



University of Pavia at iGEM 2008



# Engineering *E. coli* to multiplex and demultiplex signals

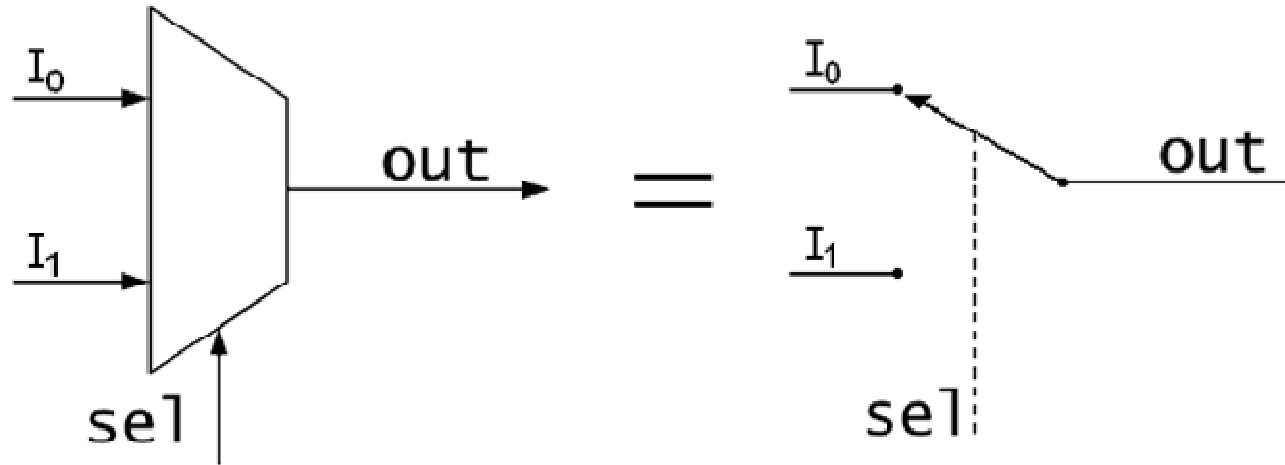


# Outline

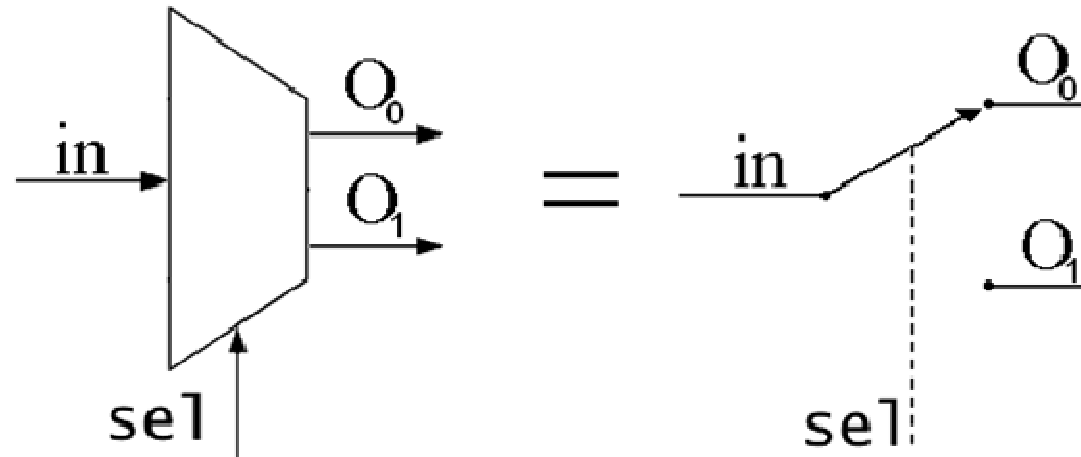
- Multiplexing and demultiplexing
- Genetic implementation of logic gates
- Genetic devices
- Results
- Applications and conclusions

