Synthetic Biology for computer scientists in 2 hours

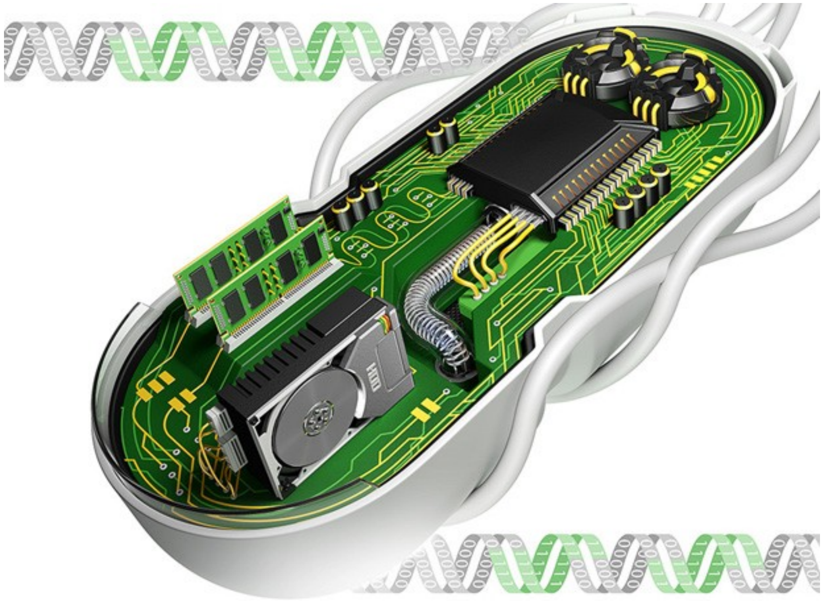

Image Courtesy of Liang Zong and Yan Liang

Inteligencia Artificial Laboratorio
POLITÉCNICA
www.lia.upm.es

ISBBC17
Universidad de Valencia
30/06/2017

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Outline of the talk

1. Basic biology concepts: DNA and gene expression
2. DNA Computing: basic concepts
3. Synthetic Biology: FAQs
4. LIA group: SynBio projects, software (BioBlocks, gro)

Outline of the talk

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DNA structure

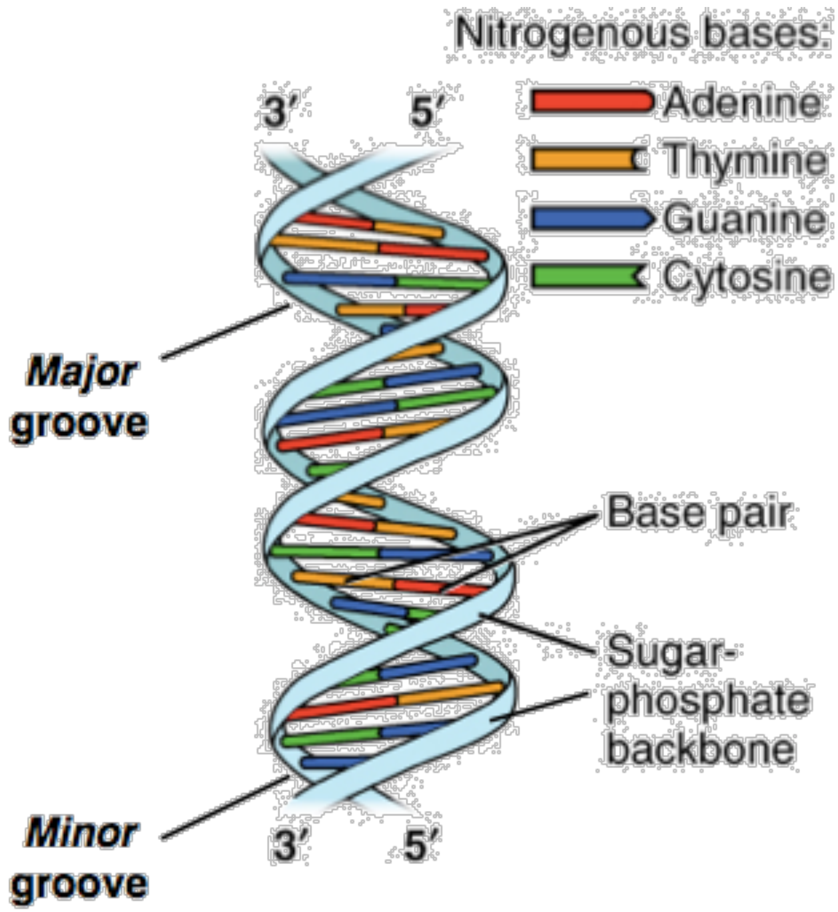
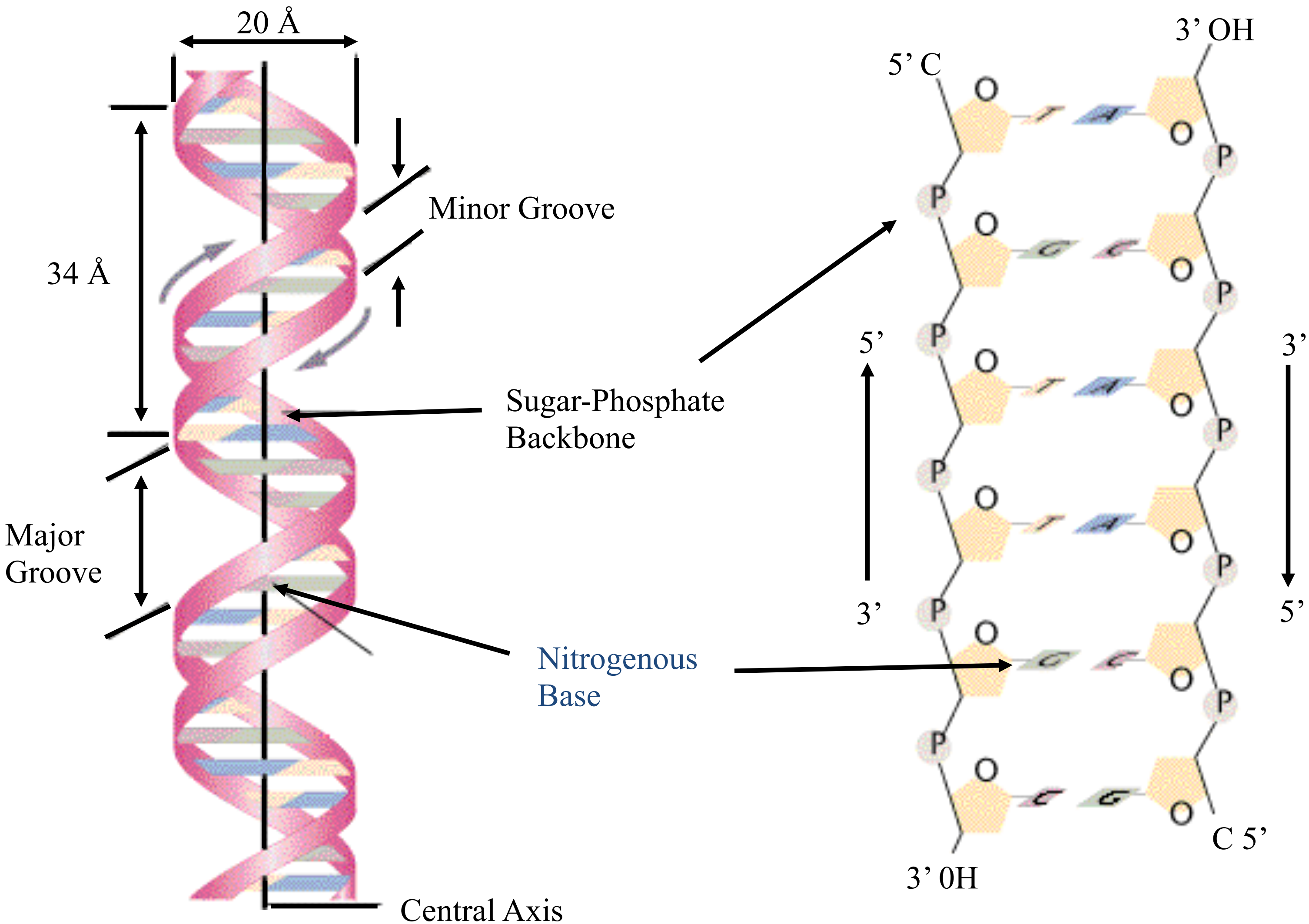


Image modified from "[DNA structure and sequencing: Figure 3]()," *by OpenStax College, Biology (*[CC BY 3.0]()*).*

