Turing meets Synthetic Biology:

Self-emerging patterns in an activator inhibitor network

UNAM CINVESTAV mexico

Cinvestav
National Laboratory of Genomics for Biodiversity

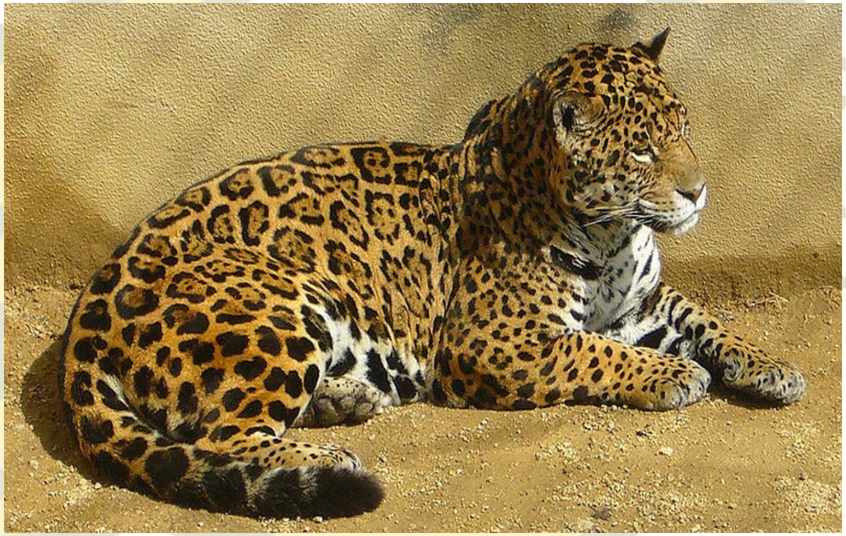
IIMAS UNAM

Turing meets Synthetic Biology:

The main goal of the project was to show that Turing patterns could be obtained by the action of an underlying genetic regulatory network

Morphogenesis and Turing Patterns

- Size and shape in nature
- Developmental biology
- Unknown general mechanisms and the role of underlying genetic regulatory networks



Turing approach

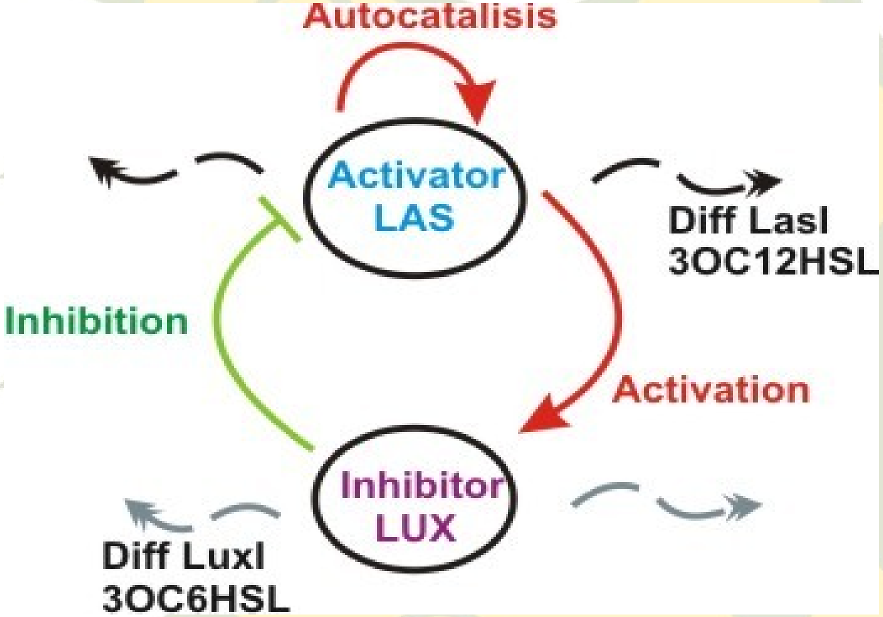
- Positional information (chemicals)
- The chemical basis of morphogenesis
  - Chemicals that interact and diffuse through the medium
  - Reaction-Diffusion systems
- Genes by themselves do not produce the pattern

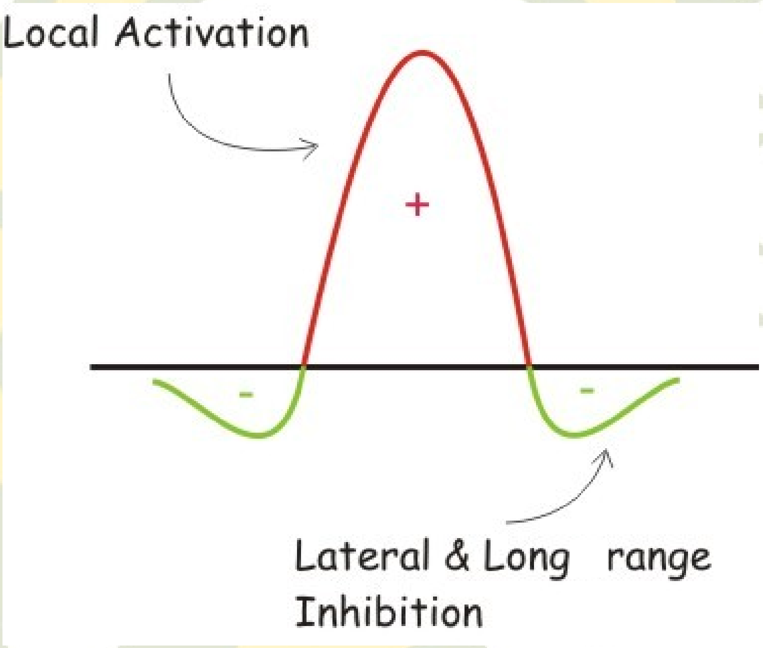
$$\frac{\partial c}{\partial t} = f(c) + D\nabla^2 c$$

$$\frac{\partial A}{\partial t} = F(A,B) + D_A\nabla^2 A$$
$$\frac{\partial B}{\partial t} = G(A,B) + D_B\nabla^2 B$$

Activator-Inhibitor

- Gierer and Meinhardt, 1972
- Local Activation and long range inhibition
- Fire and grasshoppers analogy





Conditions for pattern generation

✓The existence of at least two morphogenes with different nature that chemically interact between them and diffuse over space.

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The existence of at least two morphogenes with different nature that chemically interact between them and diffuse over space.

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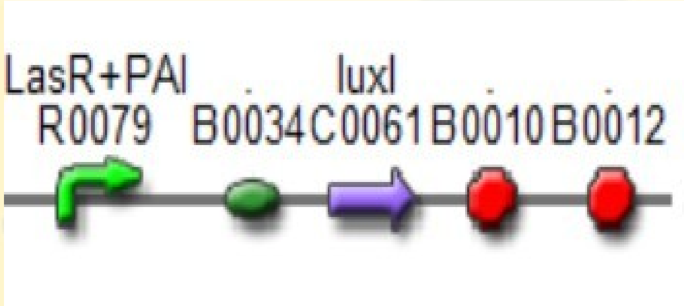
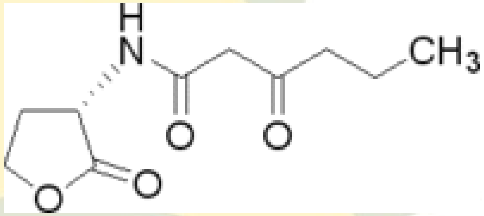
✓The starting distribution of morphogenes should not be completely homogeneous over space.

✓Local activation and long range inhibition.

