



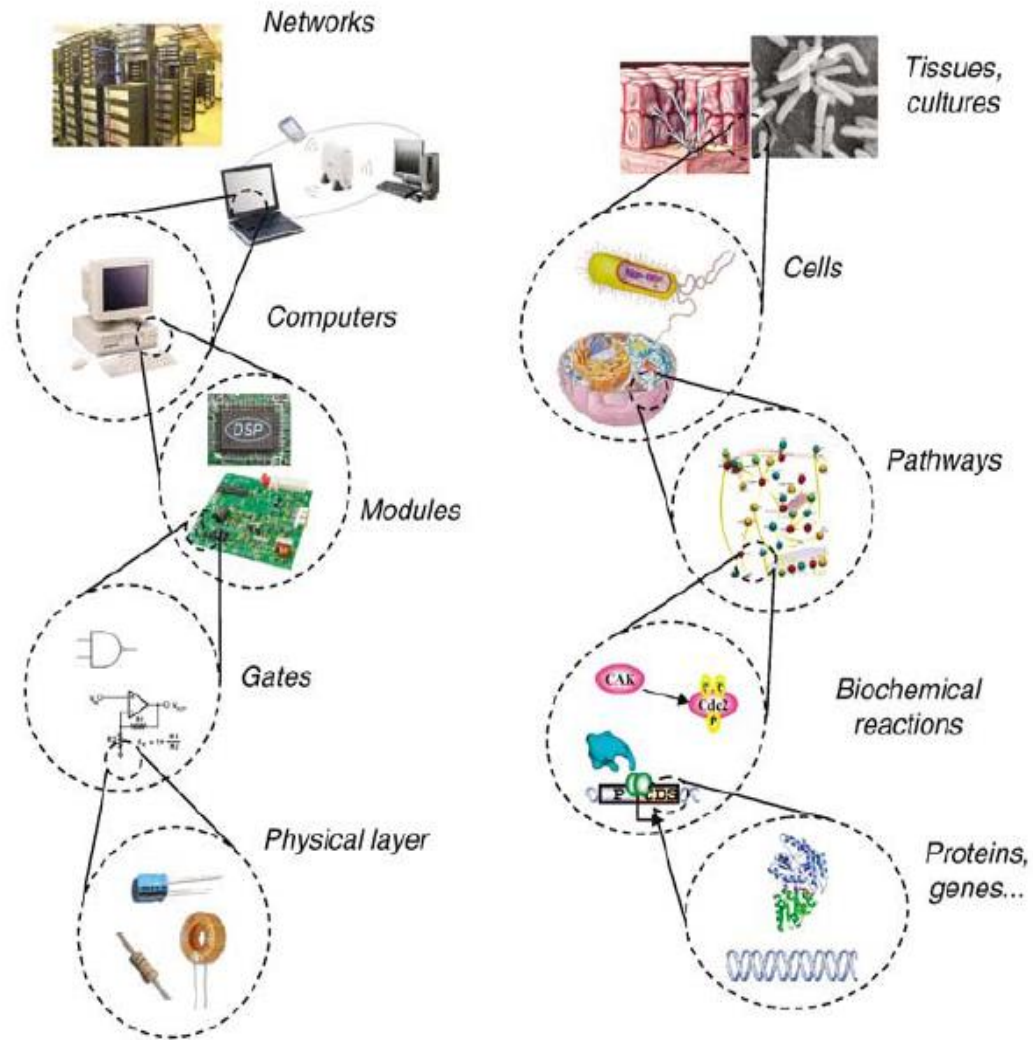
Aalto University
School of Chemical
Technology

Synthetic biology
(Course CHEM-E8125), spring 2022

Biobricks & circuits

Prof. Merja Penttilä

Analogy to electric engineering



Designing cellular functionalities

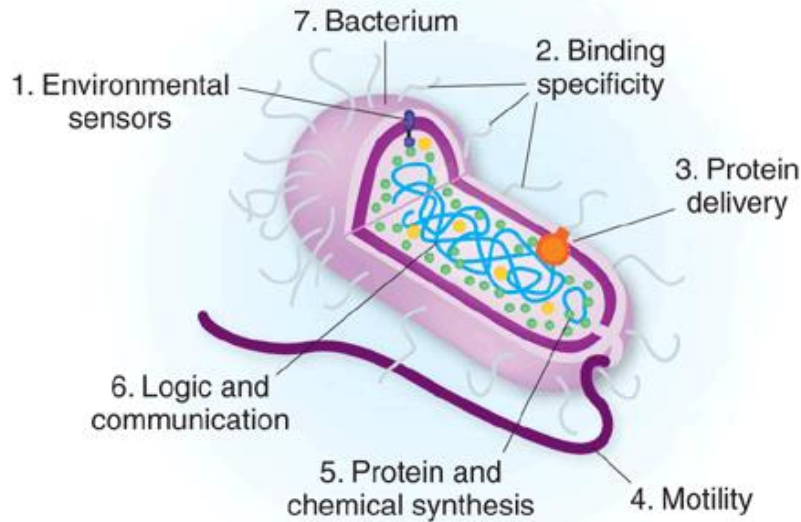
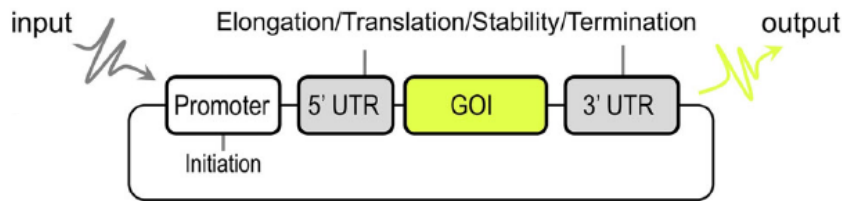
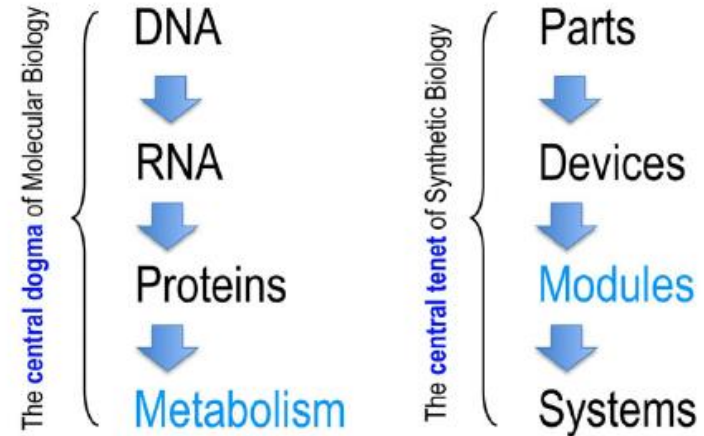


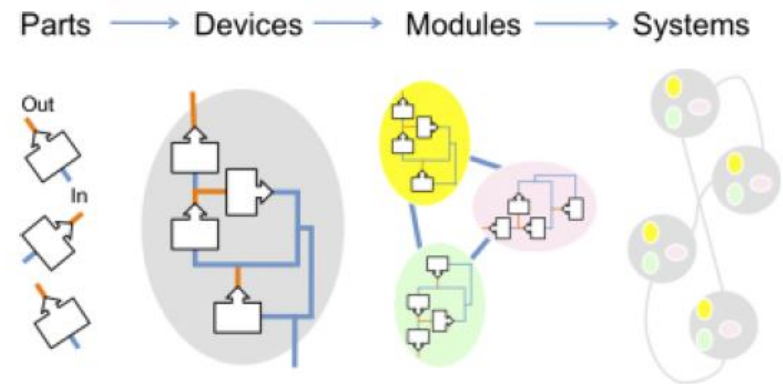
IMAGE: LIANG ZONG AND YAN LIANG

Standardization of biology

- At the beginning
Synthetic biology =
Standardized biology
- Vision >< Reality



GOI= gene of interest



BioBricks



- Biobrick – a DNA part in a standard format with known (quantifiable) function
- Form part of the iGEM competition concept
- Cloning principles allow easy and standard methods of use and sharing of BioBricks
- Documentation is an important part of BioBricks
- Inspires DIY biology, DIY bioengineering

Registry of Standard Biological Parts



tools catalog repository assembly protocols help search



Adding Parts to the Registry

The Registry's Repository contains thousands of documented parts with available DNA samples. Last year, iGEM teams submitted samples for over 1900 parts.

Be sure to add your parts and send samples to the Registry so that they can be made available to the community!

[add a part](#)
[sample submission](#)

Catalog

The iGEM Registry has over 20,000 documented parts. The Catalog organizes many of these parts by part type, chassis, function, and more. Browse for parts through the Registry Catalog or use the search menu.