# Definitions

- **Multiplexing:** one of many input signals into one output channel

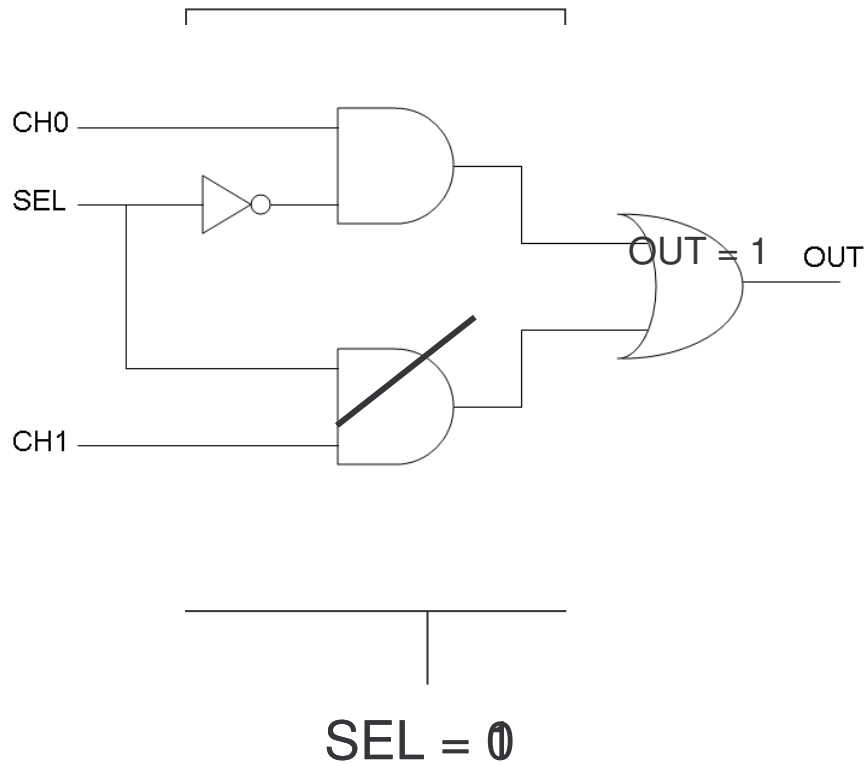


- **Demultiplexing:** one input signal into one of many output channels

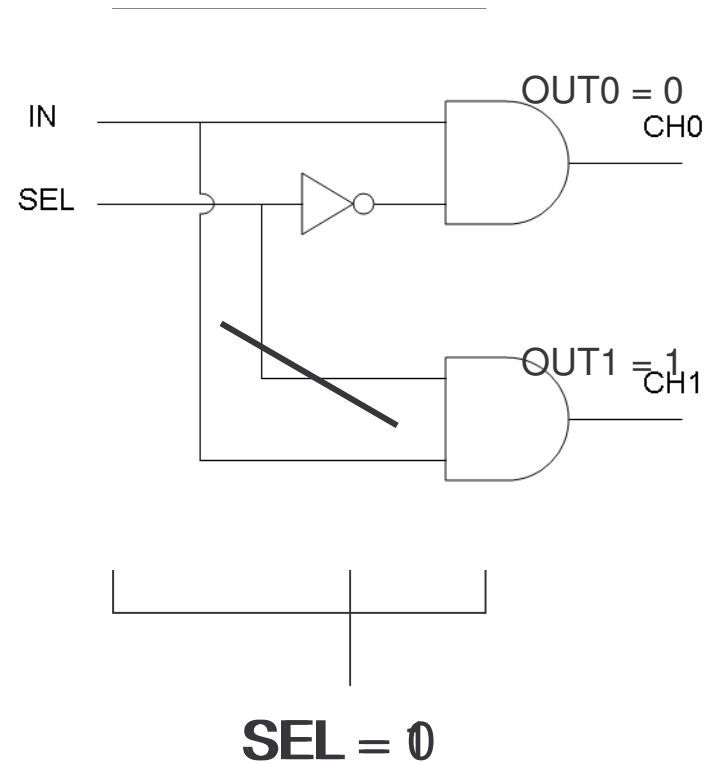


# Digital electronic devices

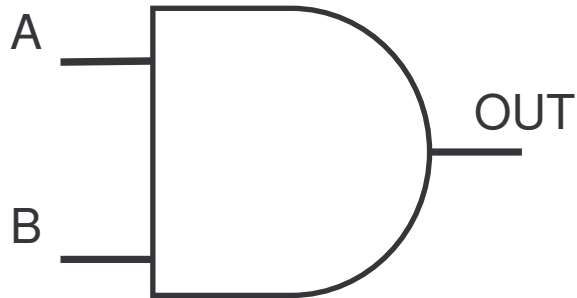
## Mux



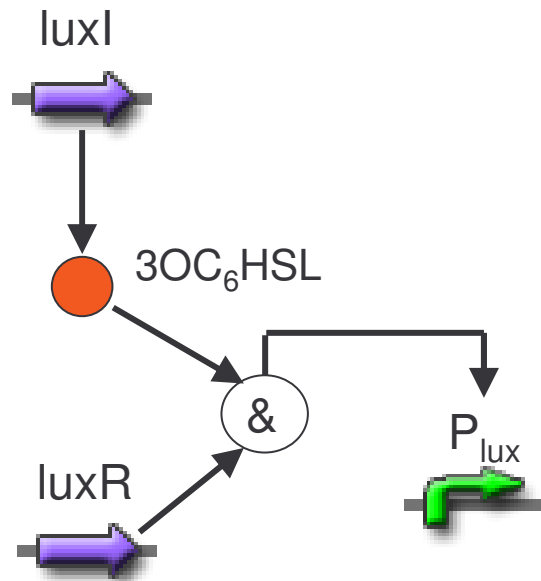
## Demux



# AND

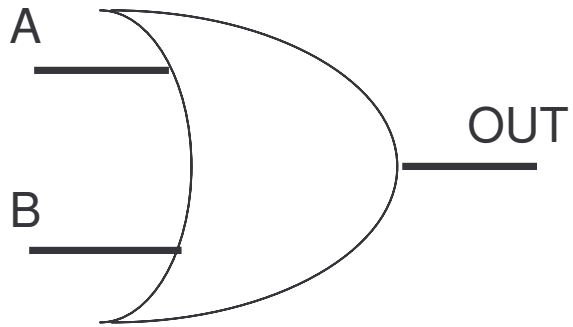


A	B	OUT
0	0	0
0	1	0
1	0	0
1	1	1

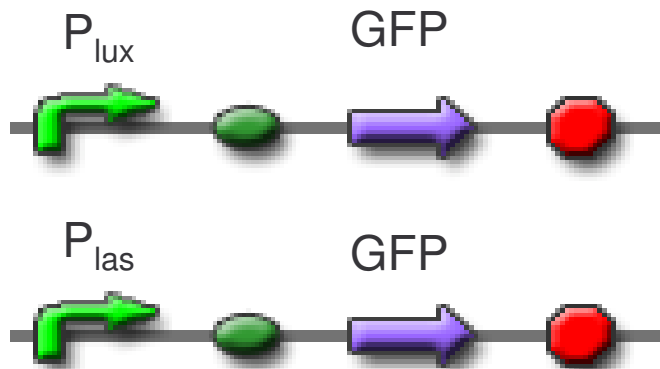


luxI	luxR	P <sub>lux</sub>
no	no	off
no	yes	off
yes	no	off
yes	yes	on

# OR

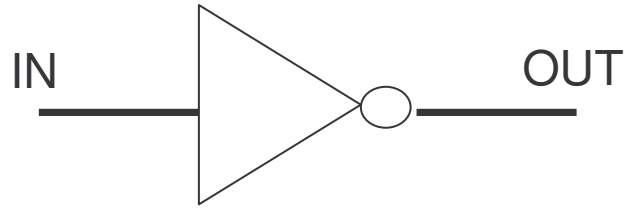