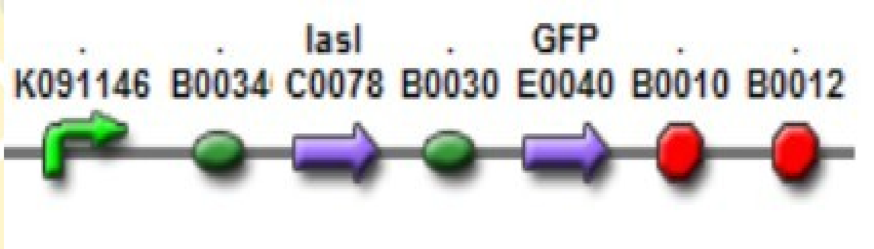
AHL Diffusion

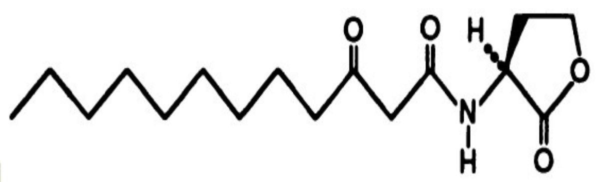
- Lux





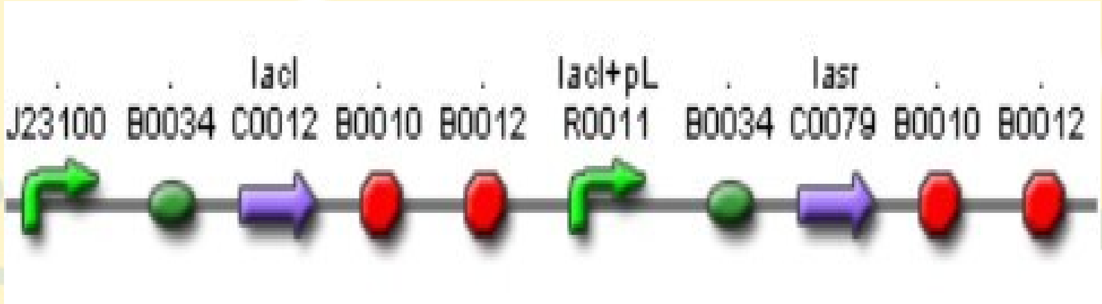
- Las



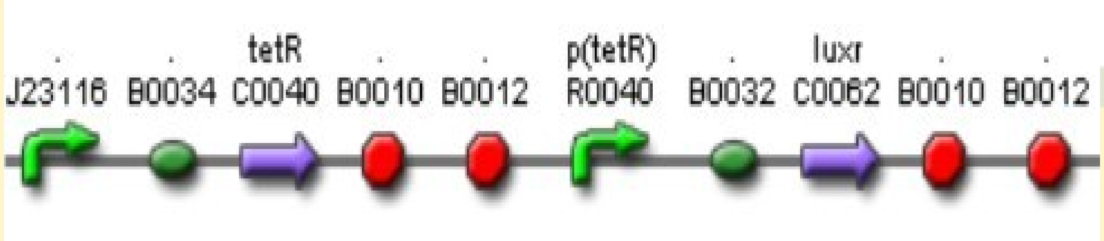


Inverters

- Lac Inverter

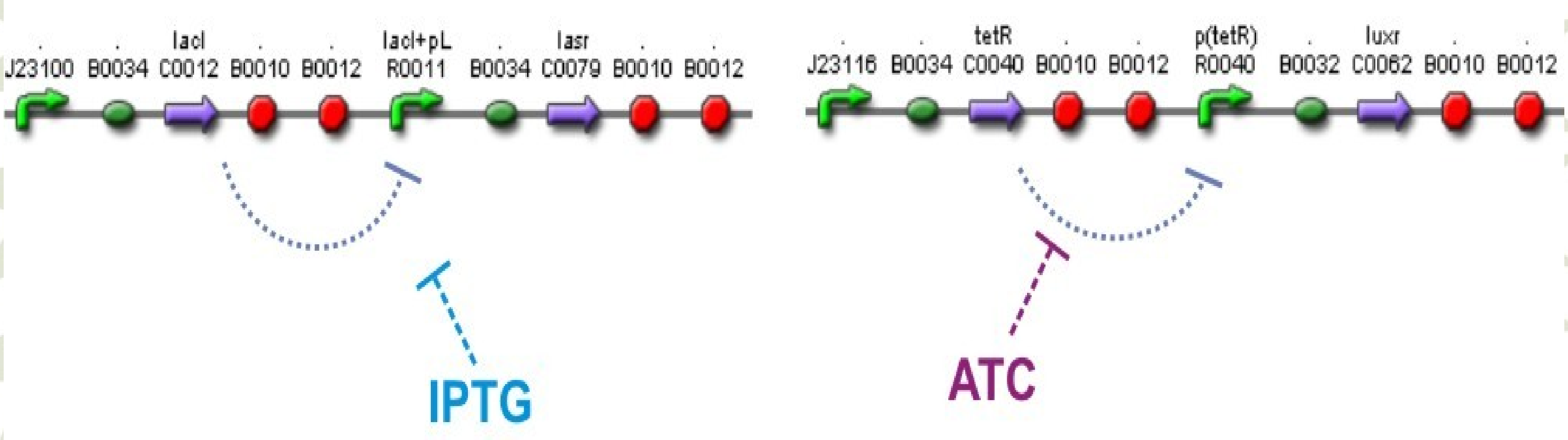


- Tet Inverter

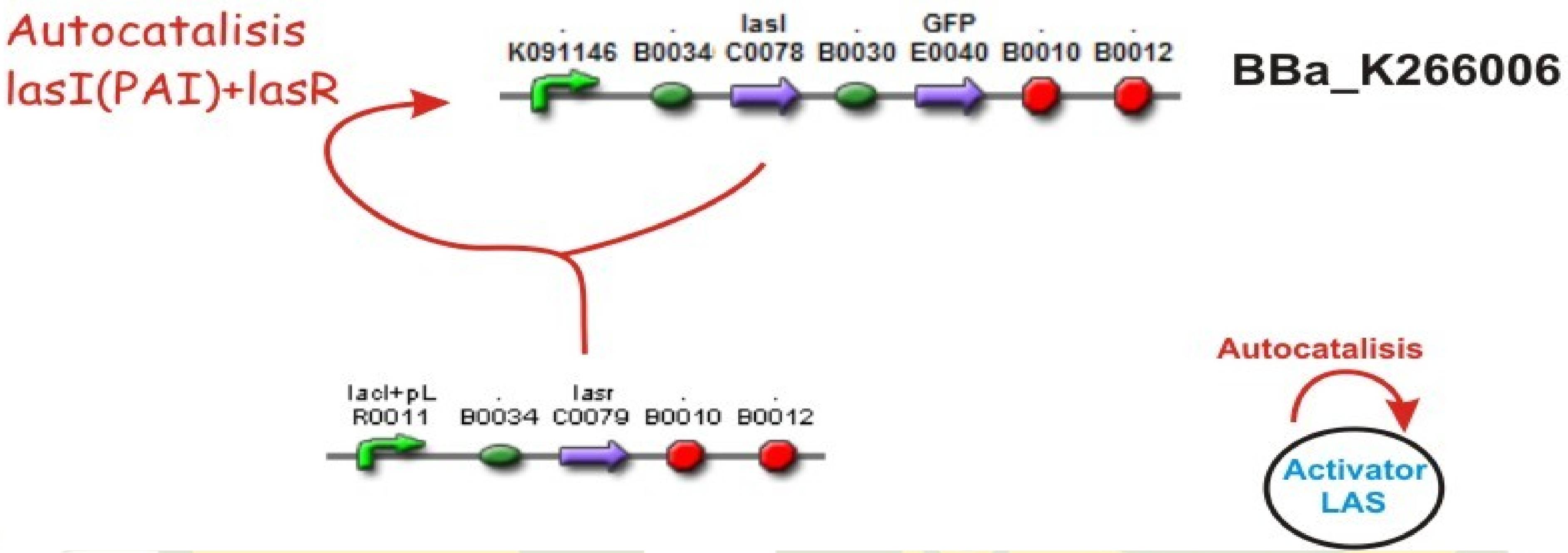


Substrates

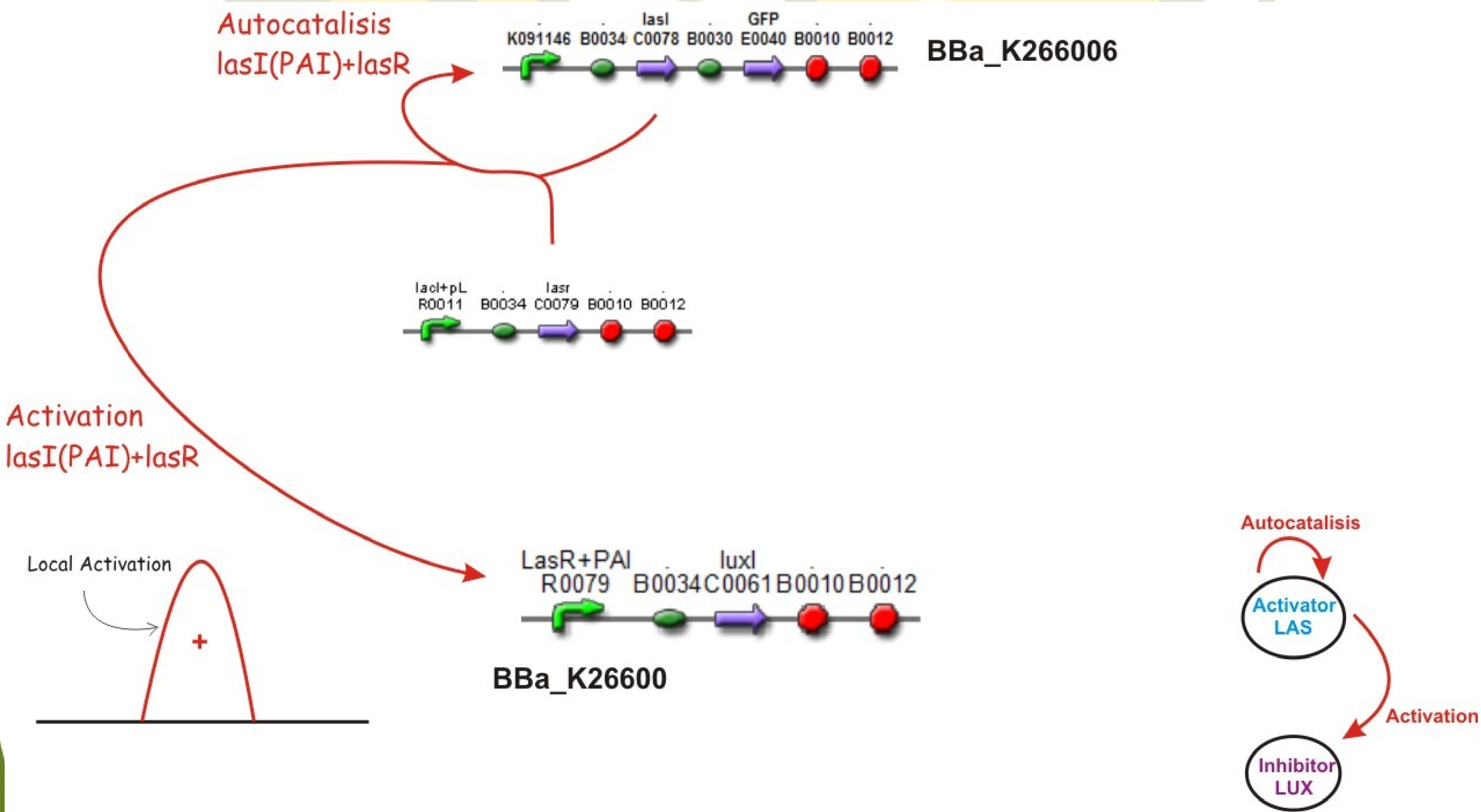
- IPTG
- ATC



Autocatalisis

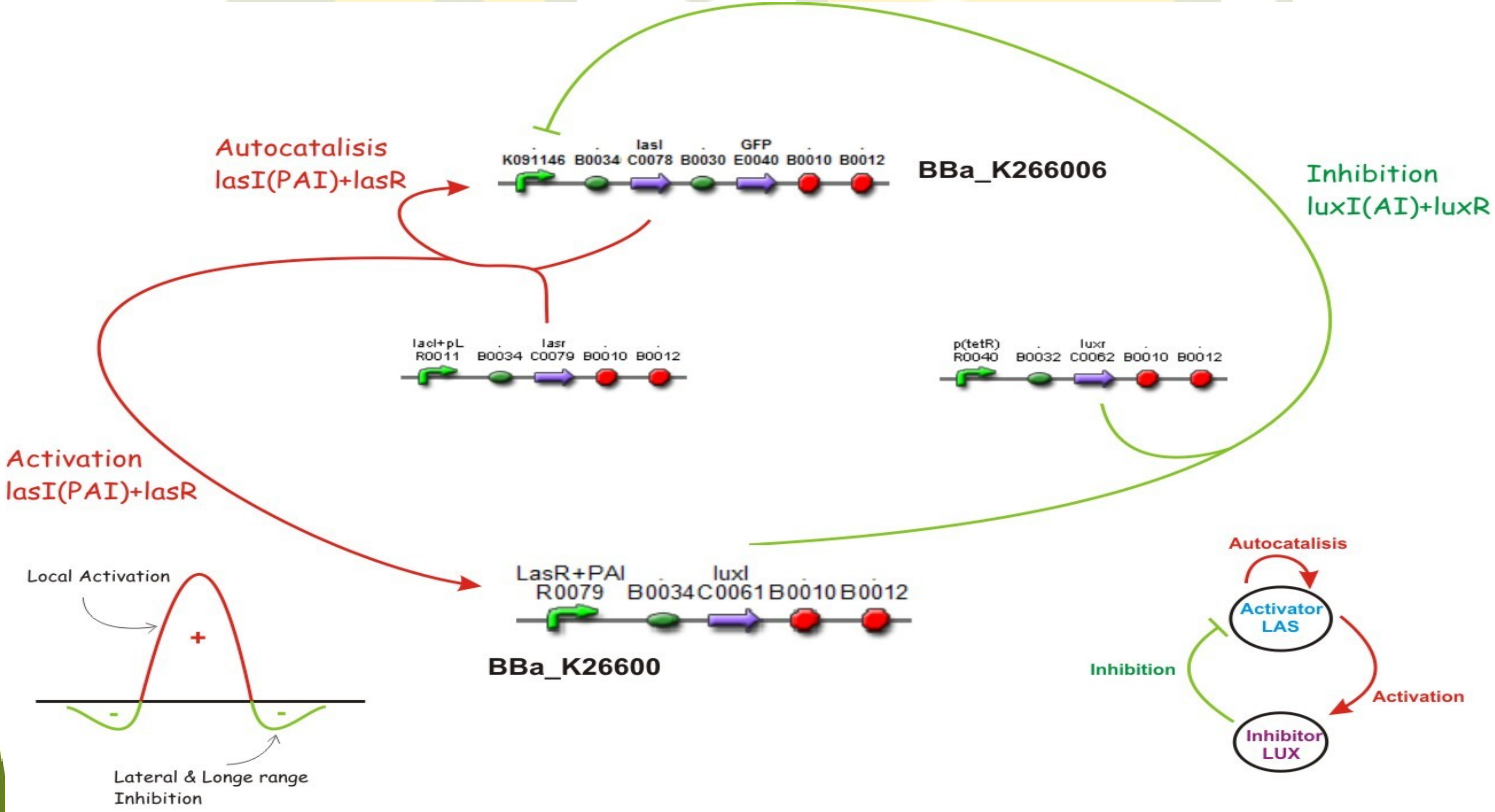


Activation

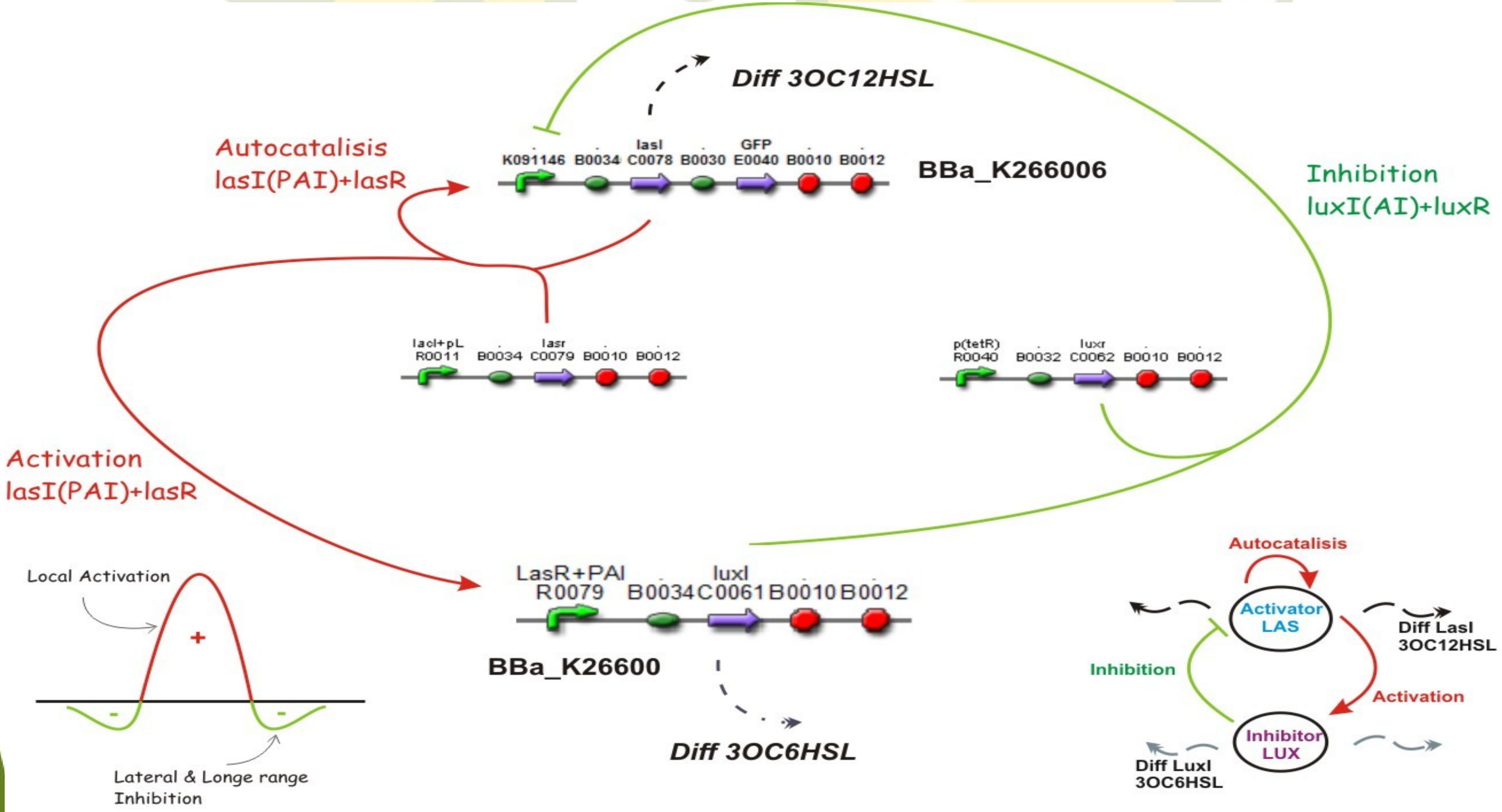


Autocatalisis
lasI(PAI)+lasR
lasI
GFP
K091146 B0034 C0078 B0030 E0040 B0010 B0012
BBa_K266006
lacI+pL
lasr