2016 DNA Distribution

The iGEM 2016 DNA Distribution has shipped! The 2017 Distribution will ship to registered teams before the beginning of summer. You may read through the 2016 Distribution Handbook to get an idea of what will be included in the 2017 kit.

Collections [updated!]

We've **updated** the Registry [part collections](#). There are part collections for reporter proteins, plant chassis, cellulose-related parts, and more. Users can discover new parts and collections and build upon what previous iGEM teams and labs have achieved.

Well domesticized chassis

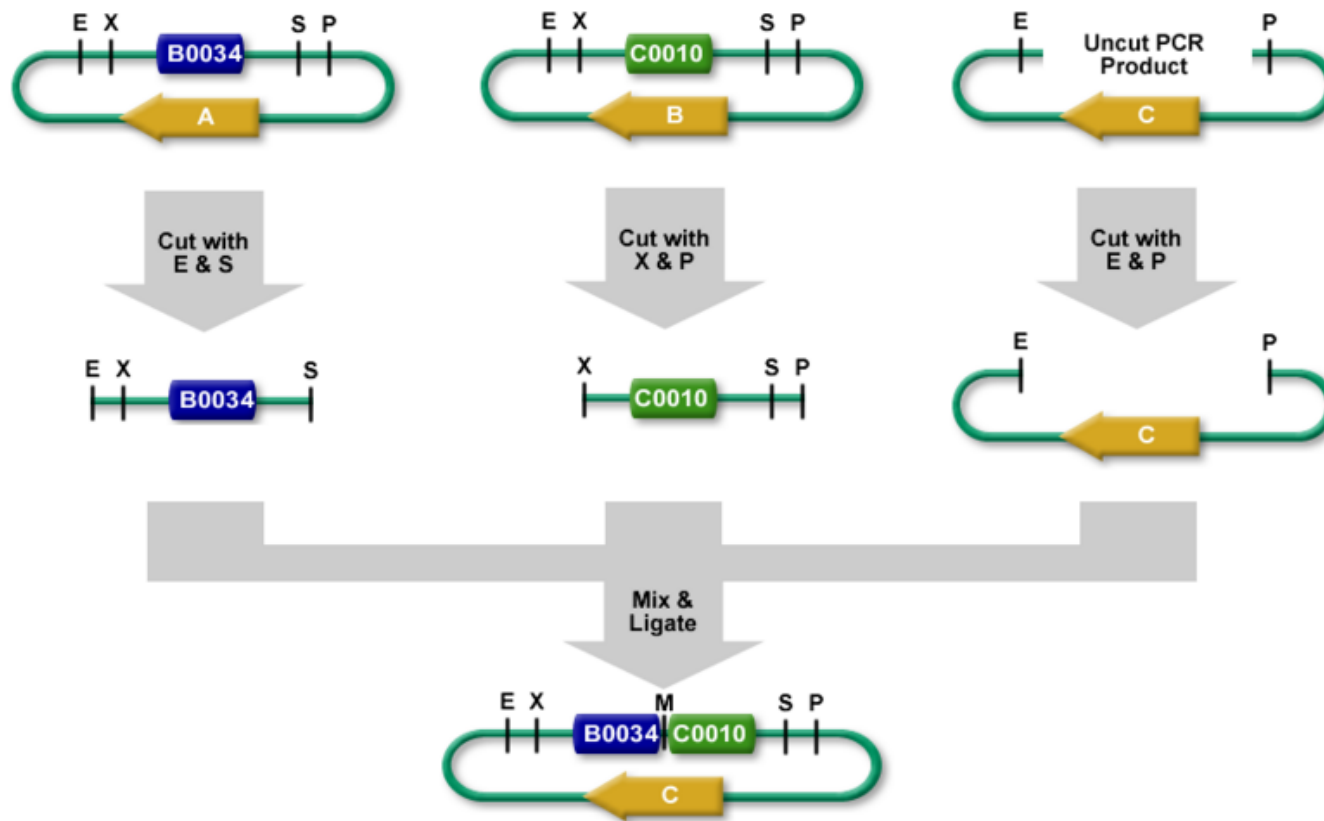
- *E. coli*, *Bacillus subtilis*, *Pseudomonas*
- Plants: <http://parts.igem.org/Collections/Plants>
- Yeasts: *Saccharomyces cerevisiae*, *Pichia pastoris*

Parts

- GOI= gene of interest (to be expressed)
- Promoters
- Terminators
- Plasmid backbones
- Chassis
- Measurement devices
- DNA parts (spacers, primer binding sites, etc..)
- Inverters
- Switches

BioBrick assembly standard RFC[10].

A strategy how to build bigger bricks from smaller parts.



EcoRI



SpeI



XbaI



PstI



Biological Building Blocks



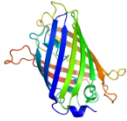
Promoter, controlling expression of a gene



Repressible promoter, active if repressor absent or inactive, binding sites for different repressors can be present



Gene, can encode signal protein or repressor



Protein, output signal



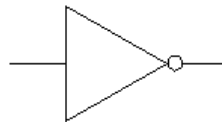
Repressor, is a protein that has binding site within promoter region



Chemical inducer, inactivating repressor

Building in/out put -> circuitries

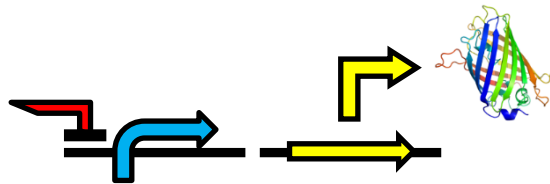
Biological NOT gate



Repressor	GFP
0	1
1	0

Truth table

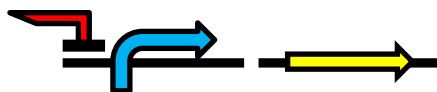
The system



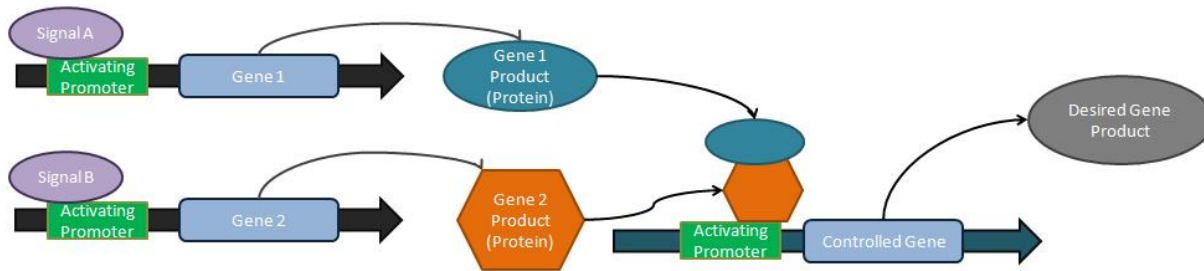
Input



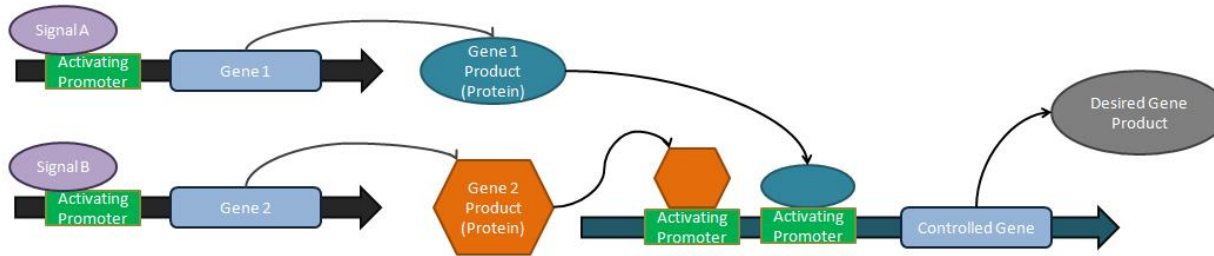
No output



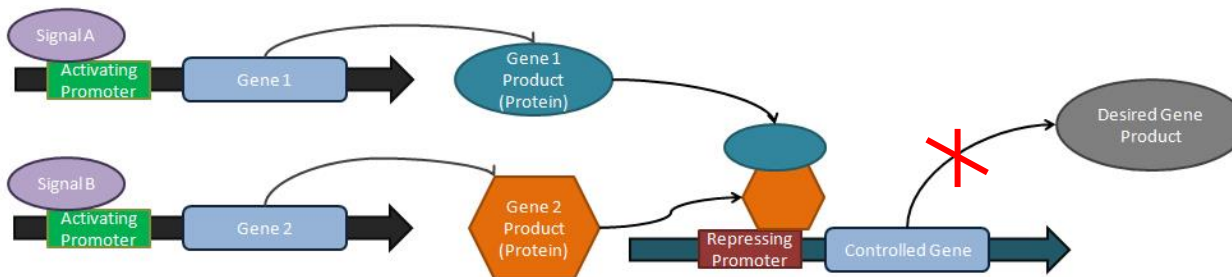
Bacterial gene expression is usually regulated by repressor proteins that bind to the promoter and prevent transcription (in eukaryotes gene regulation occurs mostly through activators).



