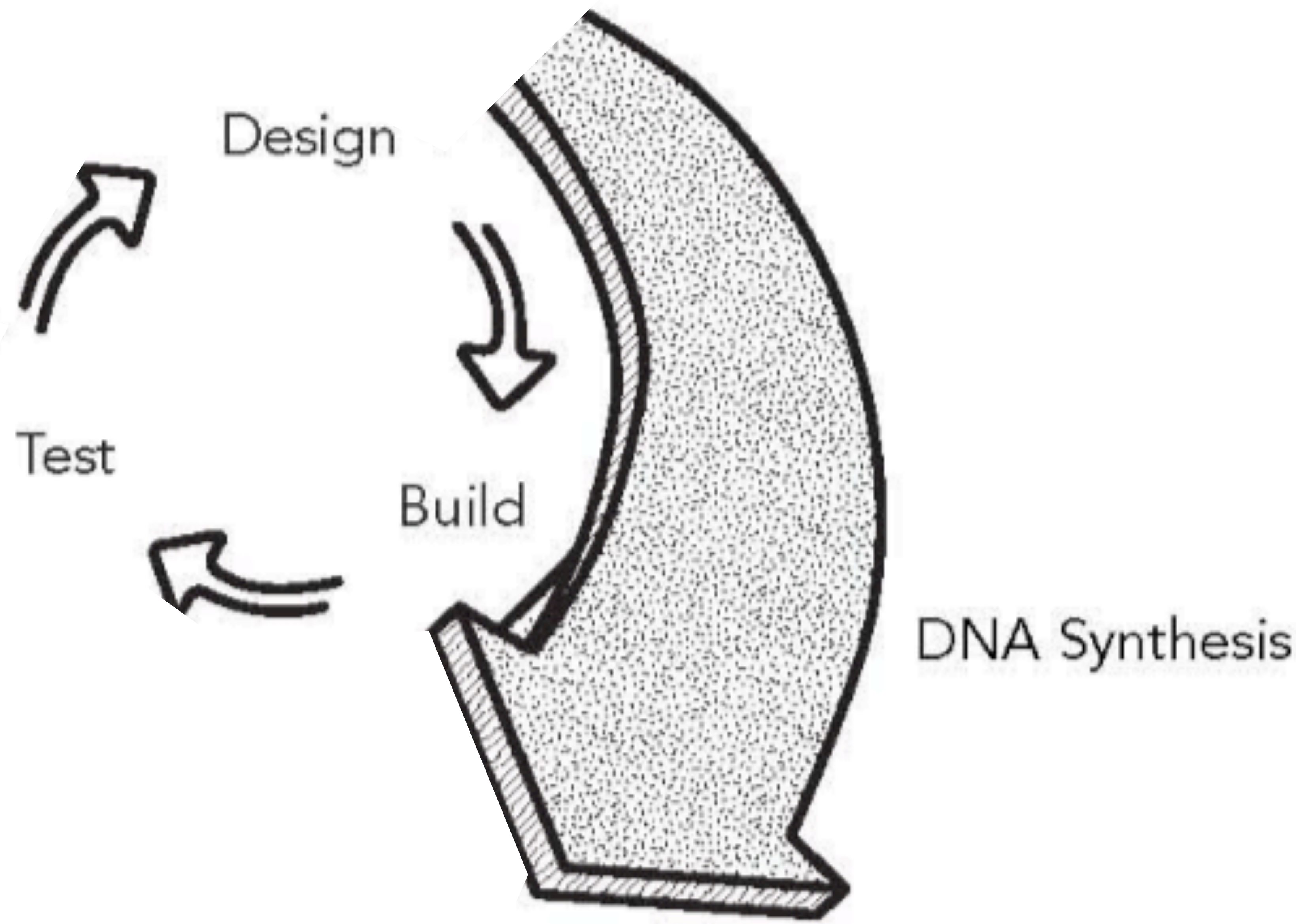
By *improvements* we meant **going around** *faster*, *smarter*, *more times in parallel*, *etc.*



Graphic from "Synthetic Aesthetics" MIT Press (2014)

By *process* we meant the **core** of the *engineering cycle*.

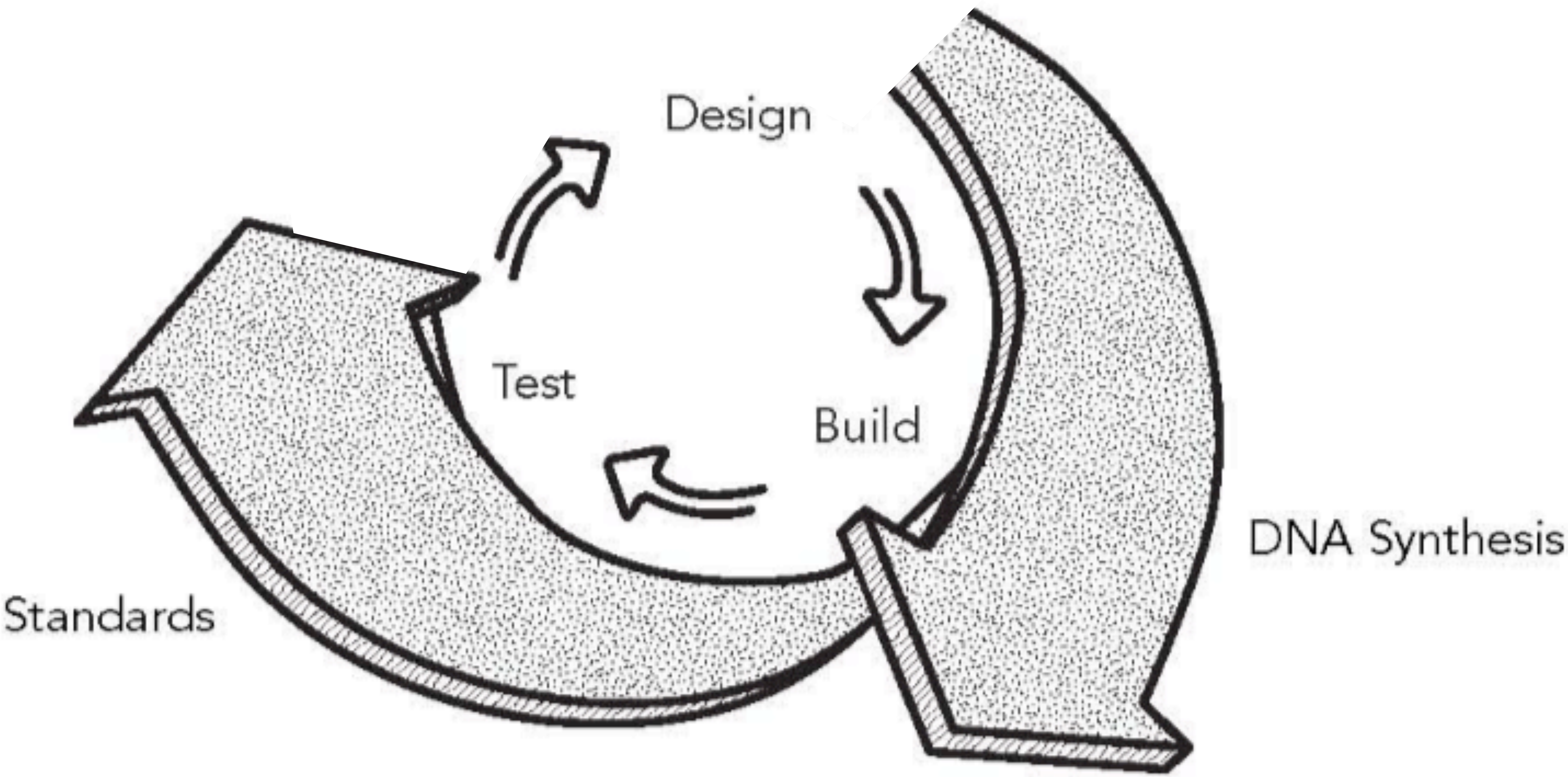
By *improvements* we meant **going around** *faster*, *smarter*, *more times in parallel*, *etc*.



Graphic from "Synthetic Aesthetics" MIT Press (2014)

By *process* we meant the **core** of the *engineering cycle*.

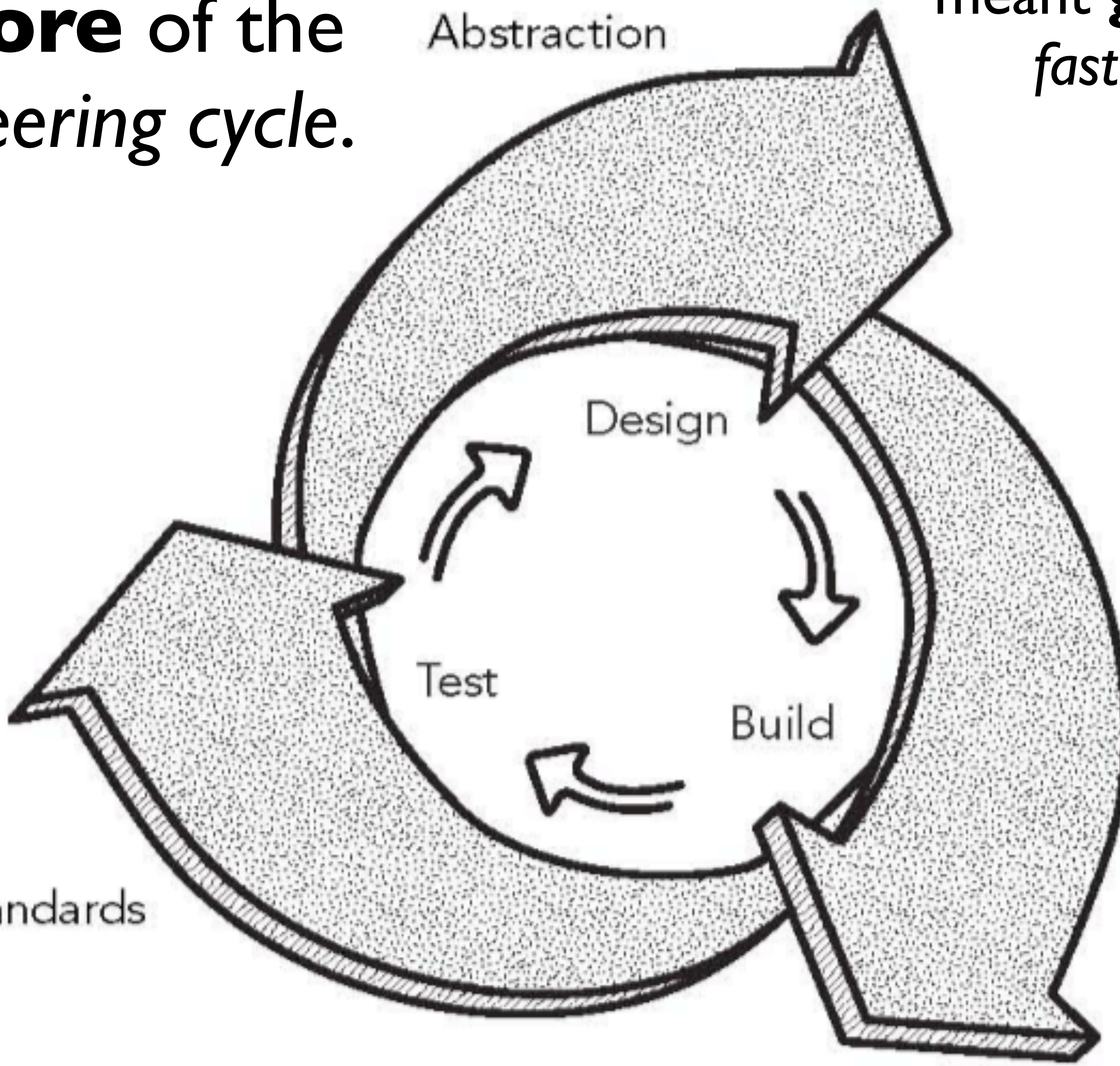
By *improvements* we meant **going around** *faster, smarter, more times in parallel, etc.*



Graphic from "Synthetic Aesthetics" MIT Press (2014)

By *process* we meant the **core** of the *engineering cycle*.

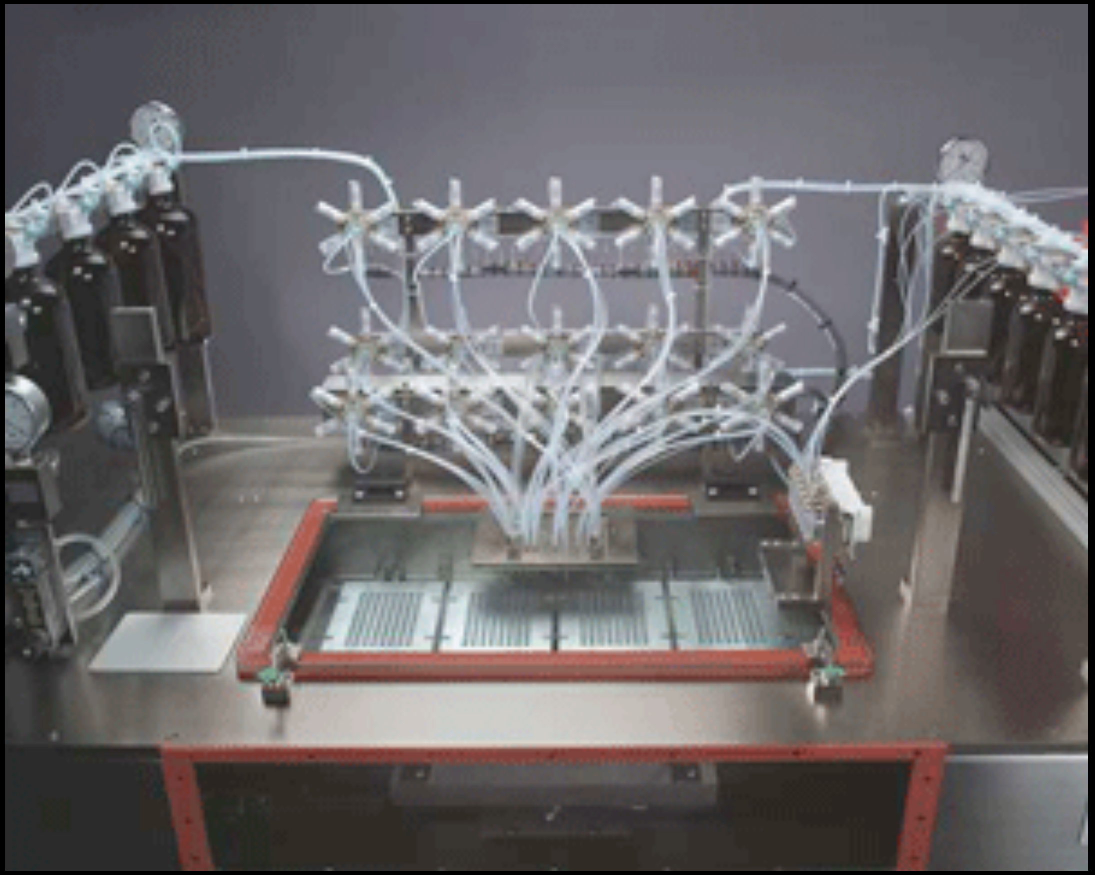
By *improvements* we meant **going around** *faster*, *smarter*, *more times in parallel*, *etc.*



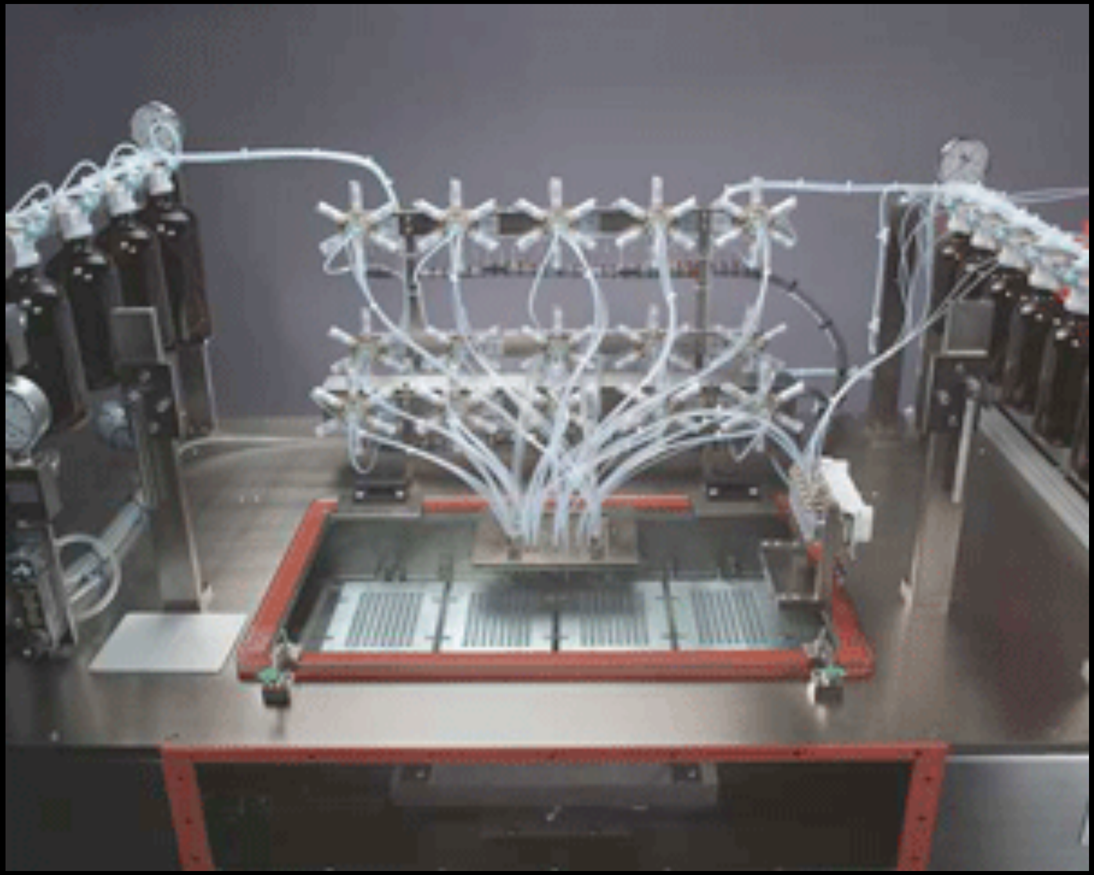
Graphic from "Synthetic Aesthetics" MIT Press (2014)


10-1010-15
dC
G009030
10.0g
GLEN RESEARCH
10-1020-5S
dG
G012013
5.0g
GLEN RESEARCH
10-1000-1S
dA
G003052
10.0g
GLEN RESEARCH
GLEN RESEARCH


10-1010-1S
dC
G009030
10.0g
GLEN
10-1020-5S
dG
G012013
5.0g
GLEN RESEARCH
10-1000-1S
dA
G003052
10.0g
GLEN RESEARCH

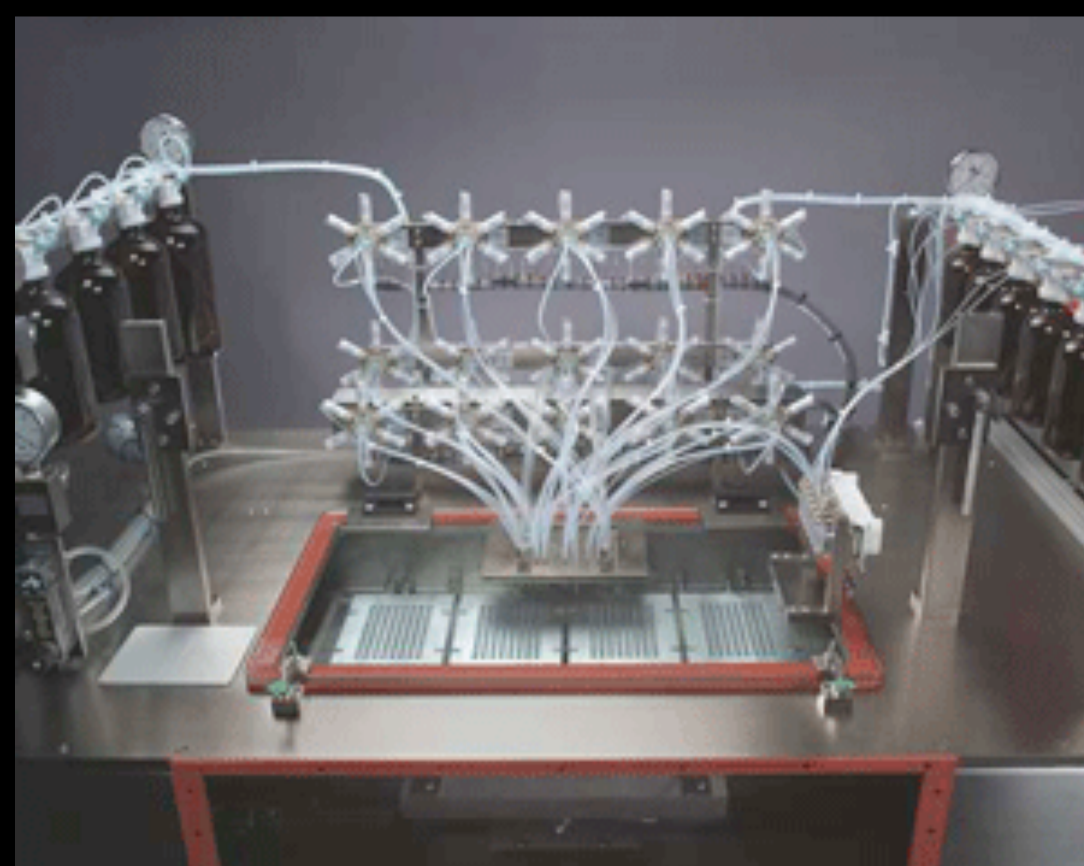


TAATACGACTCACTATAGGGAGA



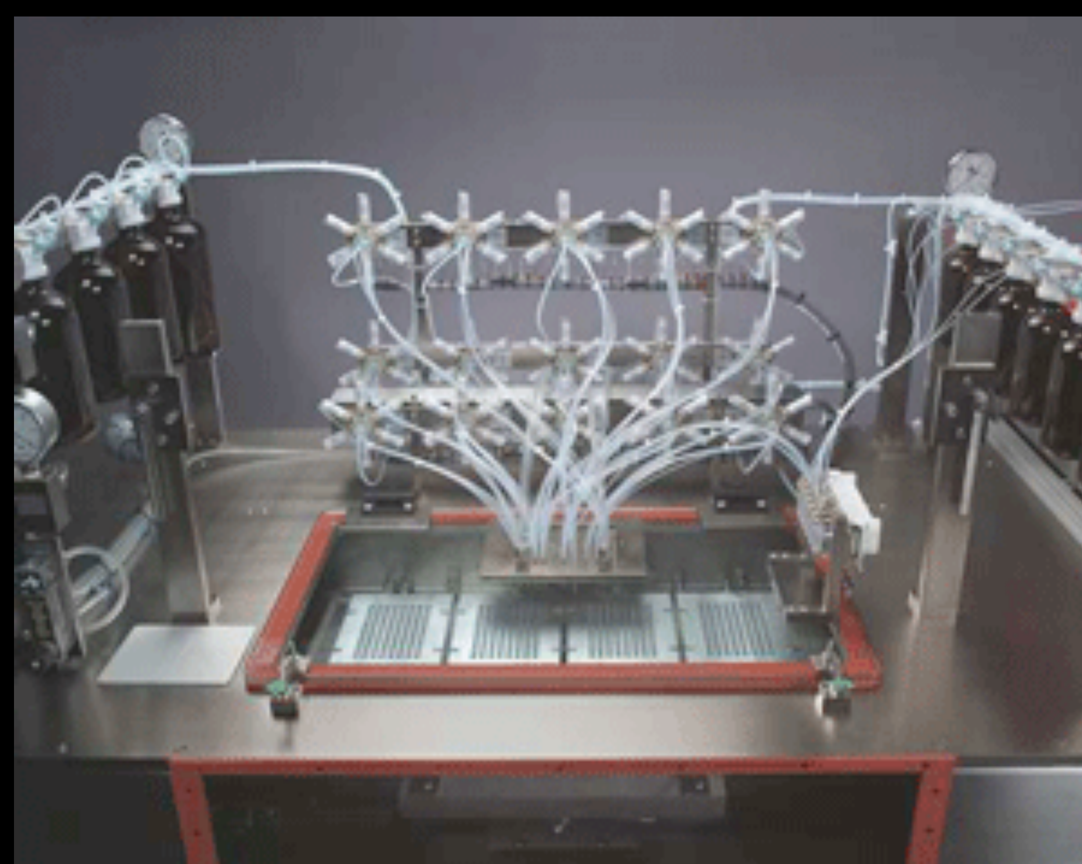
From absract information to physical, living DNA designs.

