Aalto University
School of Chemical
Technology

# *Synthetic biology*

## *(Course CHEM-E8125), spring 2019*

# *Biobricks & circuits*

Prof. Merja Penttilä

(Chris Jonkergouw)

# Designing cellular functionalities

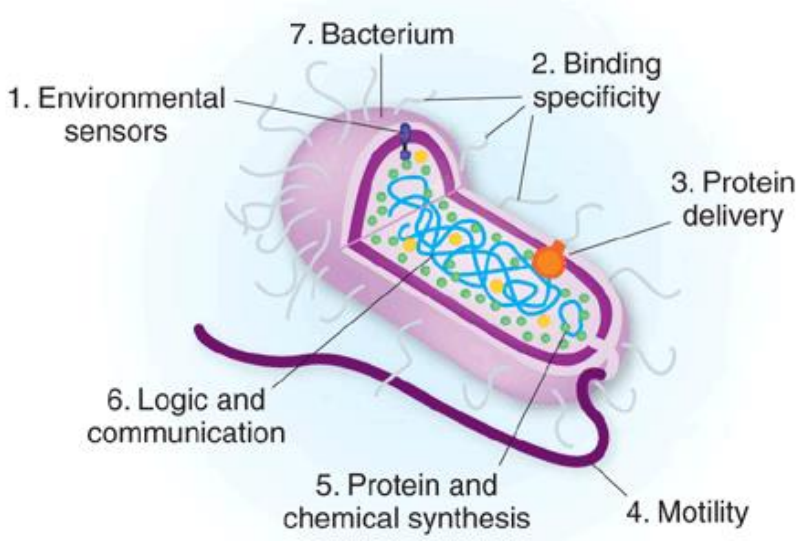
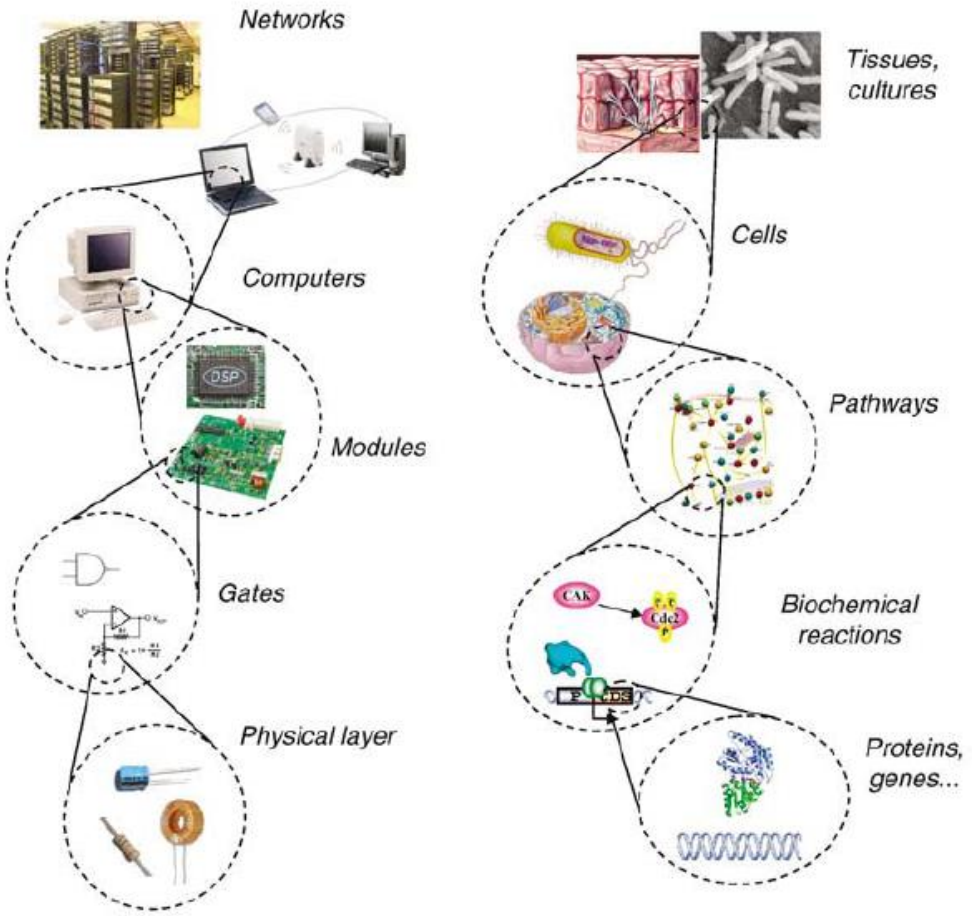


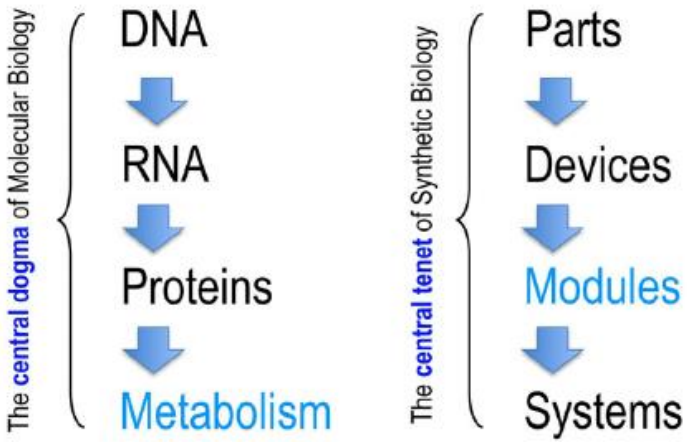

IMAGE: LIANG ZONG AND YAN LIANG

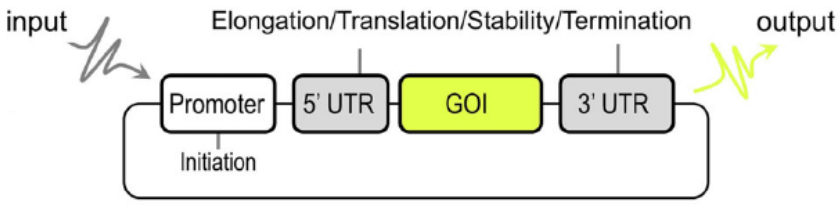
# Analogy to electric engineering

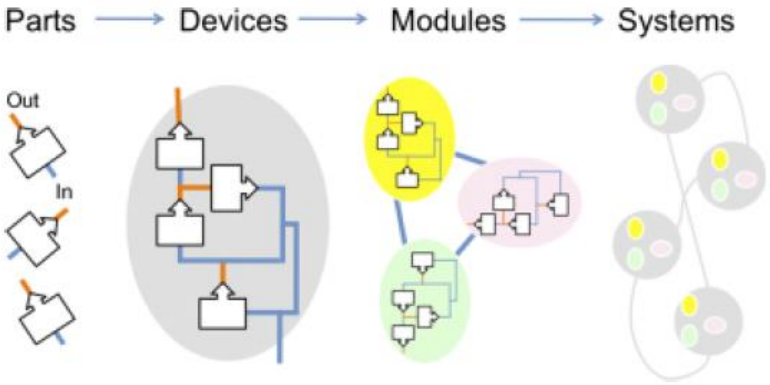


# Standardization of biology

- In the beginning
  Synthetic biology =
  Standardized biology
- vision >< reality