The logical AND gate. If Signal A **AND** Signal B are present, then the desired gene product will result. All promoters shown are inducible, activated by the displayed gene product. Each signal activates expression of a separate gene (shown in light blue). The expressed proteins then can either form a complete complex that is capable of activating expression of the output (shown), or can act separately to induce expression, such as separately removing an inhibiting protein and inducing activation of the uninhibited promoter.



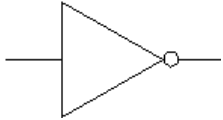
The logical OR gate. If Signal A **OR** Signal B are present, then the desired gene product will result. All promoters shown are inducible. Either signal is capable of activating the expression of the output gene product, and only the action of a single promoter is required for gene expression. Post-transcriptional regulation mechanisms can prevent the presence of both inputs producing a compounded high output, such as implementing a low binding affinity ribosome binding site.



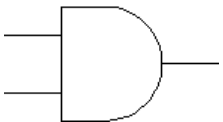
The logical Negated AND gate (= NAND gate). If Signal A **AND** Signal B are present, then the desired gene product will **NOT** result. All promoters shown are inducible. The activating promoter for the output gene is constitutive, and thus not shown. The constitutive promoter for the output gene keeps it "on" and is only deactivated when (similar to the AND gate) a complex as a result of two input signal gene products blocks the expression of the output gene.

Logic Gates

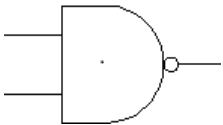
Genetic circuits can implement logic gates, logic gates can be built using genetic circuits. Logic gates are circuits in which the relationship between the input and the output is based on a certain logic.



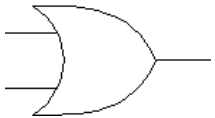
NOT: The output of a NOT gate is the inverse (opposite) of its input, so the output is true when the input is false. A NOT gate is also called an **inverter**.



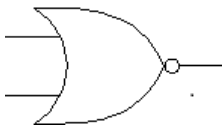
AND: The output of an AND gate is true when all its inputs are true.



NAND: The NAND gate operates as an AND gate followed by a NOT gate (repression). It acts in the manner of the logical operation "and" followed by negation. The output is "false" if both inputs are "true." Otherwise, the output is "true."



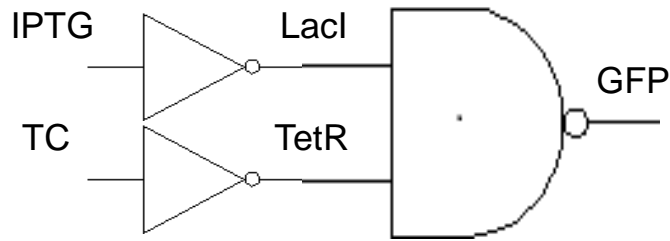
OR: The output of an OR gate is true when at least one of its inputs is true.



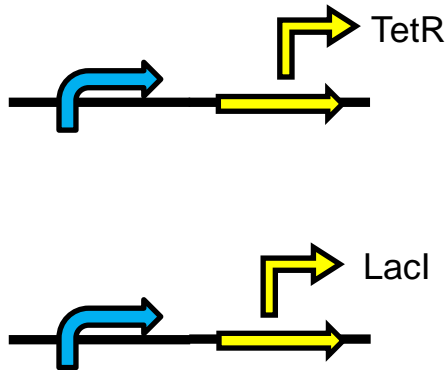
NOR: The NOR gate is a combination of an OR gate followed by an inverter. Its output is "true" only if both inputs are "false." Otherwise, the output is "false."

Note that in biology the system may not be fully on or off (unless you design it to be), and the amount and binding efficiency of the regulator to the promoter affects the output. This is also a way to finetune expression.

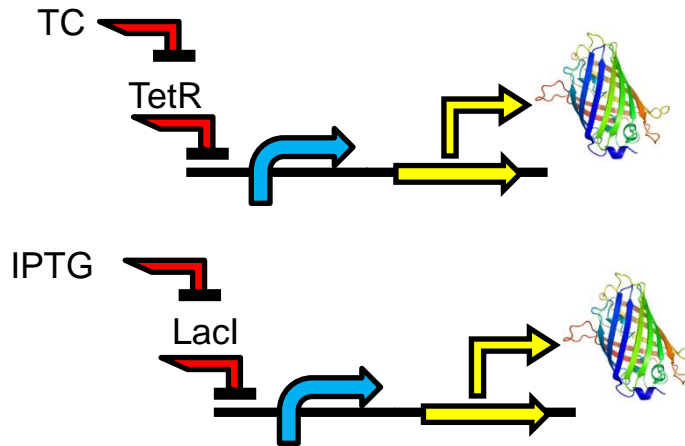
Biological OR gate



IPTG	TC	GFP
0	0	0
0	1	1
1	0	1
1	1	1



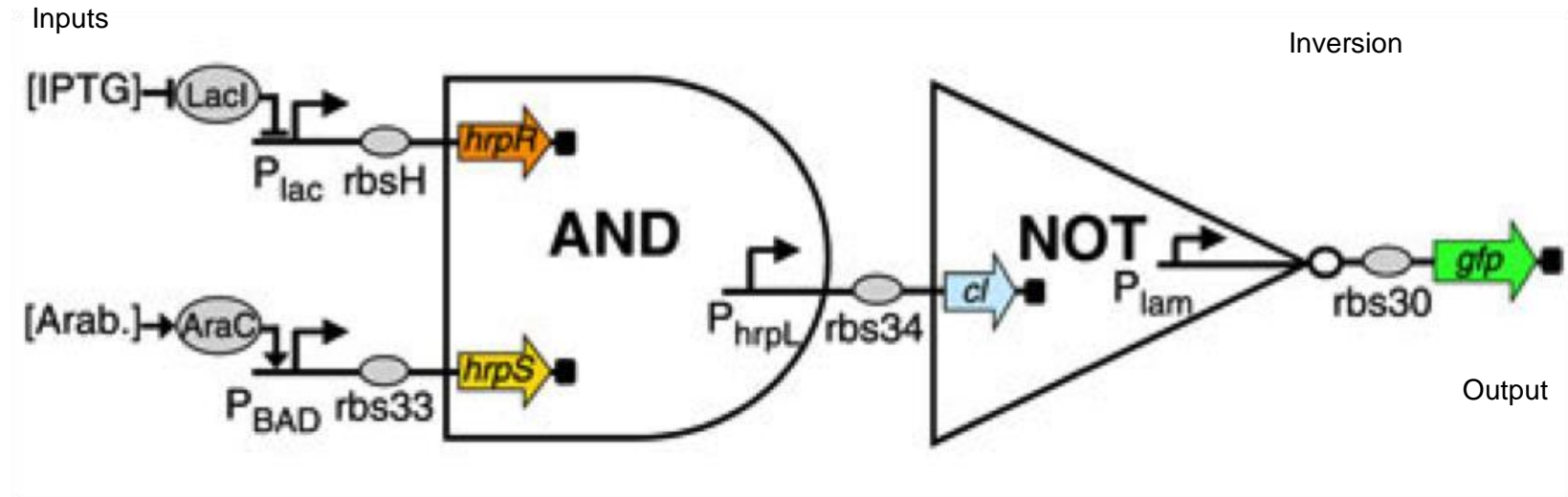
Constitutive promoter:
"On" if not repressed.



Two promoter systems, same output

TetR and LacI are bacterial repressor proteins that bind to promoters preventing transcription. TC (tetracycline) and IPTG are chemical compounds that inhibit the action of the repressors, respectively. TC and IPTG are added to the bacterial culture medium to turn on expression of the gene of interest at will.








Example of a NAND gate in more detail



IPTG induces the *lac* promoter through inactivation of the repressor LacI.
Arabinose activates the inducer AraC that activates the *BAD* promoter.

When both *hrpR* and *hrpS* bind to the *hrpL* promoter, they cause expression of the *cl* repressor of the *lam* promoter. Green fluorescent protein (GFP) is not produced.

Logic gates and truth tables

Name	NOT	AND	NAND	OR	NOR	XOR	XNOR																																																																																																
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XOR, as an example:
 X gene is not normally expressed. A OR B are needed to express X, thus they act as activators individually. But when both are present the complex forms a repressor.