R0011 B0034 C0079 B0010 B0012
Activation
lasI(PAI)+lasR
Local Activation
+
LasR+PAI
luxI
R0079 B0034 C0061 B0010 B0012
BBa_K26600
Autocatalisis
Activator
LAS
Activation
Inhibitor
LUX

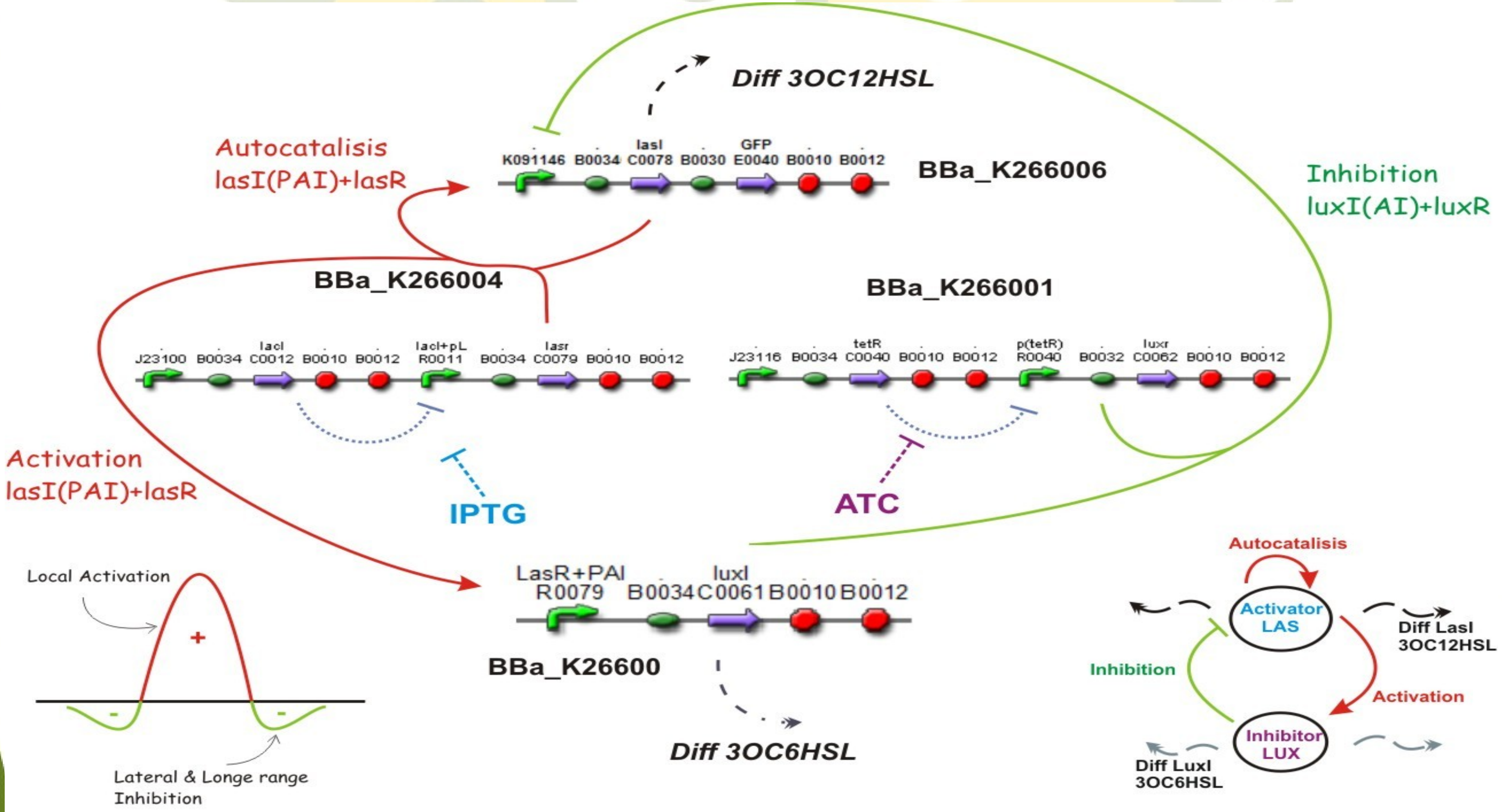
Inhibition


Autocatalisis
lasI(PAI)+lasR
K091146 B0034 lasI C0078 B0030 GFP E0040 B0010 B0012
BBa_K266006
Inhibition
luxI(AI)+luxR
lacI+pL R0011 B0034 lasr C0079 B0010 B0012
p(tetR) R0040 B0032 luxr C0062 B0010 B0012
Activation
lasI(PAI)+lasR
LasR+PAI R0079 B0034 luxI C0061 B0010 B0012
BBa_K26600
Local Activation
+
-
-
Lateral & Longe range
Inhibition
Autocatalisis
Activator
LAS
Inhibition
Activation
Inhibitor
LUX

