Complex synthetic biology: Logic to the Moon (and back)

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January 2019

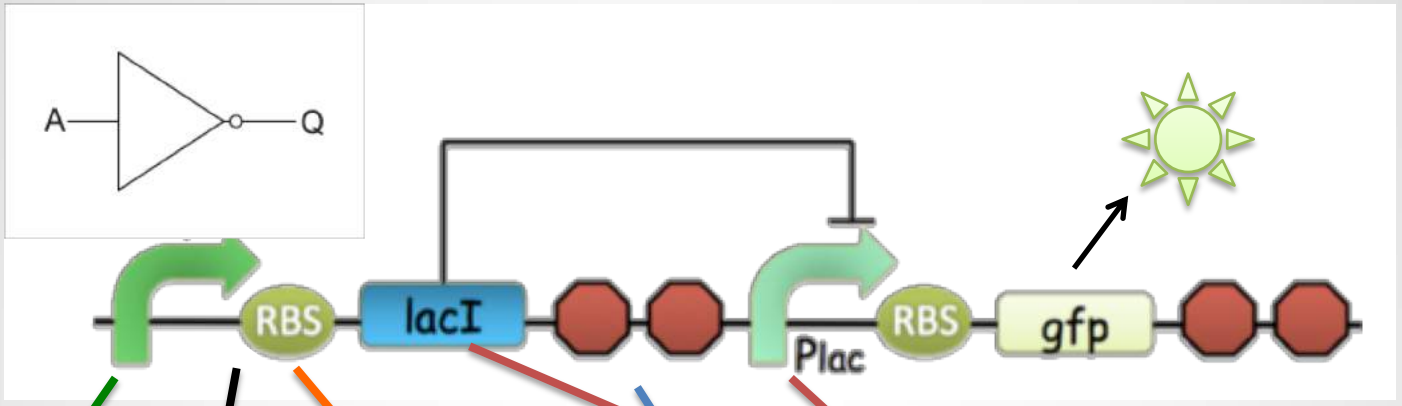
Lecture Content

- Bacterial Logic Gates from Promoters
- Circuitry that is exclusively NOT/NOR gates
- How NOT gates become NOR gates
- Cello – a Verilog for Synthetic Biology
- How Cello works and can be implemented
- Logic with the dCas9 customisable transcription factor system
- Parts performing logic for less cost to the cell

Learning Objectives

- To explain logic functions in synthetic biology
- To understand how all logic is possible with NOR gates
- To describe efforts towards predictable design
- To show how design is now automatable
- To demonstrate progress of the field towards large complex networks built with professional parts

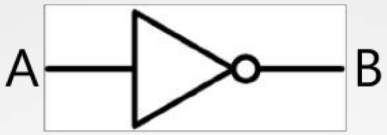
Inverter Network



- 100+ constitutive promoters
- RiboJ part or Csy4 sites to remove 5' context
- RBS custom design using RBS Calculator
- BCD method gives 50+ modular RBS parts
- 16 TetR-related TF-promoter pairs\
- ZF-TFs/ TALES in yeast
- 10000+ dCas9 options
- 600+ measured terminators
- Two terminator design models

Standard Logic Gates

NOT



A	B
1	0
0	1

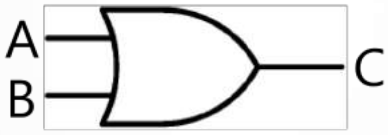
AND

A	B	C
1	1	1
1	0	0
0	1	0
0	0	0

NAND

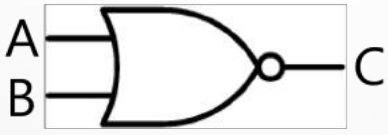
A	B	C
1	1	0
1	0	1
0	1	1
0	0	1

OR



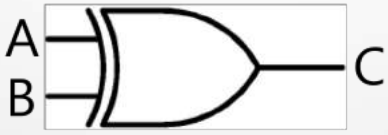
A	B	C
1	1	1
1	0	1
0	1	1
0	0	0

NOR



A	B	C
1	1	0
1	0	0
0	1	0
0	0	1

XOR

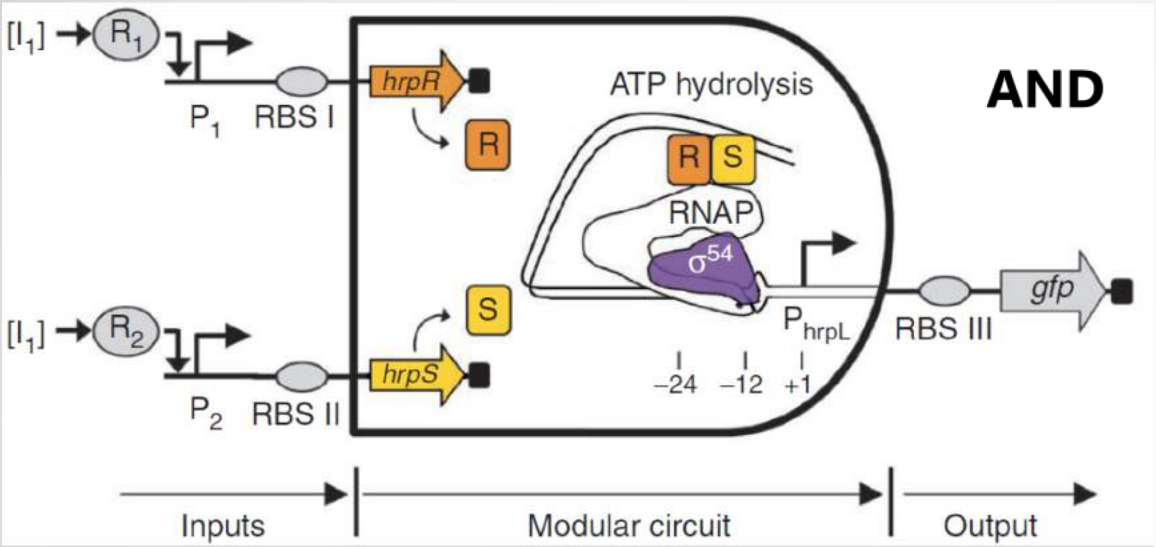


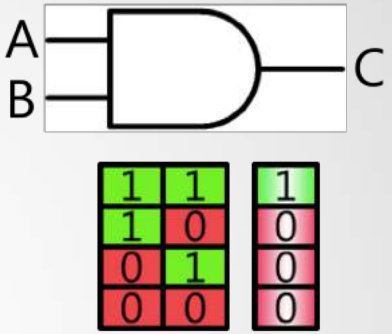
A	B	C
1	1	0
1	0	1
0	1	1
0	0	0

XNOR

A	B	C
1	1	1
1	0	0
0	1	0
0	0	1

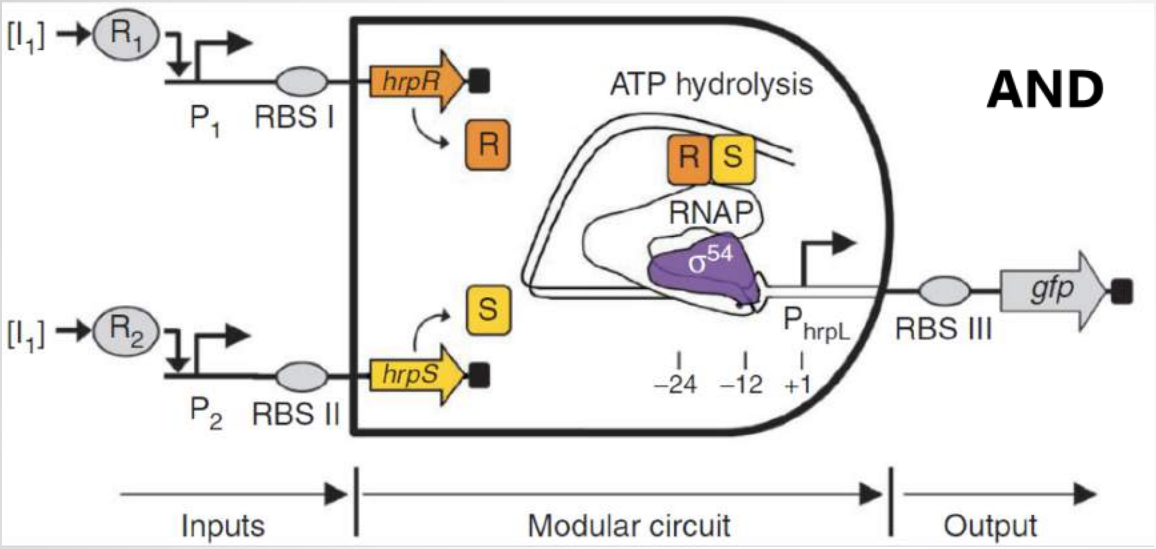
Bacterial Logic Gates - Examples

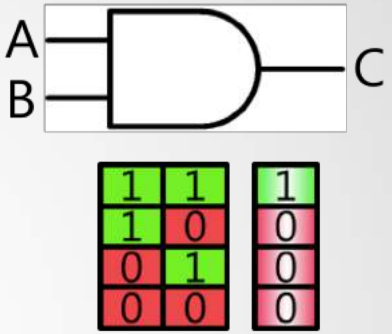


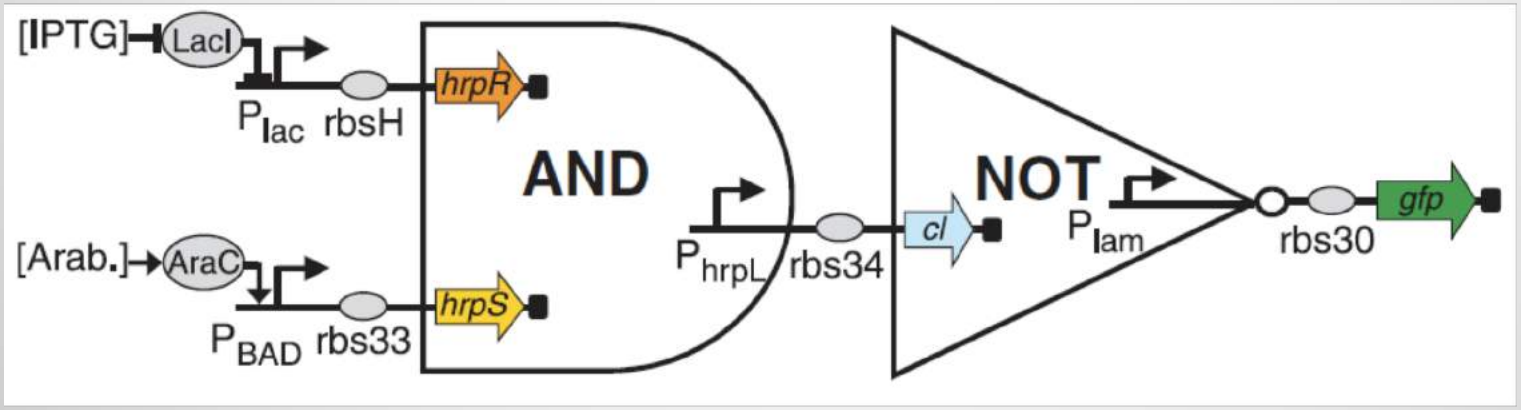


Wang *et al*. Nature Communications 2014

Bacterial Logic Gates - Examples

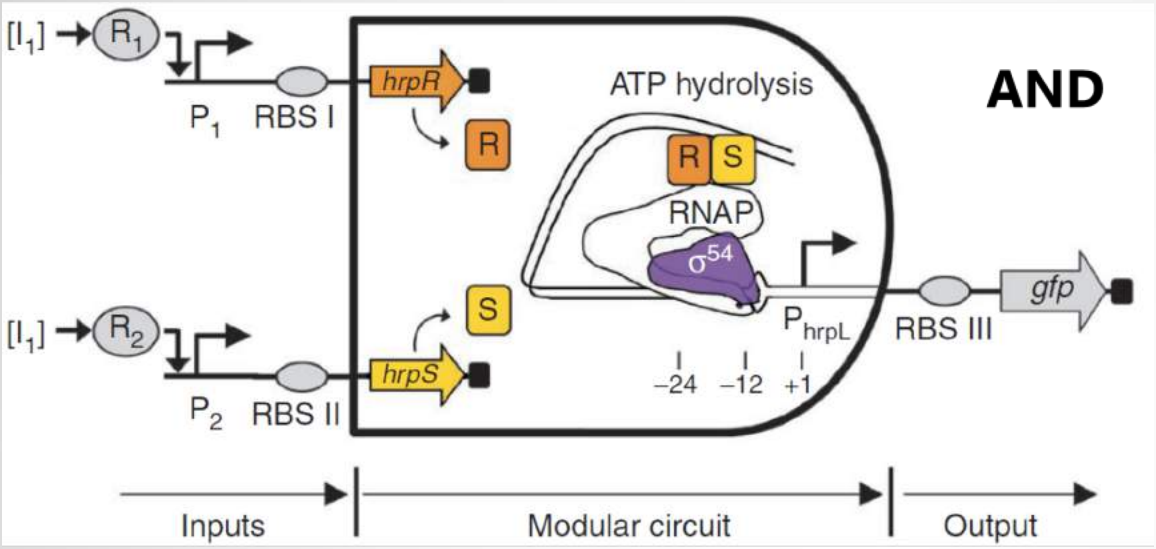


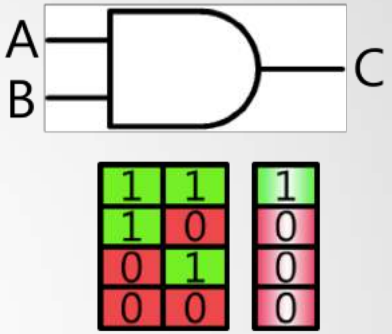


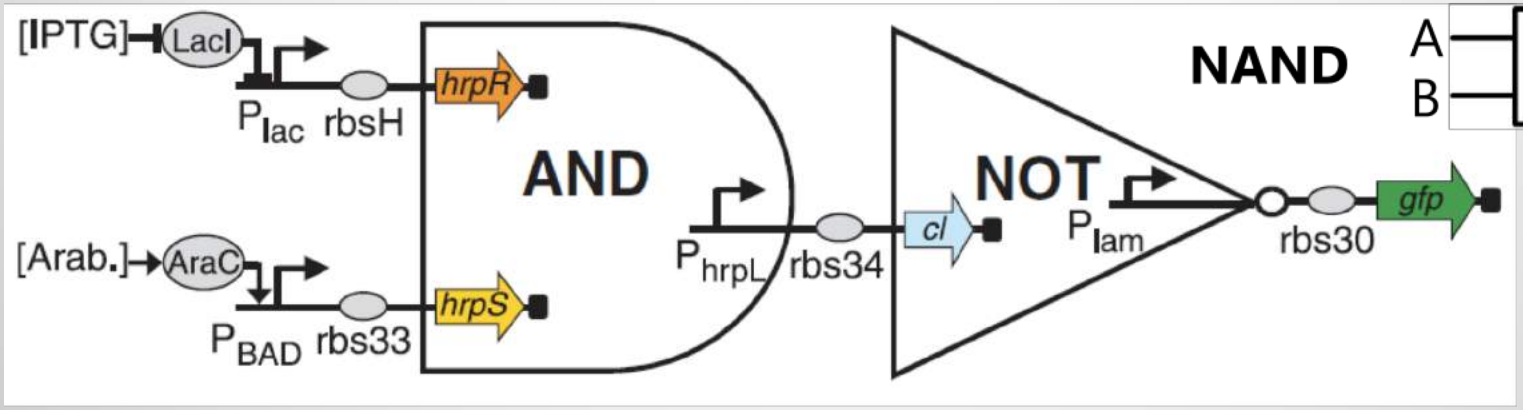


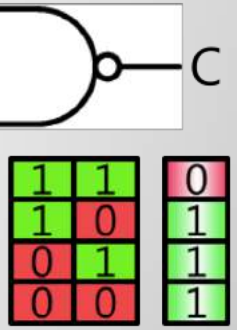
Wang *et al*. Nature Communications 2014