Toggle switch, kill switch



Example from: http://2014.igem.org/Team:Wageningen_UR



Banana plant infection by the fungus *Fusarium oxysporum* is a general concern. A design concept: an *E.coli* that would produce various antifungal agents when fusaric acid, produced by *Fusarium*, is present

Part:BBa_K1493000 - *Fusaric acid induced regulatory promoter*

http://parts.igem.org/Part:BBa_K1493000

Part:BBa_K1493000

Registry of Standard Biological Parts

[main page](#) [design](#) [experience](#) [information](#) [part tools](#) [edit](#)

Part:BBa_K1493000

Designed by: Jeremy van Baalen Group: iGEM14_Wageningen_UR (2014-10-06)



Regulatory

Not Released
Sample It's complicated
Experience: None
Not Used

[Get This Part](#)

Fusaric acid induced regulatory promoter

Promoter fusaric acid inducible

Usage and Biology

A fusaric acid efflux pump within *Pseudomonas putida* is encoded by an operon consisting of four genes. We found that this operon is controlled by a LysR-type gene (pp1262) which is located upstream of the operon. This gene inhibits the binding of RNA polymerase to the promoter in the intergenic region between pp1262 and the operon. Fusaric acid blocks this inhibition, allowing activity of the operon. (See figure 1) Hence, pp1262 and the intergenic region are isolated and put into BioBrick form, effectively acting as a Fusaric Acid inducible Promoter (FAiP).

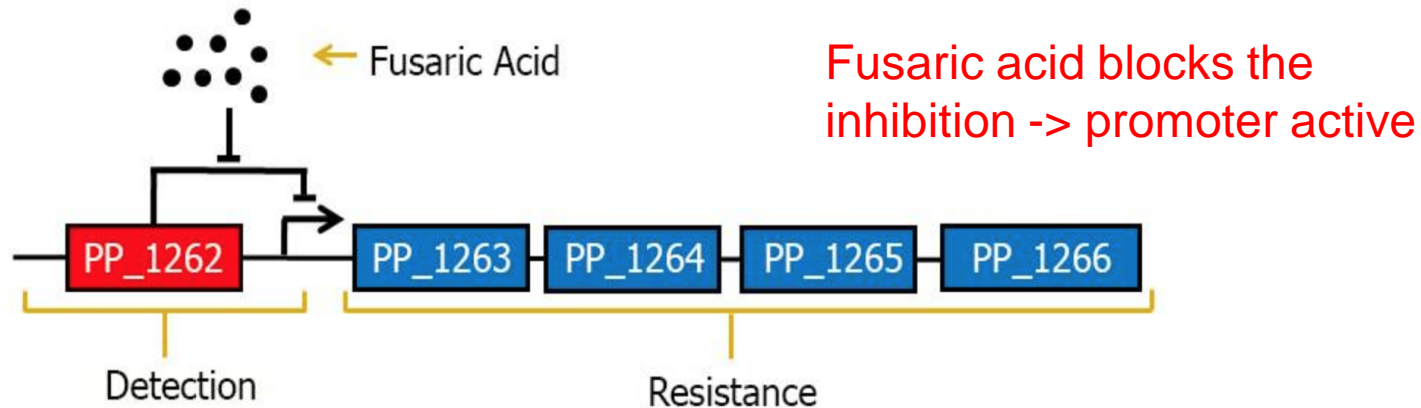


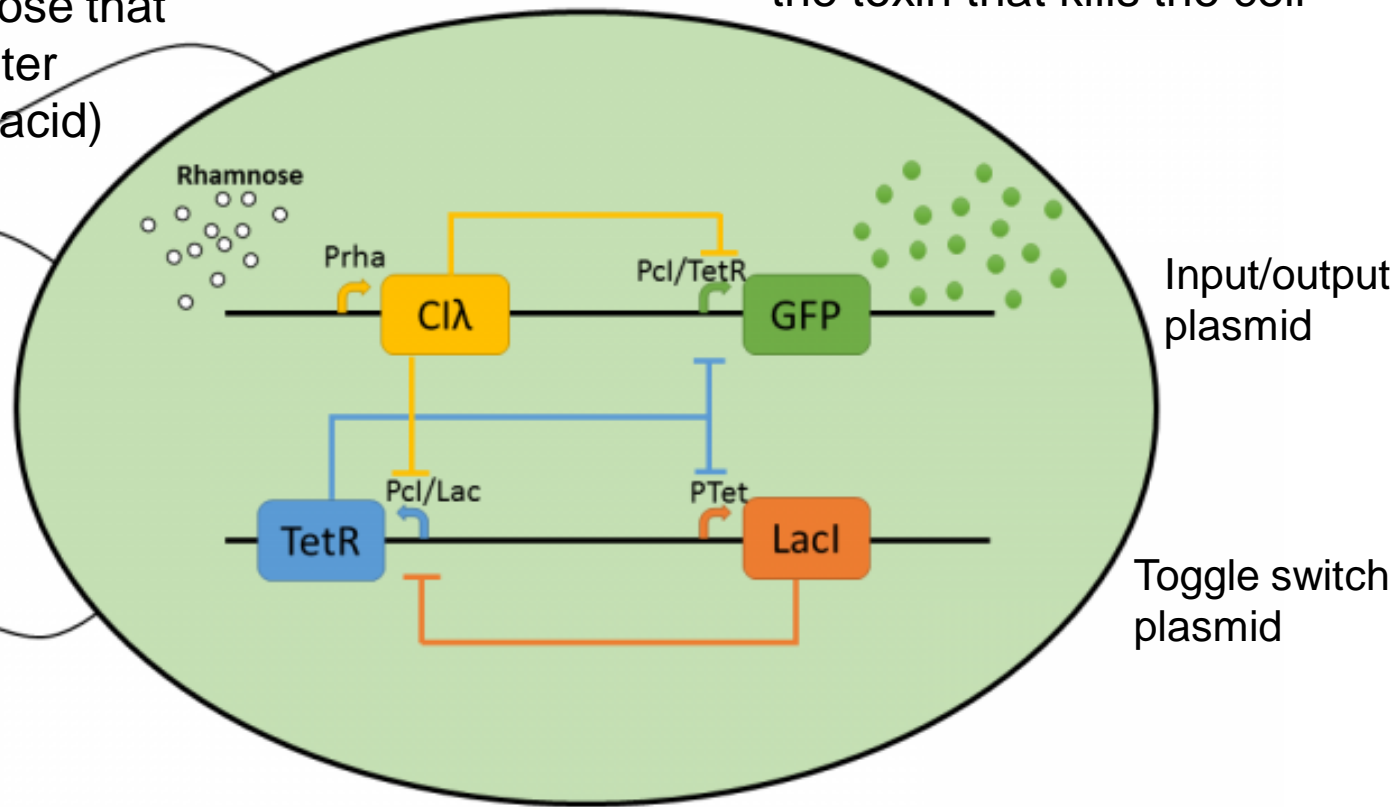
Figure 1. Fusaric acid efflux pump operon present in the genome of KT2440 *Pseudomonas putida*.

The kill switch concept

killing the antifungal agent producing *E.coli* when no fusaric acid (no *Fusarium*) is present any more