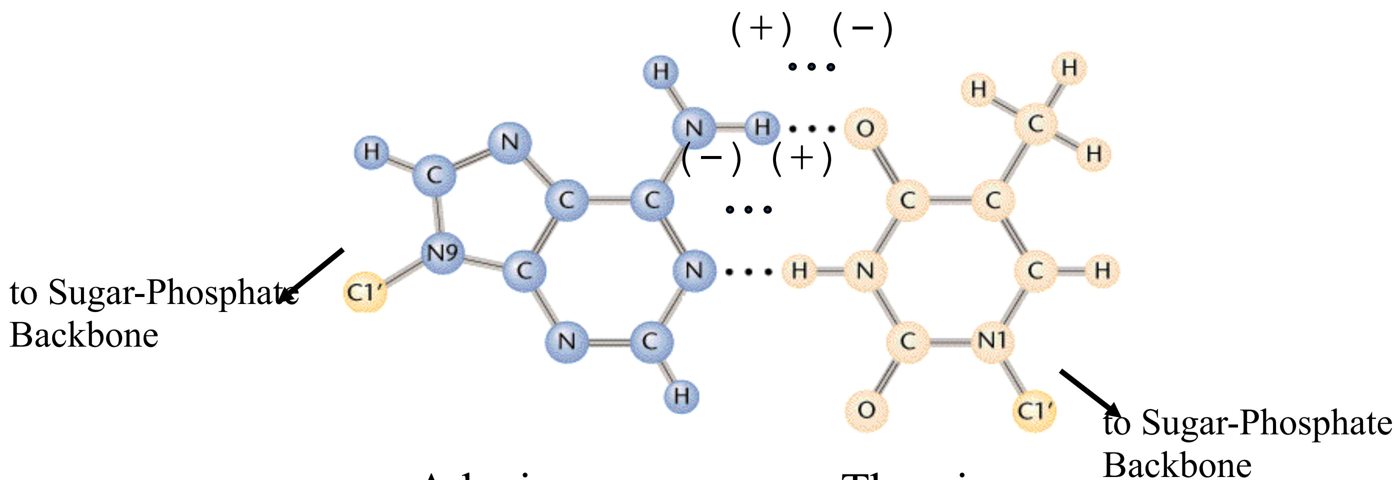
DNA structure



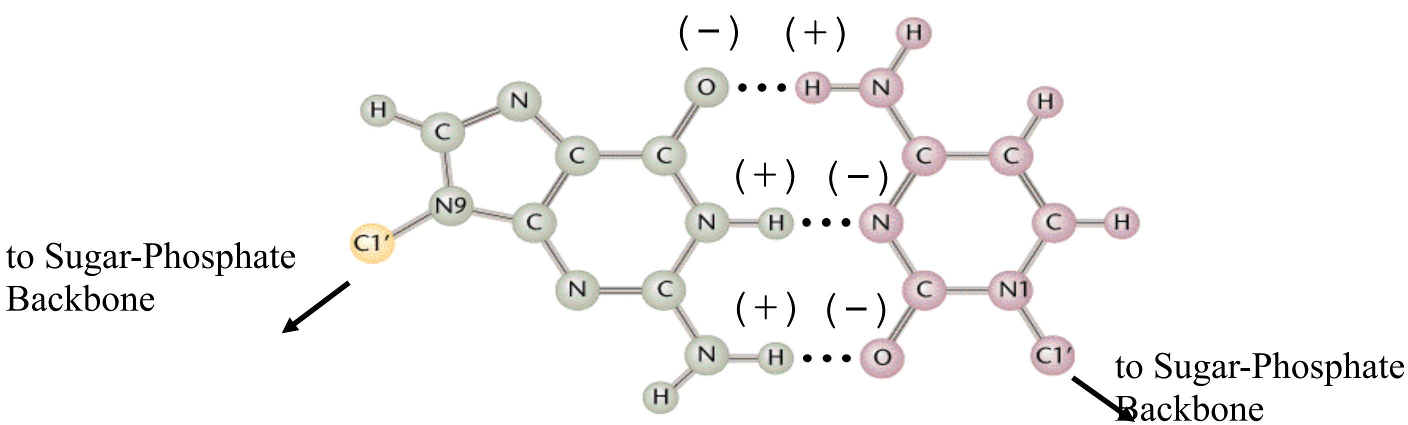
Watson-Crick complementarity: A-T C-G

Adenine – Thymine

Cytosine - Guanine

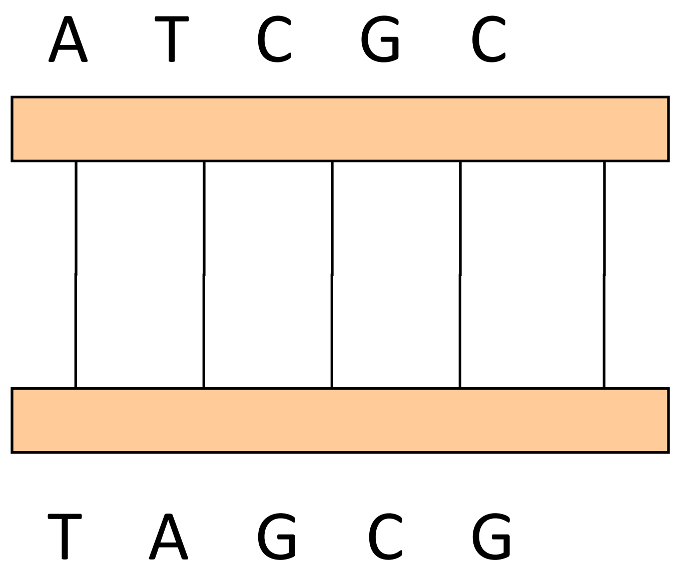


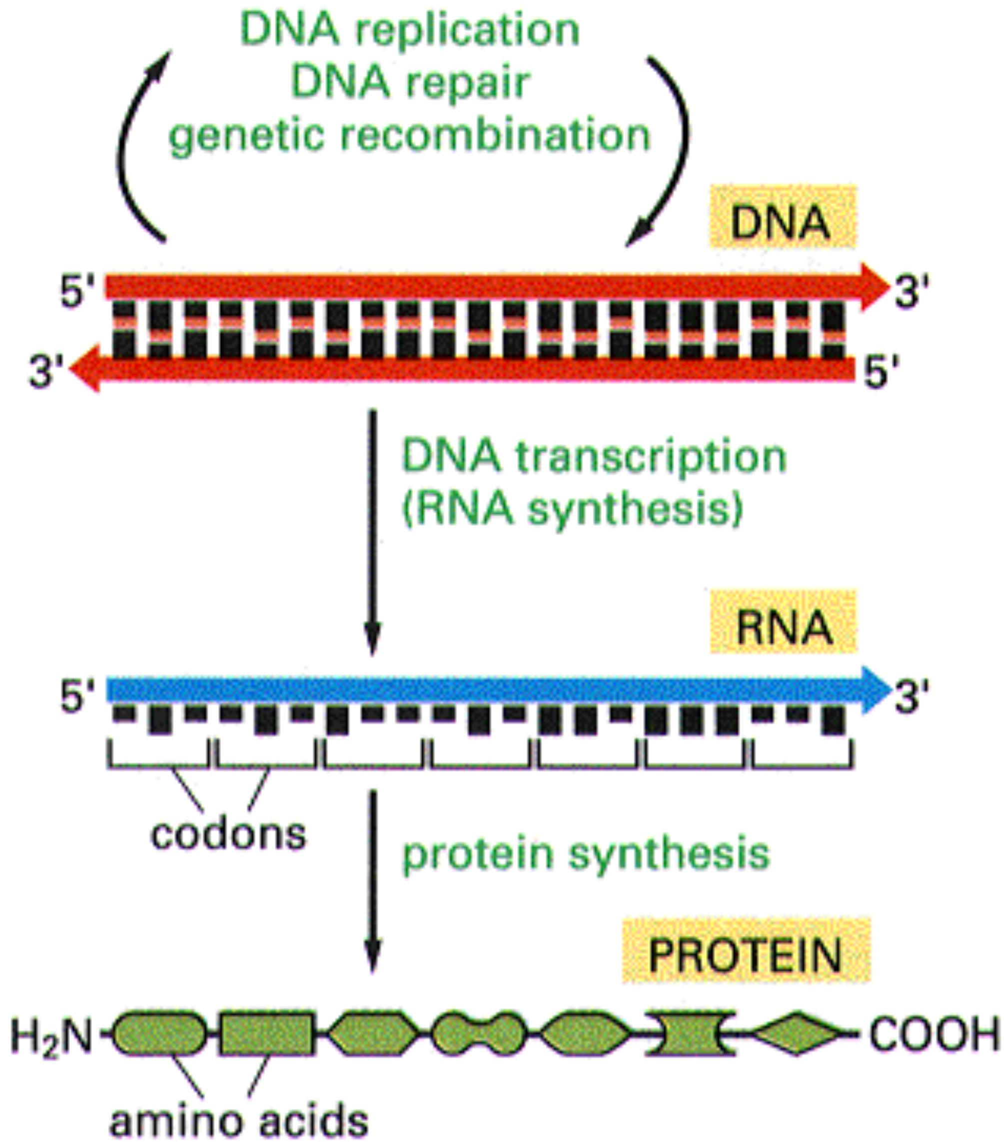
Adenine Thymine

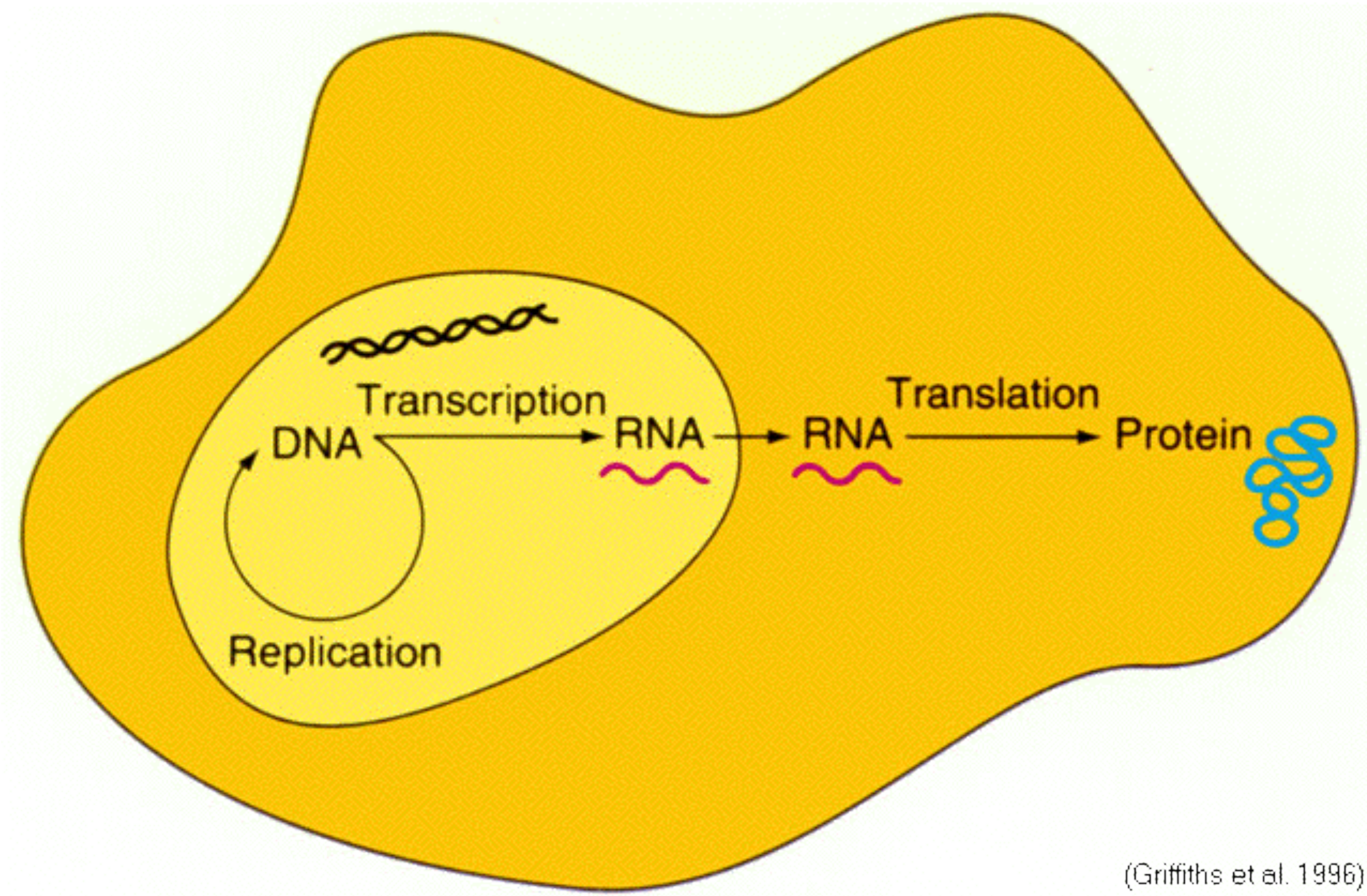


Guanine Cytosine

Hybridization and renaturation

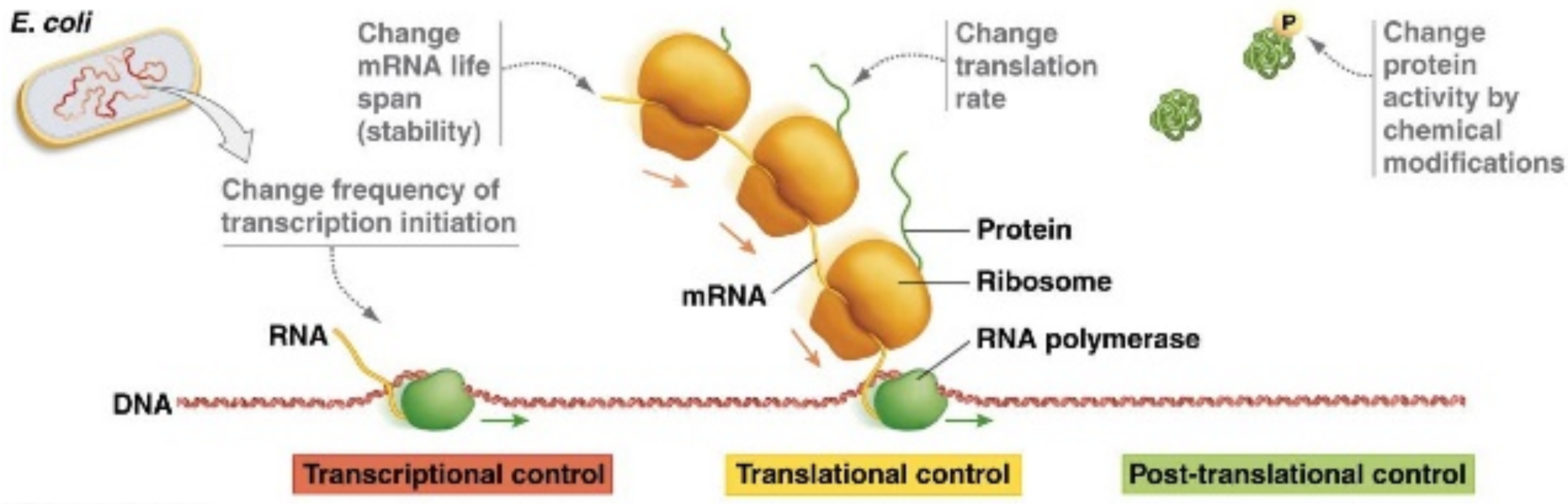



DNA replication
DNA repair
genetic recombination
DNA
5'
3'
3'
5'
DNA transcription
(RNA synthesis)
RNA
5'
3'
codons
protein synthesis
PROTEIN
H2N
COOH
amino acids



(Griffiths et al. 1996)

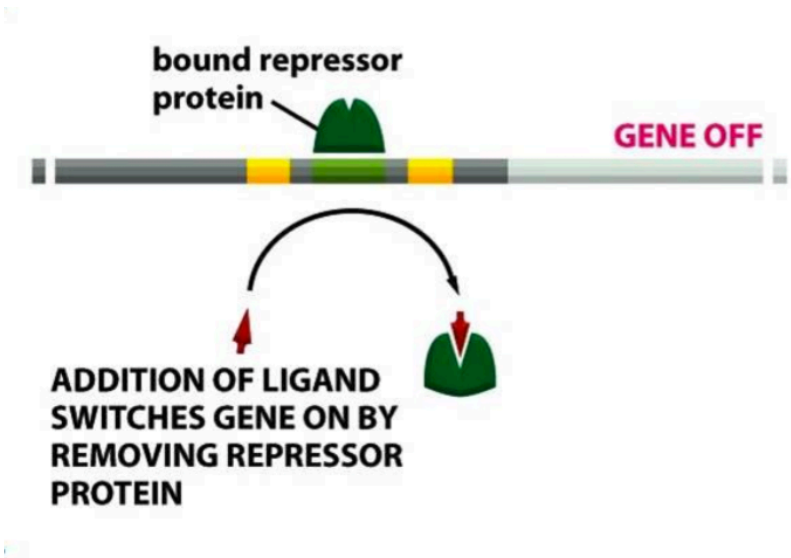
Regulation of Gene Expression

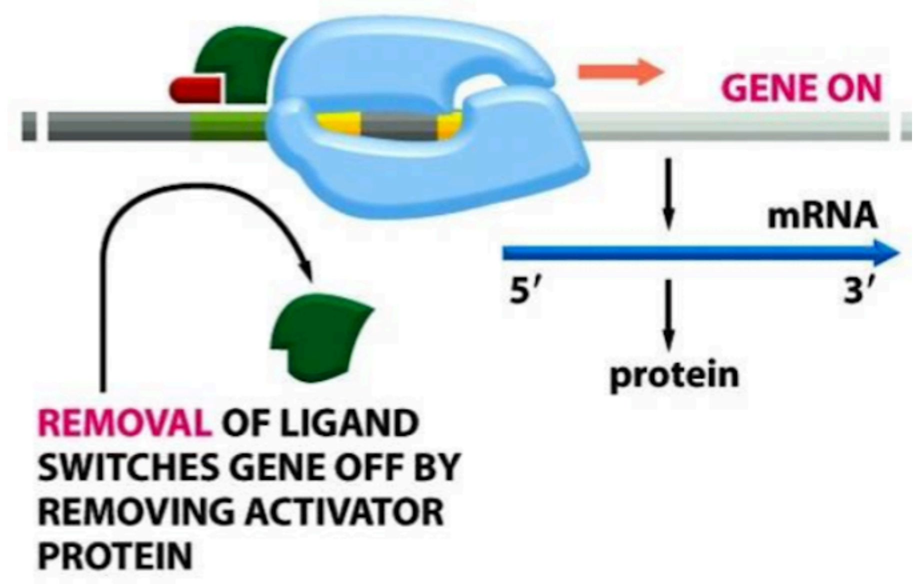


- Gene expression can be regulated:
  - During transcription (transcriptional control).
  - During translation (translational control).
  - After translation (post-translational control).

Gene expression regulation: Transcription factors and promoters

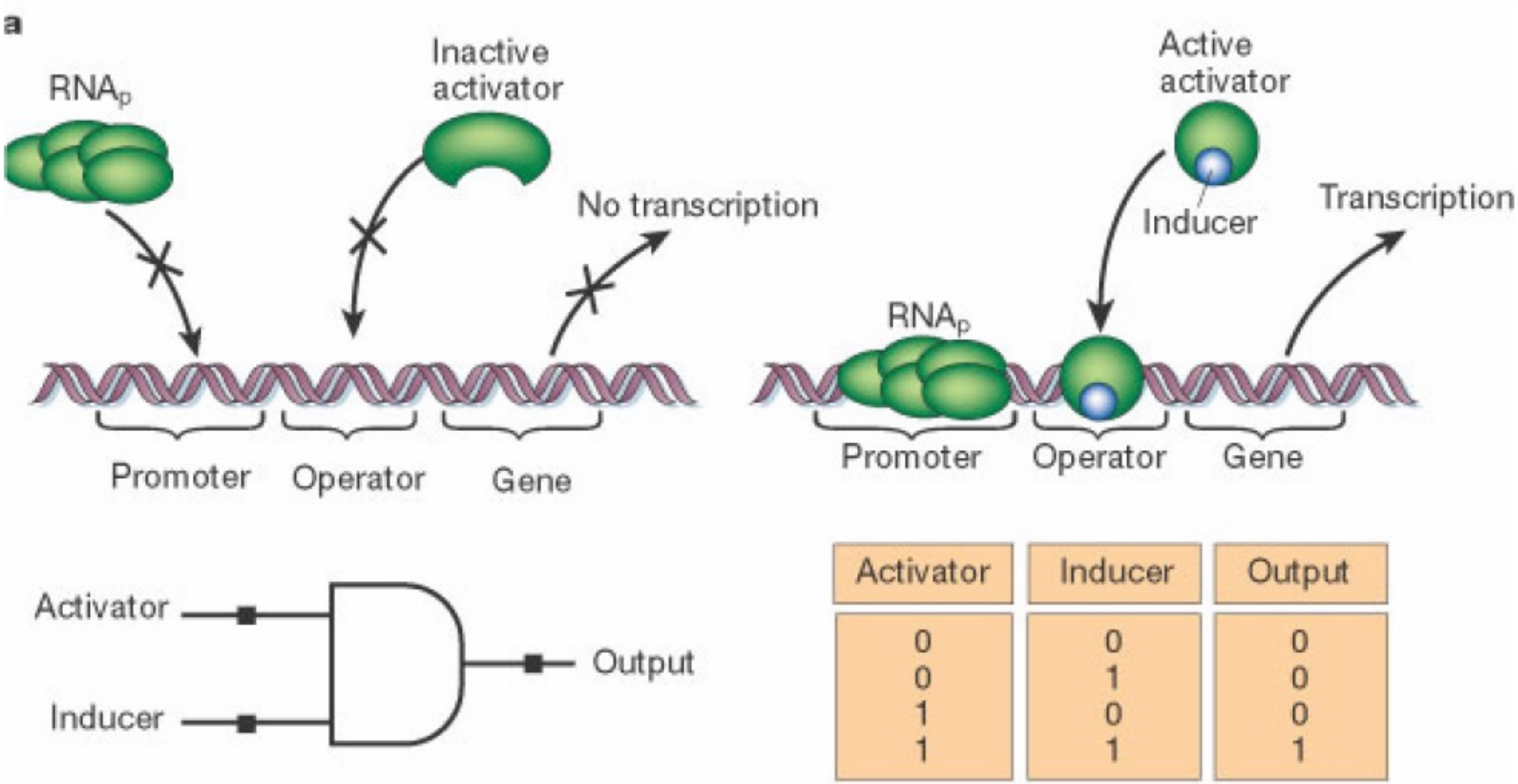
Repressor: negative regulation | **Activator: positive regulation**





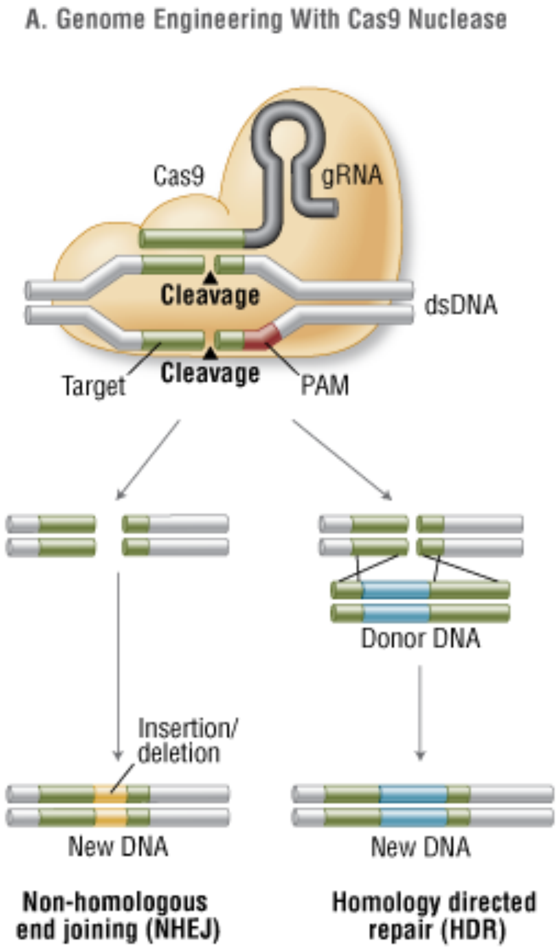
http://www.bioinformatics.utep.edu/agriculture/MEME-BEAS.php

Simple genetic circuits: AND gate



from: Hasty, McMillen, Collins, Nature 420, 224-230 (2002)