TAATACGACTCACTATAGGGAGA



From absract information to physical, living DNA designs.

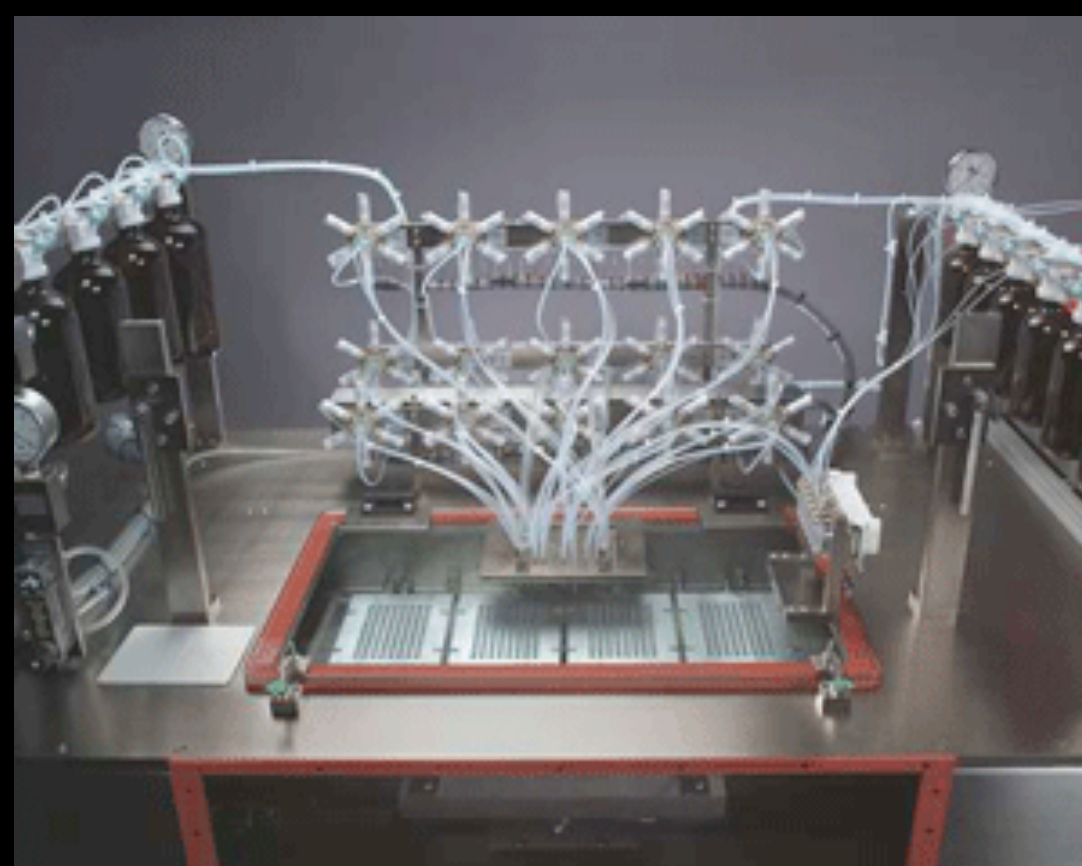
TAATACGACTCACTATAGGGAGA



2004: 10,000 bp
2010: 1,000,000 bp

From absract information to physical, living DNA designs.

TAATACGACTCACTATAGGGAGA

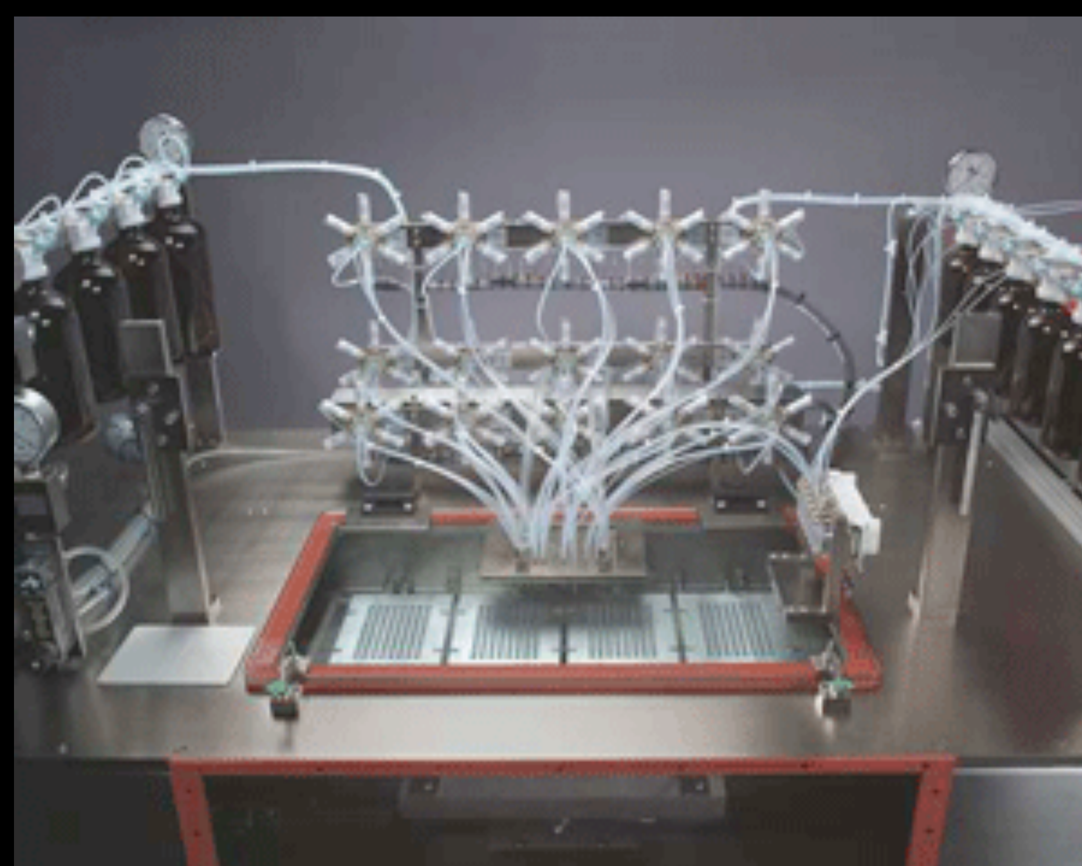


2004: 10,000 bp
2010: 1,000,000 bp
2016: 100 million?

DNA Construction = #1 Tech. of 21st Ctry.

From absract information to physical, living DNA designs.

TAATACGACTCACTATAGGGAGA



2004: 10,000 bp
2010: 1,000,000 bp
2016: 100 million?

Why does DNA synthesis matter?

Why does DNA synthesis matter?

Molecular Systems Biology (2005) doi:10.1038/msb4100025

www.molecularsystemsbiology.com

molecular systems biology

Refactoring bacteriophage T7

Leon Y Chan[1,3], Sriram Kosuri[2,3] and Drew Endy[2,*]

[1] Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA and [2] Division of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
[3] These authors contributed equally to this work
[*] Corresponding author. Division of Biological Engineering, Massachusetts Institute of Technology, 68-580, 77 Massachusetts Avenue, Cambridge, MA 02139, USA. Tel.: +1 617 258 5152; Fax: +1 617 253 5865; E-mail: endy@mit.edu

Received 15.7.05; accepted 23.7.05

Natural biological systems are selected by evolution to continue to exist and evolve. Evolution likely gives rise to complicated systems that are difficult to understand and manipulate. Here, we redesign the genome of a natural biological system, bacteriophage T7, in order to specify an engineered surrogate that, if viable, would be easier to study and extend. Our initial design goals were to physically separate and enable unique manipulation of primary genetic elements. Implicit in our design are the hypotheses that overlapping genetic elements are, in aggregate, nonessential for T7 viability and that our models for the functions encoded by elements are sufficient. To test our initial design, we replaced the left 11 515 base pairs (bp) of the 39 937 bp wild-type genome with 12 179 bp of engineered DNA. The resulting chimeric genome encodes a viable bacteriophage that appears to maintain key features of the original while being simpler to model and easier to manipulate. The viability of our initial design suggests that the genomes encoding natural biological systems can be systematically redesigned and built anew in service of scientific understanding or human intention.

Molecular Systems Biology 13 September 2005; doi:10.1038/msb4100025
Subject Categories: synthetic biology
Keywords: bacteriophage T7; synthetic biology; refactor

To build section alpha, we first cloned parts 5, 6, 7, 8, 12, 13, 14, 15, 16, 18, 20, 22, and 24 into pSB104. We cloned part 11 into pSB2K3. We cloned each part with its part-specific bracketing restriction sites surrounded by additional BioBrick restriction sites. We used site-directed mutagenesis on parts 6, 7, 14, and 20 to introduce the sites U1, U2, U3, and U4, respectively. Our site-directed mutagenesis of part 20 failed. We used site-directed mutagenesis to remove a single Eco0109I restriction site from the vector pUB119BHB carrying the scaffold Fragment 4. We cloned part 15 into this modified vector. We then cloned scaffold Fragment 4 into pREB and used serial cloning to add the following parts: 7, 8, 12, 13, 14, 16, 18, 20, 22, and 23. We digested the now-populated scaffold Fragment 4 with NheI and BclI and purified the resulting DNA. Next, we cloned parts 5 and 6 into pUB119BHB carrying scaffold Fragment 3. We used the resulting DNA for in vitro assembly of a construct spanning from the left end of T7 to part 7. To do this, we cut wild-type T7 genomic DNA with AseI, isolated the 388 bp left-end fragment, and ligated this DNA to scaffold Fragment 2. We selected the correct ligation product by PCR. We fixed the mutation in part 3 (A1) via a two-step process. First, PCR primers with the corrected sequence for part 3 were used to amplify the two halves of the construct to the left and right ends of part 3. Second, a PCR ligation joined the two constructs. We added scaffold Fragment 3 to the above left-end construct once again by PCR ligation as described above. We repaired the mutation in part 4 (A2, A3, and R0.3) following the same procedure as with part 3. We used a right-end primer containing an MluI site to amplify the entire construct, and used the MluI site to add part 7. We used PCR to select the ligation product, digested the product with NheI, and purified the resulting DNA. We isolated the right arm of a BclI digestion of wild-type T7 genomic DNA and used ligation to add the populated left-end construct and the populated Scaffold Fragment 4. We transfected the three-way ligation product into IJ1127. We purified DNA from liquid culture lysates inoculated from single plaques. We used restriction enzymes to digest the DNA and isolate the correct clones. Next, we added part 11 via three-way ligation and transfection. Because the restriction sites that bracket part 9 (RsrII) also cut wild-type T7 DNA, we needed to use in vitro assembly to add this part to a subsection of section alpha. To do this, we used PCR to amplify the region spanning parts 5–12 from the refactored genome. We cut the PCR product with RsrII and ligated part 9. We used PCR to select the correct ligation product; this PCR reaction also added a SacII site to the fragment. We digested the PCR product with SacI and SacII and cloned onto the otherwise wild-type phage. Lastly, we used the SacII site to clone part 10 onto the phage.

DNA synthesis replaces below with...

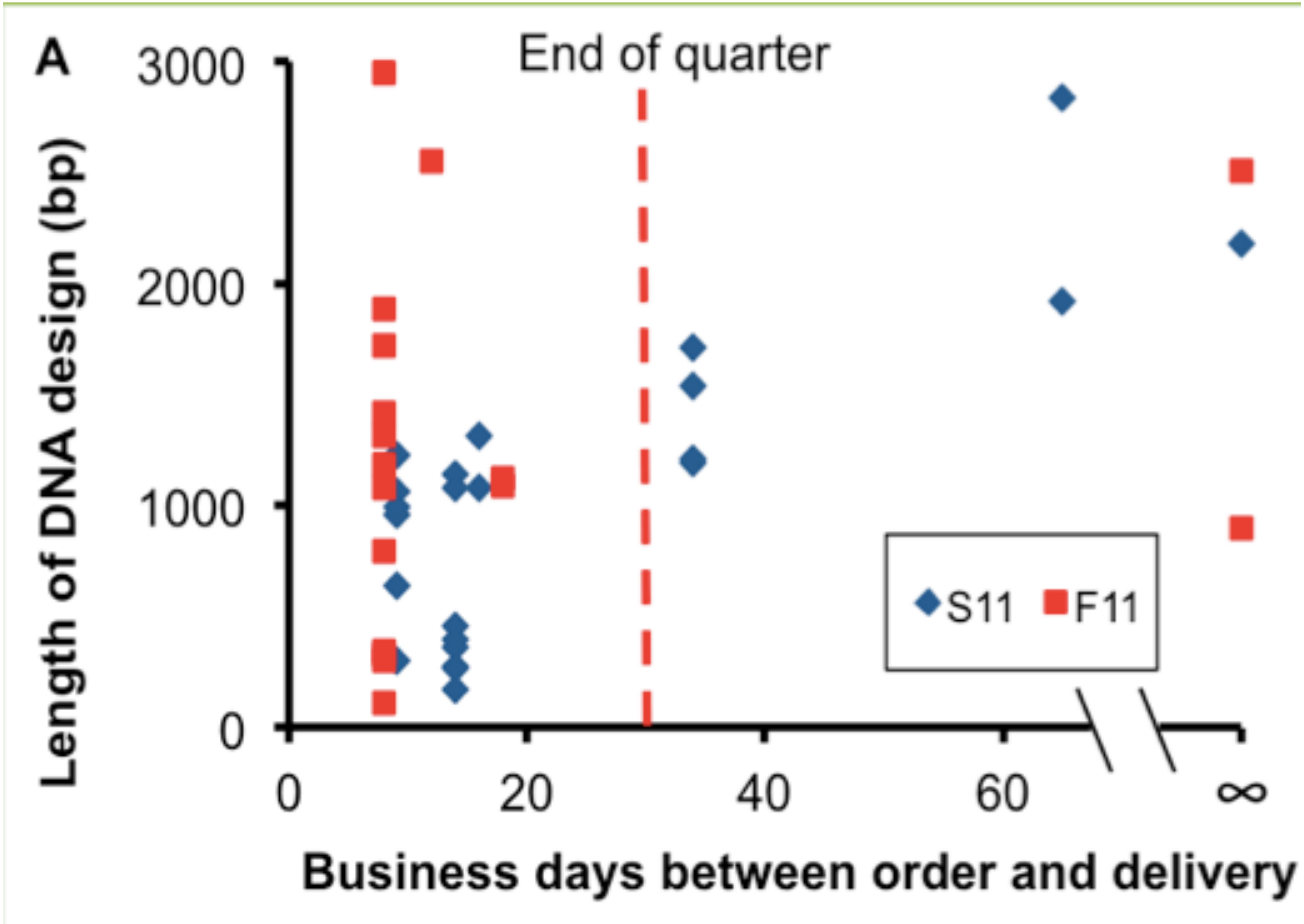
To build section alpha, we first cloned parts 5, 6, 7, 8, 12, 13, 14, 15, 16, 18, 20, 22, and 24 into pSB104. We cloned part 11 into pSB2K3. We cloned each part with its part-specific bracketing restriction sites surrounded by additional BioBrick restriction sites. We used site-directed mutagenesis on parts 6, 7, 14, and 20 to introduce the sites U1, U2, U3, and U4, respectively. Our site-directed mutagenesis of part 20 failed. We used site-directed mutagenesis to remove a single Eco0109I restriction site from the vector pUB119BHB carrying the scaffold Fragment 4. We cloned part 15 into this modified vector. We then cloned scaffold Fragment 4 into pREB and used serial cloning to add the following parts: 7, 8, 12, 13, 14, 16, 18, 20, 22, and 23. We digested the now-populated scaffold Fragment 4 with NheI and BclI and purified the resulting DNA. Next, we cloned parts 5 and 6 into pUB119BHB carrying scaffold Fragment 3. We used the resulting DNA for in vitro assembly of a construct spanning from the left end of T7 to part 7. To do this, we cut wild-type T7 genomic DNA with AseI, isolated the 388 bp left-end fragment, and ligated this DNA to scaffold Fragment 2. We selected the correct ligation product by PCR. We fixed the mutation in part 3 (A1) via a two-step process. First, PCR primers with the corrected sequence for part 3 were used to amplify the two halves of the construct to the left and right ends of part 3. Second, a PCR ligation joined the two constructs. We added scaffold Fragment 3 to the above left-end construct once again by PCR ligation as described above. We repaired the mutation in part 4 (A2, A3, and R0.3) following the same procedure as with part 3. We used a right-end primer containing an MluI site to amplify the entire construct, and used the MluI site to add part 7. We used PCR to select the ligation product, digested the product with NheI, and purified the resulting DNA. We isolated the right arm of a BclI digestion of wild-type T7 genomic DNA and used ligation to add the populated left-end construct and the populated Scaffold Fragment 4. We transfected the three-way ligation product into IJ1127. We purified DNA from liquid culture lysates inoculated from single plaques. We used restriction enzymes to digest the DNA and isolate the correct clones. Next, we added part 11 via three-way ligation and transfection. Because the restriction sites that bracket part 9 (RsrII) also cut wild-type T7 DNA, we needed to use in vitro assembly to add this part to a subsection of section alpha. To do this, we used PCR to amplify the region spanning parts 5–12 from the refactored genome. We cut the PCR product with RsrII and ligated part 9. We used PCR to select the correct ligation product; this PCR reaction also added a SacII site to the fragment. We digested the PCR product with SacI and SacII and cloned onto the otherwise wild-type phage. Lastly, we used the SacII site to clone part 10 onto the phage.

Get me my DNA!

DNA fab. displacing classical genetic engr.

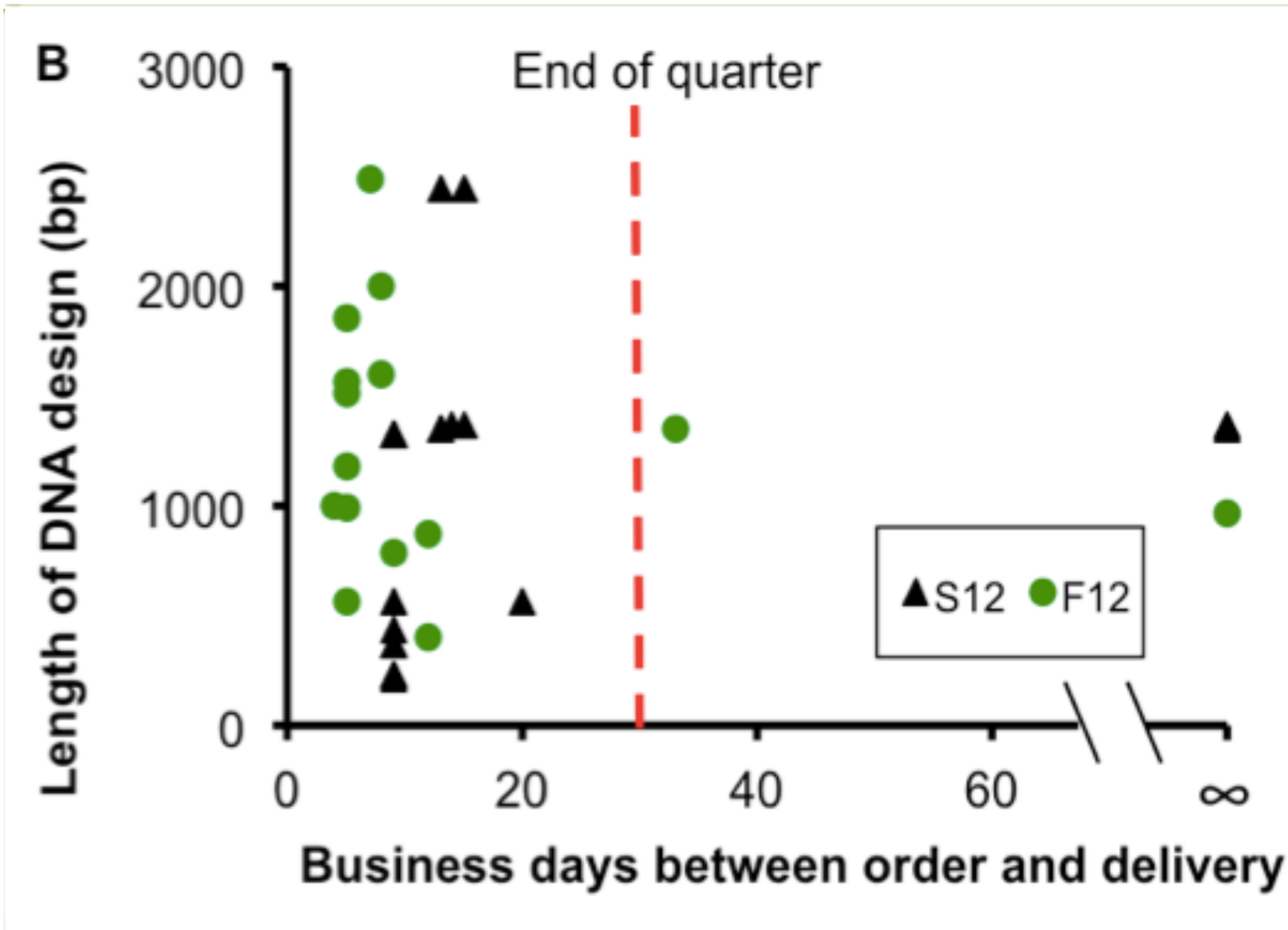
SOURCE: Joe Shih, Stanford BIOE.44

DNA fab. displacing classical genetic engr.



