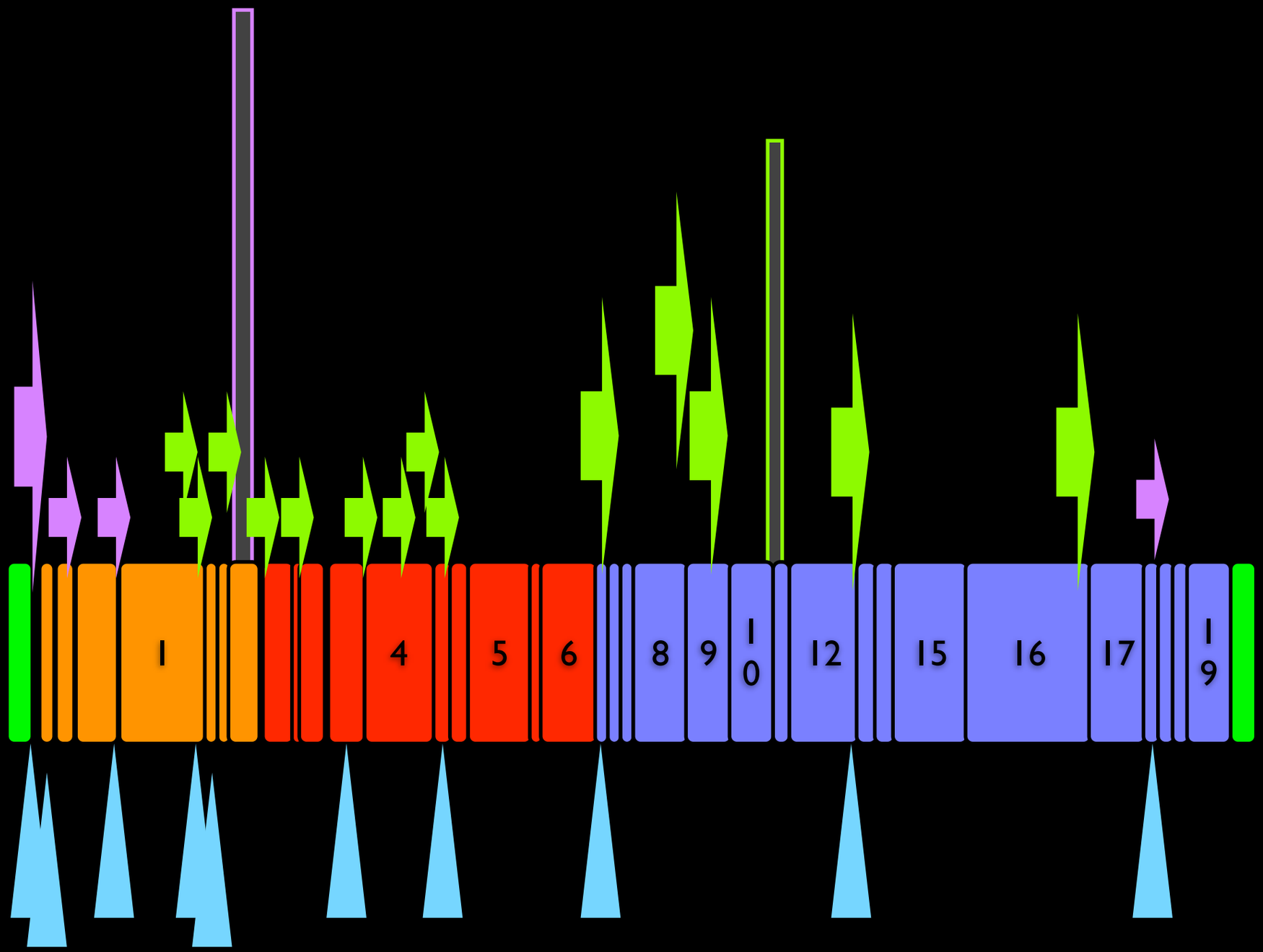
Languages & Grammars for Programming DNA

Drew Endy
MIT Biological Engineering

http://mit.edu/endy

ICSB
10 October 2006


MIT
MIT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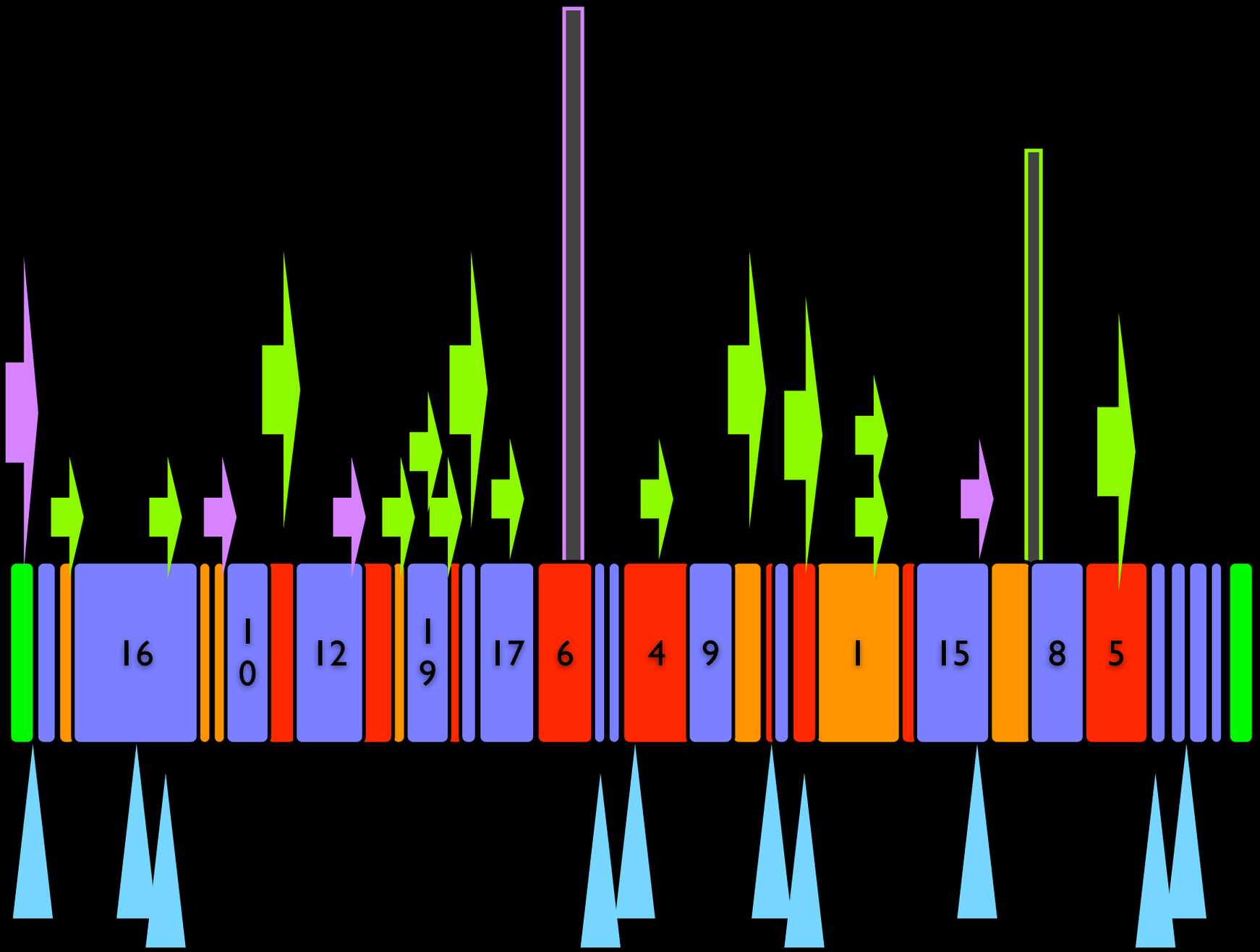
19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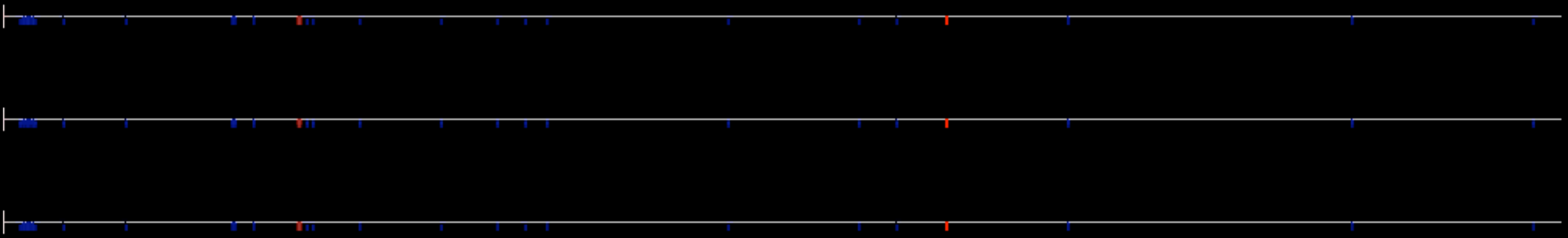