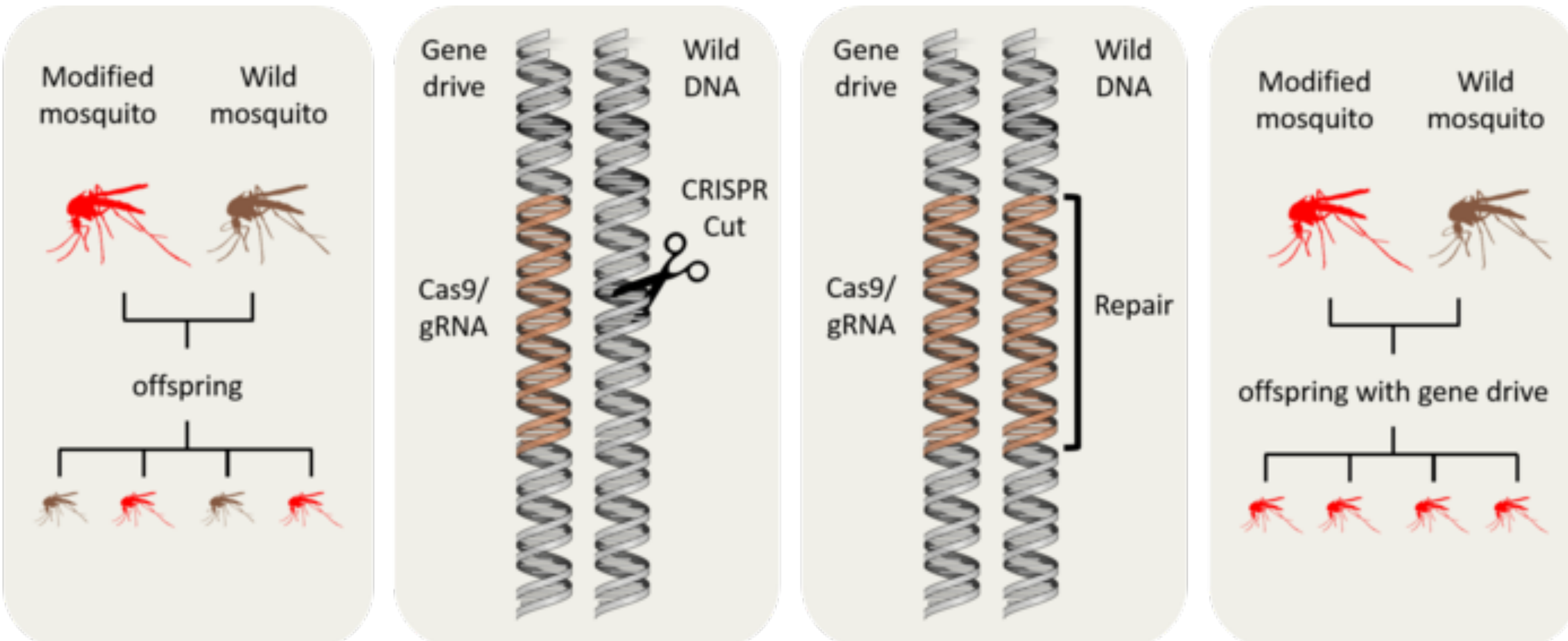
CRISPR: gene editing



CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

Figure from: http://www.neb.sg/applications/genome-editing

Gene drive

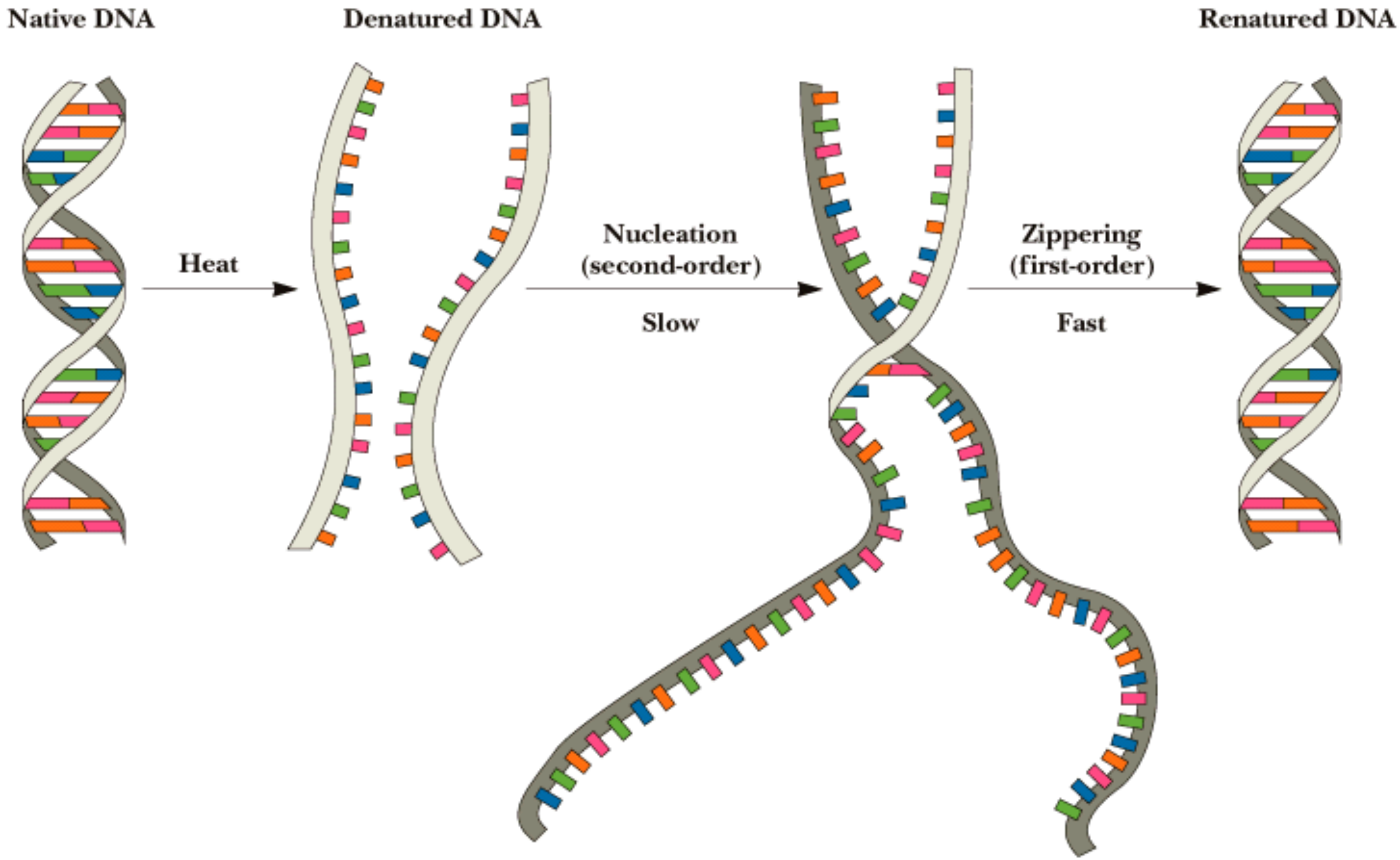

Modified mosquito
Wild mosquito
offspring
Gene drive
Wild DNA
CRISPR Cut
Cas9/ gRNA
Gene drive
Wild DNA
Cas9/ gRNA
Repair
Modified mosquito
Wild mosquito
offspring with gene drive

Operations with DNA

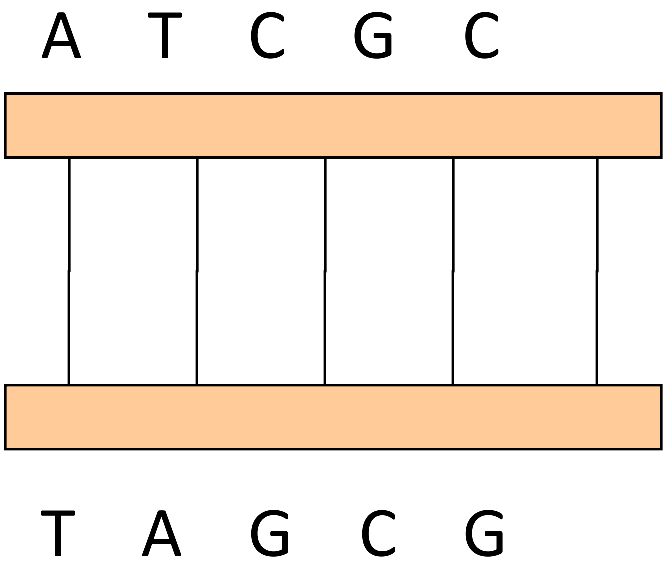
- Synthesize, sequence.
- Hybridization of complementary bases.
- Denaturation.
- Court.
- Chain separation by length. Gel electrophoresis.
- Extraction.
- Polymerization with DNA Polymerase .
- PCR amplification. DNA Photocopy.

Hybridization and renaturation

Garrett & Grisham: Biochemistry, 2/e
Figure 12.19



Hybridization and renaturation



Separation, detection, extraction

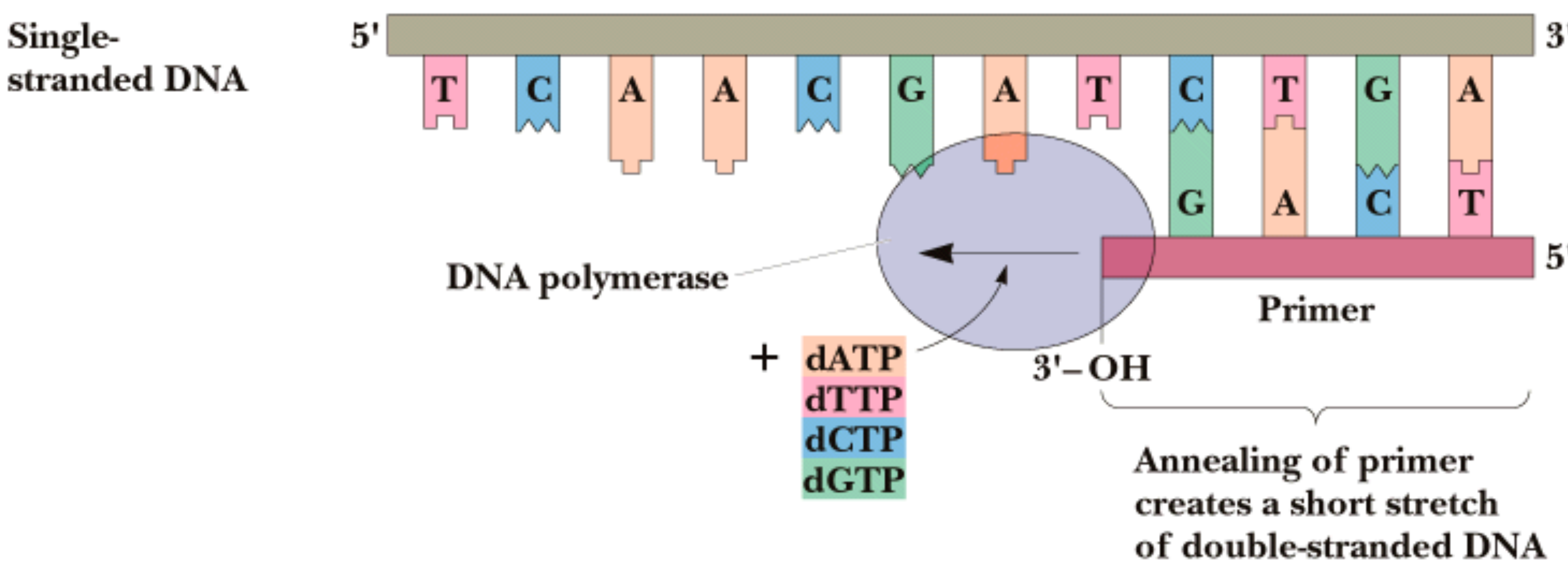
- **Hybridization probes:** “search method” or detection of specific DNA sequences.

  Probe: Small single-stranded strand complementary of the searched strand.

  1. Denaturation of the target strands .
  2. Add a `labeled’ tube and allow hybridization.
  3. Examine whether the hybridization has occured and the extraction of the pair probe.

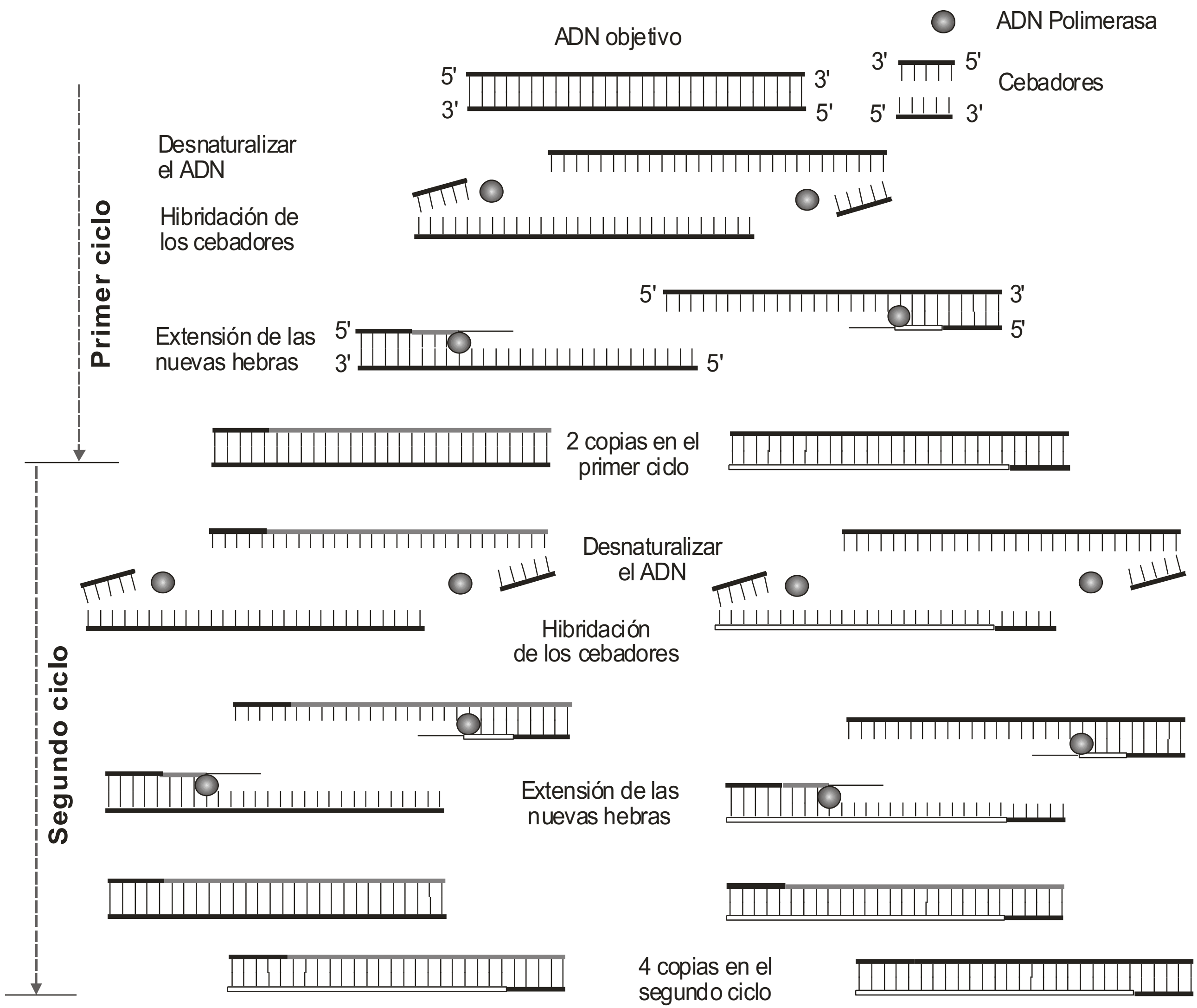
Copying a DNA sequence: DNA Polymerase

Garrett & Grisham: Biochemistry, 2/e
Figure 12.2



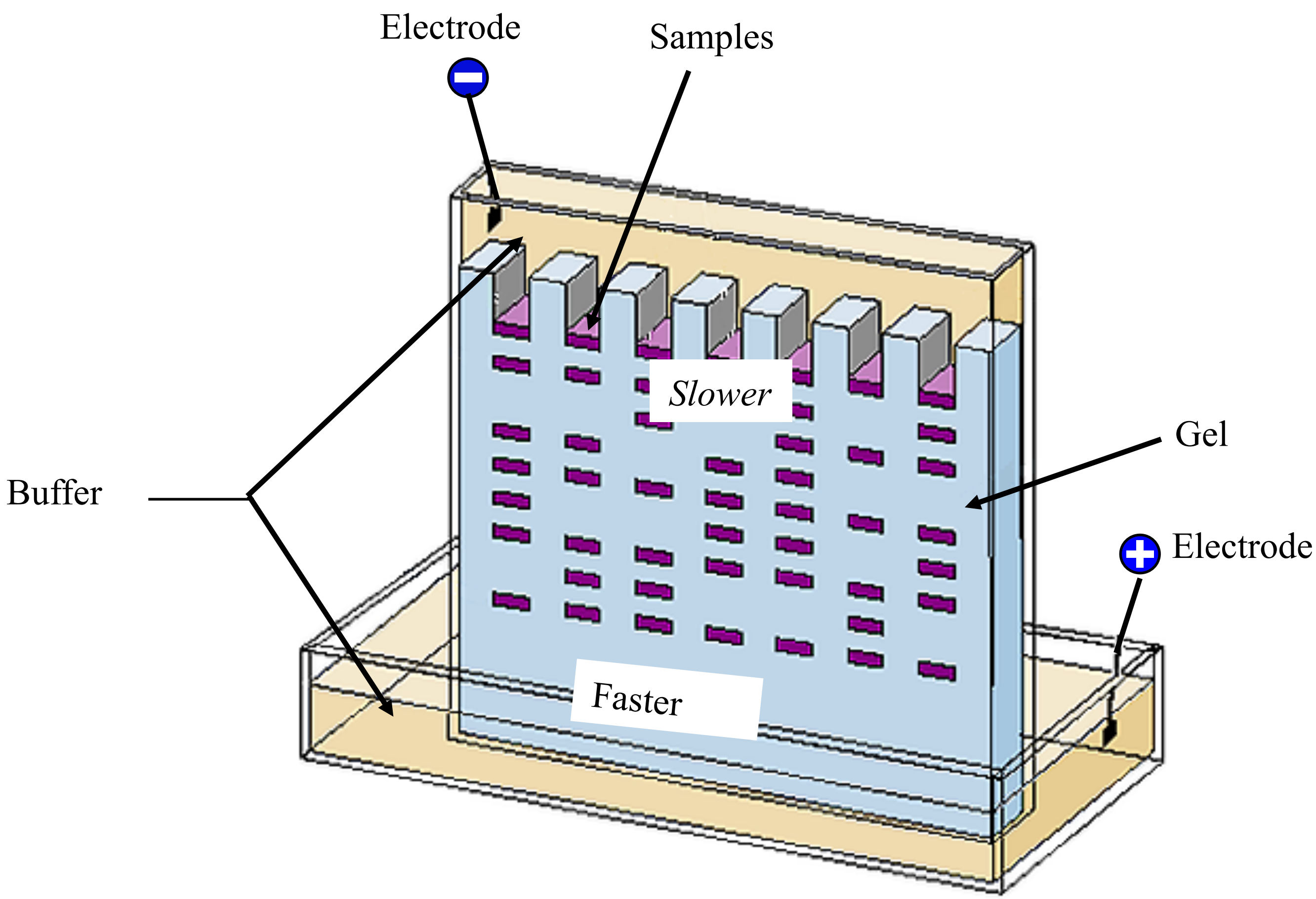
Saunders College Publishing

Polymerase chain reaction (PCR)