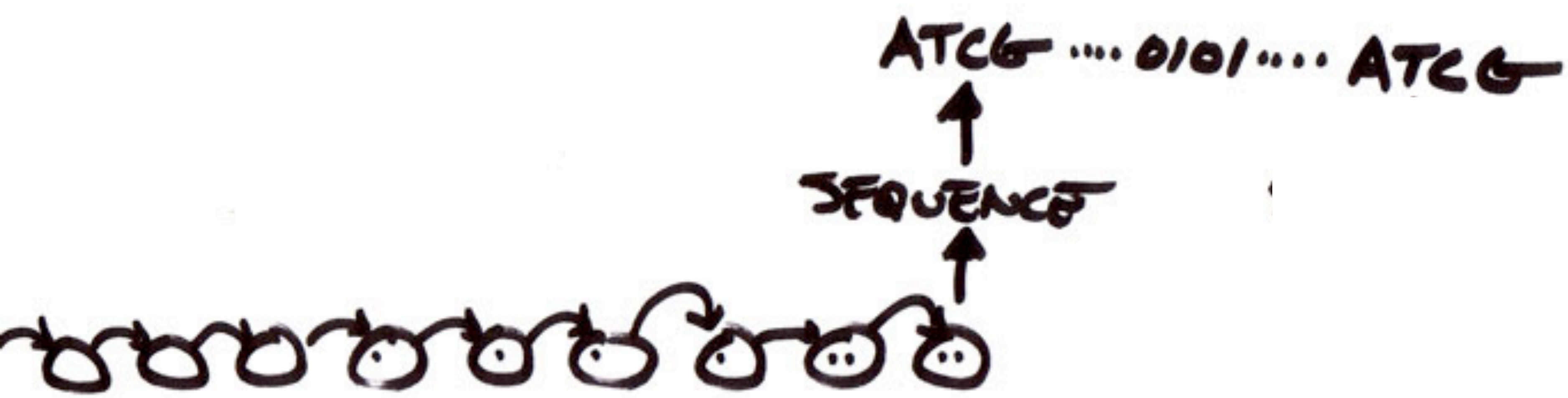
SOURCE: Joe Shih, Stanford BIOE.44

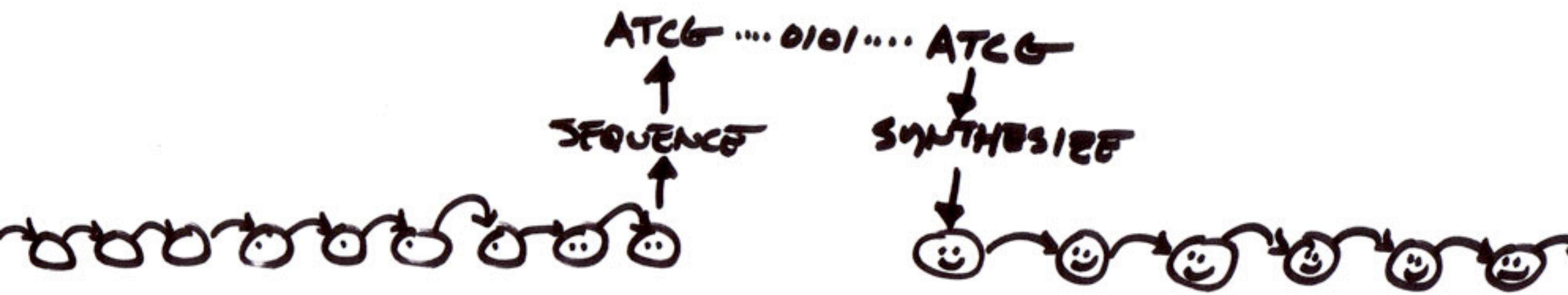
DNA fab. displacing classical genetic engr.



SOURCE: Joe Shih, Stanford BIOE.44


ATCG ....
SEQUENCE


ATCG ....0101.... ATCG
SEQUENCE


ATCG ....0101.... ATCG
SEQUENCE
SYNTHESIZE


N°5
INÉVITABLE
N°5
CHANEL
PARIS
PARFUM

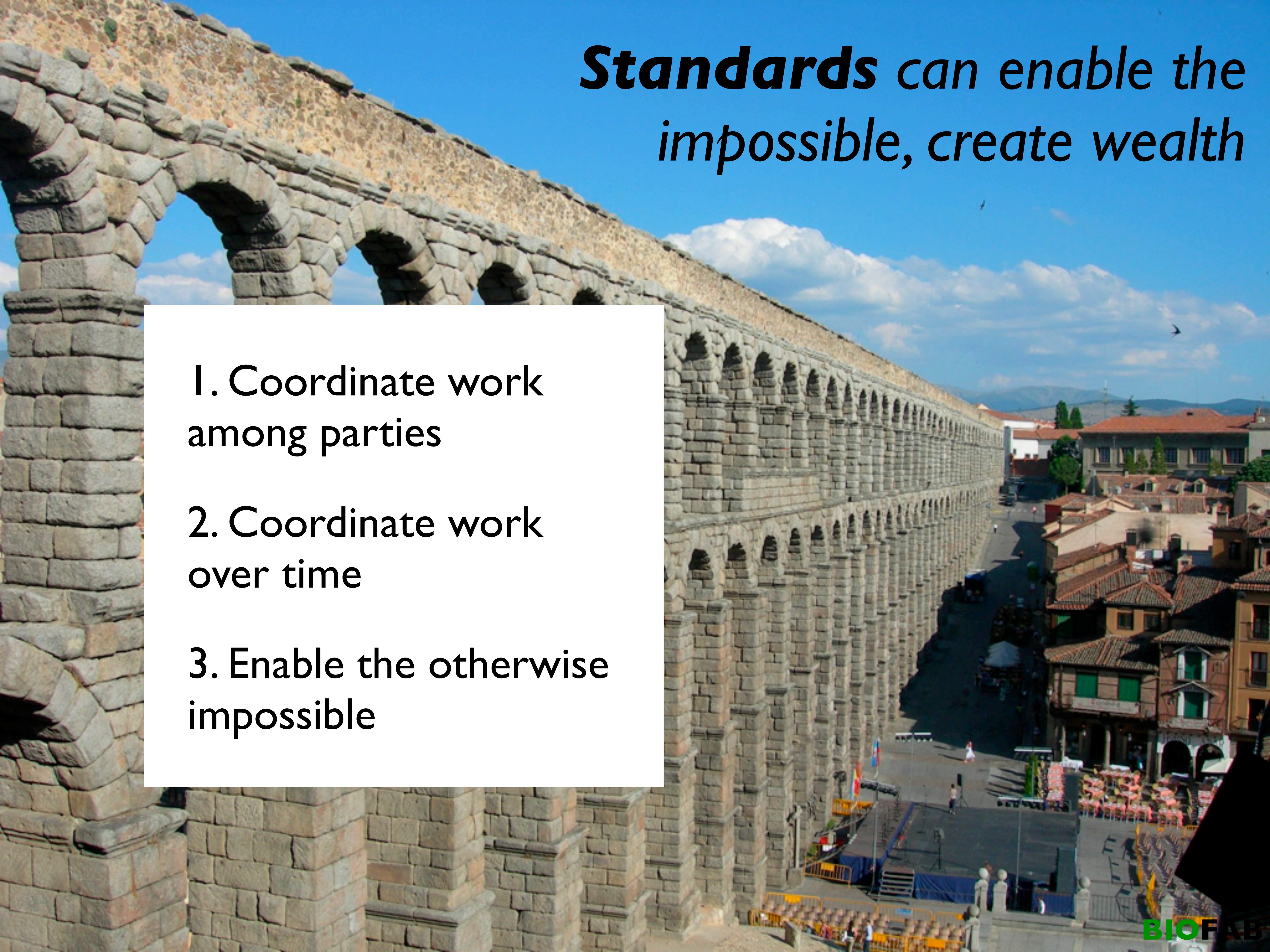

Standards *can enable the impossible, create wealth*
BIOFAB

Standards *can enable the impossible, create wealth*

1. Coordinate work among parties

2. Coordinate work over time

3. Enable the otherwise impossible



Standards *can enable the impossible, create wealth*

1. Coordinate work among parties

2. Coordinate work over time

3. Enable the otherwise impossible

E.g., some quarry rock, others assemble structure

E.g., we could fix a broken rock today, ~2000 years later

Standards in synthetic biology

Standards in synthetic biology

1. Physical genetic layout (leading to...)

Standards in synthetic biology

1. *Physical genetic layout (leading to...)*

2. *Functional composition*

Standards in synthetic biology

1. *Physical genetic layout (leading to...)*

2. *Functional composition*

3. *Metrology inside cells (measurement and reference materials)*

Standards in synthetic biology

1. *Physical genetic layout (leading to...)*
2. *Functional composition*
3. *Metrology inside cells*
   (measurement and reference materials)
4. *Representations*

"There is no such thing as a standard (biological) component, because even a standard component works differently depending on the environment. The expectation that you can type in a (DNA) sequence and can predict what a (genetic) circuit will do is far from reality and always will be."

Caltech Professor, NY Times, 2006

"There is no such thing as a standard (biological) component, because even a standard component works differently depending on the environment. The expectation that you can type in a (DNA) sequence and can predict what a (genetic) circuit will do is far from reality and always will be."

Caltech Professor, NY Times, 2006

"... reusing the same well-characterized RBS (ribosome binding site) sequence for different proteins, a common practice, is not likely to work reliably."

MIT Professor, Nature Biotechnology, 2009

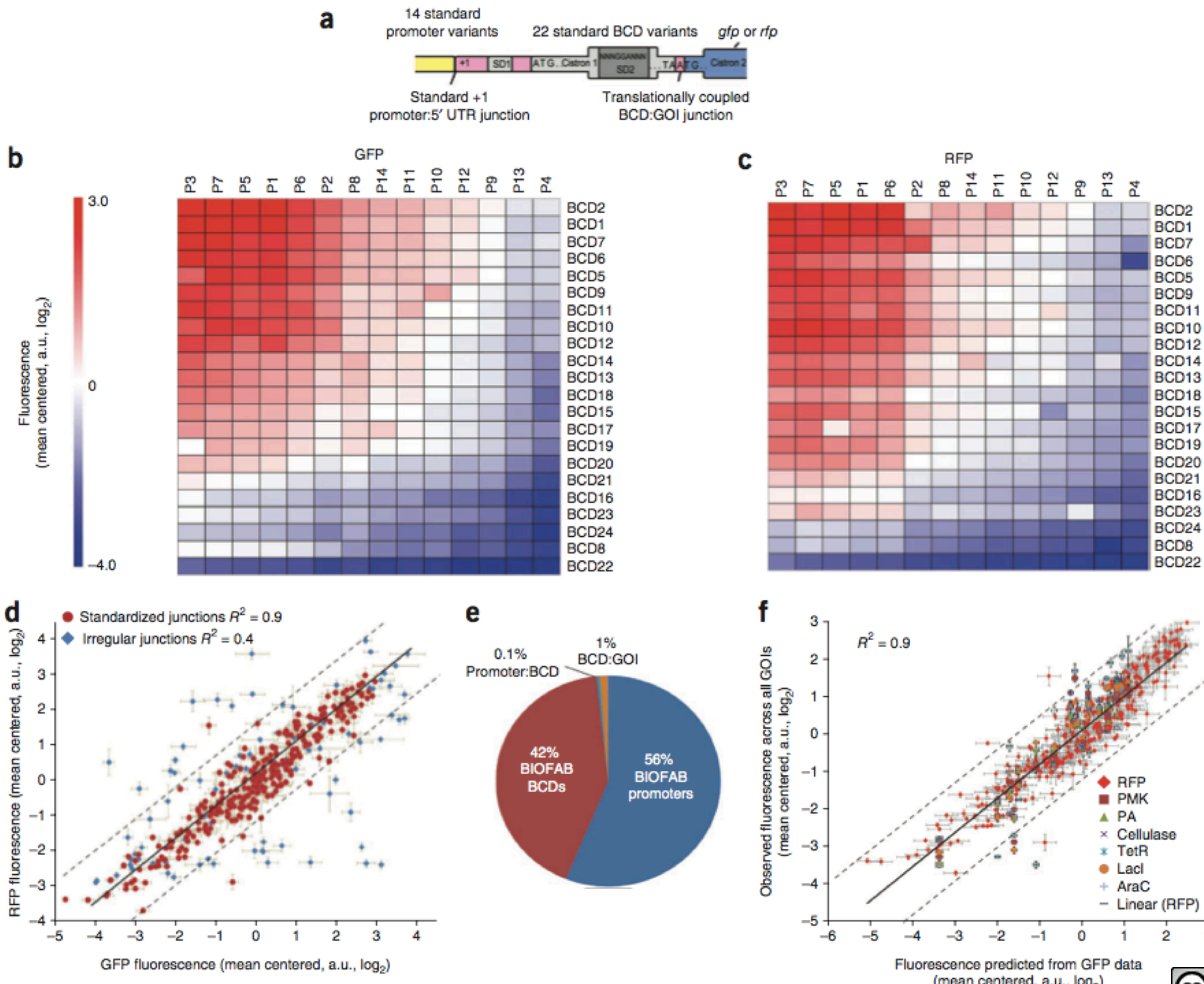


Figure 4 | Precise and reliable gene expression via standard transcription-control and translation-initiation elements. (**a**) Standard promoters produce mRNA from a common +1 nucleotide position. Translation initiation is entirely encoded by a separate and independent bicistronic design (BCD).

Abstraction to manage complexity ?%&#!!!

Abstraction to manage complexity ?%&#!!!

8 Bit Synchro.
Counter

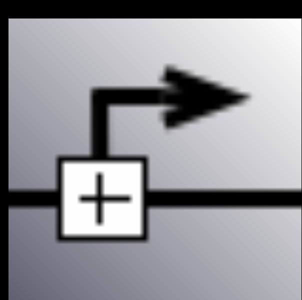
Systems = One or more devices encoding a human defined function(s).

Abstraction barrier! Do not cross!

DNA Inversion
Data Latch

Devices = One or more parts encoding a human defined function(s).

Abstraction barrier! Do not cross!



Parts = Basic biological functions encoded via DNA.

Abstraction barrier! Do not cross!

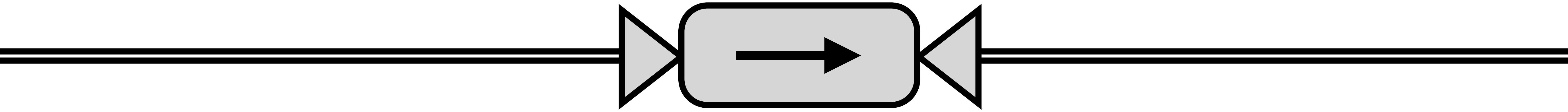
CTATAGGGAGA

DNA = Primary sequence and mater

01010101

Bonnet et al., PNAS USA, 2012

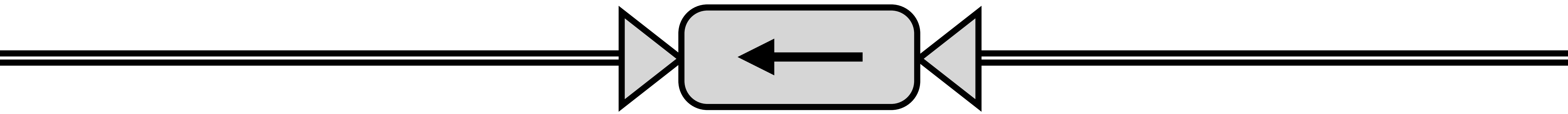
***please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage*



“flipee”

Bonnet et al., PNAS USA, 2012

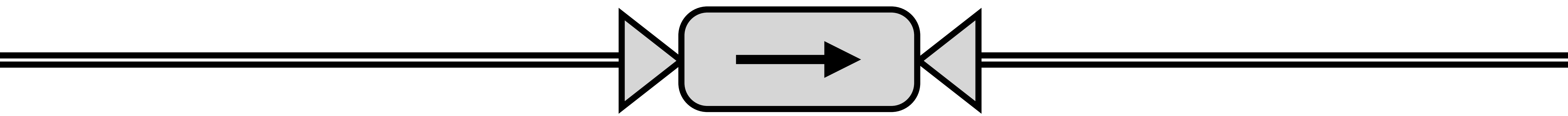
**please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage



"flipee"

Bonnet et al., PNAS USA, 2012

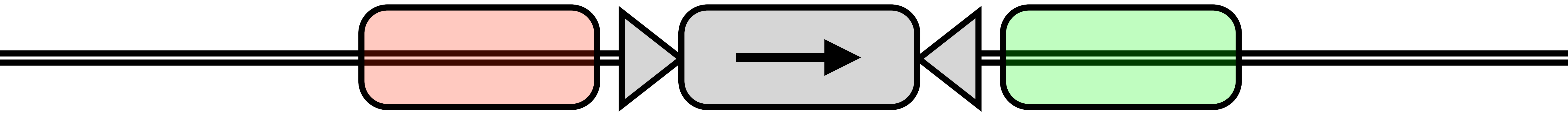
***please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage*



“flipee”

Bonnet et al., PNAS USA, 2012

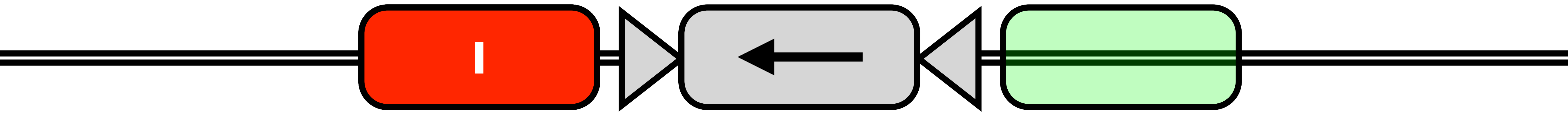
**please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage



“flipee”

Bonnet et al., PNAS USA, 2012

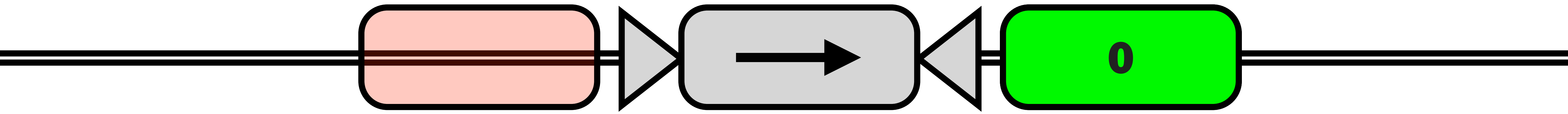
**please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage



"flipee"

Bonnet et al., PNAS USA, 2012

***please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage*

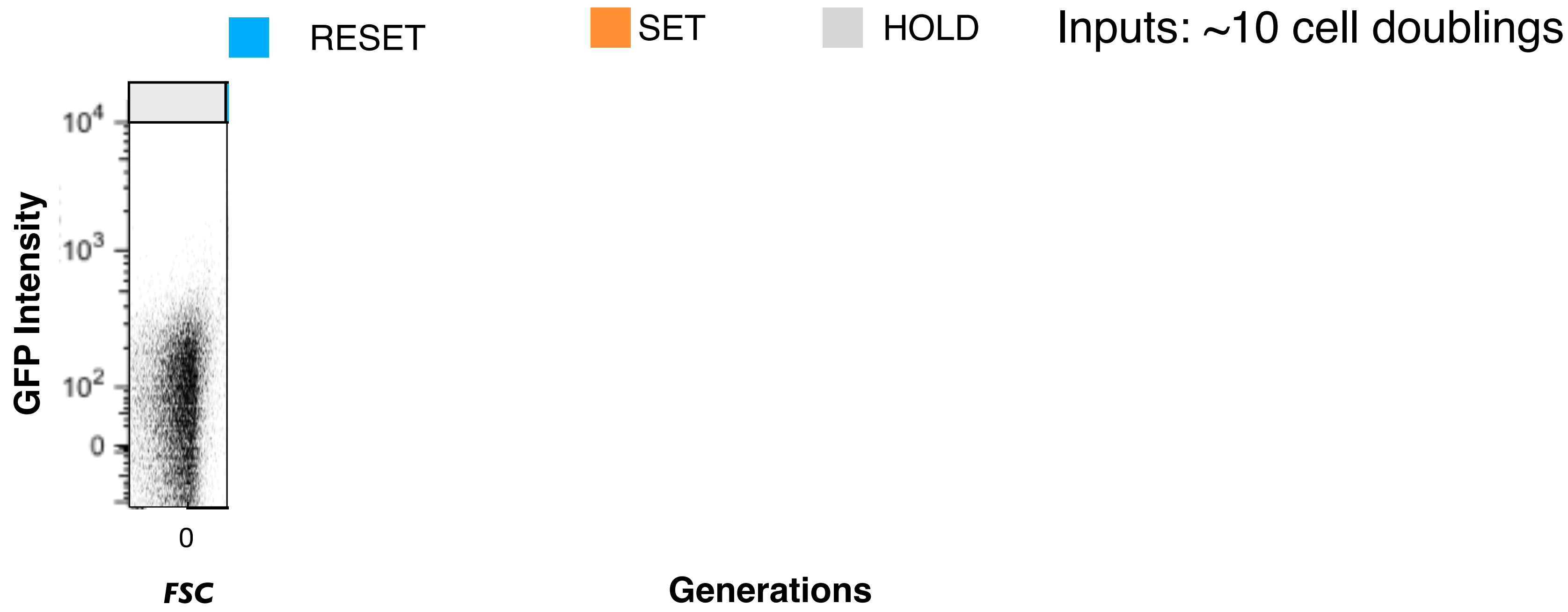


"flipee"

