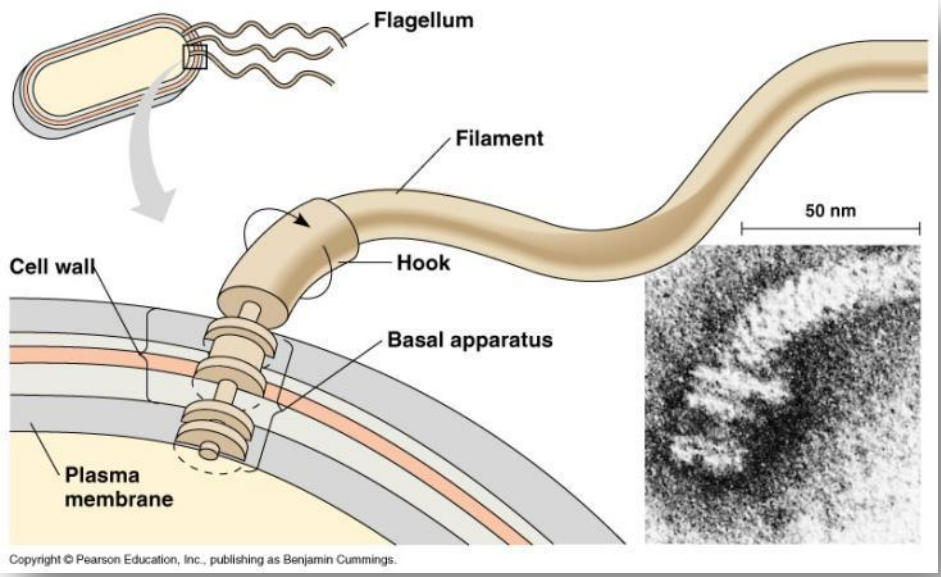
BacteriO'Clock

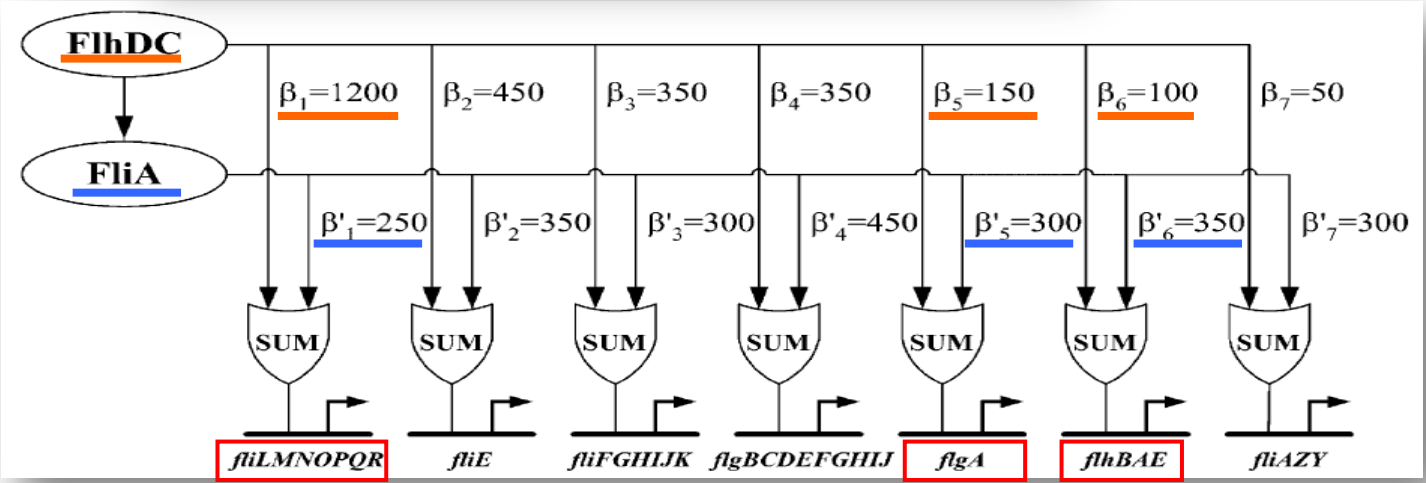
Create **chassis** for manufacturing of **biomolecular machines** that needs to be assembled in **precise order**

The Flagella Machinery

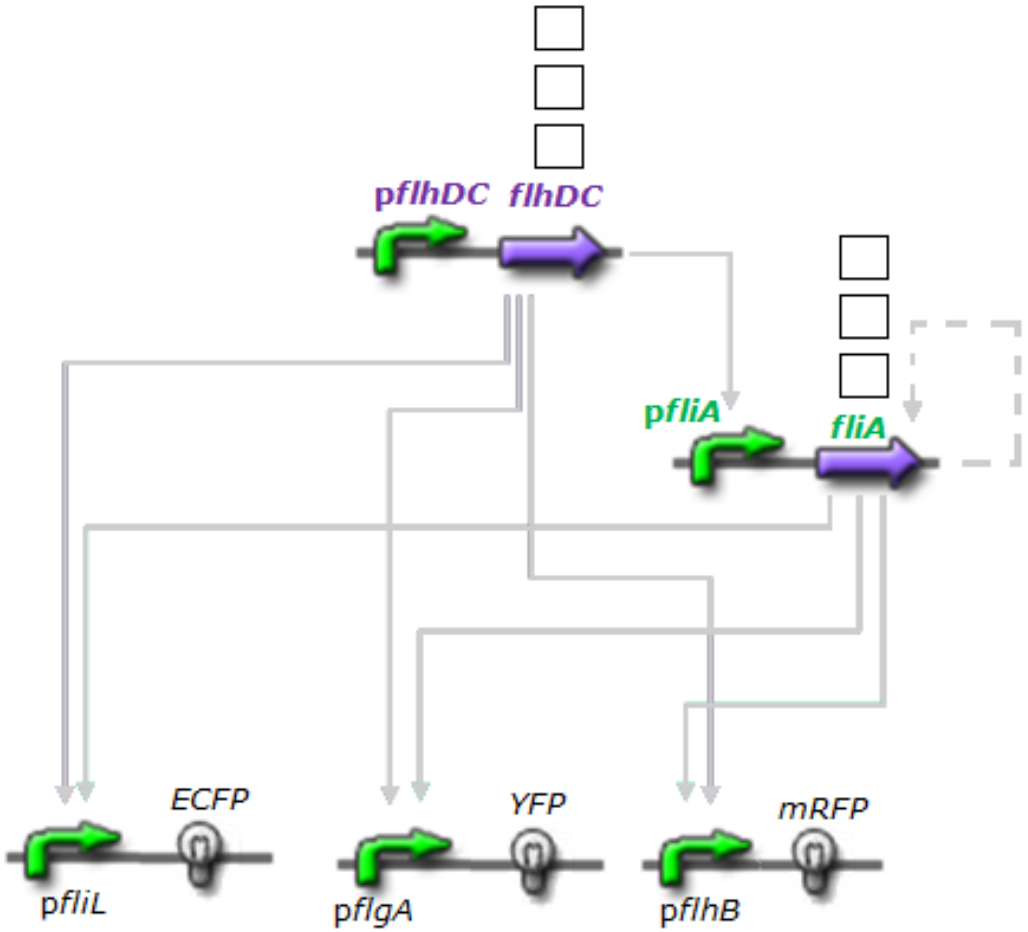
E. coli flagella is a highly complex machine



Construction based on Multiple Output FFL network motif

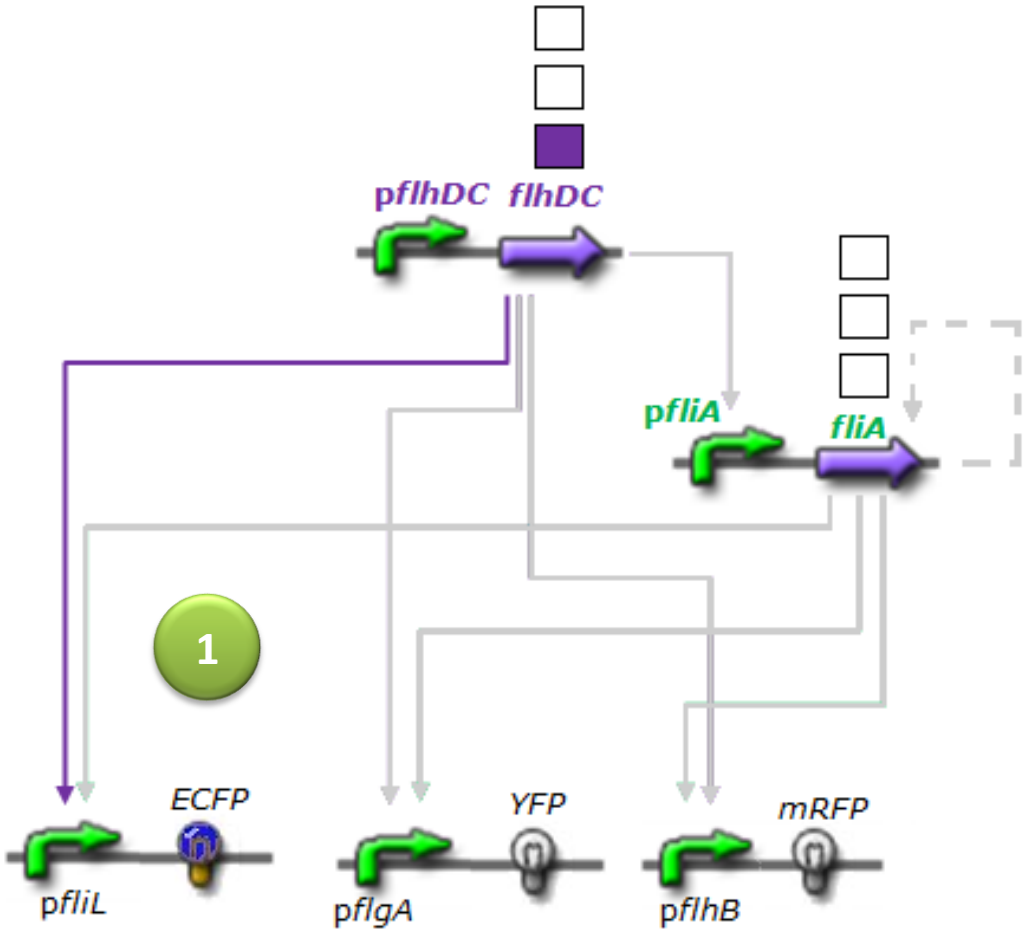


Multiple Output FFL yields FIFO behaviour



From a complex natural flagella system to a minimal synthetic gene circuit

Multiple Output FFL yields FIFO behaviour



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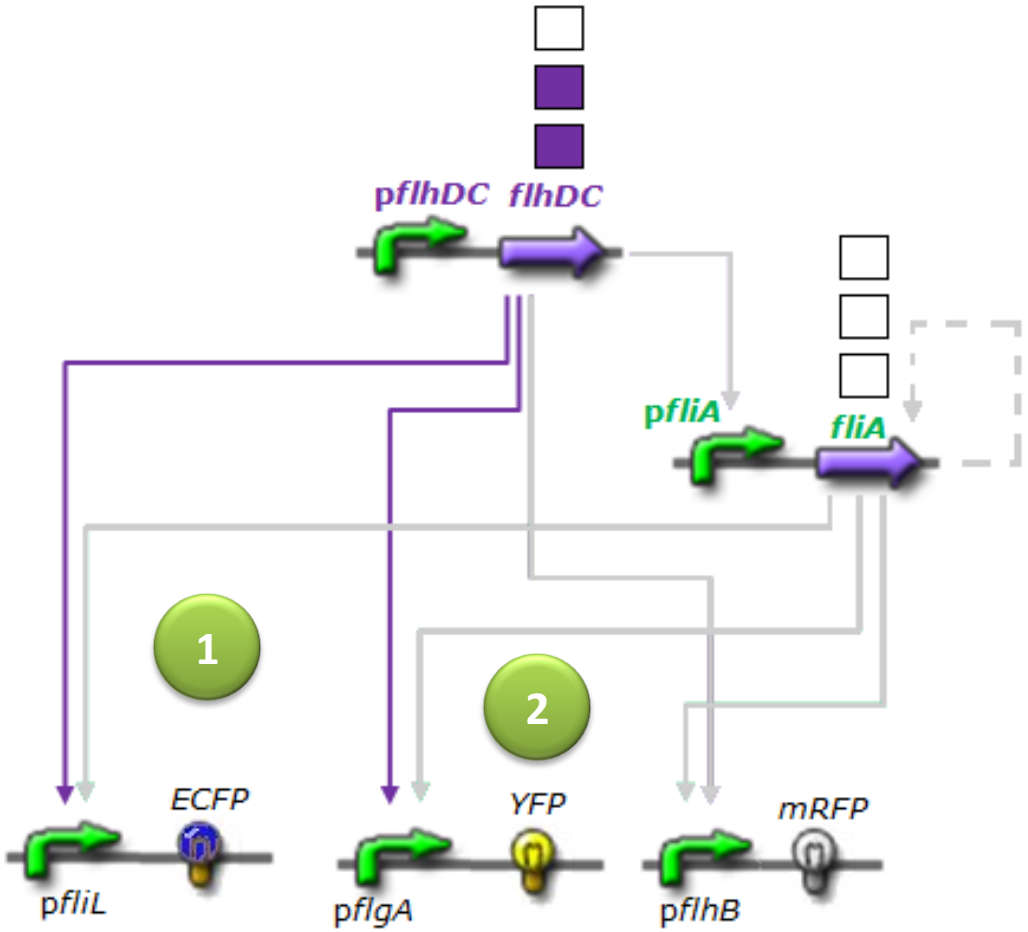