Diffusion

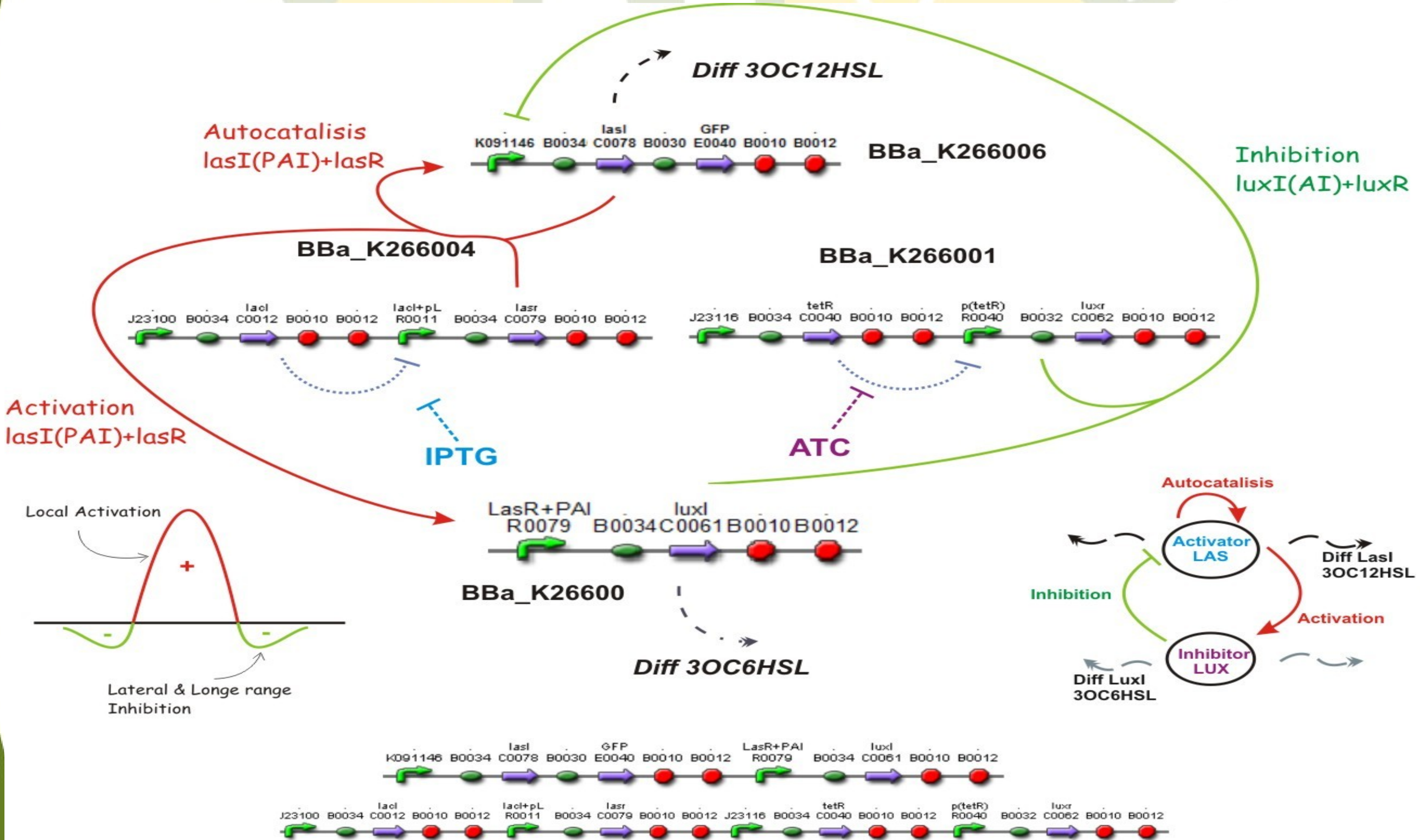

Diff 3OC12HSL
Autocatalisis
lasI(PAI)+lasR
K091146 B0034 lasI C0078 B0030 GFP E0040 B0010 B0012
BBa_K266006
Inhibition
luxI(AI)+luxR
lacI+pL R0011 B0034 lasr C0079 B0010 B0012
p(tetR) R0040 B0032 luxr C0062 B0010 B0012
Activation
lasI(PAI)+lasR
LasR+PAI R0079 B0034 luxI C0061 B0010 B0012
BBa_K26600
Diff 3OC6HSL
Local Activation
+
-
-
Lateral & Longe range Inhibition
Autocatalisis
Activator LAS
Diff LasI 3OC12HSL
Inhibition
Activation
Inhibitor LUX
Diff LuxI 3OC6HSL

Substrates



Diff 3OC12HSL
Autocatalisis
lasI(PAI)+lasR
K091146 B0034 lasI C0078 B0030 GFP E0040 B0010 B0012
BBa_K266006
Inhibition
luxI(AI)+luxR
BBa_K266004
BBa_K266001
J23100 B0034 lacI C0012 B0010 B0012 lacI+pL R0011 B0034 lasr C0079 B0010 B0012
J23116 B0034 tetR C0040 B0010 B0012 p(tetR) R0040 B0032 luxr C0062 B0010 B0012
Activation
lasI(PAI)+lasR
IPTG
ATC
Local Activation
+
-
-
Lateral & Longe range
Inhibition
LasR+PAI
R0079 B0034 luxI C0061 B0010 B0012
BBa_K26600
Diff 3OC6HSL
Autocatalisis
Activator
LAS
Diff LasI
3OC12HSL
Inhibition
Activation
Inhibitor
LUX
Diff LuxI
3OC6HSL

Complete system



AI system; las:activator, lux: inhibitor, Substrates: IPTG & ATC

Our system can generate a pattern…

…Because :

- It recognizes at least two morphogenes: *Las AHL* and *Lux AHL;*

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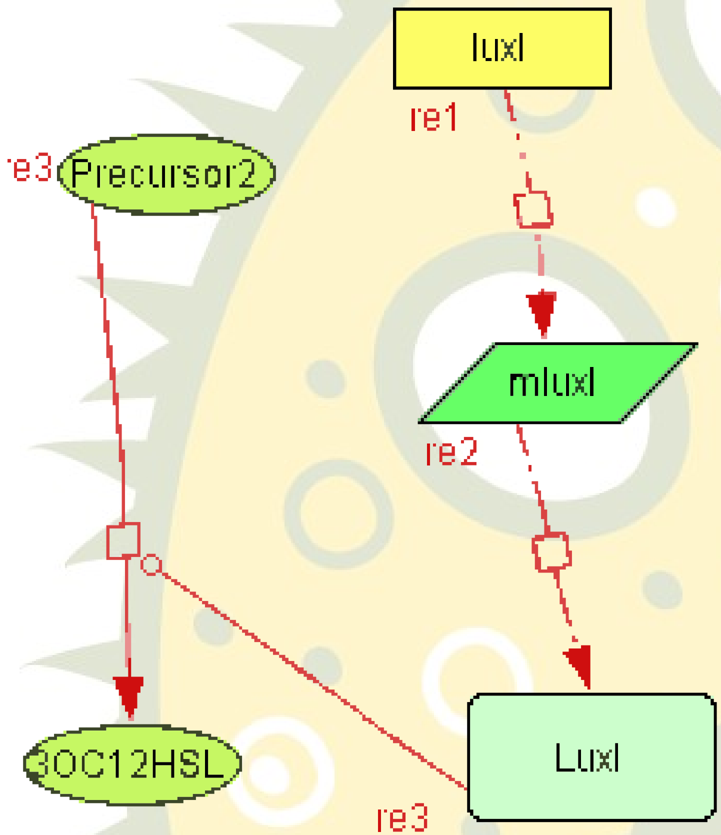
- It recognizes at least two morphogenes: *Las AHL* and *Lux AHL;*
- The chemicals diffuse with different rates;
- We can give an non-homogeneous start condition according to gradients of *IPTG* and *ATC;*

Our system can generate a pattern…

…Because:

- It recognizes at least two morphogenes: *Las AHL* and *Lux AHL;*
- The chemicals diffuse with different rates;
- We can give an non-homogeneous start condition according to gradients of *IPTG* and *ATC;*
- And the local activation and long range inhibition will happen in the media by *Lux* and *Las* Quorum sensing systems.

Single cell model: kinetic rules



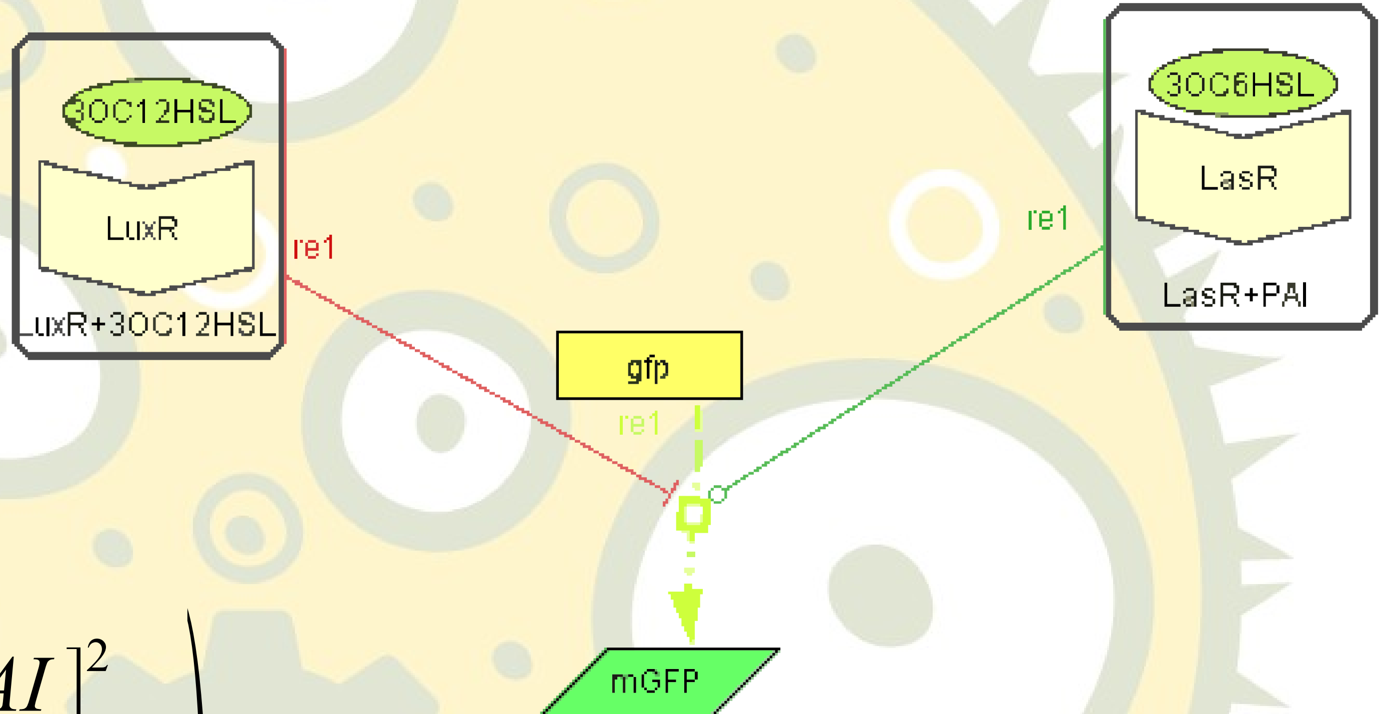
$$mluxI = k_{trans} * luxI - k_{mdeg} * mluxI$$