Bacterial Logic Gates - Examples



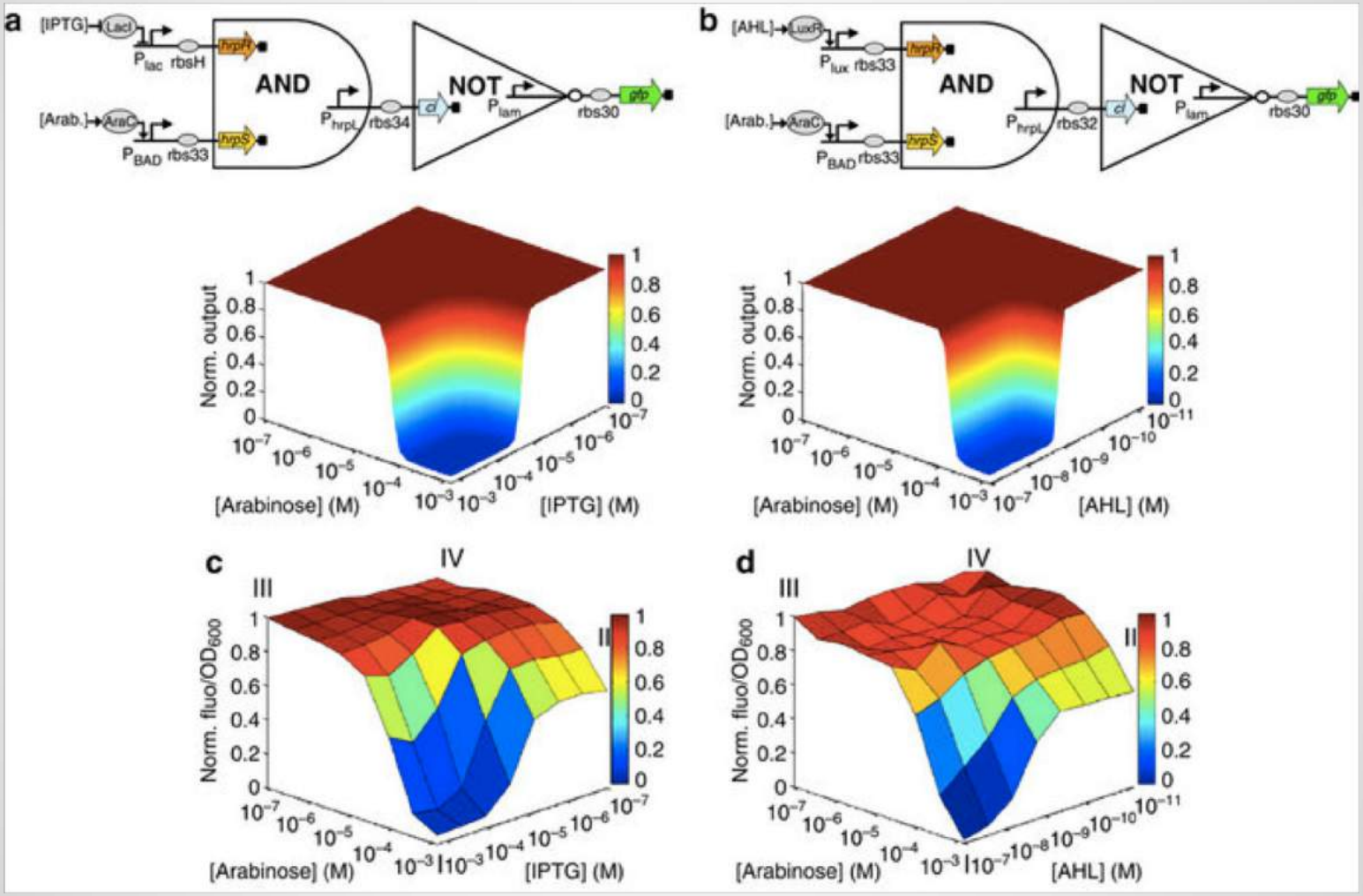






Wang *et al*. Nature Communications 2014

Bacterial Logic Gates - Examples

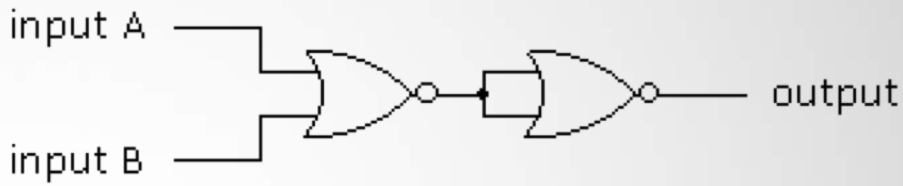


Wang *et al*. Nature Communications 2014

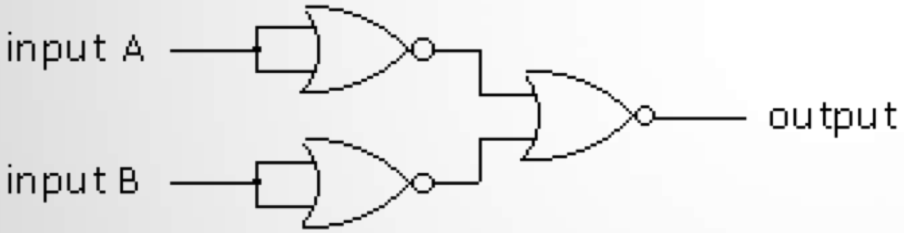
Universality of NOR gates

NOT gate (inputs joined together)

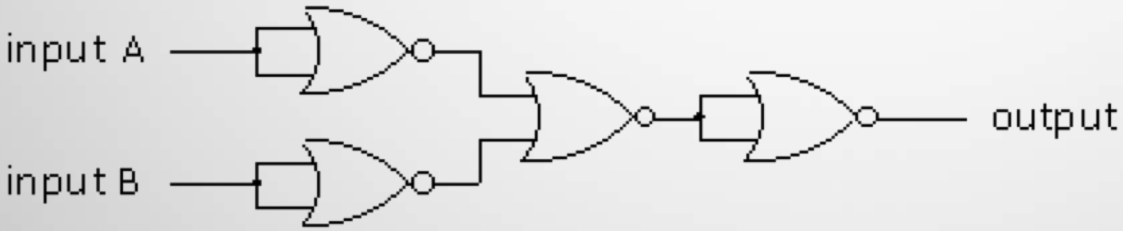
OR gate (NOR followed by NOT)



AND gate (NOT of each input followed by NOR)

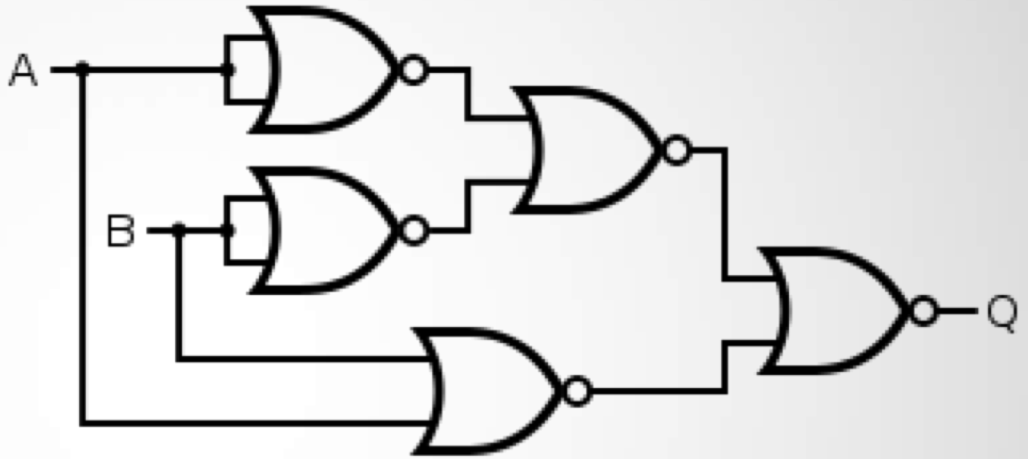


NAND gate (AND followed by NOT)

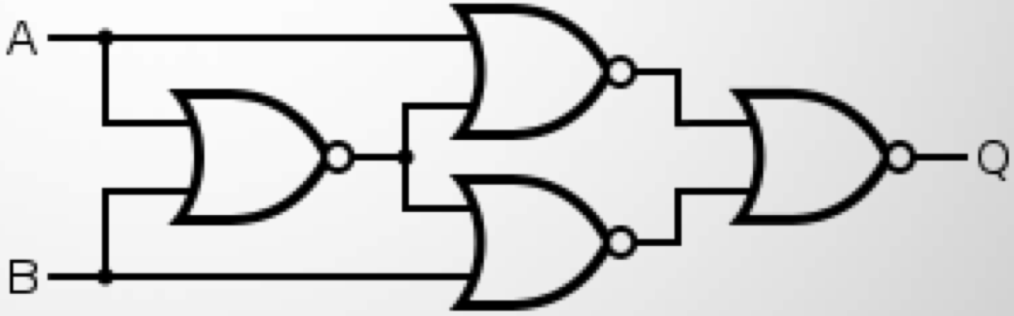


Universality of NOR gates

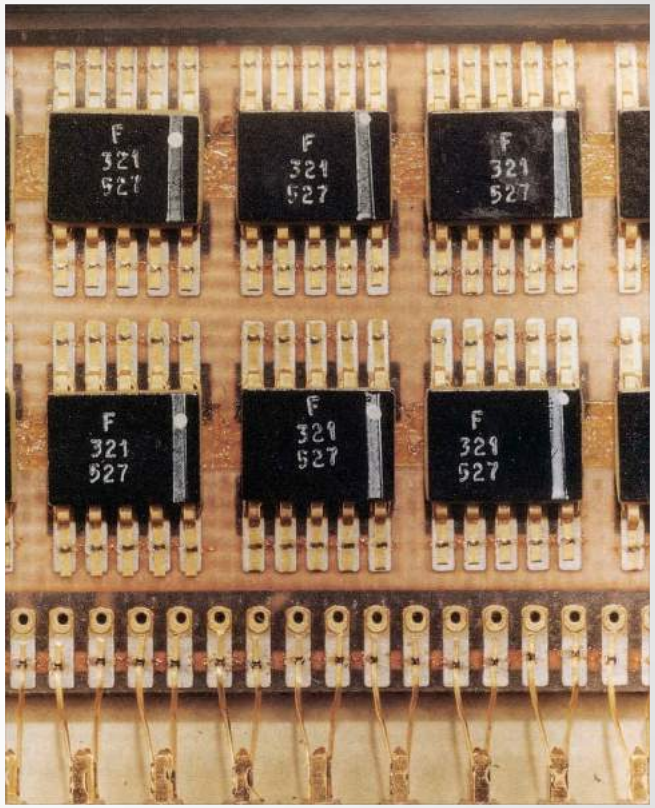
XOR



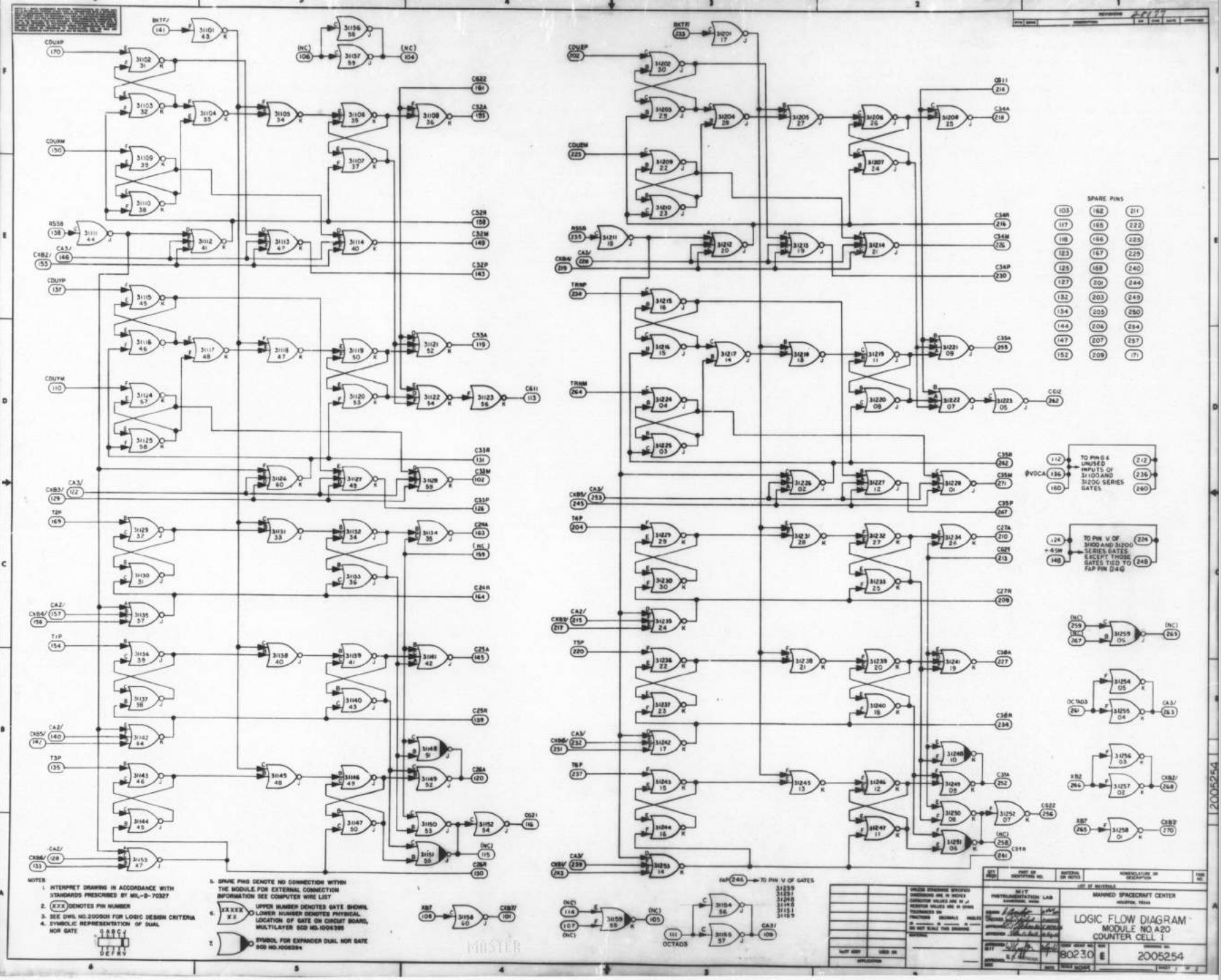
XNOR



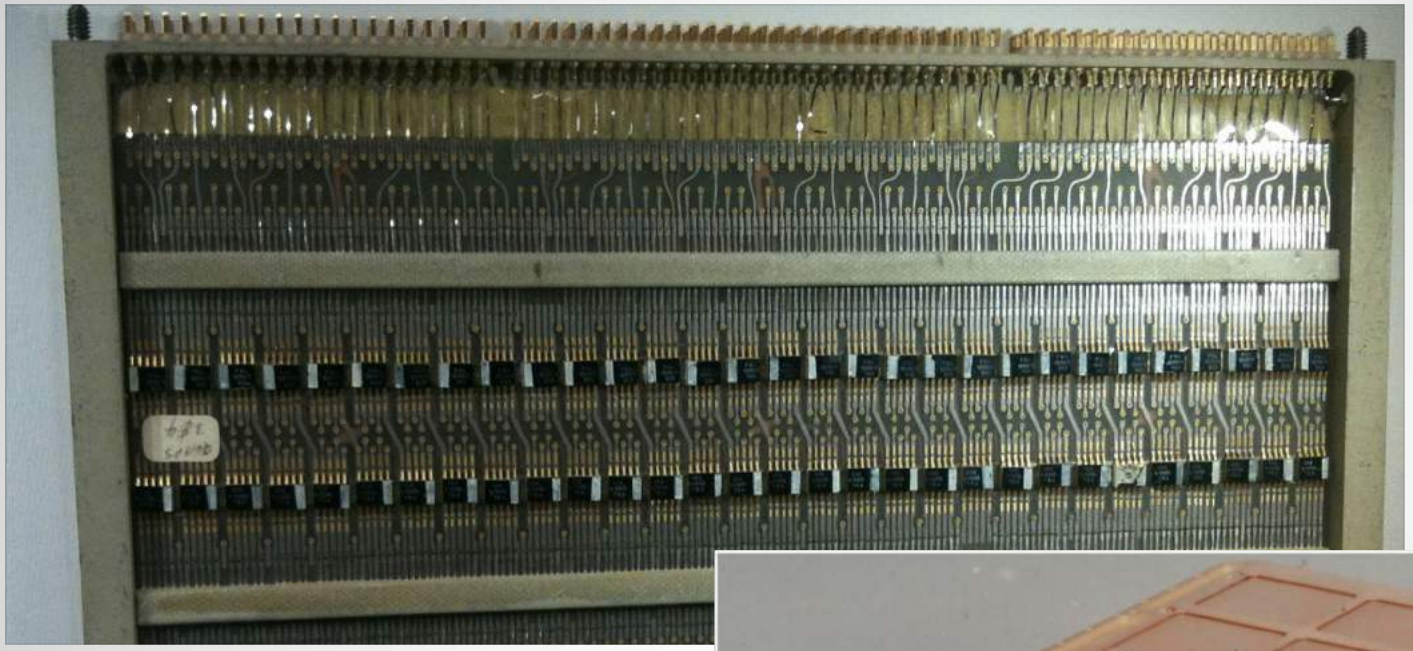
NOT/NOR-gate exclusive circuitry



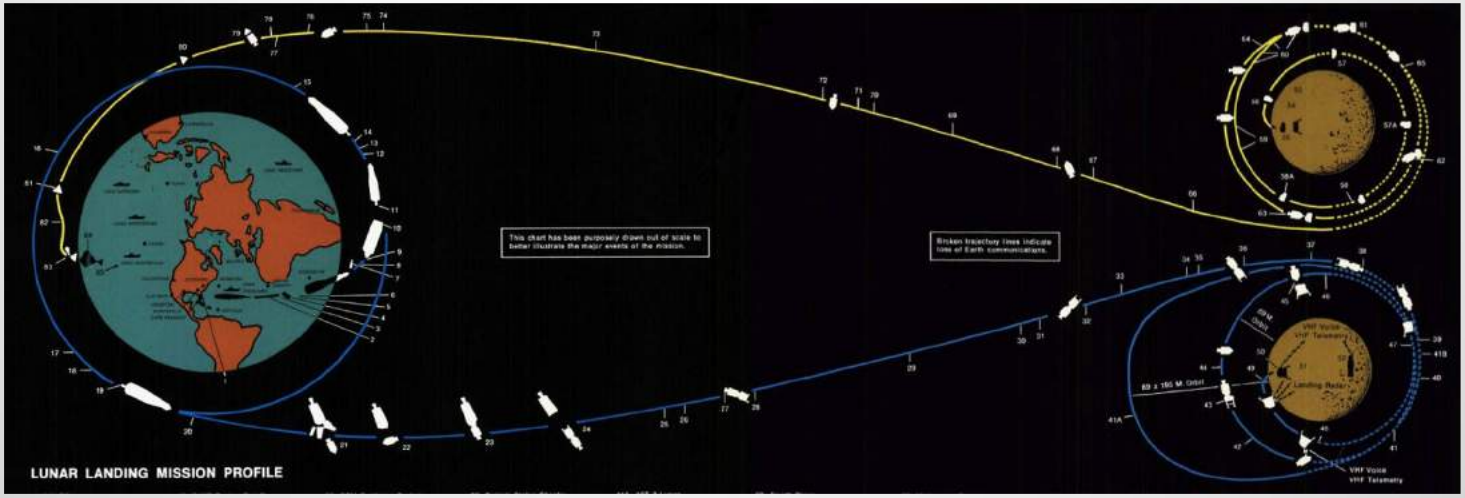
DSKY and AGC on display at the Computer History Museum (Wikipedia)