Confidence, tolerance,….Bioessays 37: 95–102, DOI 10.1002/bies.201400091

# 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



Building outside of the box: iGEM and the BioBricks Foundation.
http://dx.doi.org/10.1038/nbt1209-1099

# Registry of Standard Biological Parts

iGEM tools catalog repository assembly protocols help search BBa_


## 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.

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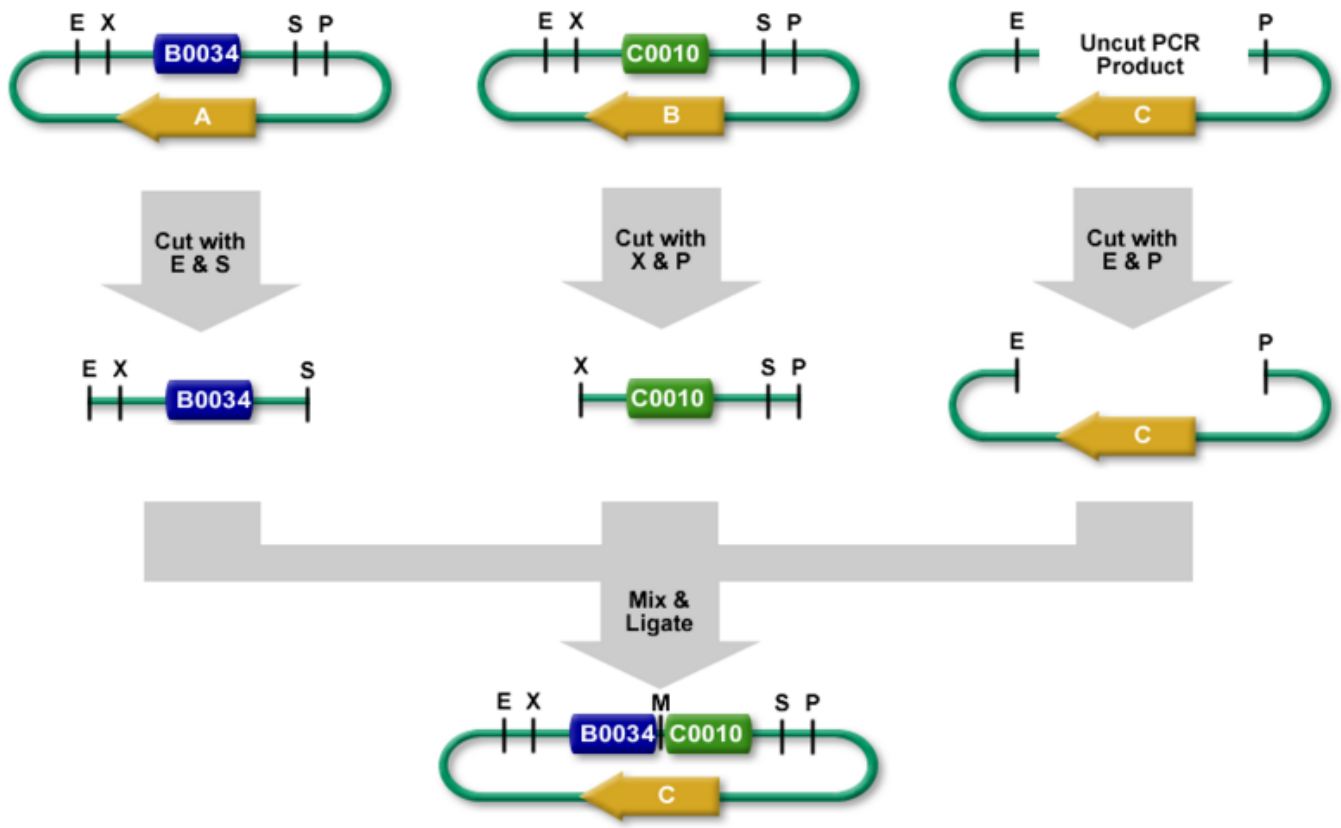
# Well domesticized chassis

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

# BioBrick assembly standard RFC[10]




| EcoRI | SpeI | XbaI | PstI |
|---|---|---|---|
| 5′...G▼AATTC...3′<br>3′...CTTAA▲G...5′ | 5′...A▼CTAGT...3′<br>3′...TGATC▲A...5′ | 5′...T▼CTAGA...3′<br>3′...AGATC▲T...5′ | 5′...CTGCA▼G...3′<br>3′...G▲ACGTC...5′ |

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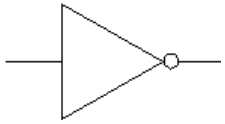
M = mutated

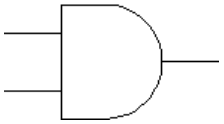
# Parts

- Promoters
- Terminators
- Plasmid backbones
- Chassis
- Measurement devices
- DNA parts (spacers, primer binding sites, etc..)
- Inverters
- Switches

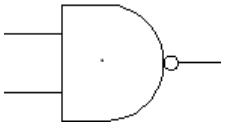
# Logic Gates



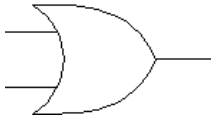
**NOT:** The output of a NOT gate is the inverse (opposite) of its input, so the output is true when the input is false. A NOT gate is also called an **inverter.**



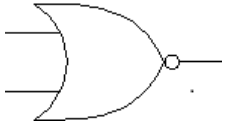
**AND**: The output of an AND gate is true when all its inputs are true.



**NAND**: The NAND gate operates as an AND gate followed by a NOT gate. 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.



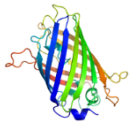
**NOR:** The NOR gate is a combination of and OR gate followed by an inverter. Its output is "true" if both inputs are "false." Otherwise, the output is "false."

