

CAST



SYSU-Software

Designer



2013-11-02

SYSU-Software

New idea...



Three obstacles...

Complex
System

Undefined
Parts

Numerous
algorithms



Conducting experiments...

Revising Design...



Experiment Failed...

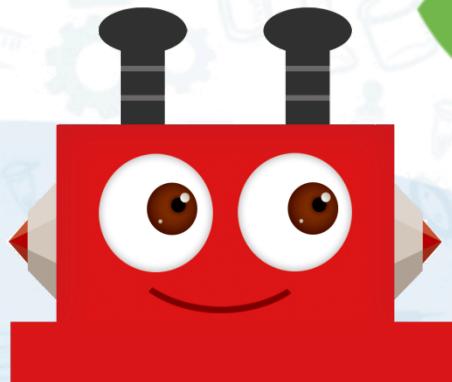


Our Vision

Apply **intelligent models and algorithms**, achieve the complex system design. Integrate various data and define standard parts, therefore direct **wet lab experiment**.



Workflow



Gene
Regulation
Network

Simulation

Wet Lab
Protocol

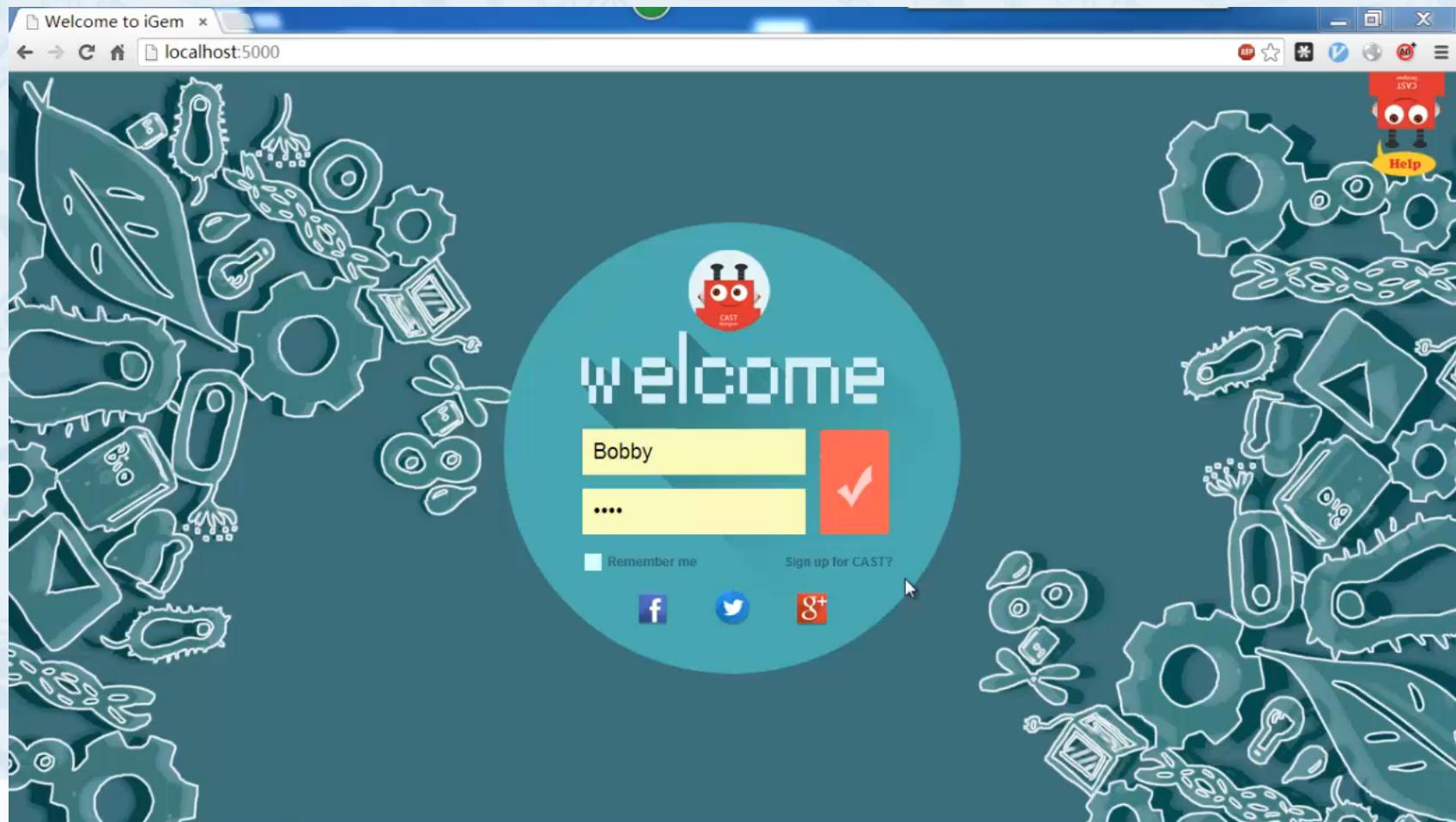
Gene
Circuit

Plasmid





CAST Designer



**Cross platform :windows, Linux, ios
Easy deposited**





Workflow



Regulation Network

Regulation x 127.0.0.1:5000/index

Regulatory Factors

Protein

Back

BBa_C0060 BBa_C0061

BBa_C0070 BBa_C0076

BBa_C0078 BBa_C0160

BBa_C0161 BBa_C0170

BBa_C0178 BBa_K091109

Shared Coding Seq

designer BY SYSU-SOFTWARE

Regulation network > Gene Circuit Plasmid Pro

Menu

Configuration