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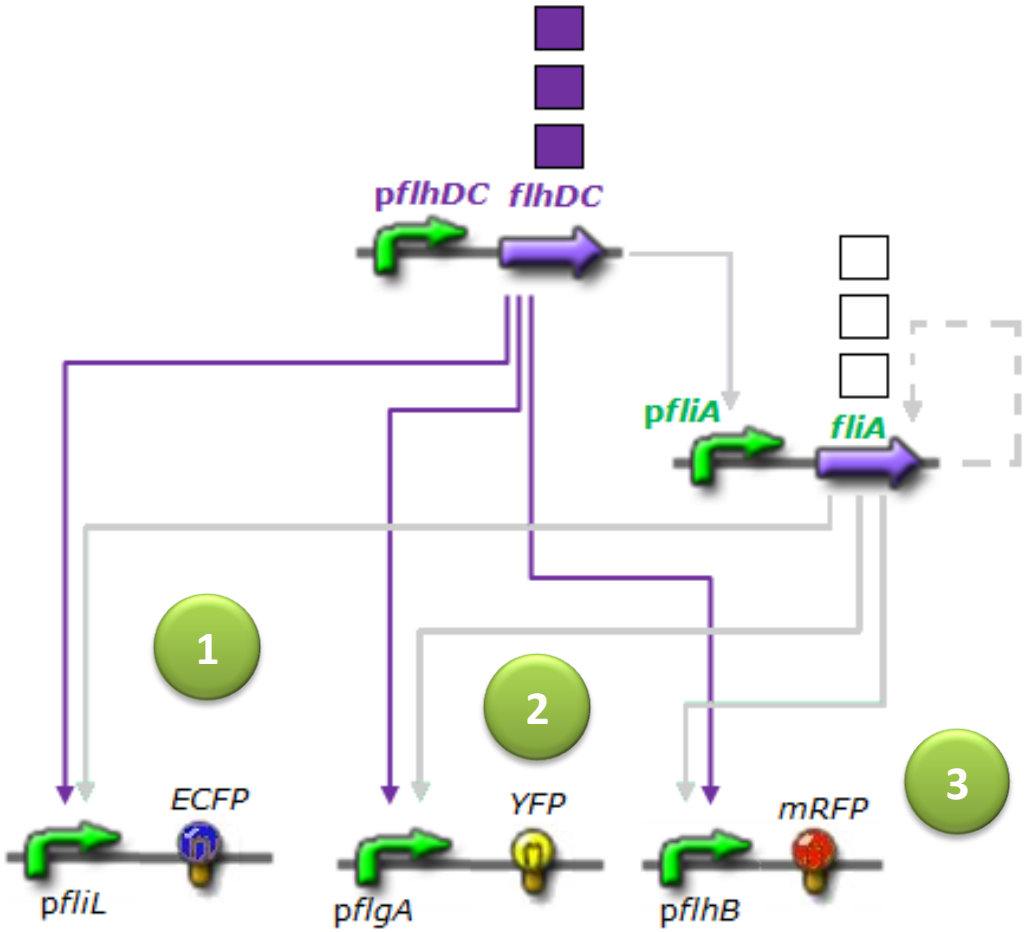

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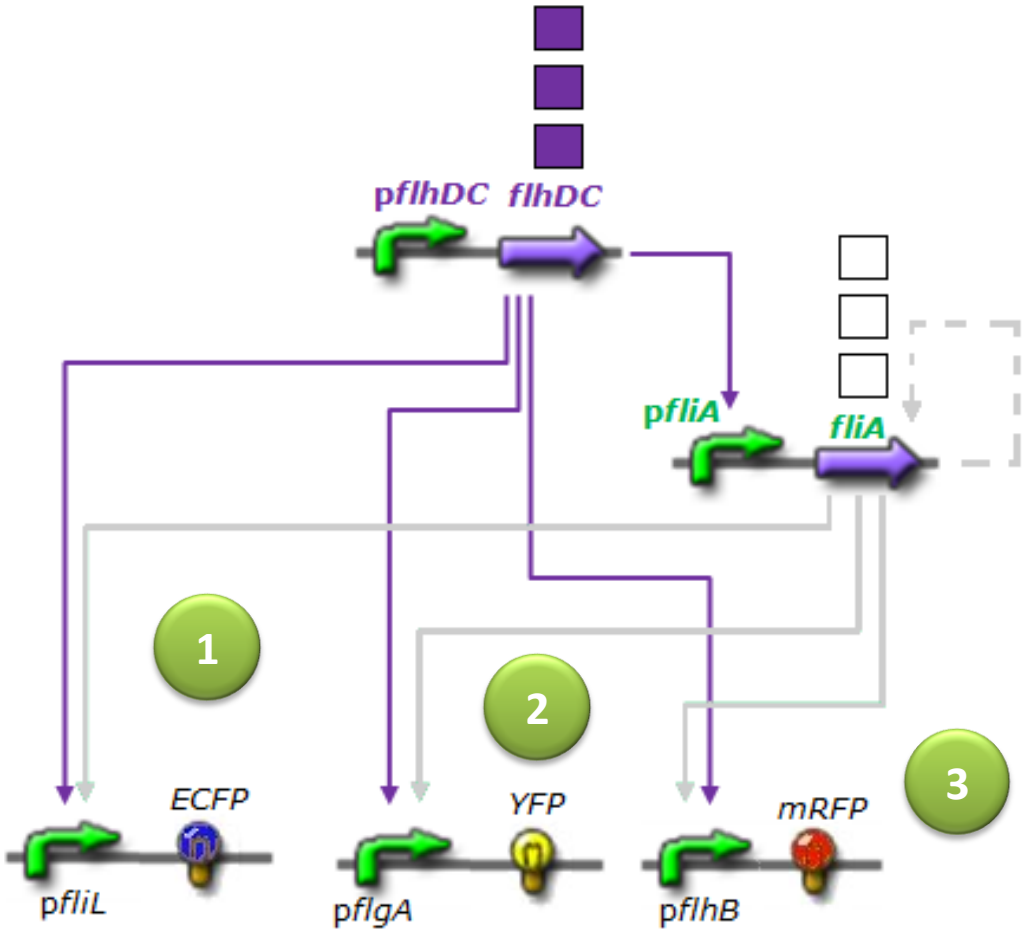

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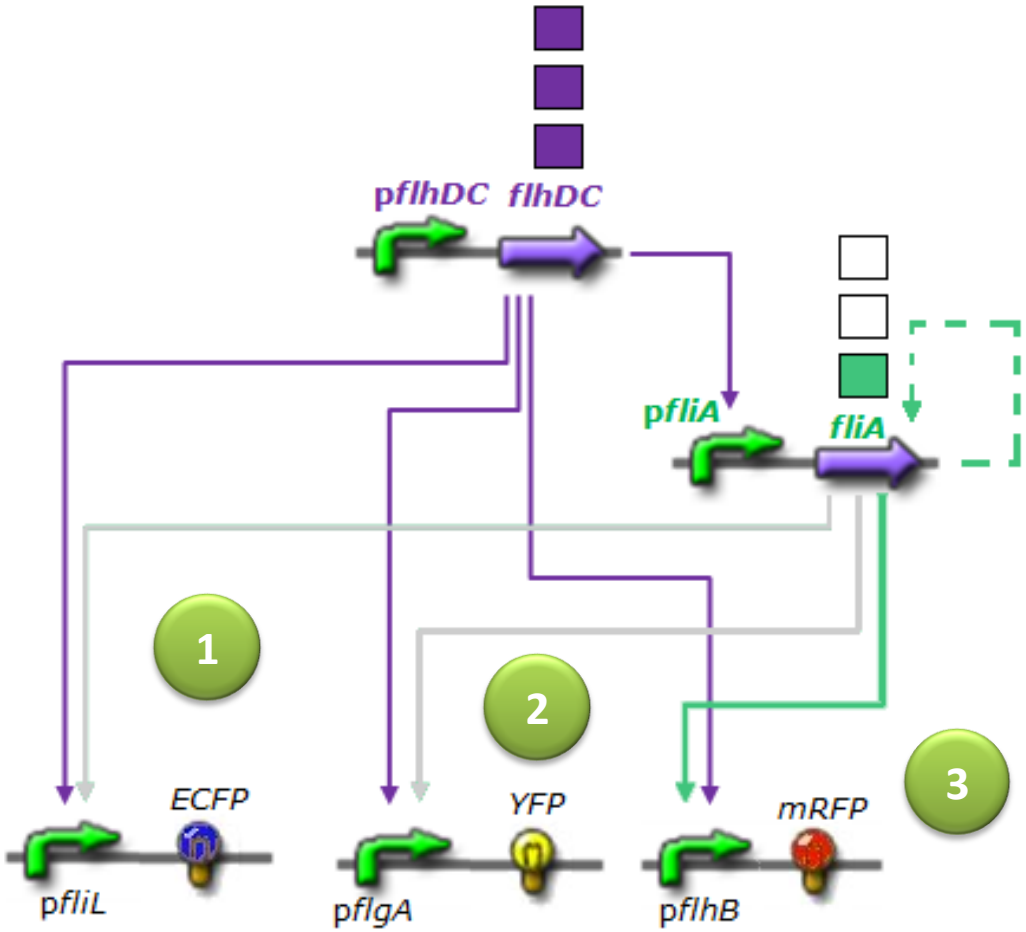
Multiple Output FFL yields FIFO behaviour