# 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

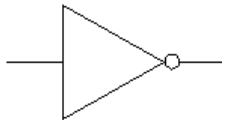


Protein, output signal

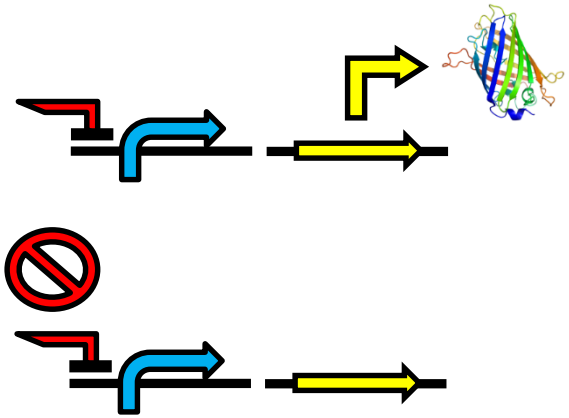
Repressor, is a protein that a has binding side within promoter region

Chemical inducer, inactivating repressor

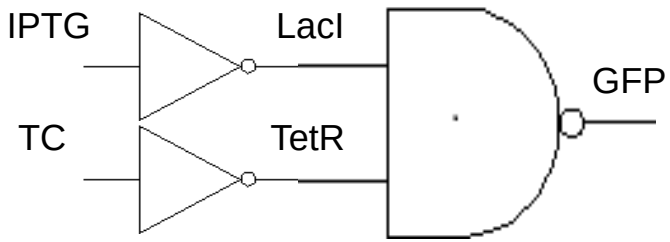
# Biological NOT gate



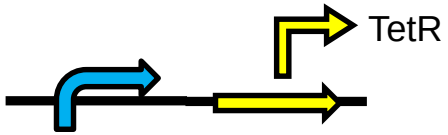
| Repressor | GFP |
|---|---|
| 0 | 1 |
| 1 | 0 |

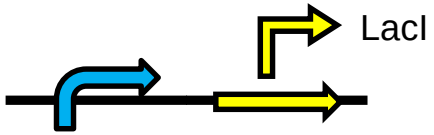


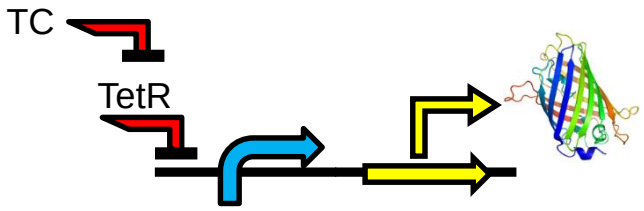
# Biological OR gate

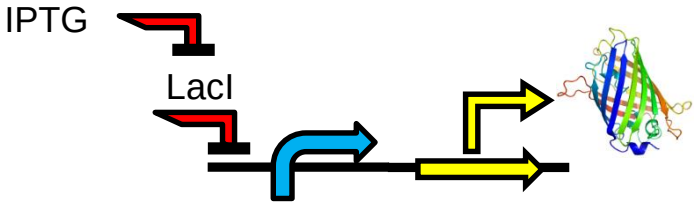



| IPTG | TC | GFP |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 1 | 1 |
| 1 | 0 | 1 |
| 1 | 1 | 1 |






TC = tetracycline

# Toggle switch, kill switch

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


Banana plant infection by ***Fusarium oxysporum*** is a general concern. An *E.coli* was developed that produced various antifungal agents when fusaric acid (produced by *Fusarium*) was 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

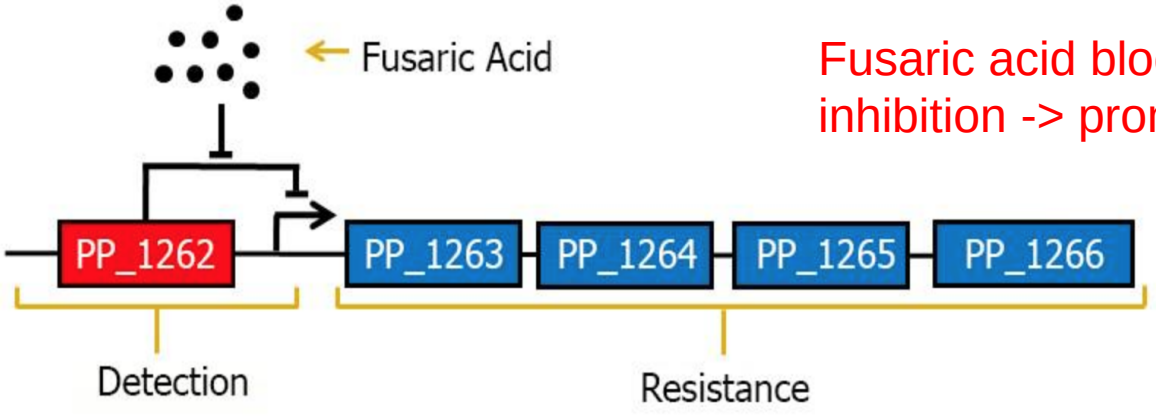
| |
|---|
| Not Released |
| Sample It's complicated |
| Experience: None |
| Not Used |
| Get This Part |