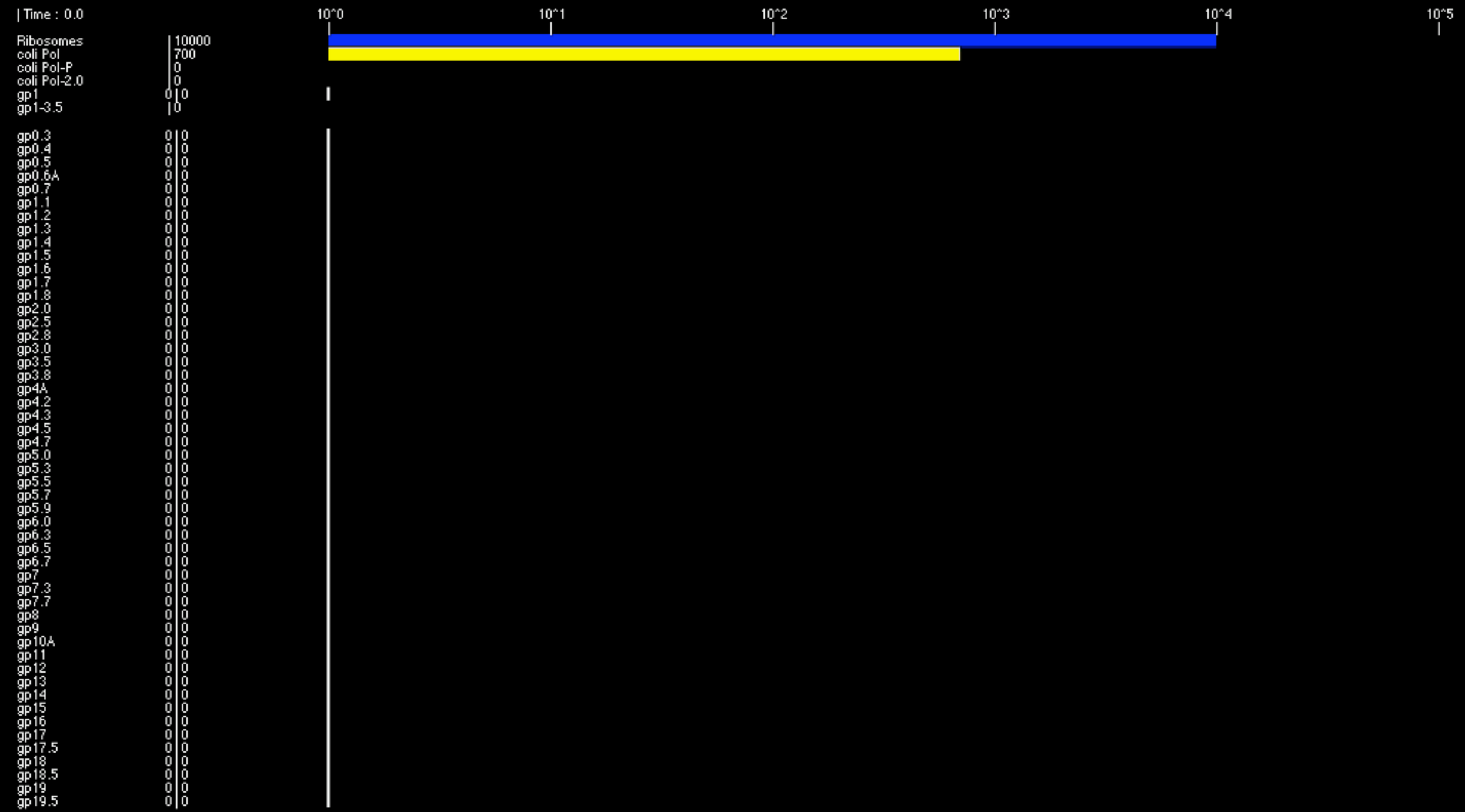
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10^1
10^2
10^3
10^4
10^5
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coli Pol | 700
coli Pol-P | 0
coli Pol-2.0 | 0
gp1 0 | 0
gp1-3.5 | 0
gp0.3 0 | 0
gp0.4 0 | 0
gp0.5 0 | 0
gp0.6A 0 | 0
gp0.7 0 | 0
gp1.1 0 | 0
gp1.2 0 | 0
gp1.3 0 | 0
gp1.4 0 | 0
gp1.5 0 | 0
gp1.6 0 | 0
gp1.7 0 | 0
gp1.8 0 | 0
gp2.0 0 | 0
gp2.5 0 | 0
gp2.8 0 | 0
gp3.0 0 | 0
gp3.5 0 | 0
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gp4.2 0 | 0
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gp4.7 0 | 0
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gp5.3 0 | 0
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gp7.7 0 | 0
gp8 0 | 0
gp9 0 | 0
gp10A 0 | 0
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gp12 0 | 0
gp13 0 | 0
gp14 0 | 0
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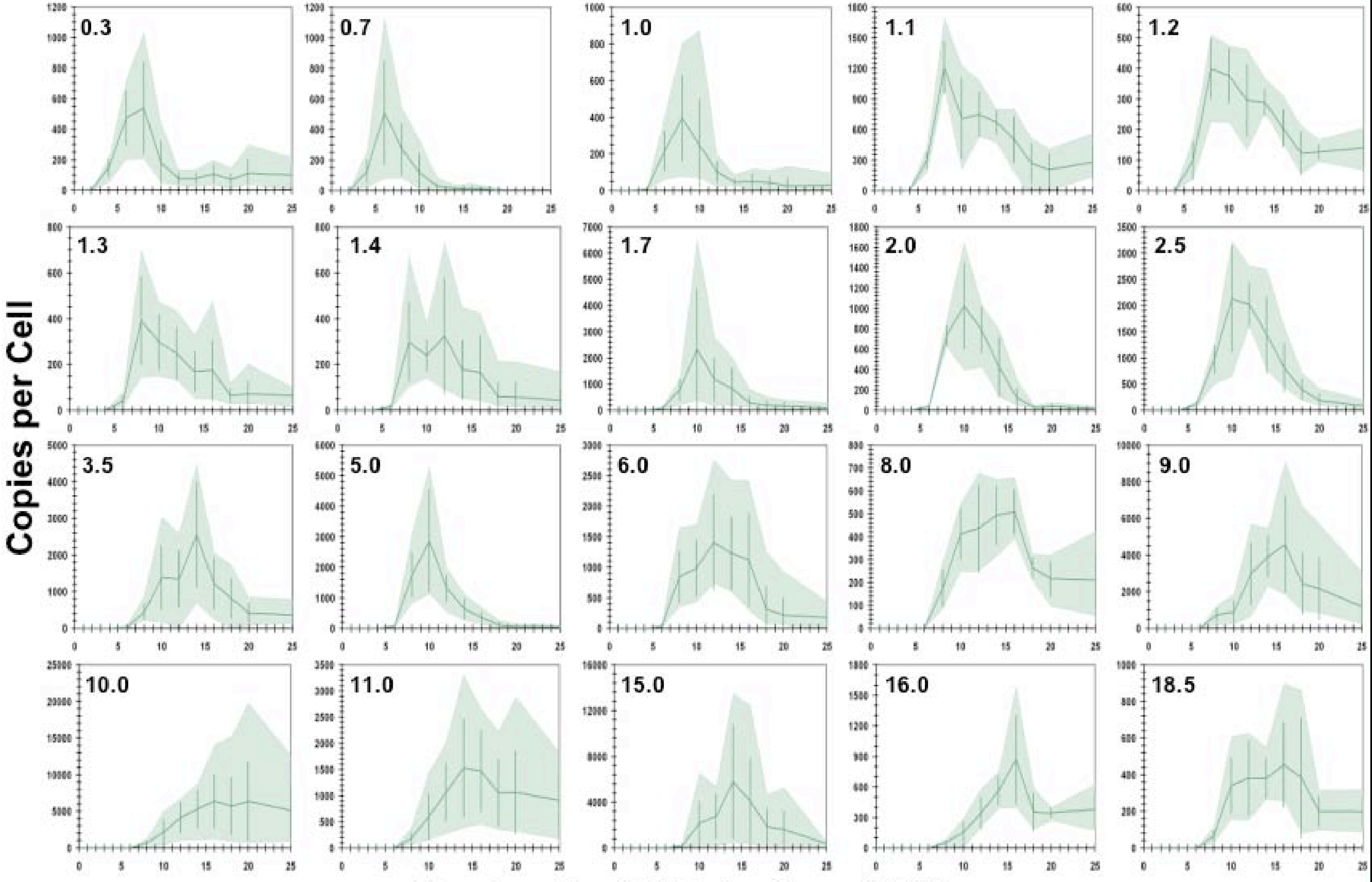
gp19.5 0 | 0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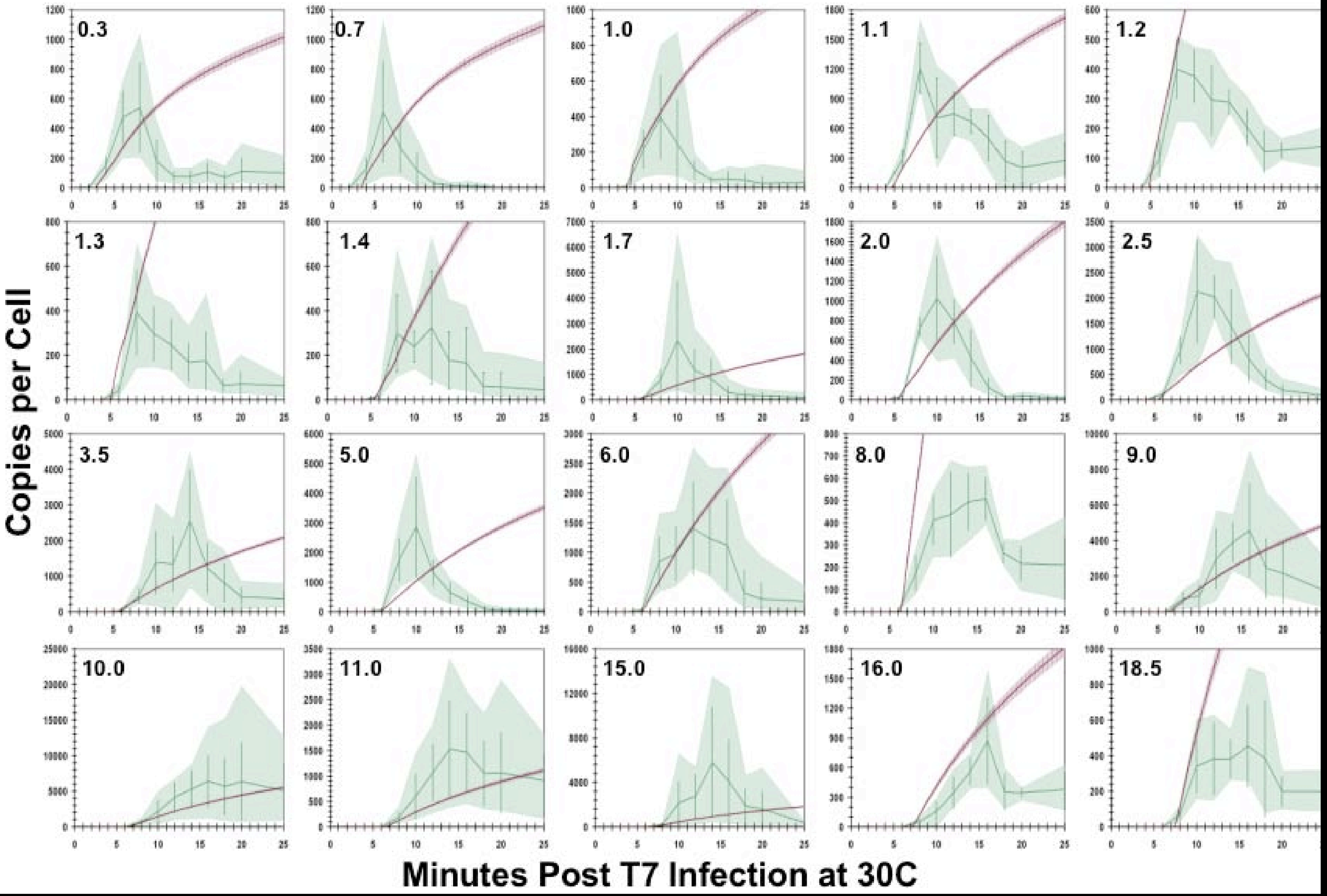
18.5
Minutes Post T7 Infection at 30C


Copies per Cell
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11.0
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16.0
18.5
Minutes Post T7 Infection at 30C

	Gene [a]	Amino acids [b]	M_r [c]	Function [d]
Class I	*0·3*	116	13,678	Inactivates host restriction
	0·4	50	5621	
	0·5	47	4744	
	0·6A	53	6201	
	0·6B	111	(13,250)	
	0·7	359	41,124	Protein kinase
	1	883	98,092	T7 RNA polymerase
	1·1	42	5180	
	1·2	84	10,059	Replication
	1·3	359	41,133	DNA ligase
Class II	*1·4*	51	5446	
	1·5	29	3174	
	1·6	86	9946	
	1·7	195	22,053	
	1·8	48	5781	
	2	63	7043	Inactivates host RNA polymerase
	2·5	231	25,562	Single-stranded DNA-binding protein
	2·8	139	15,617	
	3	148	17,040	Endonuclease
	3·5	150	16,806	Amidase (lysozyme)
	3·8	121	14,329	
	4A	566	62,656	Primase
	4B	503	55,743	Primase
	(4·1)	39	4265	
	(4·2)	112	12,653	
	4·3	70	7927	
	4·5	88	9960	
	4·7	135	15,208	
	5	704	79,692	DNA polymerase
	5·3	118	13,067	
	5·5	98	11,075	
	5·7	68	7280	Permits growth on λ lysogens
	6	347	39,995	Exonuclease
	6·3	37	4088	
Class III	*6·5*	84	9474	
	6·7	87	9207	
	7	132	15,303	Host range
	7·3	98	9937	Host range
	7·7	130	14,737	
	8	535	58,989	Head–tail protein
	9	306	33,766	Head assembly protein
	10A	344	36,414	Major head protein
	10B	397	(41,800)	Minor head protein
	11	196	22,289	Tail protein
	12	793	89,265	Tail protein
	13	138	15,852	Internal virion protein
	14	195	20,836	Internal virion protein
	15	746	84,210	Internal virion protein
	16	1318	143,840	Internal virion protein
	17	552	61,441	Tail fiber protein
	17·5	67	7391	
	18	89	10,145	DNA maturation
	(18·7)	82	9195	
	19	585	66,130	DNA maturation
	(19·2)	84	9264	
	(19·3)	56	6429	
	19·5	49	5434	