SPARE PINS
TO PIN 6 UNUSED INPUTS OF 31100 AND 31200 SERIES GATES
TO PIN V OF 31100 AND 31200 SERIES GATES EXCEPT THOSE GATES TIED TO FAP PIN (246)
NOTES
1. INTERPRET DRAWING IN ACCORDANCE WITH STANDARDS PRESCRIBED BY MIL-D-70327
2. (XXX) DENOTES PIN NUMBER
3. SEE DWG. NO. 2005091 FOR LOGIC DESIGN CRITERIA
4. SYMBOLIC REPRESENTATION OF DUAL NOR GATE
5. SPARE PINS DENOTE NO CONNECTION WITHIN THE MODULE FOR EXTERNAL CONNECTION INFORMATION SEE COMPUTER WIRE LIST
6. XX XXX UPPER NUMBER DENOTES GATE SHOWN LOWER NUMBER DENOTES PHYSICAL LOCATION OF GATE ON CIRCUIT BOARD, MULTILAYER SCD NO. 1006395
SYMBOL FOR EXPANDER DUAL NOR GATE SCD NO. 1006594
MIT INSTRUMENTATION LAB
MANNED SPACECRAFT CENTER
LOGIC FLOW DIAGRAM MODULE NO. A20 COUNTER CELL 1
80230 E 2005254
2005254

NOT/NOR-gate exclusive circuitry

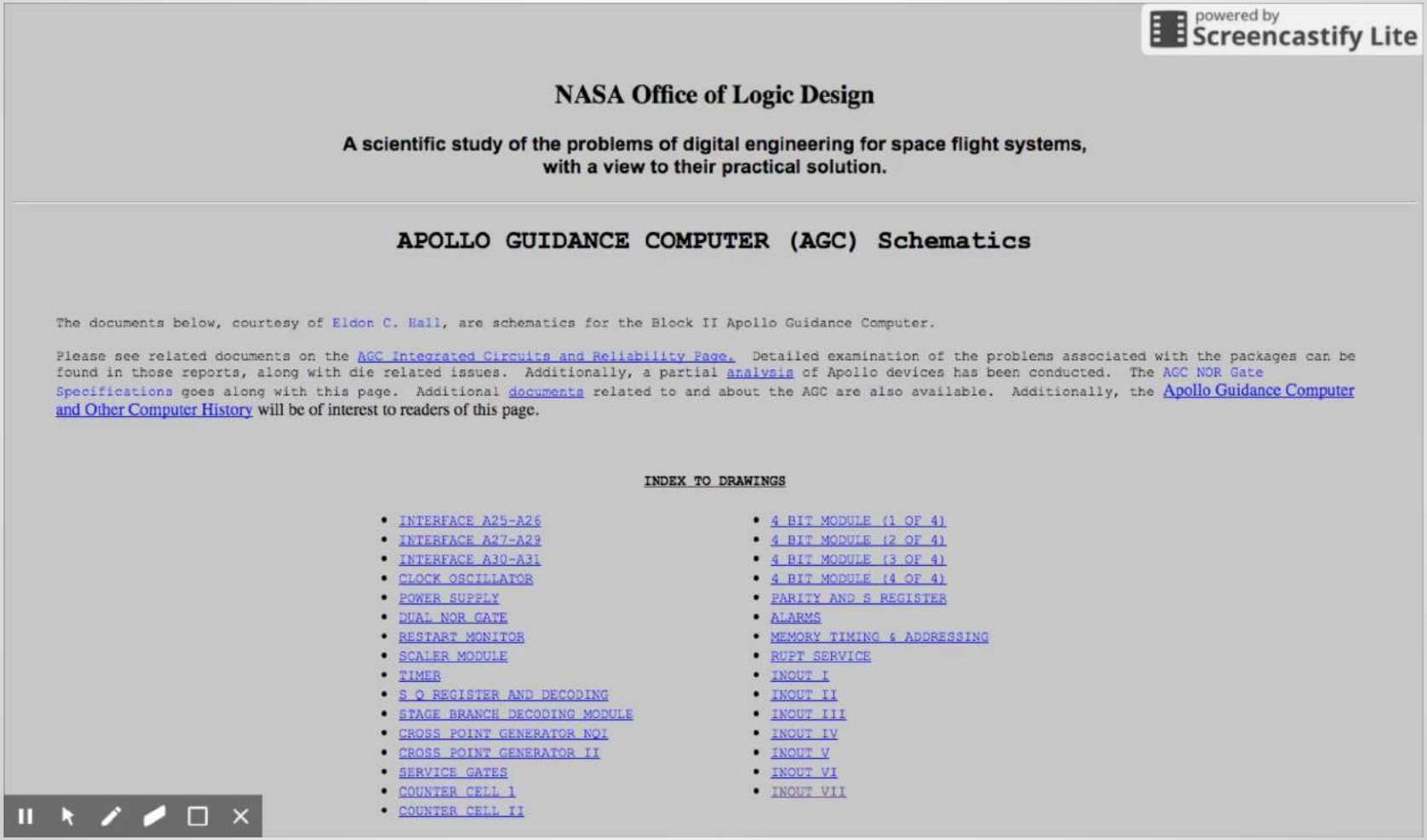


Apollo Moon Mission Circuits

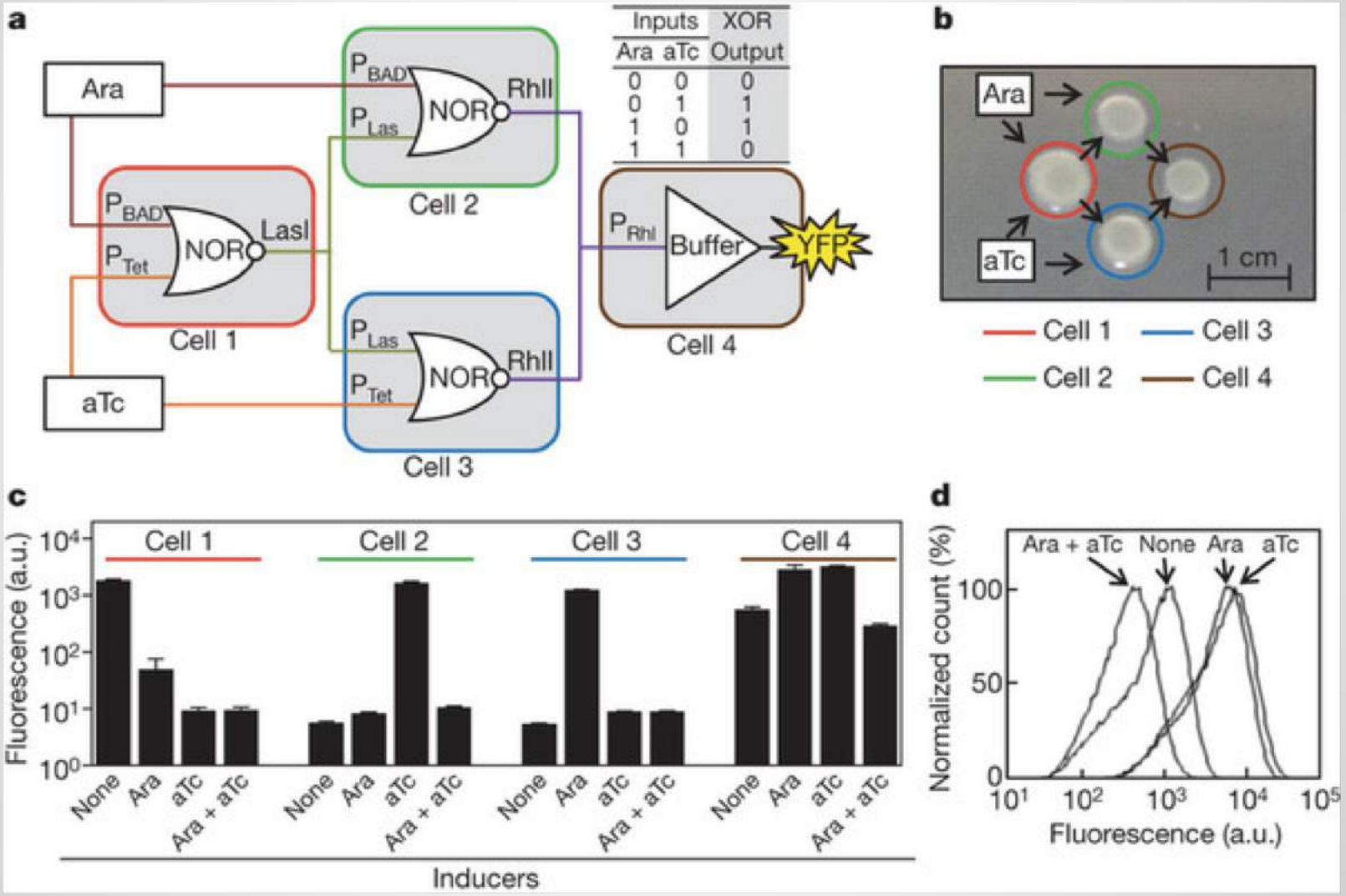


Apollo Guidance System = 5600 gates

http://klabs.org/history/ech/agc_schematics/

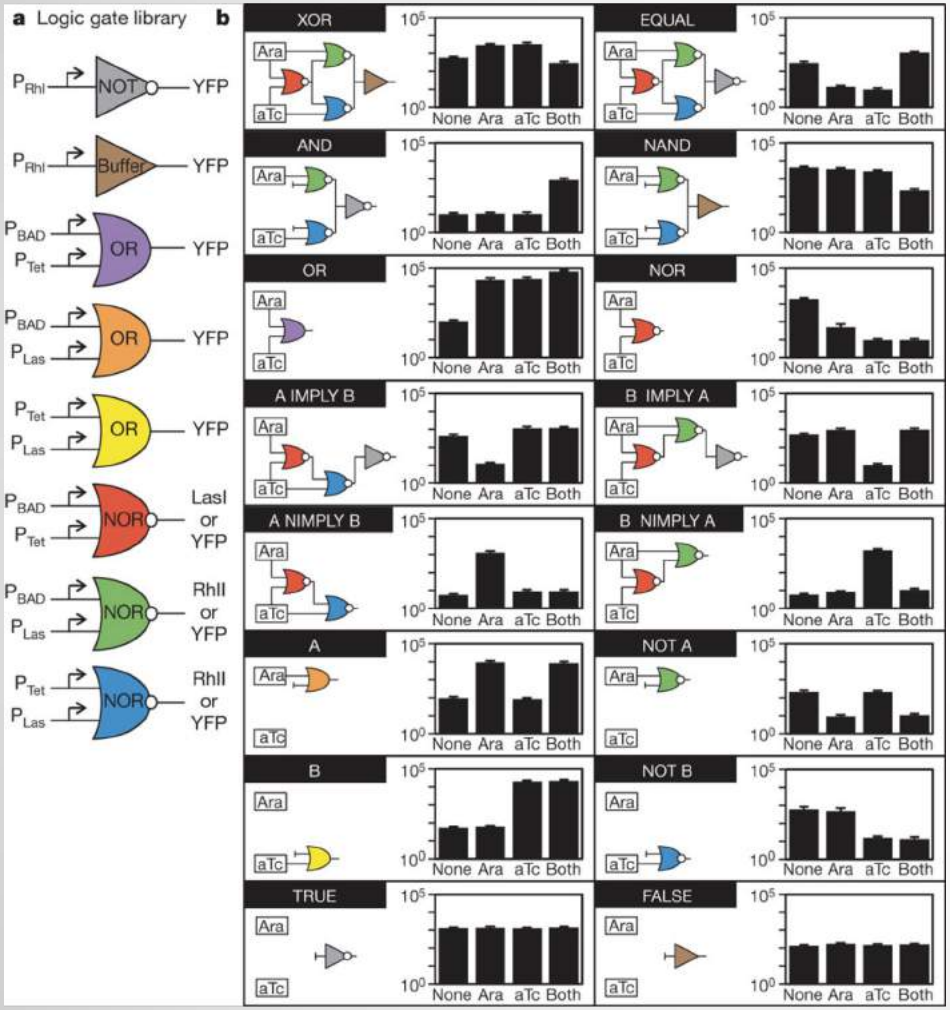


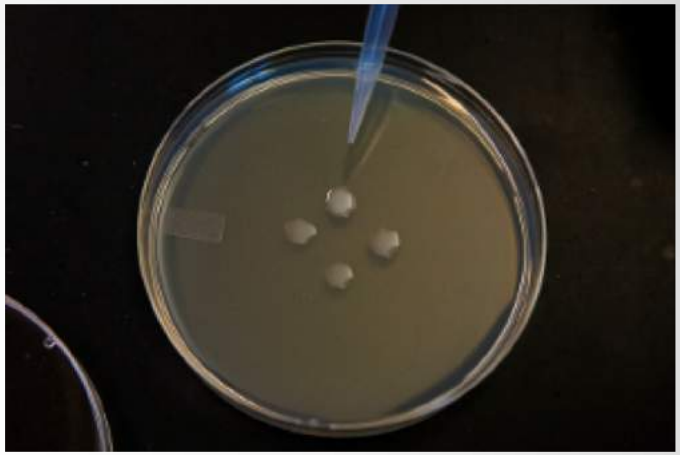
Bacterial NOR Gates - Multicellular



Tamsir *et al*. Nature 2011

Bacterial NOR Gates - Multicellular





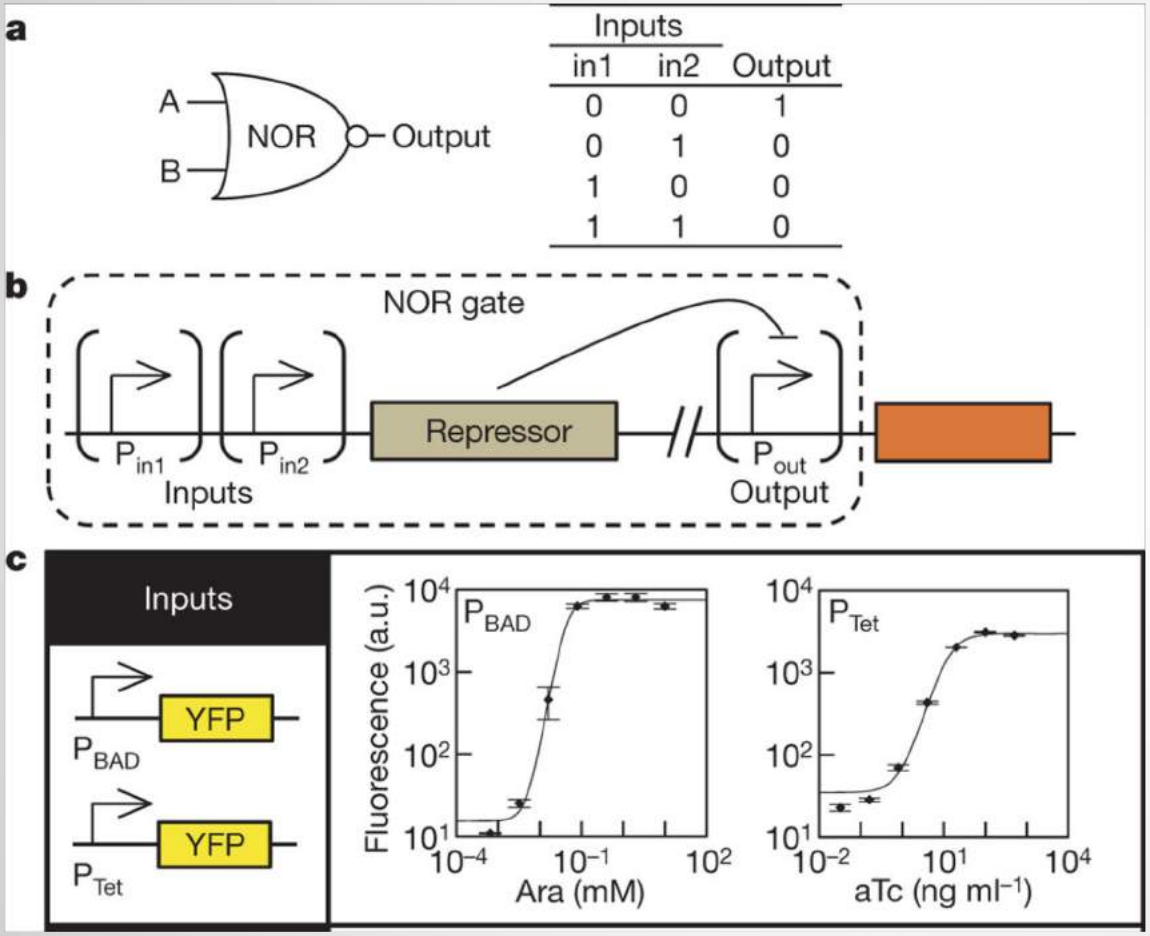
Each cell is a NOR gate with different input/outputs