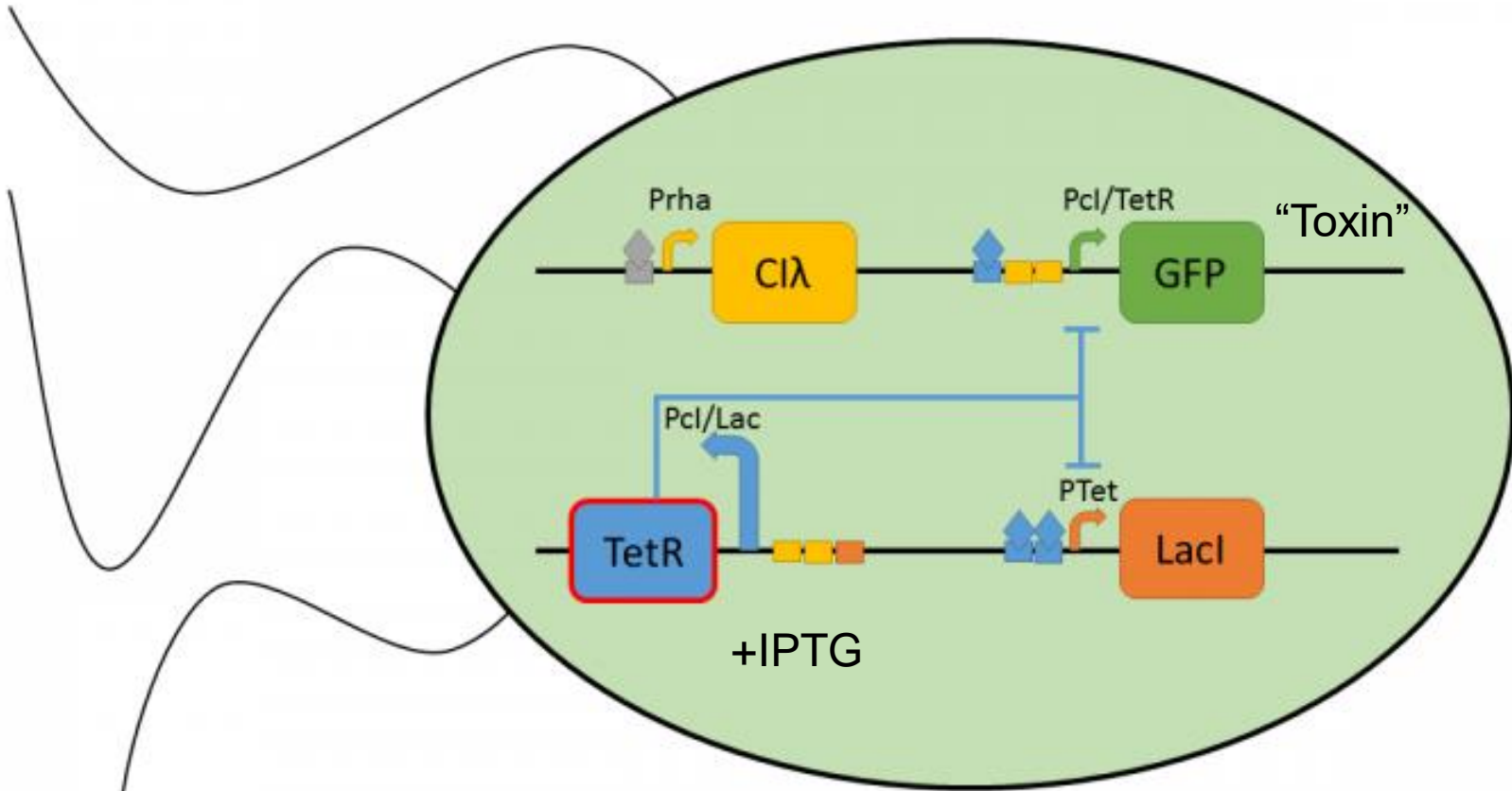
Tested with rhamnose that induces the promoter (instead of fusaric acid)

GFP; could be replaced by the toxin that kills the cell



Toggle switch in TetR – state

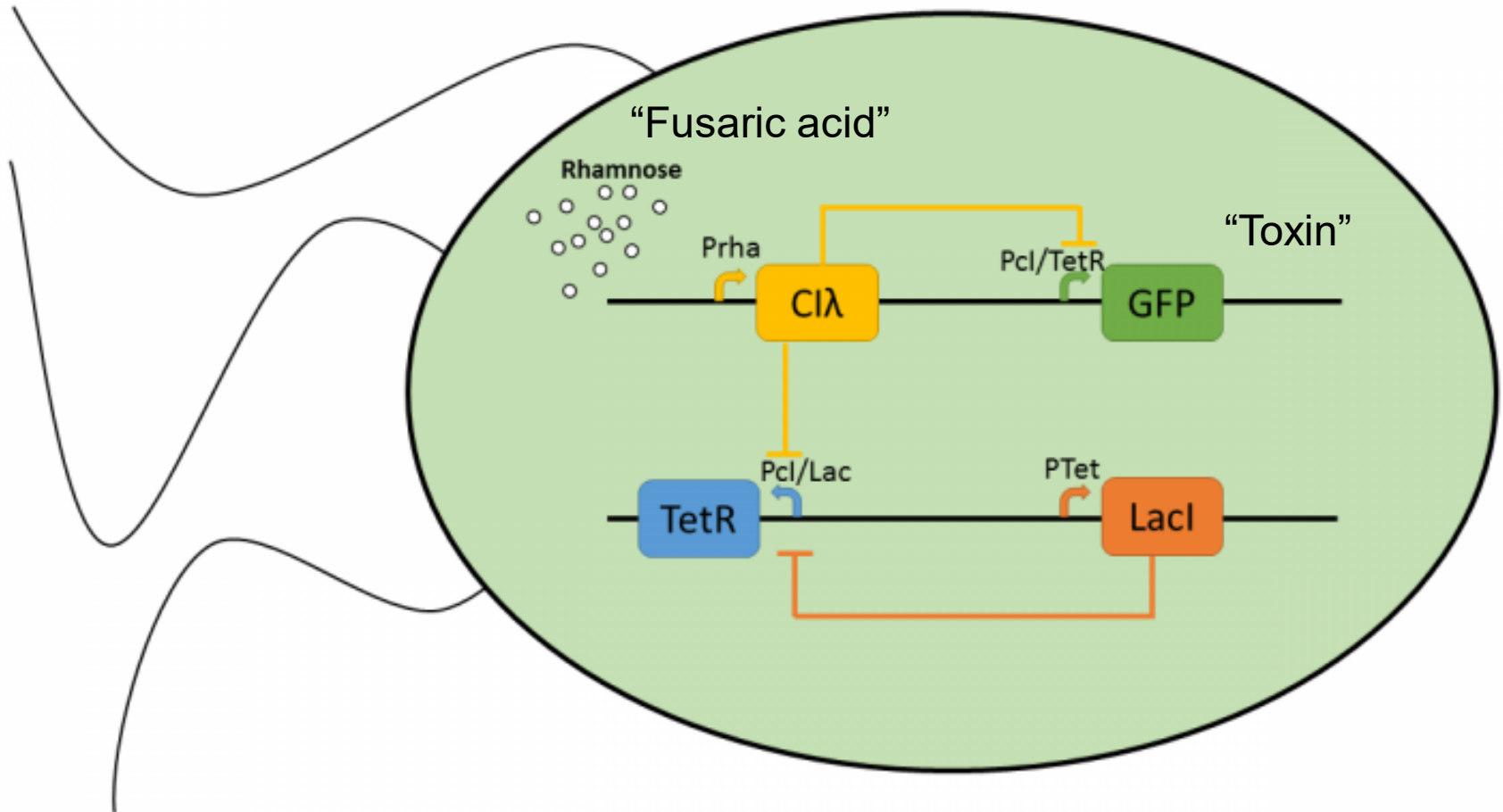
(keeping alive, propagation)