Component Config

Part_id: 3892

Part_name: BBa_C0070

Part_short_name: C0070

Part_short_desc: autoinducer synthetase for N-

Part_type: Coding

Part_status: Available

Part_results: None

Part_nickname:

Bind ➔

R

BBa_K112006

BBa_K112015

Repress

A

BBa_K112006

BBa_K112009

Activate

BBa_K112300 BBa_K108001

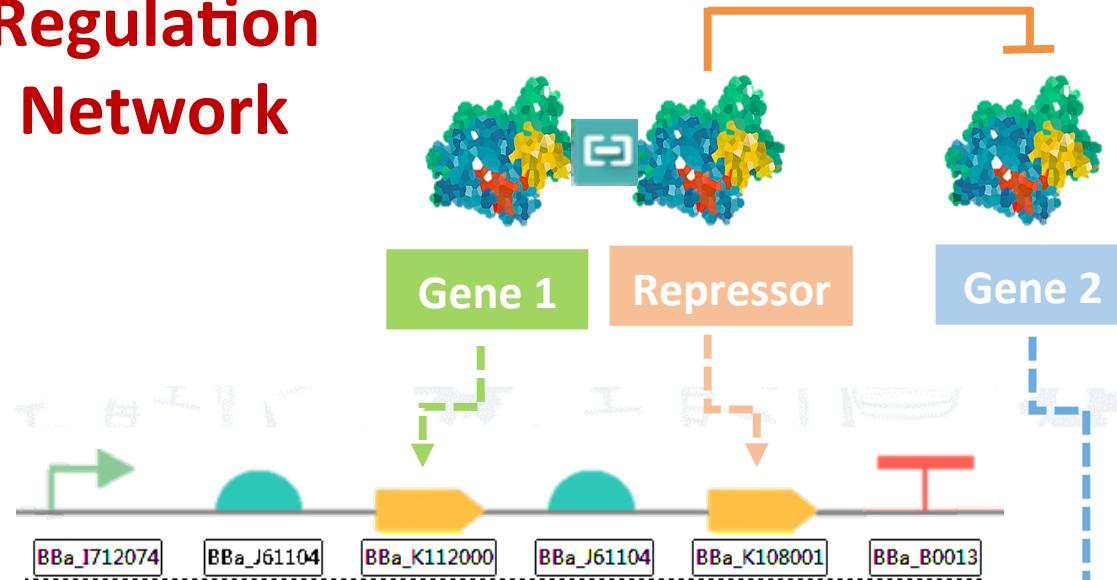
Induce

The screenshot shows the 'Regulation' tab of the BioBrick Regulation Designer software. The main area displays a regulation network diagram with four components: R, A, BBa_K112300, and BBa_K108001. Component R is labeled 'Repress' with a red arrow pointing to BBa_K112015. Component A is labeled 'Activate' with a green arrow pointing to BBa_K112009. Component BBa_K112300 is labeled 'Bind' with a red arrow pointing to BBa_K112006. Component BBa_K108001 is labeled 'Induce' with a red arrow pointing to BBa_K112300. The left sidebar lists regulatory factors and proteins, while the right sidebar shows component configuration details.