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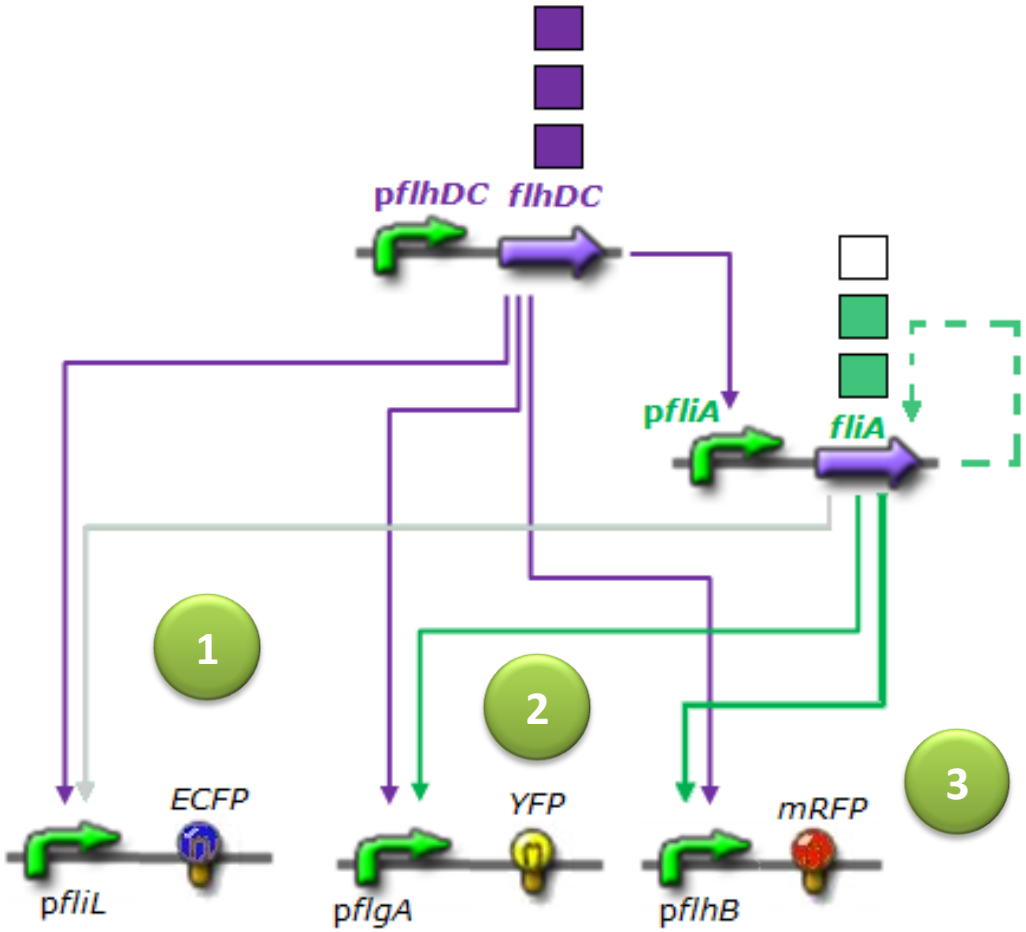
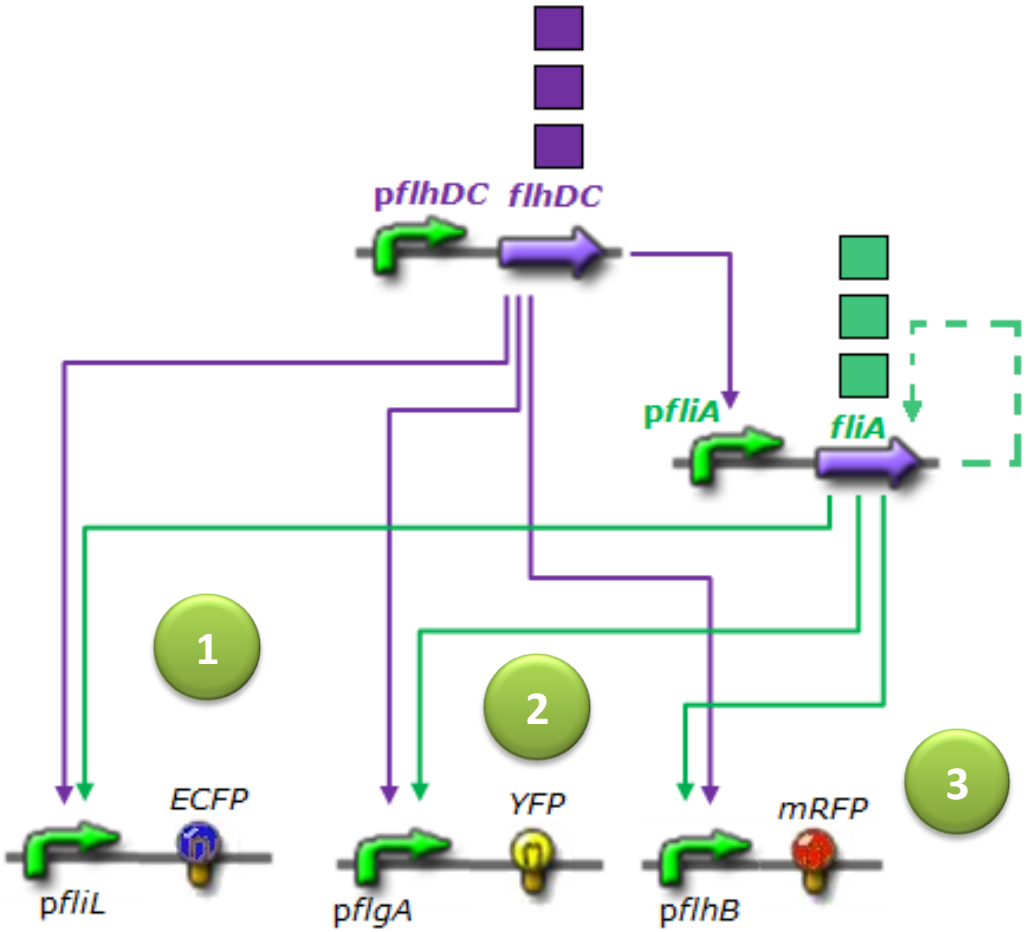
Multiple Output FFL yields FIFO behaviour



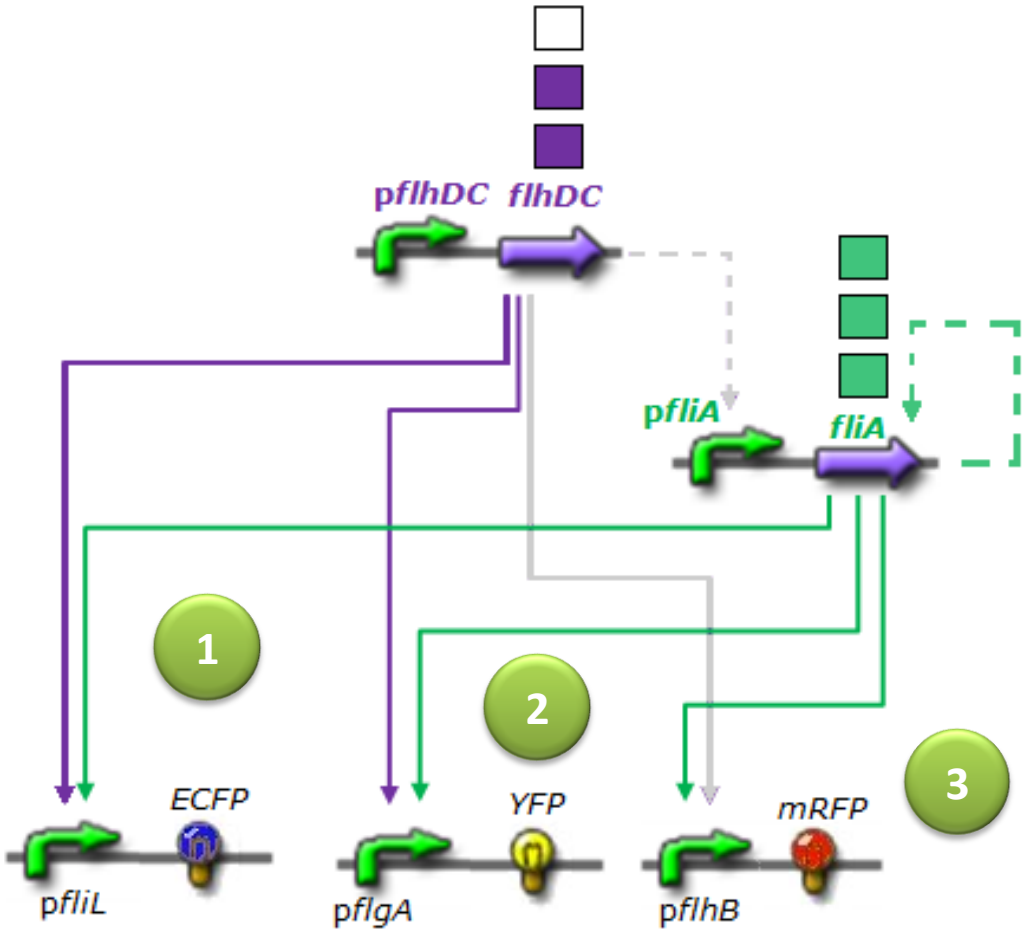
From a complex natural flagella system to a minimal synthetic gene circuit

Multiple Output FFL yields FIFO behaviour



From a complex natural flagella system to a minimal synthetic gene circuit

Multiple Output FFL yields FIFO behaviour



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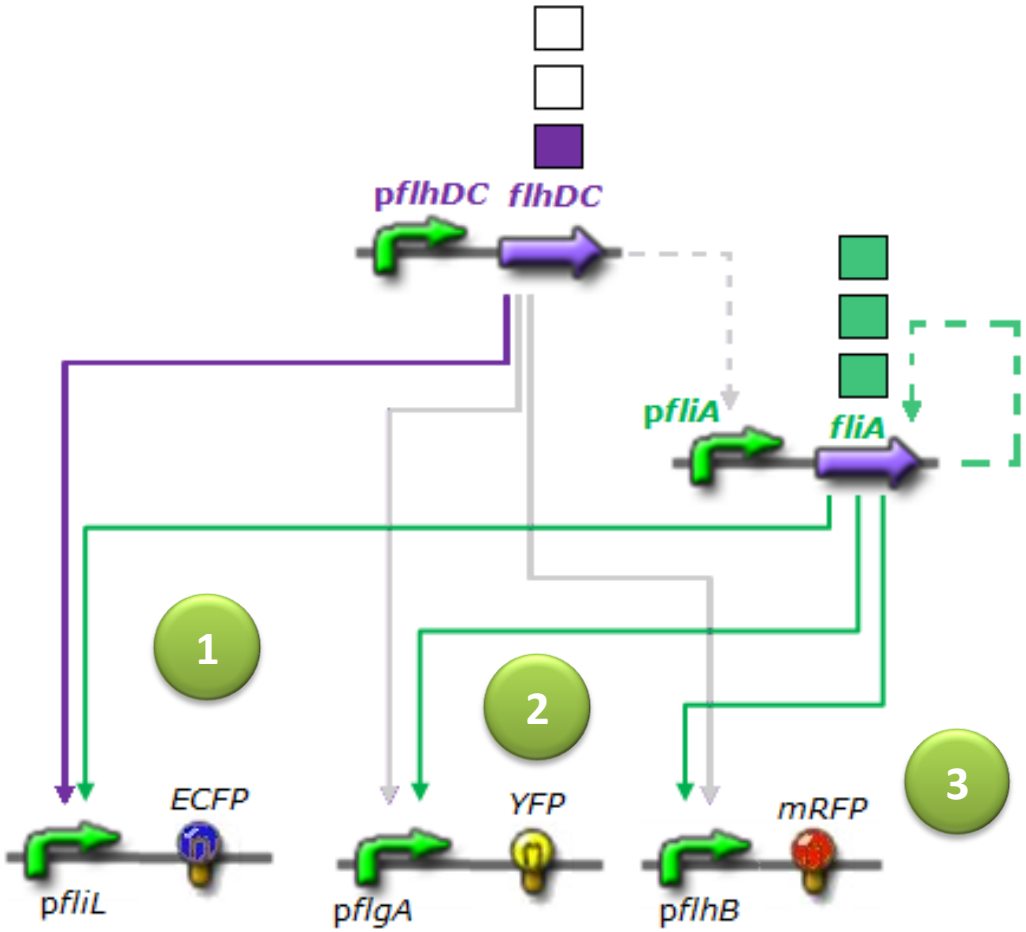