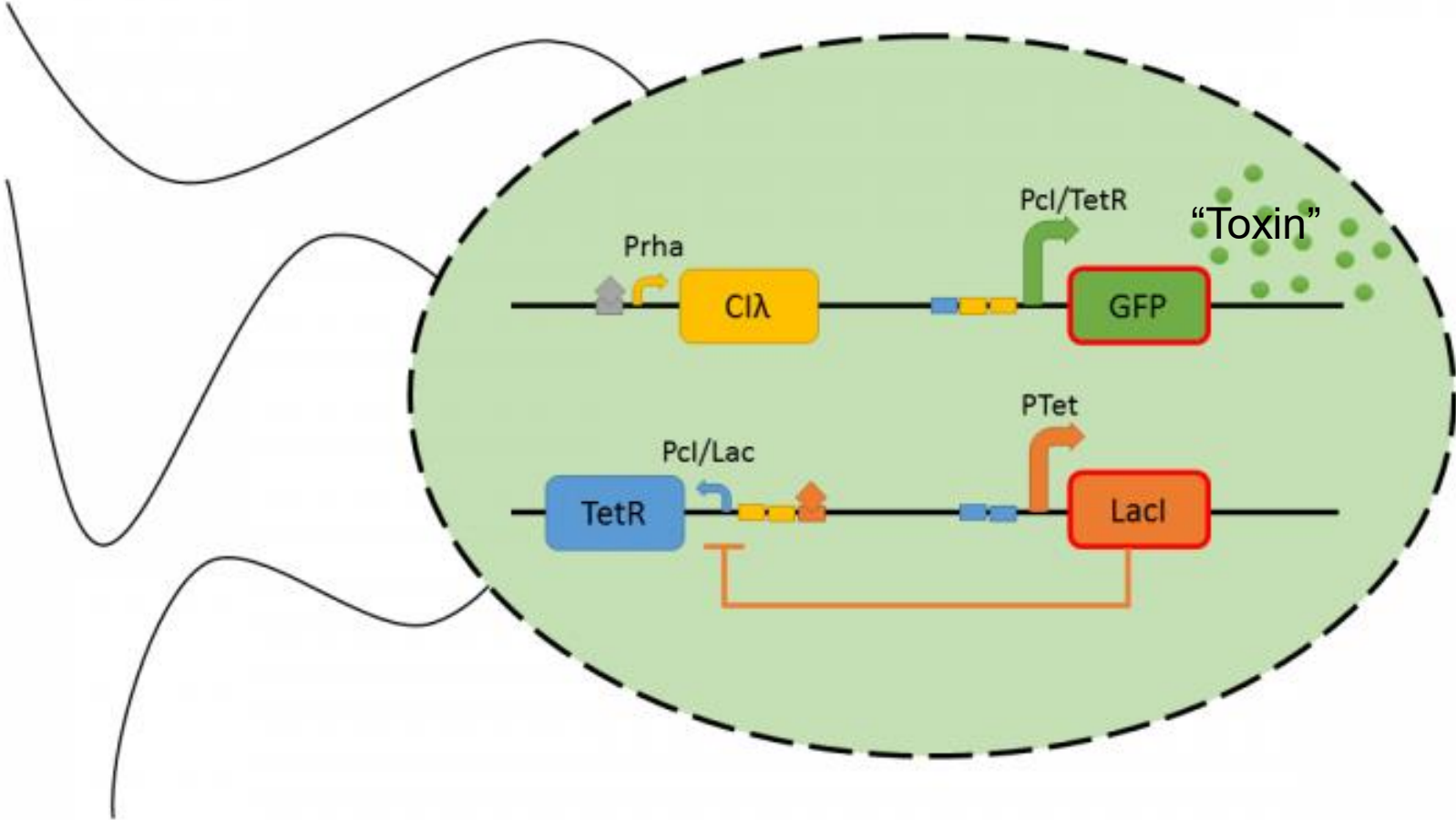
Set up the system by IPTG induction

No inducer rhamnose and no inhibitor for TetR (tetracycline or anhydrotetracycline)

Rhamnose (fusaric acid) present

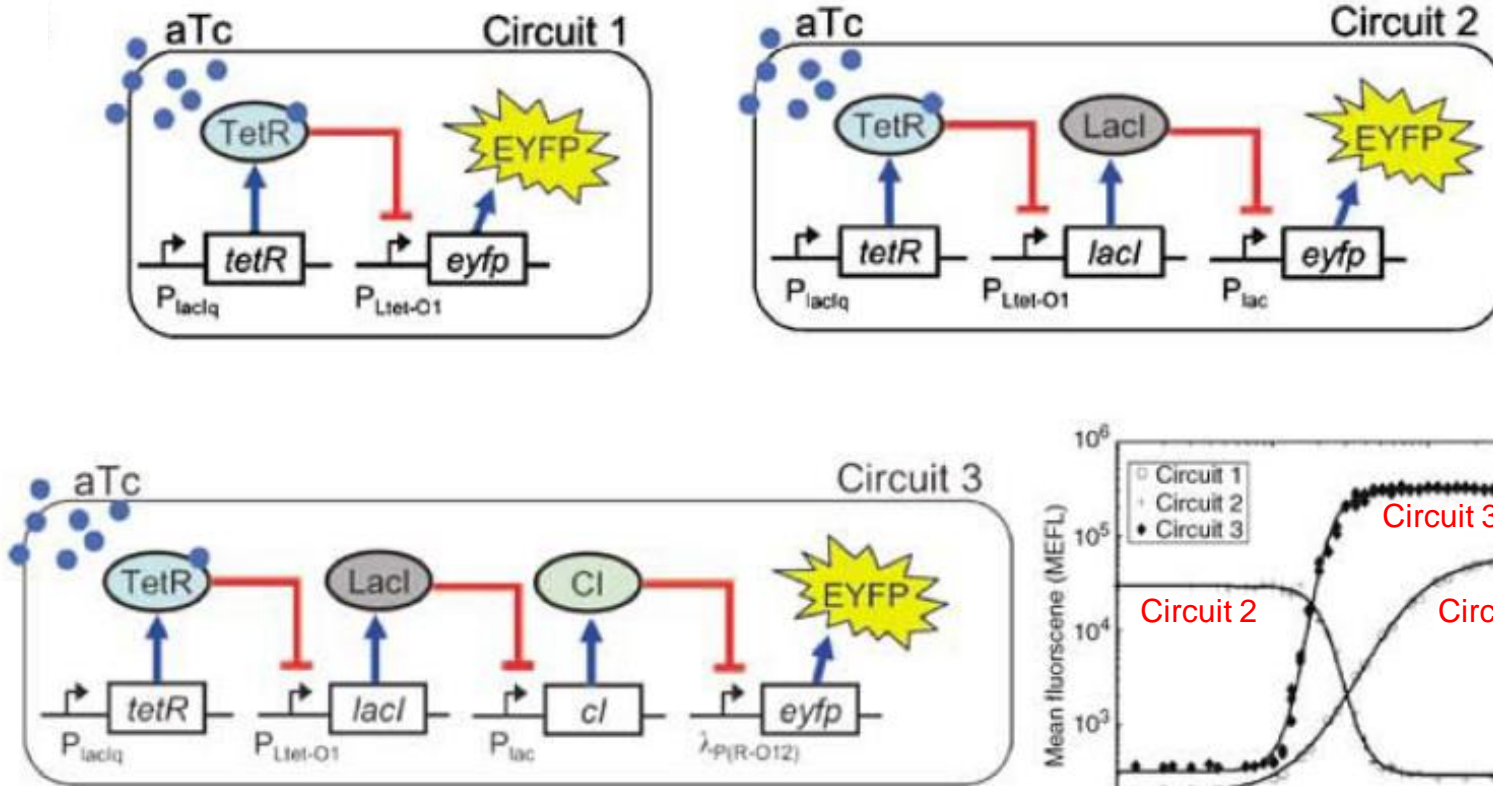


Kill switch on



Building transcriptional devices

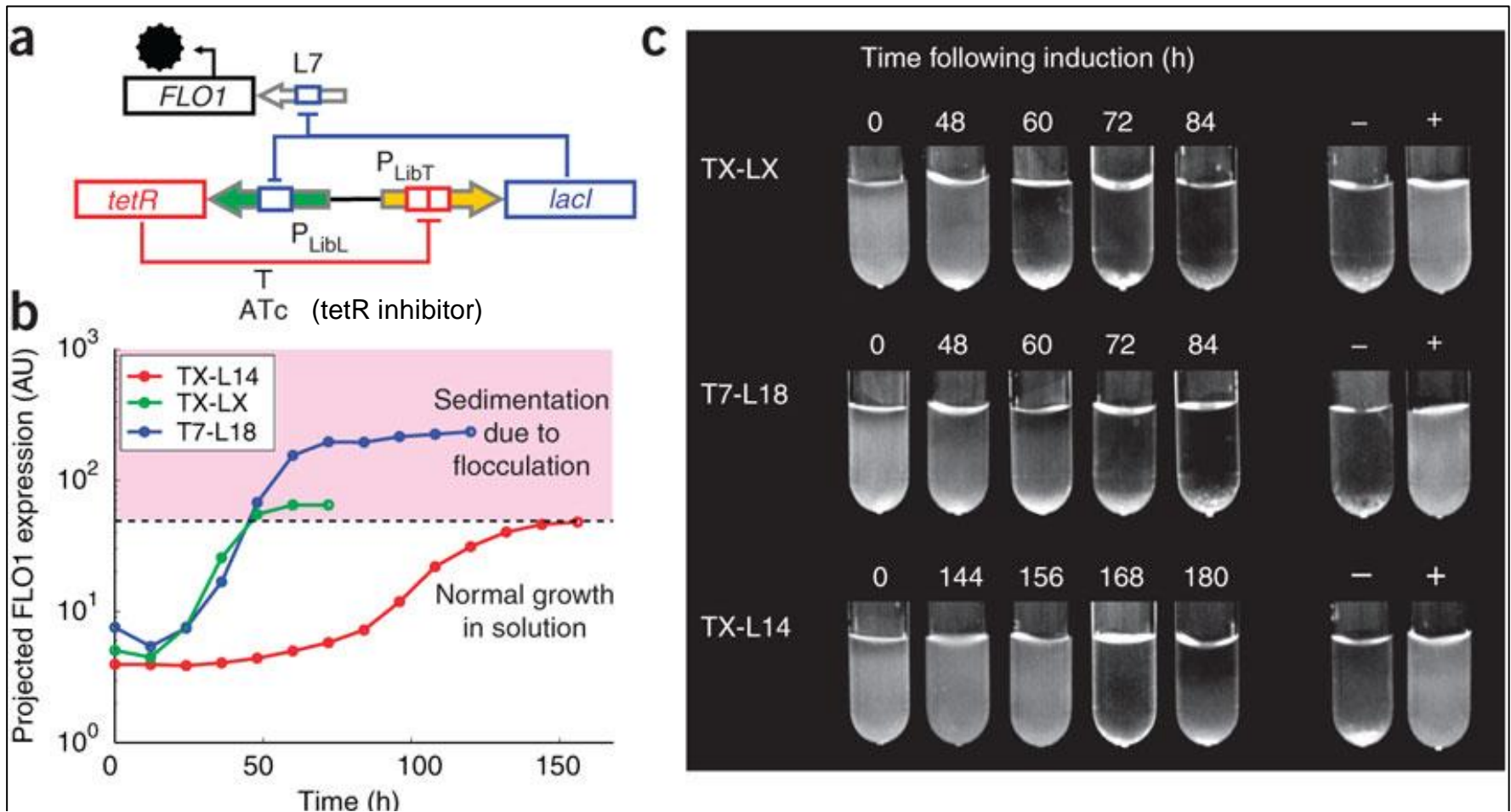
TetR, LacI and CI are bacterial/viral repressors. TetR repression is inhibited by tetracycline or its analog, anhydrotetracycline (**ATc**). *E.coli* system.