Regulation Network to Gene Circuit

Regulation
Network



Gene Circuit

Parameters & Control Panel



promoter



RBS



Plasmid
backbone



Repressor/
activator



inducer

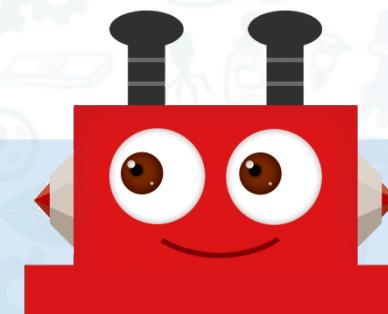
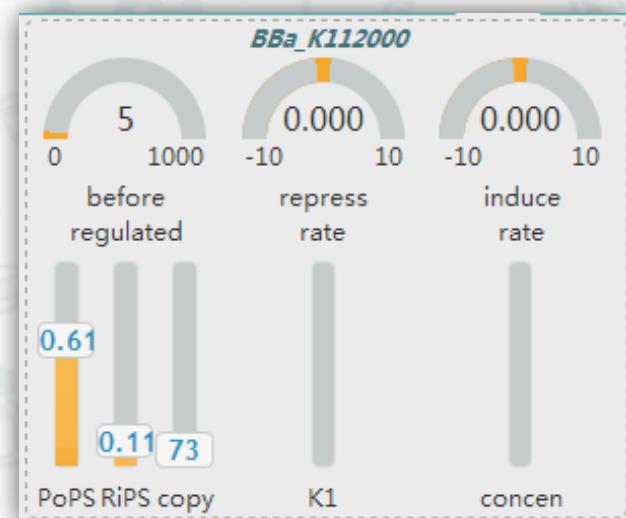
POPs