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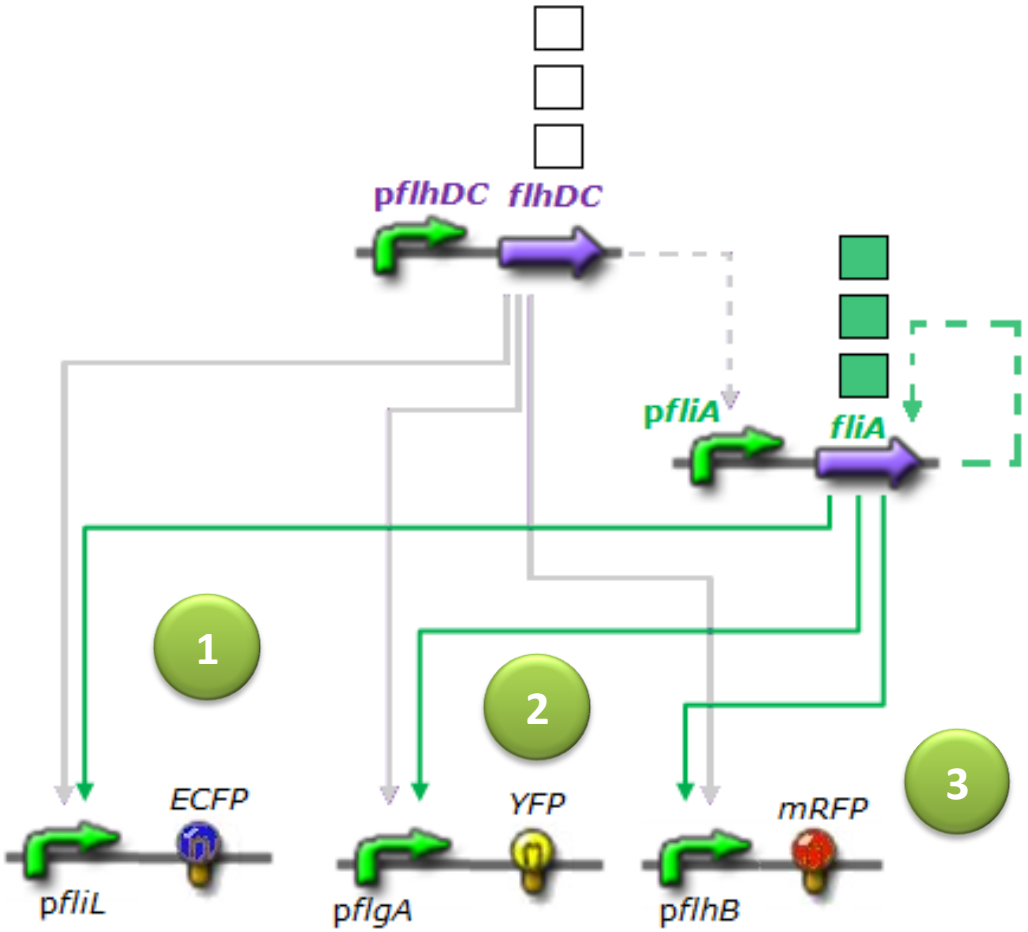
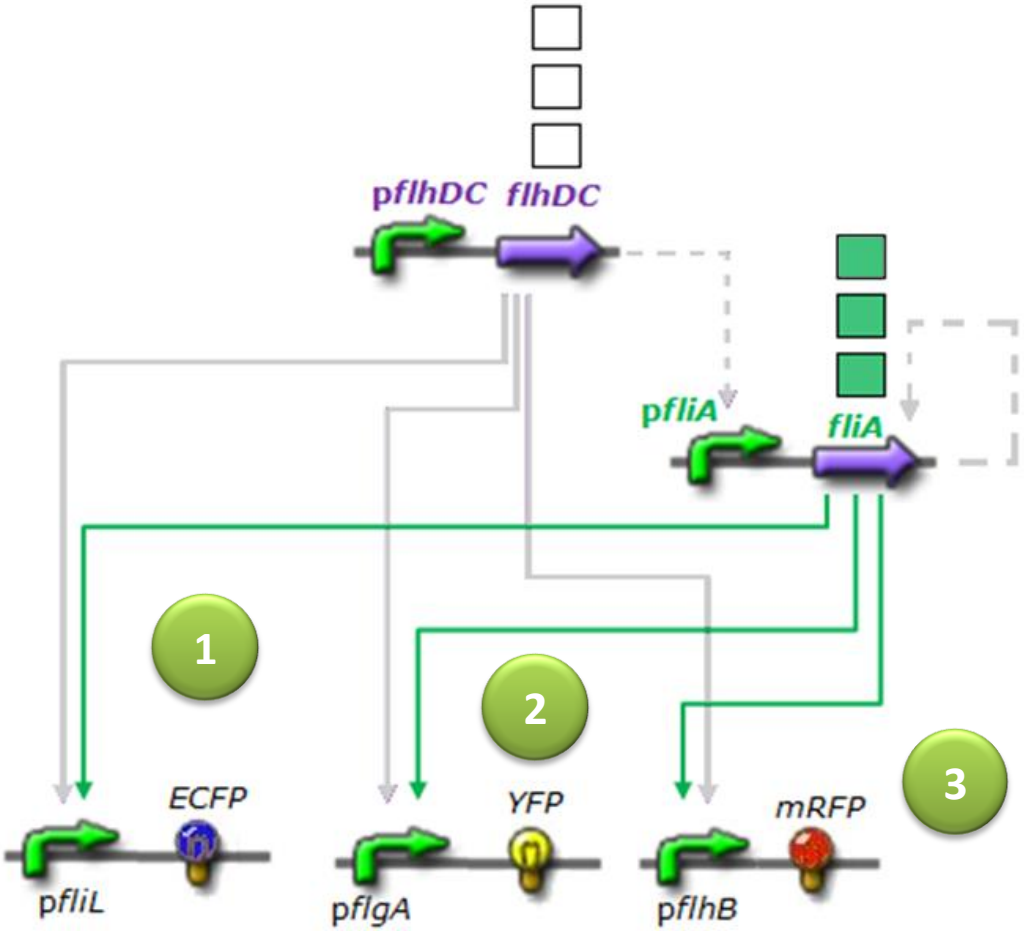


From a complex natural flagella system to a minimal synthetic gene circuit

Multiple Output FFL yields FIFO behaviour



From a complex natural flagella system to a minimal synthetic gene circuit

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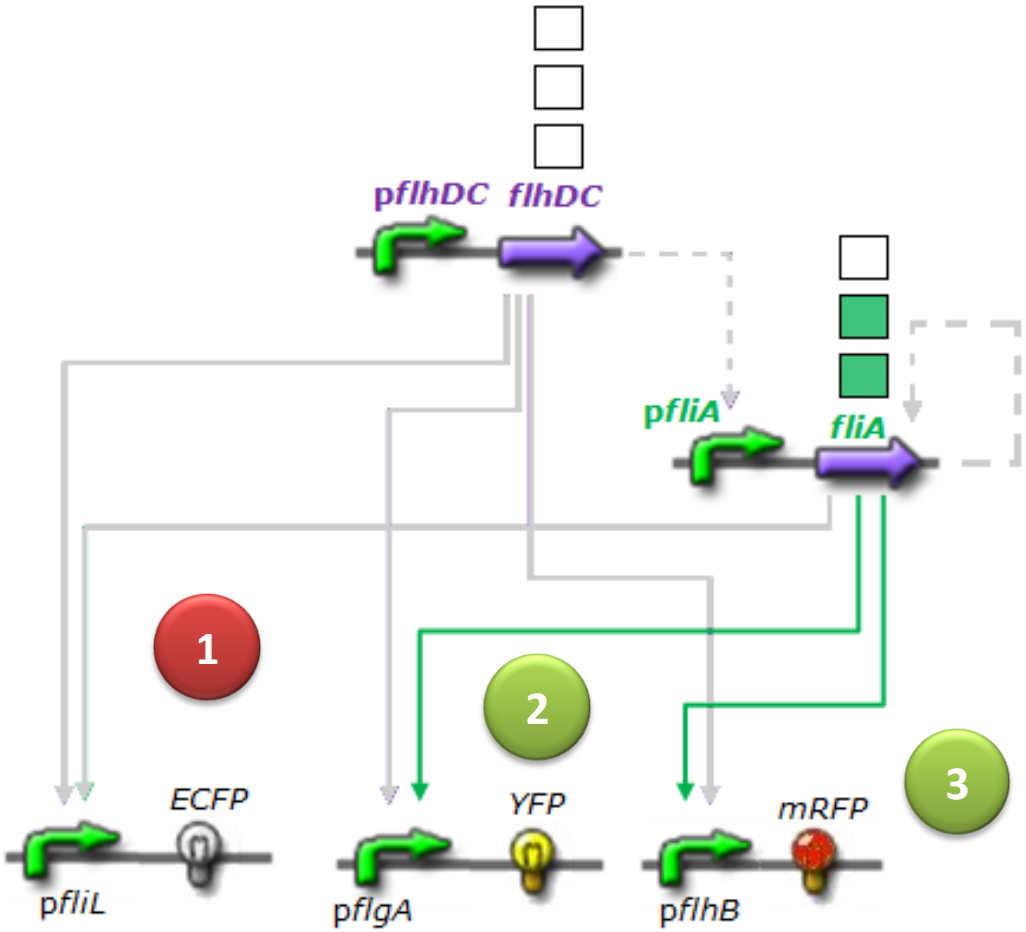

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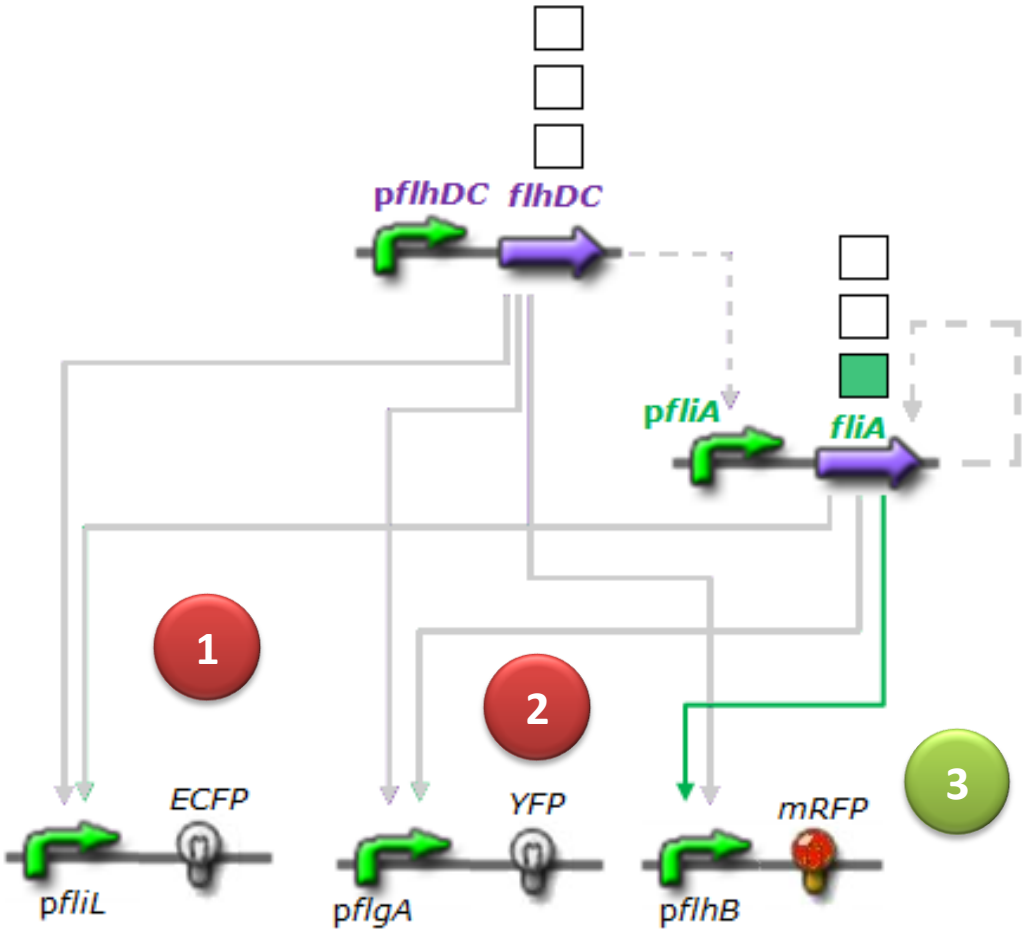
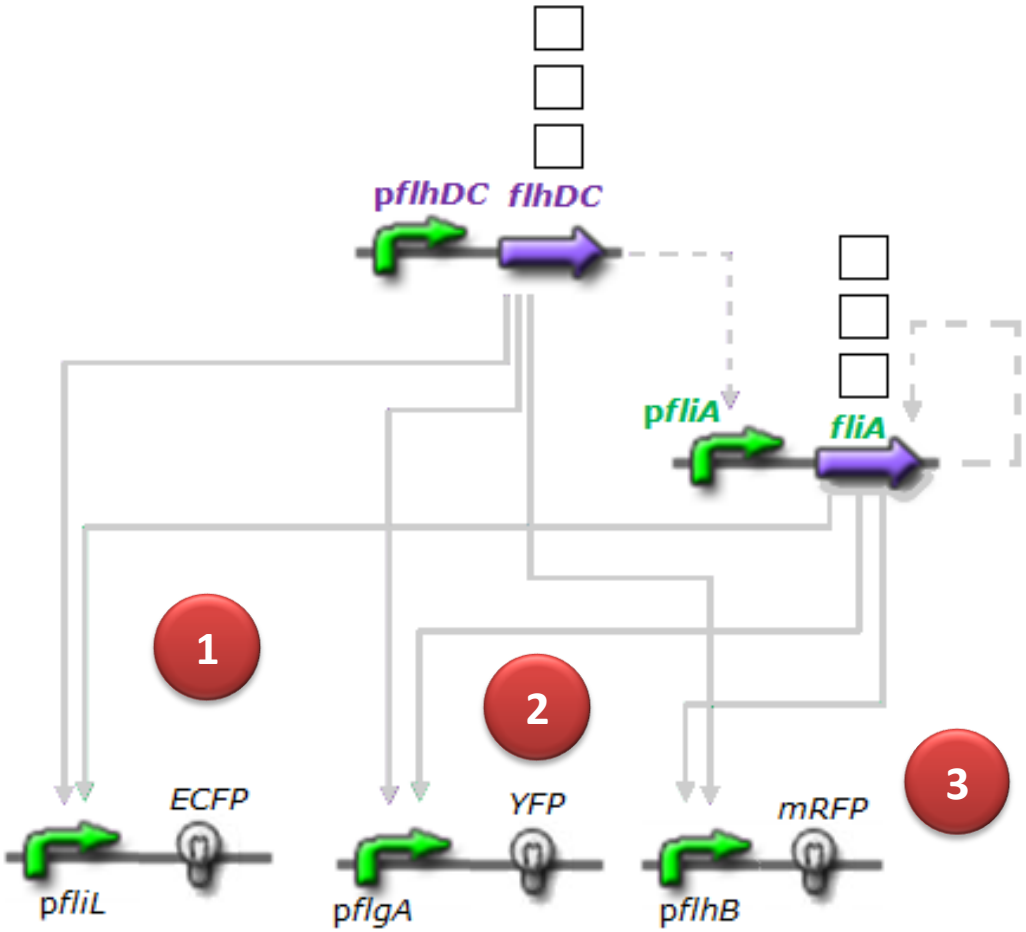