$$LuxI = k_{trad} * mluxI - k_{pdeg} * LuxI$$

$$AI = k_{cat} * luxI - k_{deg} * AI$$

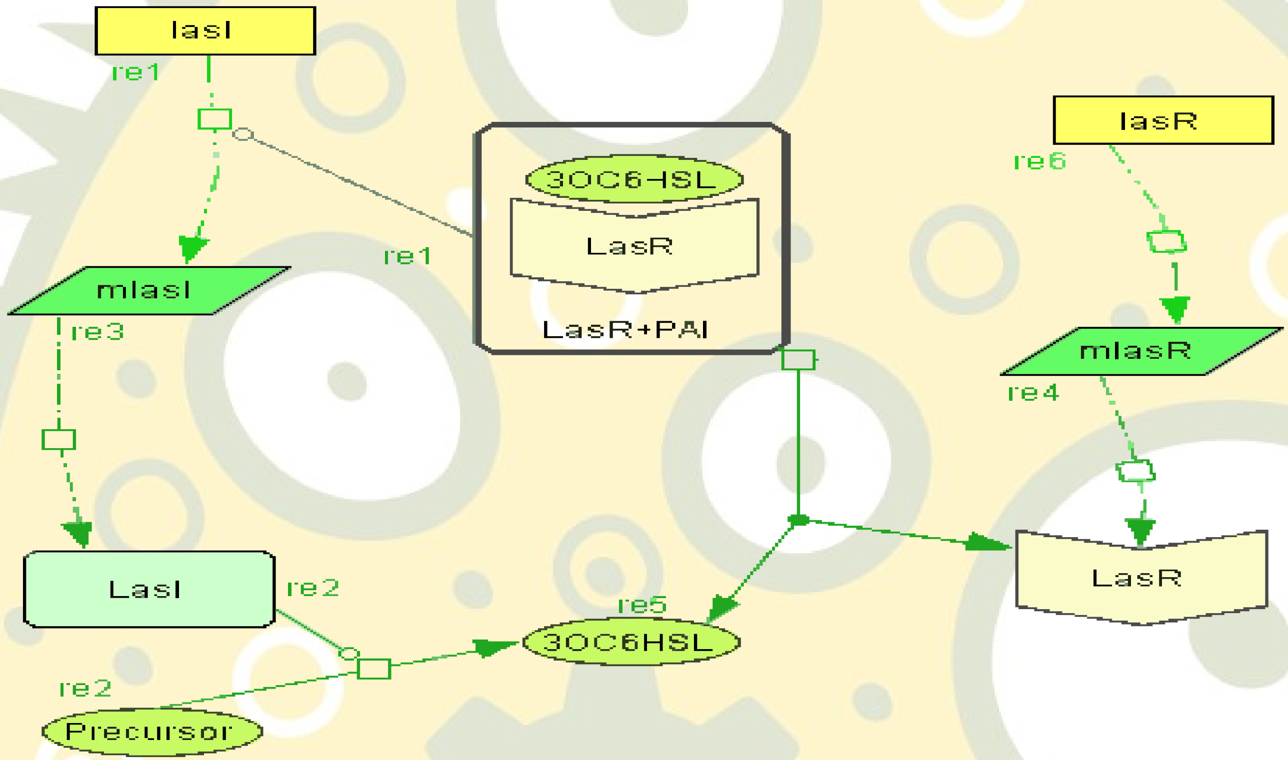
Single cell model: kinetic rules

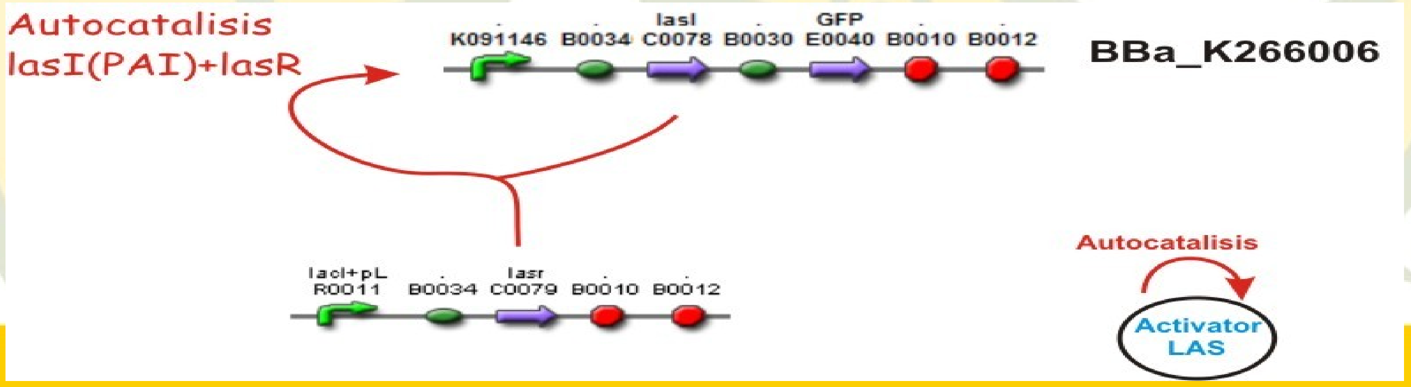
$$GFP = k_{trans} * gfp * F_1 - k_{mdeg} * mgfp$$



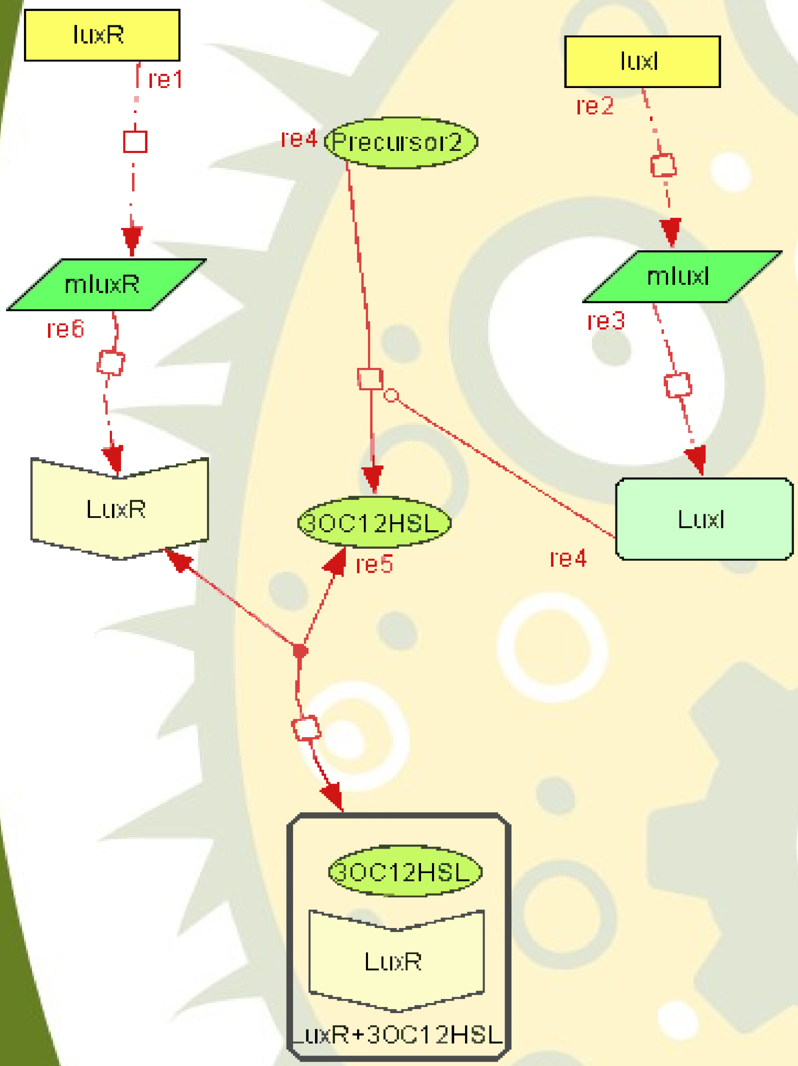
$$F_1 = \left(\frac{\frac{[LasR+PAI]^2}{k_d^2}}{1+\frac{[LasR+PAI]^2}{k_d^2}} \right) \left(\frac{1}{1+\frac{[LasR+PAI]^2}{k_d^2}} \right)$$