Gel Electrophoresis

Separation of DNA strands by length



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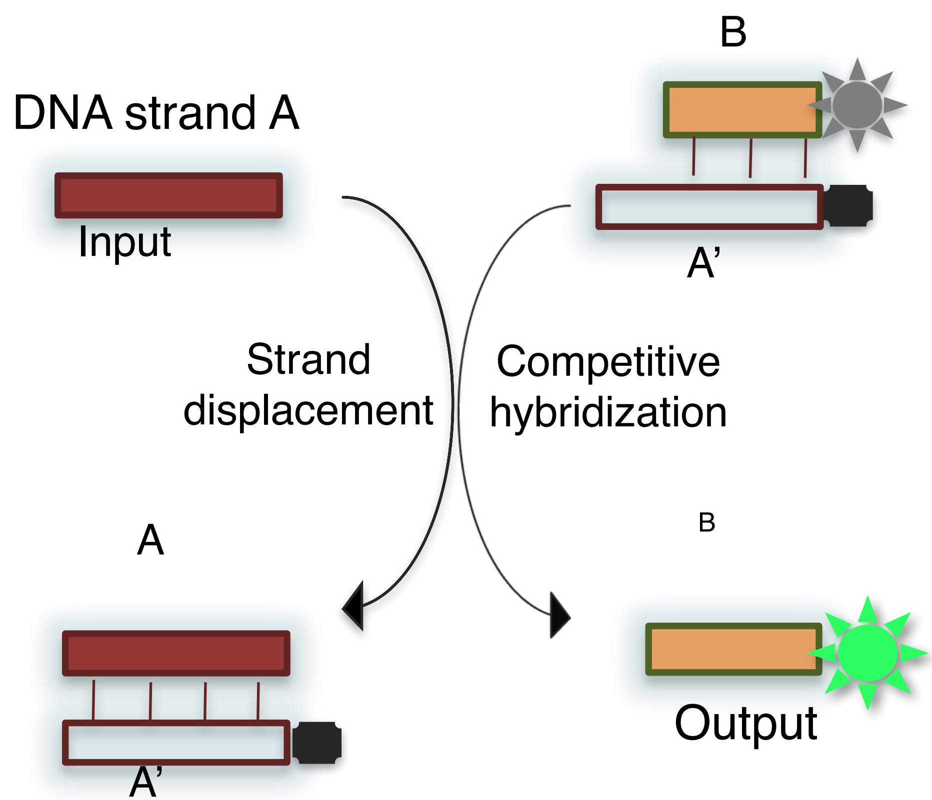
Biomolecular Computing (DNA Computing)

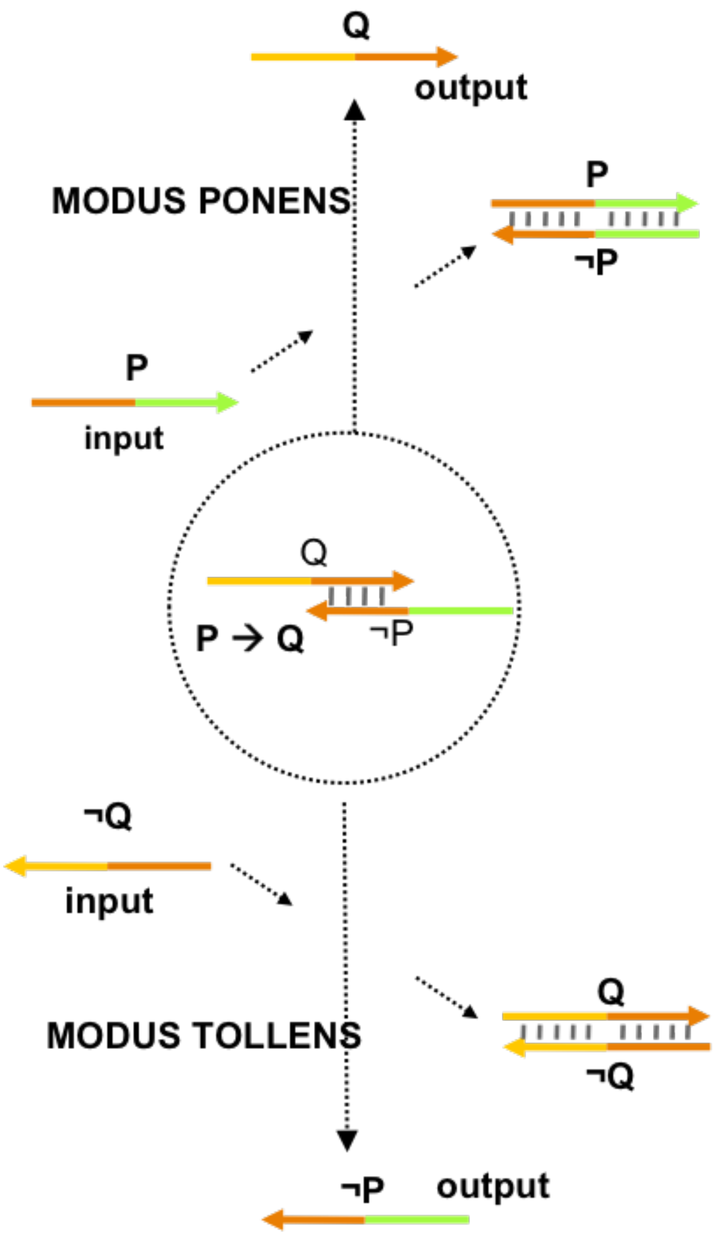
- Biomolecular Computing (DNA Computing): Design and engineering of programmable biomolecular devices with new abilities to process biomolecular information. Using biomolecules to process information encoded in biomolecules
- Why compute with DNA? To program/control biomolecular devices
- Why might be more useful a biomolecular computer than electronics? For biomolecular information processing in vitro or in vivo. To operate within a cell or a living organism.

DNA sensing based on strand displacement

Sensing a DNA/RNA strand



Logic DNA sensor/actuator



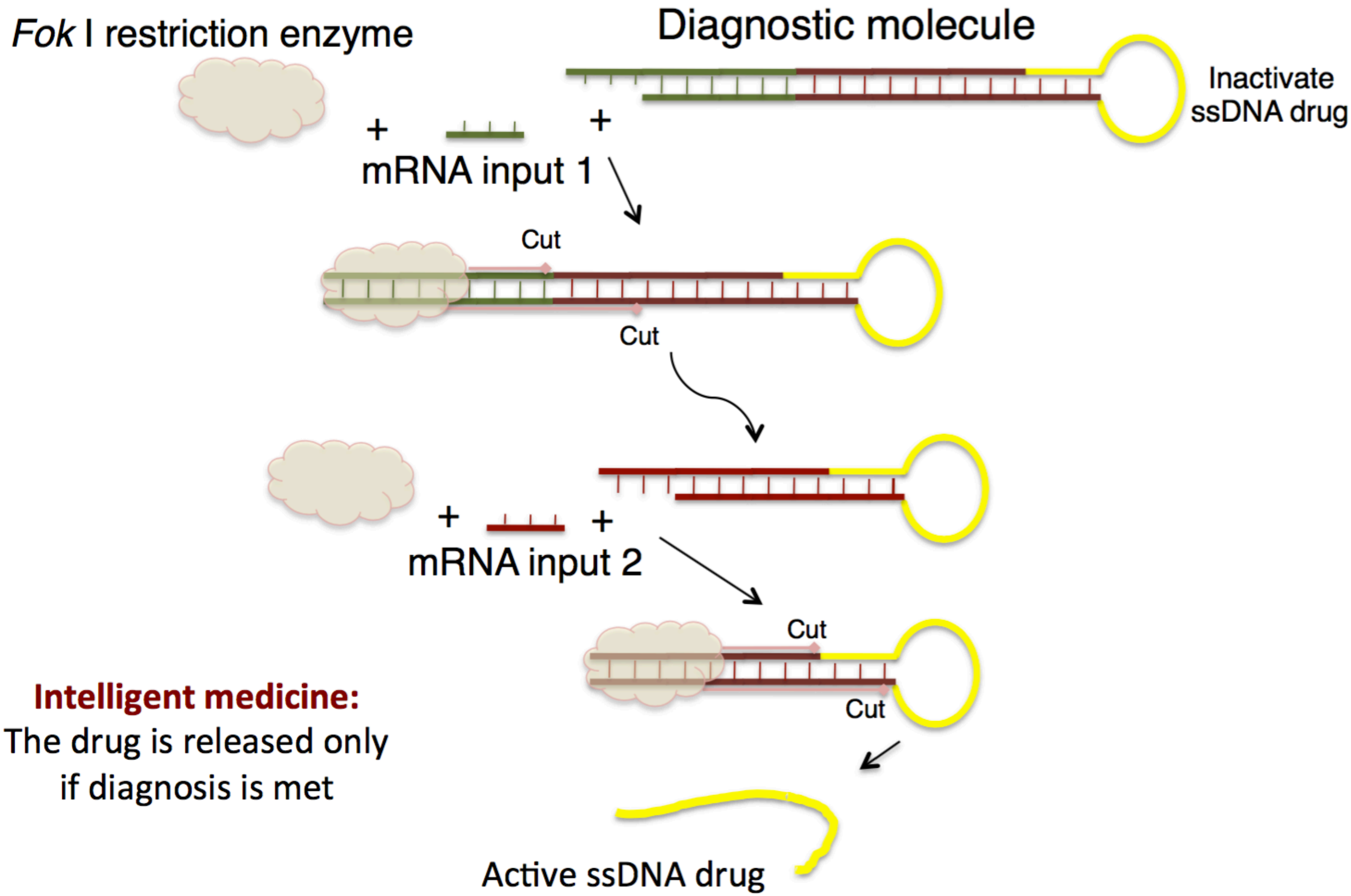
Patente del grupo LIA
http://bopiweb.com/elemento/575526/

Pioneers in DNA Computing and Synthetic Biology:
Yaakov Benenson and Ehud Shapiro. Inst. Weizmann. Israel

Benenson, Y., Paz-elizur, T., Adar, R., Keinan, E., Liben, Z., & Shapiro, E. (2001). Programmable and autonomous computing machine made of biomolecules. *Nature, 414, 430-434.*

Benenson, Y., Gil, B., Ben-Dor, U., Adar, R., & Shapiro, E. (2004). An autonomous molecular computer for logical control of gene expression. *Nature, 429, 423-429.*

DNA Computing: biomolecular automaton

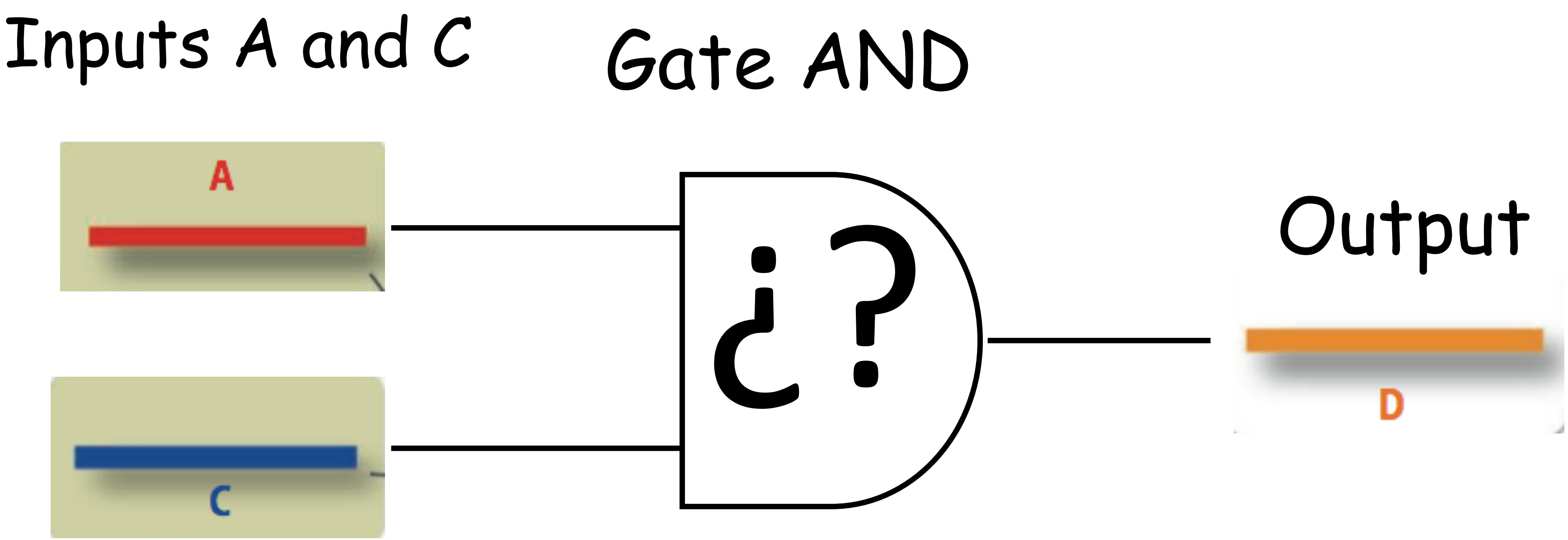


Intelligent medicine:
The drug is released only if diagnosis is met

DNA Computing: strand displacement

Designing a logic gate which processes DNA strands

AND(A,C) = *D*

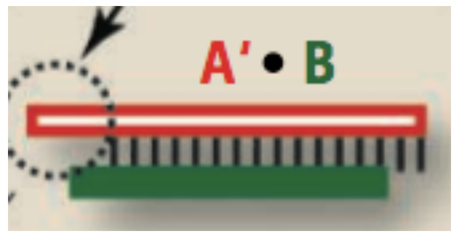


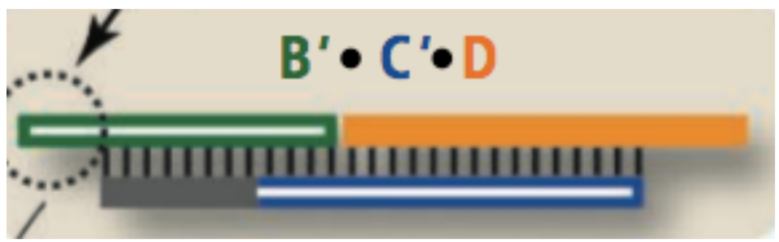
Erik Winfree, **SCIENCE** VOL 314 8 DECEMBER 2006

AND(A,C) = *D*

Inputs

AND gate





Output

Figures from: G. Seelig et al. Enzyme-Free Nucleic Acid Logic Circuits, Science, 314:1585-1588, 2006

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Synthetic Biology: FAQ list

Q1: What is Synthetic Biology?
Q2: Why was Synthetic Biology born?
Q3: When and where did Synthetic Biology start?
Pioneers in Synthetic Biology: Collins, Ellowitz, Weiss, Benenson
Q4: What is Systems Biology?
Q5: What is a gene?
Q6: What is a 0 and a 1 in Synthetic Biology?
Q7: What is a genetic circuit?
Q8: How is a genetic program written?
Q9: Where a genetic program is executed?
Simple genetic circuits: feedbacks, toggle switch, AND gate and "repressilator"
Q10: What has been happening since 2000? Any applications in the market?
Q11: What engineering principles can be applied in SB?
Q12: Is there anything special in the designing of biological systems?
Q13: Is there any "open-source" standard in order to design genetic circuits?
Q14: What are the major difficulties when programming a genetic circuit?
Q15: How to increase the complexity of genetic circuits?
Q16: Do bacteria talk?
Q17: Is it possible to transfer genetic programs between bacteria? The PLASWIRES project.
Q18: Can bacteria do the job of an engineer? Evolutionary engineering of genetic circuits: directed evolution. The EVOPROG project.

Q1: What is Synthetic Biology?

“Synthetic biology is the engineering of biology: the synthesis of complex, biologically based systems, which display functions that do not exist in nature.”

“Biology as technology” used to manufacture devices and Synthetic biological systems. And for reprogramming natural biological systems.

Natural bioware used as hardware and software to build and manufacture artificial or synthetic biological systems.

(Synthetic Biology: Applying Engineering to Biology: Report of a NEST High Level Expert Group).

Q2: Why was Synthetic Biology born?

- 90's: engineers and biologists started to work together during the Human Genome Project (1990-2003).
- 90's: engineers started to see a cell as a computer that could be programmed (inserting DNA programs).
- 2000: Read and write DNA sequences started to become cheaper.

Q3: When and where did Synthetic Biology start?