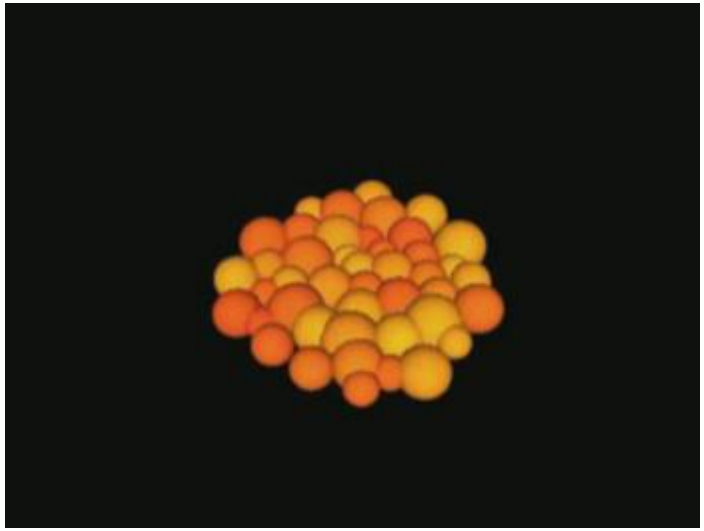
From a complex natural flagella system to a minimal synthetic gene circuit

Multiple Output FFL yields FIFO behaviour



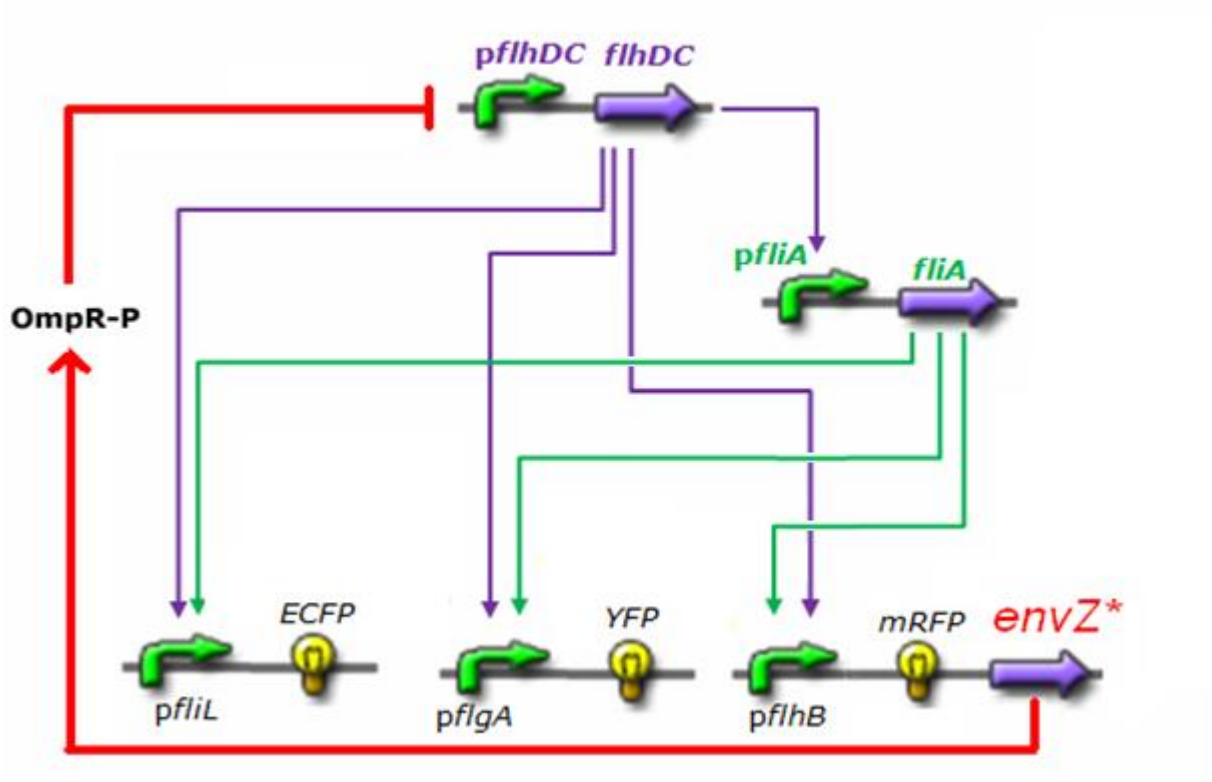
From a complex natural flagella system to a minimal synthetic gene circuit

Coordinate **expression of genes** in a **repeated** manner the **BacteriO'Clock**



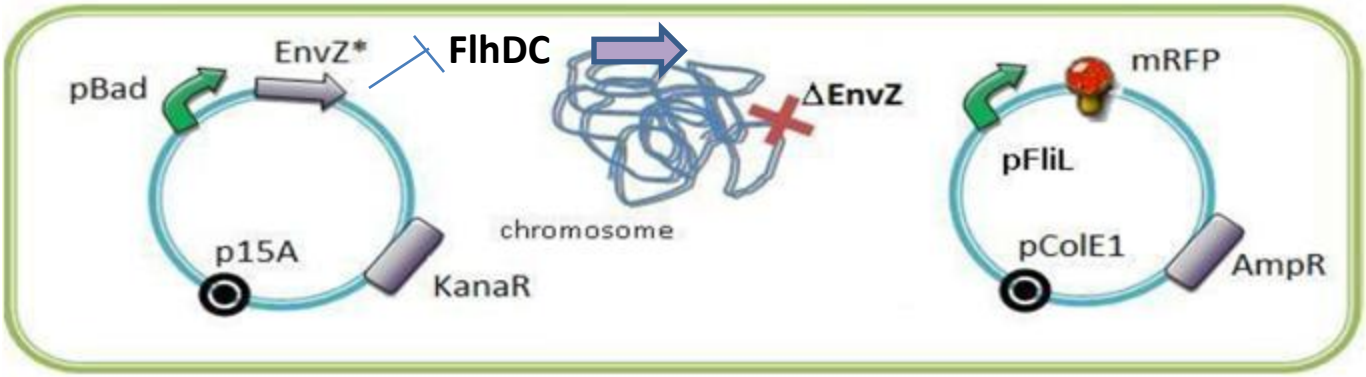
Final Design of Our Network

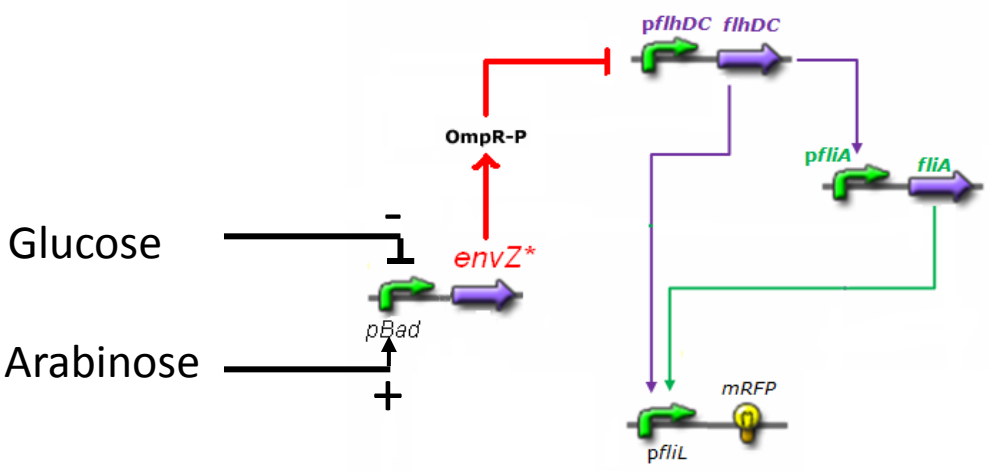
- Ensure the automatic switching ON and OFF by **adding a negative feedback loop**



Testing the Negative Feedback Loop

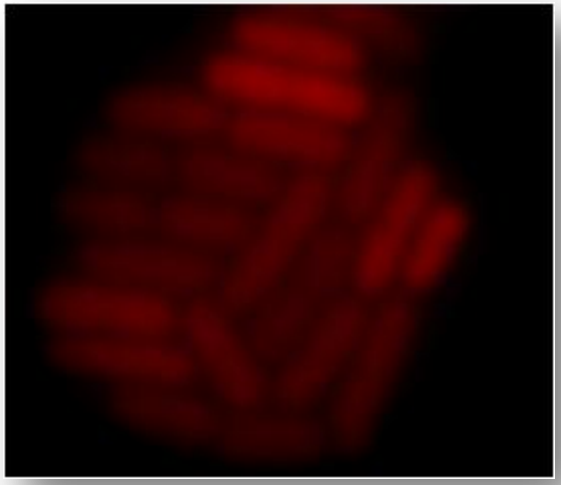
Strain used and constructions built and characterized