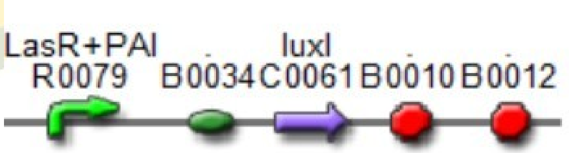
Single Cell model: Activator module

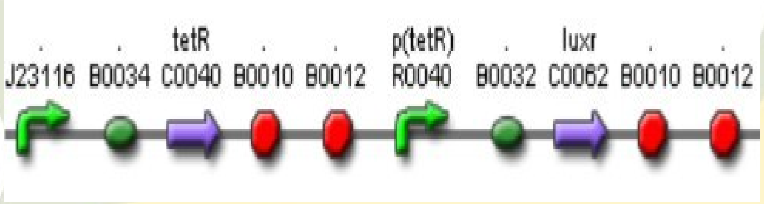




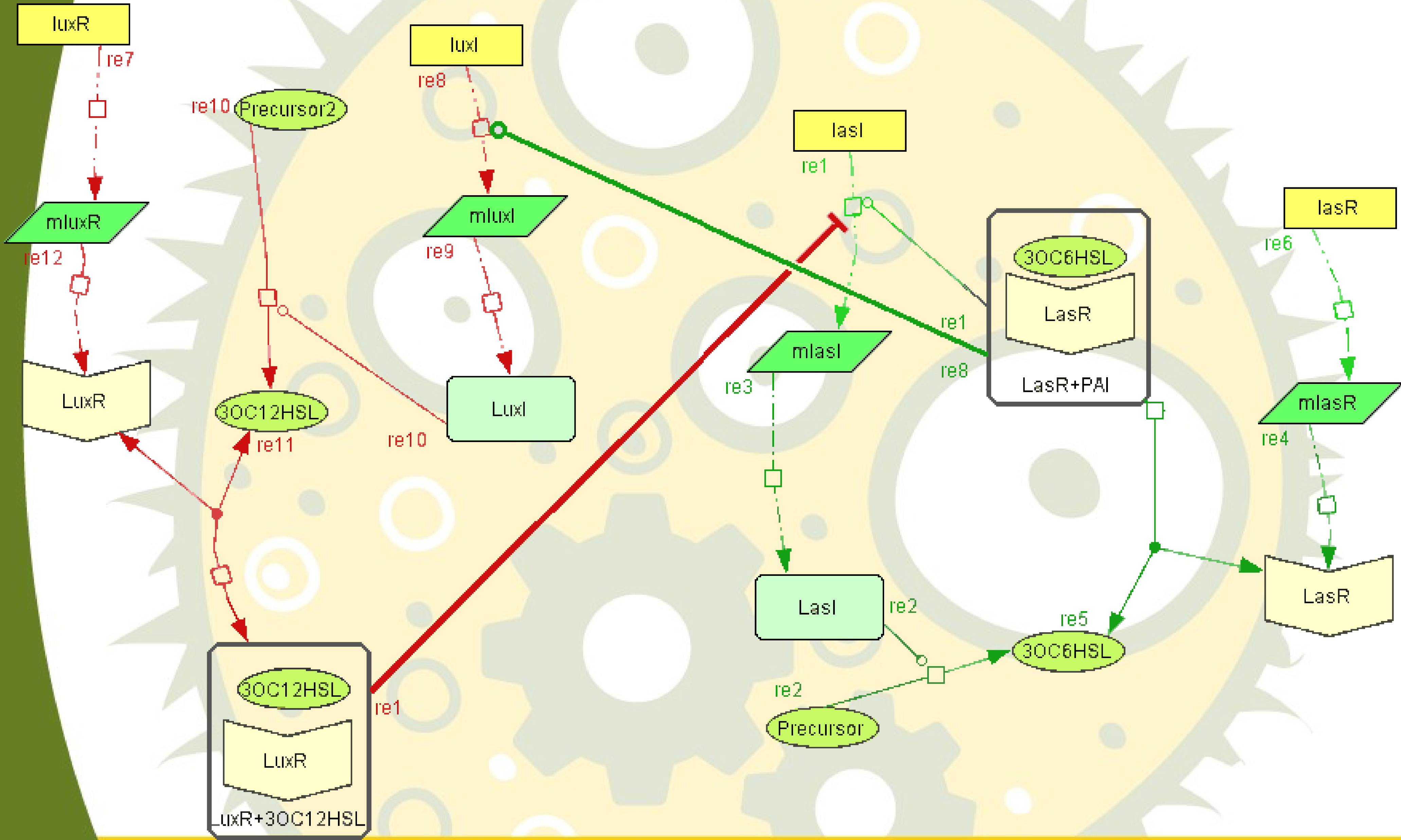
Single Cell model: Inhibitor module



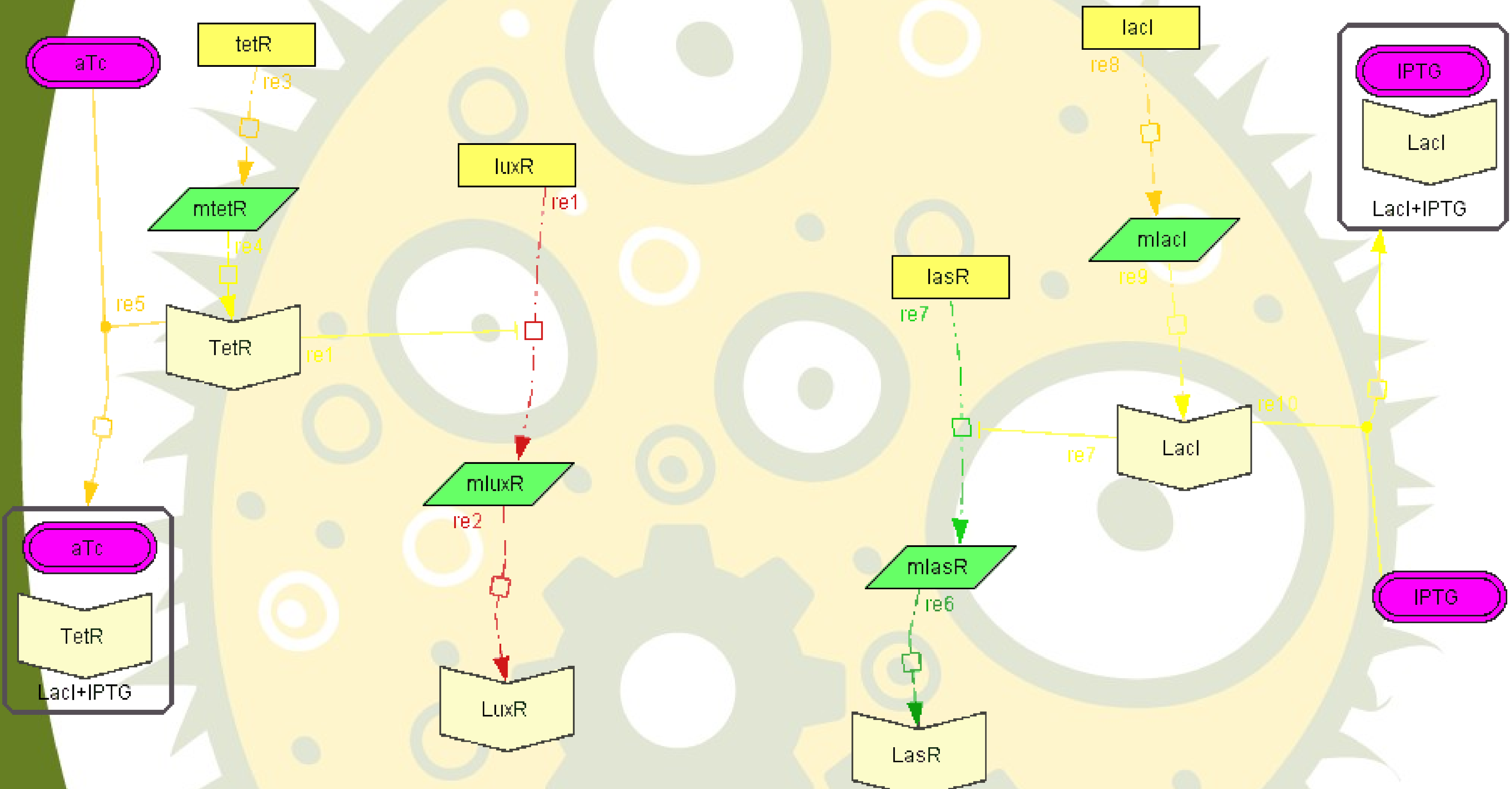


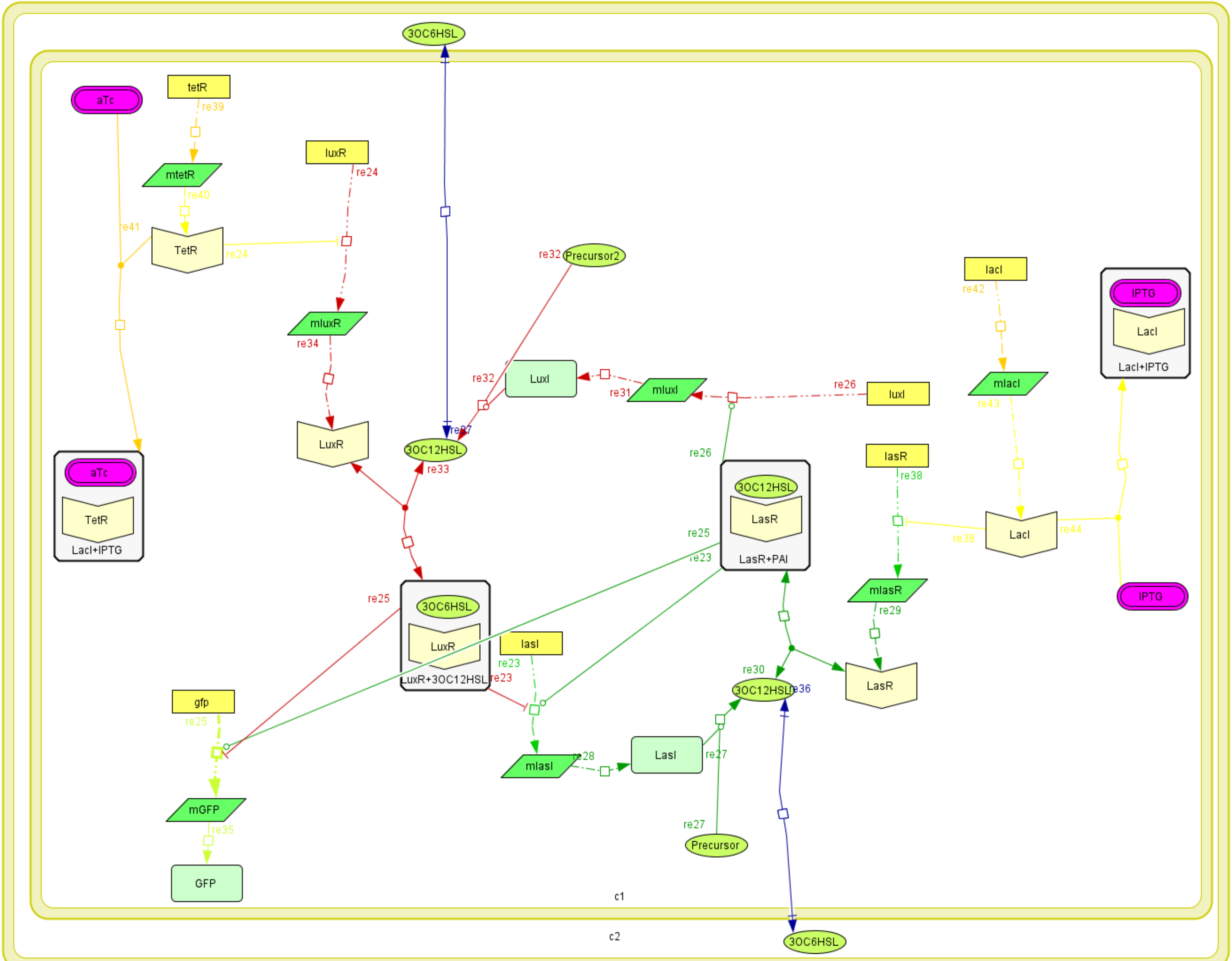


Single Cell model: Interaction

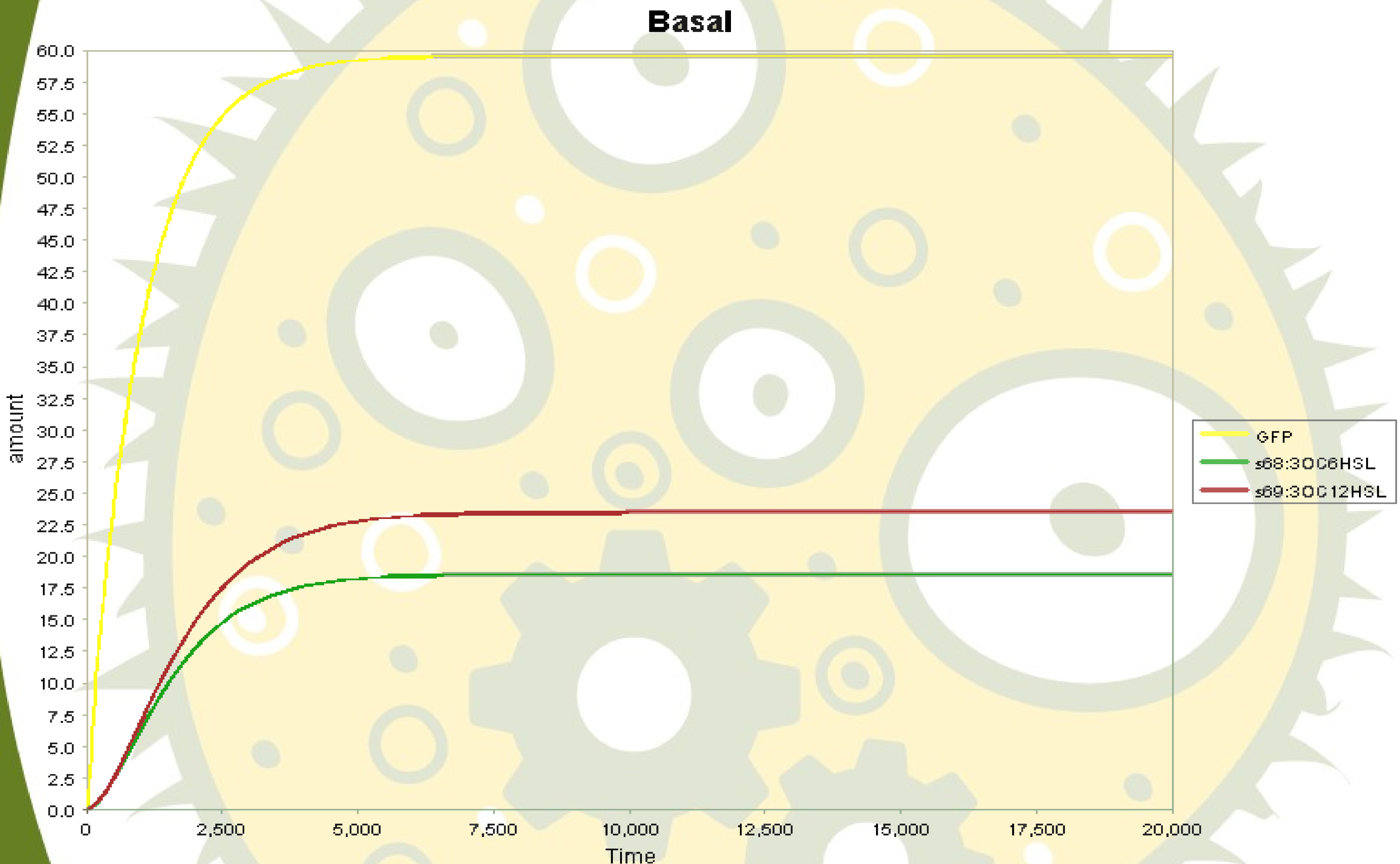


Single Cell model: Substrates modules

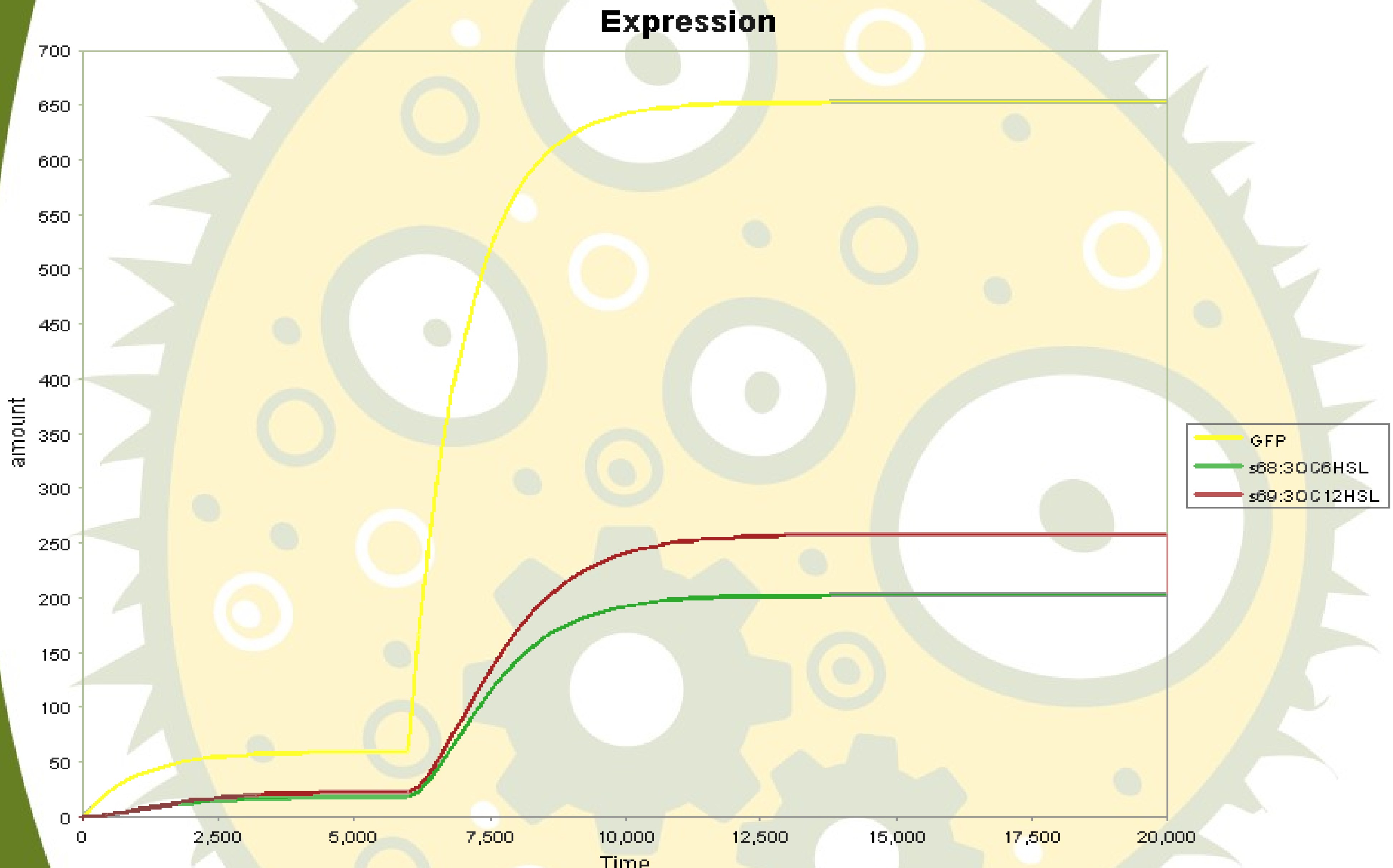



3OC6HSL
aTc
tetR
re39
mtetR
re40
re41
TetR
re24
luxR
re24
mluxR
re34
LuxR
Precursor2
re32
LuxI
re32
re31
mluxI
re26
luxI
re37
3OC12HSL
re33
aTc
TetR
LacI+IPTG
re25
3OC6HSL
LuxR
LuxR+3OC12HSL
re23
lasI
re23
re26
3OC12HSL
LasR
LasR+PAI
re25
re23
lasR
re38
re38
LacI
re44
mlasR
re29
lacI
re42
mlacI
re43
IPTG
LacI
LacI+IPTG
IPTG
re30
LasR
3OC12HSL
re36
gfp
re25
mGFP
re35
GFP
mlasI
re28
LasI
re27
re27
Precursor
c1
c2
3OC6HSL

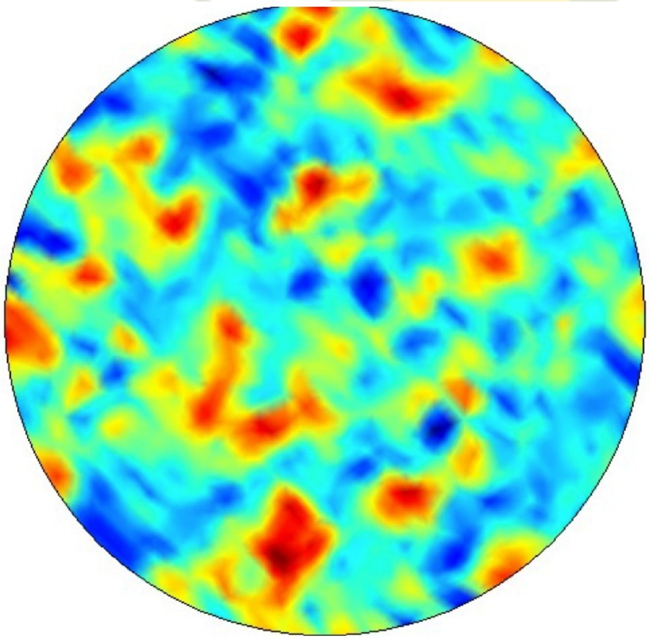