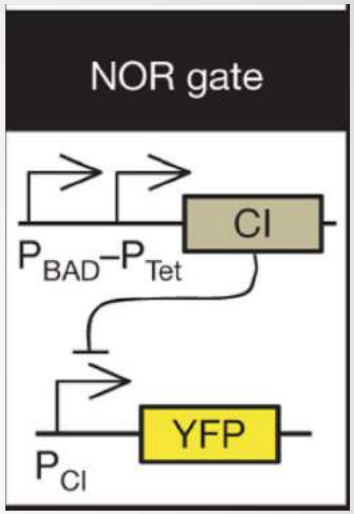
Wires are diffusible signals

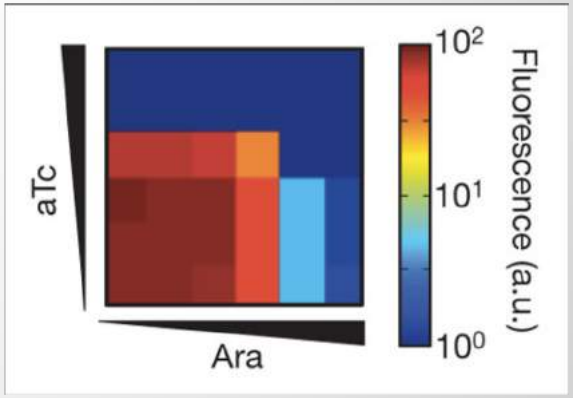
Plating 4 colonies in different places defines the logic

Tamsir *et al*. Nature 2011

Bacterial NOR Gates from NOT Gates

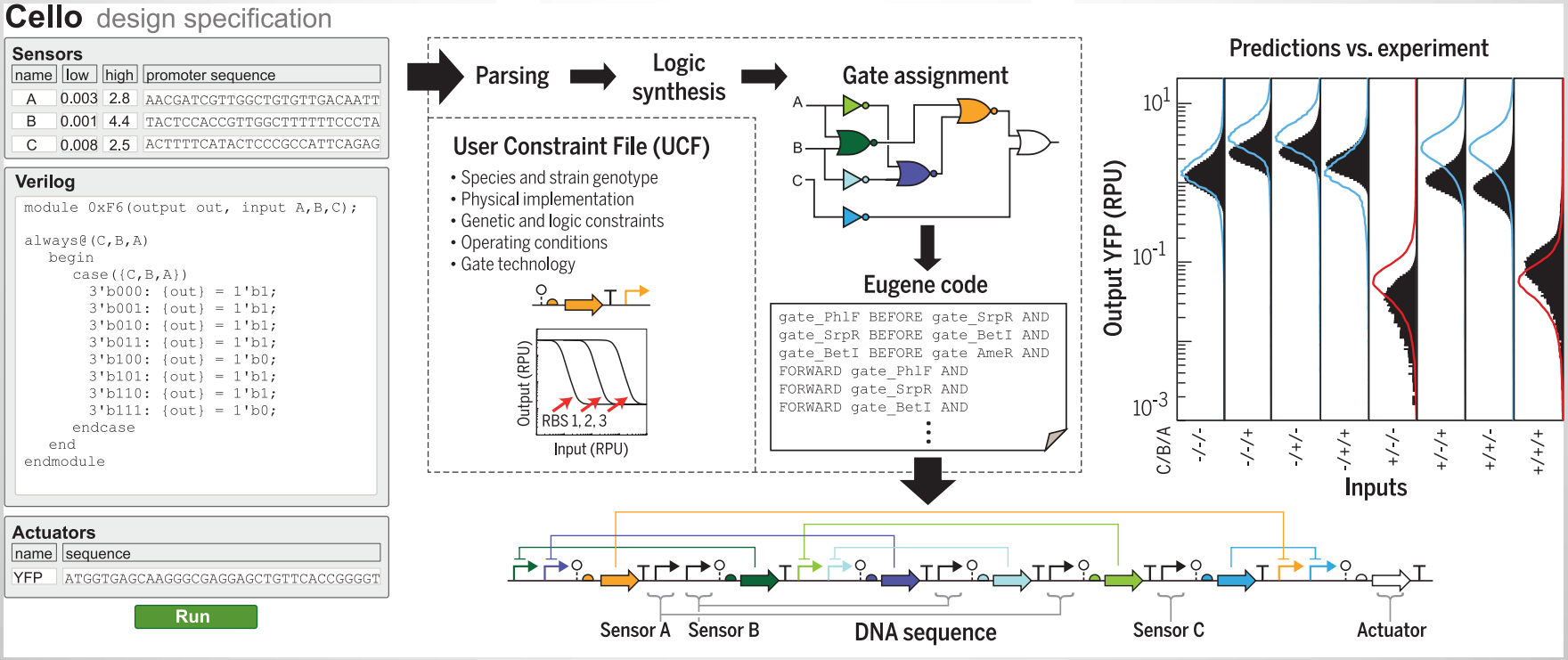




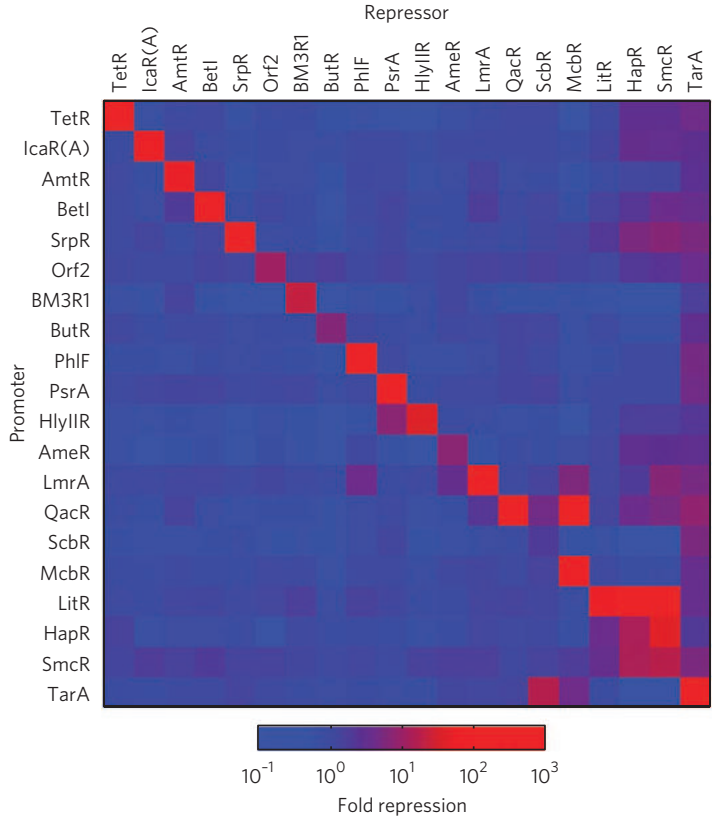


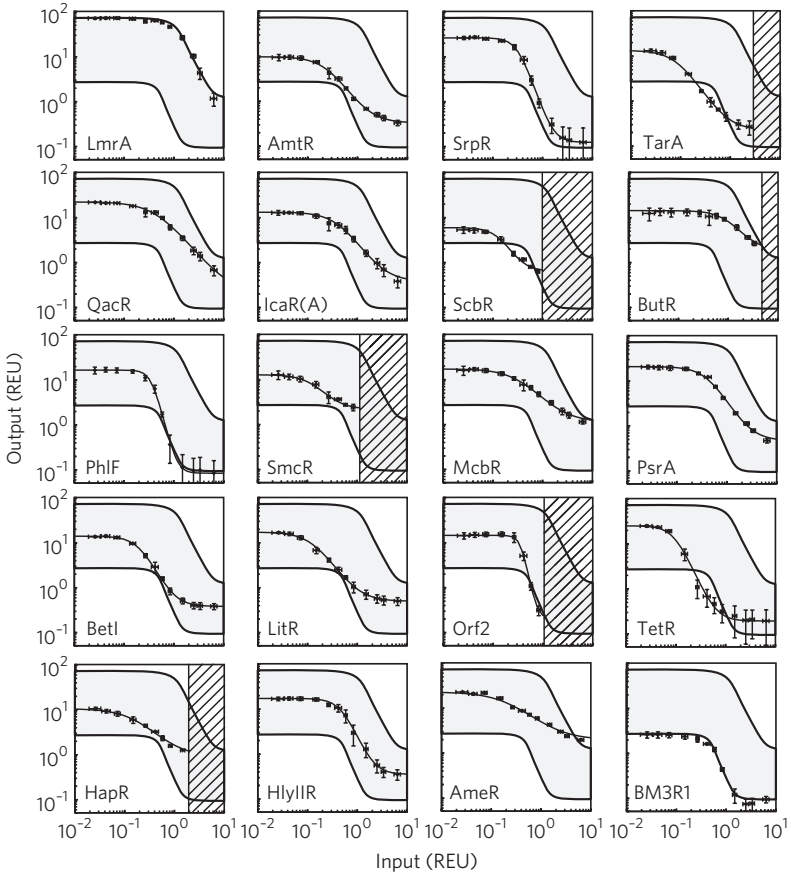
Tamsir *et al*. Nature 2011

2016: Automated Design of Complex Logic Functions… all inside an *E. coli*



Best logic in *E. coli* = 16 NOT gates

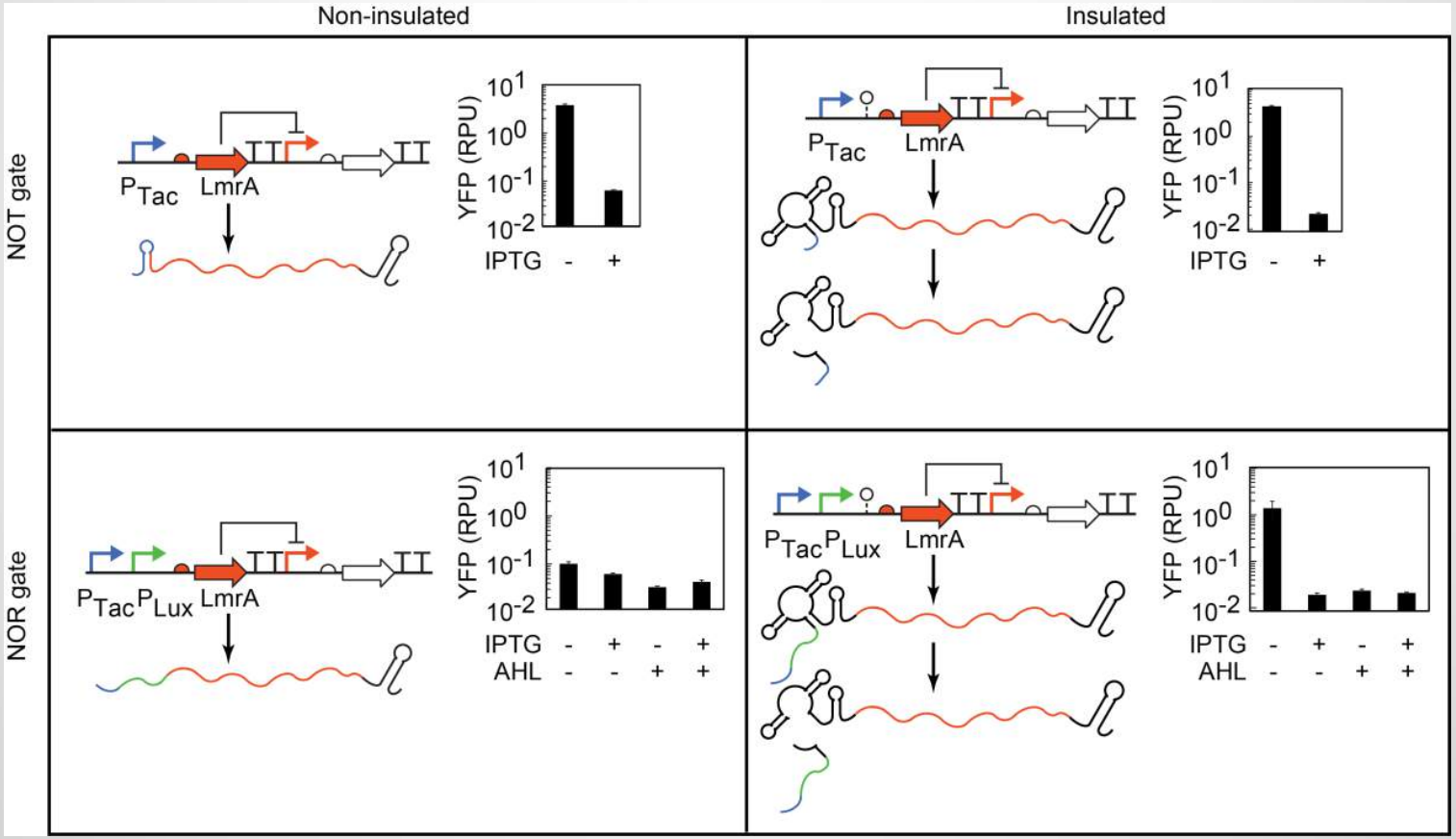




Stanton *et al*. Nature Chem Biol 2014

16 NOT gates = 256 different NORs

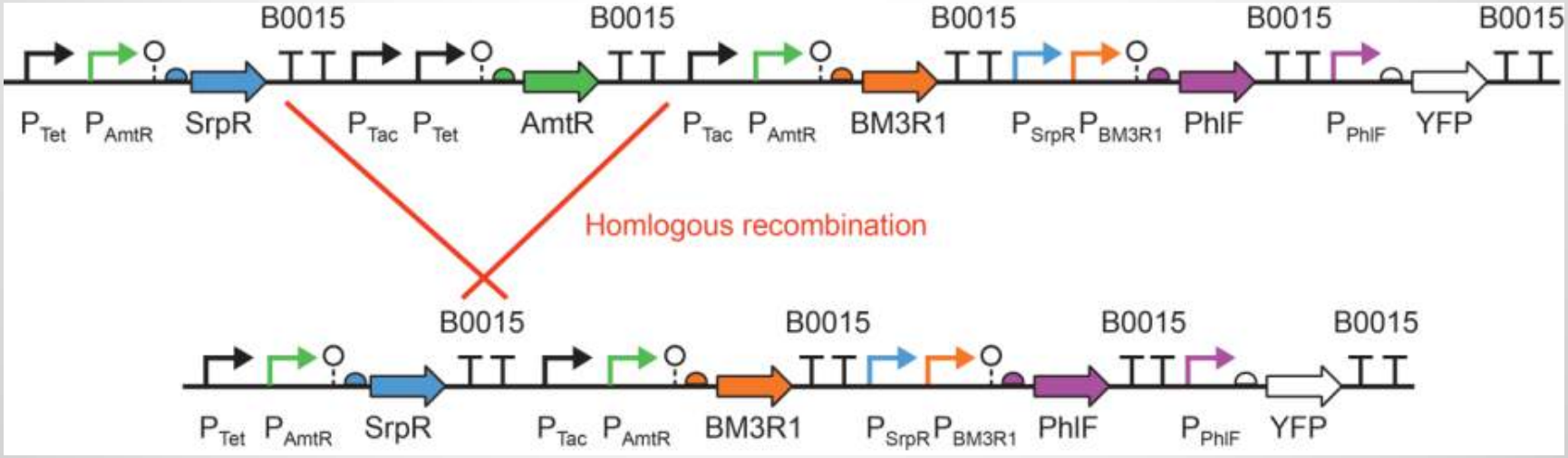
DNA circuit has to be built with RiboJ insulators so that behaviour with one promoter is the same as with two



Nielsen *et al.* Science 2016

Prevent failures: insulate & don't repeat parts

- If a plasmid has a part repeat within it, it will 'recombine' and delete out the DNA between
- Need many different versions of each part
- Use their terminator library
- Made a new RiboJ-type insulator library