A	B	OUT
0	0	0
0	1	1
1	0	1
1	1	1

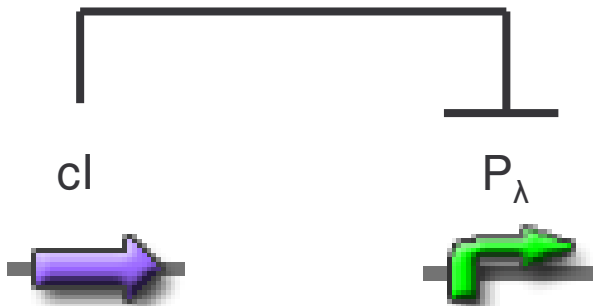


$P_{lux}$	$P_{las}$	GFP
off	off	no
off	on	yes
on	off	yes
on	on	yes

# NOT



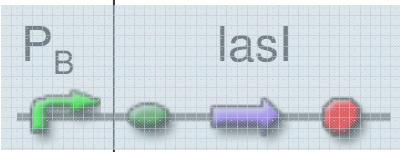
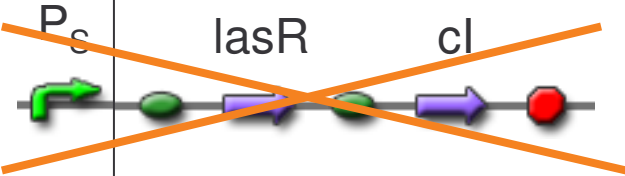
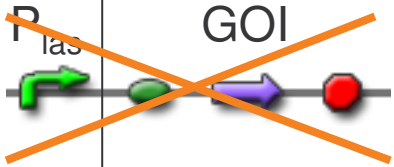
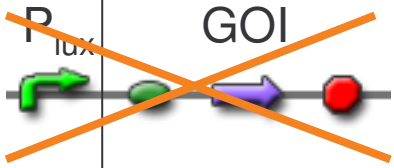
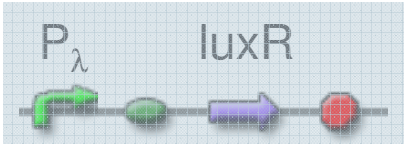
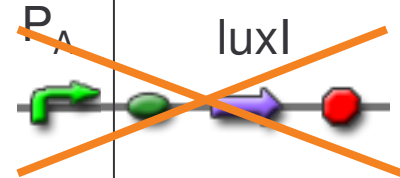
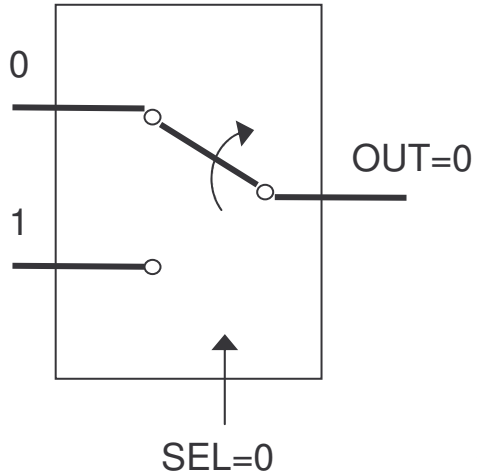
IN	OUT
0	1
1	0



cl	P <sub>λ</sub>
no	on
yes	off

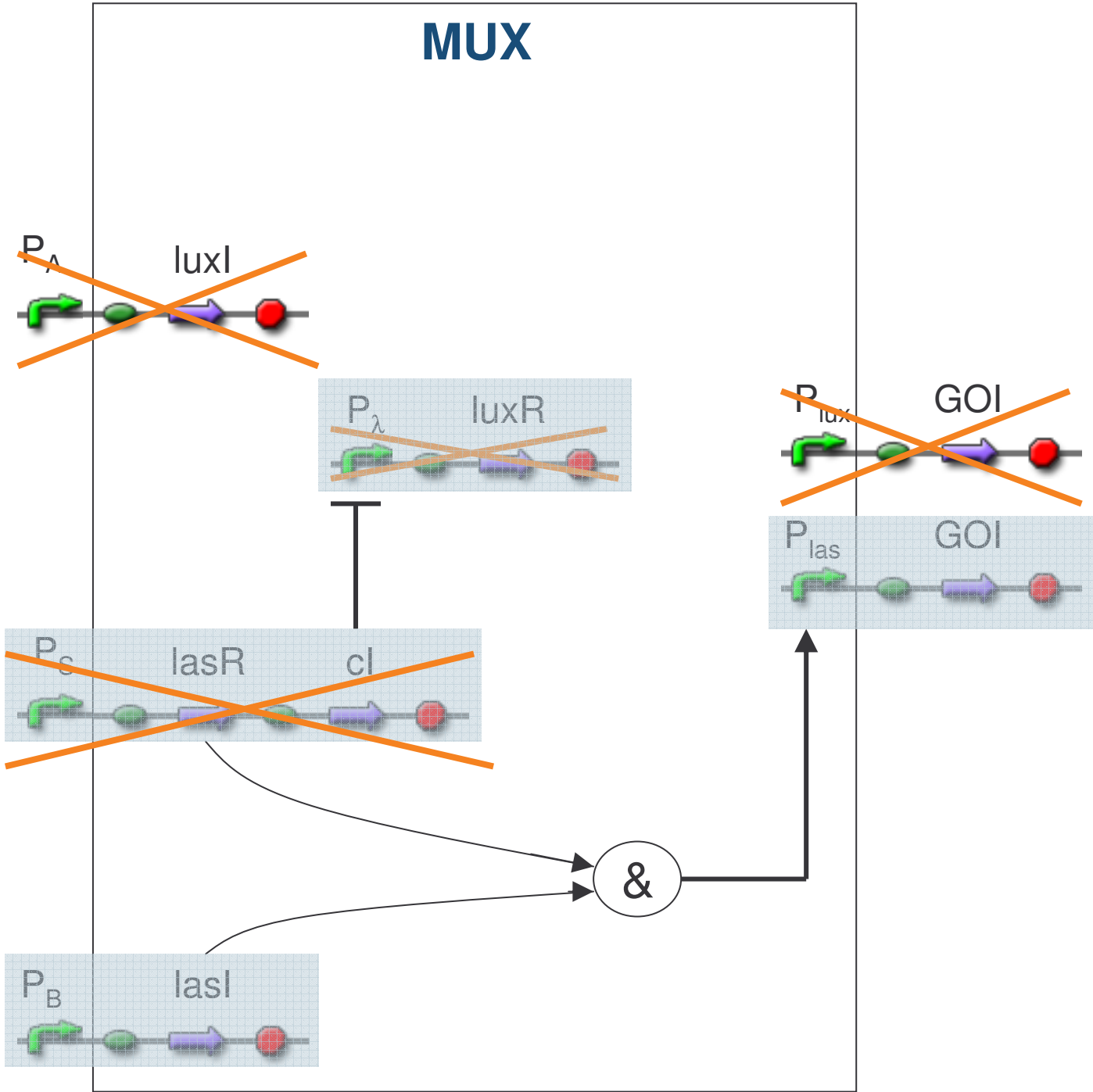
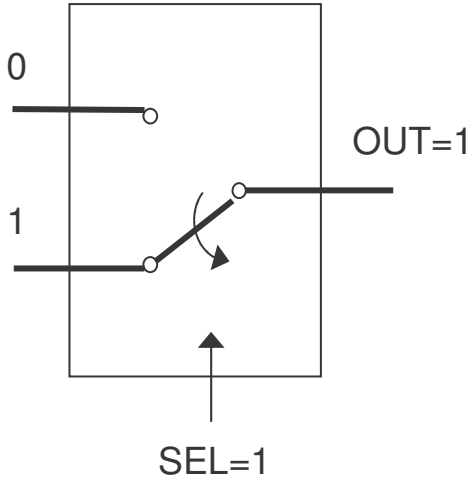
A no  
B yes  
S no