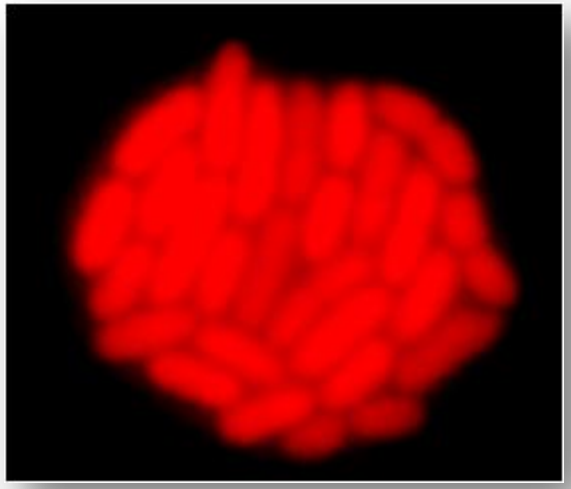


Testing Feedback Loop and Promoter Strength

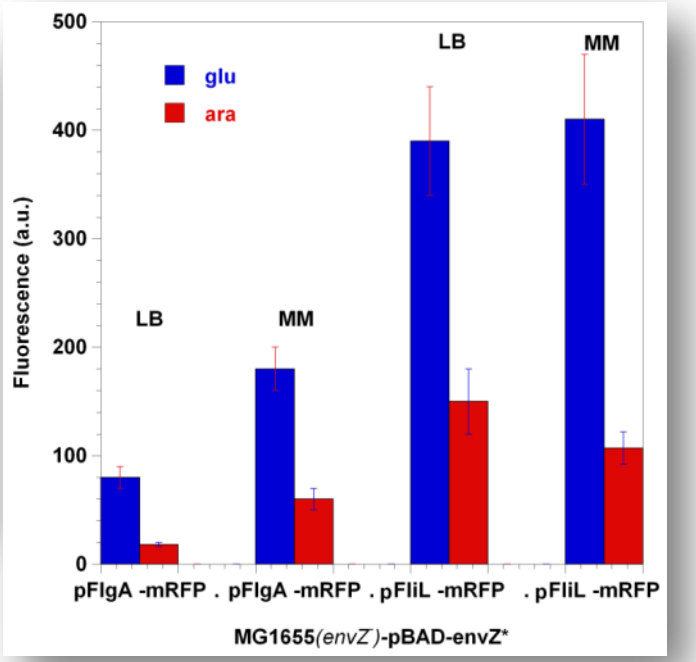
Arabinose 0.2%



Glucose 1%



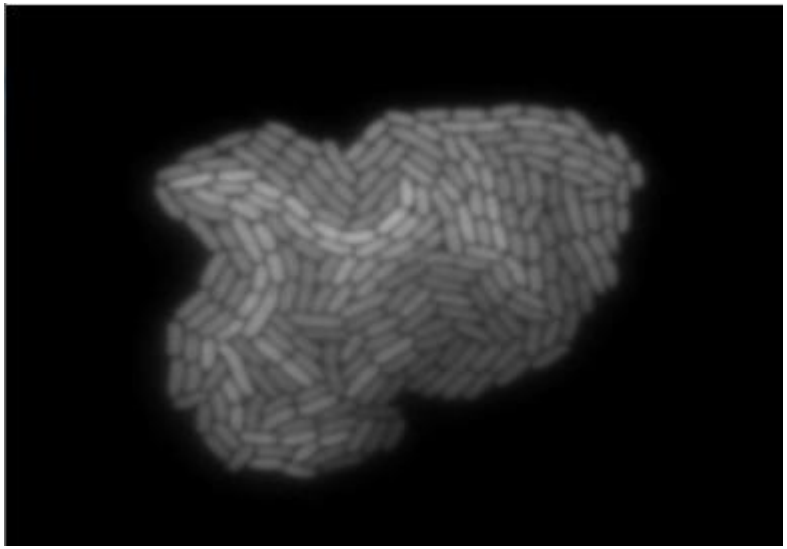
pfliL

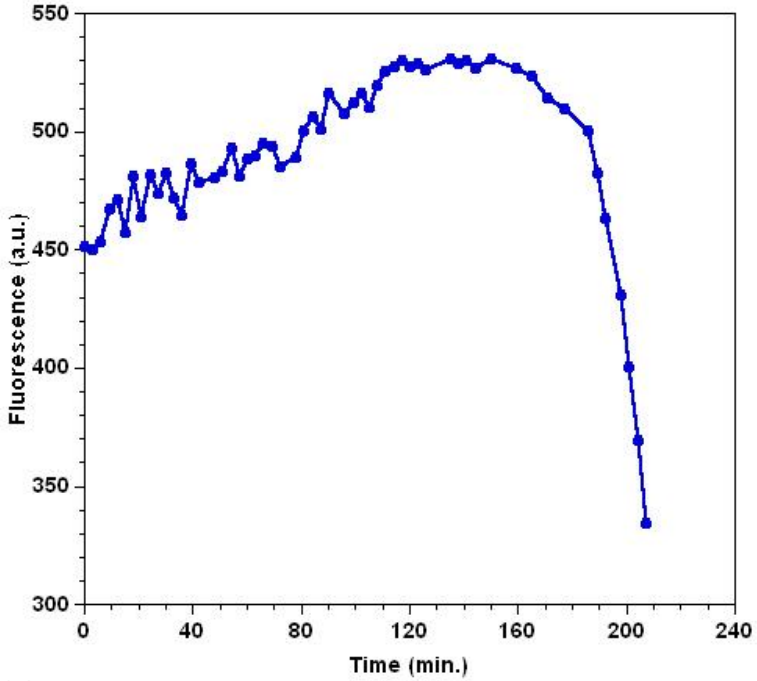


As expected **mRFP is less expressed in presence of arabinose**

Dynamical Analysis of the Switch

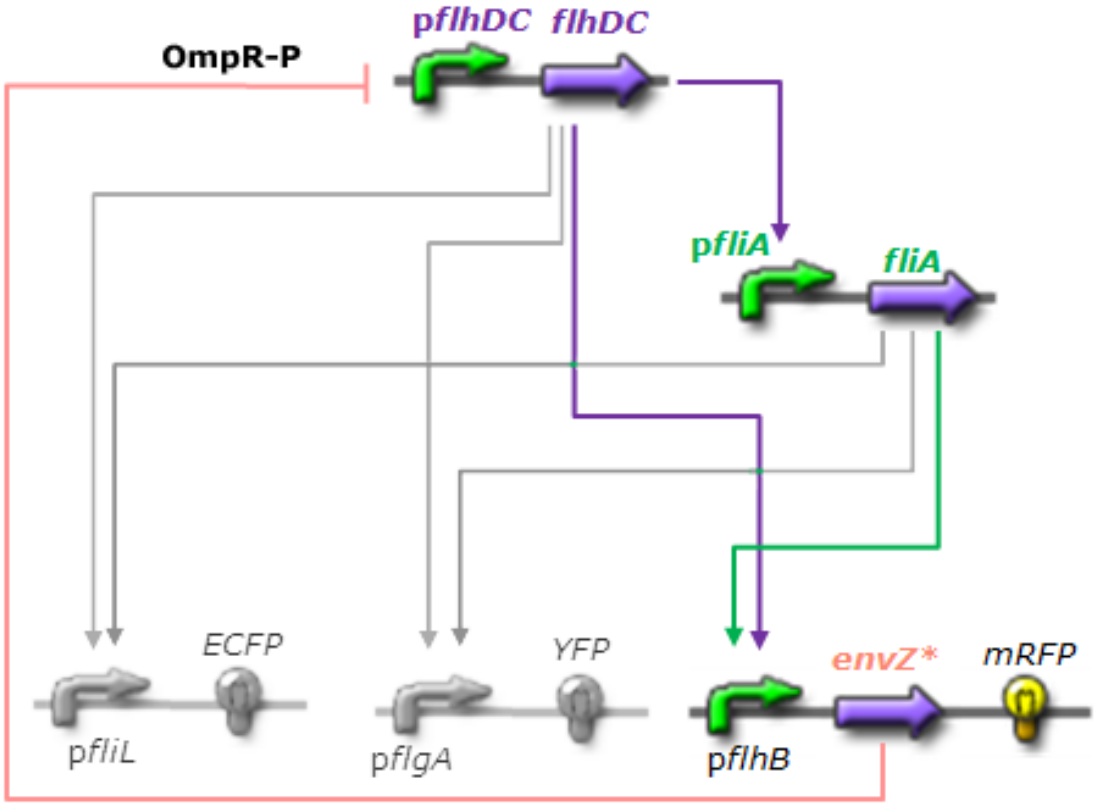
Bacteria grown in a medium containing glucose are put in presence of Arabinose





pFliL « off » step

As expected, the **fluorescence drops down** !



This system should oscillate, wouldn't it ?

Our Take on Modeling

- The goals of our approach
  - Obtain qualitative predictions by using experimentally measured parameters
  - Help biologists by improving network design

- Principle of our model :
  - Linear effect & SUM functions for flagellar genes [1]
  - Using classical Hill functions otherwise
  - Time rescaling and normalization : reduce number of unknown parameters $\frac{d[FliA]}{dt} = -\gamma_{FliA} \times [FliA] + \beta_{FliA} \times [FlhDC] + \beta'_{FliA} \times [FliA]$

[1] S. KALIR et al. 2005

Optimizing Simplicity

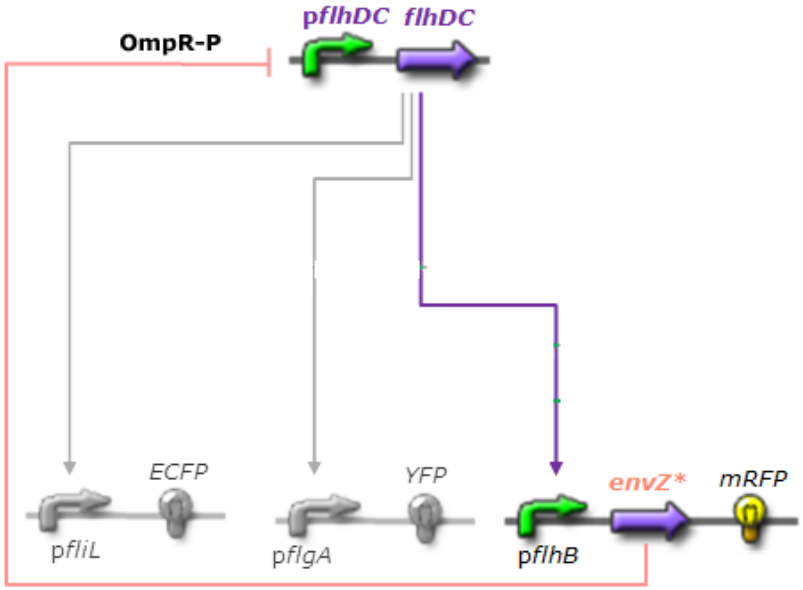
- Do you think that's too simple?
  - Models and parameters extracted from literature

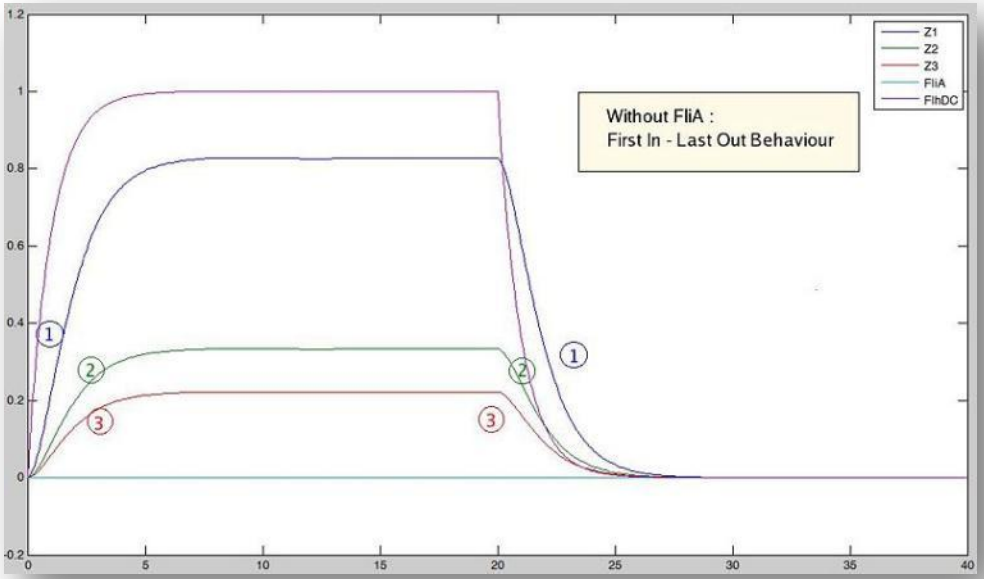
From literature	8
From normalization	2
Estimation (EnvZ*)	2
Total	12

  - Using **Akaike criteria** to discriminate between models
- We have all the information needed to develop simple experimentally-based models!

What About the FIFO?