Bonnet et al., PNAS USA, 2012

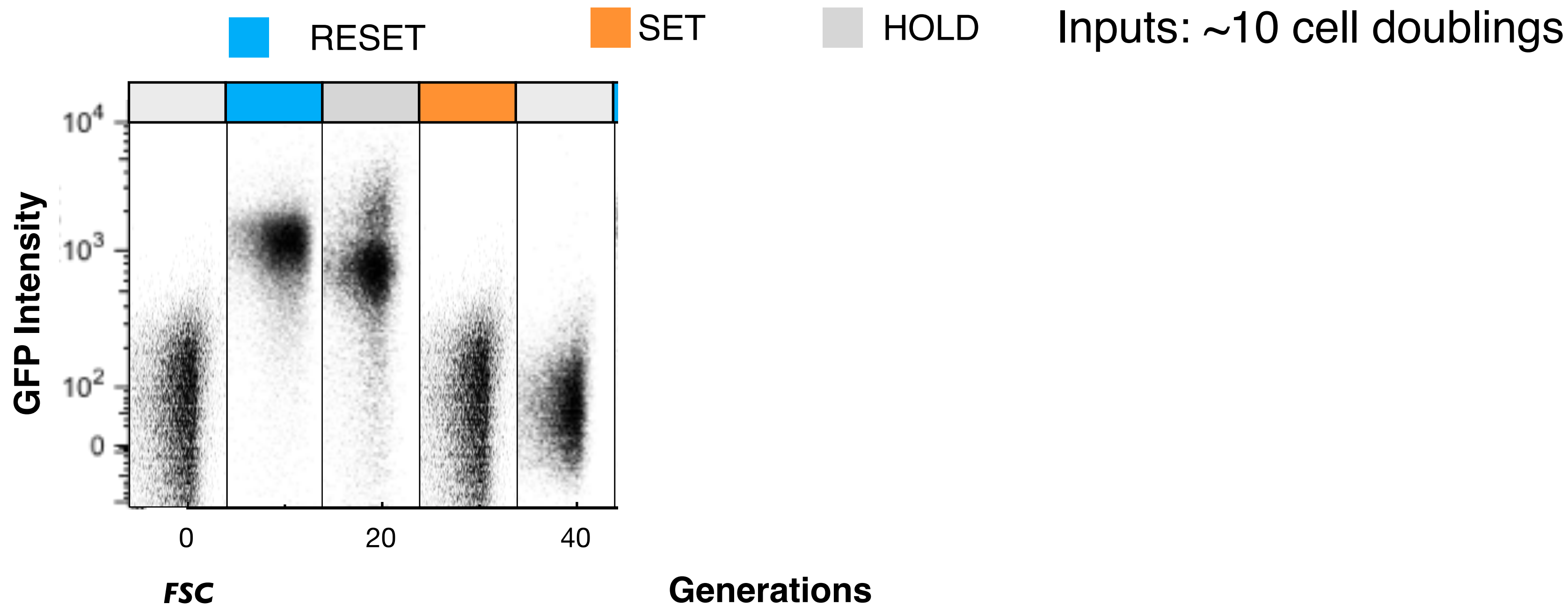
***please see Nash & Pollock (1981, 1983) thru Friedland & Lu et al. (2010+) for prior work on flipping DNA and data storage*

3 years, 750 attempts later…



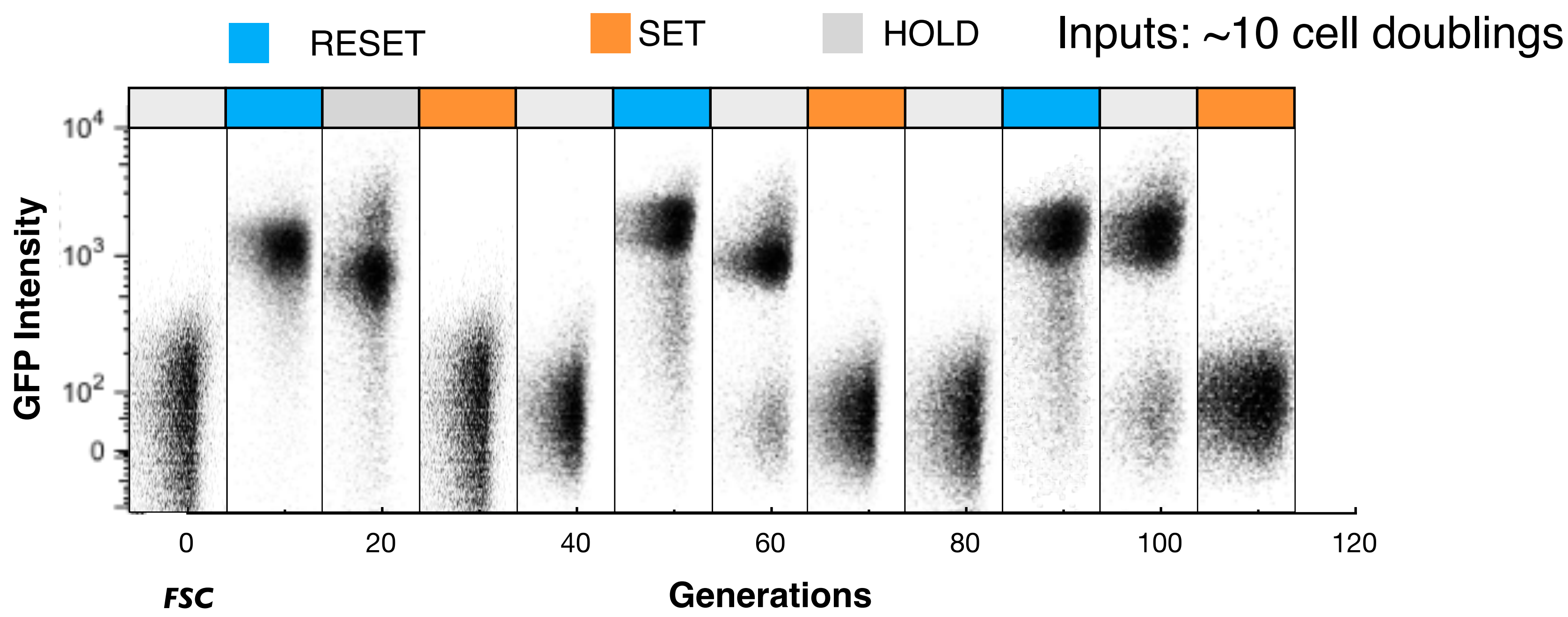
Bonnet et al., PNAS USA, 2012

3 years, 750 attempts later...



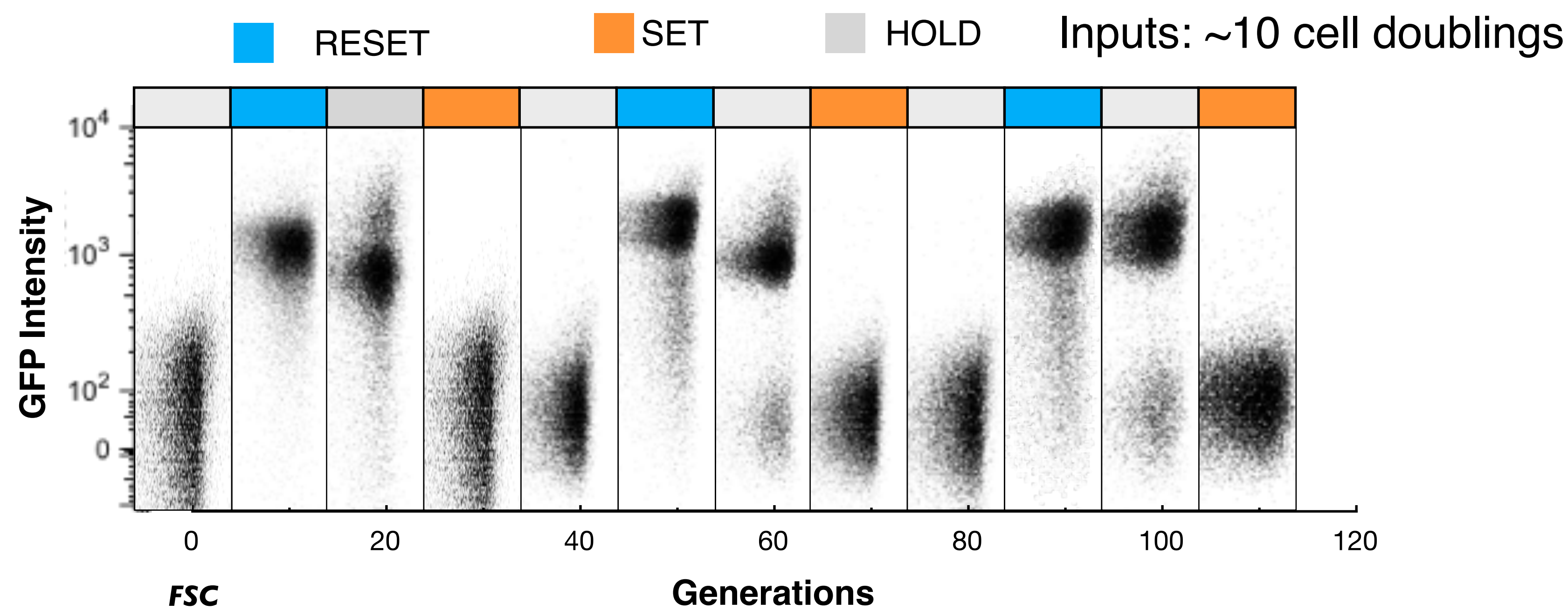
Bonnet et al., PNAS USA, 2012

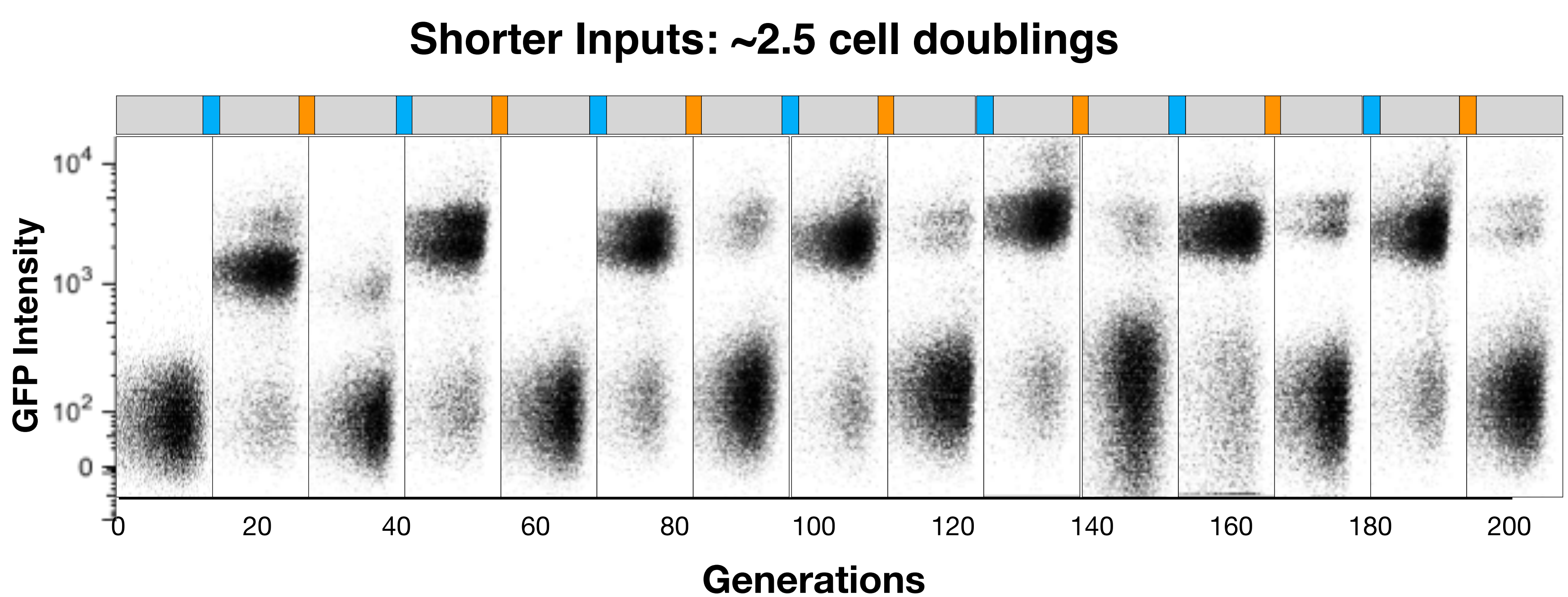
3 years, 750 attempts later...



Bonnet et al., PNAS USA, 2012

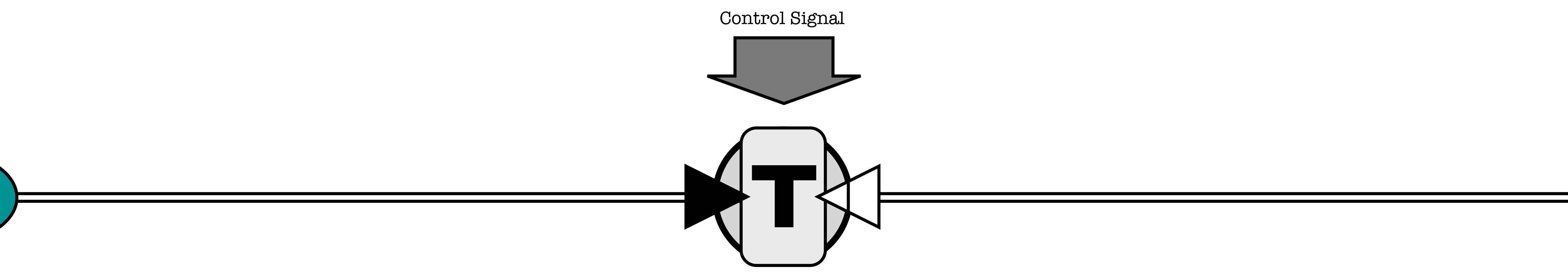
3 years, 750 attempts later...





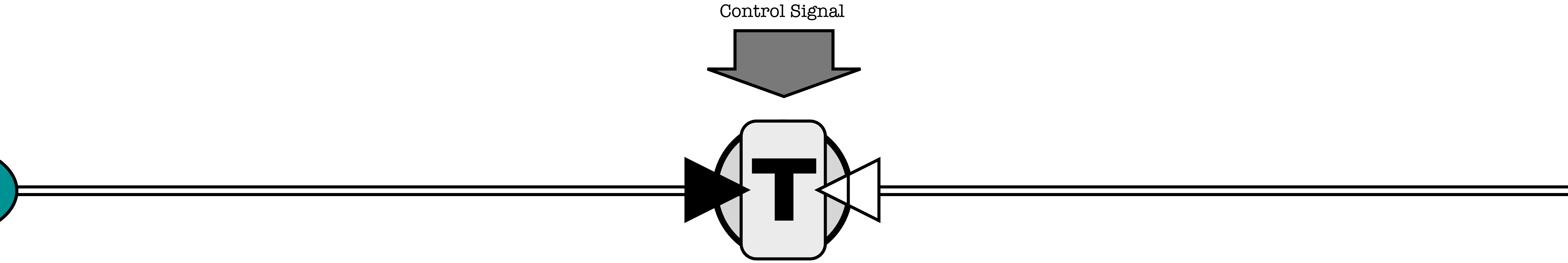
Bonnet et al., PNAS USA, 2012

But, then abstract into "transcriptors"...



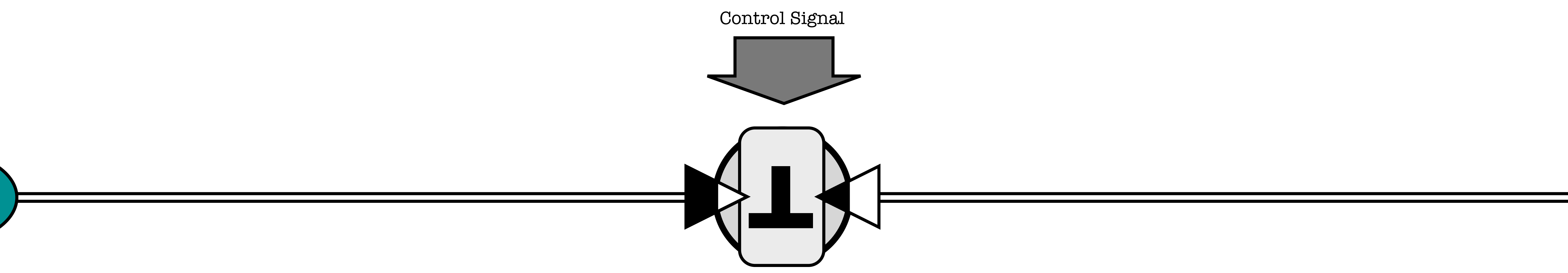
Bonnet et al., Science, 2013

But, then abstract into "transcriptors"...



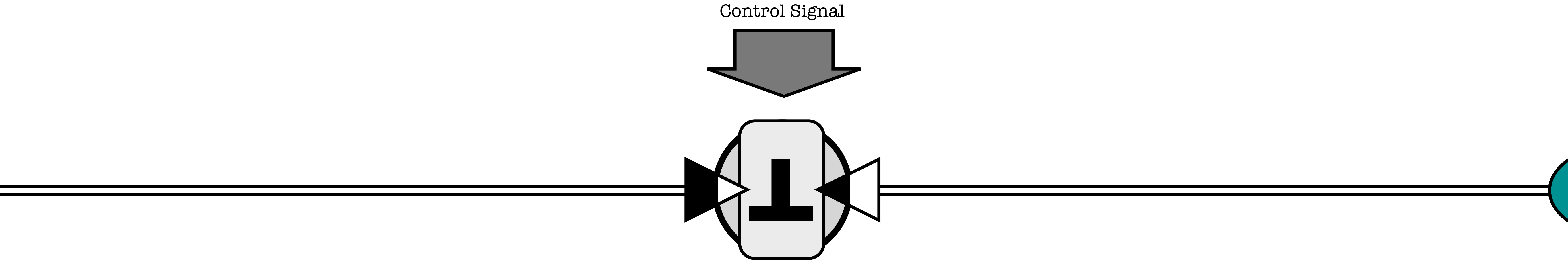
Bonnet et al., Science, 2013

But, then abstract into "transcriptors"...



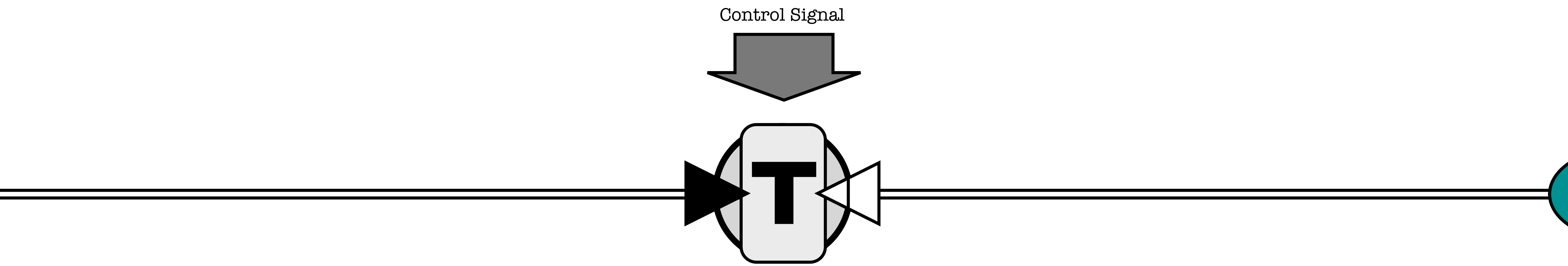
Bonnet et al., Science, 2013

But, then abstract into "transcriptors"...



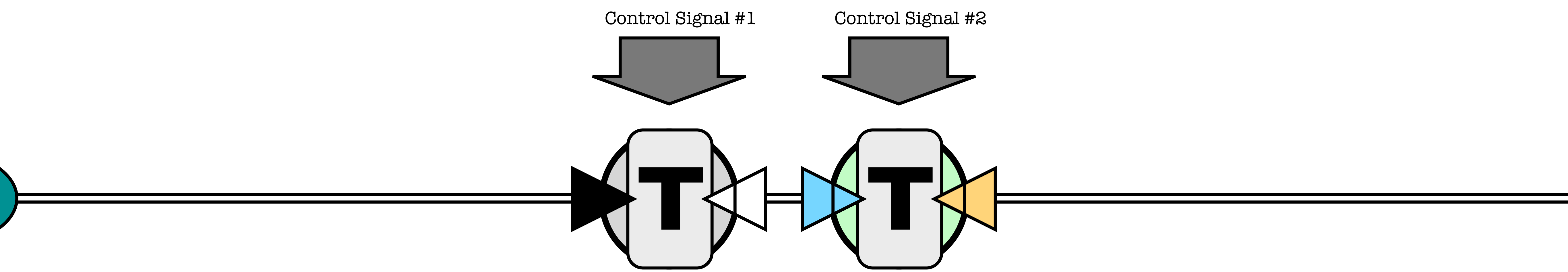
Bonnet et al., Science, 2013

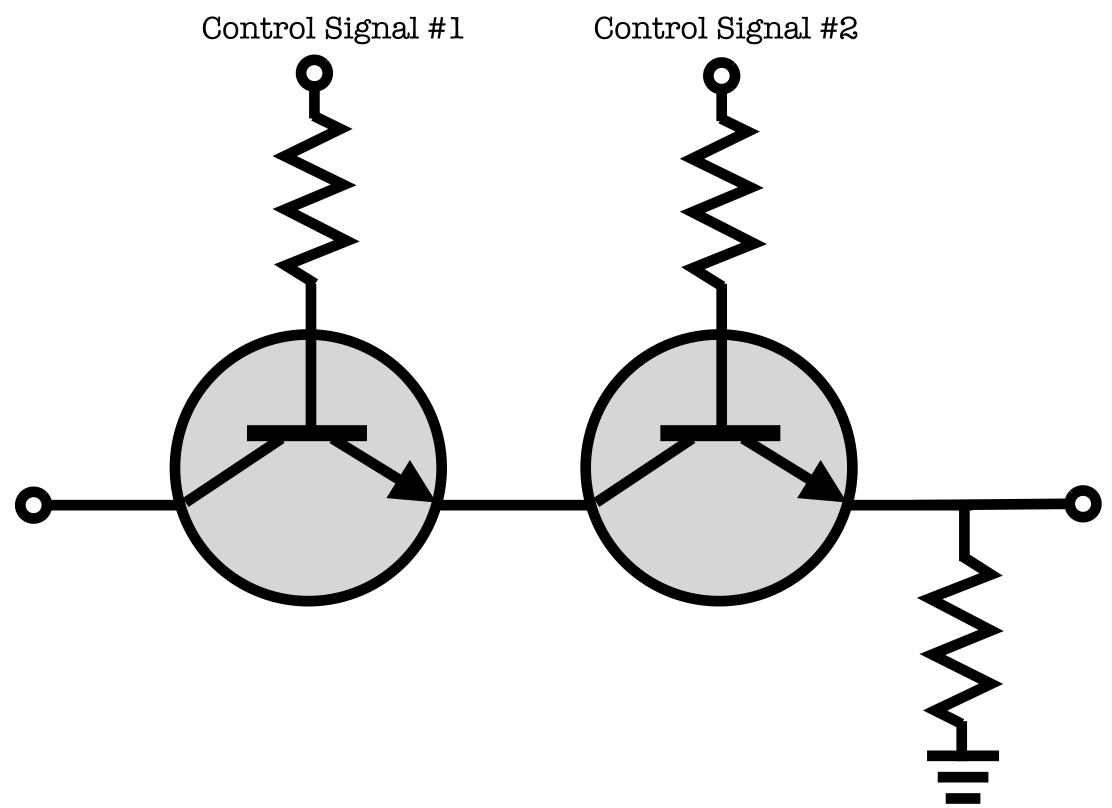
But, then abstract into "transcriptors"...