# MUX



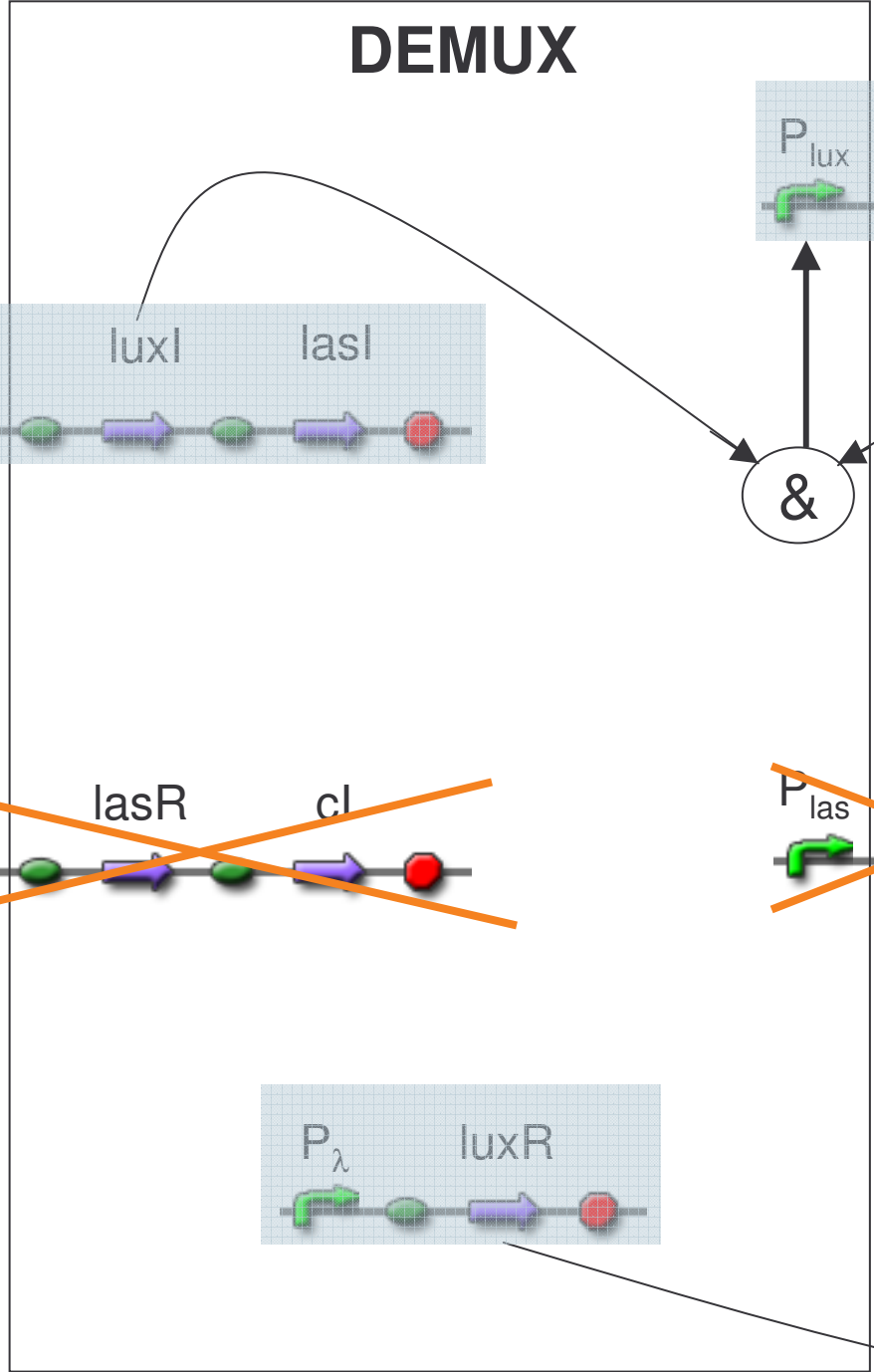
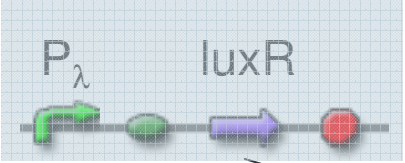
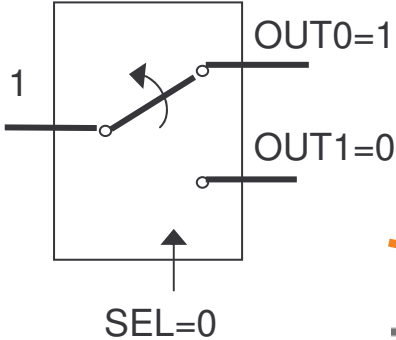
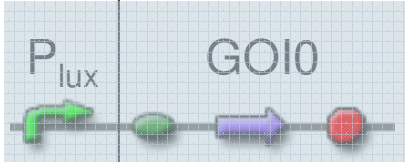