### 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).

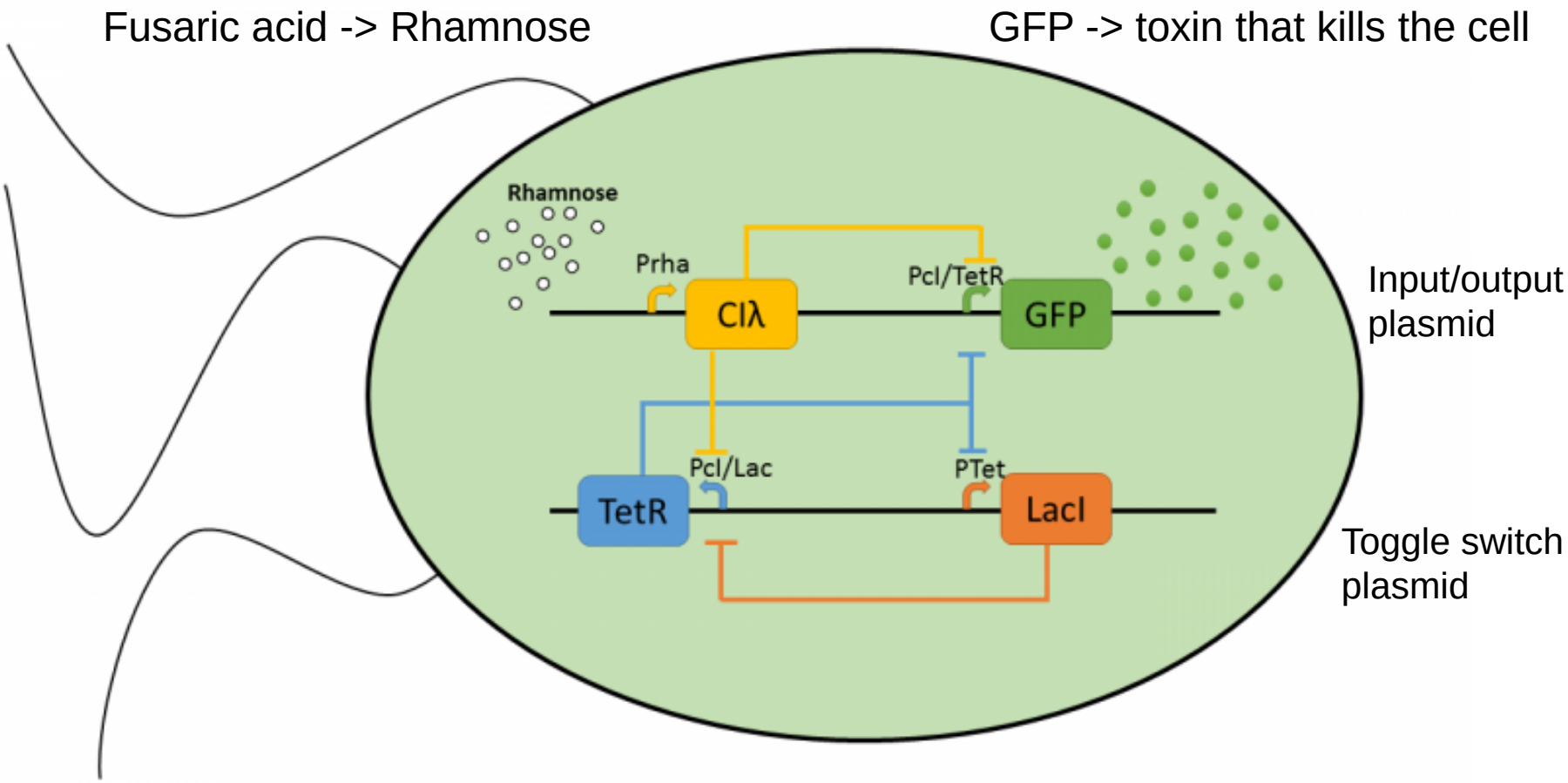



Fusaric acid blocks the inhibition -> promoter active

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

# The kill switch concept

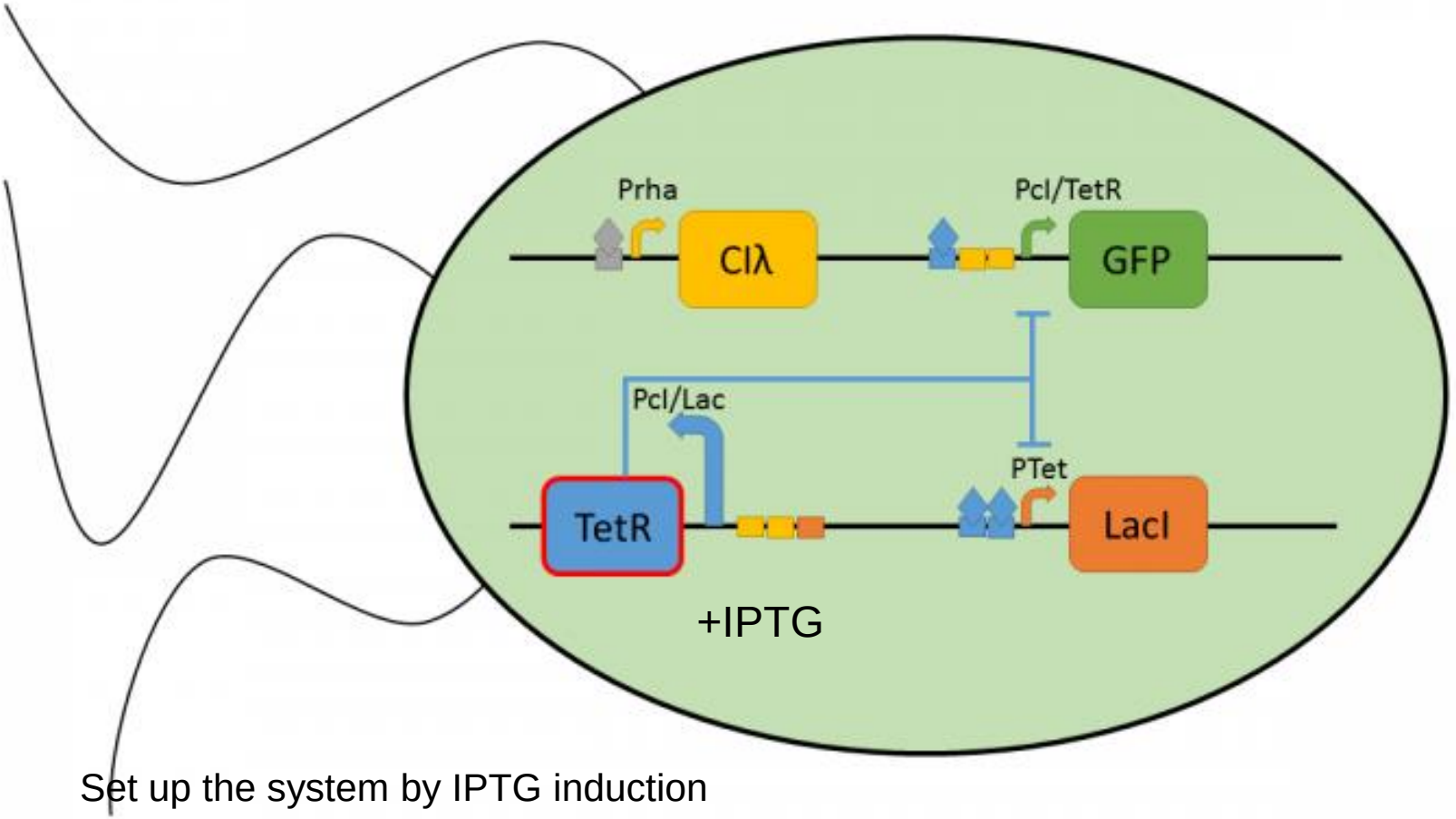
## killing the antifungal agent producing *E.coli* when no fusaric acid (no *Fusarium*) is present any more