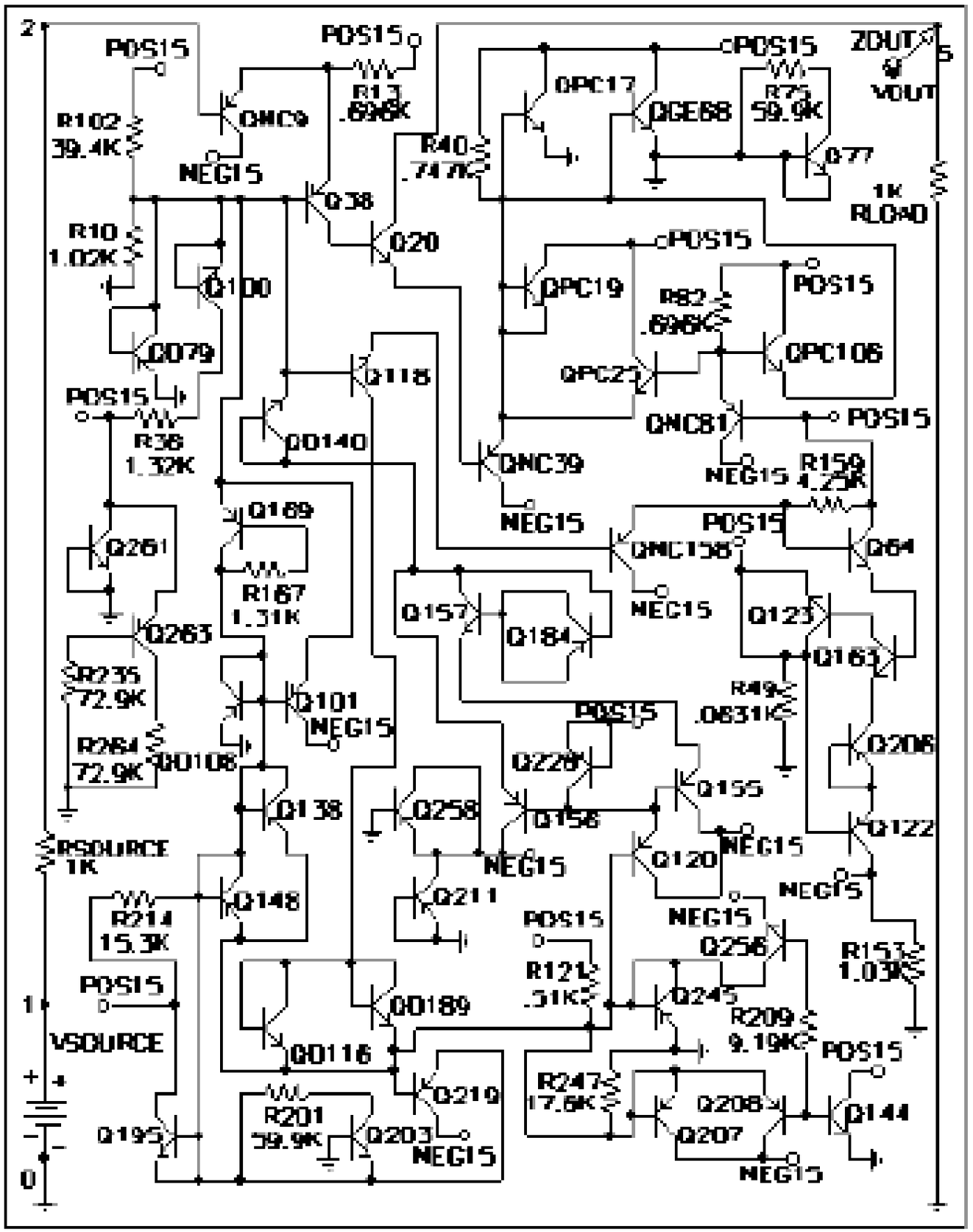
Dunn & Studier (1983)
Journal of Molecular Biology
v166 p477

Wild-type T7 genes 2.8-3

```
-------------------2.8----------------->

...acgcaaagggaggcgacatggcaggttacggcgctaaaggaatccgaaa...

<--3-RBS---><-----------------3----------------
```



J.R. Koza et al.
Automated Synthesis of Computational Circuits using GeneticProgramming,
1997 IEEE International Conference on Evolutionary Computation

----------2.8---------->

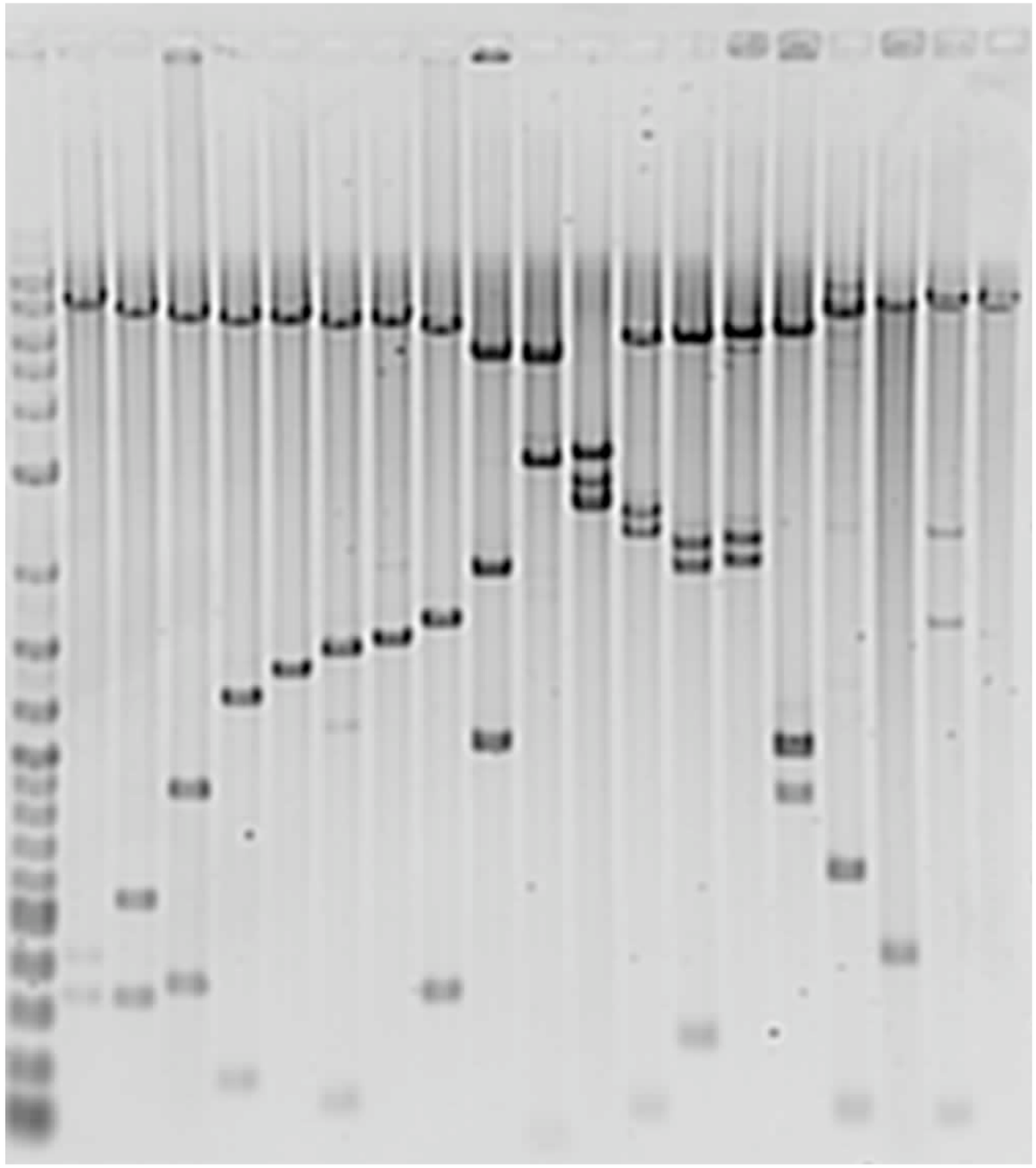
acgcaaagggaggcgacatggcaggttacggcgctaaaggaatccgaaa

<--3-RBS---><----------3----------

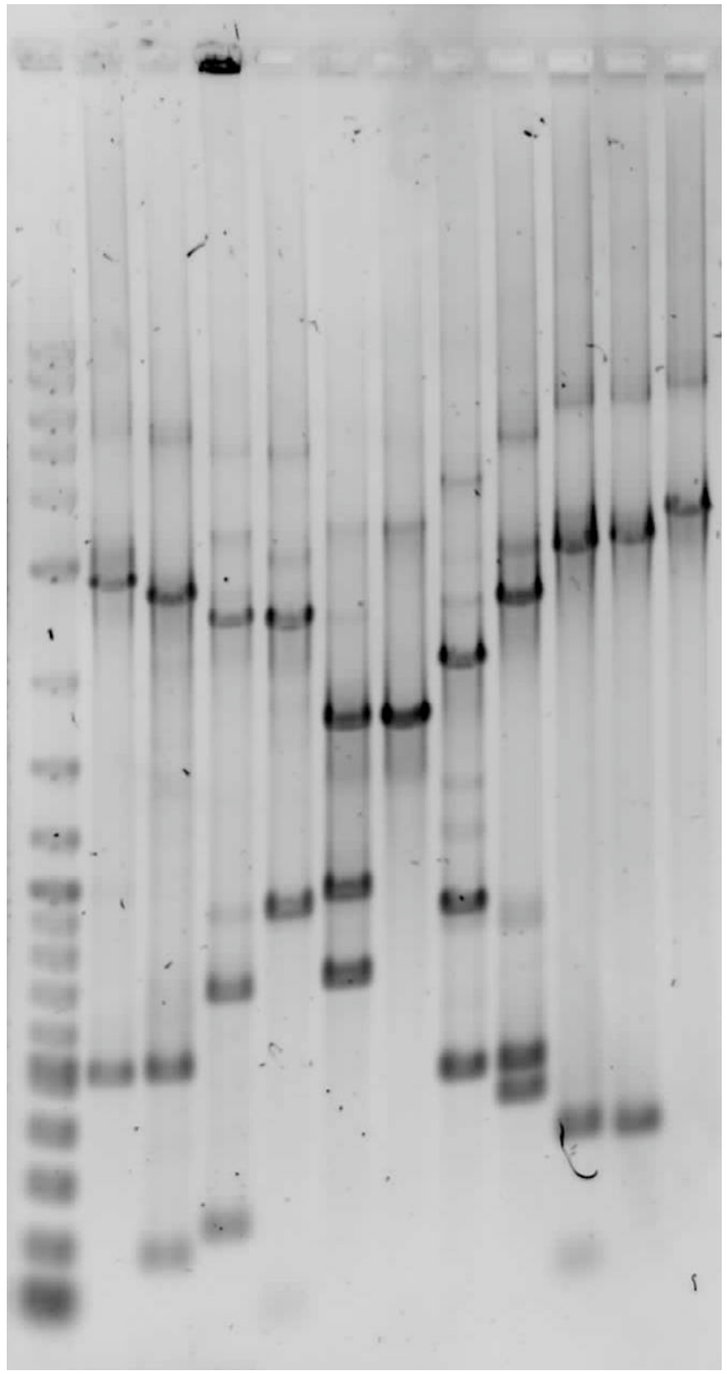
acgcaaGgggagAcgacaCggcaggttacggcgctaaggatccggccgcaaagggaggcgacatggcaggttacggcgctaaa