- Elowitz MB, Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. Nature 403: 335–338.
- Gardner TS, Cantor CR, Collins JJ (2000) Construction of a genetic toggle switch in Escherichia coli. Nature 403: 339–342.
- Becskei A, Serrano L (2000) Engineering stability in gene networks by autoregulation. Nature 405: 590–593.
- "Engineered Communications for Microbial Robotics" Ron Weiss, Tom Knight. Proceedings of the Sixth International Meeting on DNA Based Computers (DNA6), June 2000

Pioneers in Synthetic Biology:
Ron Weiss y Tom Knight. MIT – AI Lab

"Engineered Communications for Microbial Robotics" Ron Weiss, Tom Knight. Proceedings of the Sixth International Meeting on DNA Based Computers (DNA6), June 2000

Pioneers in Synthetic Biology:
M. Elowitz, J. Collins

Elowitz, M. B., & Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. *Nature, 403, 335-338.*

Gardner, T. S., Cantor, C. R, & Collins, J. J. (2000). Construction of a genetic toggle switch in *E. coli. Nature, 403, 339-342.*

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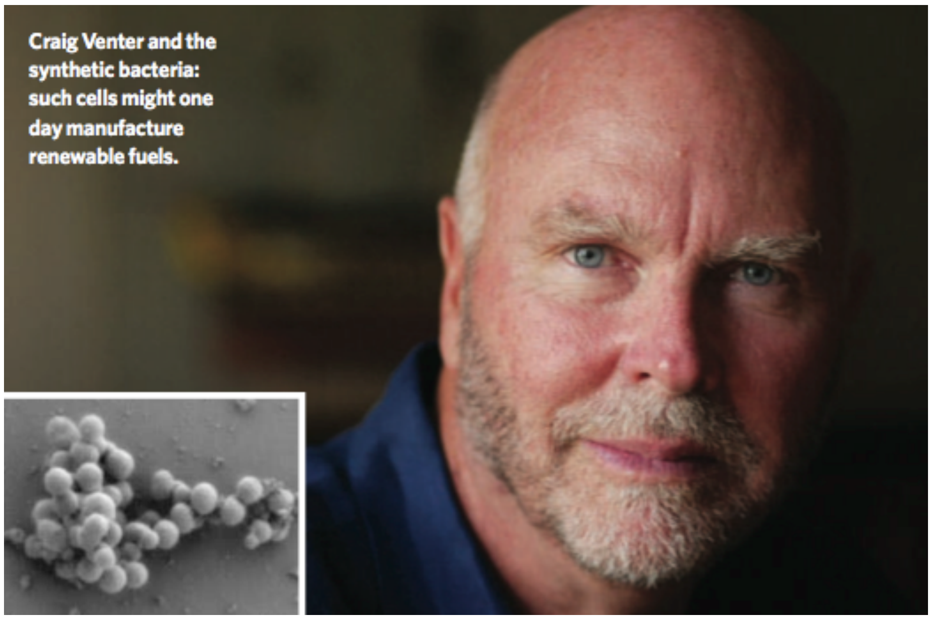
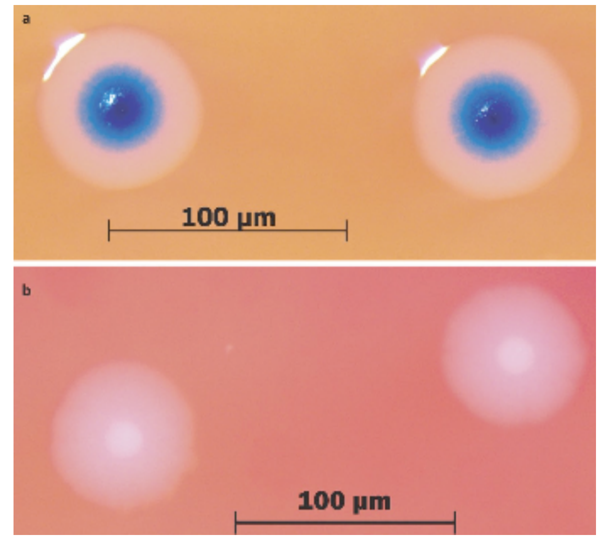
Benenson, Y., Gil, B., Ben-Dor, U., Adar, R., & Shapiro, E. (2004). An autonomous molecular computer for logical control of gene expression. *Nature, 429, 423-429.*

Pioneer in Synthetic Biology: John Craig Venter





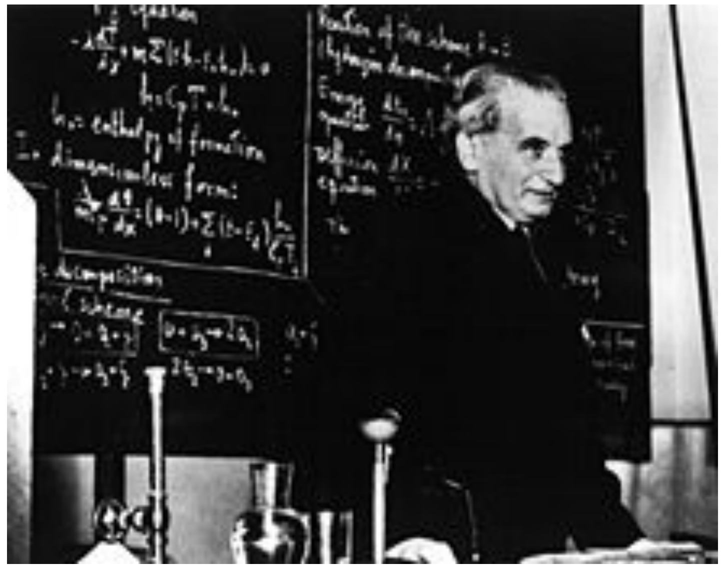
Mycoplasma Mycoides JCVI-syn 1.0

Mycoplasma mycoides JCVI-syn1.0

Gibson, D. G., J. I. Glass, C. Lartigue, V. N. Noskov, R.-Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z.-Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison III, H. O. Smith, and J. C. Venter. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. Science, Published online May 20 2010.

J. Craig Venter INSTITUTE

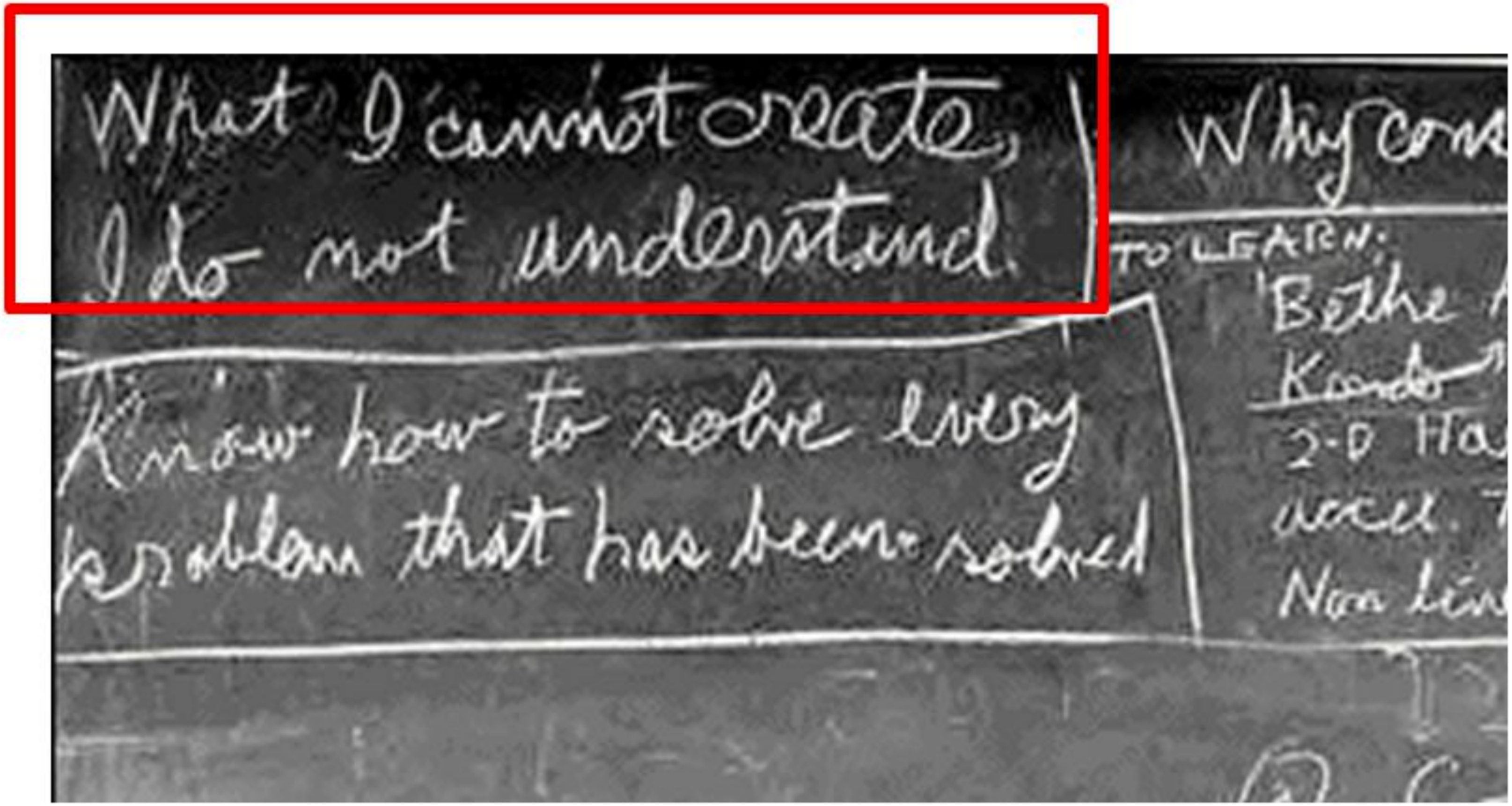
Q4: Systems Biology and Synthetic Biology: Science and Engineering. Analysis and Synthesis.



A Scientist discovers that which exists;
an Engineer creates that which never was.
-- Theodore von Karman

“What I cannot create, I do not understand”

Richard Feynman



Systems Biology and Synthetic Biology: Biology as Science and as Technology

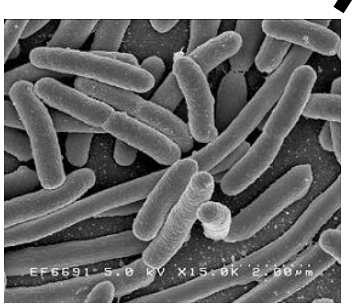
Reverse-engineering and Forward engineering Biology

Knowledge & understanding
models

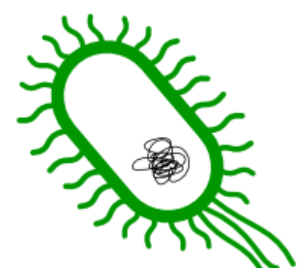
Science
Systems Biology

Engineering
Synthetic Biology

Natural organisms



Engineered organisms



Q5: What is a gene?

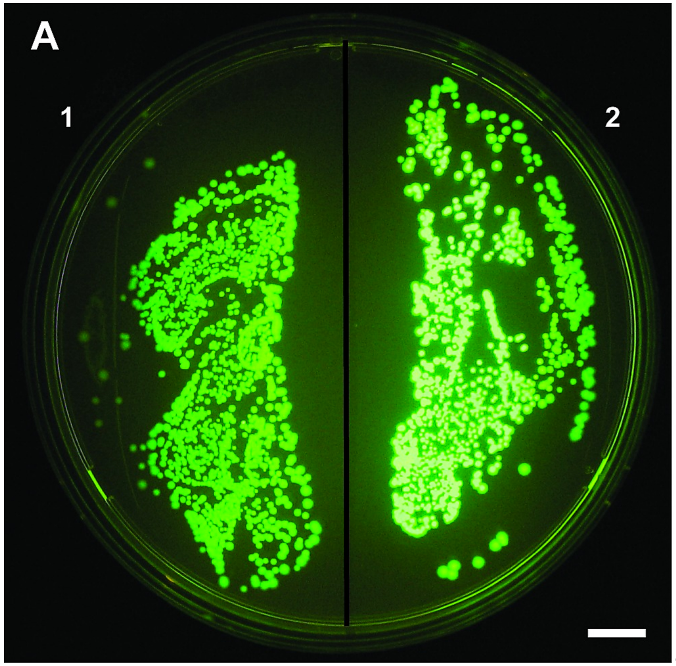
- A program (DNA sequencing) with the instructions to build a biological machine: a protein. A gene is the software to build a biological hardware (a protein).
- Gene expression: DNA->RNA->Protein.
- Gene activation (ON/OFF) can be controlled and regulated through other proteins. Genes show a digital, binary behavior.
- The regulatory area of a gene activation is called promoter area. It can be activable or repressible. We can combine genes with different promoters.

Q6: What is an 0 and an 1 in synthetic biology?

- Low concentration of a biomolecule = 0
- High concentration of a biomolecule = 1

How is the output of a genetic circuit displayed?

Fluorescent proteins: GFP, RFP, YFP



http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0014274

Q7: What is a genetic circuit?

It is a genetic program that is executed in a cell.

Input: Proteins

Circuit: One or more (Promoter+Gene)

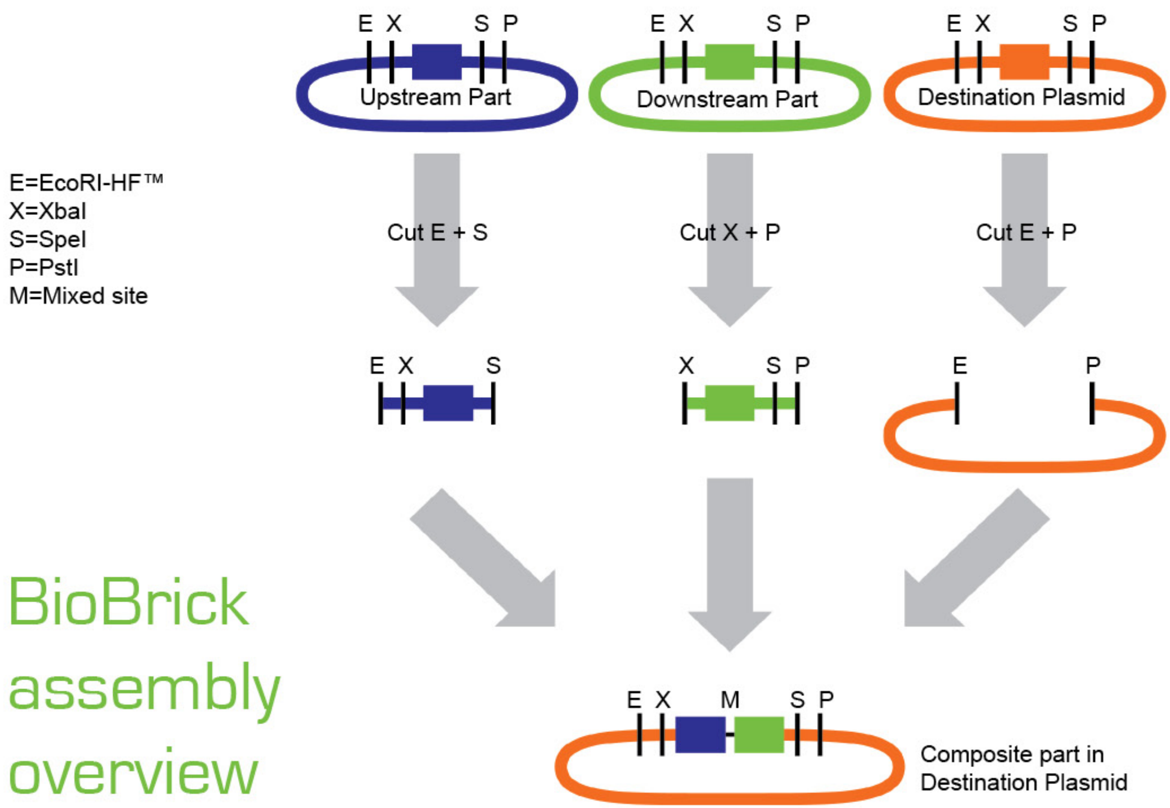
Output: Protein

Genes show a digital, binary behavior (ON/OFF).
Input/Output proteins are considered binary variables (0/1).
Thus, we use the models of the digital/Boolean circuits and Boolean logic gates.

The simplest genetic circuits are the logic gates with 1-input (YES, NOT gate) and the feedbacks

Q8: How is a genetic program written?

- In a DNA circular strand called plasmid.

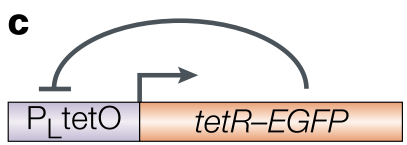


Q9: Where is a genetic program executed?

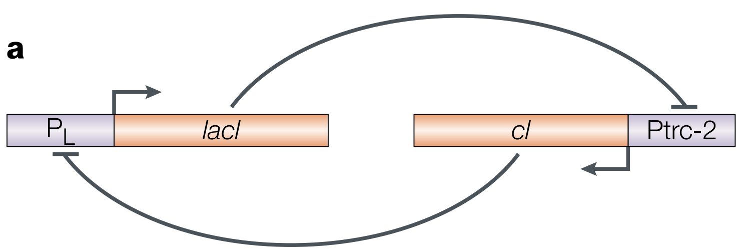
- In a biological processor called “cell”.
- The PC/Apple of biology: biologists work with bacteria called E. Coli. Its operating system (chromosome) contains some 4.6 M base pairs and some 4K genes.

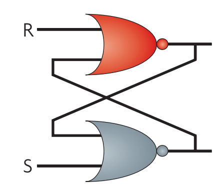
Simple genetic circuits: feedbacks, toggle switch, AND gate and "repressilator"

- The gene Gi produces the protein i; if this protein i regulates its own expression we have: positive feedback or negative feedback.