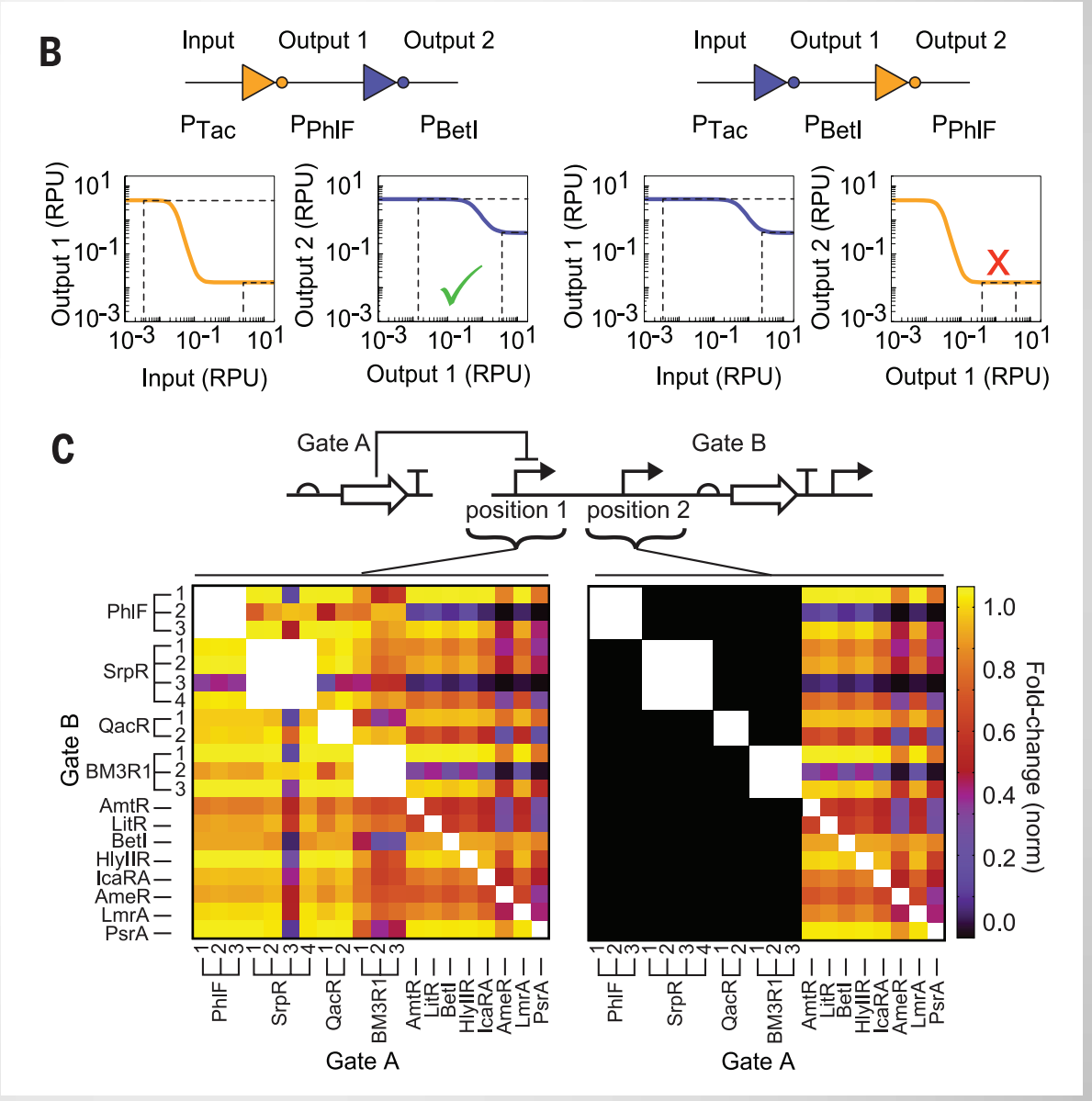


Nielsen *et al.* Science 2016

Some NOR gates are better than others

Range of outputs of one gate needs to match with the other gate's input range

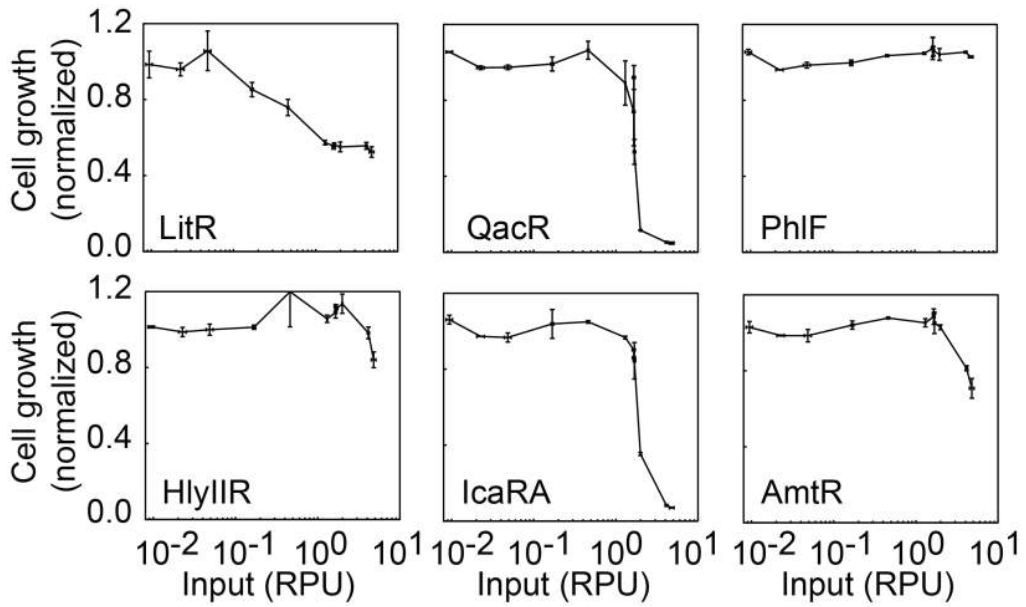
Testing many combinations reveals ones with the best ranges and those not to use



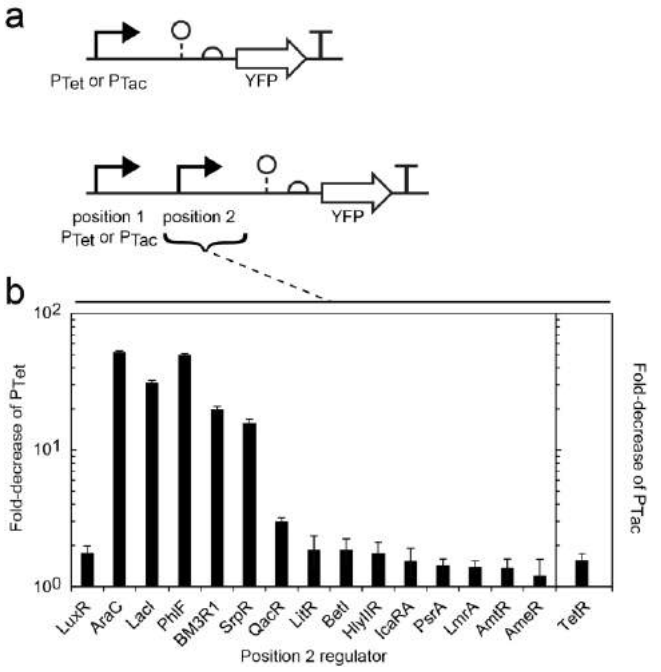
Nielsen *et al.* Science 2016

Some combinations are terrible to use

Toxic Transcription Factors

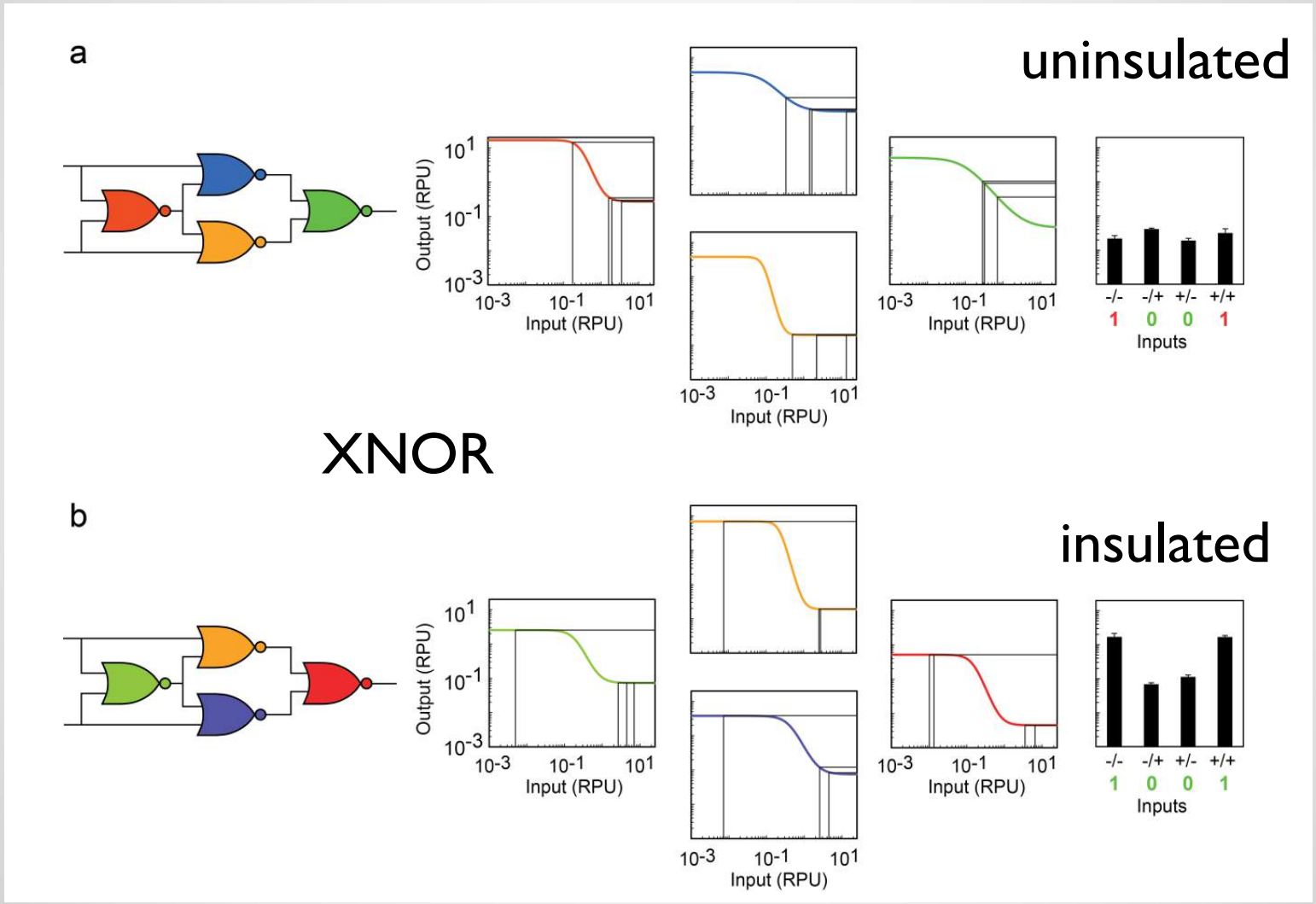


Roadblock Promoters



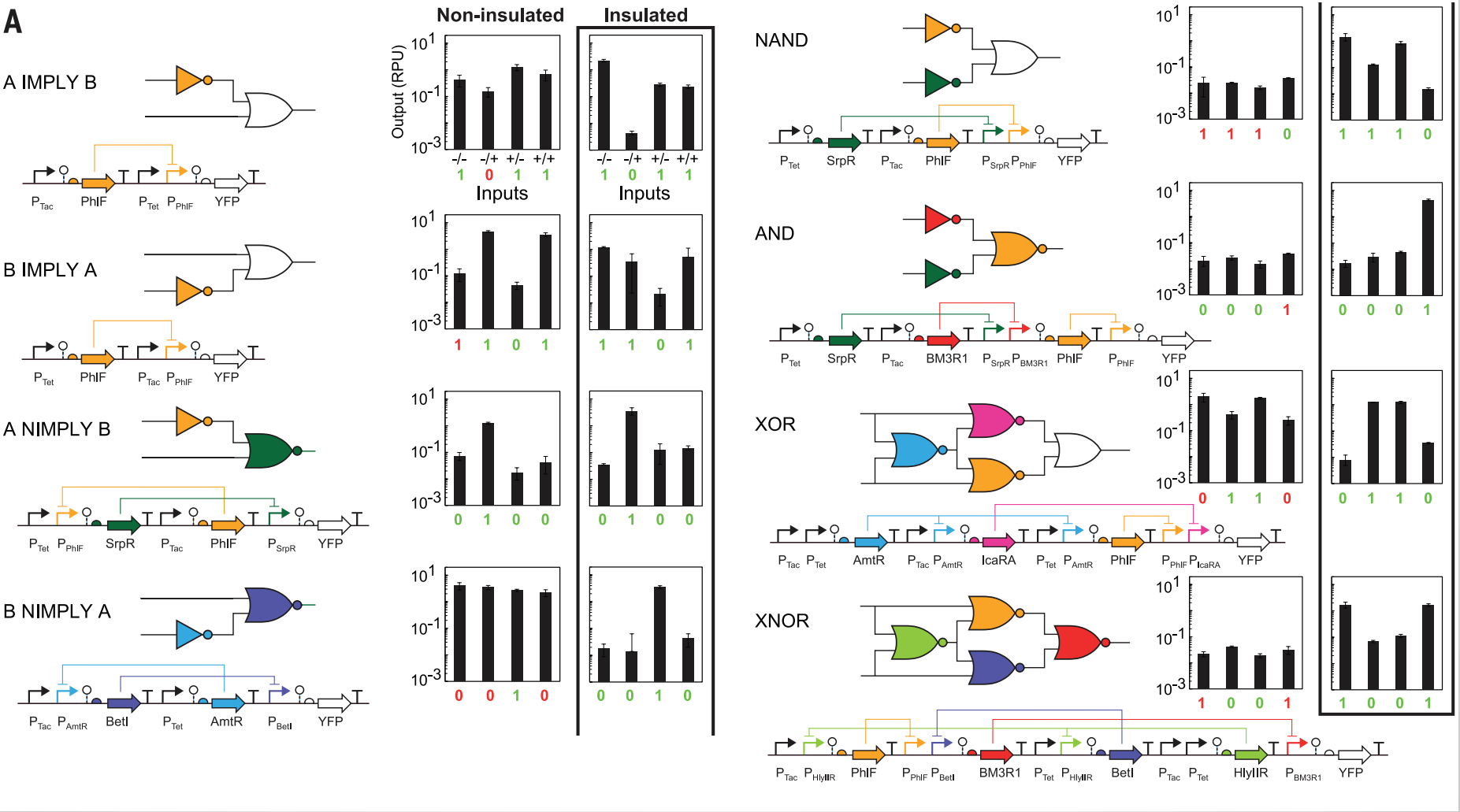
Nielsen *et al.* Science 2016

Insulating and choosing best parts works



Nielsen *et al.* Science 2016

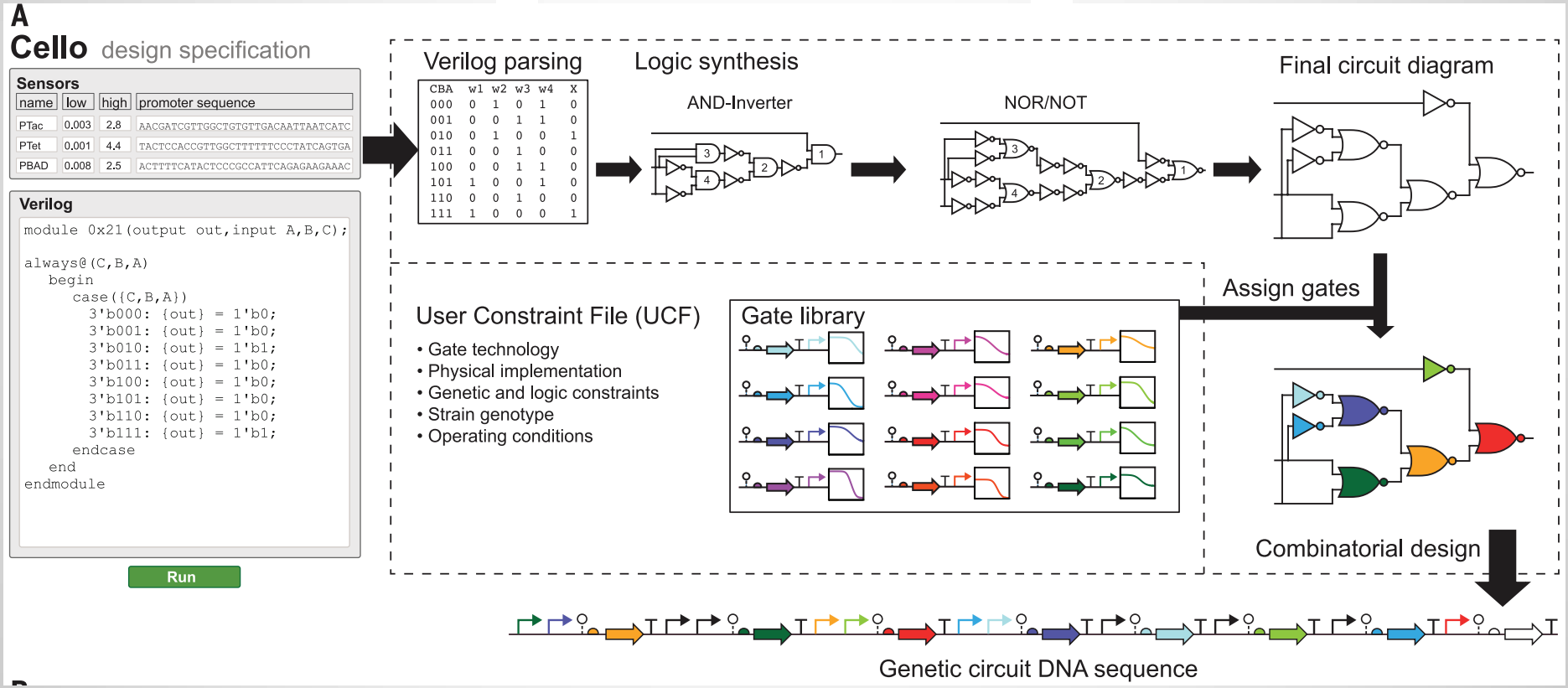
Insulating and choosing best parts works