Demonstration of standardized in/output. Modelling of cascades.

Orthogonal control circuit in yeast using *E.coli* parts

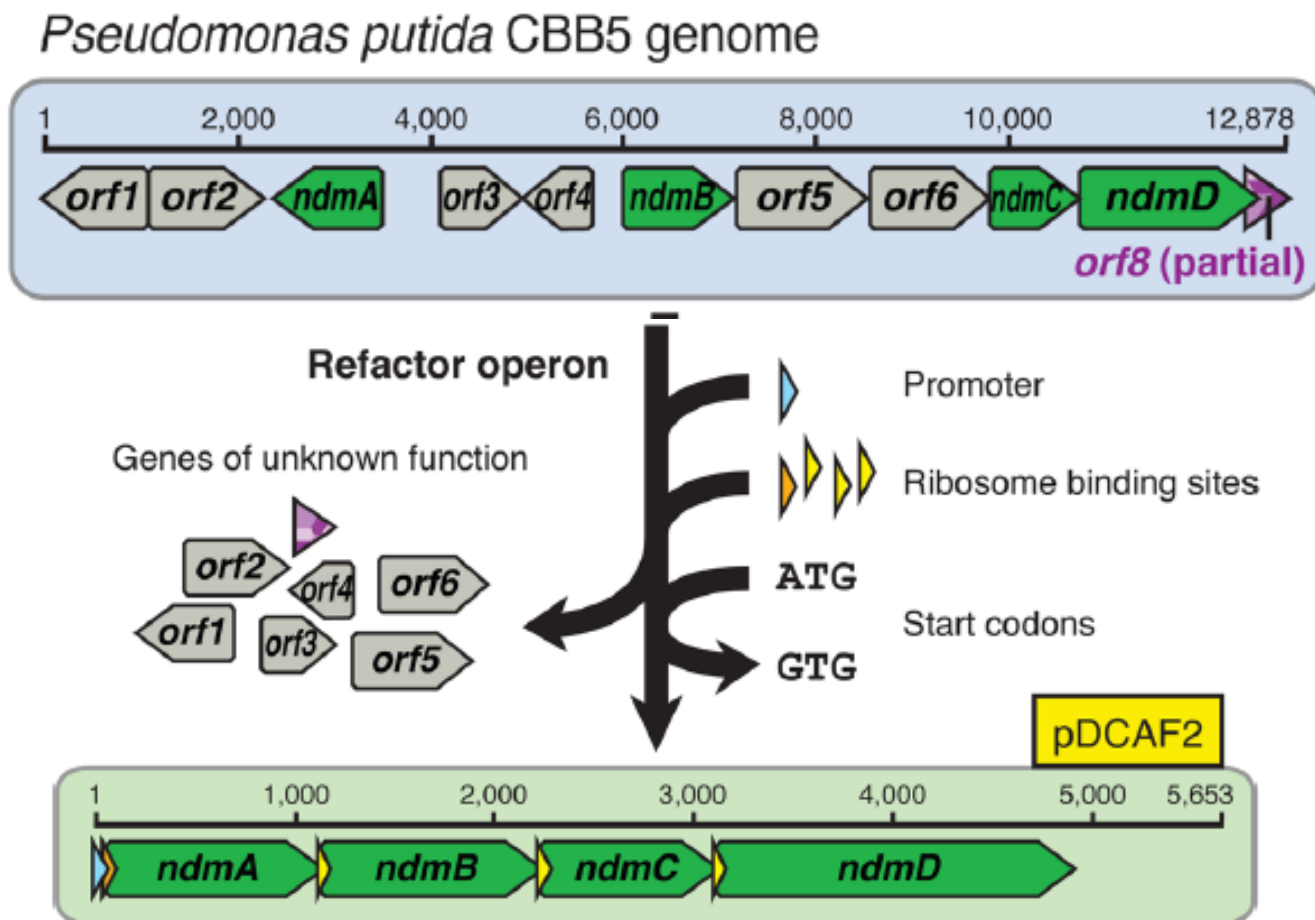
Ellis et al. Nat Biotechnol. 2009; 27(5): 465–471. doi:10.1038/nbt.1536.



Control of yeast sedimentation (flocculation) with anhydrotetracycline (ATc) controllable expression circuit after product formation to aid product recovery and cell removal

Refactoring operons

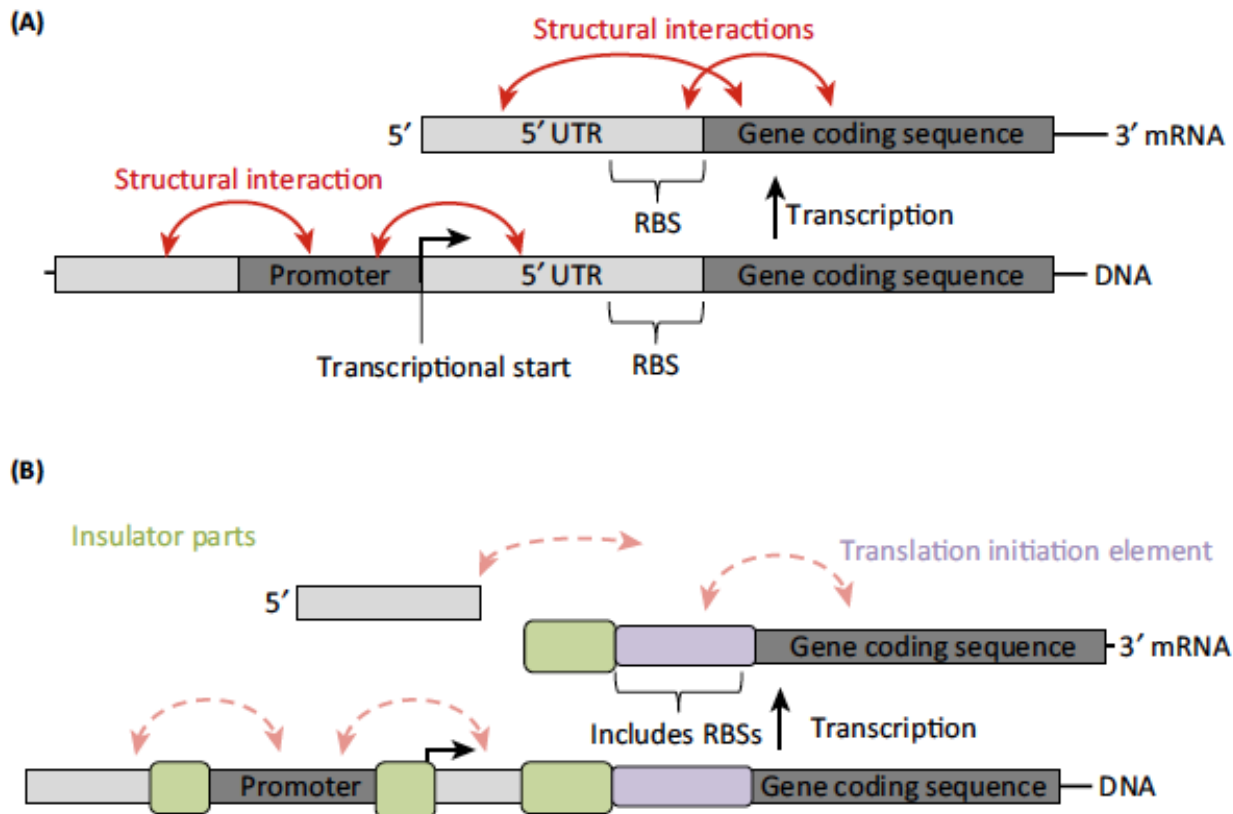
Caffeine operon from *Pseudomonas* to *E.coli*