----------2.8---------><D28R|D29L><--3RBS------><----------3----

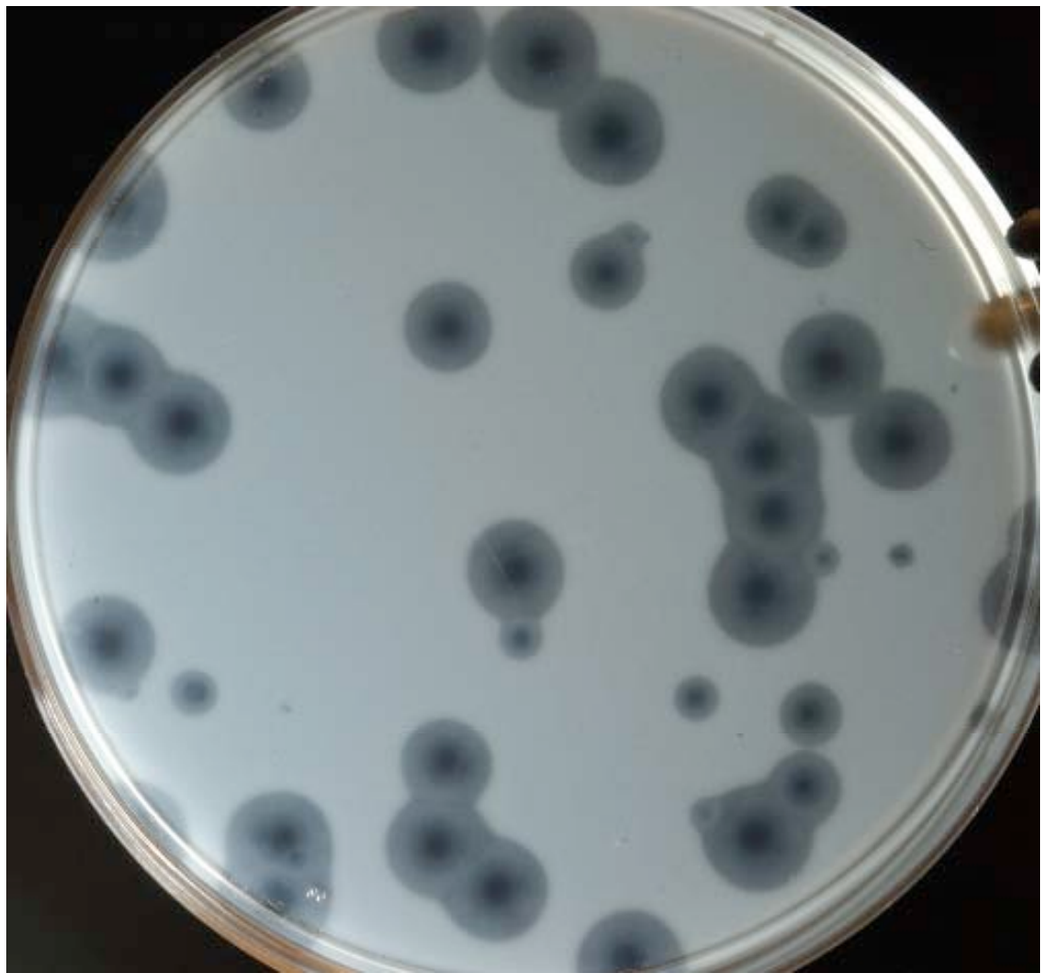
Section *alpha*
(1 → 8,311 bp)



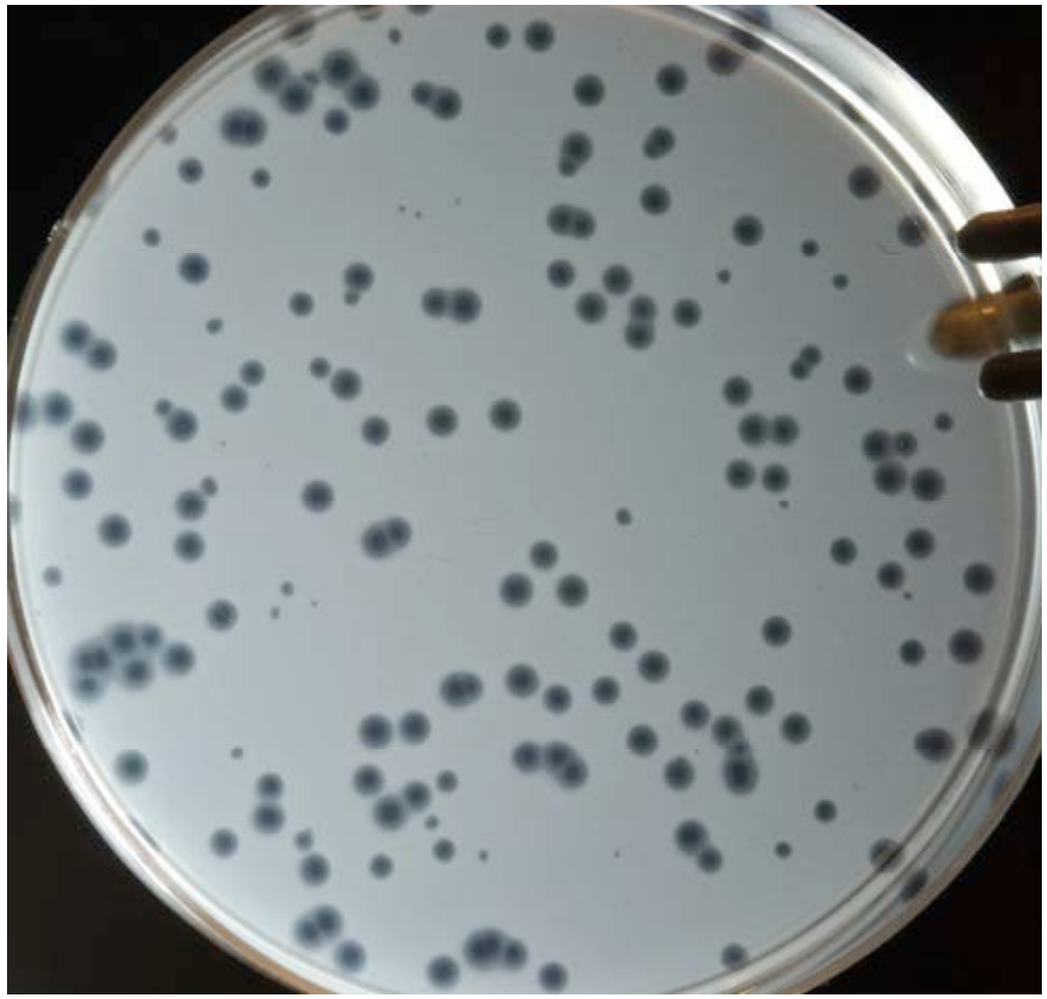
Section *beta*
(8,311 → 12,179 bp)



Wild-Type T7 ($T7^{+}$)



$T7.1_{[1\text{-}12,179]}:T7^{+}{}_{[11,516\text{-}39,937]}$



the engineering of biology

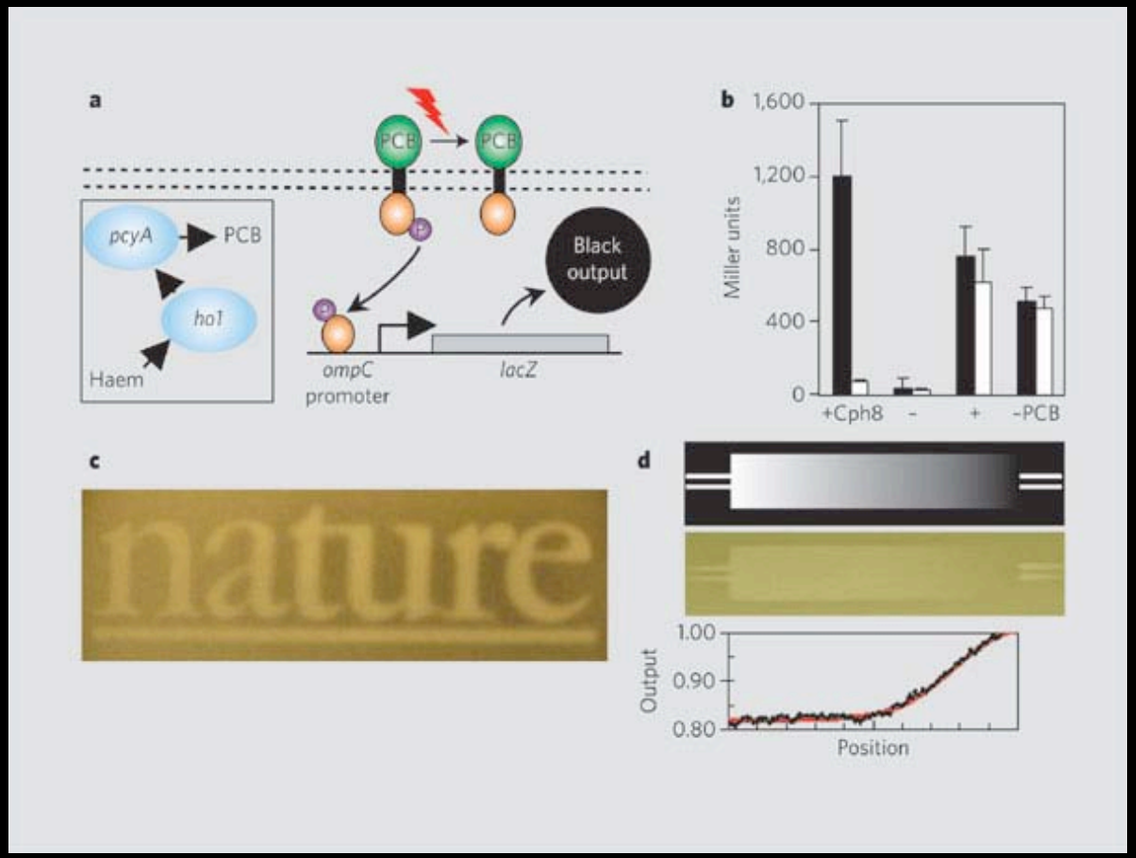
Vol 438 | 24 November 2005 nature

BRIEF COMMUNICATIONS

Engineering *Escherichia coli* to see light

These smart bacteria 'photograph' a light pattern as a high-definition chemical image.