A no  
B yes  
S yes



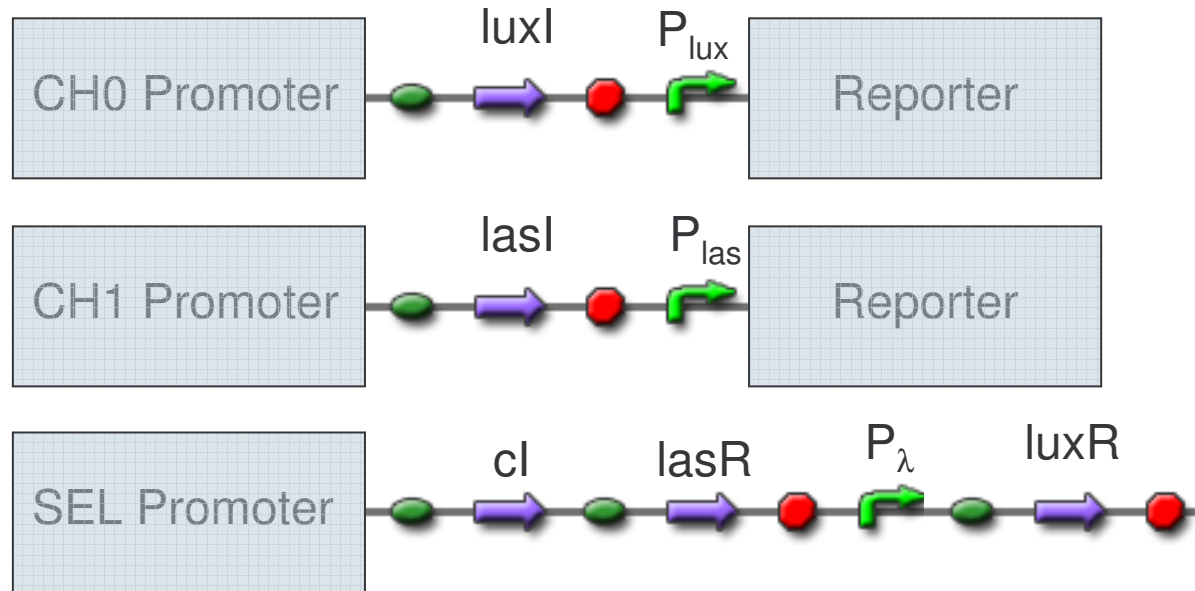
I yes  
S no

# DEMUX

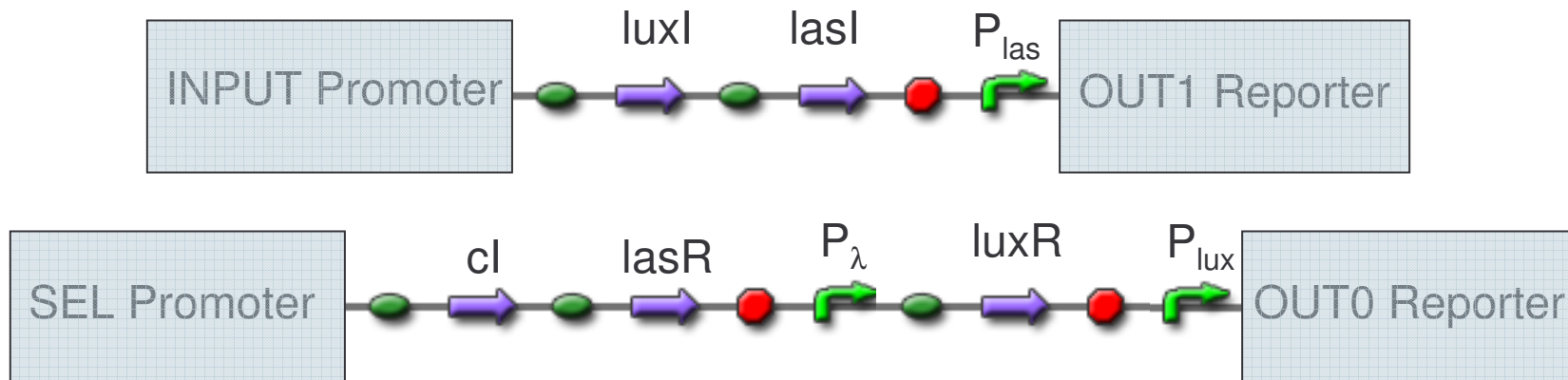


# Final devices

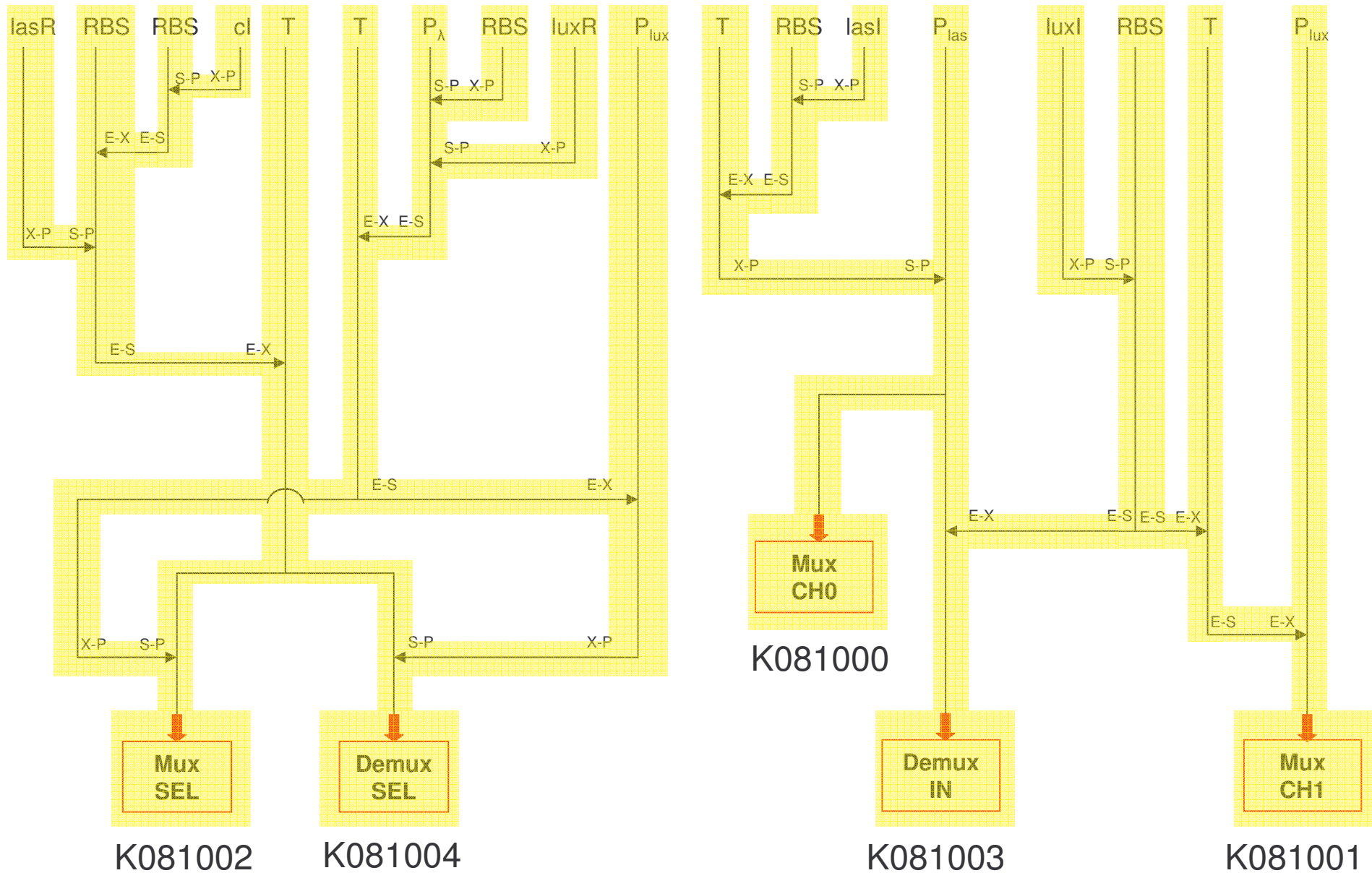
## MUX:



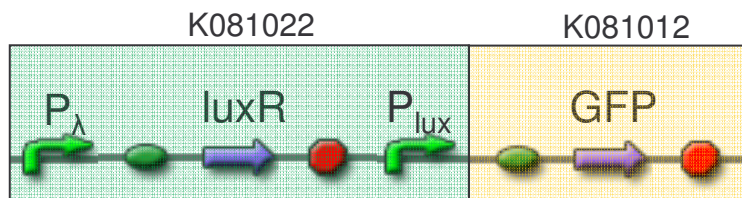
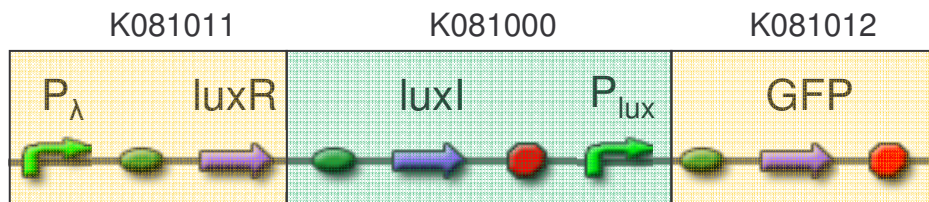
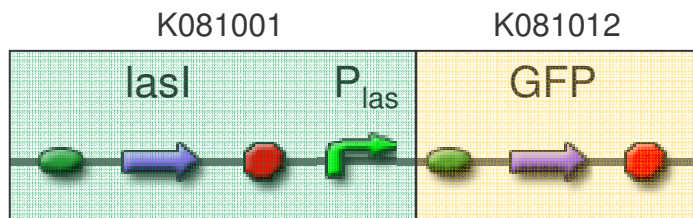
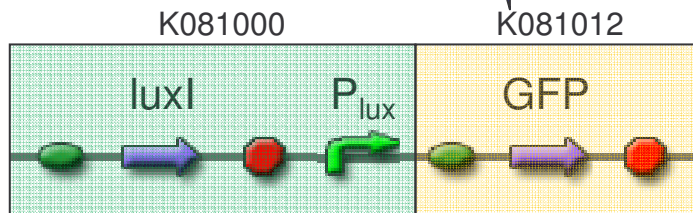
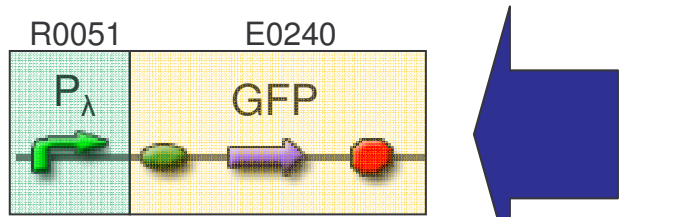
## DEMUX:



# Assembly schema



# On/off experiments



# Truth table validation

AND (*lux* system)

<b>luxI</b>	<b>luxR</b>	<b>P<sub>lux</sub></b>
No	No	Off
No	Yes	Off
Yes	No	Off
Yes	Yes	On

AND (*las* system)

<b>lasI</b>	<b>lasR</b>	<b>P<sub>las</sub></b>
No	No	Off
No	Yes	Off
Yes	No	Off
Yes	Yes	On