FlhDC being the only regulator…

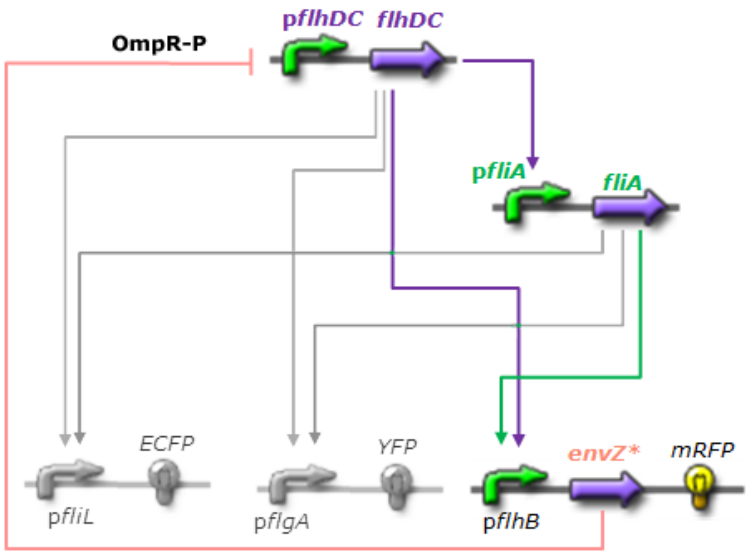


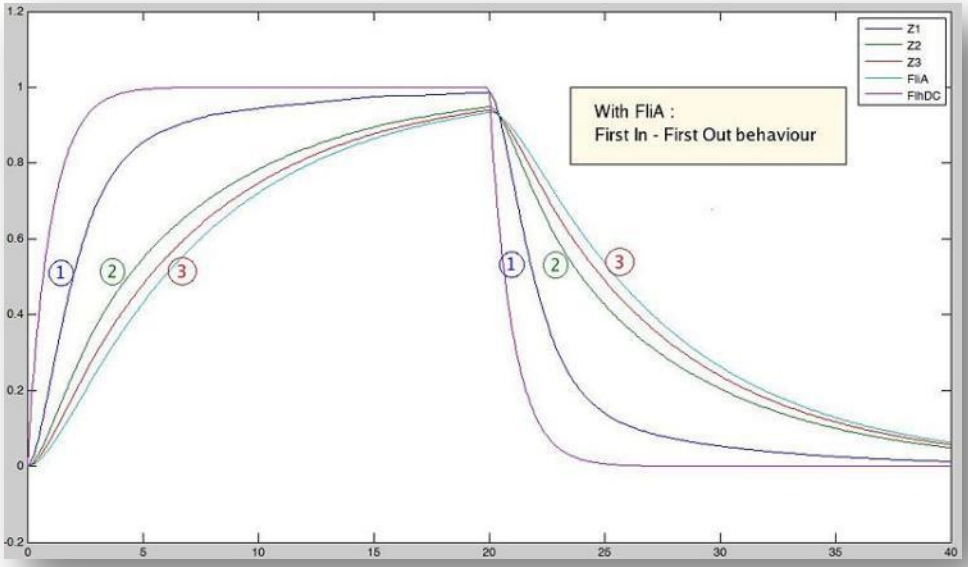


Hey, it's a LIFO! ☹

What about the FIFO?

FlhDC and FliA : FFL-based control



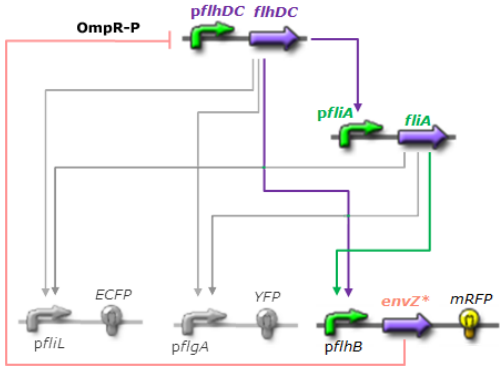


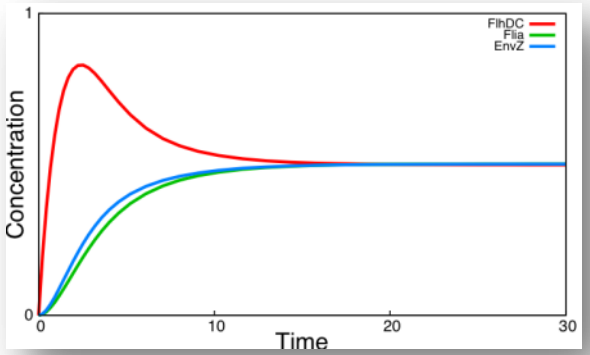
Parameters and models from the publications :

It worked as a FIFO !

What About the Oscillations?

- With original parameters

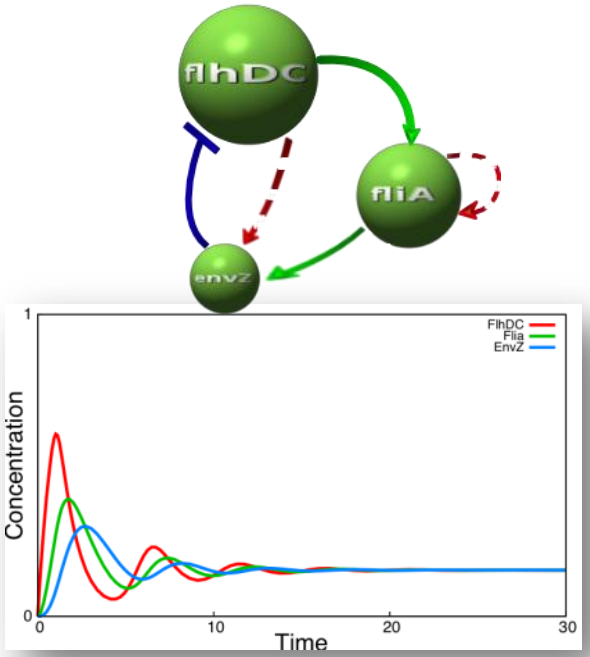




- It does not oscillate

  (+ solved analytically !)

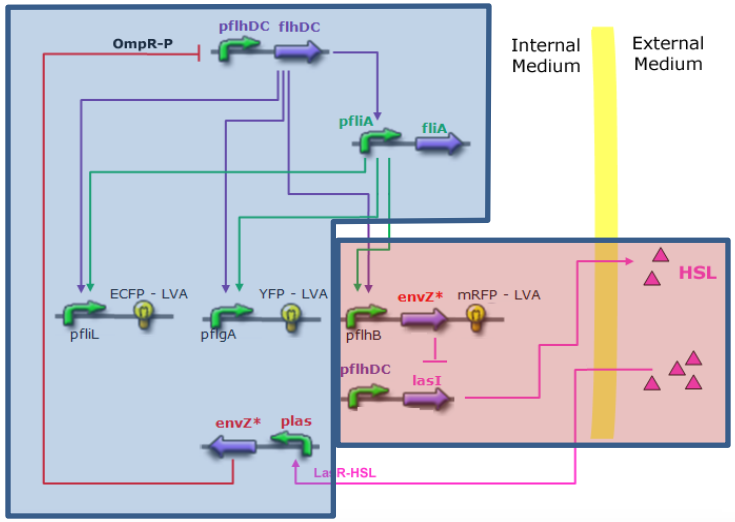
- Oriented parameter exploration (zoom on the key points)



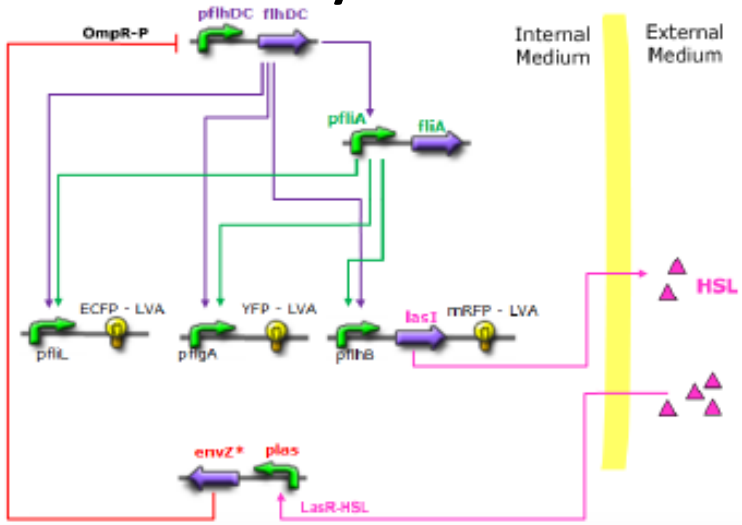
- Damped oscillations
- Three indications for biologists

Improvements with Quorum Sensing

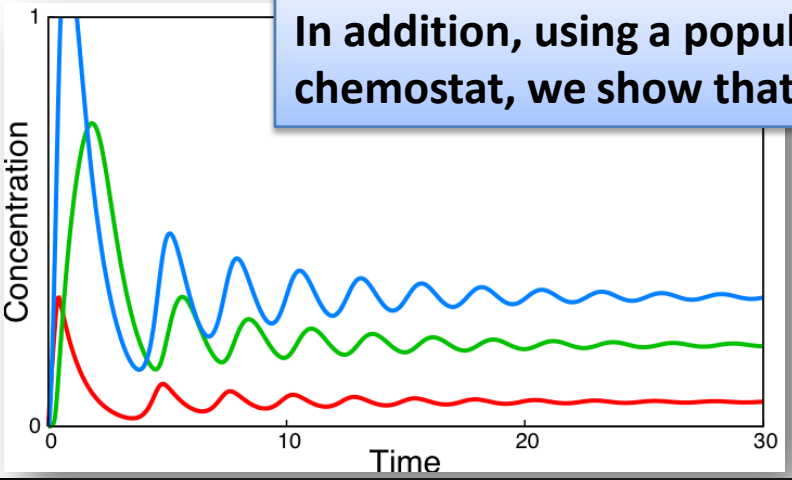
Coupled Oscillators

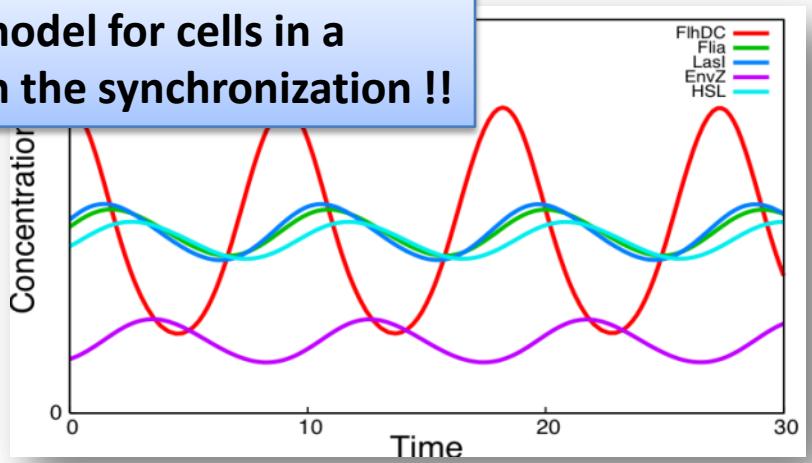


Final System



In addition, using a population model for cells in a chemostat, we show that we win the synchronization !!





« Virtual Lab »
Modeling for Fine Tuning

A modular and predictive model based on systematic parts characterizations

Proposal of an assay for generic characterization

A Bottom-Up Approach

Building of a modular and predictive model :

(1) Decomposition of the system

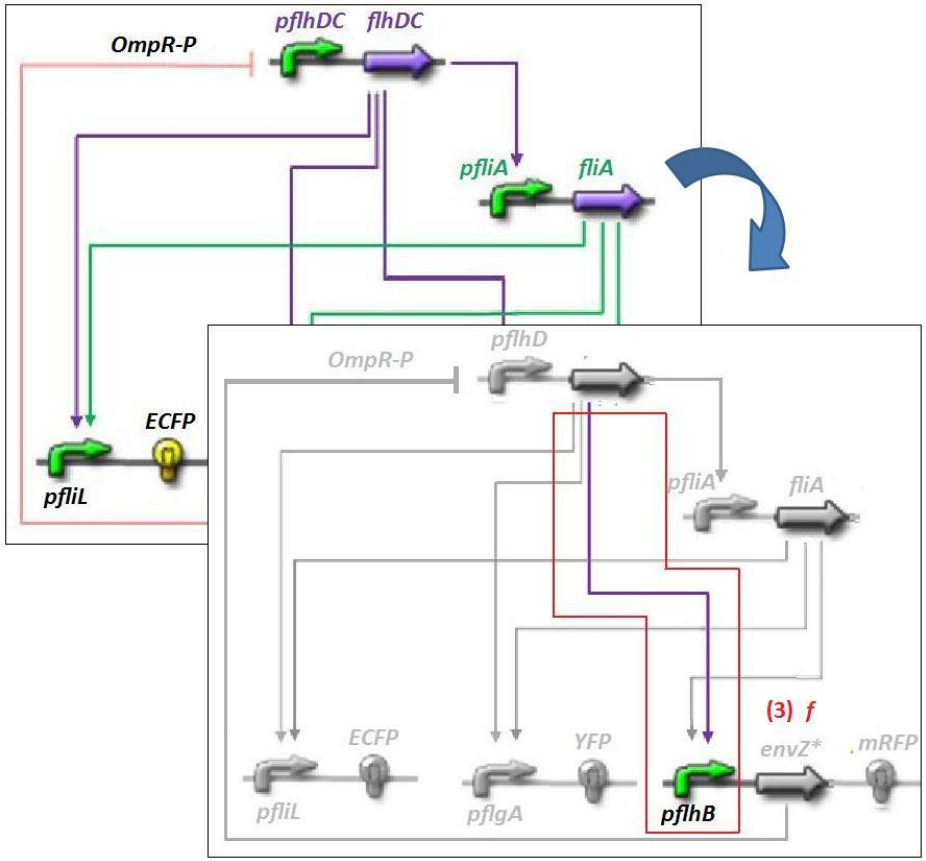
(2) Modeling of each part

(3) Experimental measurements

(4) Implementation in an integrated computational environment

Decomposition of the System

Small and independent modules, treated as «Black Boxes» : outputs= *functions* of inputs