Bonnet et al., Science, 2013

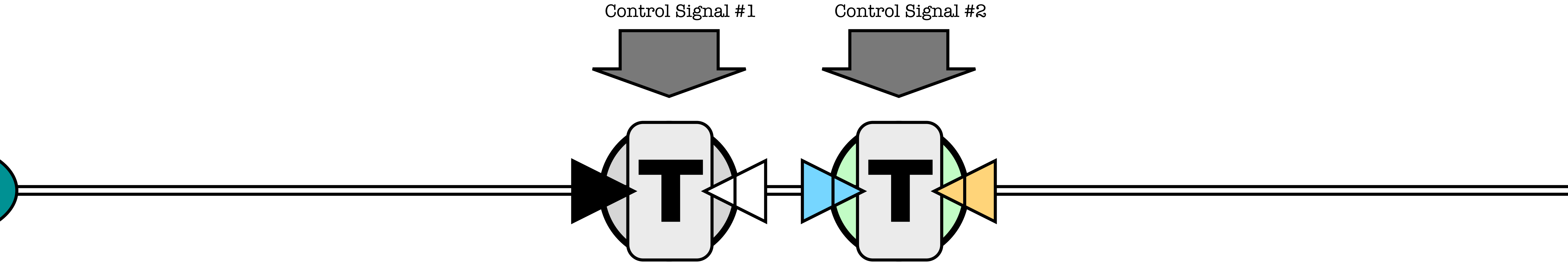
AND

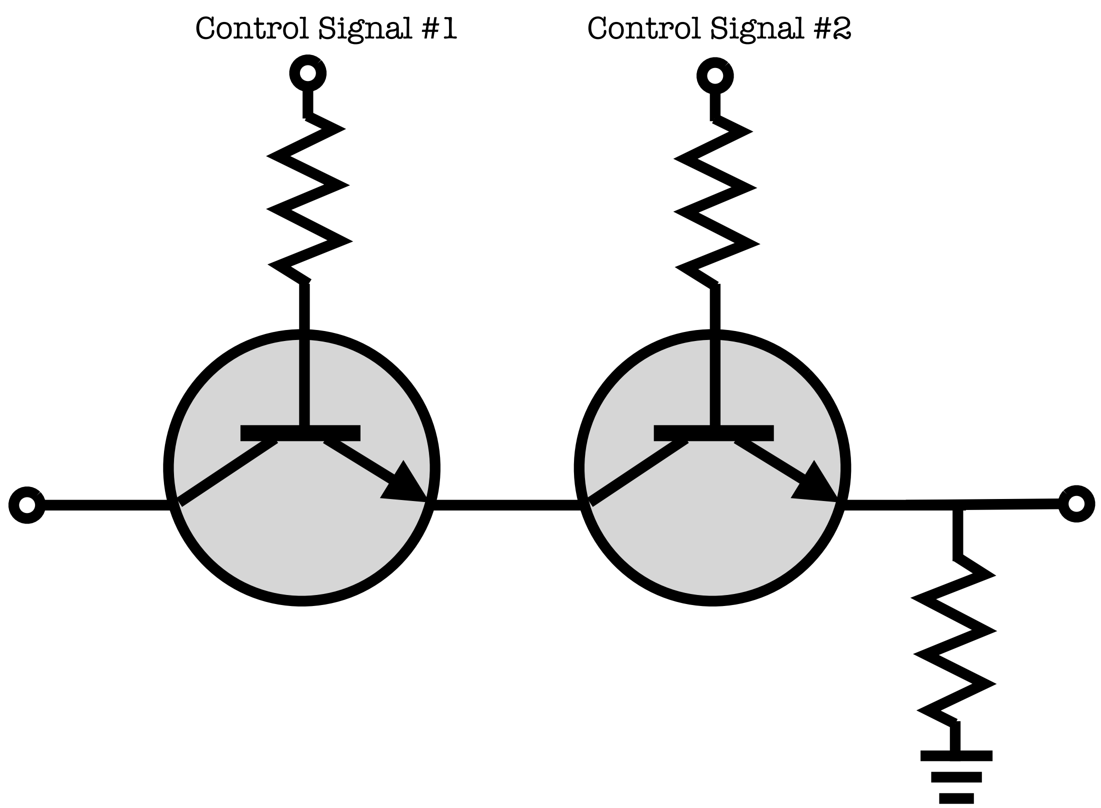




Bonnet et al., Science, 2013

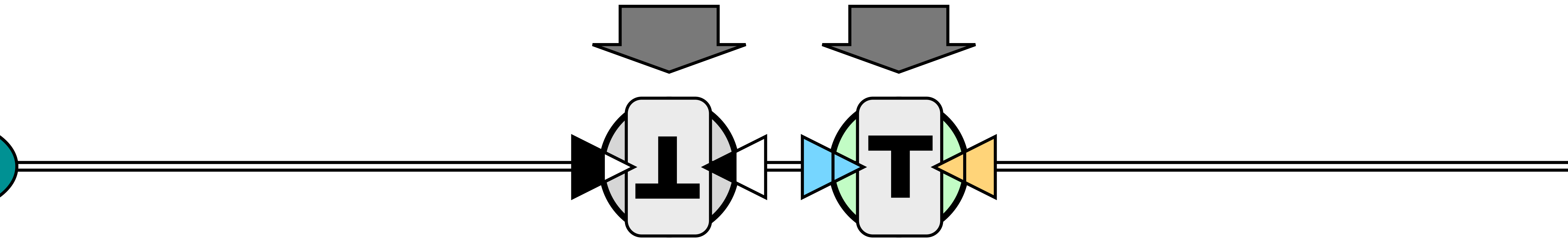
AND

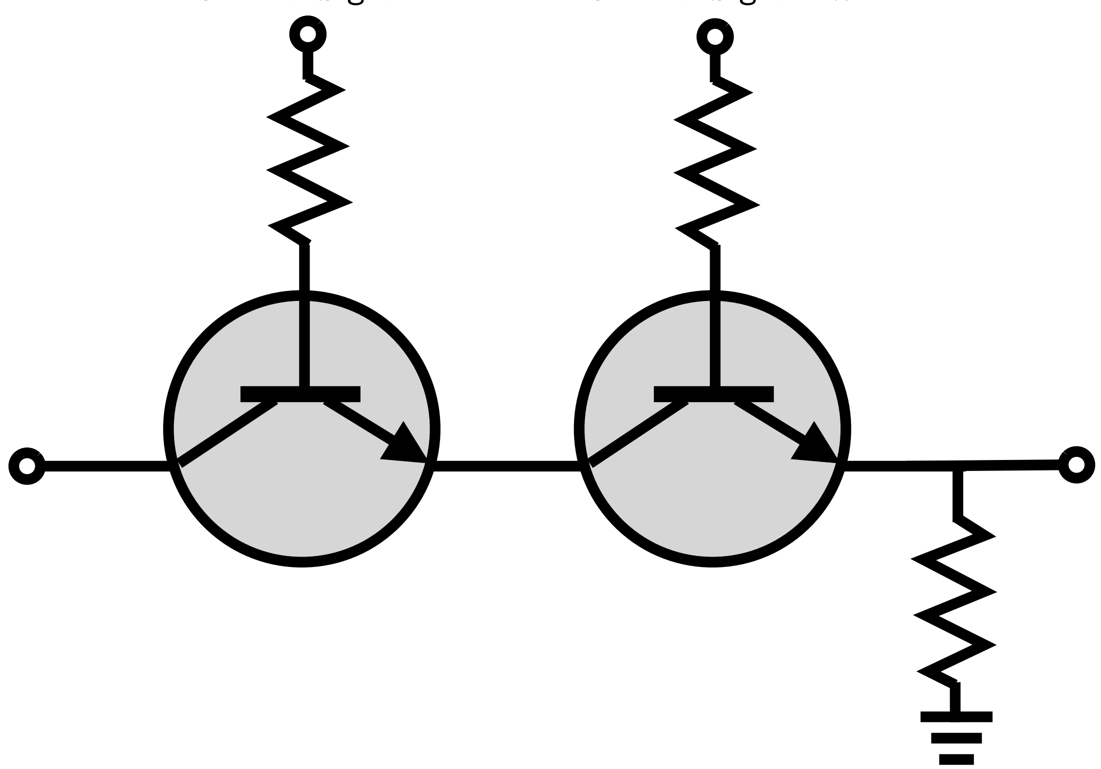




Bonnet et al., Science, 2013

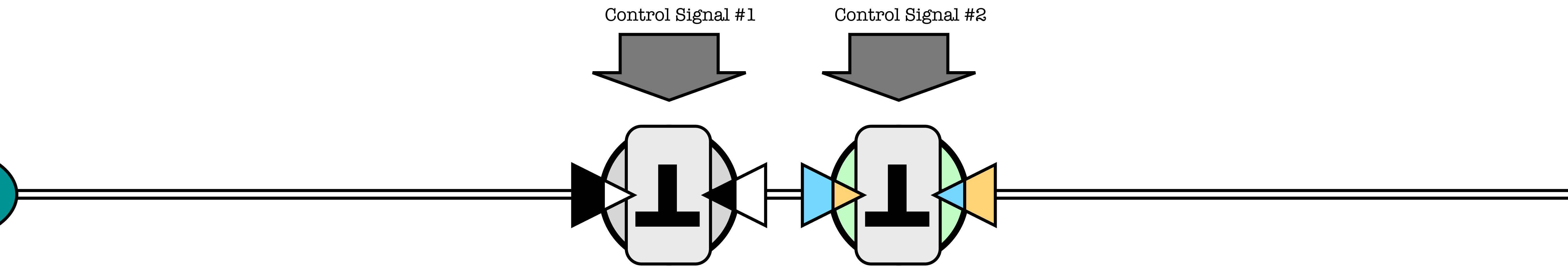
AND

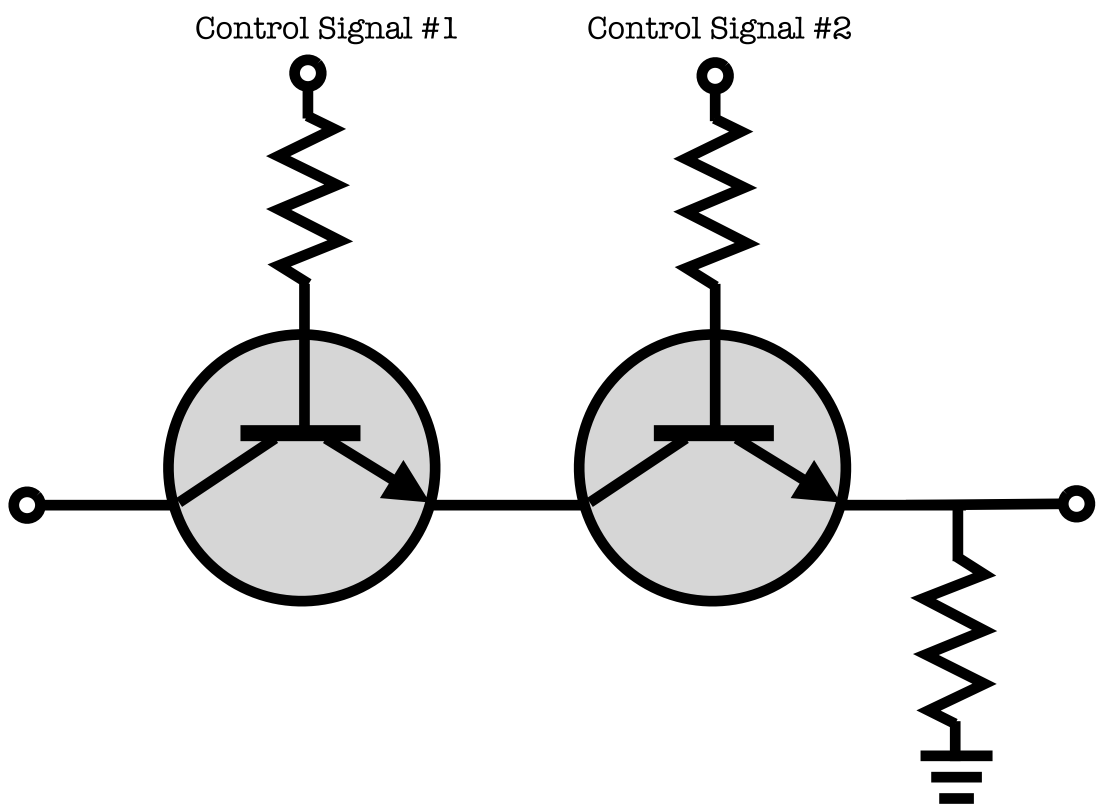




Bonnet et al., Science, 2013

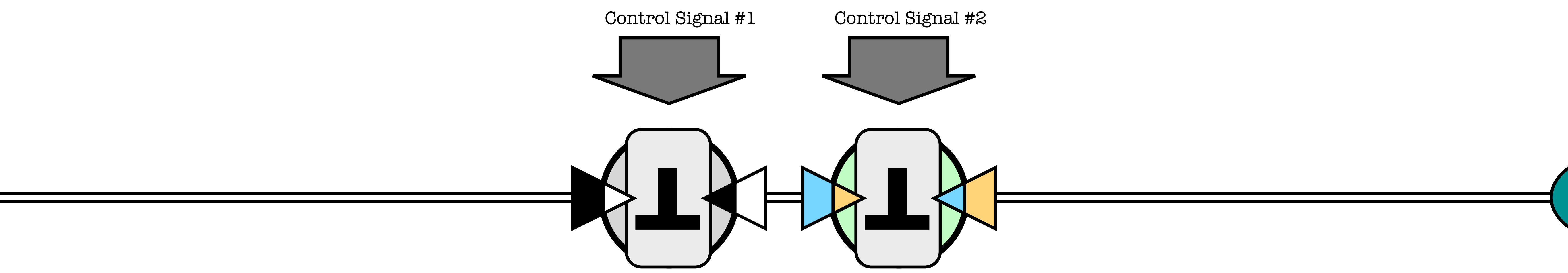
AND

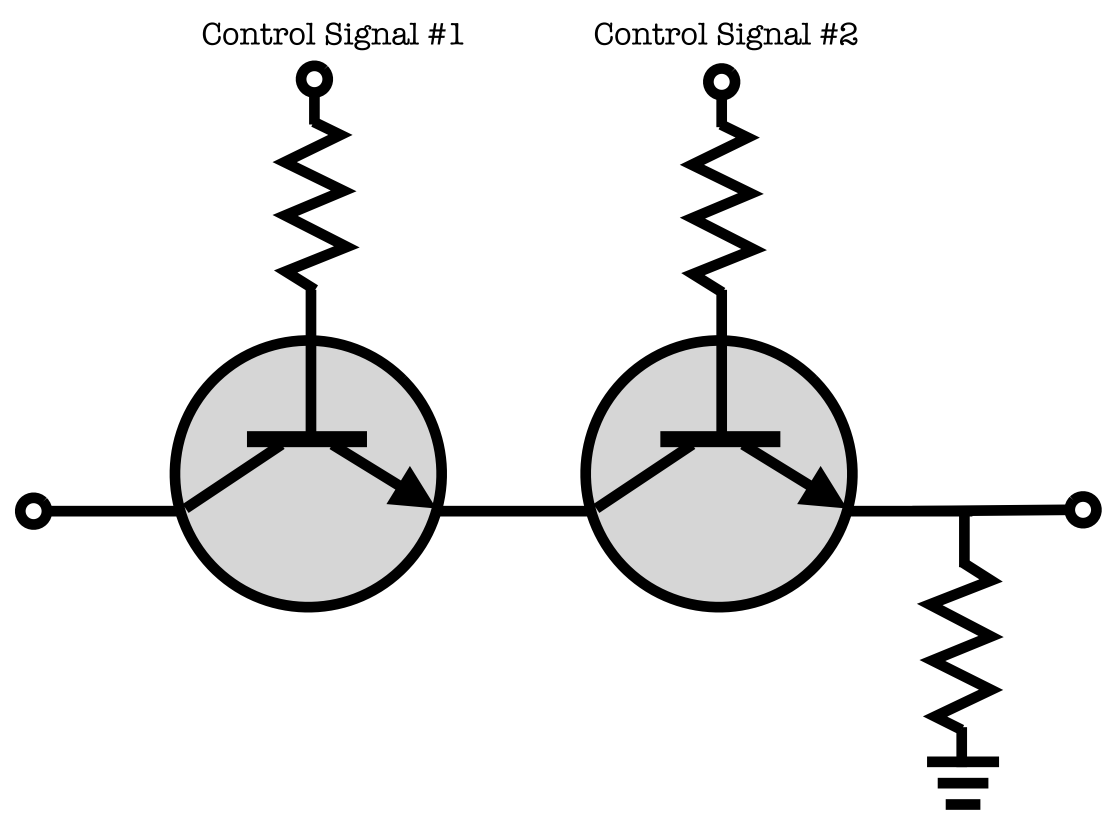




Bonnet et al., Science, 2013

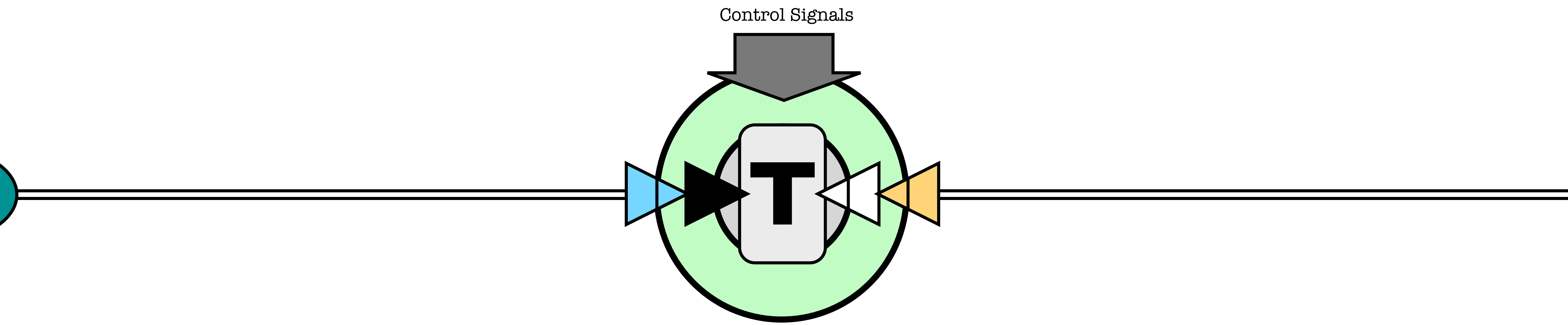
AND





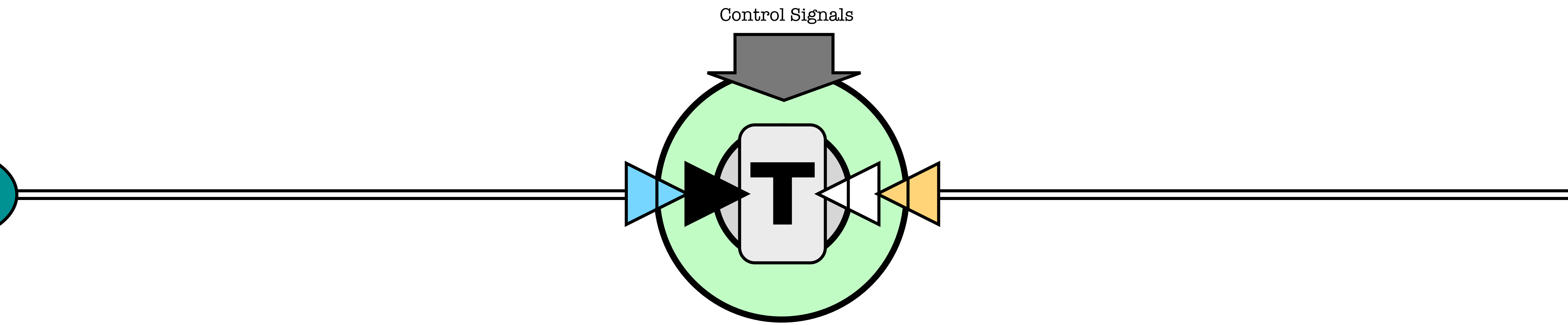
Bonnet et al., Science, 2013

XOR



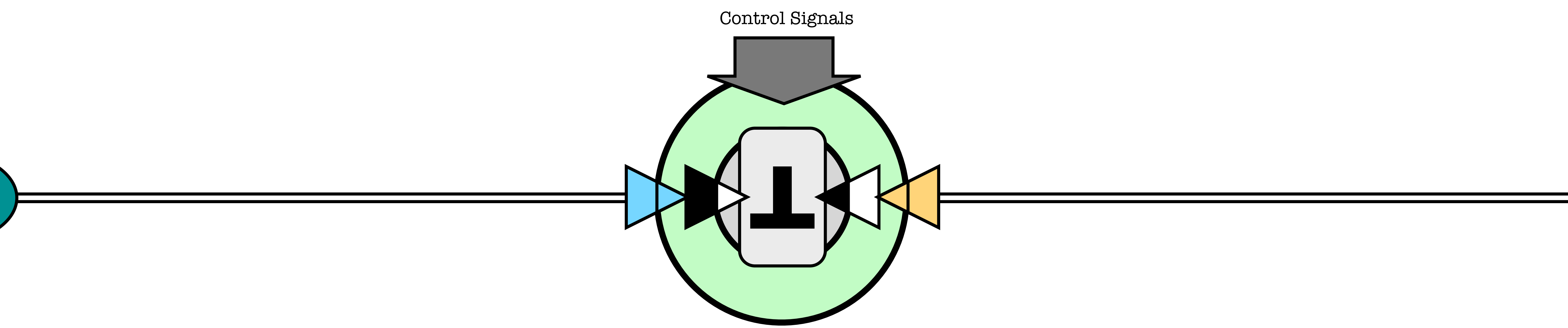
Bonnet et al., Science, 2013

XOR



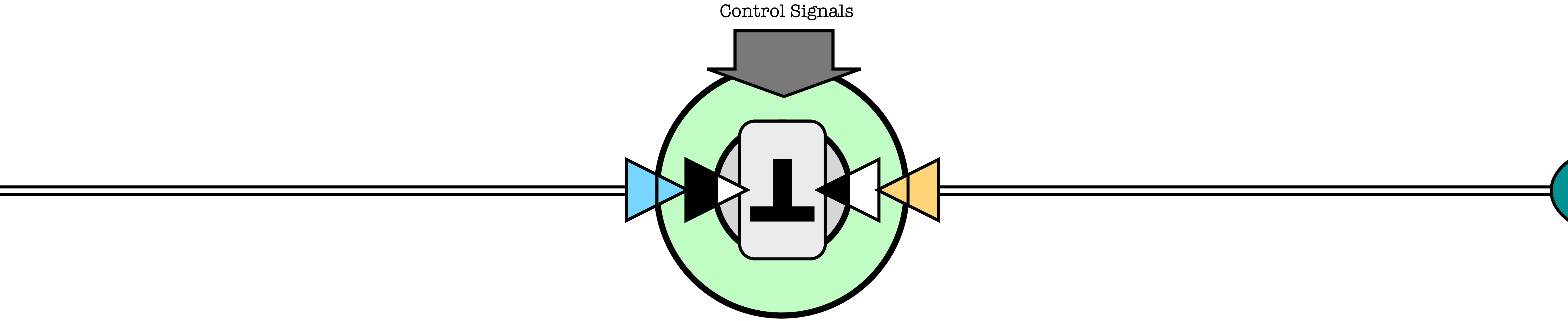
Bonnet et al., Science, 2013

XOR



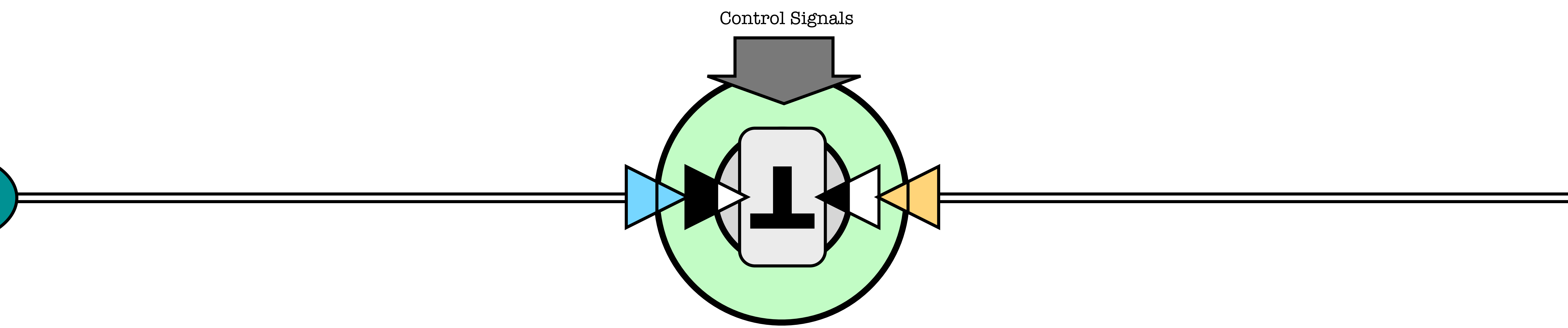
Bonnet et al., Science, 2013

XOR



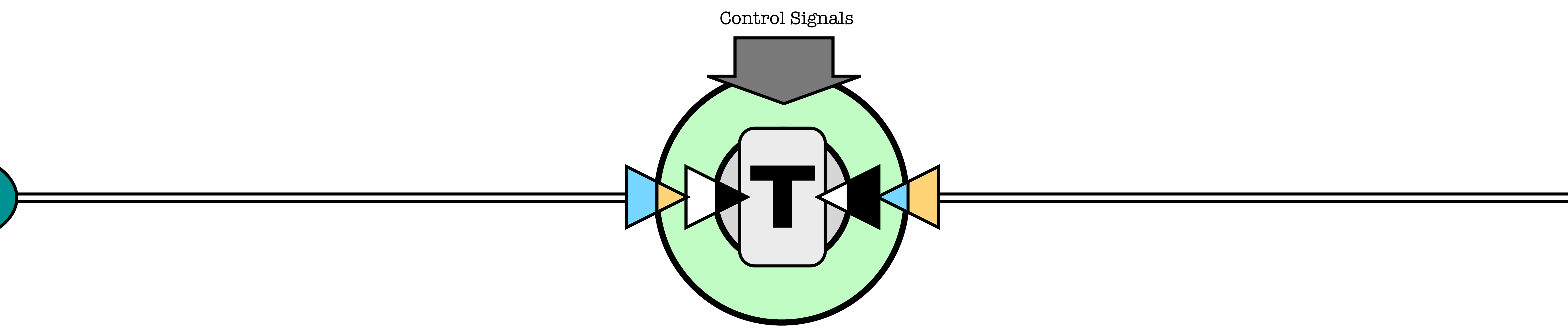
Bonnet et al., Science, 2013

XOR



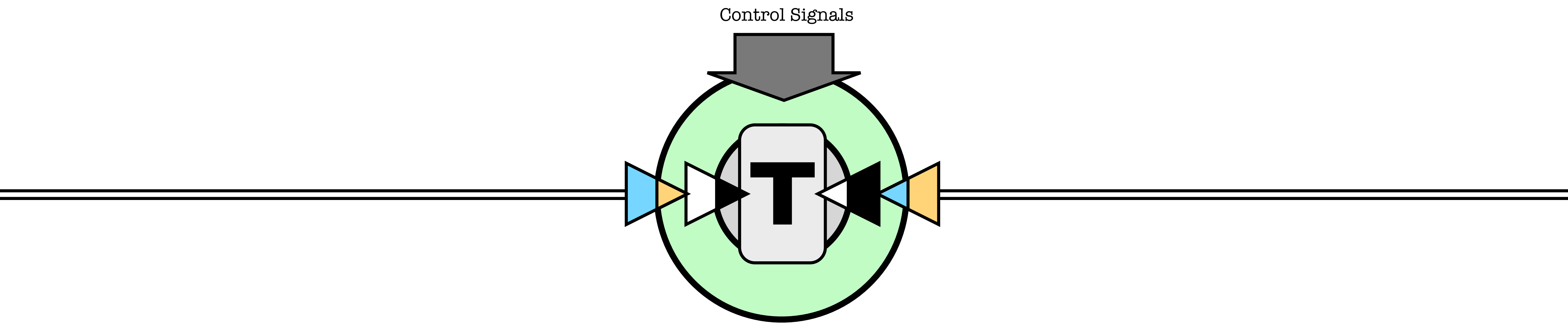
Bonnet et al., Science, 2013

XOR



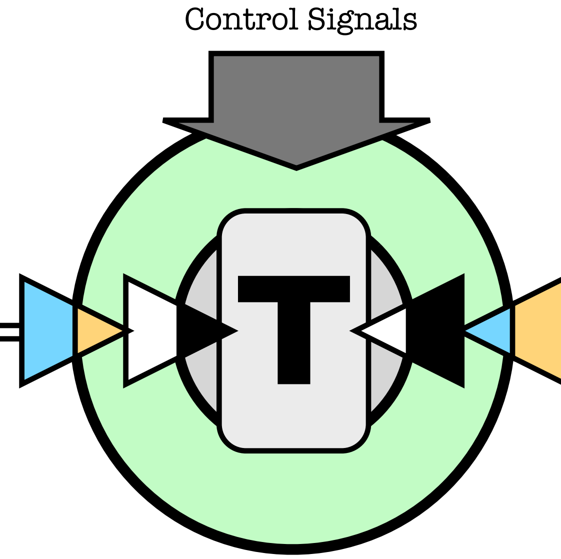
Bonnet et al., Science, 2013