Anselm Levskaya*, Aaron A. Chevalier†, Jeffrey J. Tabor†, Zachary Booth Simpson†, Laura A. Lavery†, Matthew Levy†, Eric A. Davidson†, Alexander Scouras†, Andrew D. Ellington†‡, Edward M. Marcotte†‡, Christopher A. Voigt*§||



doi:10.1016/j.jmb.2005.10.076 J. Mol. Biol. (2006) 355, 619–627

JMB

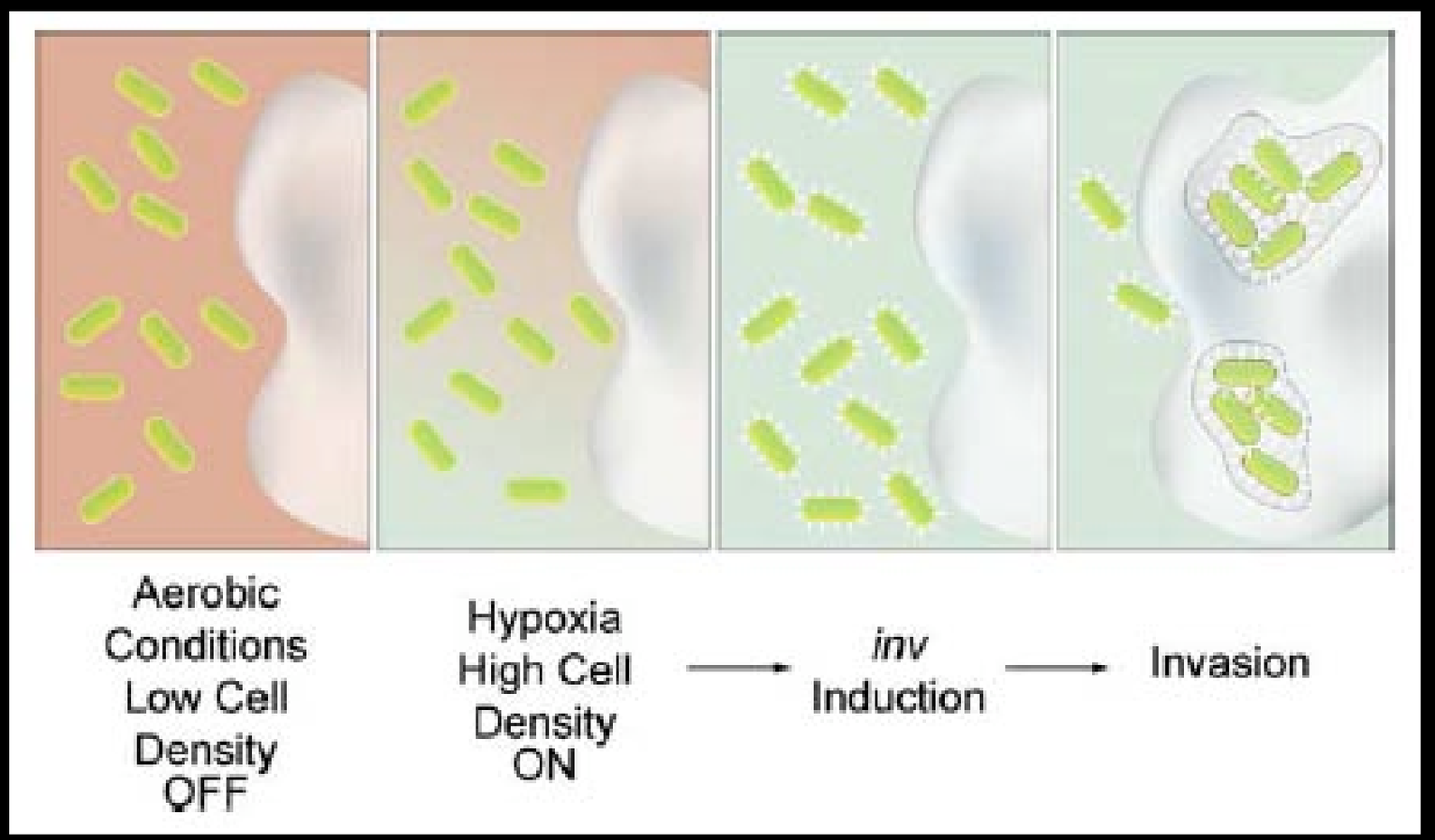
Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria

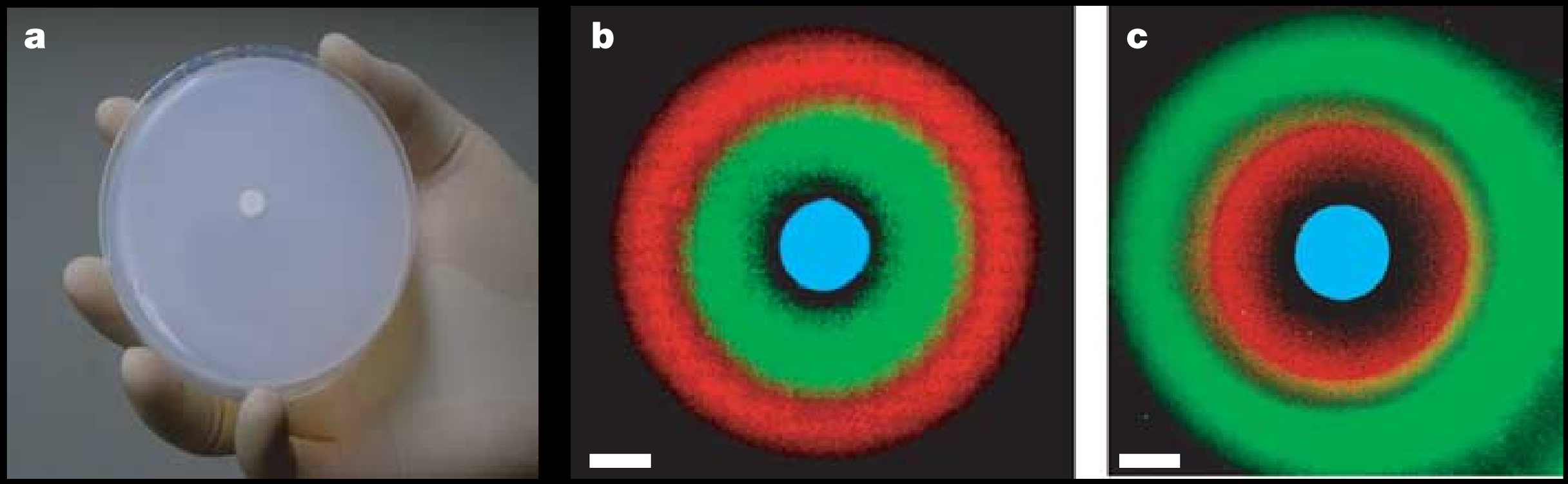
J. Christopher Anderson[1,3], Elizabeth J. Clarke[3], Adam P. Arkin[1,2*] and Christopher A. Voigt[2,3]



A synthetic multicellular system for programmed pattern formation

Subhayu Basu[1], **Yoram Gerchman**[1], **Cynthia H. Collins**[3], **Frances H. Arnold**[3] **& Ron Weiss**[1,2]

[1]*Department of Electrical Engineering and* [2]*Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA*
[3]*Division of Chemistry and Chemical Engineering, California Institute of Technology 210-41, Pasadena, California 91125, USA*



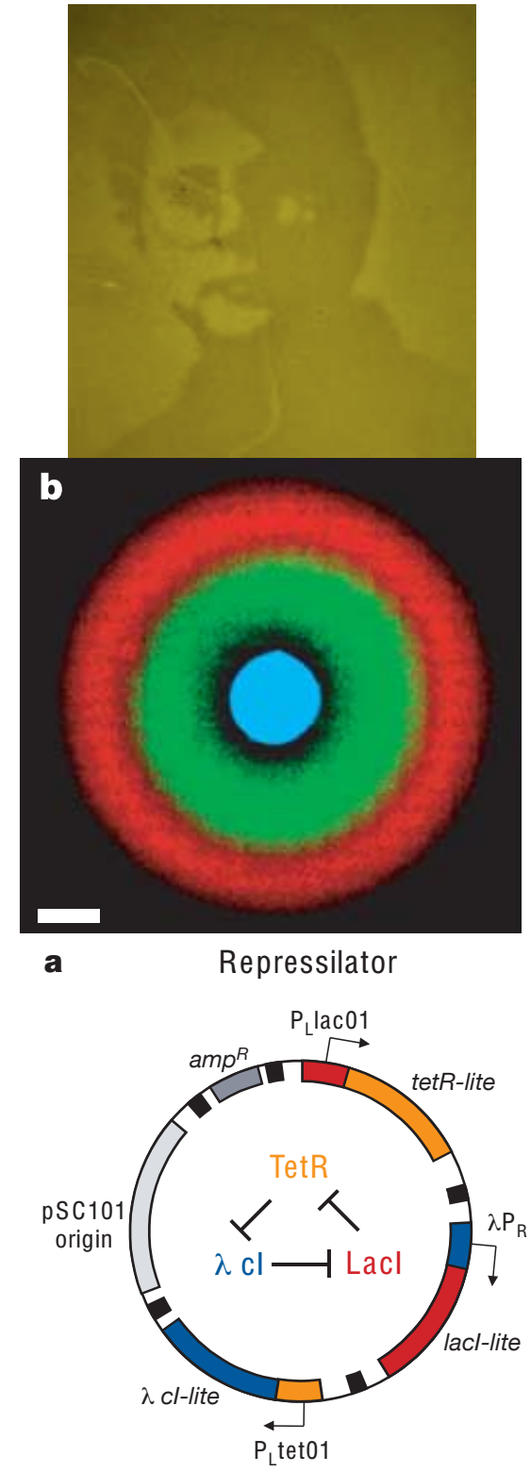
A synthetic oscillatory network of transcriptional regulators

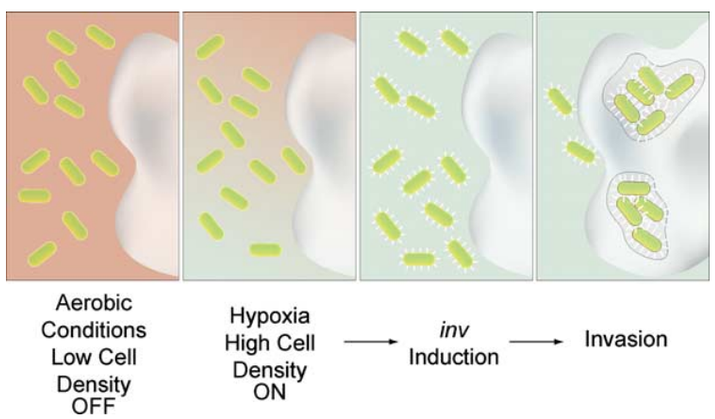
Michael B. Elowitz & Stanislas Leibler

Design & Construction

Devices???

Systems???





1. Recombinant DNA
2. PCR
3. Automated sequencing
4. DNA synthesis
5. Standardization
6. Abstraction

DNA Synthesis


GLEN RESEARCH
10-1030-5S
10-1010-1S
10-1020-5S
10-1000-1S

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 this DNA!

Abstraction

DNA is genetic material.

TAATACGACTCACTATAGGGAGA

Parts are basic biological f(x)'s encoded via genetic material.