Modeling of Each Part

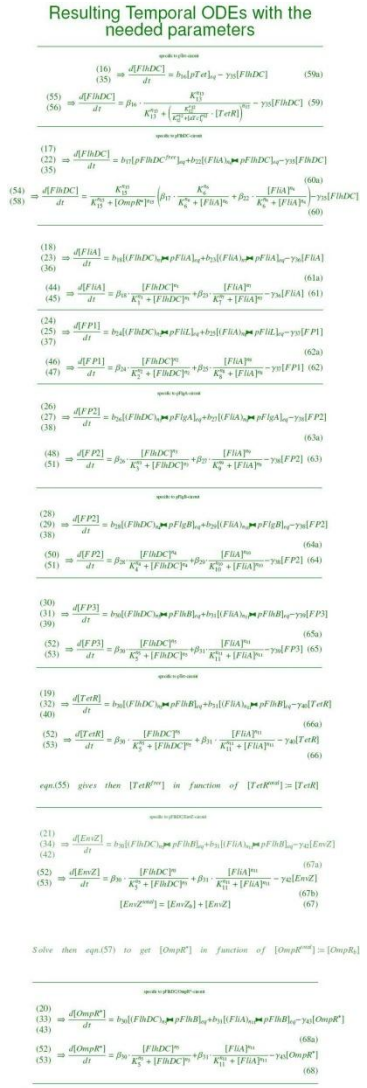
Analytical expressions for each black box

Model is a compromise between:

- Level of details considered for molecular reactions (favors predictibility)
- Available experimental data

Complex mathematical development:

- Can be simplified by the black box approach
- Simplification renders the model specific



Experimental Measurements

- Our black boxes =

  Input: Transcription Factor (TF) concentration

  Output: Promoter Activity

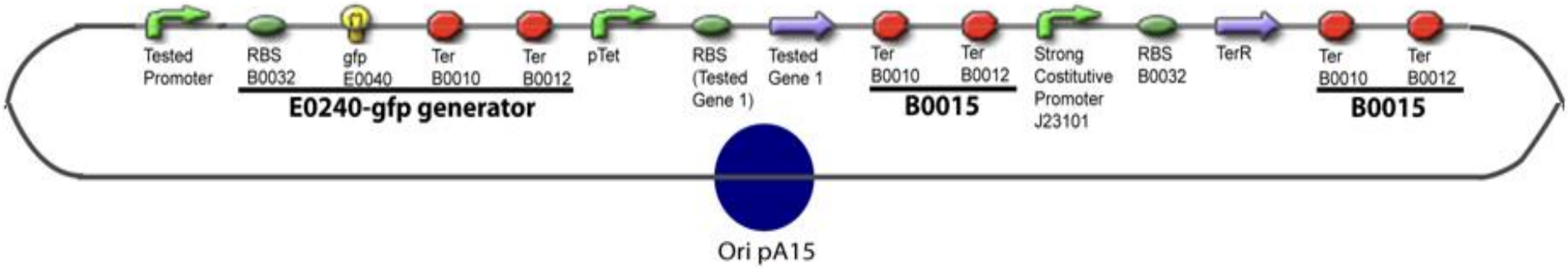
  → construction of a generic plasmid that allows

  – Controlling TF concentration with inducible promoters

  – Measuring promoter activity with fluorescence

- Measurements in specific conditions for improved predictability

  But: Bio-Brick Compatible !!



Integrated Modeling Environment

- Experimental data + Analytical Expressions

Least-square ↓ *Optimization Program*

Estimated Parameters

- Towards a **Virtual Lab**: Final modular program allowing *in silico* experiments :

★ ***Save time and money!*** ★

Parts Realized

Paris 2008 iGEM Team Parts

Favorite Paris 2008 iGEM Team Parts

-?-	Name	Description
S	BBa_K136023	Composite
S	BBa_K136024	Composite
S	BBa_K136027	Composite
S	BBa_K136028	Composite
S	BBa_K136029	Composite
S	BBa_K136030	Composite
S	BBa_K136037	Composite
S	BBa_K136039	Composite
S	BBa_K136044	Intermediate
S	BBa_K136046	Coding
S	BBa_K136047	Intermediate
S	BBa_K136048	Intermediate
S	BBa_K136049	Composite
S	BBa_K136050	Intermediate
S	BBa_K136051	Composite
S	BBa_K136052	Intermediate
S	BBa_K136053	Composite
S	BBa_K136054	Composite
S	BBa_K136055	Composite
S	BBa_K136056	Intermediate
S	BBa_K136057	Intermediate
S	BBa_K136058	Intermediate
S	BBa_K136059	Composite
	BBa_K136060	Plasmid
	BBa_K136064	Composite
	BBa_K136065	Composite
	BBa_K136066	Composite
	BBa_K136069	Composite
	BBa_K136070	Composite

Part:BBa_K136066

Designed by Banos Dhayer Group: iGEM08_Paris

araC/pBad - EnvZ*

inducible expression of EnvZ* by the araC/pBad. EnvZ is a transmembrane histidine kinase that, in response to osmotic changes, phosphorylates OmpR the natural inhibitor of flhDC promotor the master regulator of the flagella synthesis. EnvZ* is here a mutated version which constitutively phosphorylates OmpR.

Motility assay in a MG1655 (envZ-) strain. From left to right: no plasmid, pSB3K3-pBAD is quite leaky, there is a clear difference between it's activation and it's repr motility.

Sequence and Features

Part:BBa_K136006:Experience

Designed by Kok-Phen YAN Group: iGEM08_Paris

This experience page is provided so that any user may enter their experience using this Please enter how you used this part and how it worked out.

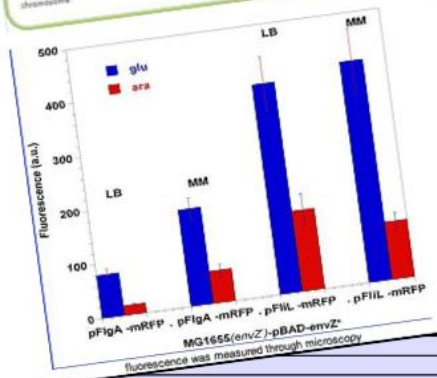
Applications of BBa_K136006

MG1655 (envZ-) was transformed with pSB3K3-BBa_K136066 (pBAD-envZ*) and

expression of mRFP1.

EnvZ (expressed from the pBAD) naturally represses flhDC through the activat

expression of the flagella class 2 genes (fliL, figA, flhB...). We measure here



Part:BBa_K136046

Designed by alexandra bouaziz Group: iGEM08_Paris

envZ*

EnvZ is a transmembrane histidine kinase that, in response to osmotic changes, phosphorylates OmpR the natural i of the flagella synthesis. EnvZ* is here a mutated version which constitutively phosphorylates OmpR. The resulting pr repression of FlhDC expression and then absence of motility for the bacteria.

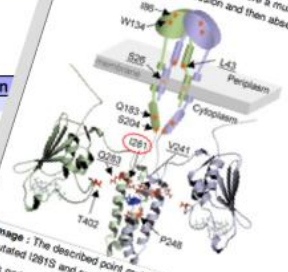


Image : The described point mutations of EnvZ in motility deficient strains of E. coli (Giraud et al. PLoS Genet. 2008). The mutated EnvZ mutated I281S and present the more severe phenotype.

This part was successfully used in the composite part BBa_K136066

A site EcoRI is present in the part which doesnt comply with the Biobrick standard. We will soon provide a new version of this part without th

Sequence and Features

EcoRI site found in the sequence at base pair 316.

	flhB promoter + WT RBS (K136009) - EnvZ*	1513
	flhB promoter + WT RBS - GFP	929
	araC/pBad - EnvZ*	2569
	fliL promoter (+WT RBS) - EnvZ*- GFP generator	2397
	flhB Promoter (+ WT RBS) - EnvZ* - GFP generator	2446

Conclusion

- We designed a **chassis** that can be used to **build complex genetically engineered machines**
- We **tested experimentally** that our promoters and the negative feedback loop behaved as predicted
- Thanks to **our models we tested and improved our system**. In particular, the synchronization at population level was added to maintain the oscillations
- **We developed a new standard tool** for parallel characterization of complex BioBricks
- *Perspectives*

Perspectives

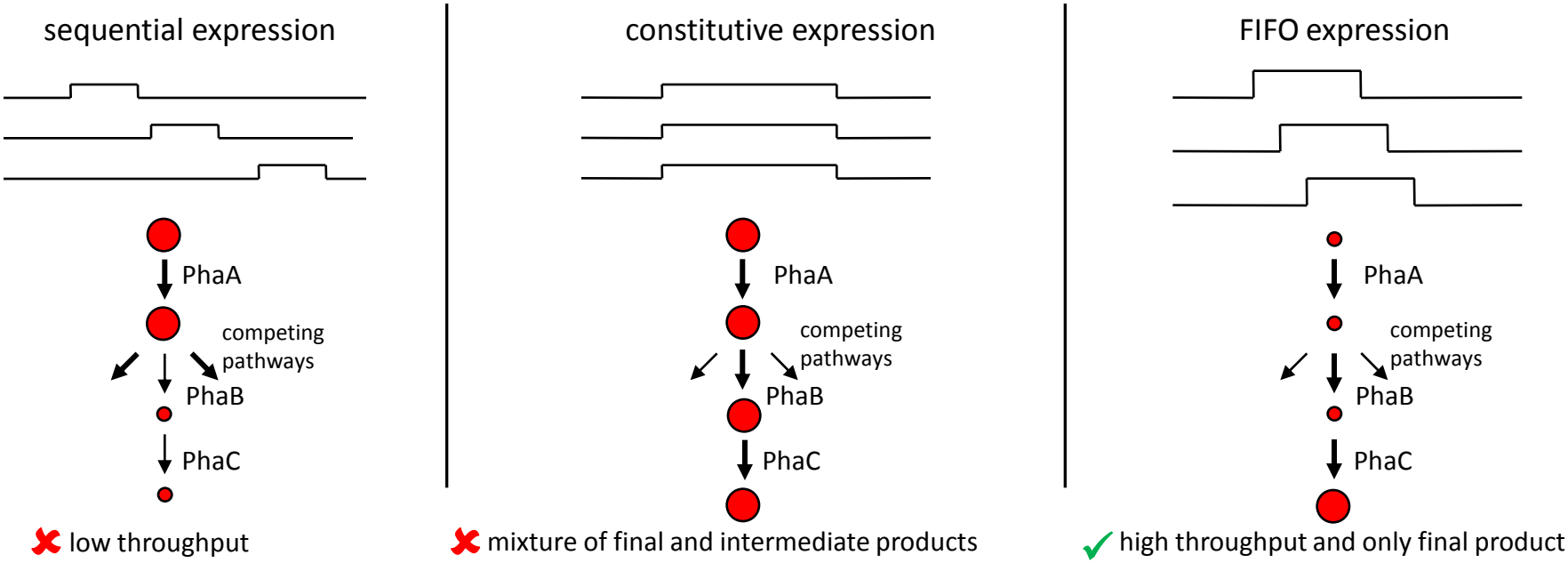
- Control of injectisome construction

  Injectisome is a nanomachine that injects bacterial proteins into eucaryotic cells

  Injectisome shares many similarities with flagellar system at gene level

- Optimization of biosynthetic pathways

  Example of an environmentally-friendly plastic: polyhydroxyalkanoate synthesis



The Paris Team

Have a look at our poster!

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Special Acknowledgement

Thank you for your attention!

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