http://2014.igem.org/Team:Wageningen_UR/project/kill-switch

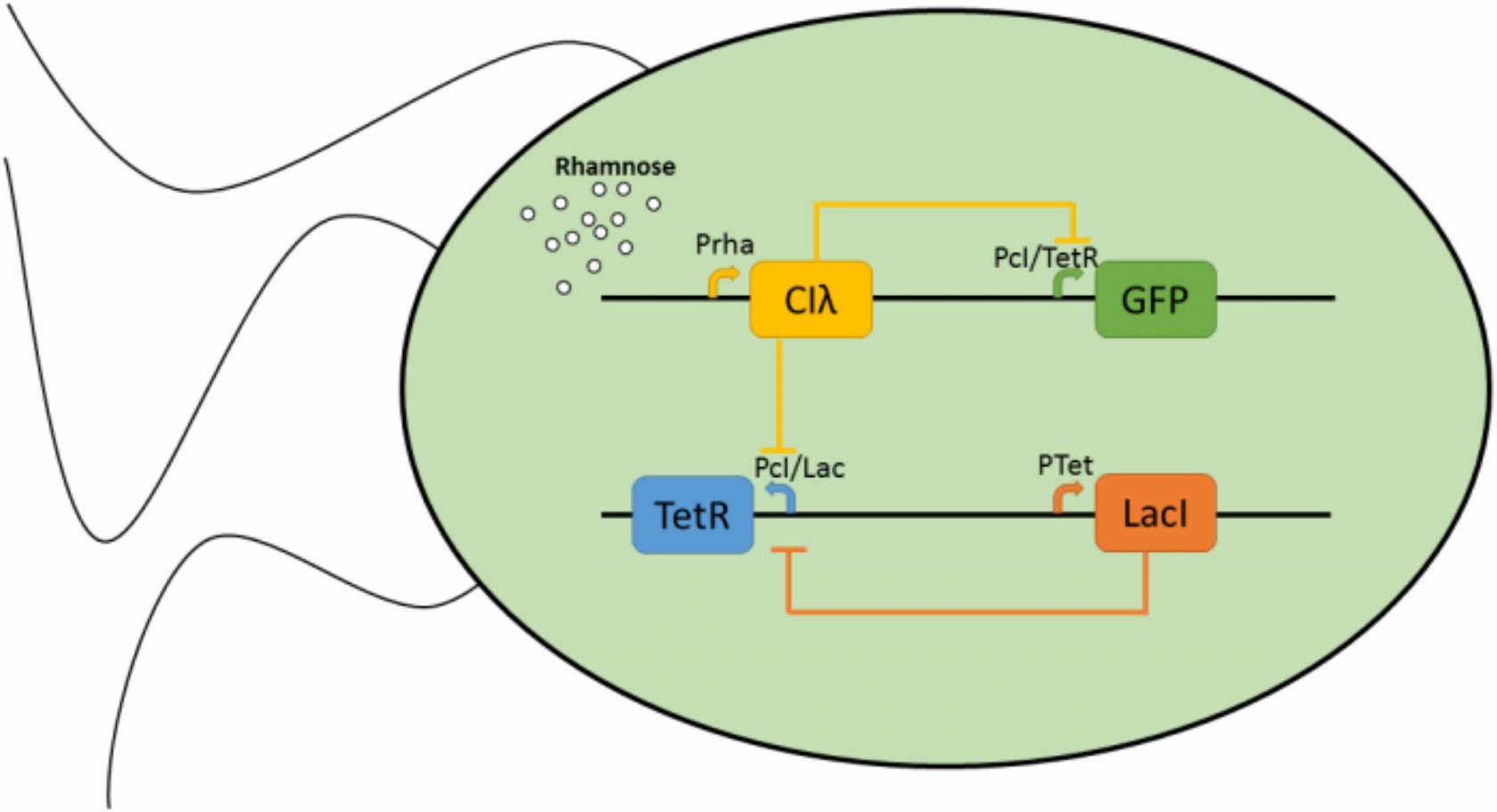
# Toggle switch in TetR - state




Set up the system by IPTG induction

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

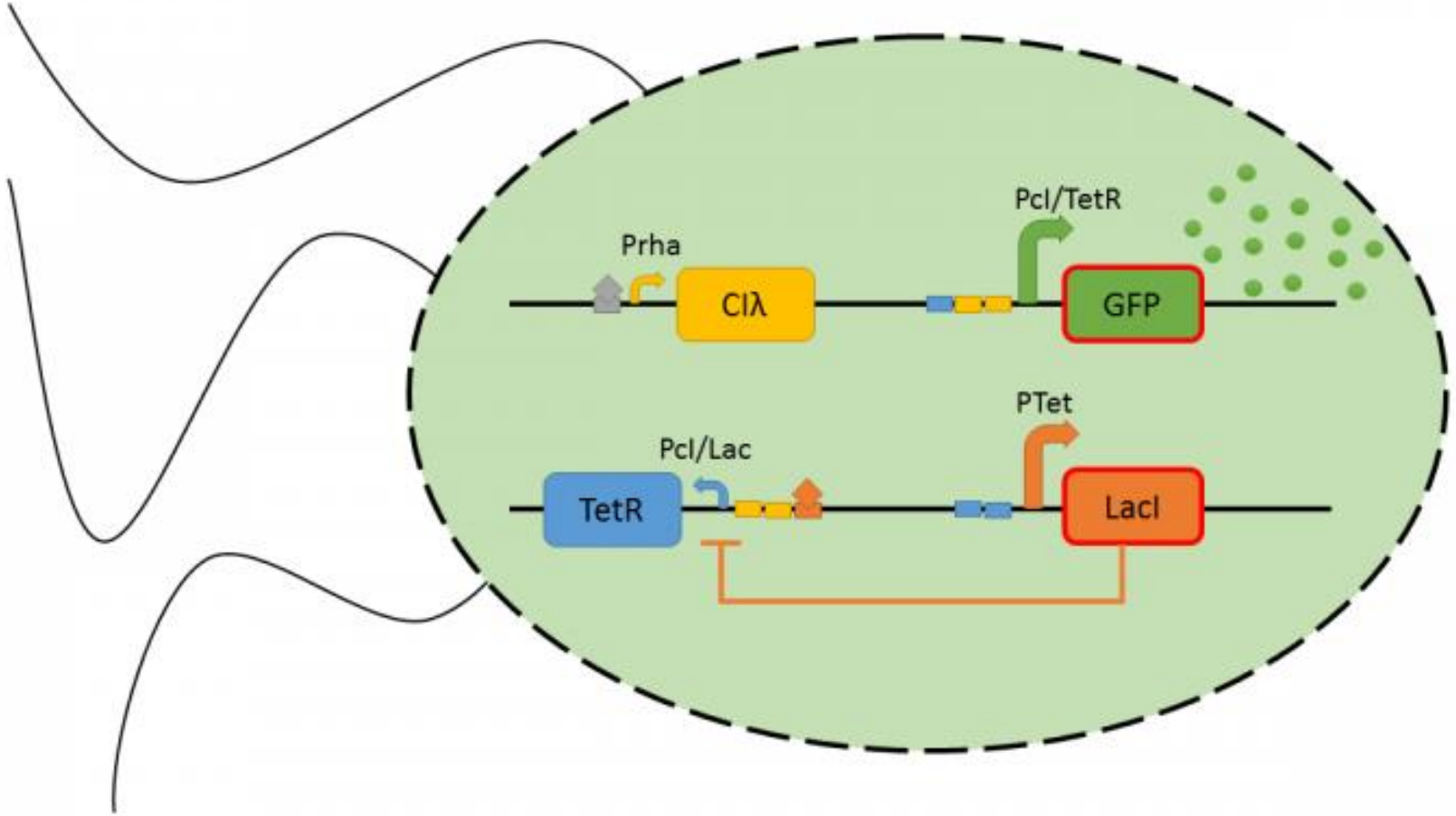
# Rhamnose (fusaric acid) present






http://2014.igem.org/Team:Wageningen_UR/project/kill-switch

# Kill switch on

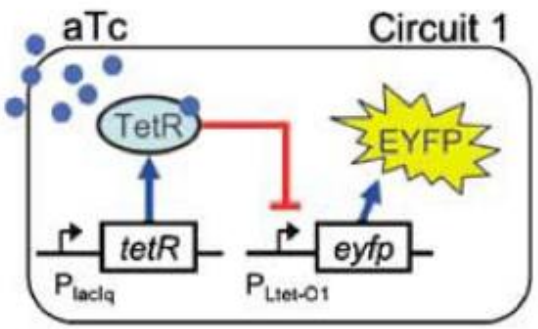


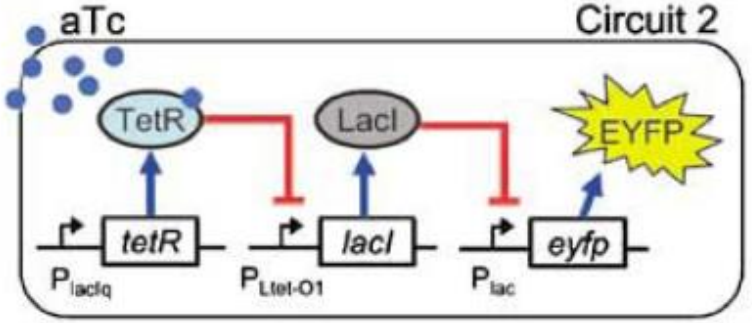


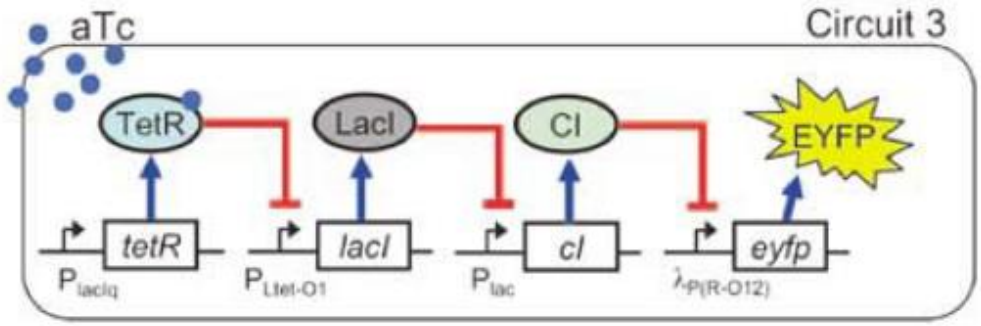

http://2014.igem.org/Team:Wageningen_UR/project/kill-switch