RIPs

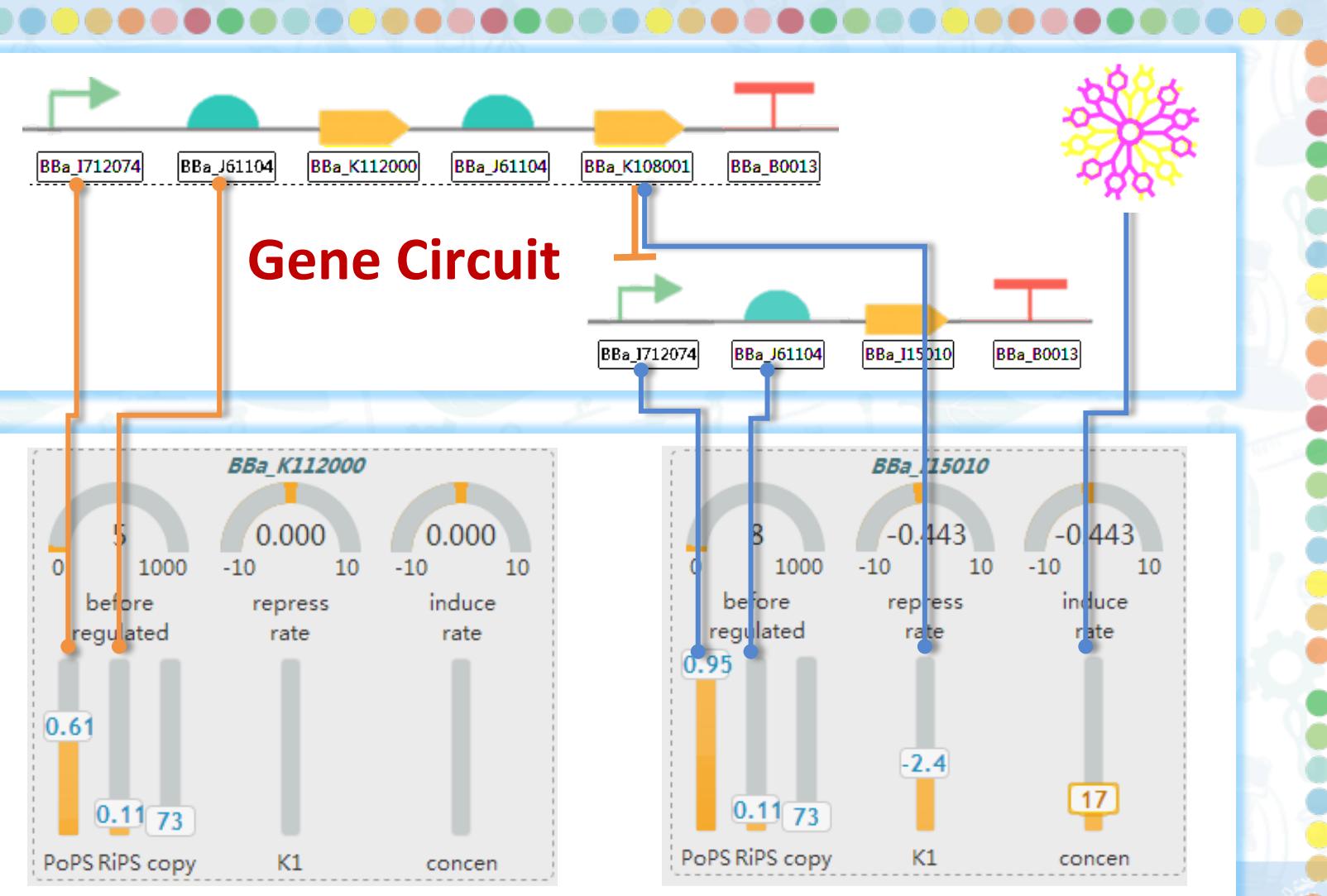
Copy
number

K_1

Concentration



Parts & Dynamic Parameters



Gene Circuit

C.A.S.T designer
BY SYSU-SOFTWARE

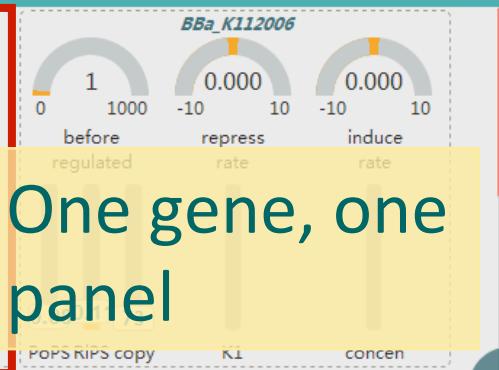
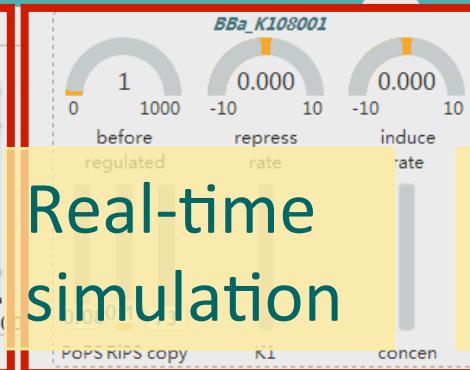
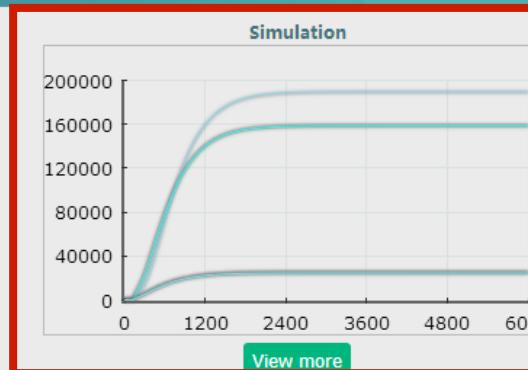
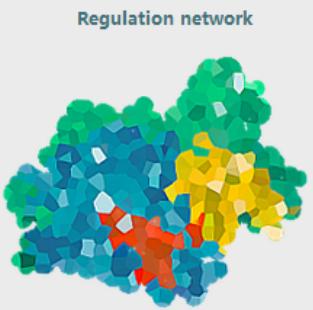
Regulation network