R0083

Type: **Promoter**
Family: **Protein:DNA**
Activity: **2 PoPS (max.)**
Cell Type: **Any**
Requires: **C0083**
Temp: **< Tm**
Issues: **None**
License: **Public**

Devices provide human-defined f(x)'s using one or more parts.

Q005 I
(INVERTER)

Systems provide human-defined f(x)'s using one or more devices.

Systems

Devices

Parts

DNA

Standards

ON A SYSTEM OF

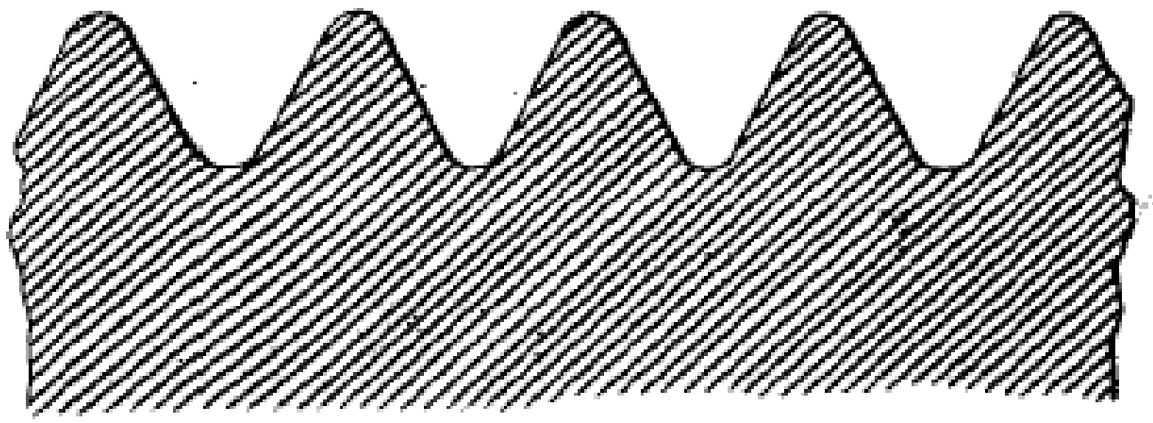
SCREW THREADS AND NUTS.

By WILLIAM SELLERS.

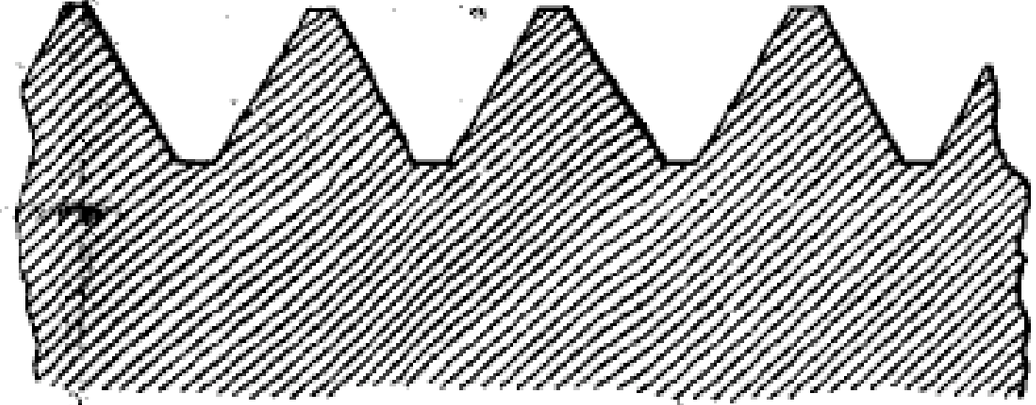
[Read before the FRANKLIN INSTITUTE, April 21, 1864.]

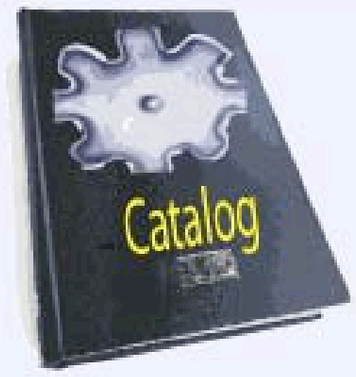
The importance of a uniform system of screw threads and nuts is so generally acknowledged by the engineering profession, that it needs no argument to set forth its advantages; and in offering any plan for their acceptance, it remains only to demonstrate its practicability and its superiority over any of the numerous special proportions now used by the different manufacturers. In this country no organized attempt has as yet been made to establish any system, each manufacturer having adopted whatever his judgment may have dictated as the best,

— FIG. 2 —



— FIG. 3 —





Registry of Standard Biological Parts

jump to part

BBa_

navigation

- Main Page
- Browse Part Types
- iGEM Wiki
- Community portal
- Recent changes
- Recent part changes

resources

- User Accounts
- Add a Part
- Part Searches
- DNA Repositories
- Sequence Analysis
- Assembly Tool
- Help

search

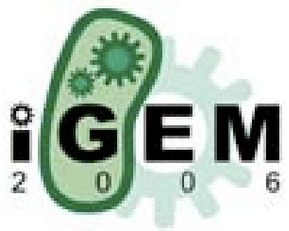
Go Search

toolbox

- What links here

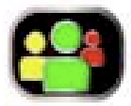
Browse Parts by Type

Browse Parts by School



Featured Parts

Help & Documentation



Users & Groups

Registry Toolbox

Add a part

Search Parts

DNA Repositories

Sequence Analysis

Latest News

- [8/01/06] We have contact information for the creators of parts. You can access this information when you access "Hard Information" of a part.
- [8/01/06] A table made for yeast parts is now available on the Part Types page

Report any bugs here | Request new features here | See new features here

BBa_F2620

3OC$_6$HSL → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 5 October 2006

Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC$_6$HSL is controlled by a tetR regulatable operator (BBa_R0040). Device input is 3OC$_6$HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a logical AND function.

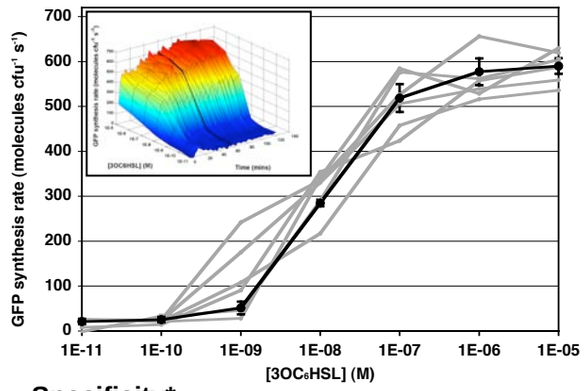
Characteristics

Input Swing: **0.1 to 1000** nM 3OC$_6$HSL, exogenous
Output Swing: **21±3 to 590±9** GFP molecules cfu^{-1} s^{-1}
Switch Point: **10 nM** 3OC$_6$HSL, exogenous
LH Response: **9.7 min** ($t_{50\%}$), **17 min** ($t_{90\%}$)

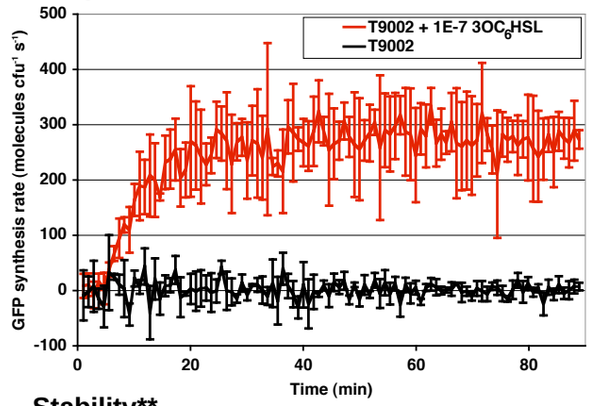
Key Components

BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator

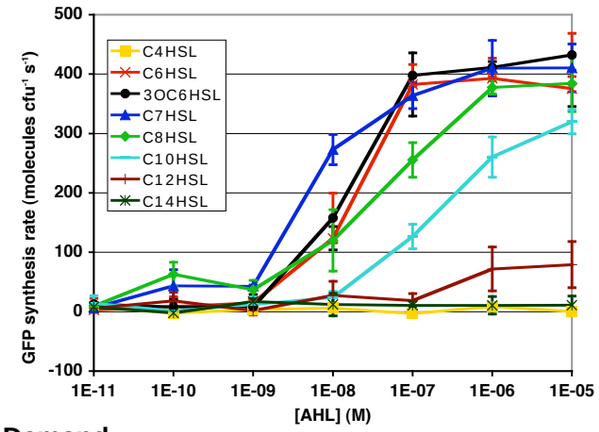
Transfer Function*



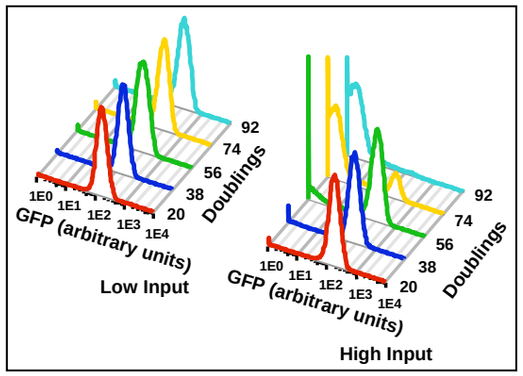
Response Time*



Specificity*



Stability**



Demand

Translation Demand: 5040/141600 charged tRNA cfu^{-1} s^{-1}
(low/high input) 336/9449 ribosomes sequestered cfu^{-1}

Compatibility

Chassis: Compatible with *MC4100*, *MG1655*, and *DH5α*
Plasmids: Compatible with *pSB3K3* and *pSB1A2*
Devices: Compatible with *E0240*, *E0430* and *E0434*
Crosstalk with systems containing *TetR (C0040)*
Signaling: Crosstalk with input molecules similar to *3OC$_6$HSL*

Stability

Genetic: >**92/74** replication events*
Performance: >**92/74** replication events*

Conditions (abridged)

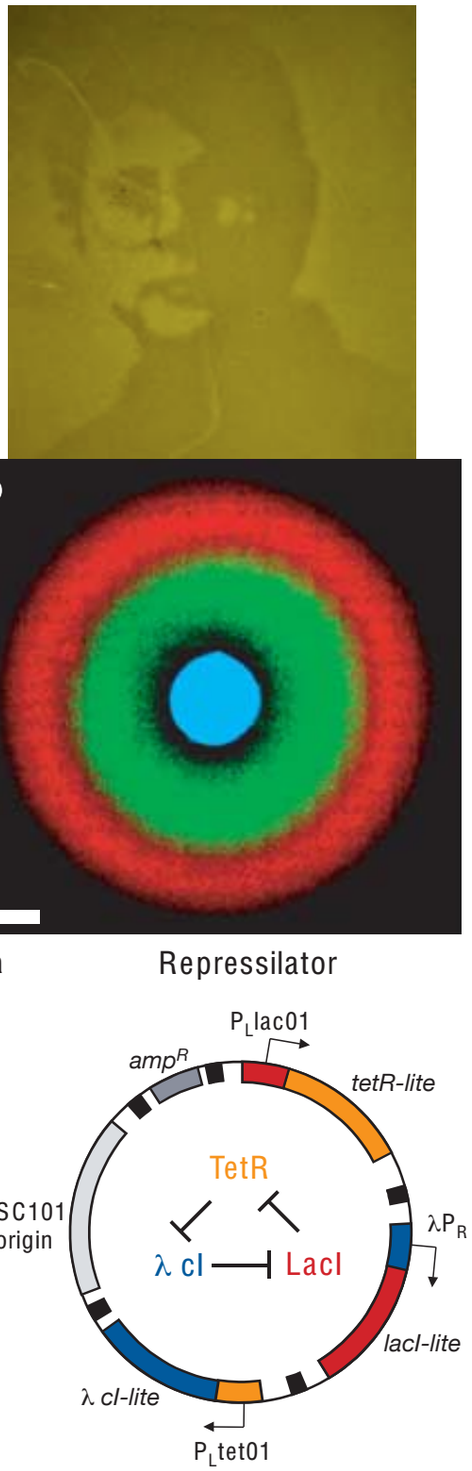
Output: Indirect via *BBa_E0240*
Vector: pSB3K3
Chassis: MG1655
Culture: Supplemented M9, 37ºC
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