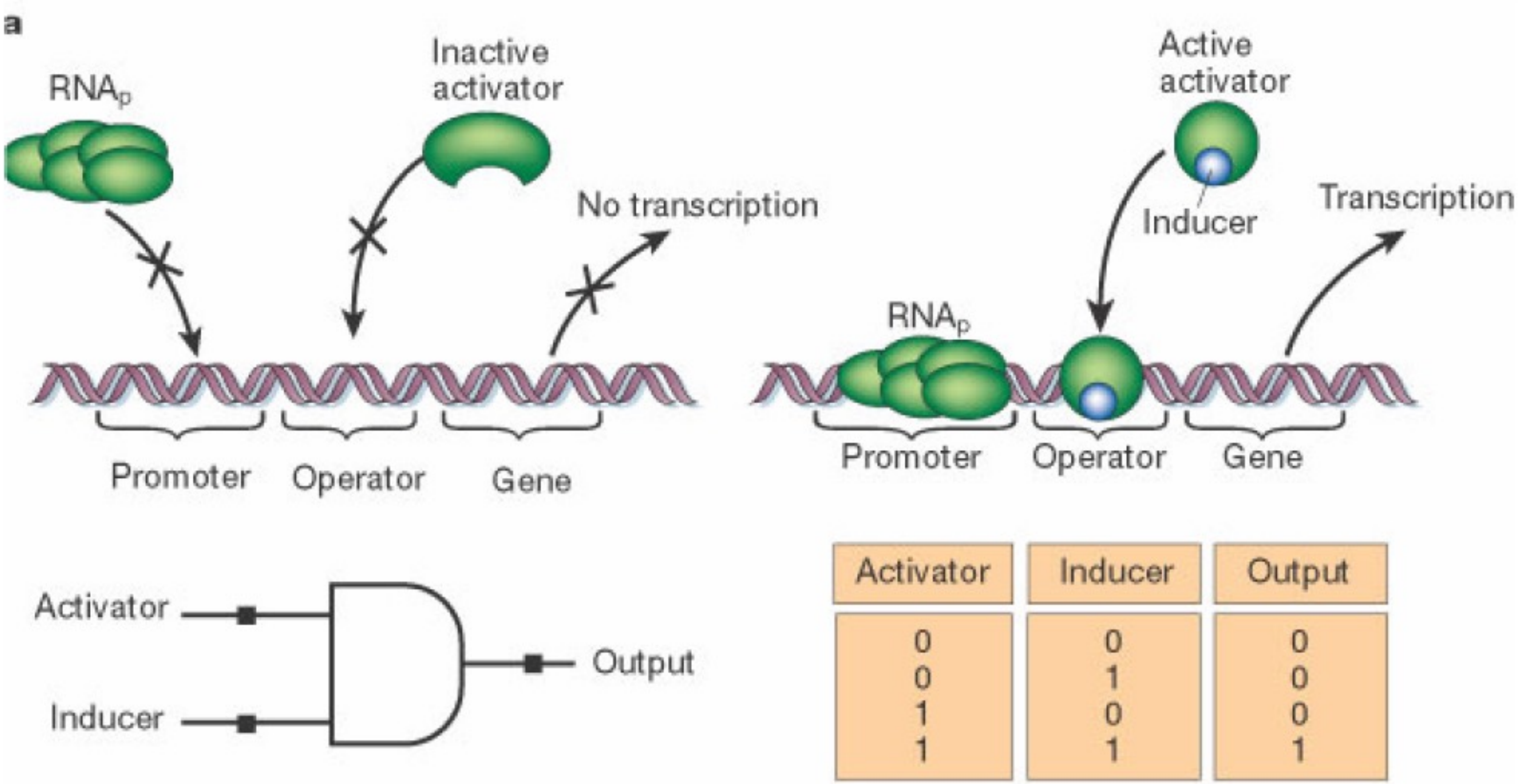


- Toggle switch: The gene i (the protein i) inhibits the gene j and vice versa: the gene j (its protein j) inhibits the gene i. Two genes that are mutually inhibited.





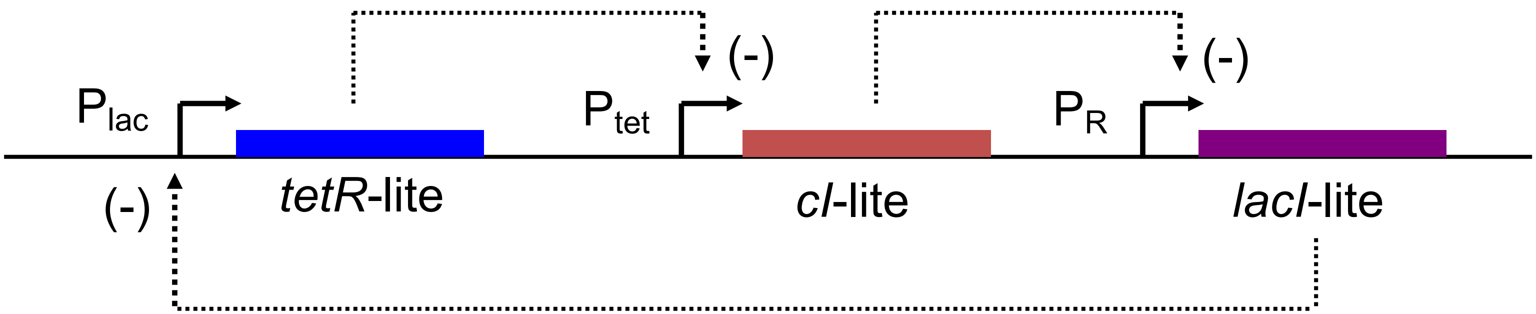
Simple genetic circuits: AND gate

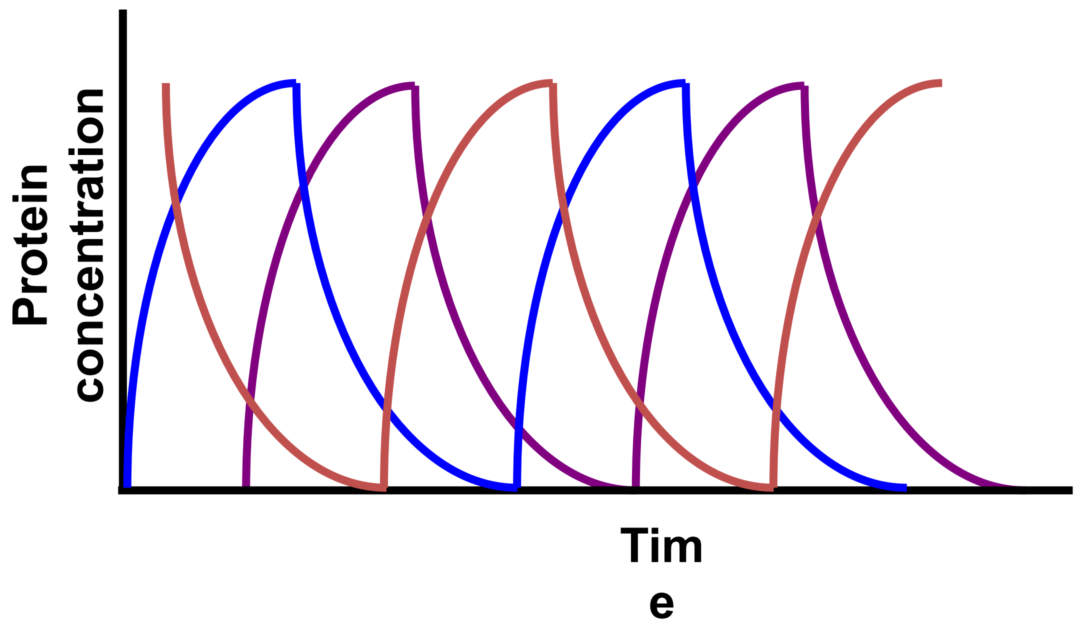


Activator	Inducer	Output
0	0	0
0	1	0
1	0	0
1	1	1

from: Hasty, McMillen, Collins, Nature 420, 224-230 (2002)

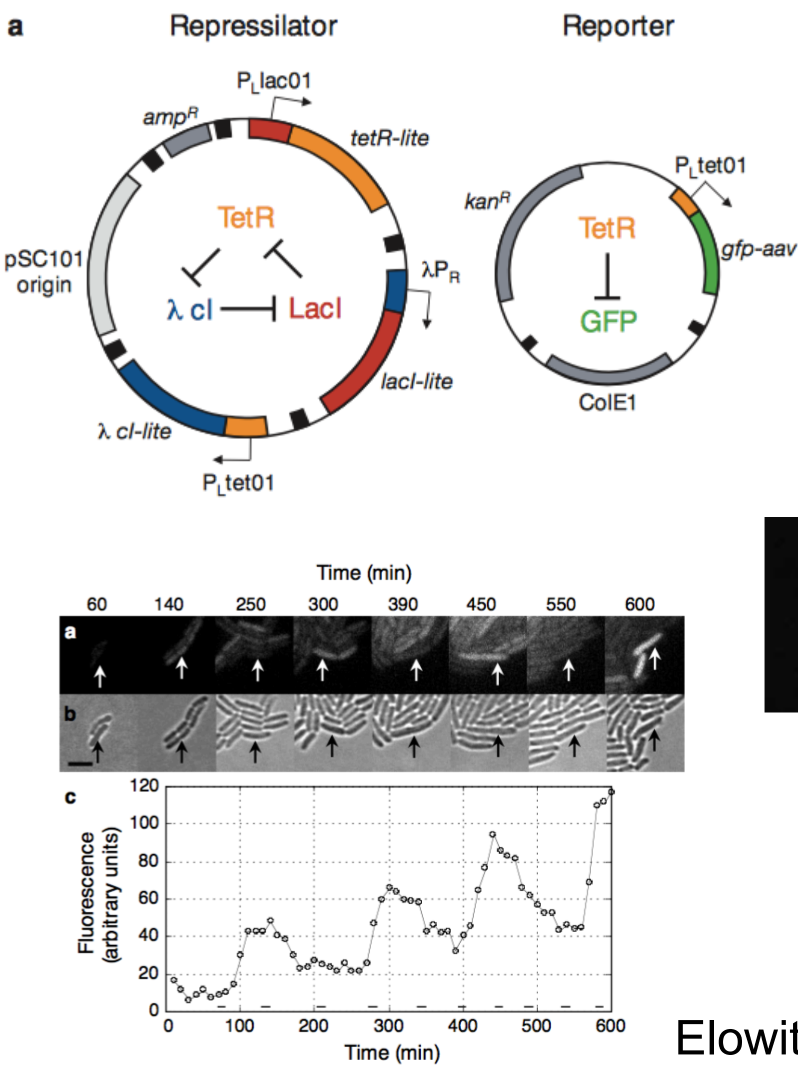
Synthetic oscillator composed by 3 genes: "Repressilator"





Elowitz & Leibler. 2000. *Nature* **403**:335-8

Repressilator: a genetic oscillator

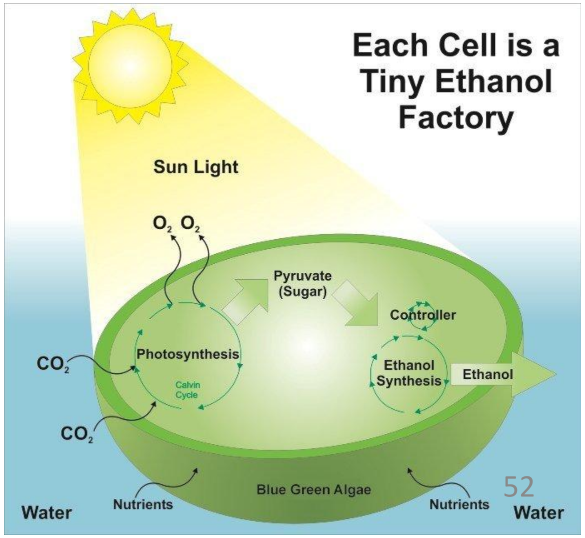


Elowitz & Leibler. 2000. *Nature* **403**:335-8

Q10: Are there already applications in the market?

- Synthetic vaccine against malaria: Artemisia (J. Keasling)
- Biofuel: Cells which convert light/sugar into ethanol.
- Design of medicines, synthetic chemistry, cellular sensors.

- Engineers+Biologists: “Making biology easier to program”.
- Jay Keasling: “Everything that a plant can produce can be produced with a microbe”.



Q11: What engineering principles can be applied in SynBio?

- abstraction, hierarchy, modularity, standardization, encapsulation.

Q12: Is there anything special in the designing of biological systems?

New design principles: Engineering by Directed Evolution

- Components and devices evolve. Modularity is a desire not always a reality. The devices reproduce and die. They are able to self-repair and self-organize.

Q13: Is there any "open-source" standard in order to design genetic circuits? Yes. Biobricks

BioBricks FOUNDATION

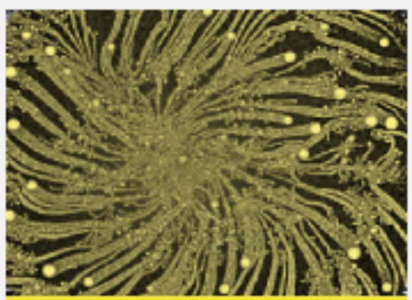
Biotechnology in the public interest

The Synthetic Biology Network

OPENWETWARE.ORG

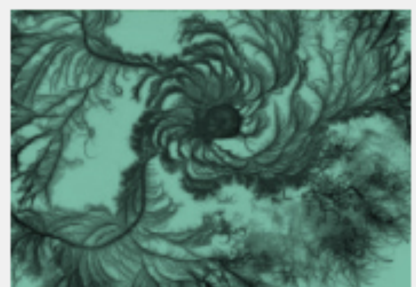
- Protocols
- Lab Notebooks
- Courses

SYNTHETICBIOLOGY.ORG



- Conferences
- Labs
- Courses

PARTSREGISTRY.ORG



- Catalog of parts & devices
- DNA repositories
- Users & groups

IGEM.ORG

- What is IGEM?
- Start a team
- 2011 teams

Q14: What are the major difficulties when programming a genetic circuit?

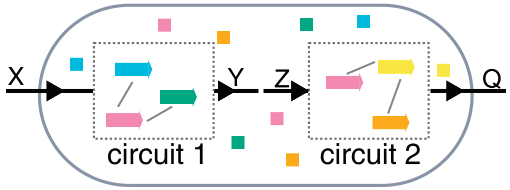
- Life hardware that reproduces and sometimes fails.
- Software that replicates and sometimes mutates.
- Noise, crosstalk: bacteria confuse signals.
- A 1 is not always a 1. A 0 is not always a 0.
- “Mismatch impedance problem”.
- Not very happy bacteria: metabolic load.

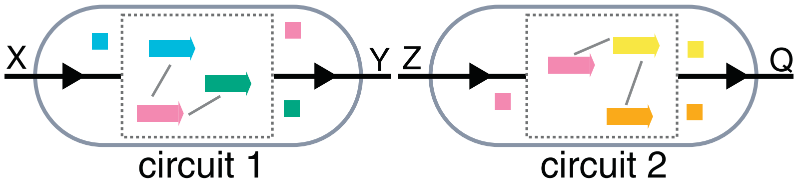
Q15: How to increase the complexity of the genetic circuits?

From synthetic biology to synthetic ecology

From intracellular circuits to multicellular circuits
Multicellular systems programming: Parallel and distributed computation.
Intercellular communication engineering.
Bacterial communication protocols:

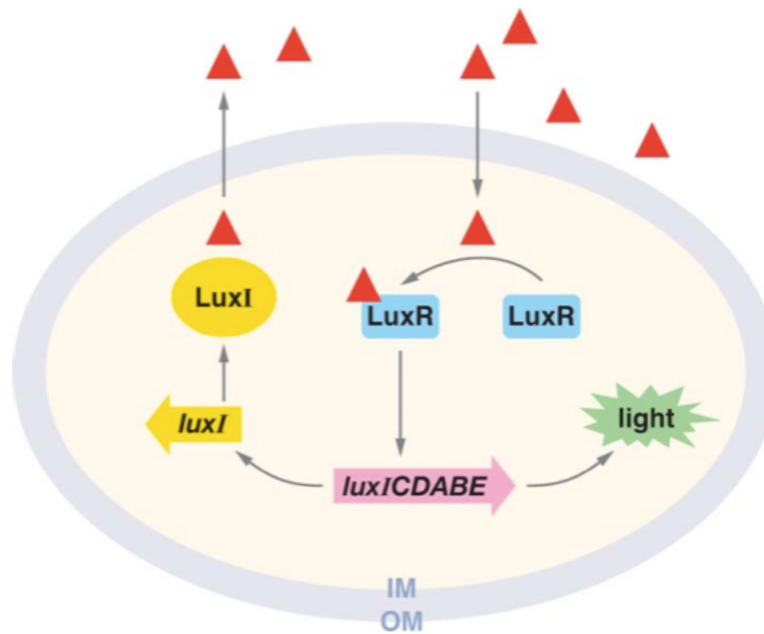
1. Quorum sensing and
2. Conjugation





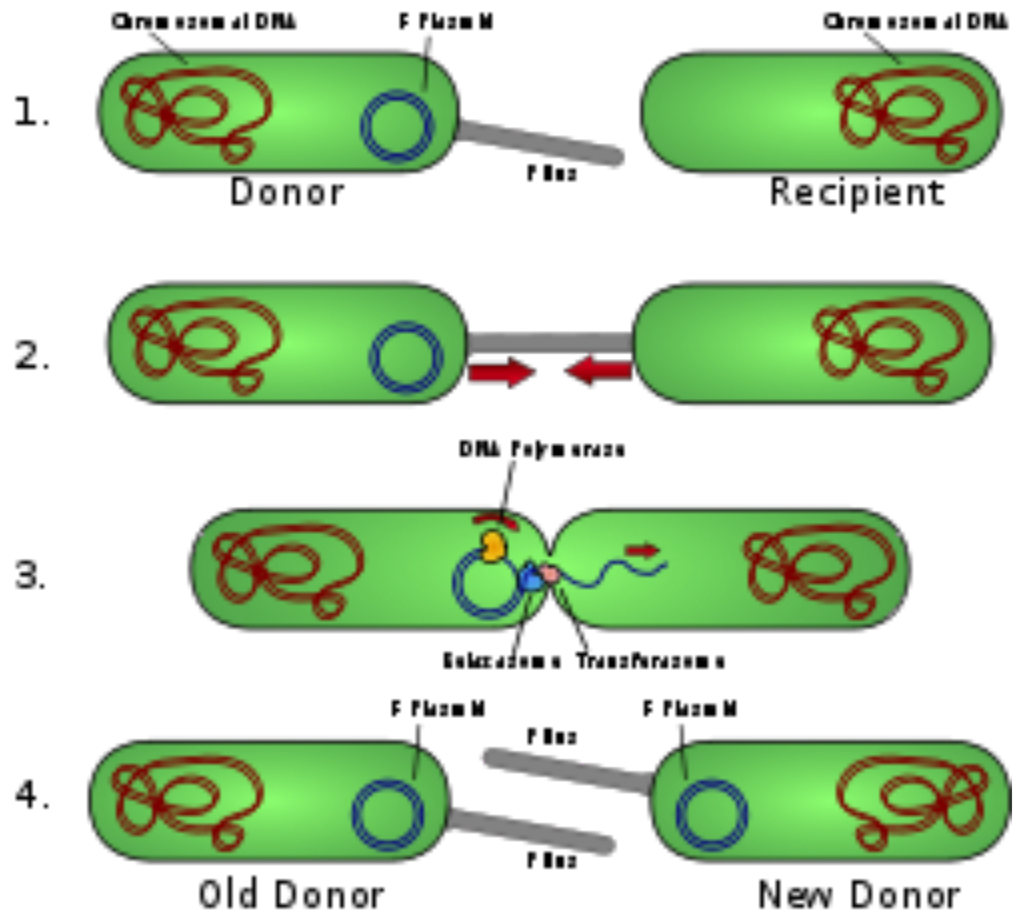
Q16: Do bacteria talk? Quorum Sensing: V. Fischeri and the squid of Hawai

image credit: news.wisc.edu



Waters, C.M. & Bassler, B.L. Quorum sensing: cell-to-cell communication in bacteria. Annual Review of Cell and Developmental Biology 21, 319-346 (2005).