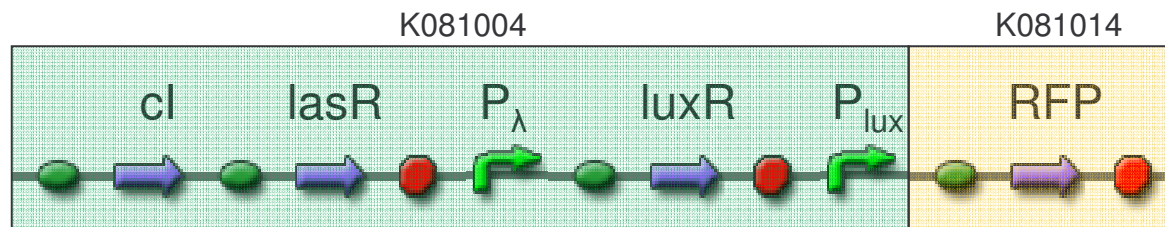
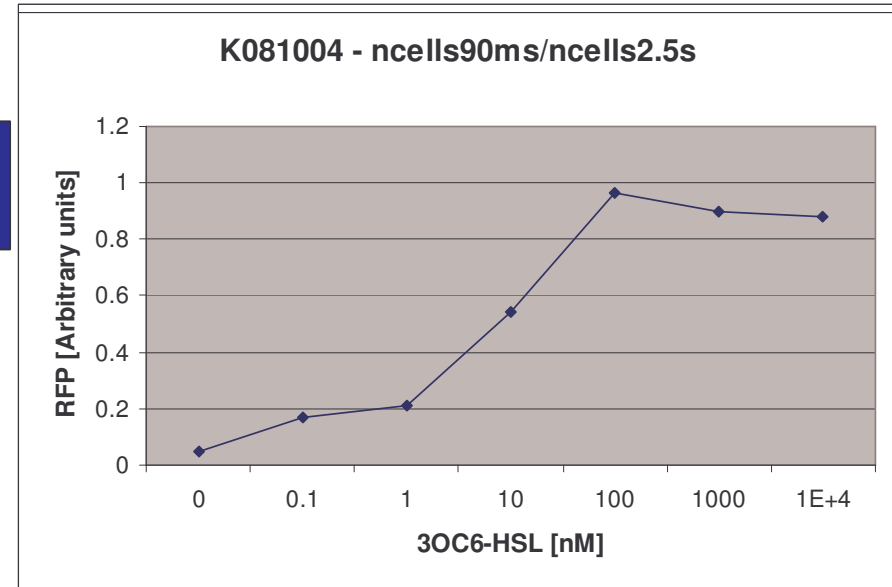
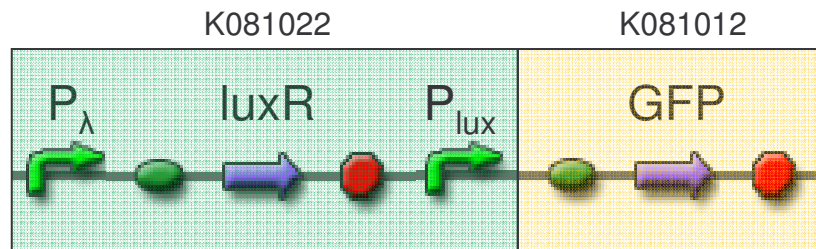
NOT ( $\lambda$ -*cl* system)

<b>cl</b>	<b>P<sub><math>\lambda</math></sub></b>
No	On
Yes	Off

OR (gene under two independent promoters)

<b>P<sub>lux</sub></b>	<b>P<sub>las</sub></b>	<b>GFP</b>
Off	Off	No
Off	On	Yes
On	Off	Yes
On	On	Yes

# Quantitative experiments



# Modeling

## Bound molecule

$$Prob = \frac{[T]^n}{K_{50}^n + [T]^n}$$

[T]: transcription factor

$K_{50}$ : dissociation constant

$n$ : cooperativity constant

[m]: mRNA

$\delta$ : mRNA degradation rate

$a$ : leakage factor

[P]: protein

$\alpha$ : protein production rate

$\beta$ : protein degradation rate

## Free molecule

$$Prob = 1 - \frac{[T]^n}{K_{50}^n + [T]^n} = \frac{K_{50}^n}{K_{50}^n + [T]^n}$$

## Regulated transcription

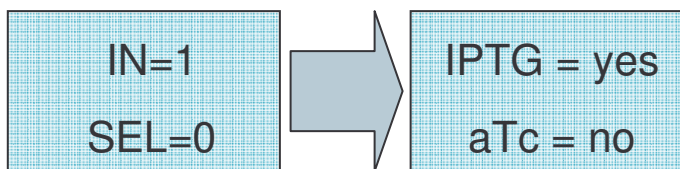
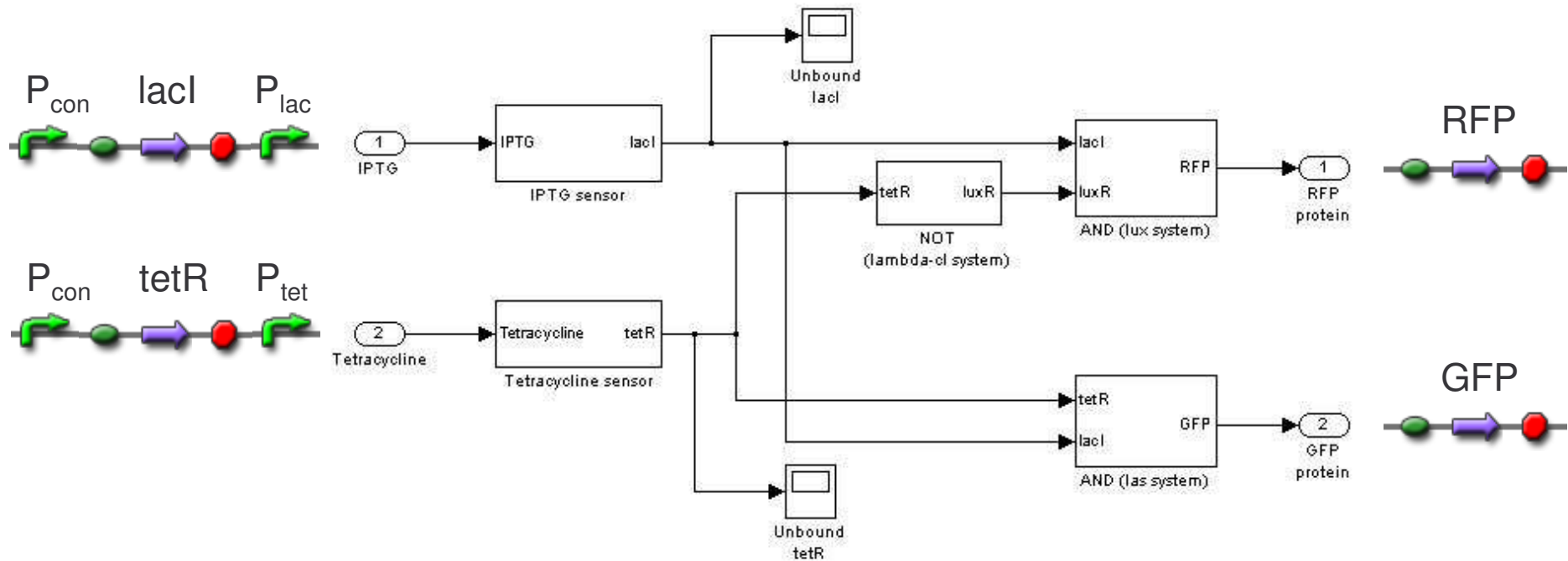
$$\frac{d[m]}{dt} = V_{max} \{a + (1 - a) Prob\} - \delta[m]$$