Nielsen *et al.* Science 2016

Design can now be rational and automatic

http://www.cellocad.org/



Nielsen *et al.* Science 2016

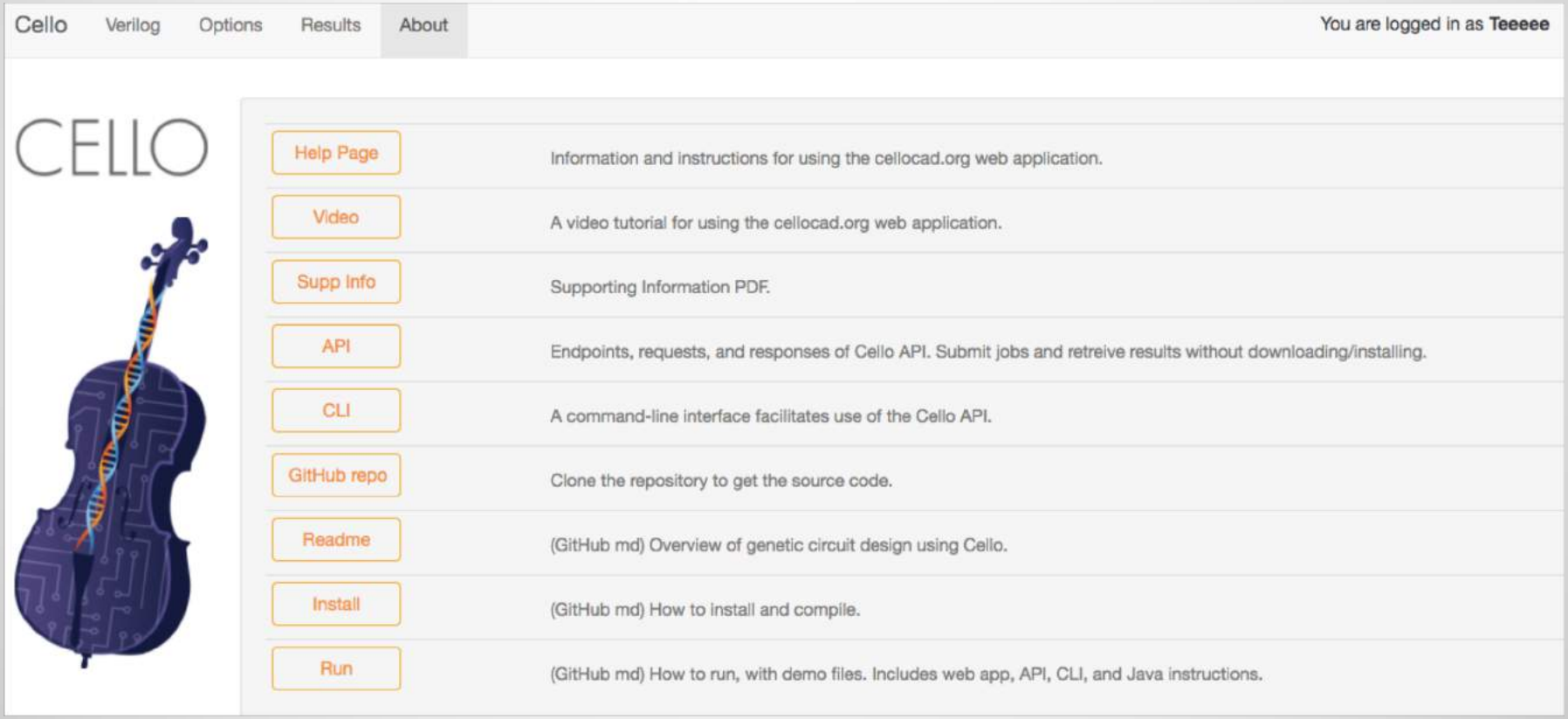
Verilog: design language for hardware

What is Verilog?

https://www.youtube.com/watch?v=q1QwC3YIHG0

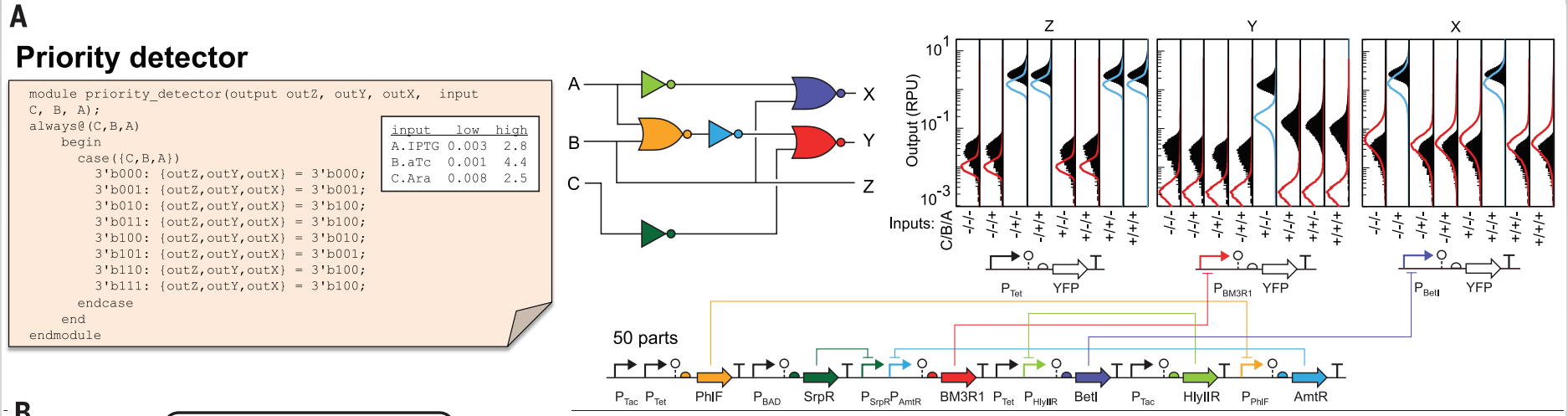
Cello: Verilog for *E. coli* logic circuit design

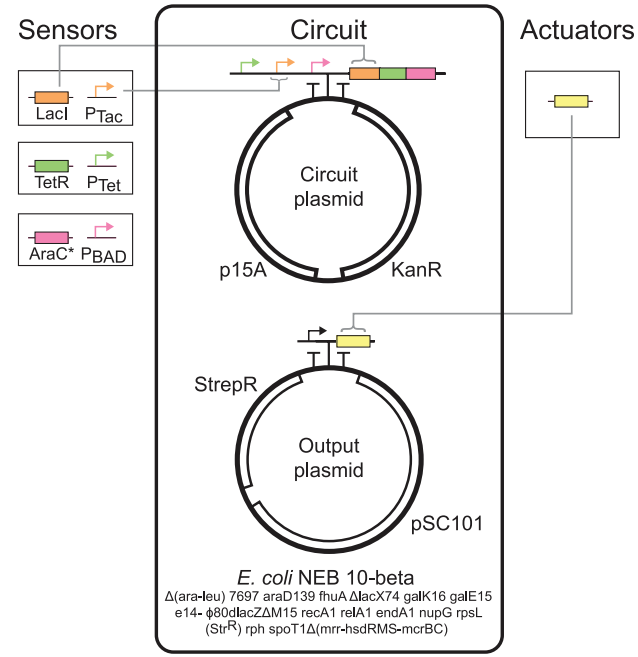
CelloCAD http://www.cellocad.org/



Nielsen *et al.* Science 2016

Cello: Verilog for *E. coli* logic circuit design

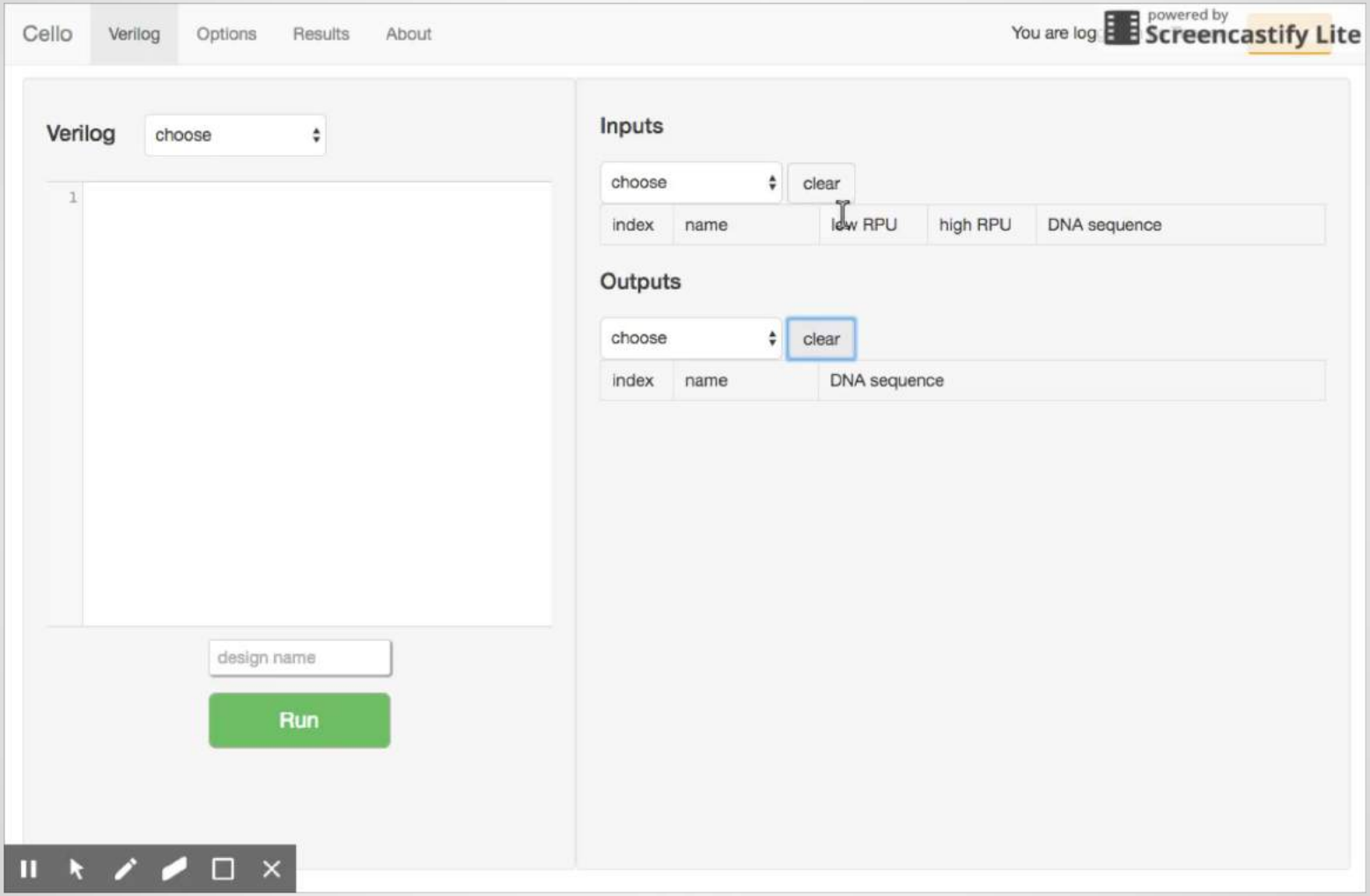




- Inputs are sensors (for inducers)
- Outputs are fluorescent proteins or actuator transcription factors
- Actuators can be regulators that change *E. coli* behaviours