Single cell model

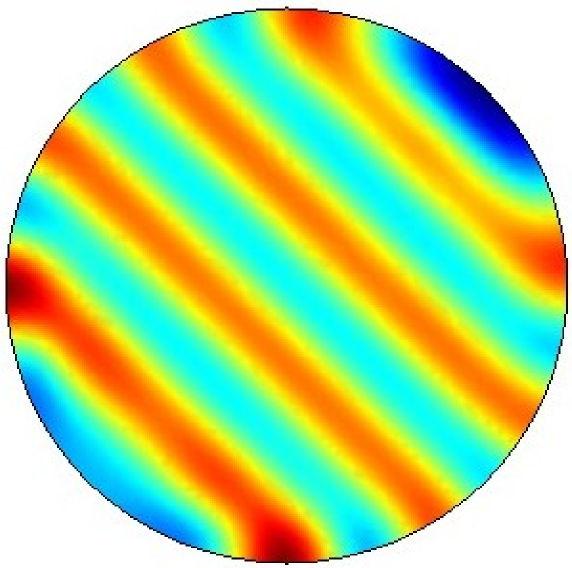


Single Cell model: Full system



Classical model with estimated diffusion constants





Based on Einstein's equations

$$D = \frac{RT}{6\pi NkP}$$

$$k^* = k(1+\phi)$$

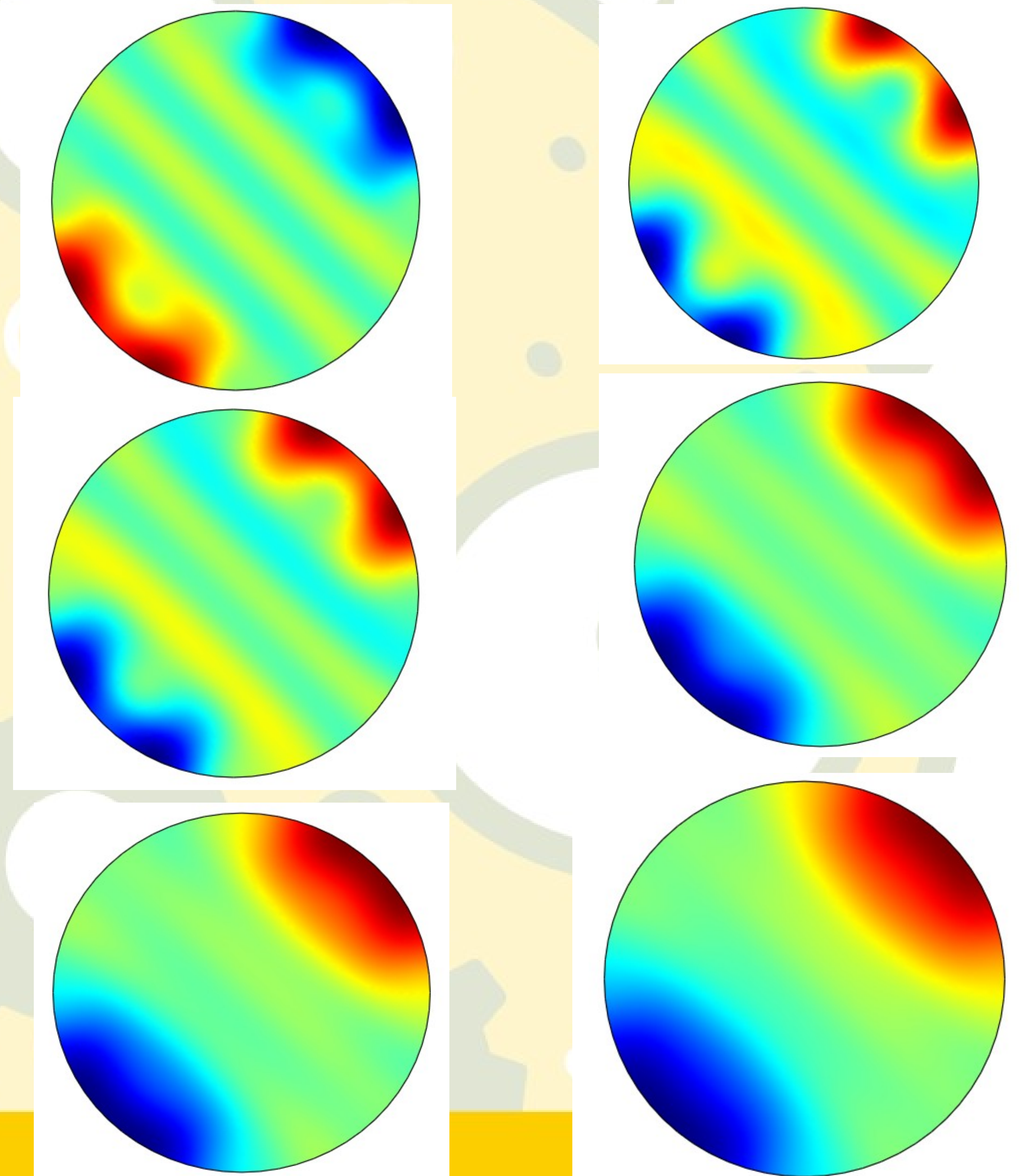
and bibliographical search we estimated the following constants:

$$D_{PAI} = 0.47 \times 10^{-5} \frac{cm^2}{s} \qquad D_{AI} = 0.608 \times 10^{-5} \frac{cm^2}{s}$$

with the Gierer and Meinhardt kinetics and an inhomogeneous initial condition we obtained this patterns.

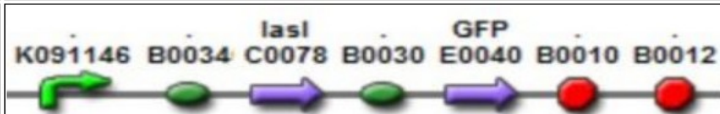
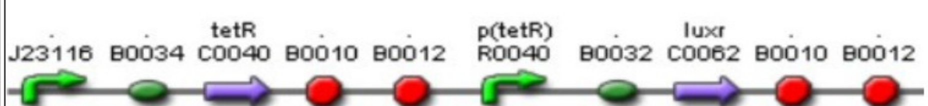
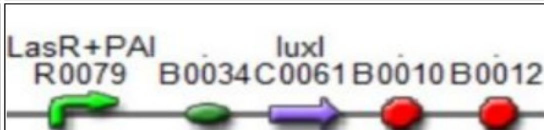
Spatial model from the single cell aproach

- When simulated con Comsol Multiphysics software with the reaction diffusion equations we obtained these results.
- The initial condition was non-homogeneous.