## Protein production

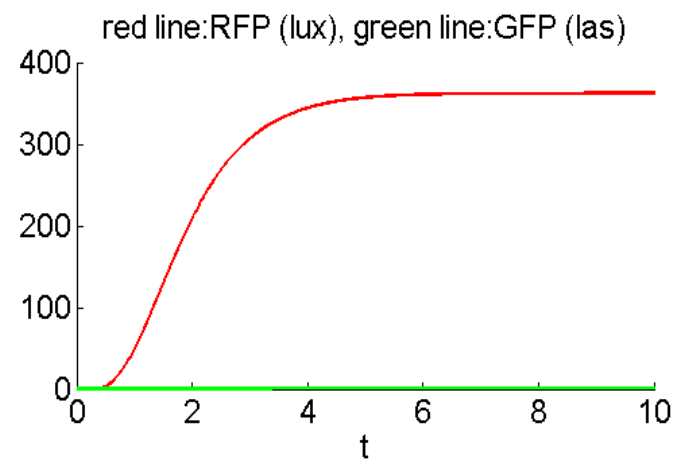
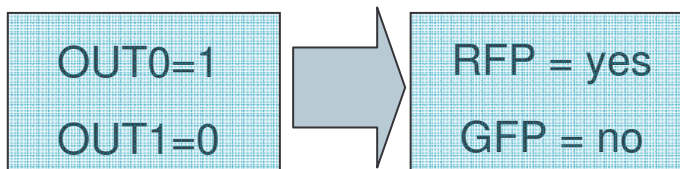
$$\frac{d[P]}{dt} = \alpha[m] - \beta[P]$$



# Simulation

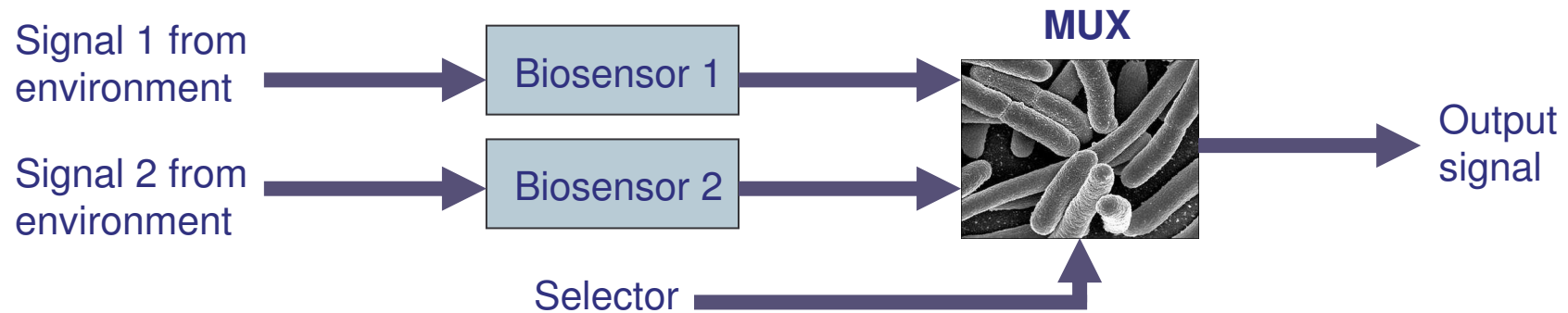


Expectation:

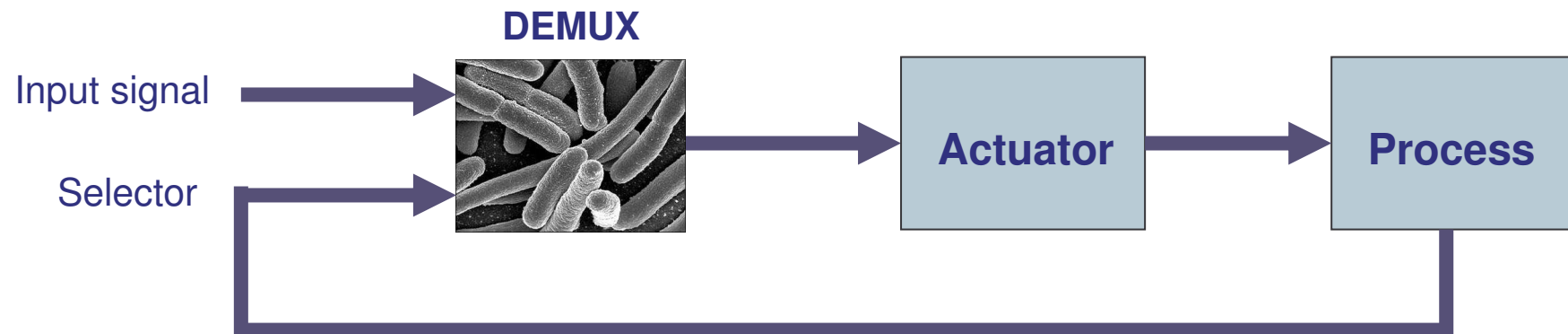


# Applications

## ➤ MUX: Signal integration in biosensors



## ➤ DEMUX: Controlled actuators



# Conclusions

- 20 amplified from Spring 2008 Distribution
- 27 BioBrick parts submitted to the Registry
- 5 final devices completed
- 8 successful experiments to validate circuits behavior
- Synthetic Biology is now at University of Pavia!!!

# UNIPV-Pavia iGEM 2008 Team



<b>Instructors:</b>	<b>Paolo Magni Maria Gabriella Cusella</b>
<b>Advisor:</b>	<b>Daniela Galli</b>
<b>Students:</b>	<b>Lorenzo Pasotti Mattia Quattrocelli</b>

## Acknowledgements

- **Bologna iGEM 2008 Team (friendly collaboration)**
- **Riccardo Bellazzi (Bioinformatics research unit leader)**
- **Giovanni Magenes (Center for Tissue Engineering director)**
- **Mario Stefanelli (Biomedical Informatics Lab director)**
- **Laura Benedetti (Molecular Biology Lab manager)**
- **Alessandra Tiengo (poster layout)**
- **Wet & dry lab people (meeting and discussion)**
- **Grazia Bruttocao (University of Pavia press office director)**
- **Radio Campus Pavia**