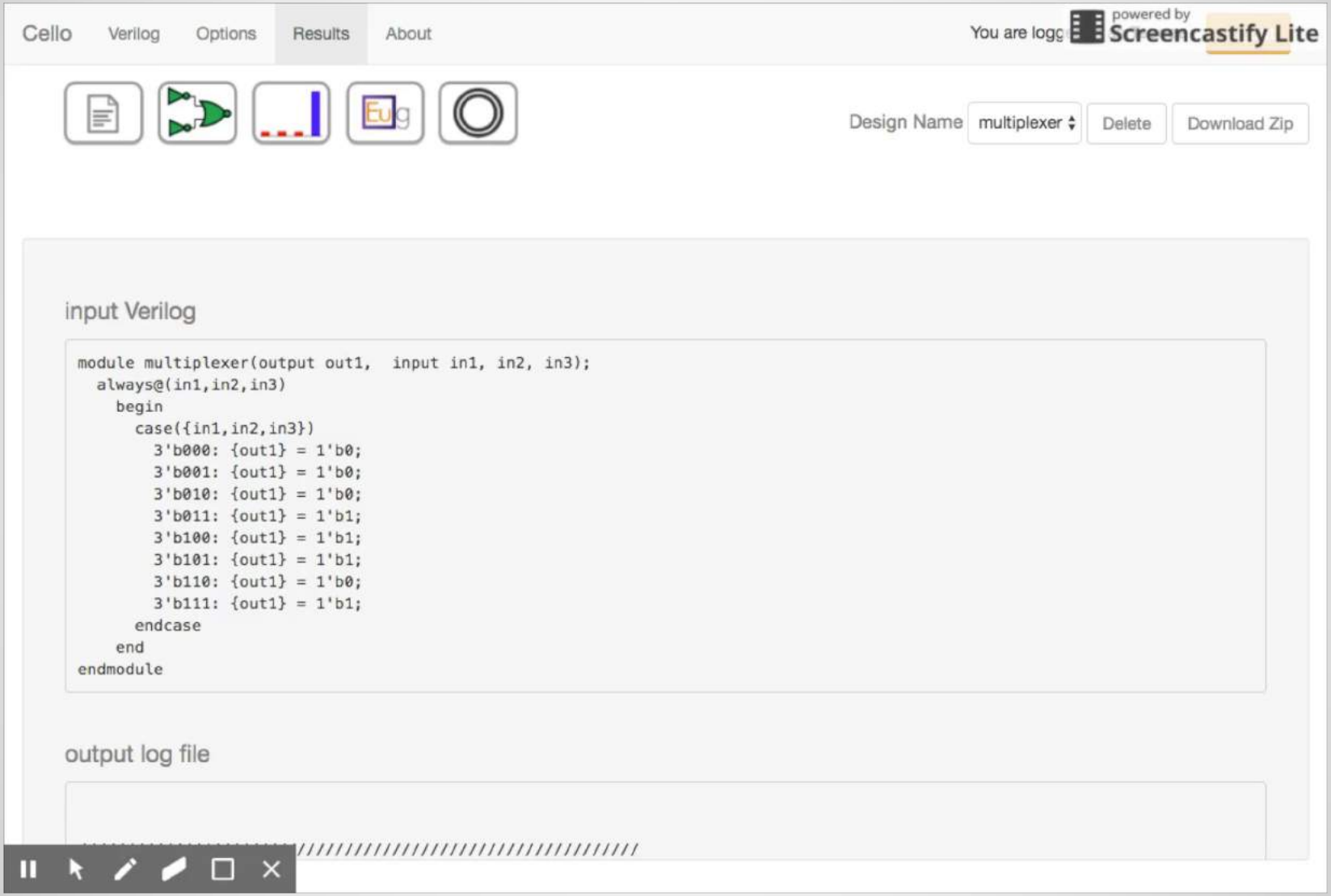
Nielsen *et al.* Science 2016

Cello: Inputs and Coding



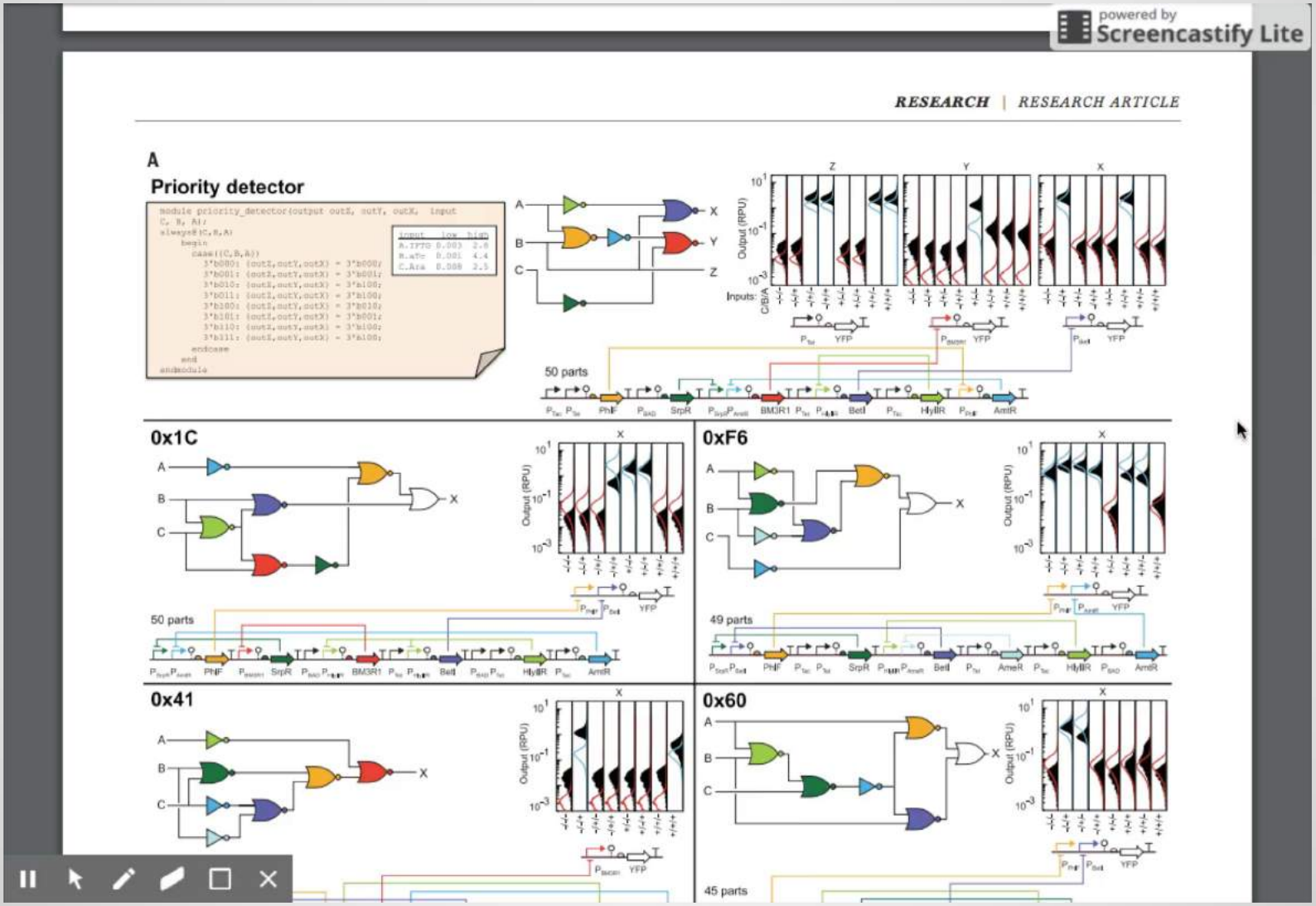
Nielsen *et al.* Science 2016

Cello: Results



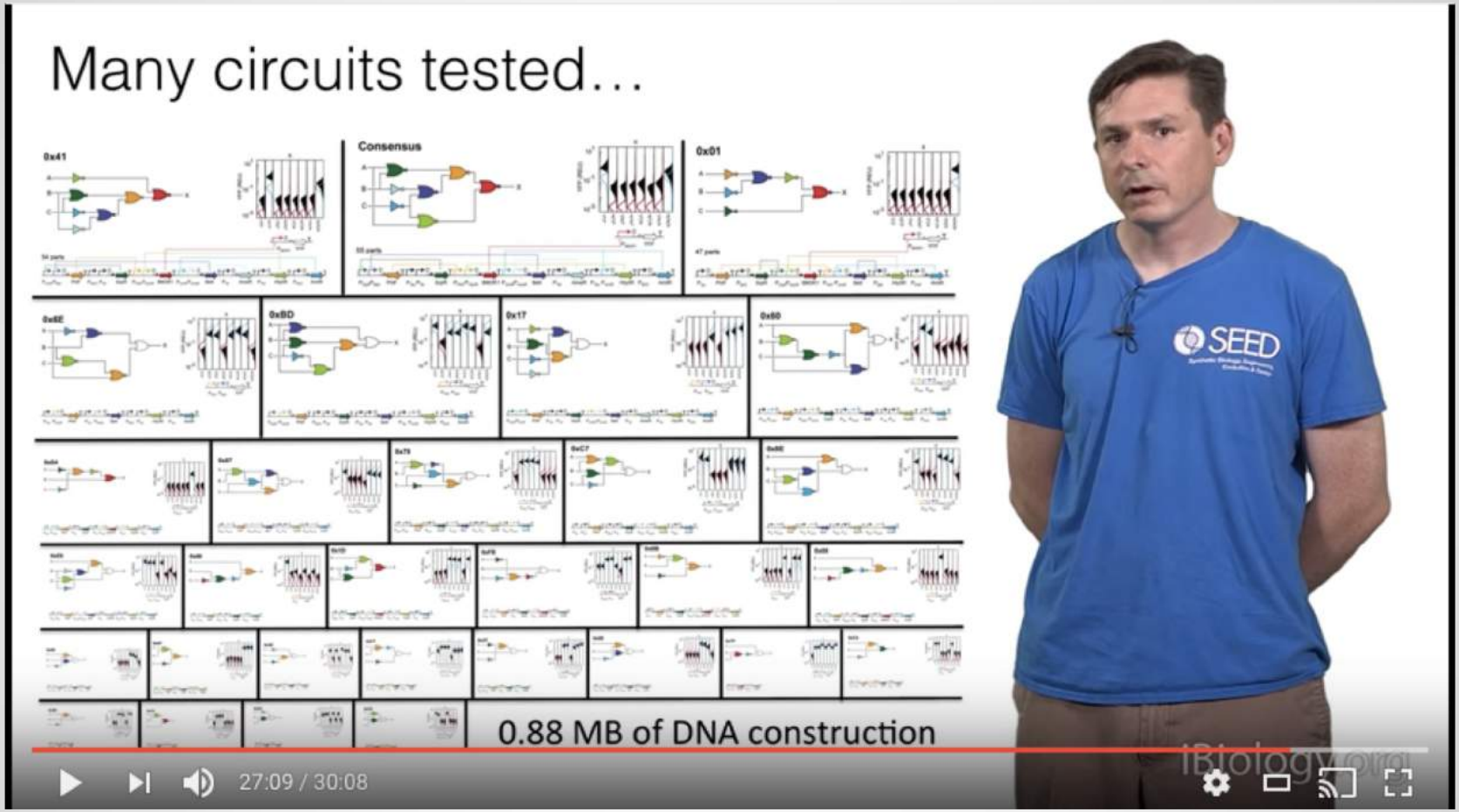
Nielsen *et al.* Science 2016

Cello: Possibilities



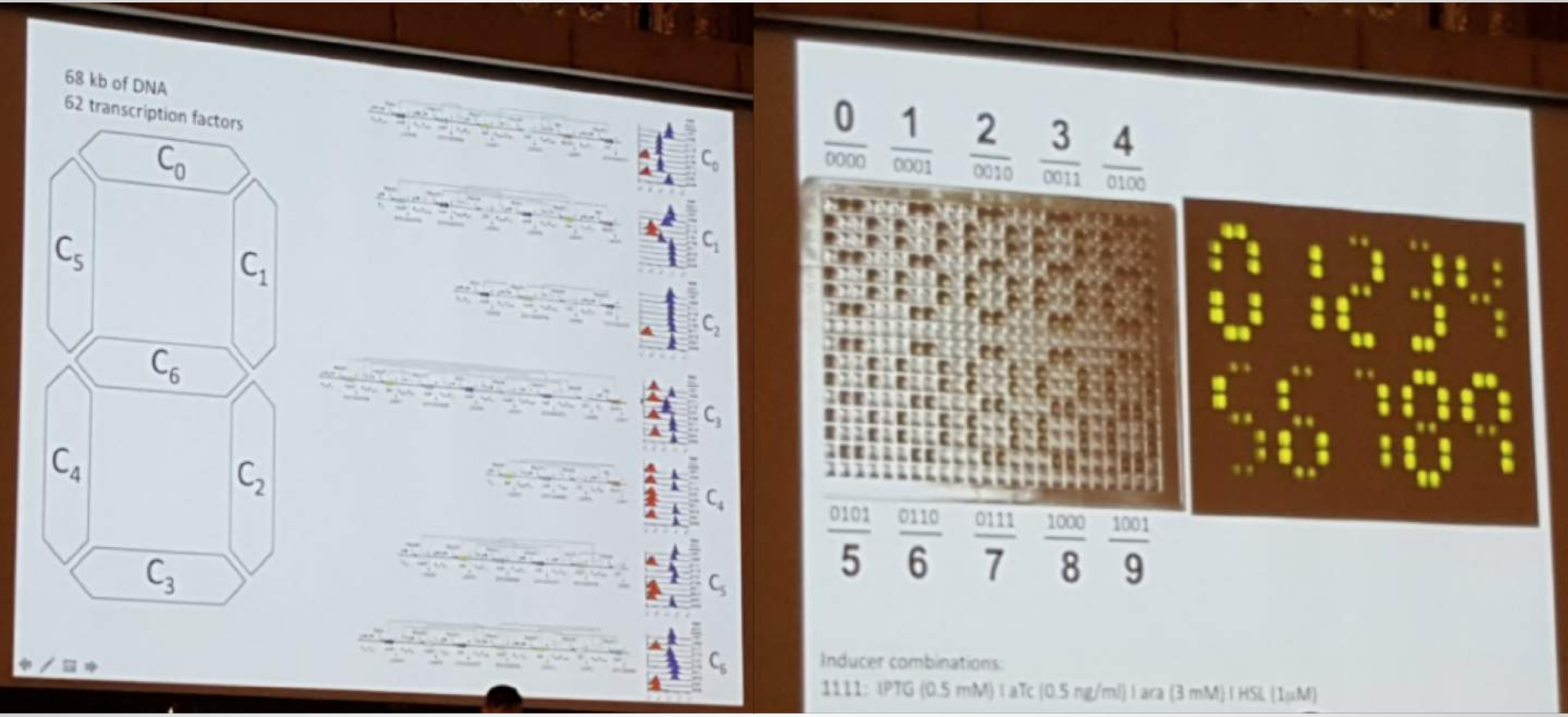
Nielsen *et al.* Science 2016

Automatable Circuit Design Done!



https://www.youtube.com/watch?v=INttxYdGHs4

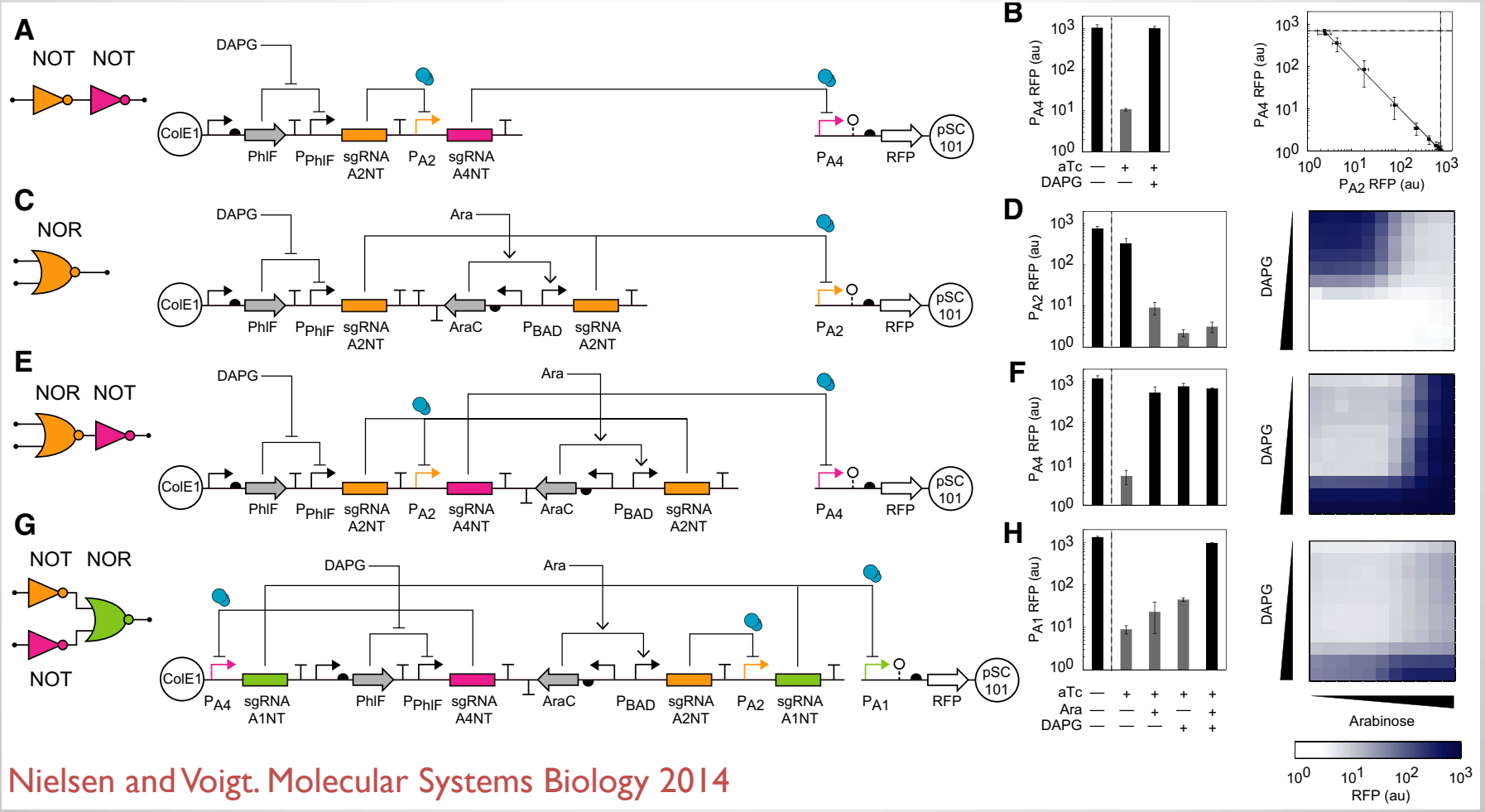
What they're doing with Cello now…



68 kbp DNA logic for LCD number display

Can also do logic with CRISPRi

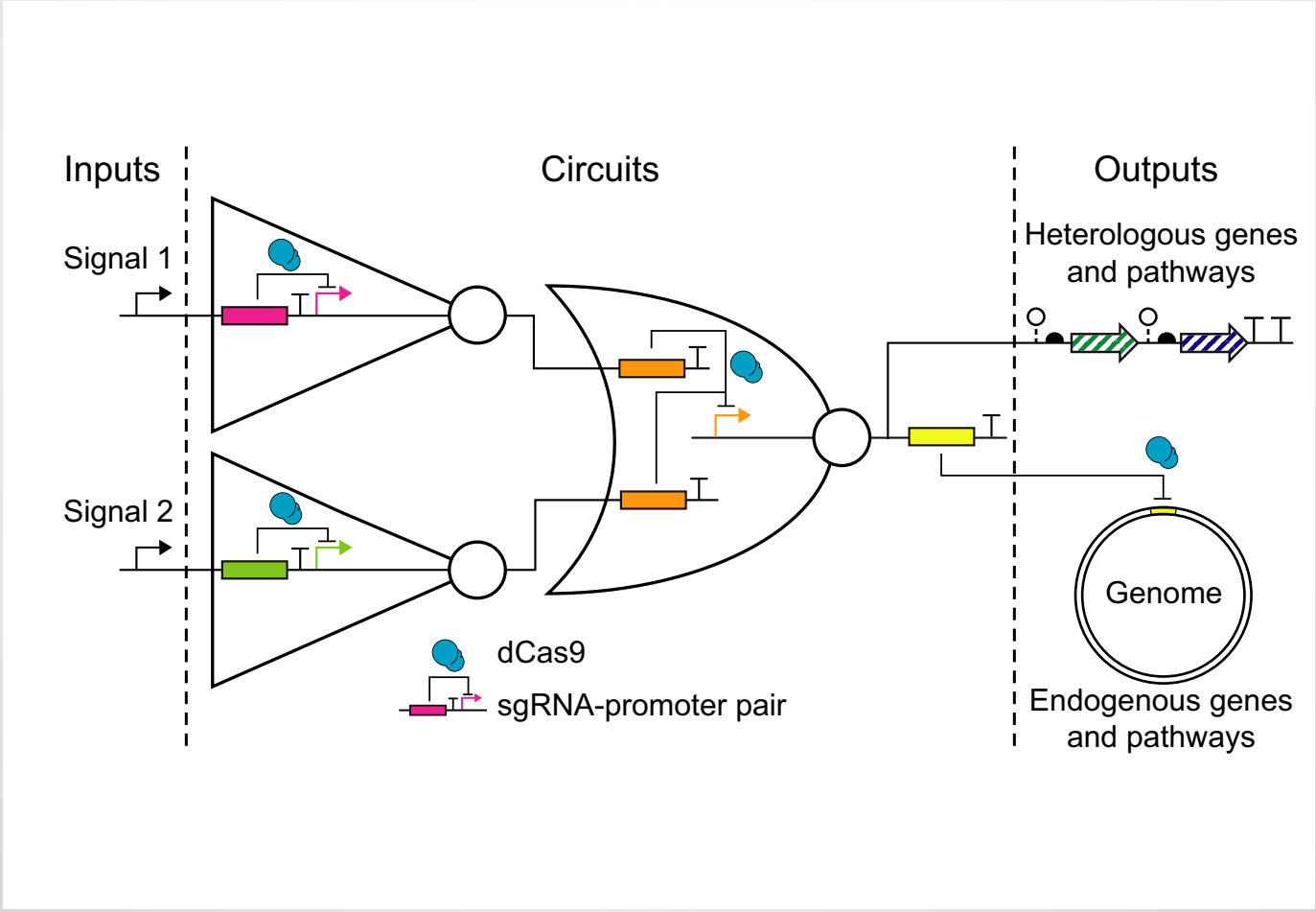
CRISPRi regulation can be combined and cascaded to make logic gates