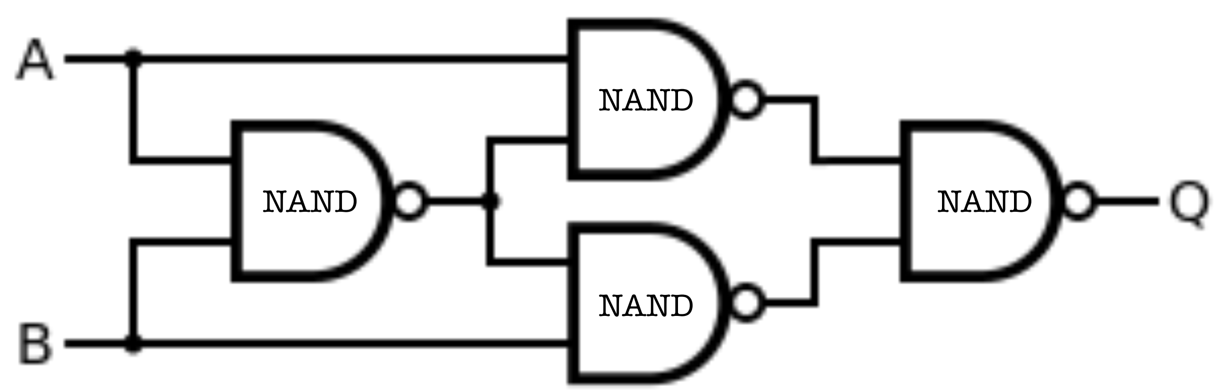
XOR



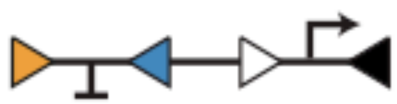
Bonnet et al., Science, 2013

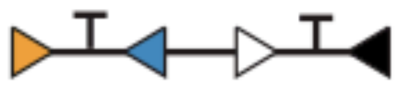
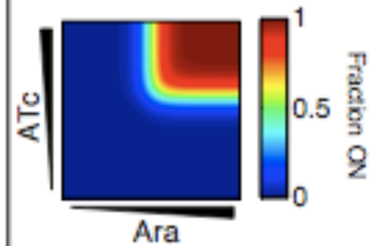
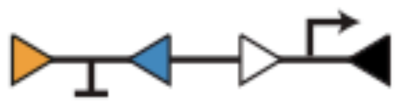
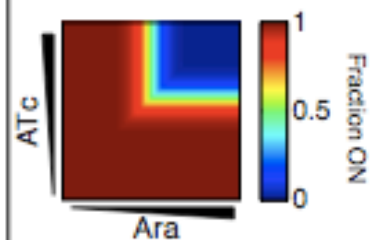
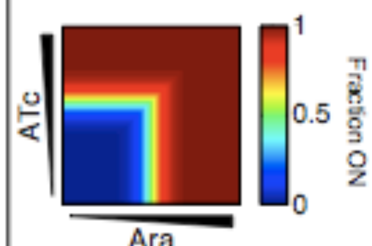
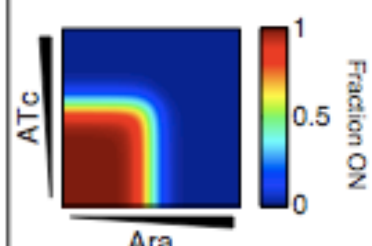
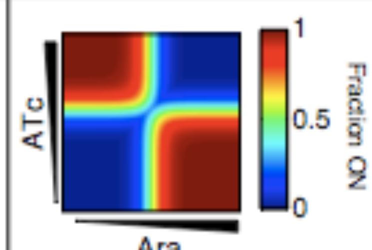
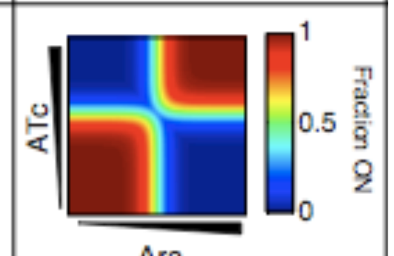
XOR



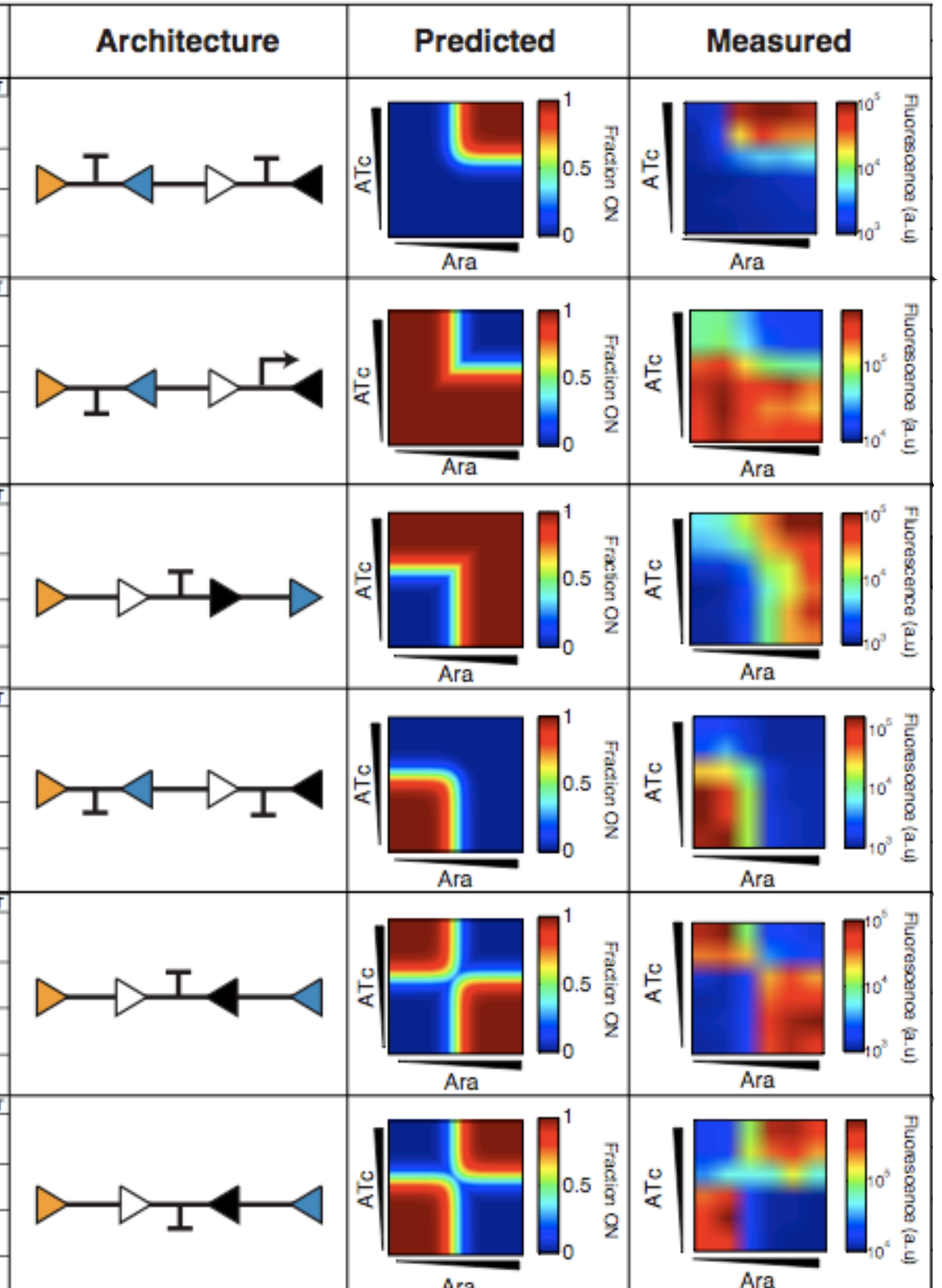


Bonnet et al., Science, 2013

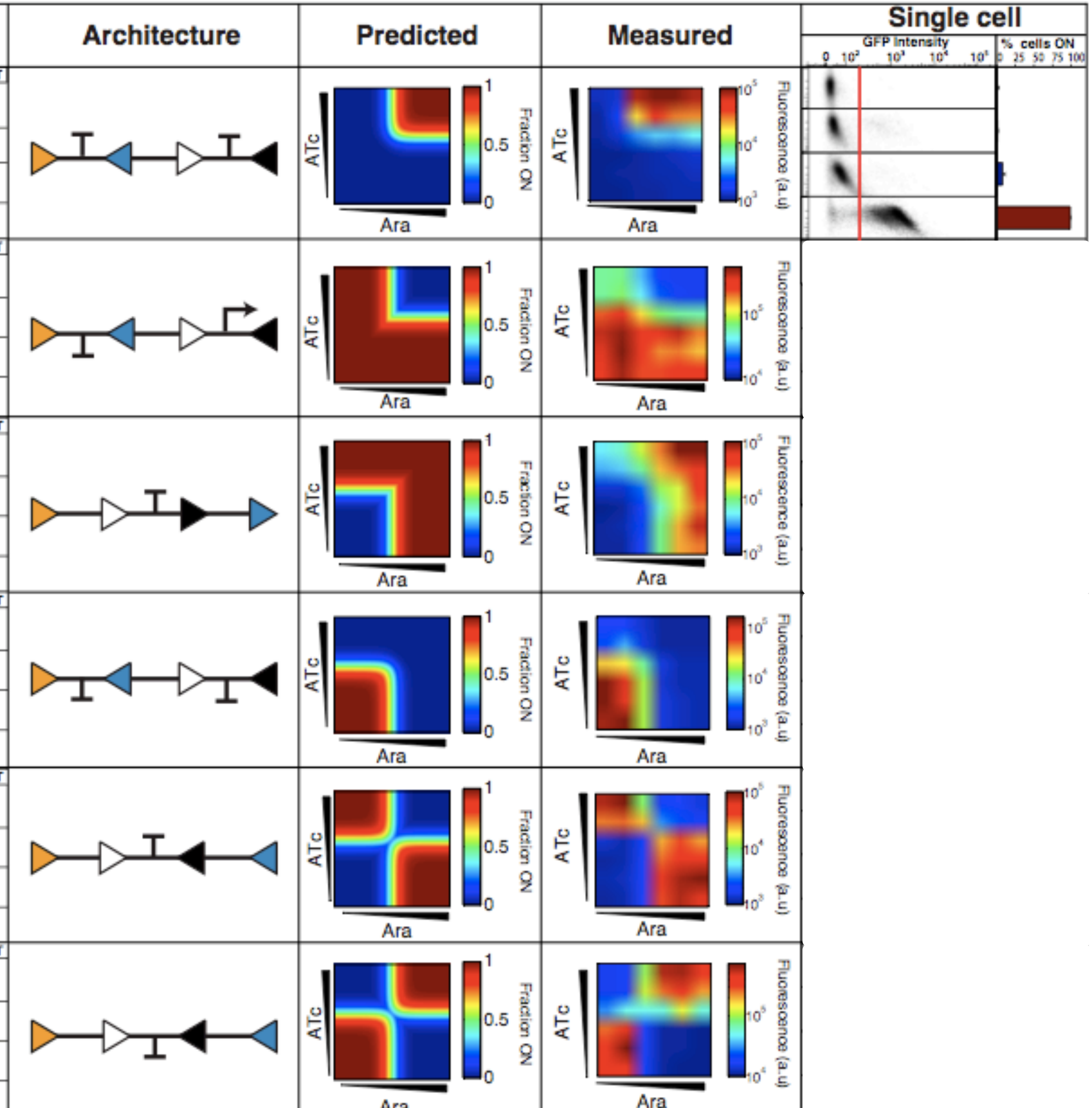
Gate	Truth Table ARA	ATC	OUT	Architecture
AND	0	0	0	
	1	0	0	
	0	1	0	
	1	1	1	
NAND	0	0	1	
	1	0	1	
	0	1	1	
	1	1	0	
OR	0	0	0	
	1	0	1	
	0	1	1	
	1	1	1	
NOR	0	0	1	
	1	0	0	
	0	1	0	
	1	1	0	
XOR	0	0	0	
	1	0	1	
	0	1	1	
	1	1	0	
XNOR	0	0	1	
	1	0	0	
	0	1	0	
	1	1	1	

Gate	Truth Table: ARA	ATC	OUT	Architecture	Predicted
AND	0	0	0		
	1	0	0		
	0	1	0		
	1	1	1		
NAND	0	0	1		
	1	0	1		
	0	1	1		
	1	1	0		
OR	0	0	0		
	1	0	1		
	0	1	1		
	1	1	1		
NOR	0	0	1		
	1	0	0		
	0	1	0		
	1	1	0		
XOR	0	0	0		
	1	0	1		
	0	1	1		
	1	1	0		
XNOR	0	0	1		
	1	0	0		
	0	1	0		
	1	1	1		