E.coli lacI gene has a GTG start codon

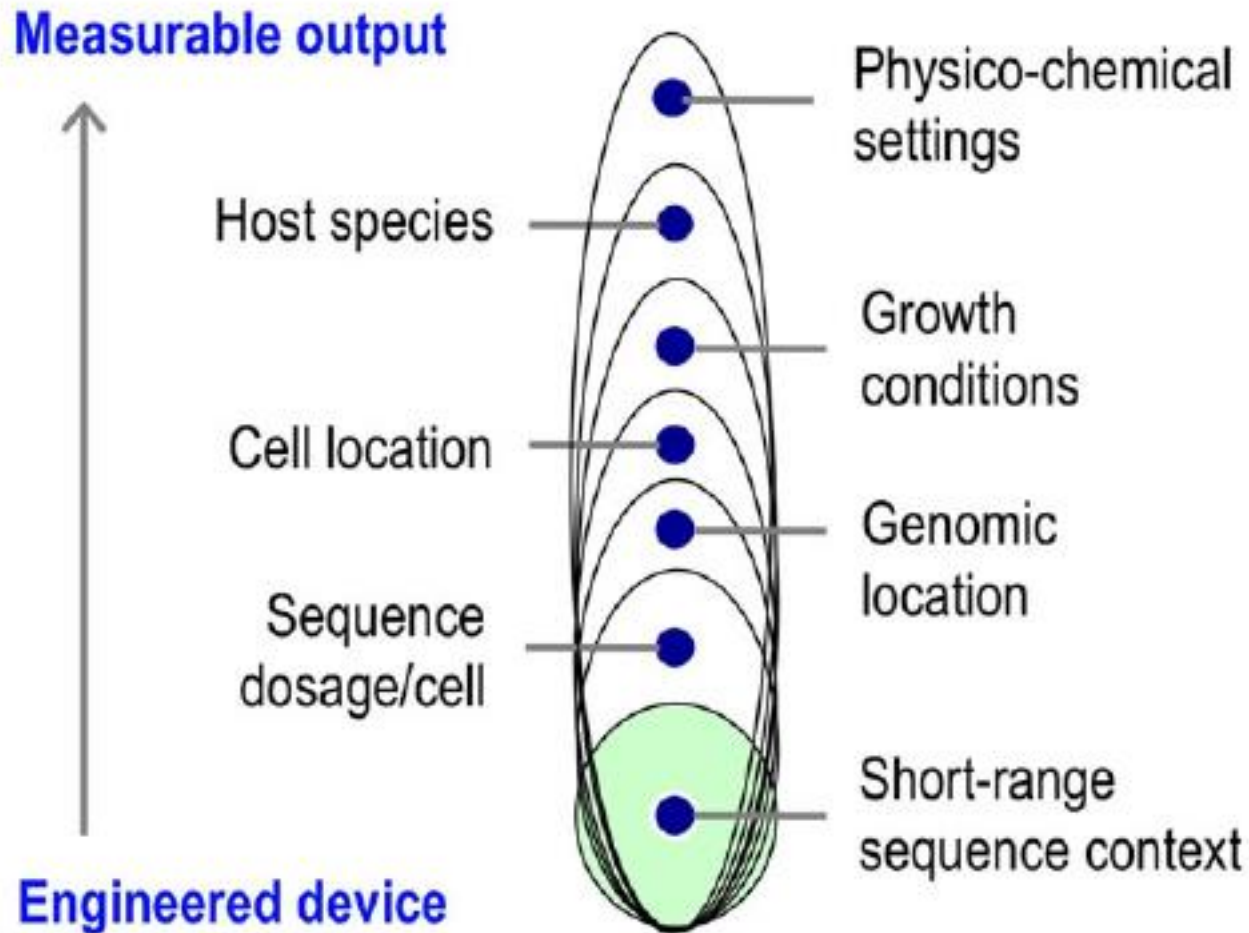
Robustness of parts

- Switching time of toggle switch
- Sensitivity of cascade, interactions between parts



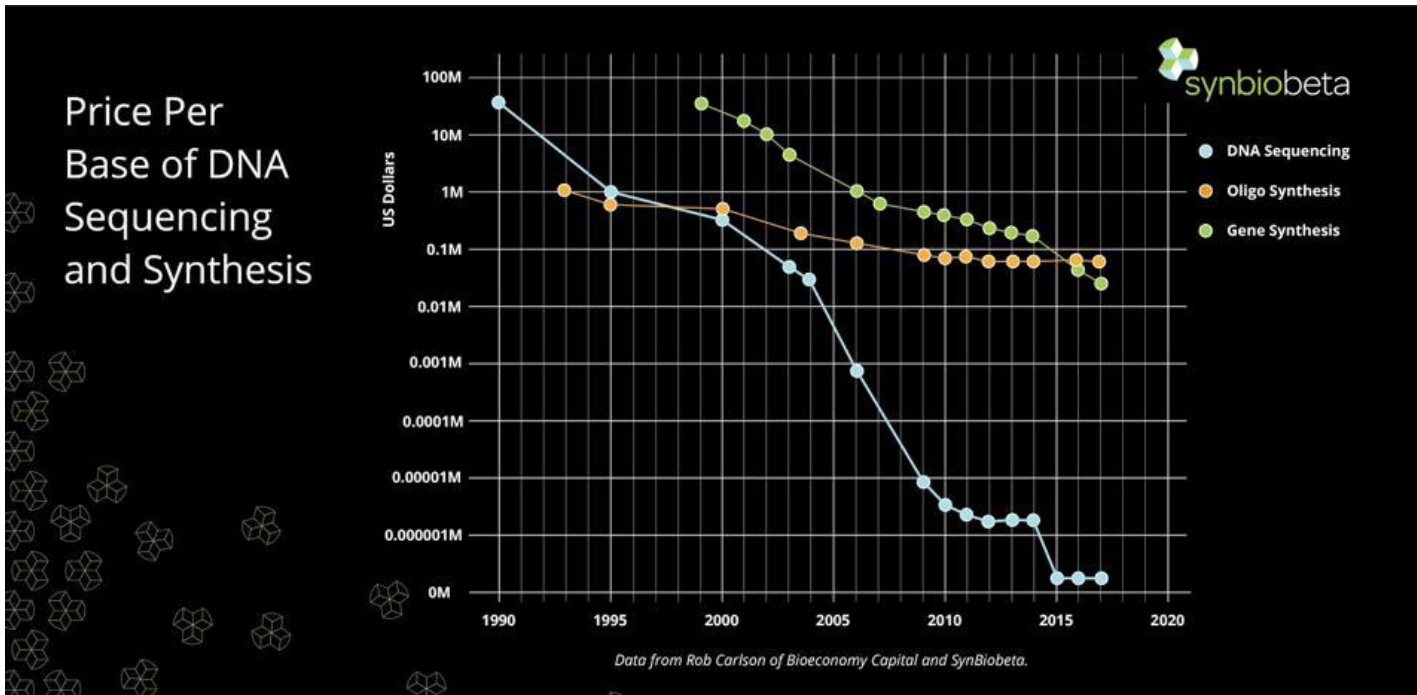
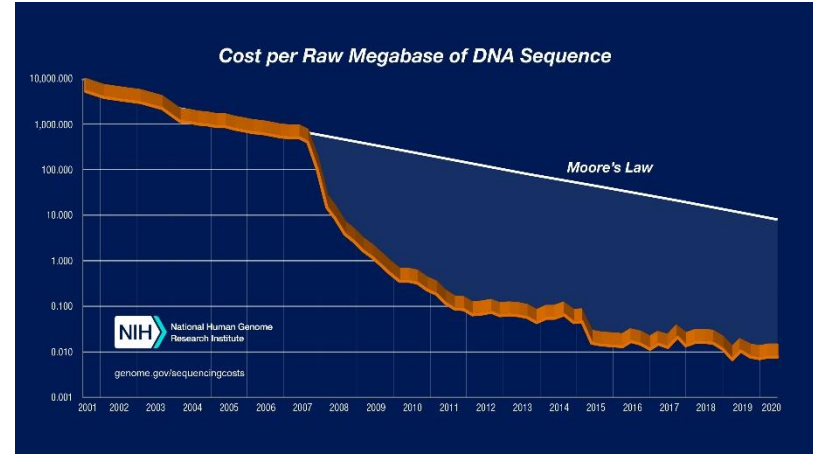
TRENDS in Biotechnology

Functionality and context



- <http://parts.igem.org> - very useful
- BioBricks enable controllability and modelling of predicted function of even complex cascades
- Problems: Cloning inefficiency, cloning scars and frame shifts
- The cost of gene synthesis goes down dramatically. Long pieces of DNA can be designed and synthesized. Reduces the need to build modules from BioBricks.

The future of BioBricks (or normal cloning) ?



Group work - BioBricks

- Design a sensor based on standard parts for input and parts for outputs (+ circuit variations) using the iGEM registry for standard parts.
- Describe the idea of what kind of a sensor you want to build and why. Identify the selected parts (iGEM code numbers), how they work, how you assemble them (assembly standard) and how the system works in the off/on state. Show the design in the way you have seen in the course lectures. Give the truth table of your circuit design.
- Send the presentations to merja.penttila@vtt.fi by noon the 25.3. the latest.
- Max 15min for presentation, followed by discussion. Present as a group. Be clear, speak slowly.