Q17: Is it possible to transfer genetic programs between bacteria? Yes. Through plasmid conjugation



From Wikipedia

PLAS WIRES

European project PLASWIRES: “Engineering Multicellular Biocircuits: Programming Cell-Cell Communication Using Plasmids' as WIRES” www.plaswires.eu

PLASWIRES' main goal: To show how to program a parallel distributed living computer using conjugative plasmids as wires between cellular processors.

Q18: Can bacteria do the job of an engineer? Evolutionary engineering of genetic circuits: directed evolution

From rational design to evolutionary design

Directed evolution: Evolution in the Lab.

Library of possible genetic circuits. Which one has the best behavior?

Manual solution: Examine one by one each genetic circuit.

Automatic in vivo solution: Program bacteria to select autonomously the best circuits among all the library. (BACTOCOM and EVOPROG projects).

EVOPROG

European project EVOPROG: www.evoprog.eu
"General-Purpose Programmable Evolution Machine on a Chip"

EVOPROG's main goal

construct a general-purpose programmable evolution machine able to quickly evolve new biomolecules or phenotypes in bacterial cells.

Some intro material (to read)

If you prefer to read:

- Tinkering with Life. By Jef Akst.The Scientist, October 1, 2011.
- http://www.the-scientist.com/?articles.view/articleNo/31193/title/Tinkering-With-Life/
- Synthetic Life. By W. Wayt Gibbs. Scientific American, May 2004. https://www.researchgate.net/profile/Wayt_Gibbs/publication/8577265_Synthetic_Life/links/0deec51e989f241ab5000000/Synthetic-Life.pdf
- Engineering Life. By the Bio FAB group. Scientific American, June 2006. http://www.synbiosafe.eu/uploads/pdf/SciAm_BioFab_2006_06.pdf
- Synthetic Biology: Bits and pieces come to life. James Collins Nature 483, S8–S10 (01 March 2012) doi:10.1038/483S8a (free full access) http://www.nature.com/nature/journal/v483/n7387_supp/full/483S8a.html
- Q&A: Circuit capacity. James Collins Nature 483, S11 (01 March 2012) doi:10.1038/483S11a http://www.nature.com/nature/journal/v483/n7387_supp/full/483S11a.html

Some intro material (to watch)

If you prefer to watch (Videos):

- Conferencia en Chile 2013 de Alfonso R. Patón: "Biología Programable: 18 FAQs sobre Biología Sintética de un informático". https://www.youtube.com/watch?v=jp7IF8uOxyE
- Slides in pdf available from our web page: http://www.lia.upm.es/index.php/intro-to-syn-bio
- Synthetic Biology - intro video by Jim Collins. (12 minutes). https://www.youtube.com/watch?v=X01MK7MIEwA
- Synthetic Biology Explained (6 minutes). https://www.youtube.com/watch?v=rD5uNAMbDaQ
- Synthetic Biology - intro video (3 minutes). https://www.youtube.com/watch?v=xOx3B2Z_qqE
- Jugando con Biobloques - conferencia de Manu Giménez en TEDxUBA (10 minutos) http://www.tedxuba.org/videos/tedxuba-2013/jugando-con-biobloques http://youtu.be/8I5mqniN

Outline of the talk

1. Basic biology concepts: DNA and gene expression
2. DNA Computing: basic concepts
3. Synthetic Biology: FAQs
4. **LIA group: software (BioBlocks, gro)**

LIA members

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- Professors:
  - Alfonso Rodríguez-Patón
  - Andrei Paun
  - Iván Pau
  - Daniel Manrique
- PostDocs:
  - Xiangxiang Zeng
- PhD students:
  - Martín Gutiérrez
  - Vishal Gupta
  - Paula Gregorio
  - Guillermo Pérez del Pulgar
  - Marcos Rodríguez
  - Antonio García
- Master students:
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  - Sandra Sáez

PLAS WIRES
EVOPROG
SEVENTH FRAMEWORK PROGRAMME
GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Software developed in LIA

- **BioBlocks**: programming protocols made easier
- **GRO**: Multicell 2D bacterial simulator

- Open-source software available in our web

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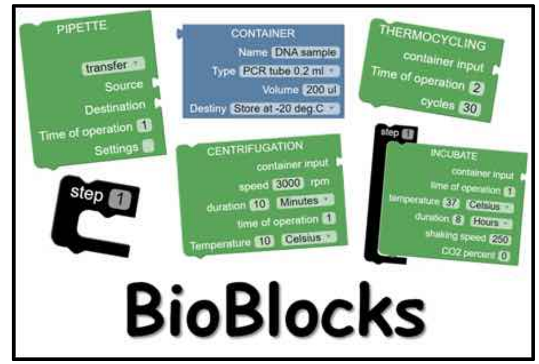
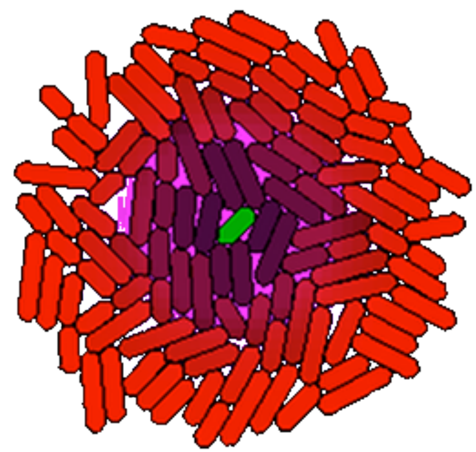
We work on two scales

Scale

High-level
protocols/programs



Multicellular simulations



GRO

ACS Synthetic Biology

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Technical Note

‹ Previous Article

BioBlocks : Programming protocols in biology made easier

Vishal Gupta, Jesus Irimia, Ivan Pau, and Alfonso Rodriguez-Paton

ACS Synth. Biol., Just Accepted Manuscript
DOI: 10.1021/acssynbio.6b00304
Publication Date (Web): January 4, 2017

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Research Article

‹ Previou

A New Improved and Extended Version of the Multicell Bacterial Simulator gro

Martín Gutiérrez[*†‡], Paula Gregorio-Godoy[†], Guillermo Pérez del Pulgar[†], Luis E. Muñoz[†], Sandra Sáez[†], and Alfonso Rodríguez-Patón[*†]
[†] Departamento de Inteligencia Artificial, ETSIINF, Universidad Politécnica de Madrid, 28040 Madrid, Spain
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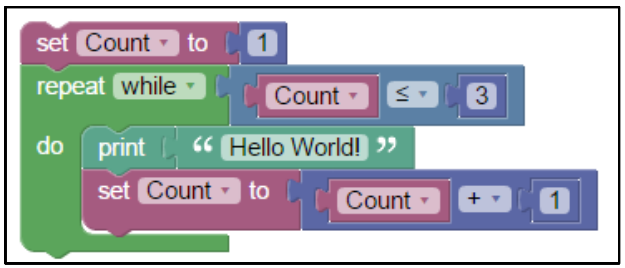
ACS Synth. Biol., Article ASAP
DOI: 10.1021/acssynbio.7b00003
Publication Date (Web): April 24, 2017

BioBlocks is based on Google's Blockly and MIT Scratch

Blockly and Scratch are block-based tools to teach children programming!



Language: JavaScript ▾

```
var Count;


Count = 1;
while (Count <= 3) {
  window.alert('Hello World!');
  Count = Count + 1;
}
```

http://vps159.cesvima.upm.es/software/Bioblocks

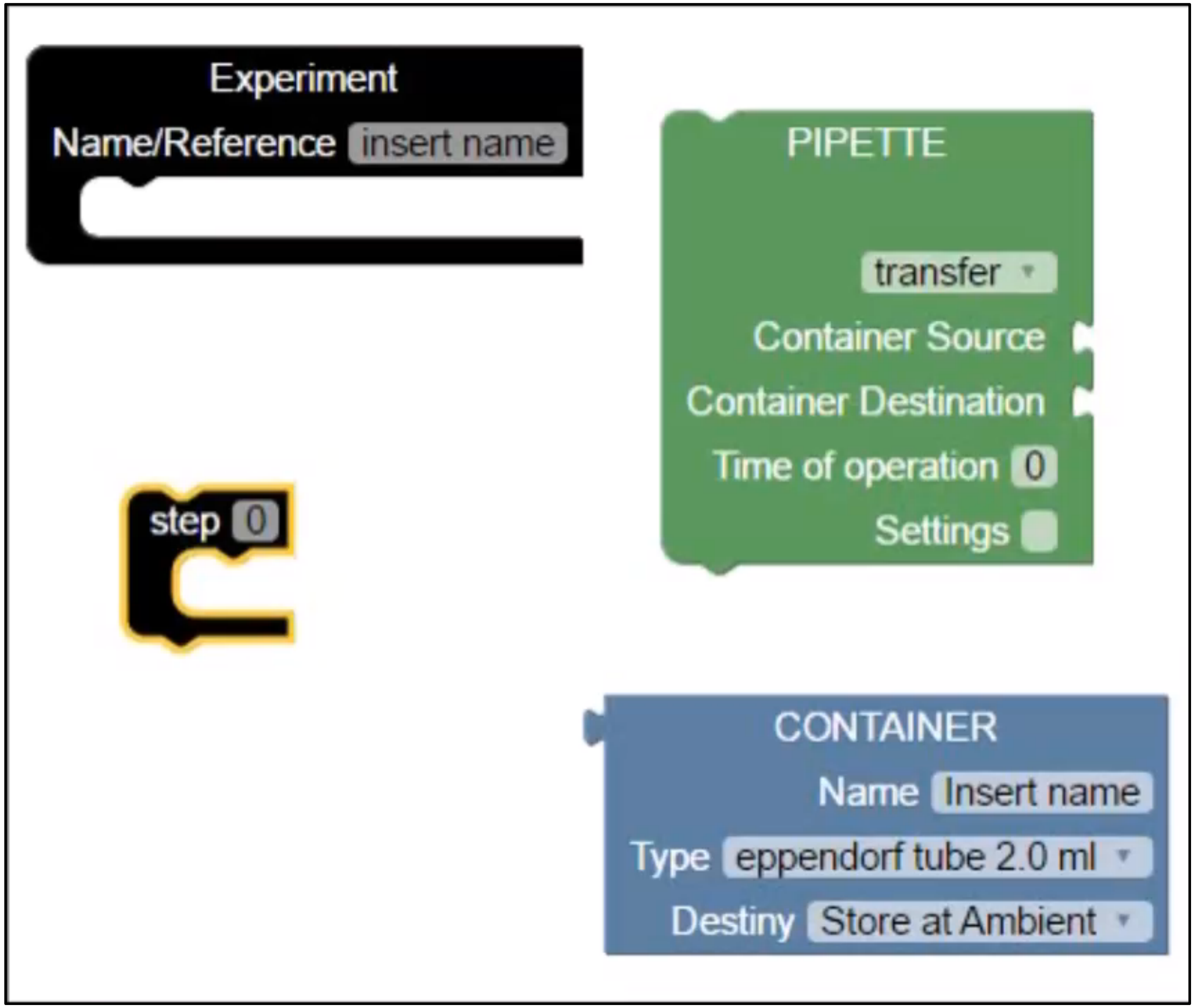
BioBlocks: A visual language for protocol Specification

- Consists of :