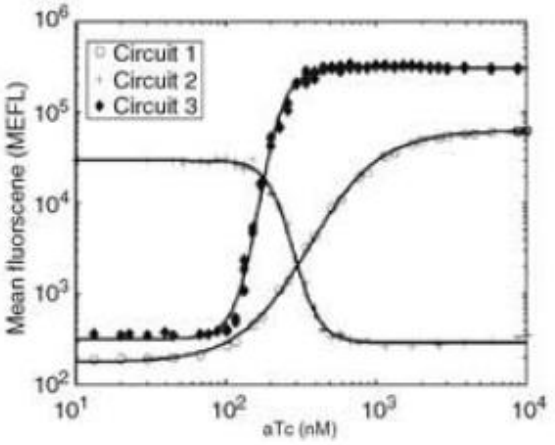
# Building transcriptional devices

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




Demonstrati-on of stan-dardized in/output. Modelling of cascades.

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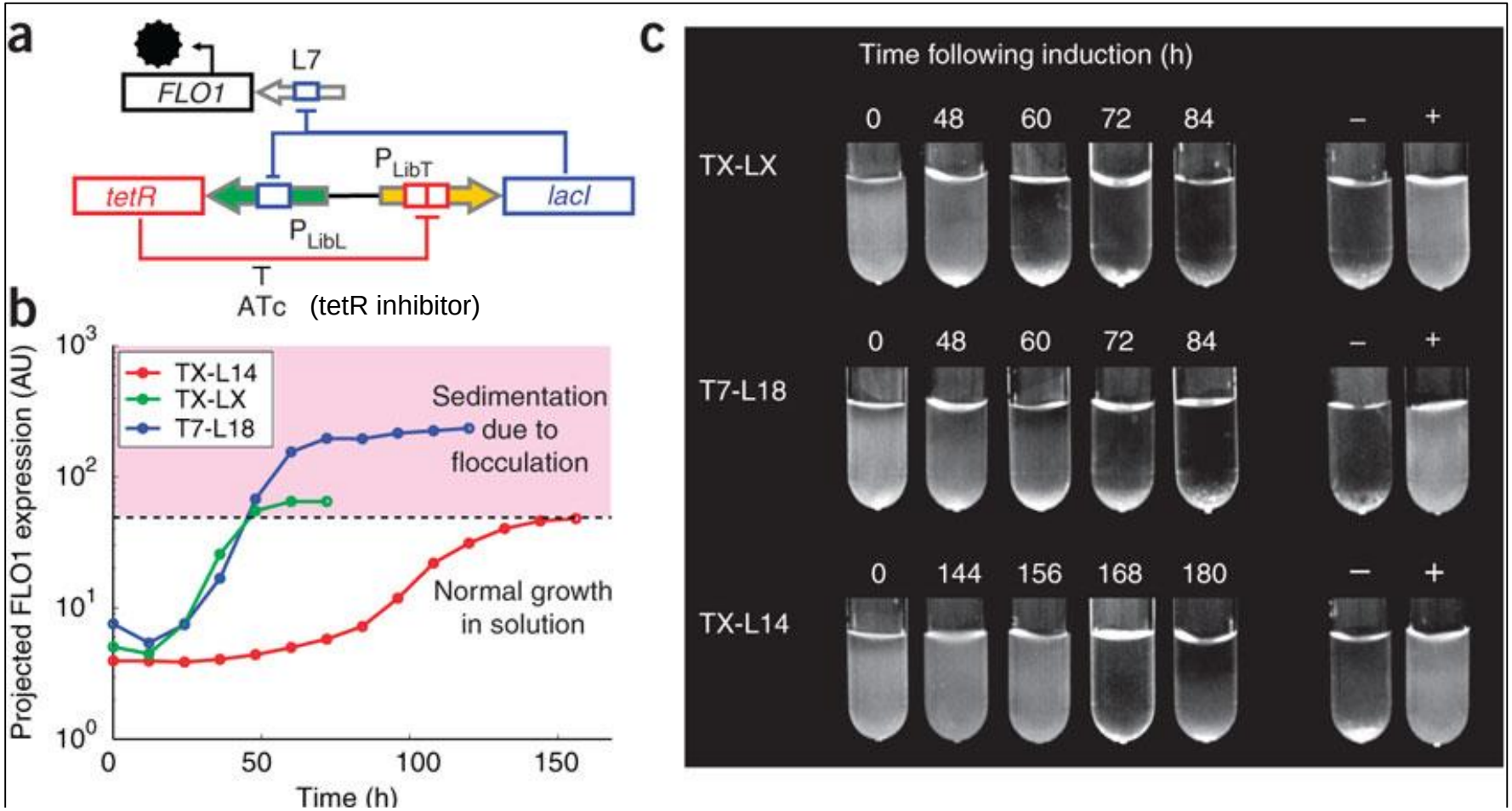
Ultrasensitivity and noise propagation in a synthetic transcriptional cascade; www.pnas.org/cgi/doi/10.1073/pnas.0408507102

# Control circuit in yeast using *E.coli* parts

- inputs, outputs, receivers, transmitters
- standardisation, reproducibility
- quantification & measurements
- mathematical modelling