Gene Circuit

Plasmid

Protocol

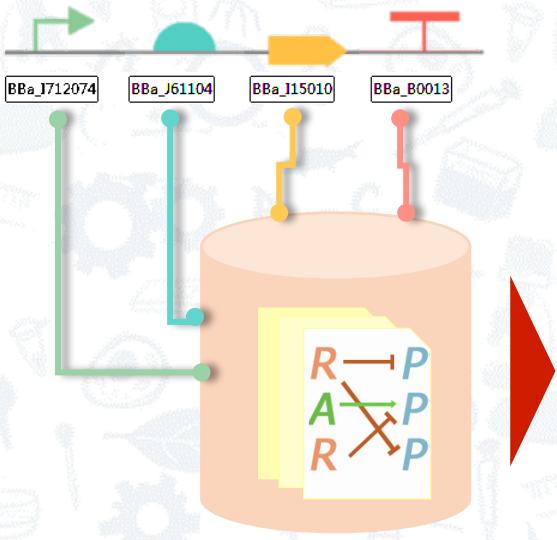
Simulation



Undo Redo



Back-end Workflow



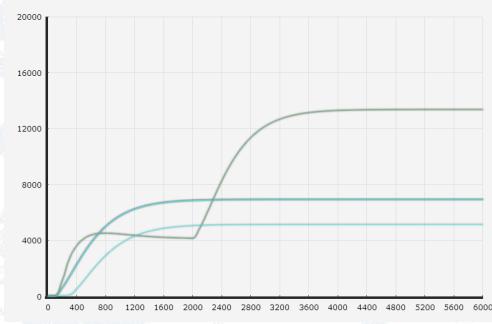
Data repository

DnaComponent [
uri: http://sbol.org/
displayId: plasmid
name: plasmid
description: undefined
annotations:[
SequenceAnnotation [-
SequenceAnnotation [-
SequenceAnnotation [-
SequenceAnnotation [-
]
]
DnaSequence [-
]
]

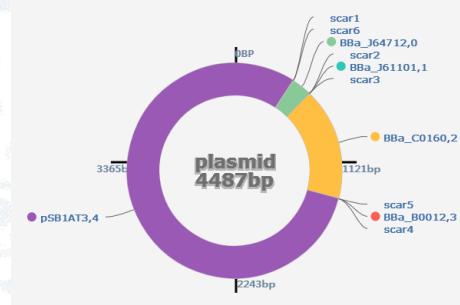
DnaComponent [
uri: http://sbol.org/
displayId: plasmid
name: plasmid
description: undefined
annotations:[
SequenceAnnotation [bioEnd: 68
bioStart: 22
strand: +
uri: http://sbols.org/
subComponent [
DnaComponent [
description: T7 P
displayId: 10177
name: BBa_I712074
type: Regulatory
uri: http://partsregistry.org/BBa_I712074
LeakageRate": "0.000000"
]
DnaSequence [
uri: http://sbols.org/
nucleotides: GAATTCCGGGCGCTTC
TACTAGAGaaagaaggacatACTAGAGat
ttcgctgttaatagtagatcattaacaccagg
acctatttttagatagacataggatggccaga
]

Standard SBOL format

$$\frac{d[mRNA_1]}{dt} = \text{CopyNumber}_1 (\text{MPPromoter}_1 - \text{LeakageRate}_1) + \text{LeakageRate}_1 - \text{DegRatemRNA}_1 [mRNA_1]$$
$$\frac{d[Protein_1]}{dt} = \text{MPRBS}_1 [mRNA_1] - \text{DegRatePro}_1 [\text{Protein}_1]$$
$$\frac{d[mRNA_2]}{dt} = \text{CopyNumber}_2 \frac{\text{MPPromoter}_2 - \text{LeakageRate}_2}{1 + \left(\frac{[\text{Protein}_1]}{K_1} \right)^{\text{HillCoef}}}} + \text{LeakageRate}_2 - \text{DegRatemRNA}_2 [mRNA_2]$$
$$\frac{d[Protein_2]}{dt} = \text{MPRBS}_2 [mRNA_2] - \text{DegRatePro}_2 [\text{Protein}_2]$$