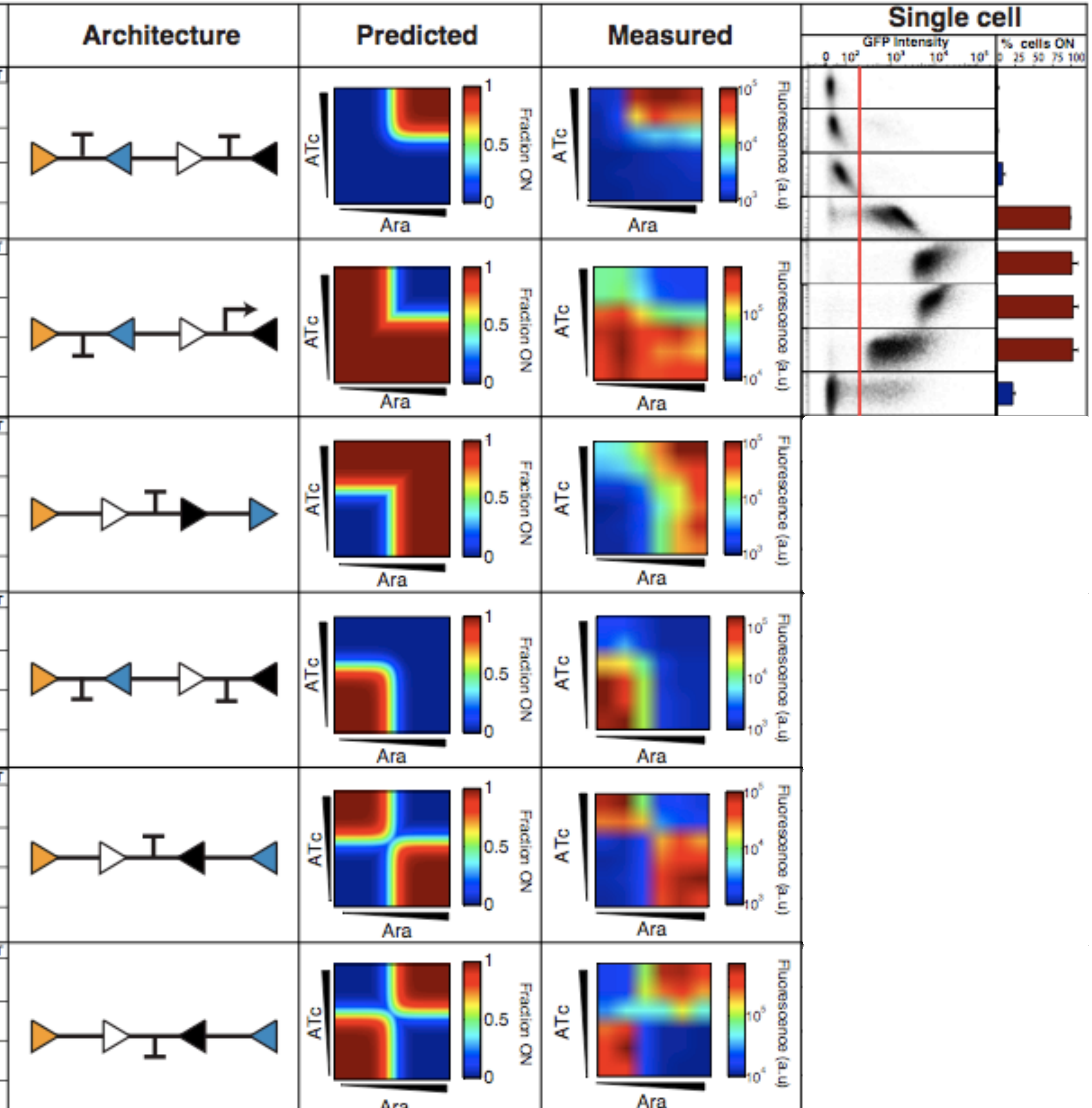
Gate	Truth Table ARA	ATC	OUT
AND	0	0	0
	1	0	0
	0	1	0
	1	1	1
NAND	0	0	1
	1	0	1
	0	1	1
	1	1	0
OR	0	0	0
	1	0	1
	0	1	1
	1	1	1
NOR	0	0	1
	1	0	0
	0	1	0
	1	1	0
XOR	0	0	0
	1	0	1
	0	1	1
	1	1	0
XNOR	0	0	1
	1	0	0
	0	1	0
	1	1	1

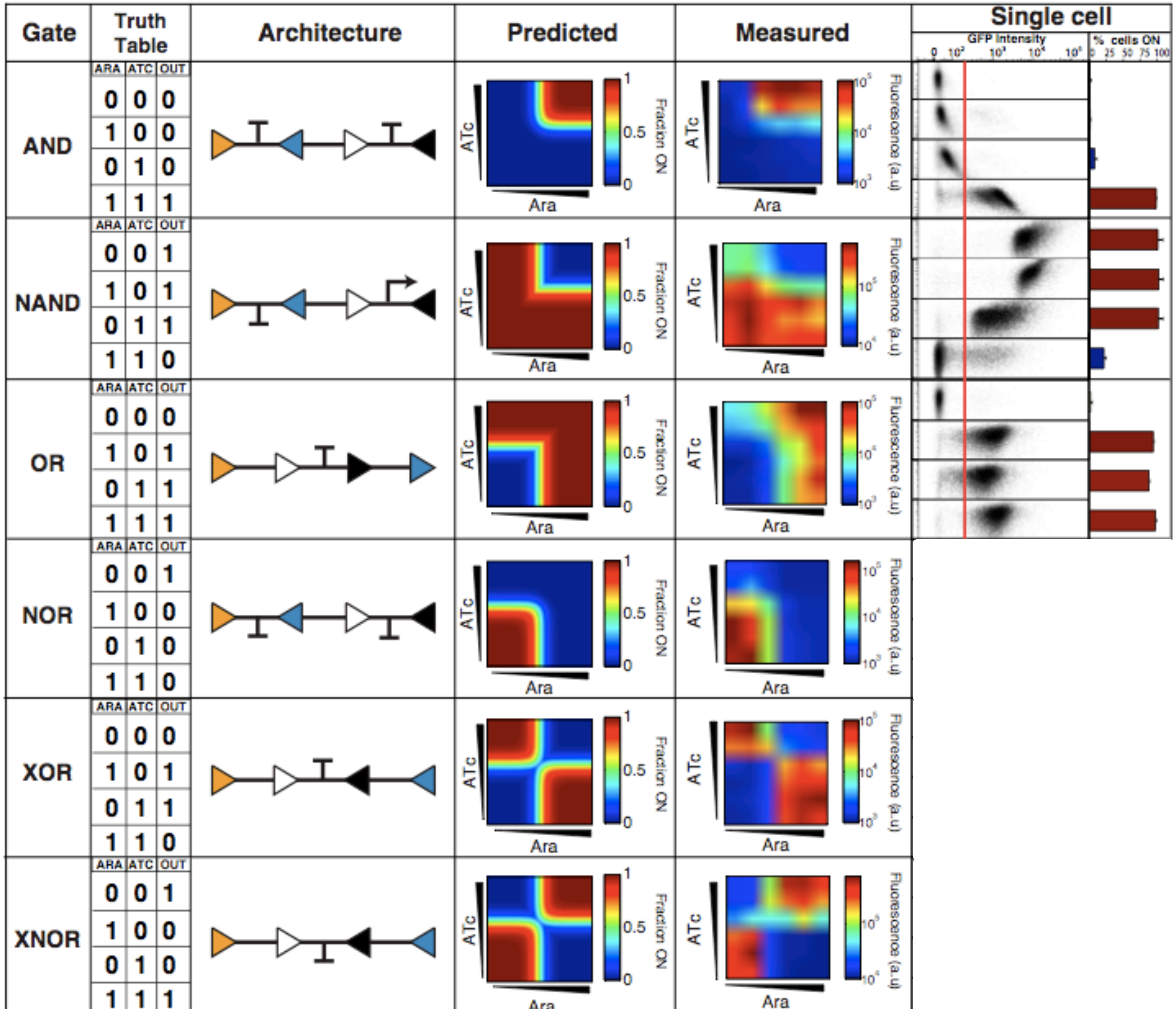


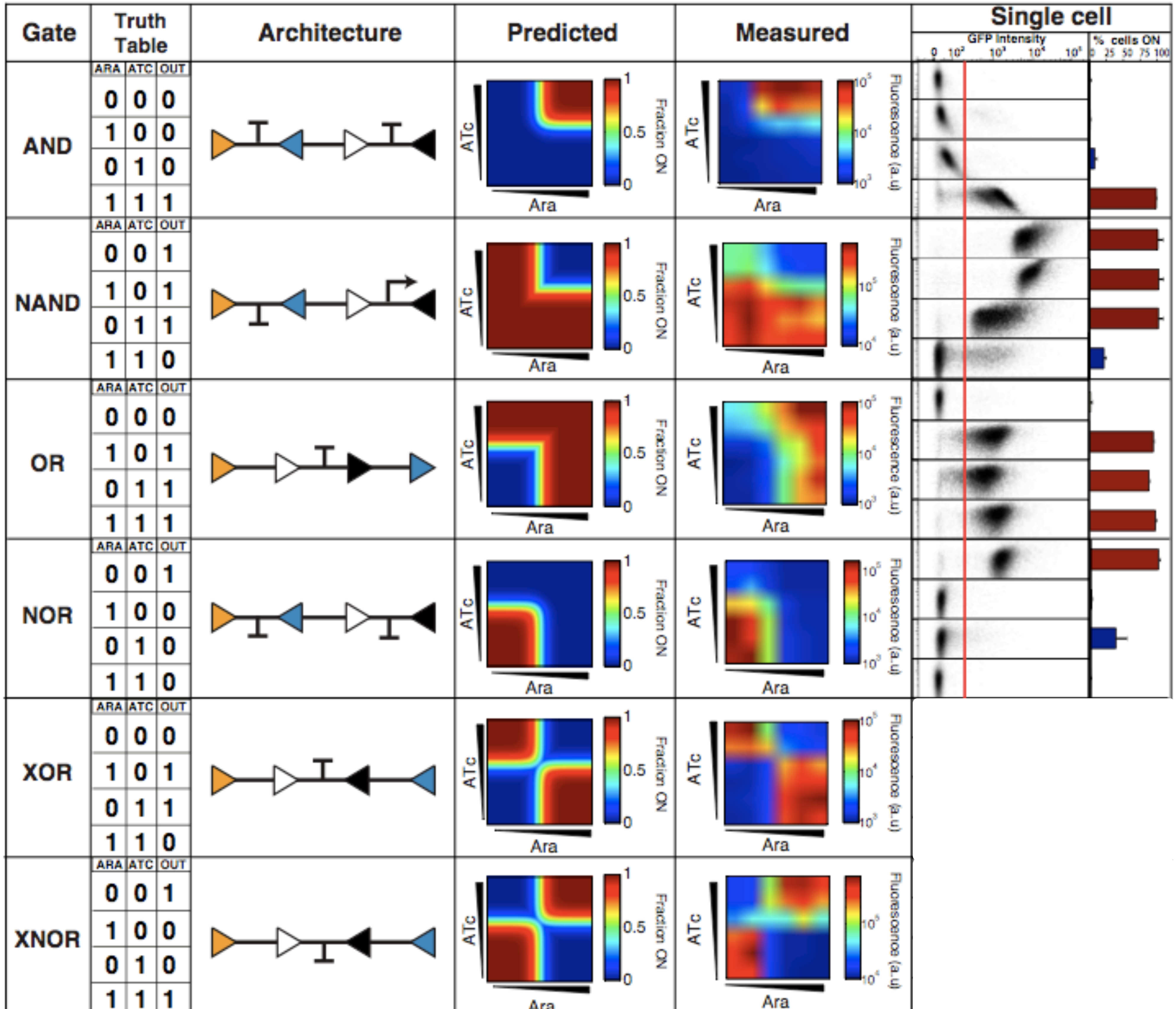
Gate	ARA	ATC	OUT
AND	0	0	0
	1	0	0
	0	1	0
	1	1	1
NAND	0	0	1
	1	0	1
	0	1	1
	1	1	0
OR	0	0	0
	1	0	1
	0	1	1
	1	1	1
NOR	0	0	1
	1	0	0
	0	1	0
	1	1	0
XOR	0	0	0
	1	0	1
	0	1	1
	1	1	0
XNOR	0	0	1
	1	0	0
	0	1	0
	1	1	1

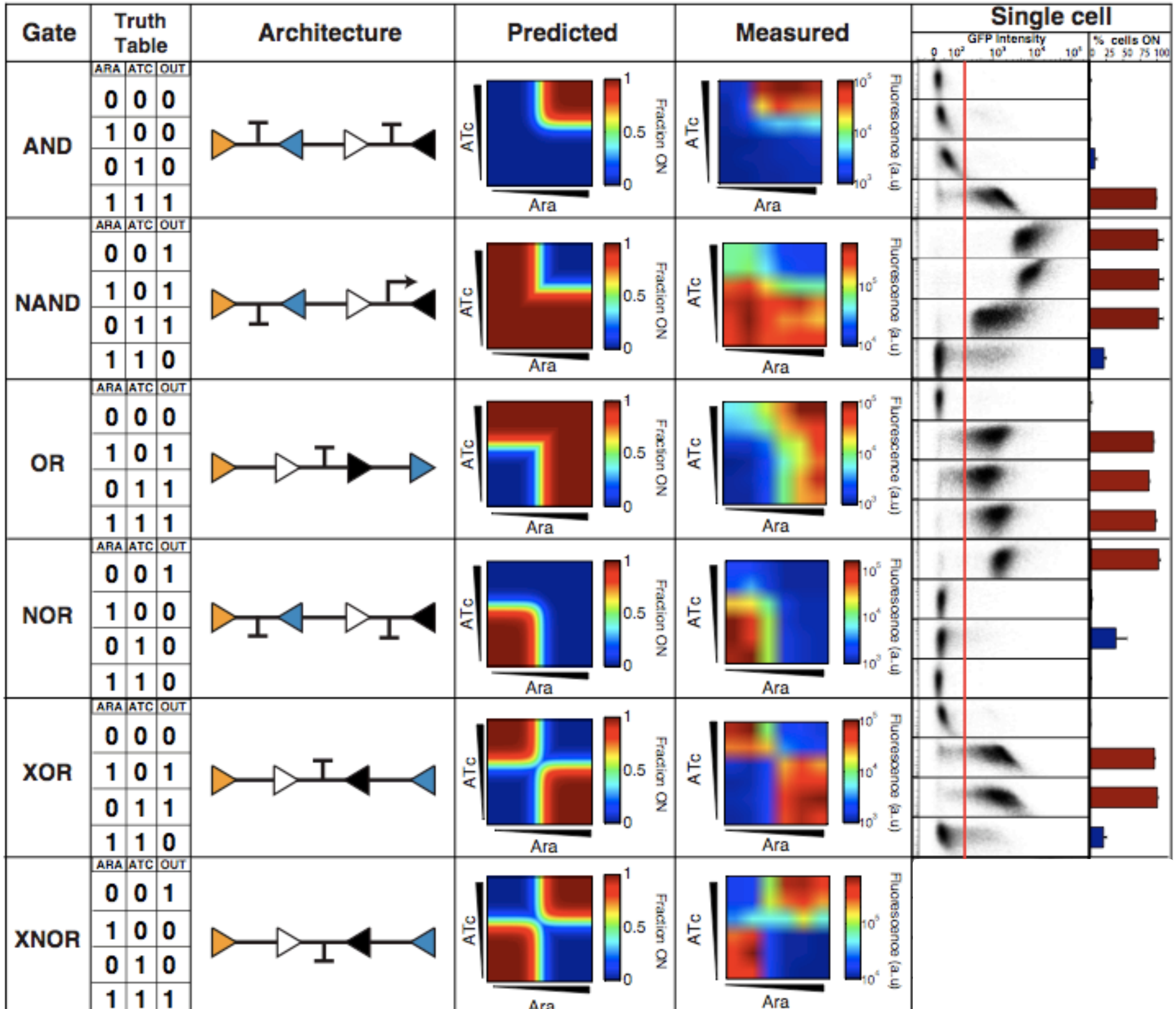


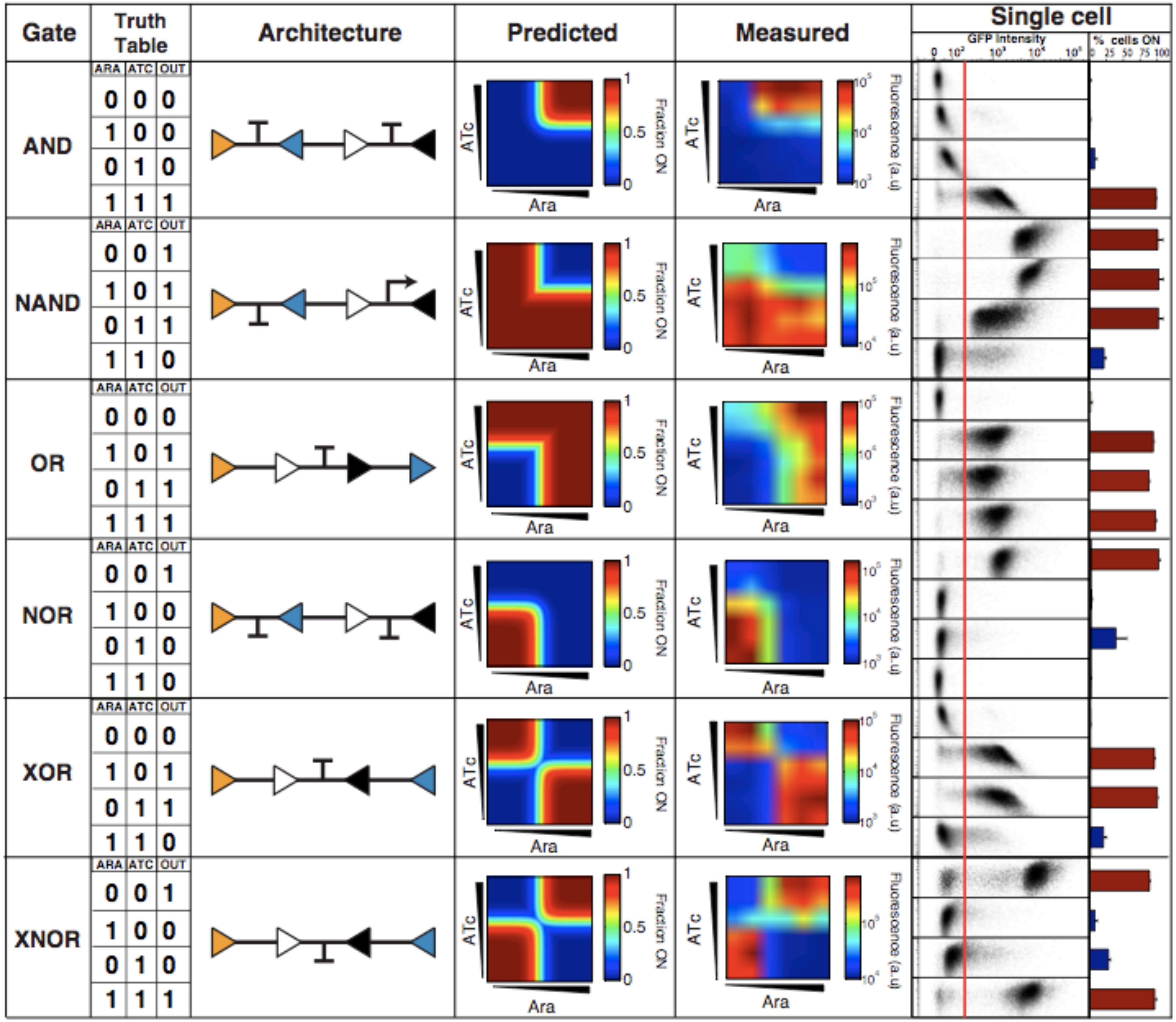
Gate	ARA	ATC	OUT
AND	0	0	0
	1	0	0
	0	1	0
	1	1	1
NAND	0	0	1
	1	0	1
	0	1	1
	1	1	0
OR	0	0	0
	1	0	1
	0	1	1
	1	1	1
NOR	0	0	1
	1	0	0
	0	1	0
	1	1	0
XOR	0	0	0
	1	0	1
	0	1	1
	1	1	0
XNOR	0	0	1
	1	0	0
	0	1	0
	1	1	1



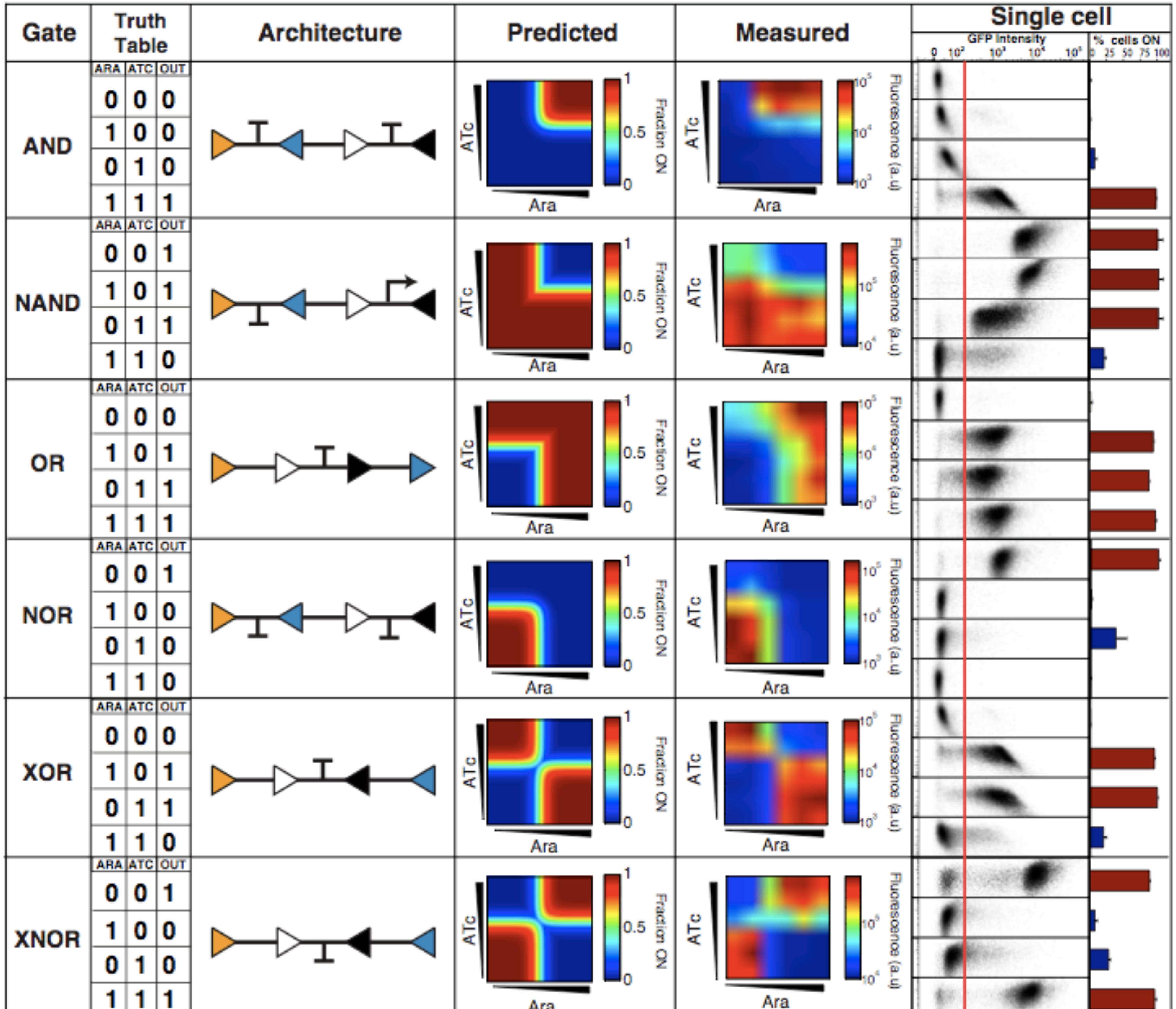

Gate
Truth Table
Architecture
Predicted
Measured
Single cell
GFP intensity
% cells ON
AND
ARA ATC OUT
0 0 0
1 0 0
0 1 0
1 1 1
NAND
ARA ATC OUT
0 0 1
1 0 1
0 1 1
1 1 0
OR
ARA ATC OUT
0 0 0
1 0 1
0 1 1
1 1 1
NOR
ARA ATC OUT
0 0 1
1 0 0
0 1 0
1 1 0
XOR
ARA ATC OUT
0 0 0
1 0 1
0 1 1
1 1 0
XNOR
ARA ATC OUT
0 0 1
1 0 0
0 1 0
1 1 1
ATc
Ara
Fraction ON
Fluorescence (a.u)