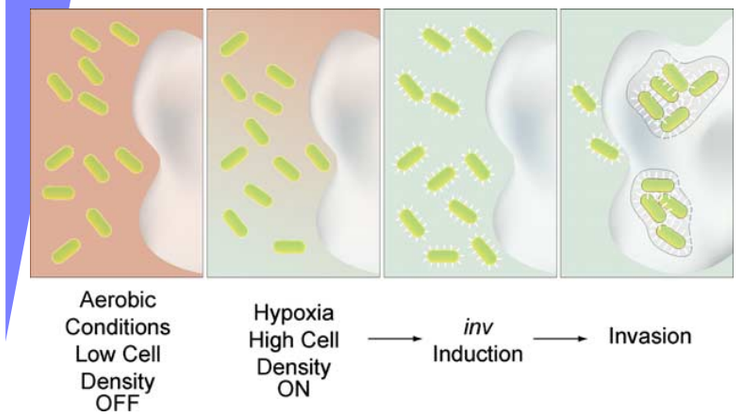
Signaling Devices

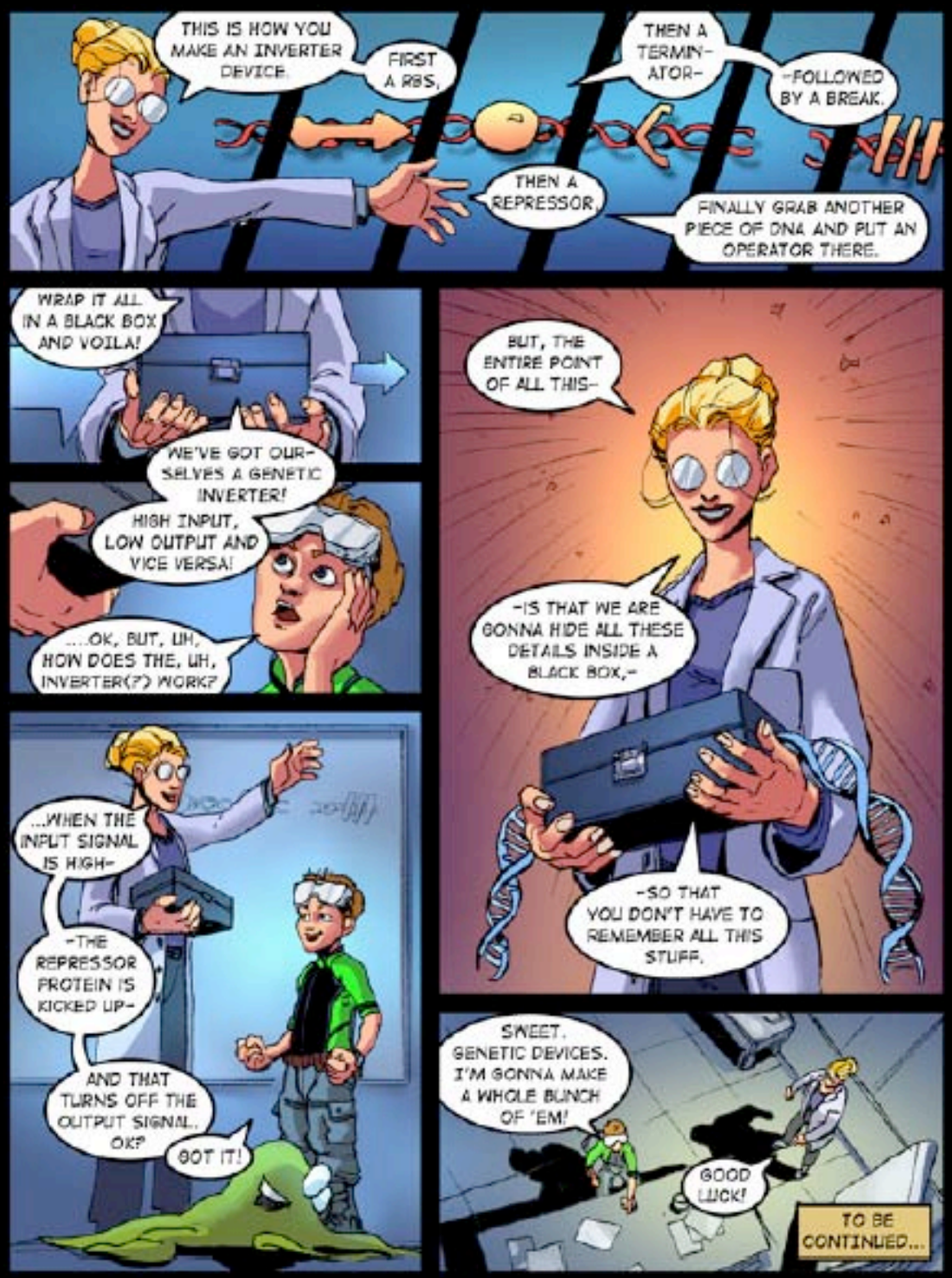
Design

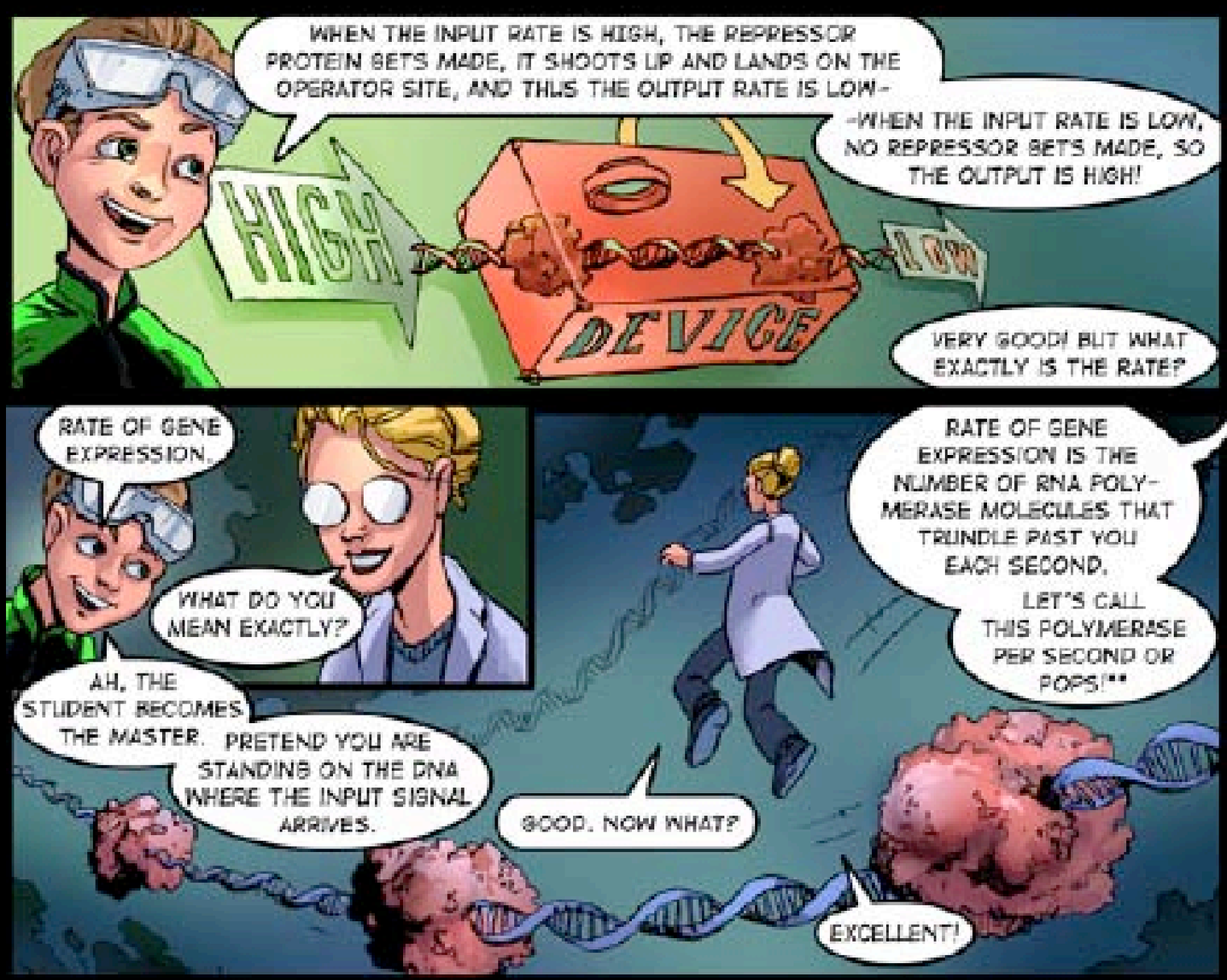
Construction

Devices and Systems









Endy D, Deese I, Wadey C
Adventures in Synthetic Biology
Nature 24 November 2005
http://openwetware.org/wiki/Adventures

Designs on life

Earlier this month, students from around the world locked horns in competition. Their challenge was to build functioning devices out of biological parts. Erika Check finds out how they got on.

Even if you're thinking big, you usually have to start small. Especially, as a group of Swiss students found, when big means counting to infinity. The team was drawing up a blueprint for the world's first counting machine made entirely of biological parts. Although they had their sights on loftier numbers, they opted to go no higher than two. If the plan worked, it would be a proof-of-principle for a much larger tallying device.

The group, from the Federal Institute of Technology (ETH) in Zurich, was one of 17 teams unveiling their projects at the first international Intercollegiate Genetically Engineered Machine (iGEM) competition, held at the Massachusetts Institute of Technology (MIT) in Cambridge on 5 and 6 November. The event attracted students from all over the world to design and build machines made entirely from biological components such as genes and proteins. They drew up grand designs for bacterial Etch-a-Sketches, photosensitive t-shirts, thermometers and sensors. And if none of the designs succeeded completely, that was more because of the limitations of the nascent science of synthetic biology than any lack of enthusiasm, creativity or hard work.

Synthetic biology aims to merge engineering approaches with biology. Researchers working at the most basic level are copying simple biological processes, such as the production of a protein from a gene. They break the process down into its component elements, such as a gene and the pieces of DNA

E. CHECK

Bidding for glory: teams from the ETH in Zurich (top), Cambridge, UK, (bottom right) and Massachusetts at the first International Intercollegiate Genetically Engineered Machine competition.

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iGEM - The international Genetically Engineered Machine competition

iGEM is an international arena where student teams compete to design and assemble engineered machines using advanced genetic components and technologies. Learn more.