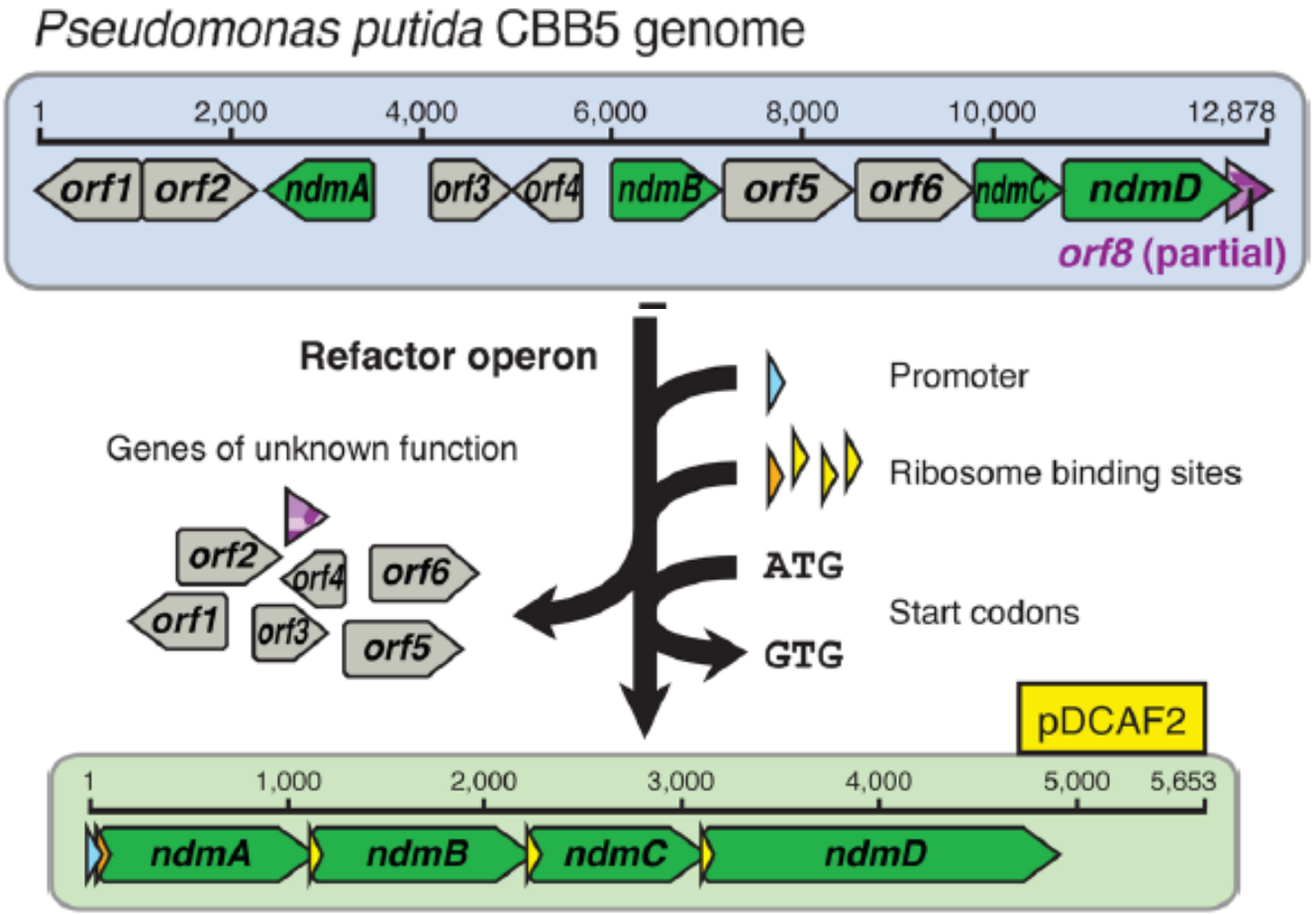
Ellis et al. Nat Biotechnol. 2009; 27(5): 465–471. doi:10.1038/nbt.1536.




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

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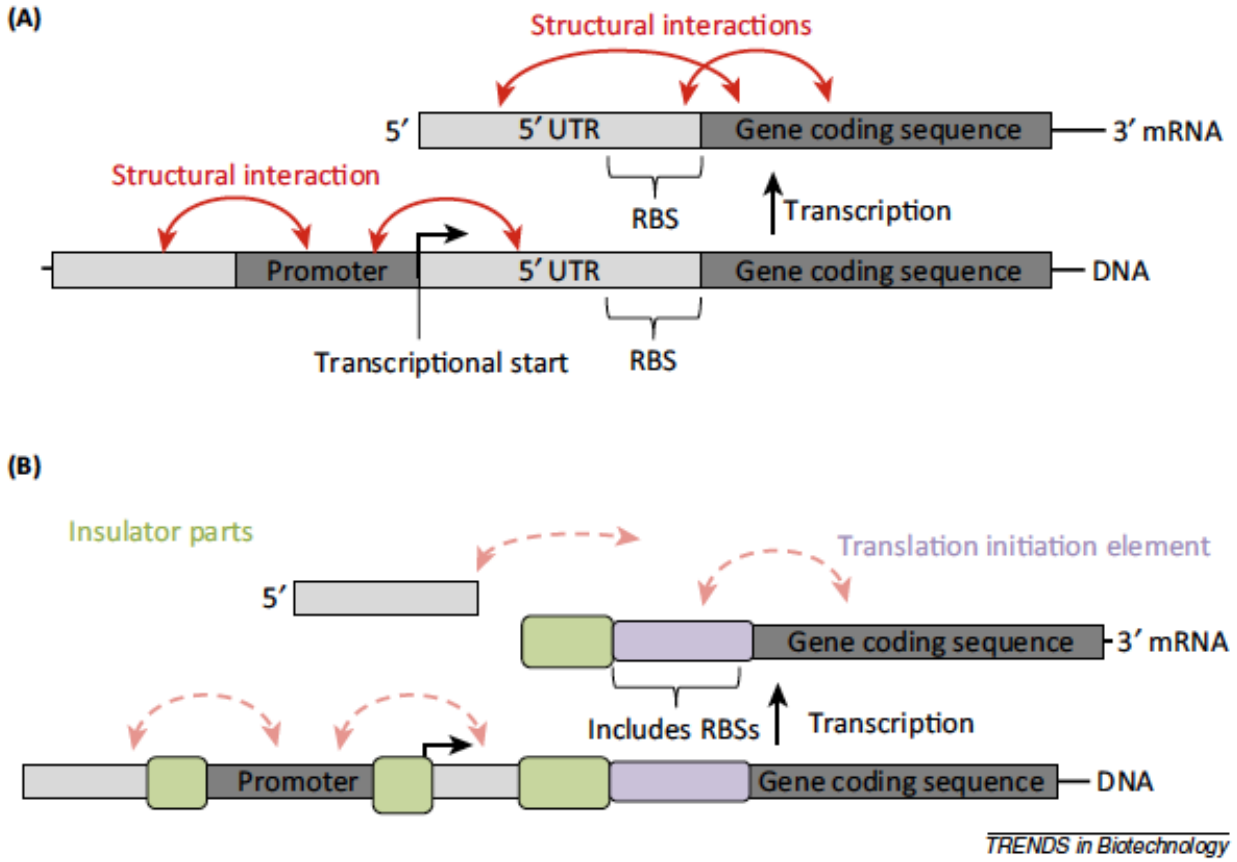
Caffeine degradation to xanthine, precursor for guanine synthesis. Decaffeination and Measurement of Caffeine Content by….
ACS Synth. Biol. 2013, 2, 301−307