Model and algorithm



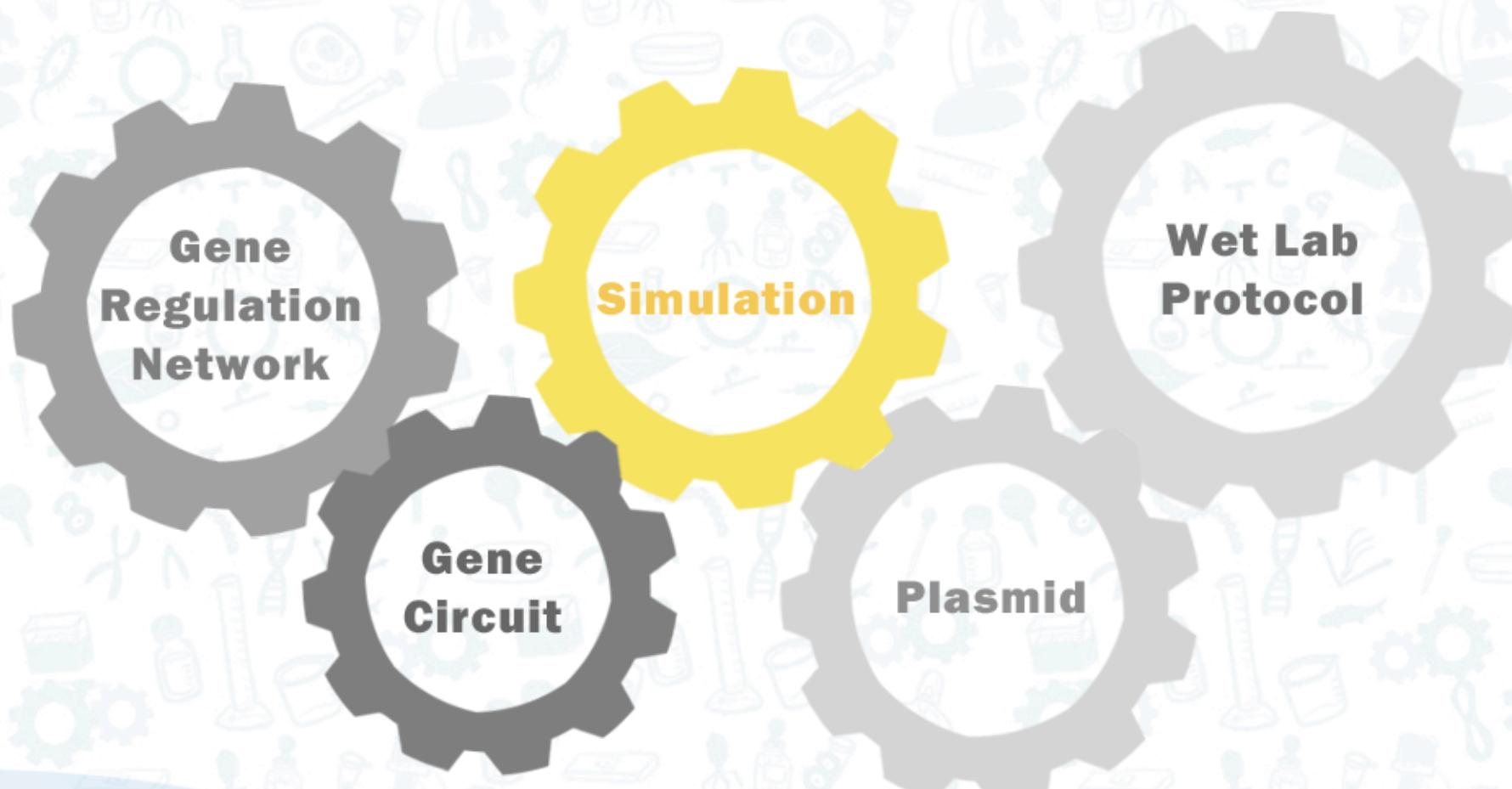
Plasmid Sequence



Protocol



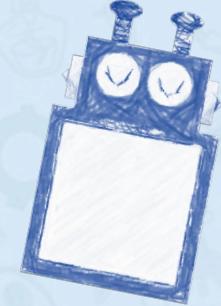
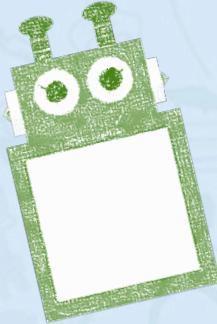
Workflow



Simulation

1. Deterministic, stochastic and time delay simulations
2. Focus on the interested curve
3. Add inducer at any time
4. Based on powerful models and algorithms





Challenges in Modeling

Challenge I : Complex of synthetic circuits

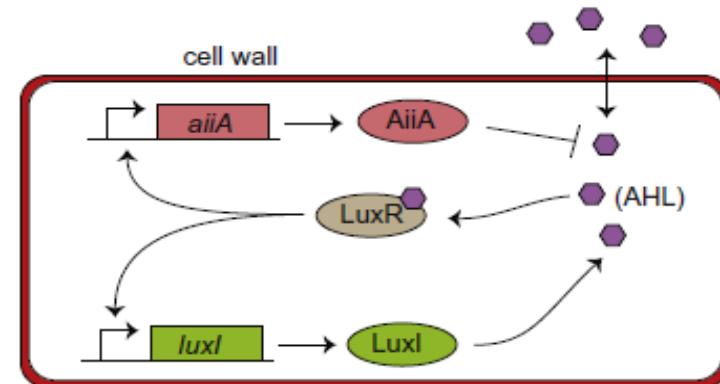
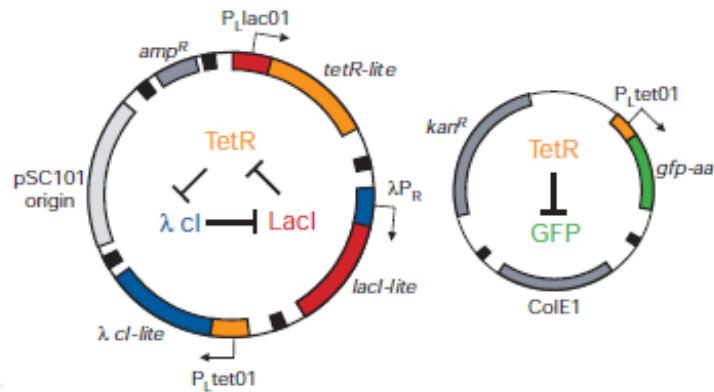
Challenge II : Evaluation of regulator strength