Gate
Truth Table
Architecture
Predicted
Measured
Single cell
GFP intensity
% cells ON
AND
NAND
OR
NOR
XOR
XNOR
ARA ATC OUT
AND: 0 0 0 / 1 0 0 / 0 1 0 / 1 1 1
NAND: 0 0 1 / 1 0 1 / 0 1 1 / 1 1 0
OR: 0 0 0 / 1 0 1 / 0 1 1 / 1 1 1
NOR: 0 0 1 / 1 0 0 / 0 1 0 / 1 1 0
XOR: 0 0 0 / 1 0 1 / 0 1 1 / 1 1 0
XNOR: 0 0 1 / 1 0 0 / 0 1 0 / 1 1 1
ATc
Ara
Fraction ON
Fluorescence (a.u)


Gate
Truth Table
Architecture
Predicted
Measured
Single cell
GFP intensity
% cells ON
AND
NAND
OR
NOR
XOR
XNOR
ARA ATC OUT
AND: 0 0 0 / 1 0 0 / 0 1 0 / 1 1 1
NAND: 0 0 1 / 1 0 1 / 0 1 1 / 1 1 0
OR: 0 0 0 / 1 0 1 / 0 1 1 / 1 1 1
NOR: 0 0 1 / 1 0 0 / 0 1 0 / 1 1 0
XOR: 0 0 0 / 1 0 1 / 0 1 1 / 1 1 0
XNOR: 0 0 1 / 1 0 0 / 0 1 0 / 1 1 1
ATc
Ara
Fraction ON
Fluorescence (a.u)


Gate
Truth Table
Architecture
Predicted
Measured
Single cell
GFP intensity
% cells ON
AND
NAND
OR
NOR
XOR
XNOR
ARA ATC OUT
AND: 0 0 0 / 1 0 0 / 0 1 0 / 1 1 1
NAND: 0 0 1 / 1 0 1 / 0 1 1 / 1 1 0
OR: 0 0 0 / 1 0 1 / 0 1 1 / 1 1 1
NOR: 0 0 1 / 1 0 0 / 0 1 0 / 1 1 0
XOR: 0 0 0 / 1 0 1 / 0 1 1 / 1 1 0
XNOR: 0 0 1 / 1 0 0 / 0 1 0 / 1 1 1
ATc
Ara
Fraction ON
Fluorescence (a.u)

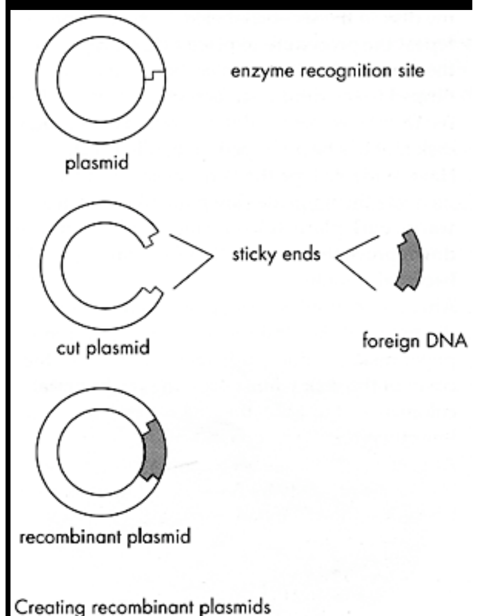
Boolean Integrase Logic (BIL) gates



From genetic engineering to synthetic biology

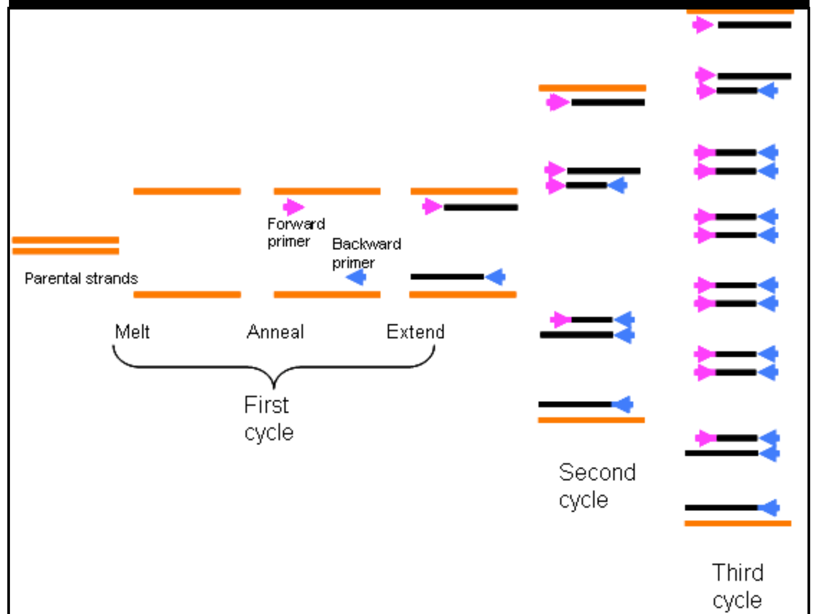
Genetic Engr. **=**

Recombinant DNA



Basic "Cut" & "Paste"

Polymerase Chain Reaction



Amplify & Make Simple Changes

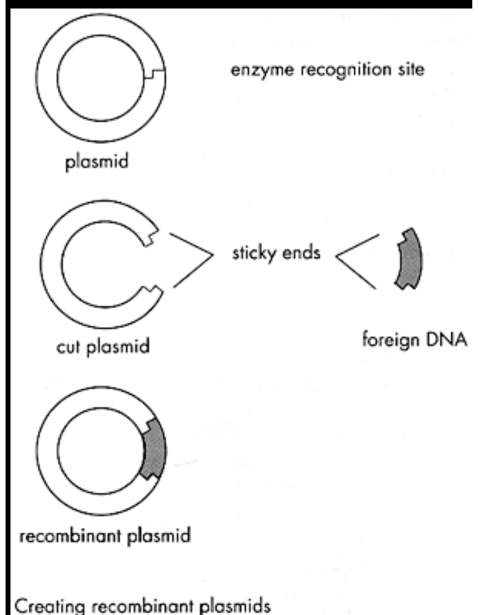
DNA Sequencing

Read Out the Genetic Code

From genetic engineering to synthetic biology

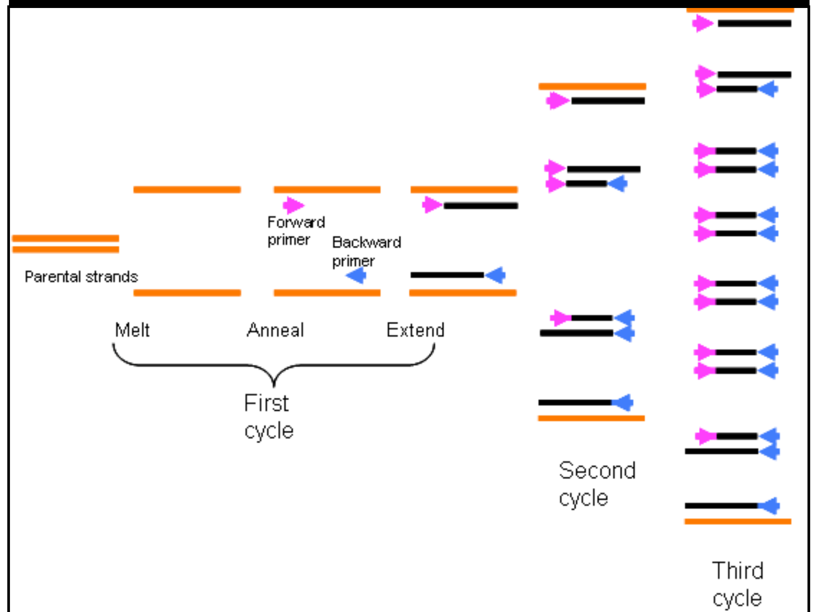
Genetic Engr. **=**

Recombinant DNA



Basic "Cut" & "Paste"

Polymerase Chain Reaction



Amplify & Make Simple Changes

DNA Sequencing

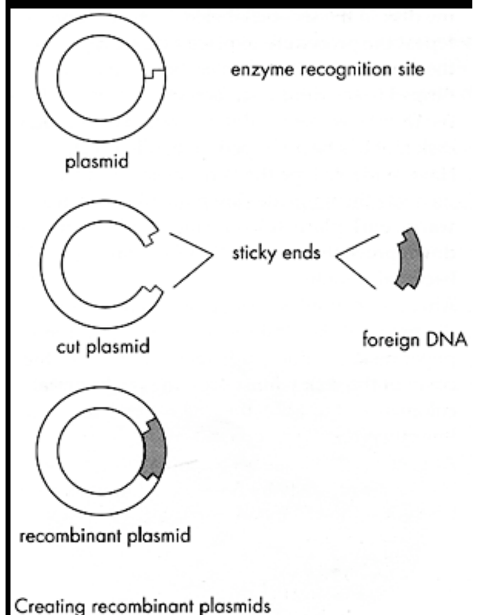
Read Out the Genetic Code

Synth. Biology **=**

From genetic engineering to synthetic biology

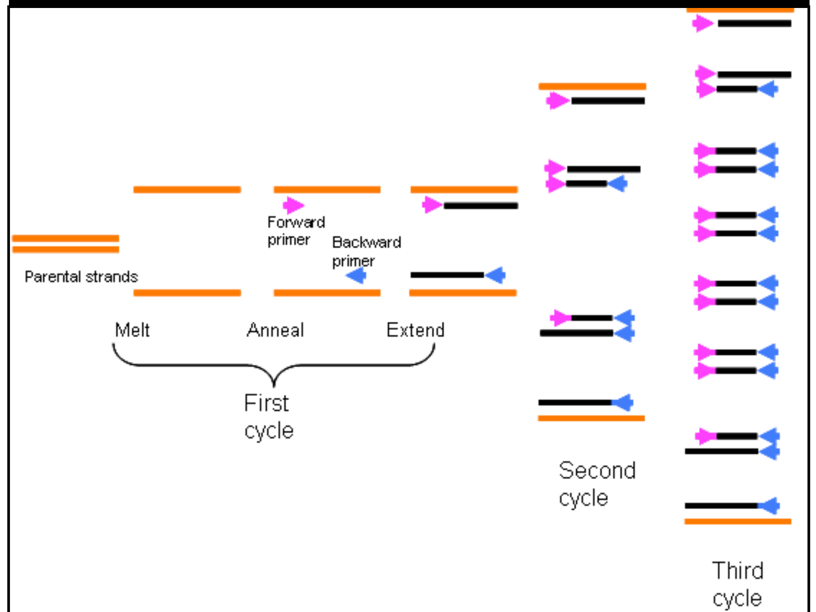
Genetic Engr. =

Recombinant DNA



Basic "Cut" & "Paste"

Polymerase Chain Reaction



Amplify & Make Simple Changes

DNA Sequencing

Read Out the Genetic Code

Synth. Biology =

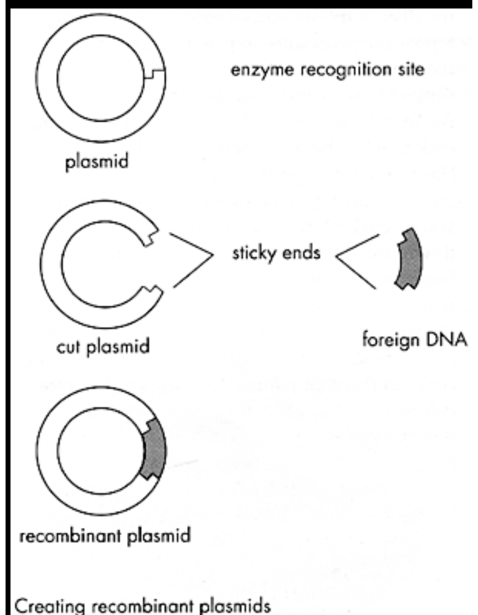
Synthesis

Decoupling of design & fabrication, leading to CAD and EDA.

From genetic engineering to synthetic biology

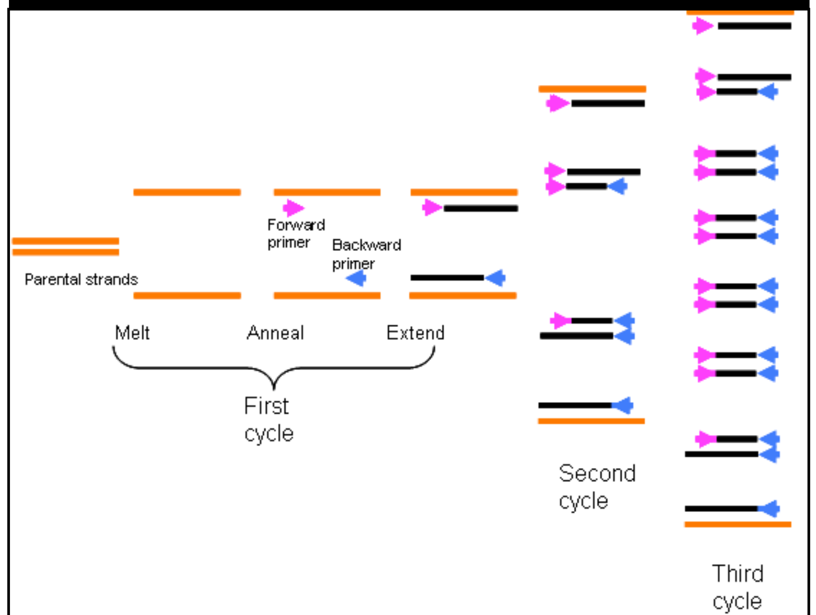
***Genetic Engr.* =**

Recombinant DNA



Basic "Cut" & "Paste"

Polymerase Chain Reaction



Amplify & Make Simple Changes

DNA Sequencing

Read Out the Genetic Code

***Synth. Biology* =**

Synthesis

Decoupling of design & fabrication, leading to CAD and EDA.

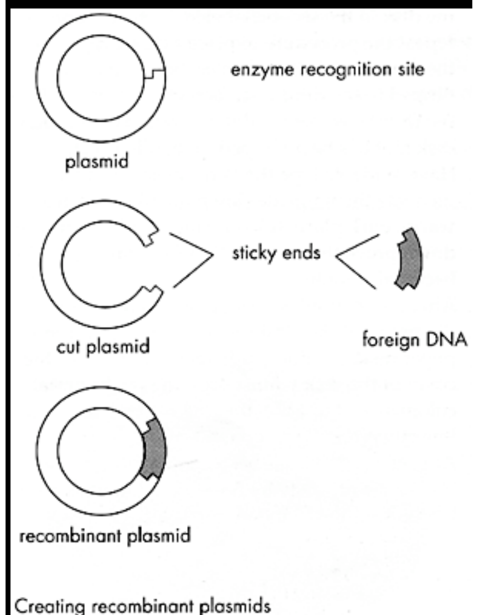
Abstraction

Engineered simplicity enabling many component systems.

From genetic engineering to synthetic biology

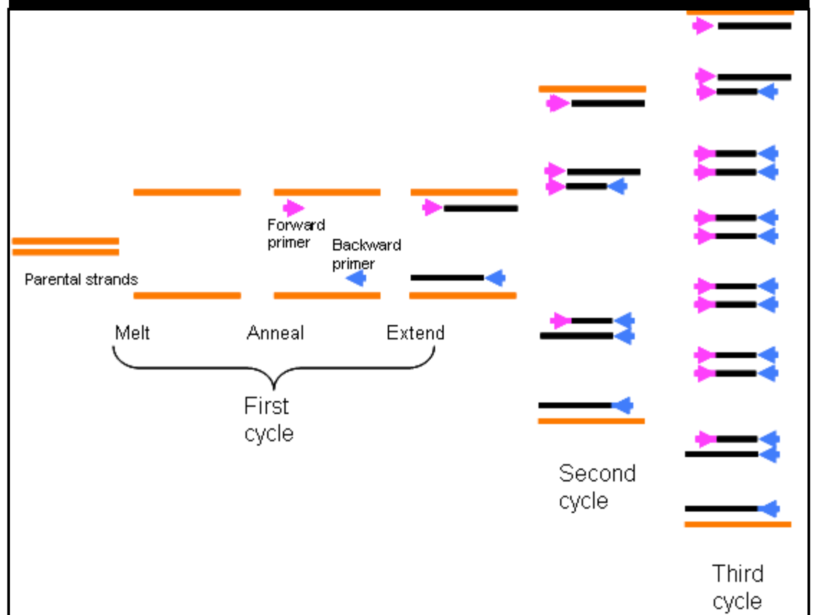
***Genetic Engr.* =**

Recombinant DNA



Basic "Cut" & "Paste"

Polymerase Chain Reaction



Amplify & Make Simple Changes

DNA Sequencing

Read Out the Genetic Code

***Synth. Biology* =**

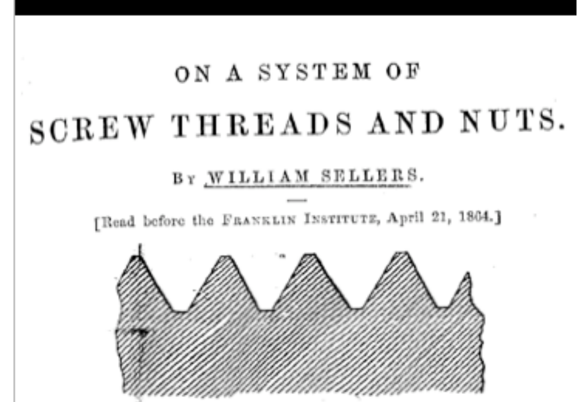
Synthesis

Decoupling of design & fabrication, leading to CAD and EDA.

Abstraction

Engineered simplicity enabling many component systems.

Standardization



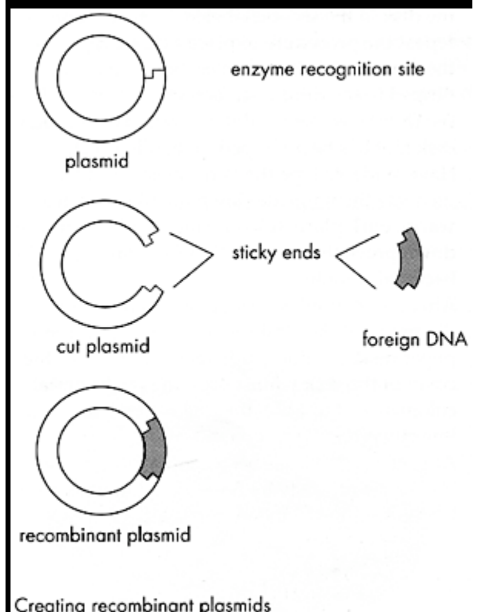
ON A SYSTEM OF
SCREW THREADS AND NUTS.
By WILLIAM SELLERS.
[Read before the Franklin Institute, April 21, 1864.]

Refined genetic components supporting "off the shelf" reuse.

From genetic engineering to synthetic biology

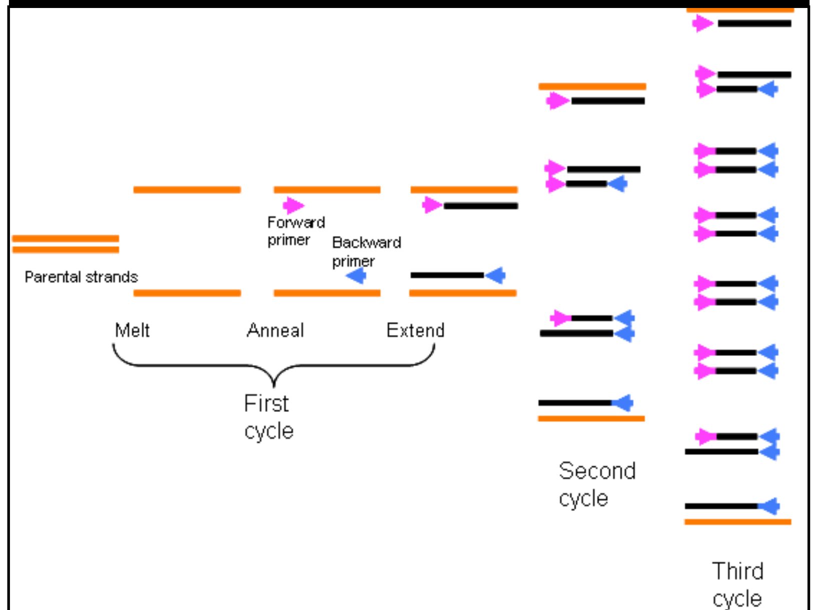
***Genetic Engr.* =**

Recombinant DNA



Basic “Cut” & “Paste”

Polymerase Chain Reaction



Amplify & Make Simple Changes

DNA Sequencing

Read Out the Genetic Code

***Synth. Biology* =**

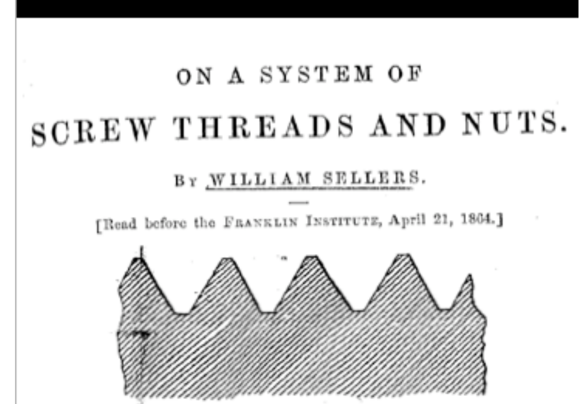
Synthesis

Decoupling of design & fabrication, leading to CAD and EDA.

Abstraction

Engineered simplicity enabling many component systems.

Standardization


ON A SYSTEM OF
SCREW THREADS AND NUTS.
By WILLIAM SELLERS.
[Read before the Franklin Institute, April 21, 1864.]

Refined genetic components supporting “off the shelf” reuse.

…