# Robustness of parts

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

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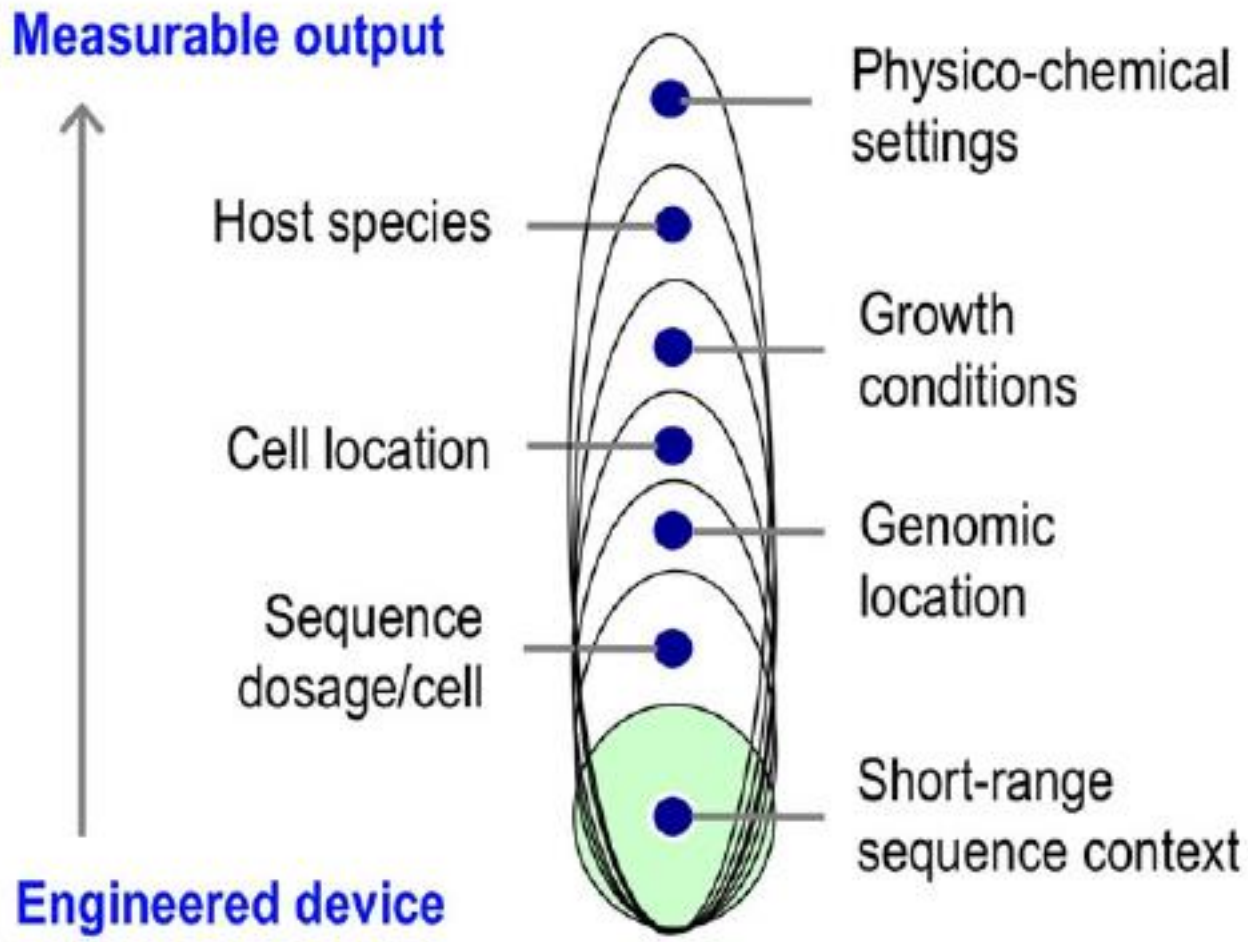
Modularity, context-dependence
Trends in Biotechnology, February 2015, Vol. 33, No. 2

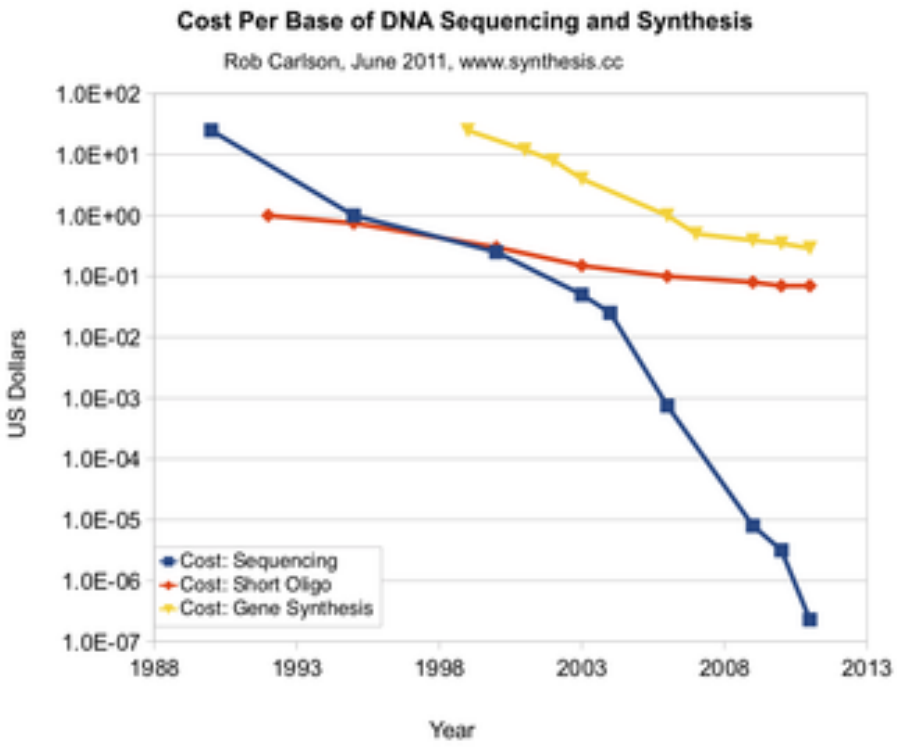
# Functionality and context




Confidence, tolerance,….Bioassays 37: 95–102, DOI 10.1002/bies.201400091

# The future of Biobricks ?

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



# Group work - BioBricks

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