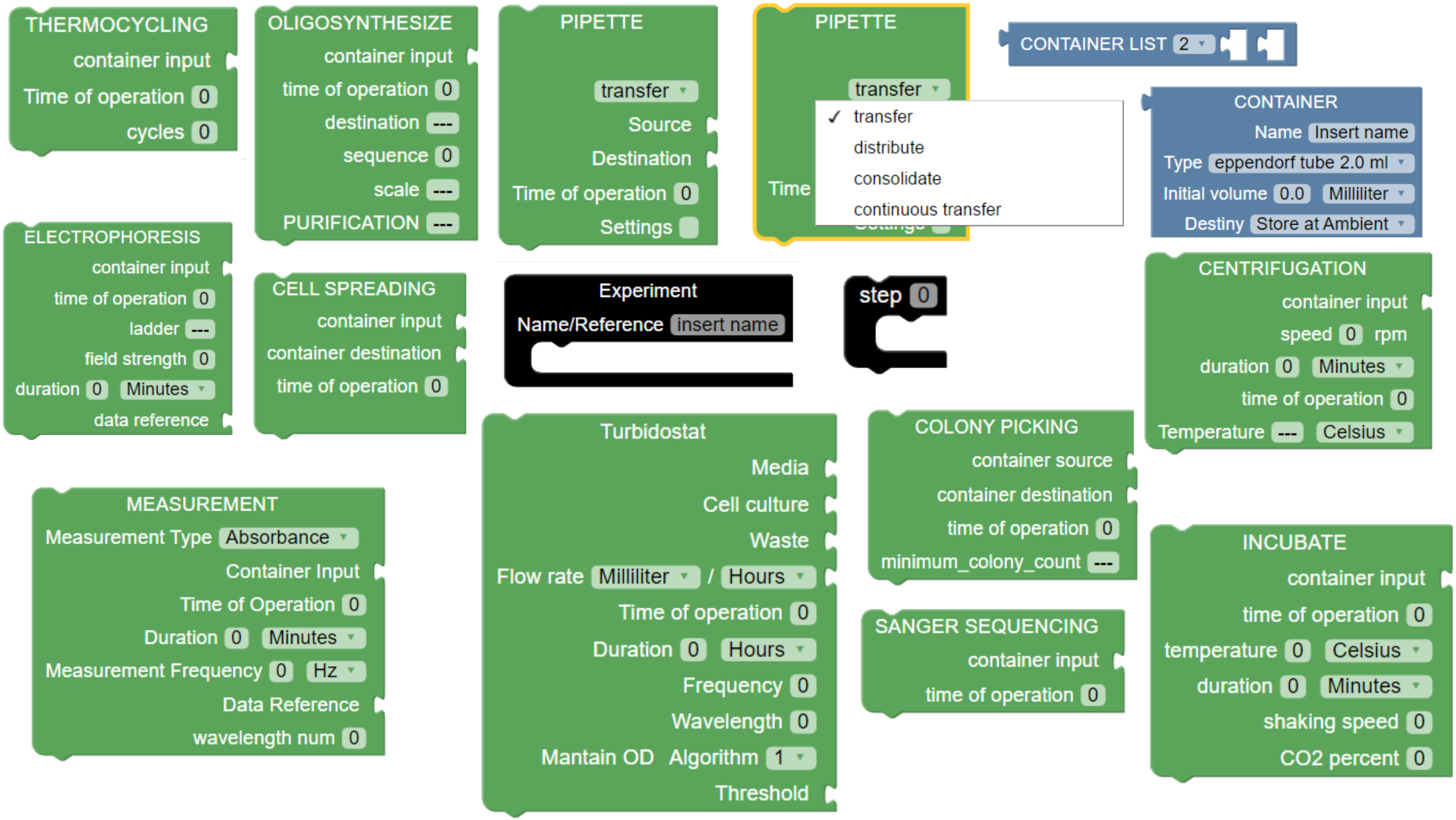
  -Organization Blocks

  -Operation Blocks

  -Container Blocks



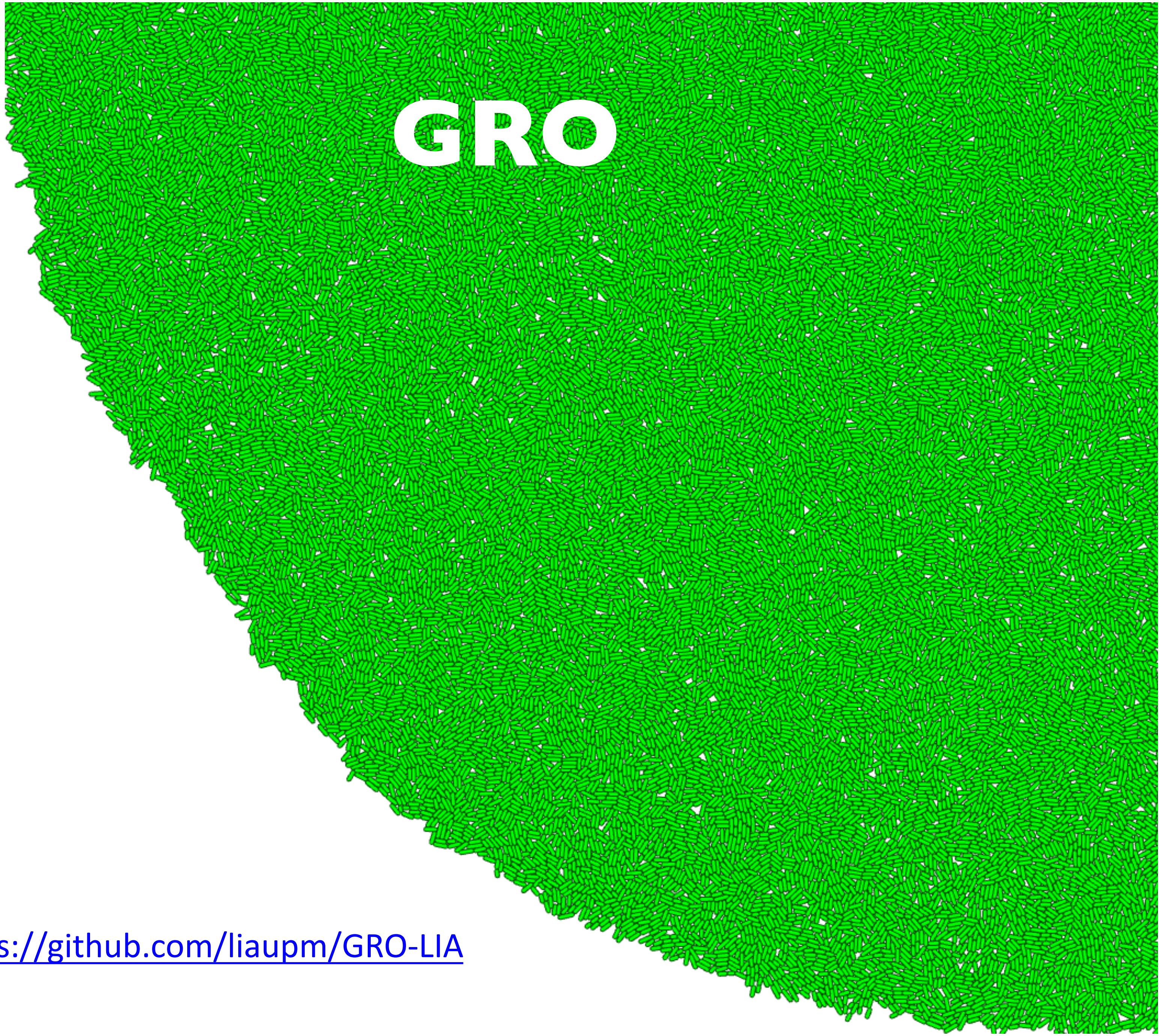
BioBlocks language



Blocks from the BioBlocks Library

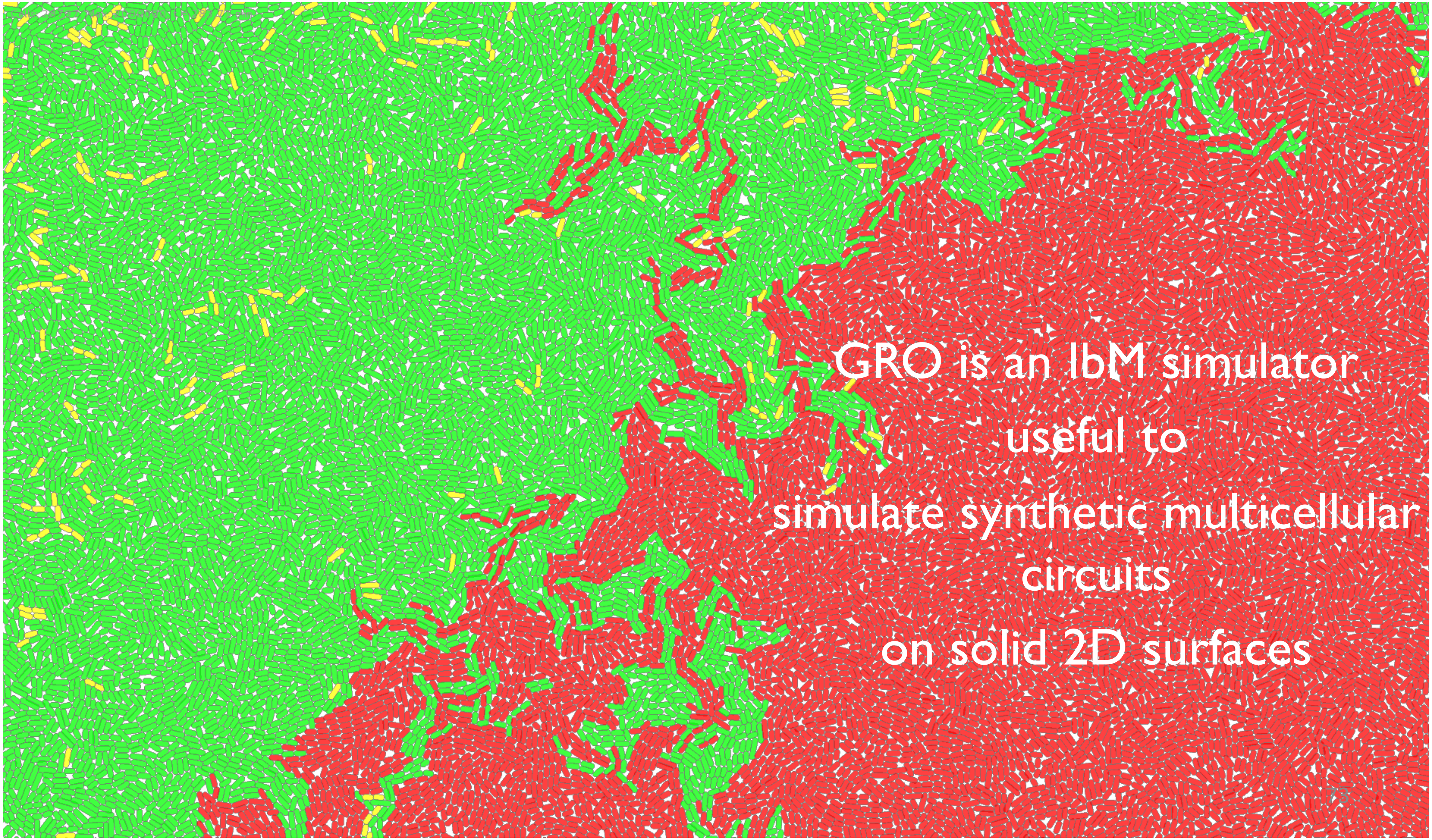
http://vps159.cesvima.upm.es/software/Bioblocks

GRO



https://github.com/liaupm/GRO-LIA

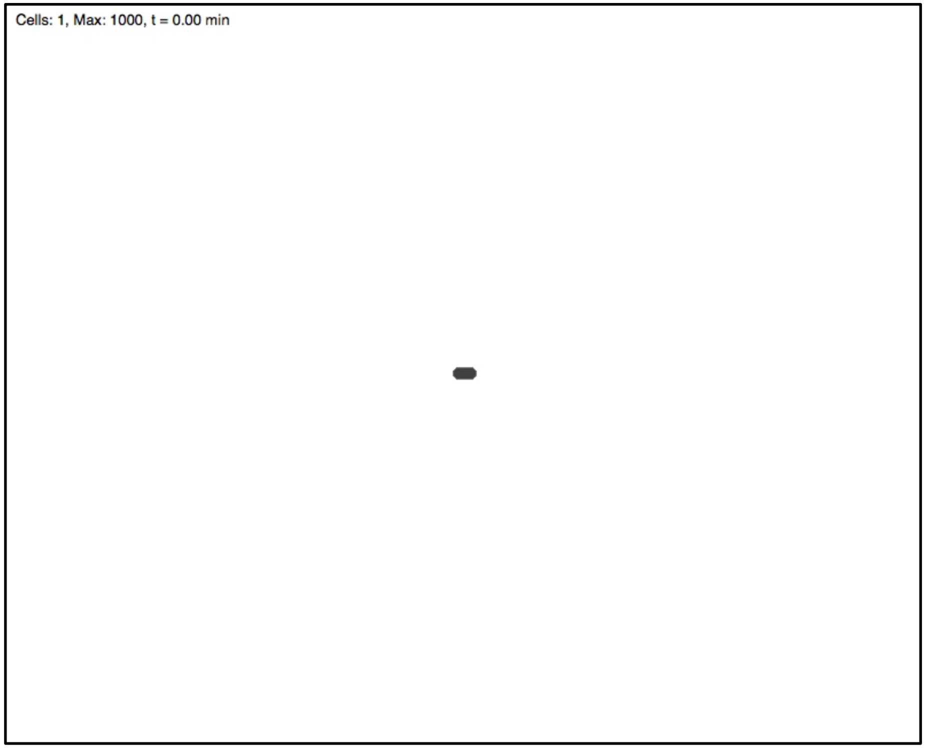
GRO, the cell programming language



GRO, the cell programming language

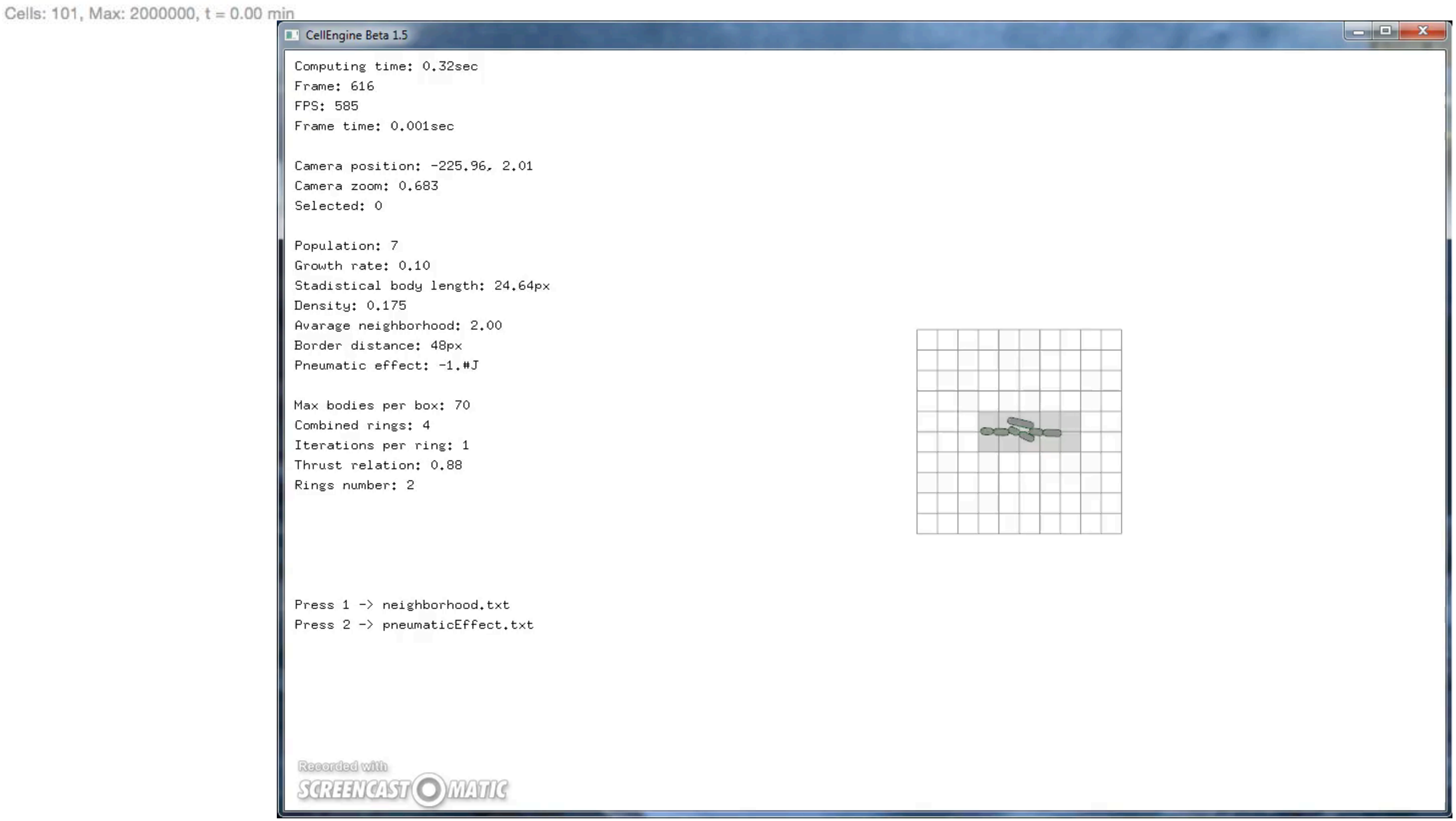
- Developed at Klavins Lab (University of Washington) http://depts.washington.edu/soslab/gro/

- GRO is mainly concerned with studying bacterial colony growth in 2D and multicellular behaviors based on signals.



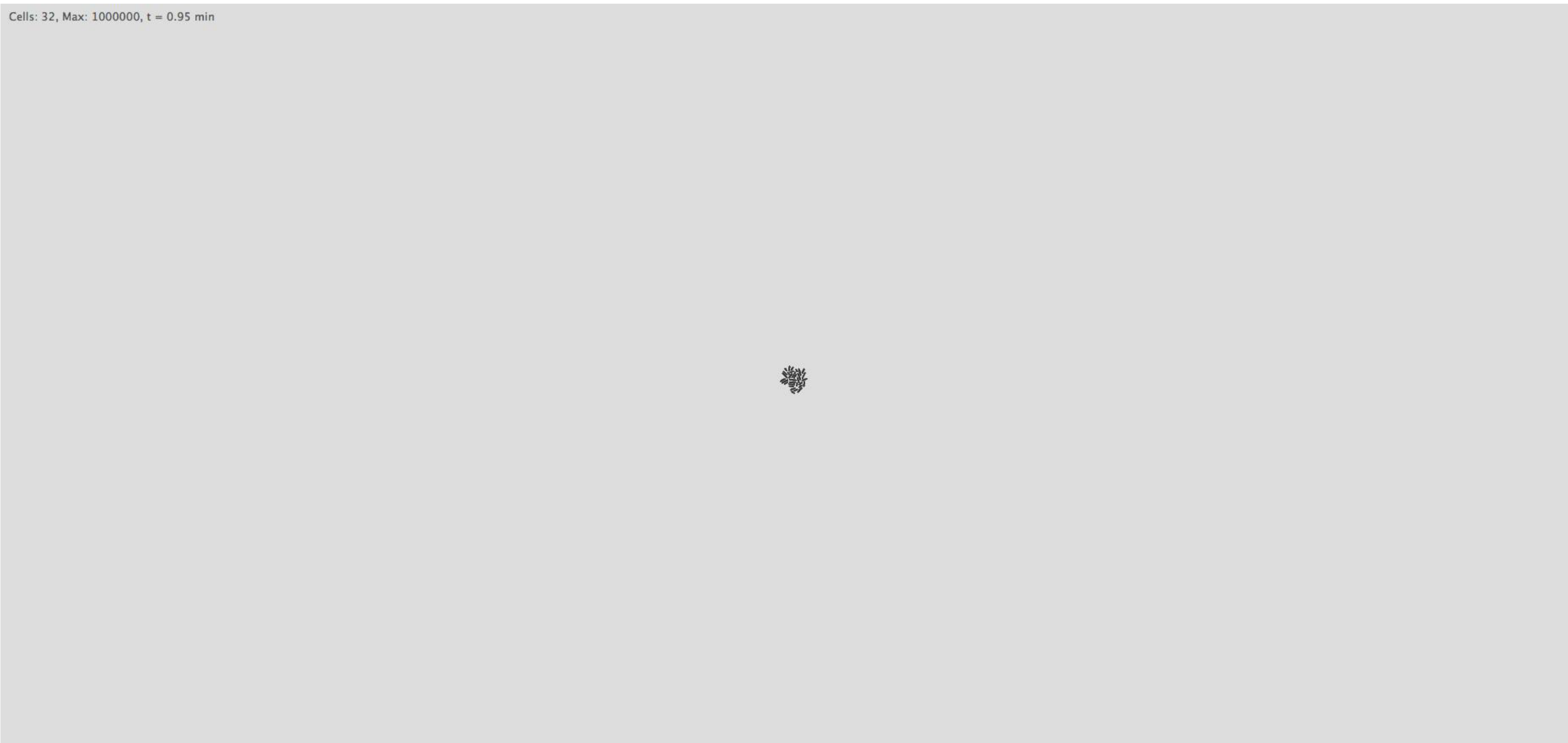
https://github.com/liaupm/GRO-LIA

Cell orientation in gro



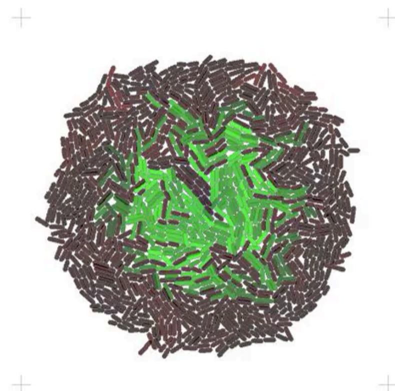
https://github.com/liaupm/GRO-LIA

A multicellular edge detector



A multicellular bullseye

Cells: 1122, Max: 2000000, t = 71.80 min



THANK YOU!

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