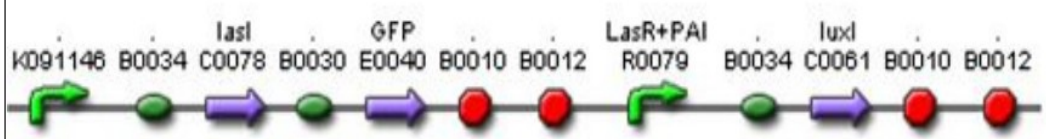
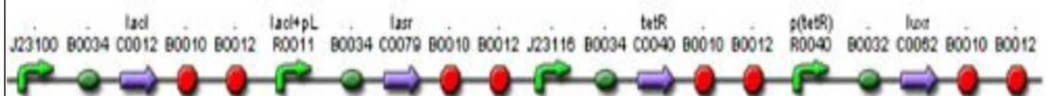
Experimental Implementation

- *E. coli* cells with the biobrick system
- Four modules:

Module and Status	Biobrick Name	Type	Image	Description
Mod 1 Sent	BBa_K266006	Las AHL		BBa_K091146 promoter (PAI+LasR inducible & AI+LuxR repressible) controls the policistronic expresion of LasI enzyme and GFP, double terminator.
Mod 2 Sent	BBa_K266004	Lac inverter		Constitutive promoter J23100 with Lac system inverter controlling the expression of LasR.
Mod 3 Sent	BBa_K266001	Tet inverter		J23100 constitutive promoter directs the expression of tetracycline repressor (TetR). TetR binds to pTet regulatory region resulting in a negative control of the production of LuxR. The whole system acts as an Inverter of Tet system controlling LuxR expression. TetR repression is inhibited by the addition of tetracycline or its analog, aTc.
Mod 4 Sent	BBa_K266000	Lux AHL		This biobrick has a promoter inducible by PAI+LasR (BBa_R0079) I.e. positive regulation and produces LuxI enzyme (BBa_F1610). This enzyme produces 3OC6HSL (AI).

Experimental Implementation

- *E. coli* cells with the biobrick system
- Two plasmids with different resistance

Status	Biobrick Name	Image	Description
Sent	BBa_K266007		Complex Quorum sensing circuit that receives the signal of PAI+LasR and AI+LuxR to control the production of LuxI and LasI enzymes.
Pending	BBa_K2660010		Tet constitutive inverter controlling LasR expression and Lac constitutive inverter controlling LuxR expression.

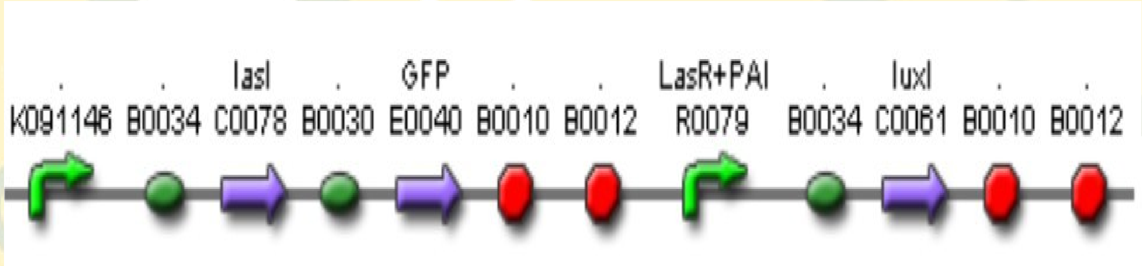
Biobricks in the registry

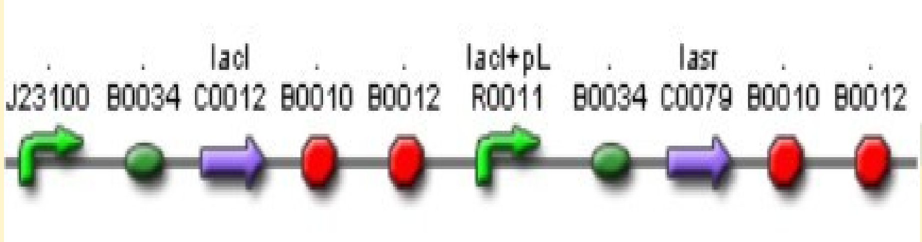
- 11 new biobricks, standard assembly 10
- Inverters, protein generators, and AHL senders

Status	Biobrick Name	Image	Description
Sent	BBa_K266002		LasR coding region and a double terminator.
Sent	BBa_K266005		BBa_K091146 promoter (PAI+LasR inducible & AI +LuxR repressible) controls the expresion of LasI enzyme, no terminator.
Sent	BBa_K266008		The BBa_J23100 constitutive promoter with Lac systems inverter.
Sent	BBa_K266009		The BBa_J23100 constitutive promoter with Lac systems inverter and strong RBS.
Sent	BBa_K2660011		POPS regulated and Lac inverter system with strong RBS.

Activator Test

- LasR inverter controlled by pLac and IPTG
- GFP and LasI controlled by PAI + LasR





Activator Test

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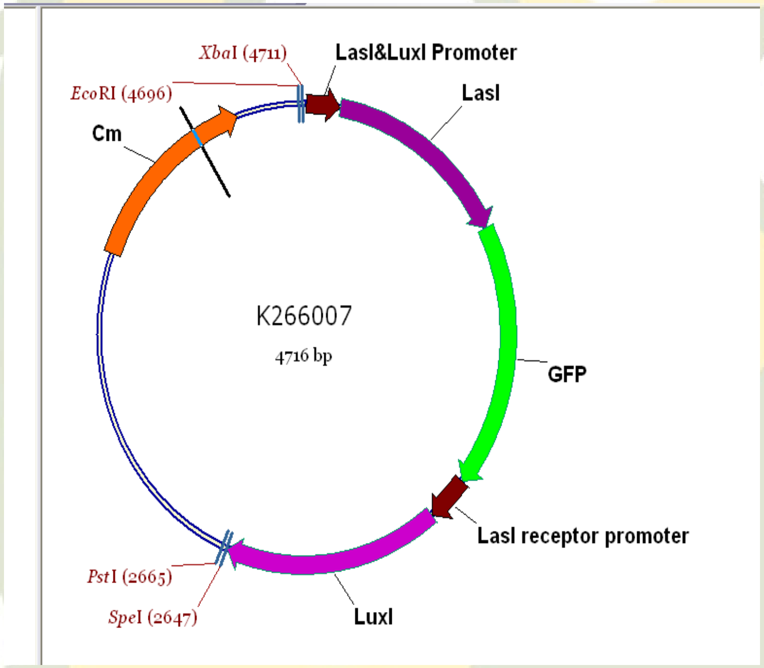
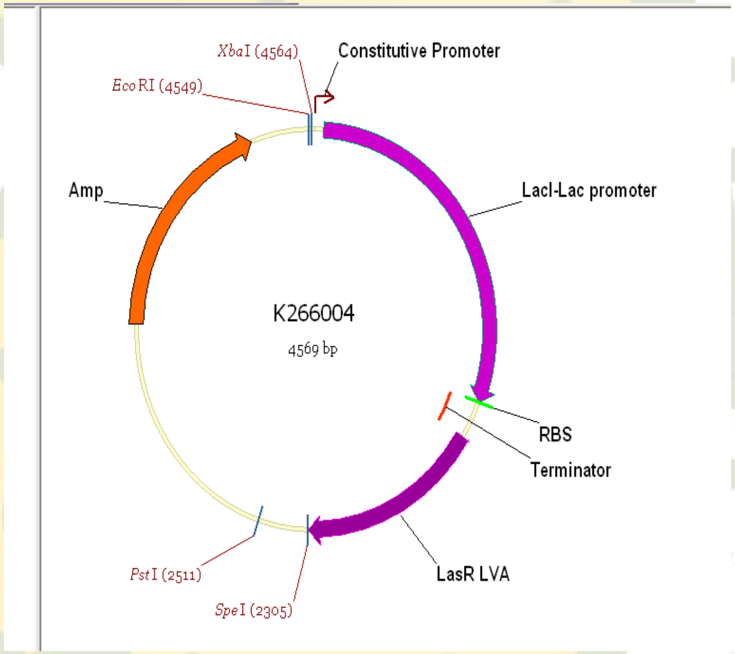
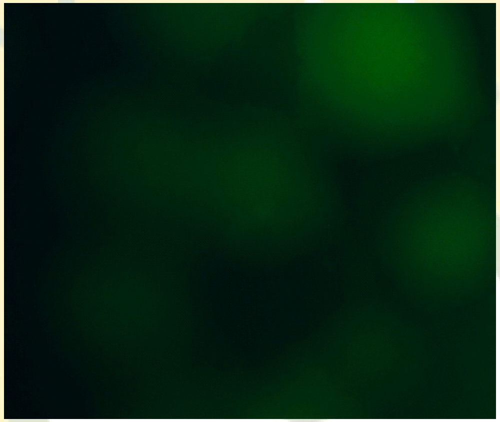


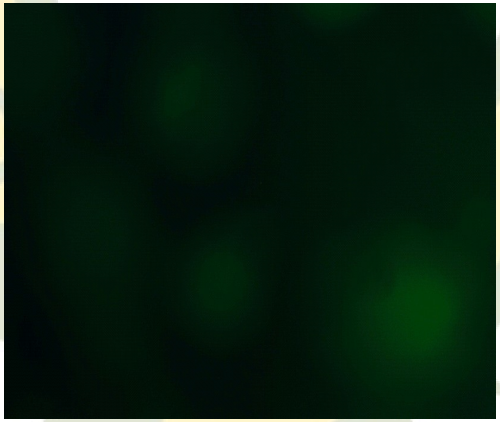


Testing the system

- Activator module with basal GFP and Activator cells with IPTG

NO IPTG





IPTG

Progress

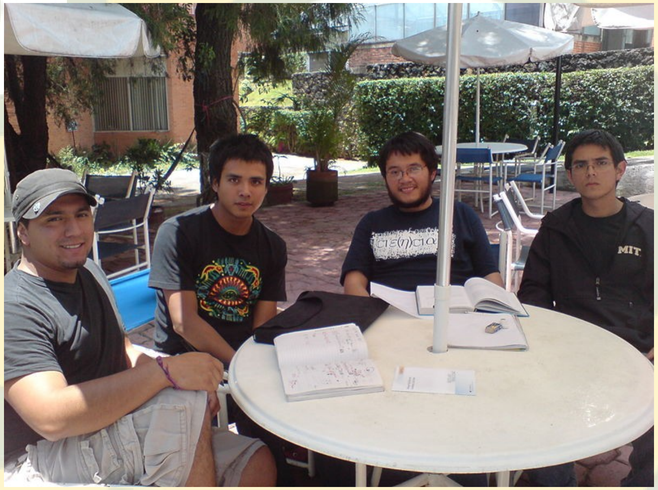
- Biobrick system 90% ready
- 1 ligation to finish
- Relation between IPTG and GFP expression
- Functional activator module

Difficulties

- 1 month delay in the Biobricks distribution
- Lack of Spe1
- Reactives delivery time

Collaboration

- LCG-UNAM-MEXICO & IPN-UNAM-MEXICO



Conclusions

- We built a synthetic biobrick network of activator-inhibitor type that gives the cells the potential to differentiate according to morphogenes and substrates gradients by expressing GFP
- Qualitative requeriments to produce a pattern
- Activator module working
- Modeling plays a crucial role

Conclusions

- We will be able to reproduce non-trivial behavior given by simple physical mechanisms

- We used synthetic biology to test the biological viability of theoretical models

Future work & Perspectives

- Coupling Inhibitor module
- Implementation of gradients of IPTG and ATC

- Effective morphogenes
- Eucariotic tissues
- Mice melanocites
- :D

New initiatives

- Collect local bacteria with interesting features that can be used in synbio applications
- Identify and isolate specialized functions
- Biosensors

THANK YOU!