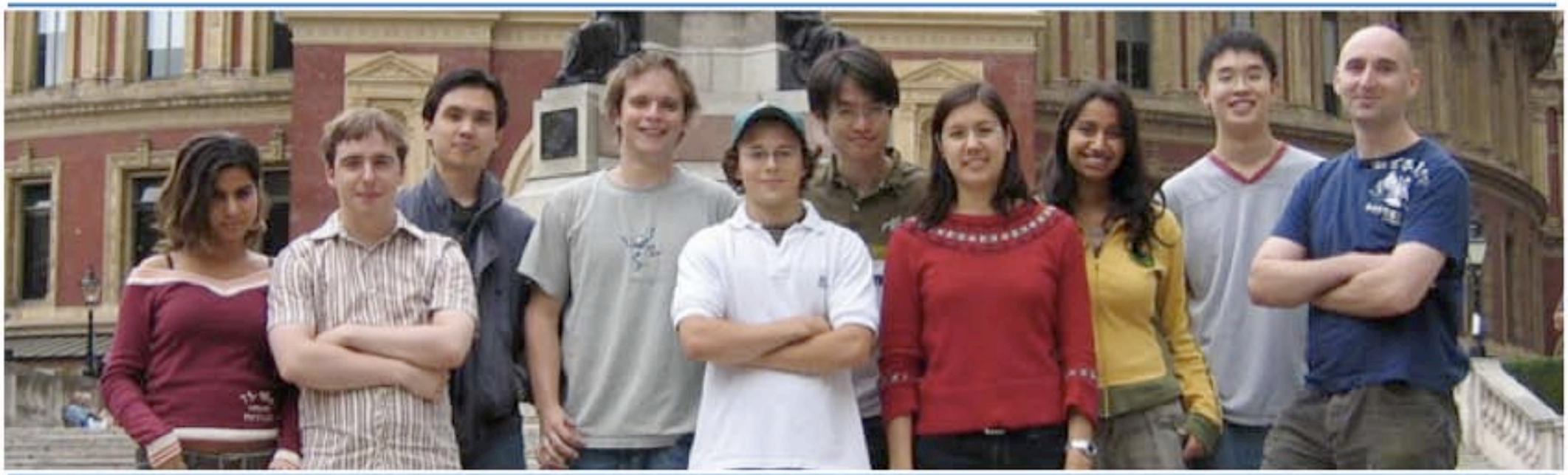
Meet the 38 teams participating in 2006

See teaching resources to learn about our unique methods

Ready to build? Go to the Registry for BioBrick parts and tools

The Ambassador Program helps teams around the globe succeed

Give and get help information and FAQs here.



The Imperial College iGEM Team: Deepti Aswani, Tom Hinson, Chueh-Loo Poh, John Chattaway, Jonathan Wells, Jiongjun Bai, Christin Sander, Farah Yohra, John Sy, Vincent Rouilly

Current Events

- Hotel@MIT discounted rooms: see the Jamboree page for details.
- The Boston-area schools had a get together on July 28th - find out more
- The UK teams had a meeting in Cambridge on July 24th/25th - check the report
- Browse or contribute to iGEM pictures or videos. *Media wanted!*

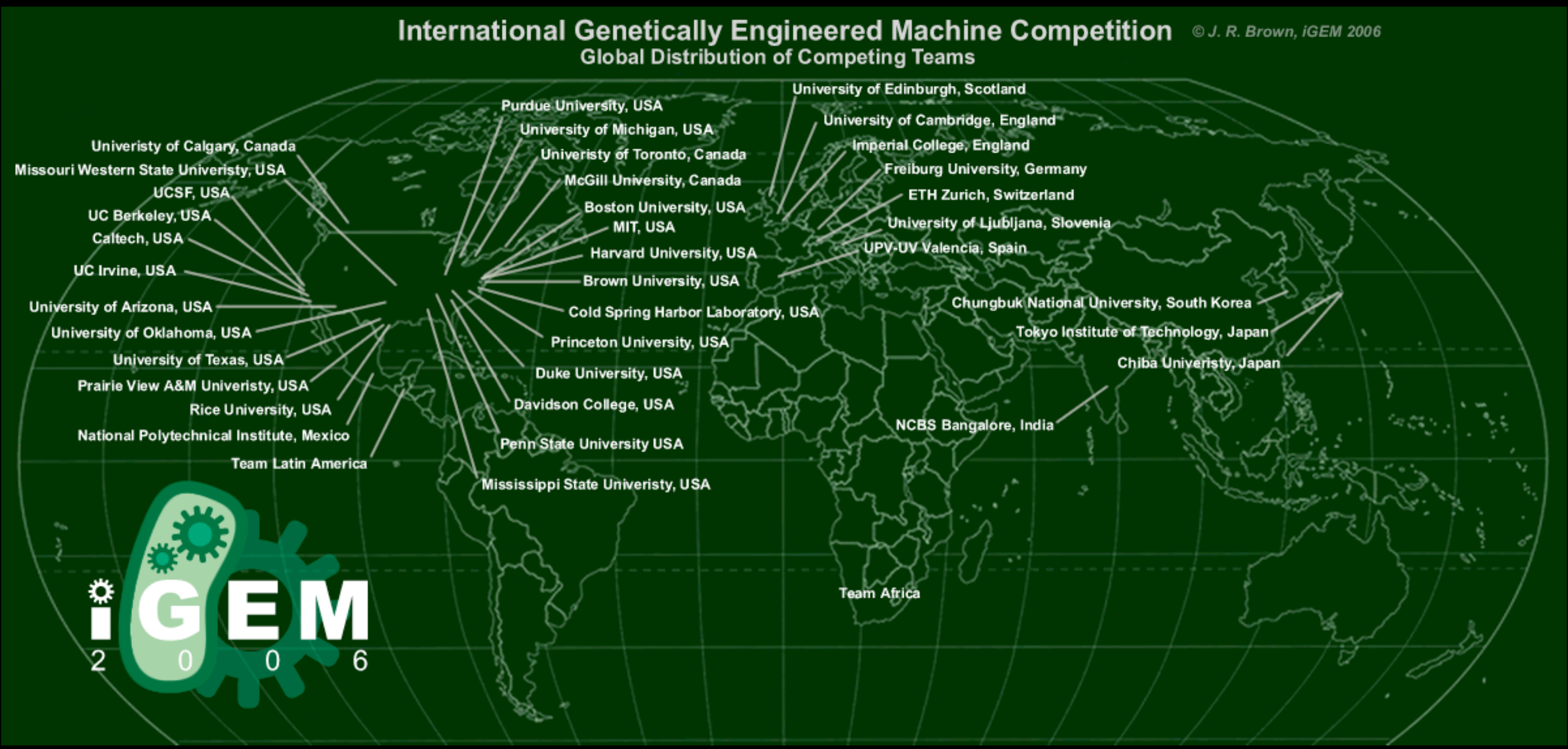
iGEM News

News reports focusing on iGEM and the Registry from the greater scientific community and beyond...

- >> Synthetic Biology: Life 2.0 the Economist 8/31/2006
- Organism from Scratch (in german) Neue Zürcher Zeitung, Switzerland - 08/24/06
- Imperial pioneers new biological engineering parts Imperial College News 08/11/06
- New center to focus on synthetic biology MIT News Office 08/04/06

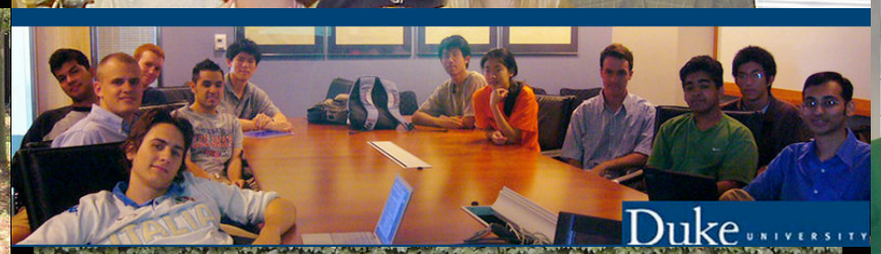
International Genetically Engineered Machine Competition

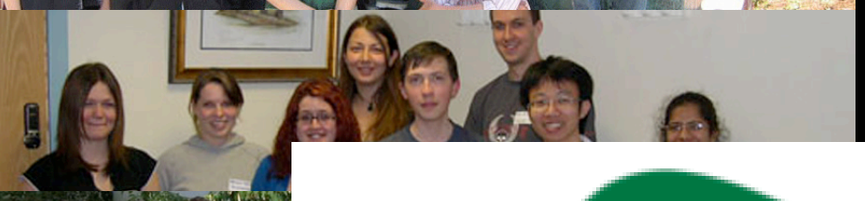
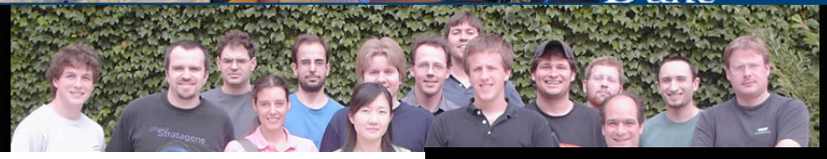
Global Distribution of Competing Teams

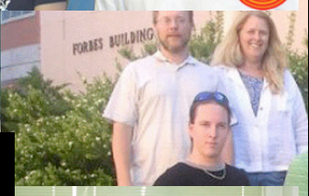


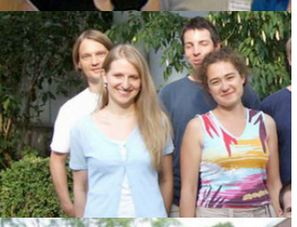
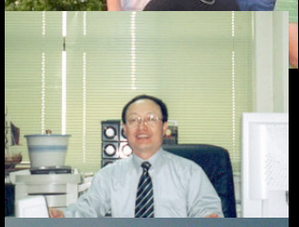
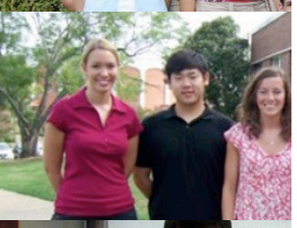




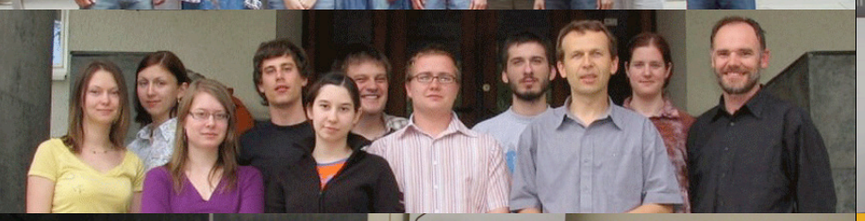


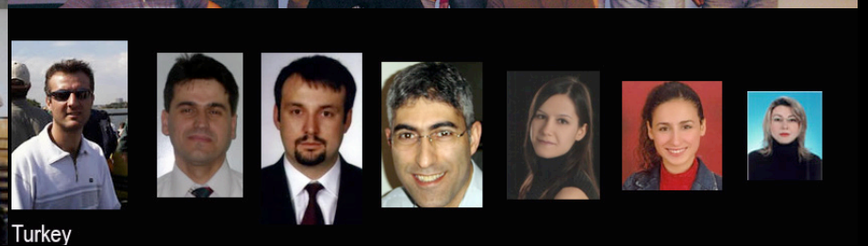









2006 iGEM Jamboree
4-5 November
MIT Building 32

We can make it much easier to engineer biology

We have a responsibility to help lead the overwhelmingly constructive development and application of future biological technologies.

Thank you!