Nielsen and Voigt. Molecular Systems Biology 2014

Applying CRISPRi logic in *E. coli*

CRISPRi logic can be used to control synthetic or genomic genes



Applying CRISPRi in *E. coli*

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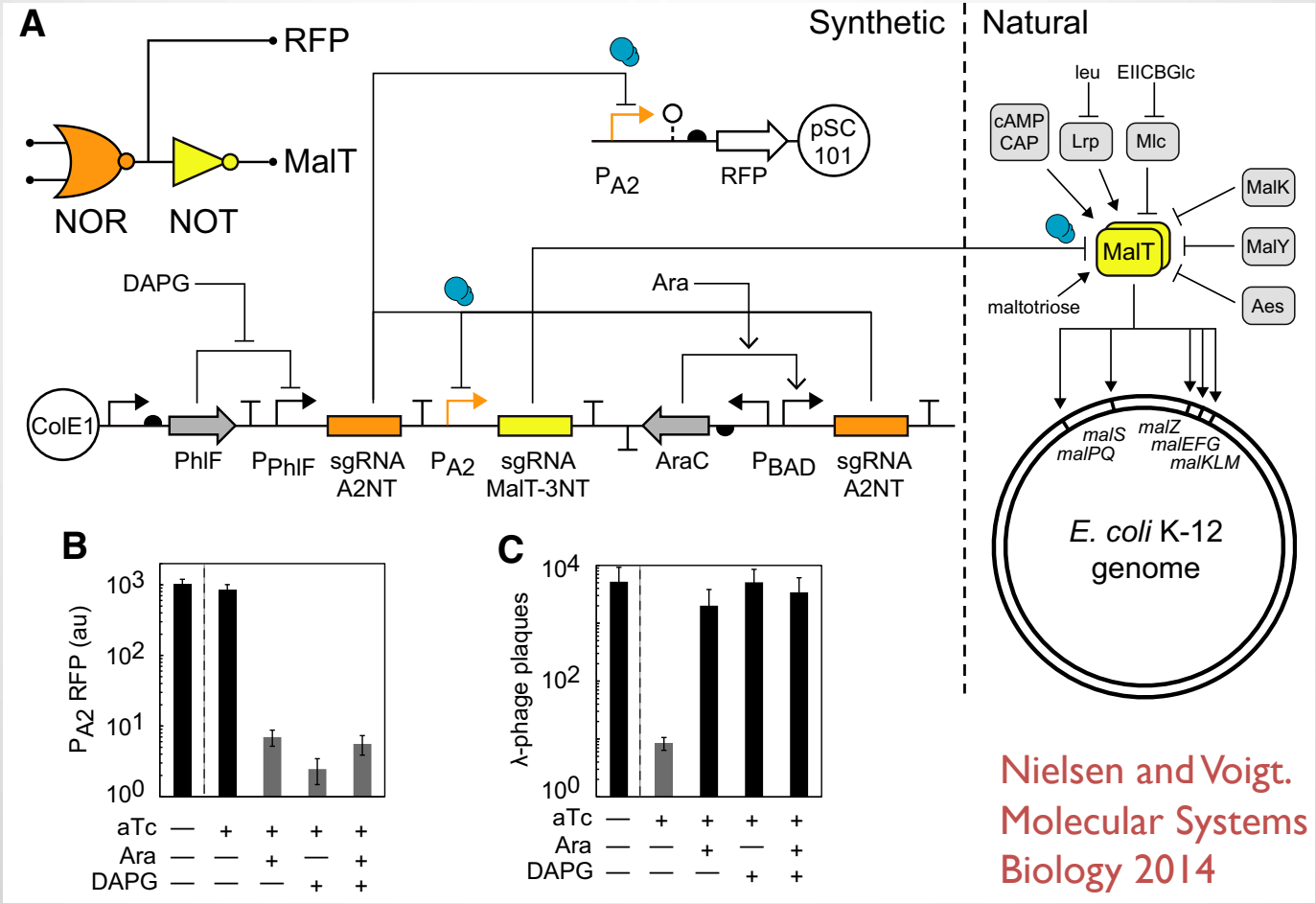


Nielsen and Voigt. Molecular Systems Biology 2014

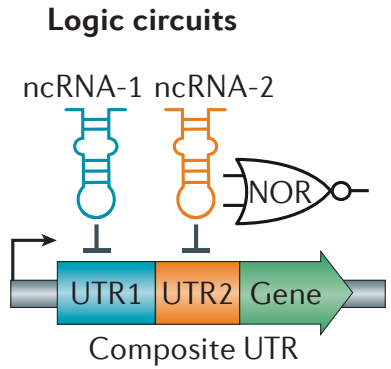
100+ logic gates in a cell?

- Scalable parts doesn't fully solve the problem…
- Bacteria are efficient cells optimised for growth and extra DNA programs are a 'burden' that slows growth and impairs performance
- Two solutions to reduce overloading a cell:

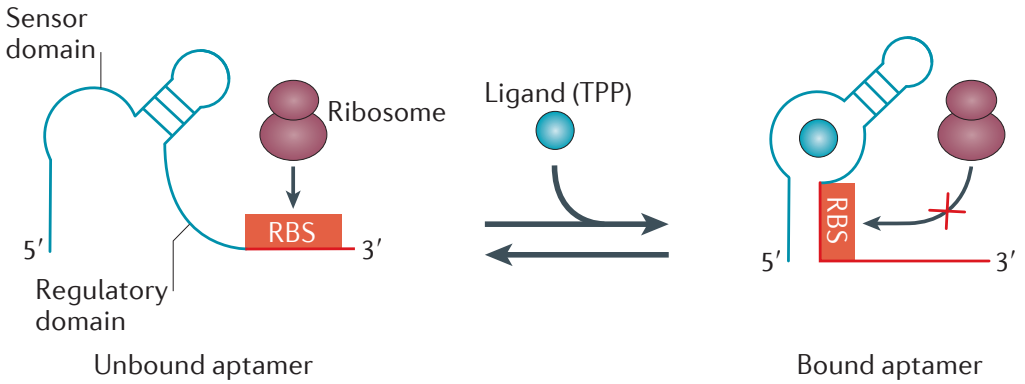
1. Use programs that are less 'costly' to the cell
2. Distribute program among a population:
   i.e. multicellular genetic networks

Less 'costly' programs: RNA devices

- DNA→RNA is much less work for a cell than DNA→RNA→Protein (translation is a major cost)
- RNA parts can substitute for protein regulators and sensors
- e.g. Riboregulators, Riboswitches (and CRISPRi)



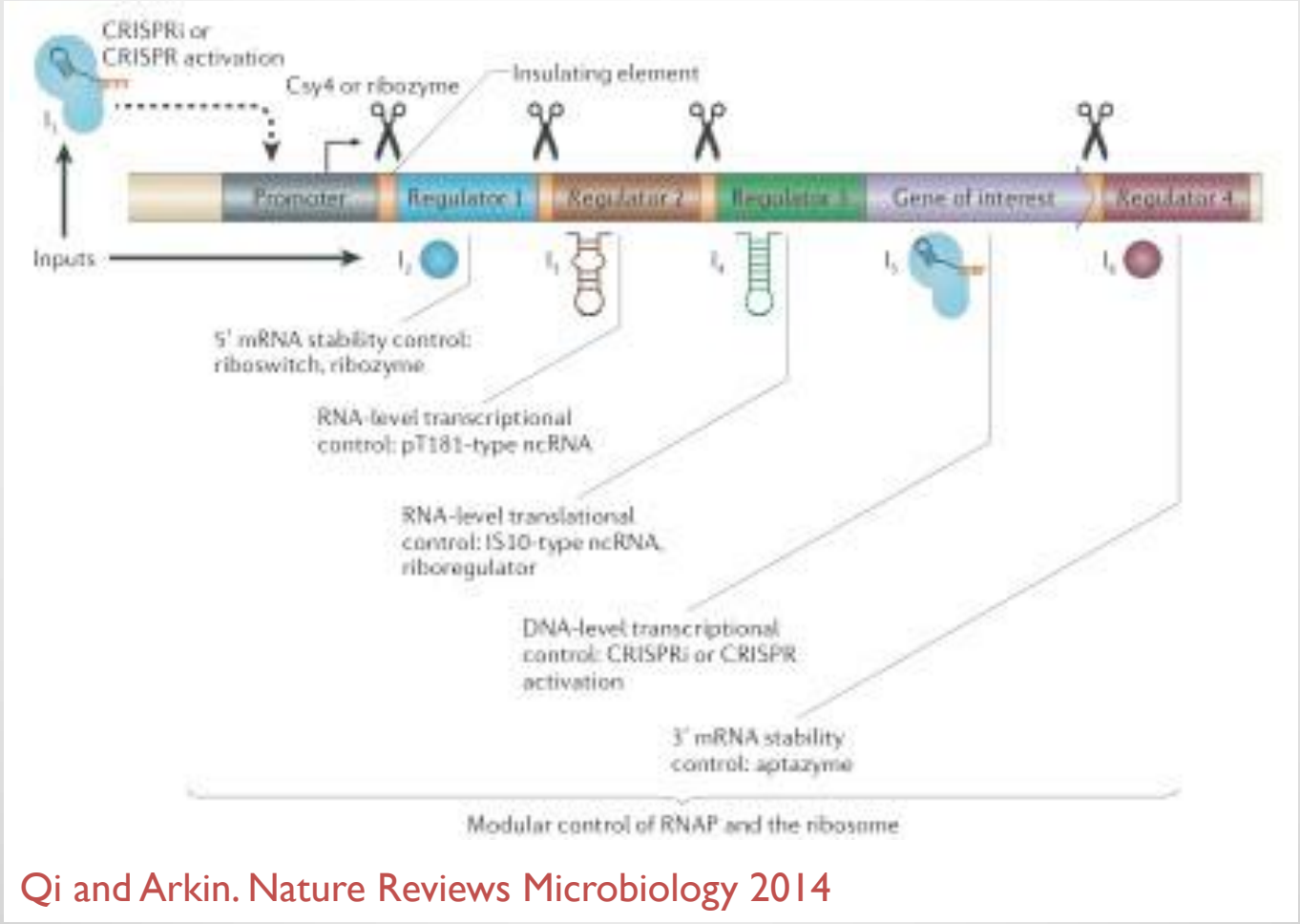
Riboregulators



Riboswitch (also known as an aptamer)

Less ‘costly’ programs: RNA devices

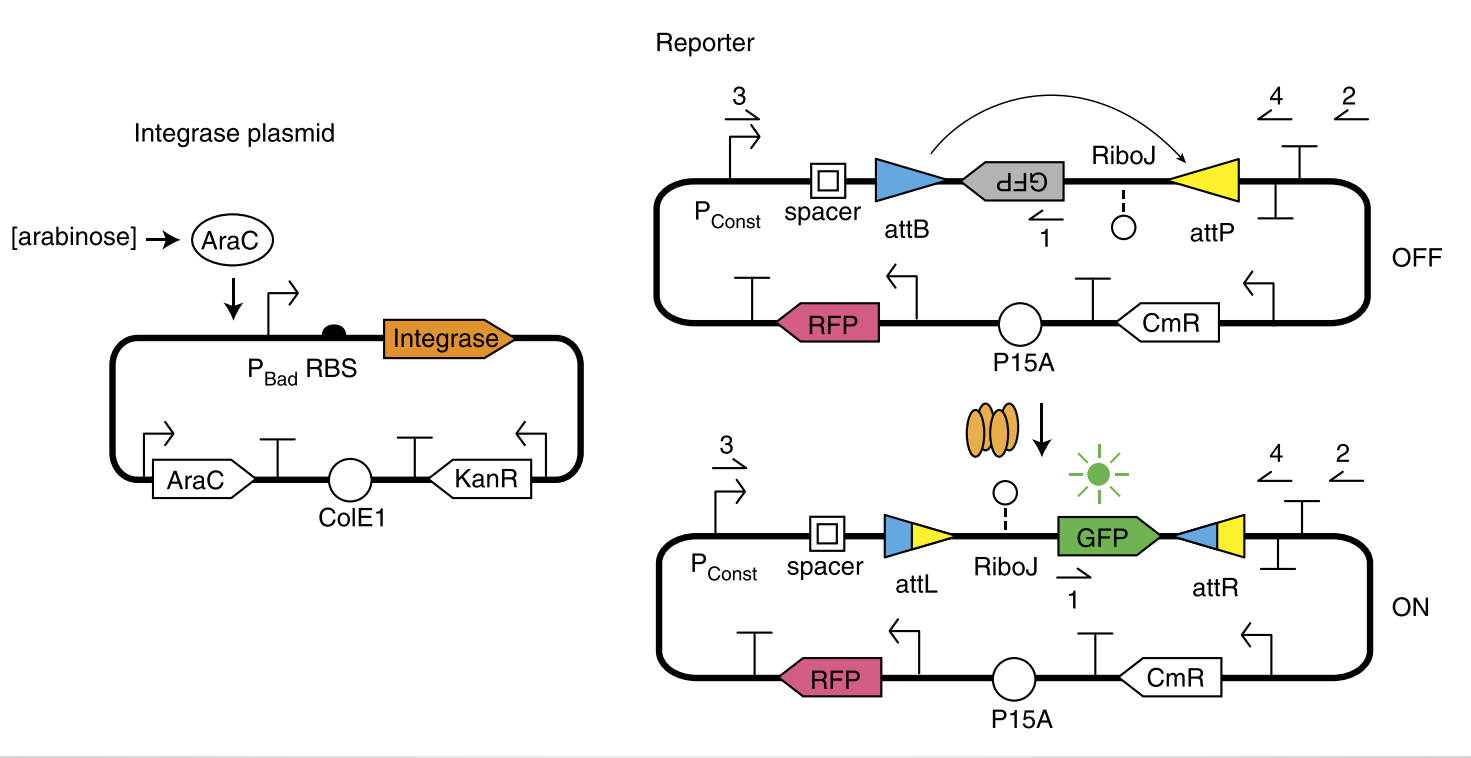
Many different ways to tune gene expression with RNA



Qi and Arkin. Nature Reviews Microbiology 2014

Less ‘costly’ programs: DNA switches

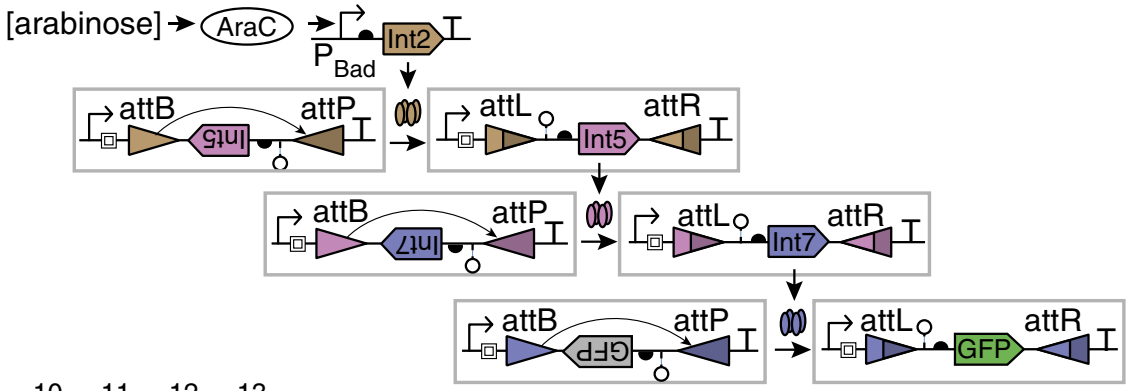
- DNA recombination can be used to write memory physically into the DNA sequence
- DNA region inverts when an *integrase* is induced

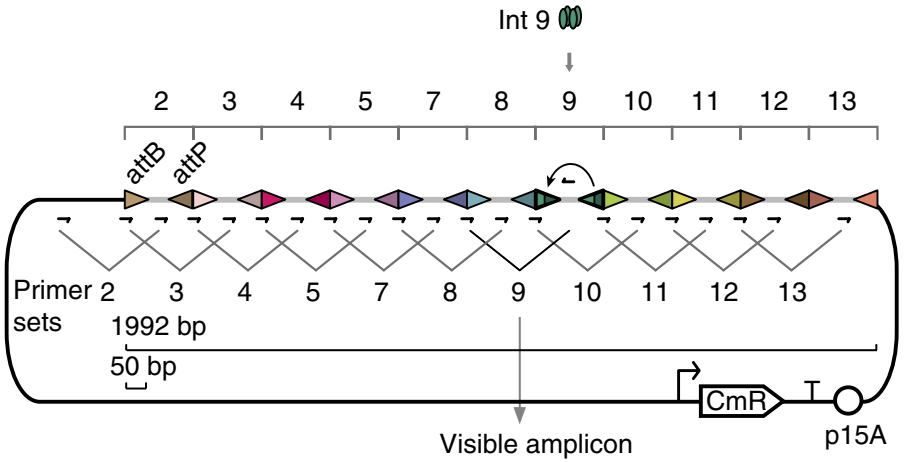


Less ‘costly’ programs: DNA switches

With 11 different integrases you can encoded >1 byte of digital information (2^{11} different events)

Nesting of integrase genes between recombination sites





Final layout of DNA is a record of what inducers came in which order

Yang, Nielsen *et al.* Nature Methods 2014

Summary

- Bacterial logic is possible at promoters
- NOR gate logic in multicellular systems and now also within *E. coli*
- Logic scales if well-characterised and insulated
- Cello means design is fully-automated.
- CRISPRi is a game-changer for regulation
- As things get more complex, non-protein parts are needed to allow lower-cost

Example Exam Questions

- Give examples of logic functions implemented in *E. coli* by synthetic biologists
- How does Cello work?
- Outline the need for insulators and characterisation for automating logic circuit design?
- Describe the challenges of making a 12-gate logic network in *E. coli*

Key References

Original Research

Genetic Circuit Design Automation

Nielsen *et al*. Science 2016

Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'

Tamsir, Tabor and Voigt Nature 2011

CRISPRi Logic Gates

Nielsen and Voigt. Molecular Systems Biology 2014

Further References

Principles of Gene Circuit Design (REVIEW)
Brophy and Voigt. Nature Methods 2014

RNA-based parts and devices (REVIEW)
Qi and Arkin. Nature Reviews Microbiology 2014

Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology
Wang *et al*. Nature Communications 2011

DNA recombinases for >1byte of memory
Yang, Nielsen *et al*. Nature Methods 2014