Challenge III : The fluctuations of the circuits

Challenge IV: Inconsistent parameters for simulation

Challenge I

Complex of Synthetic Circuits



Solutions: improved ordinary differential equations (ODEs)

1. Combine regulatory and metabolic ODEs as well as Hill Equations
2. Multi-level modeling

Features:

1. Experimental-oriented (copy number & leakage rate diversity)
2. Classification



Regulation and Metabolic Networks

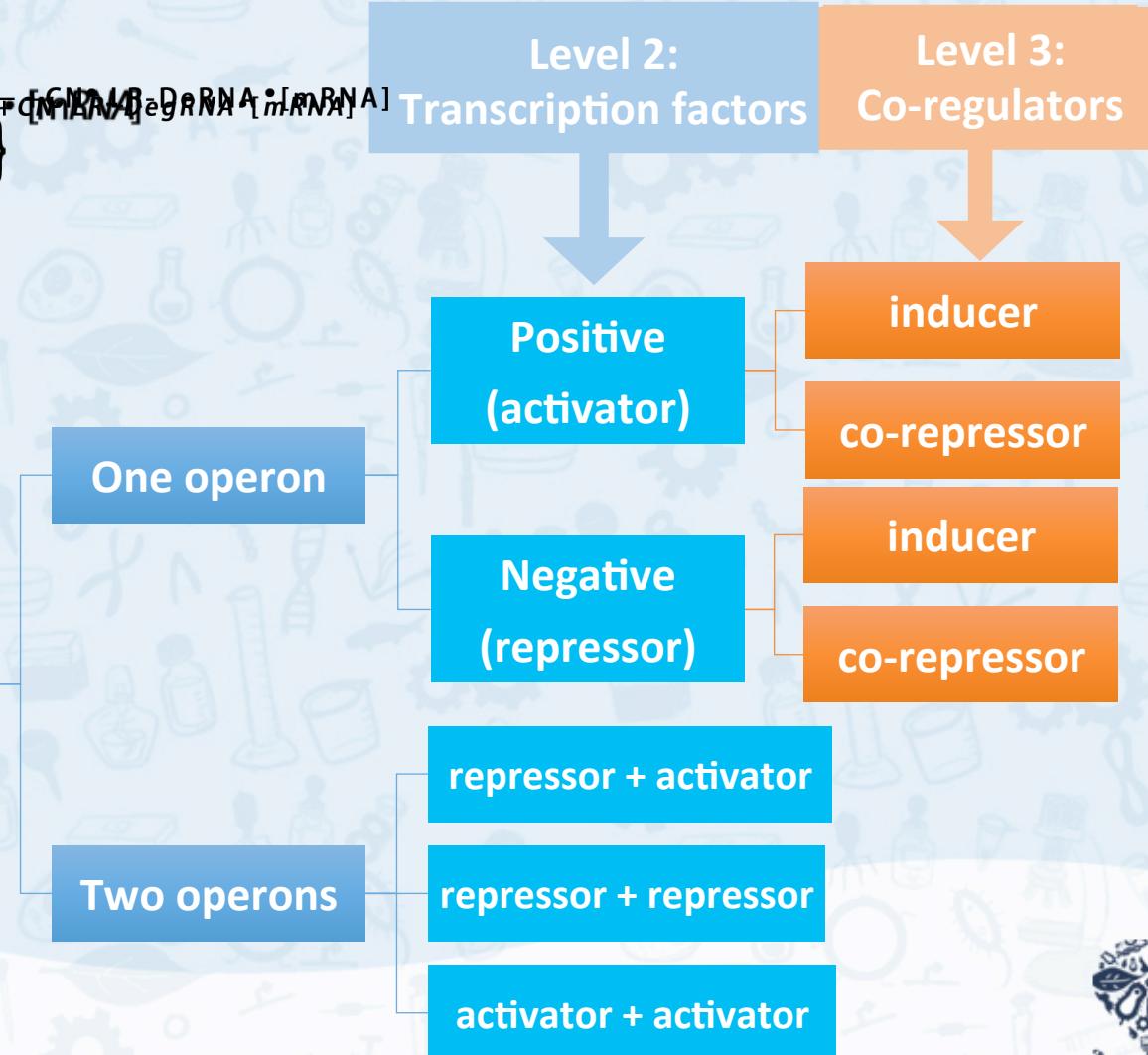
Promoters

$$\frac{d[\text{mRNA}]}{dt} = \frac{\text{CN} \cdot (\text{TS} + \text{RR})}{1 + \left\{ \left(\frac{[\text{R}] [\text{R}]}{K_1 K_1 + ([\text{I}])^{n_2}} \right) \right\}} [\text{mRNA}]$$

promoters

Level 1:
No regulations

constitutive
inducible





Regulation and Metabolic Networks

CDSes, RBSes and terminators

$$d[\text{Protein}]/dt = \text{TE} \times \text{TerE} \times [\text{mRNA}] - \text{DegPro}[\text{Protein}]$$

Metabolic networks: enzymatic reactions

$$d[P]/dt = V_{\text{max}} [S] / K_m + [S]$$

$$d[\text{protein}]/dt = V_{\text{max}} [S] / (K_m + [S])$$

$$d[\text{protein}]/dt = V_{\text{max}} [S] / K_m (1 + [I] / K_I) + [S]$$

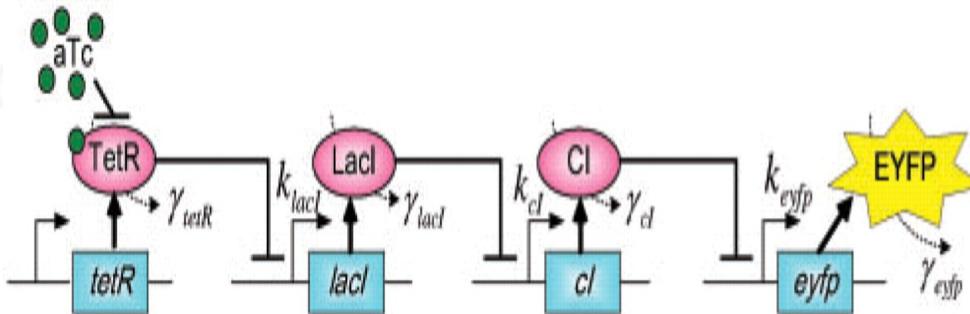


Challenge II

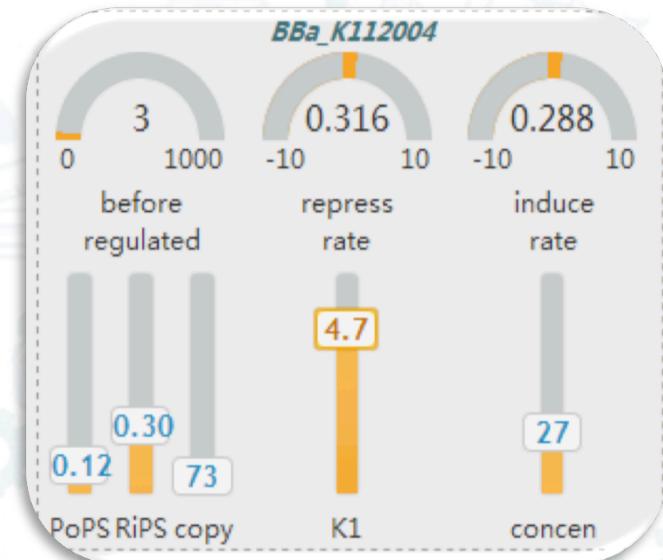
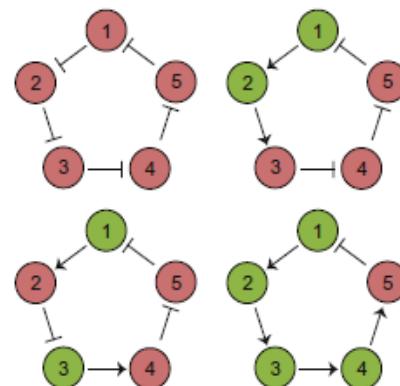
Evaluation of regulator strength

New Iteration and Optimization Algorithms

Cascades



Cyclic circuits

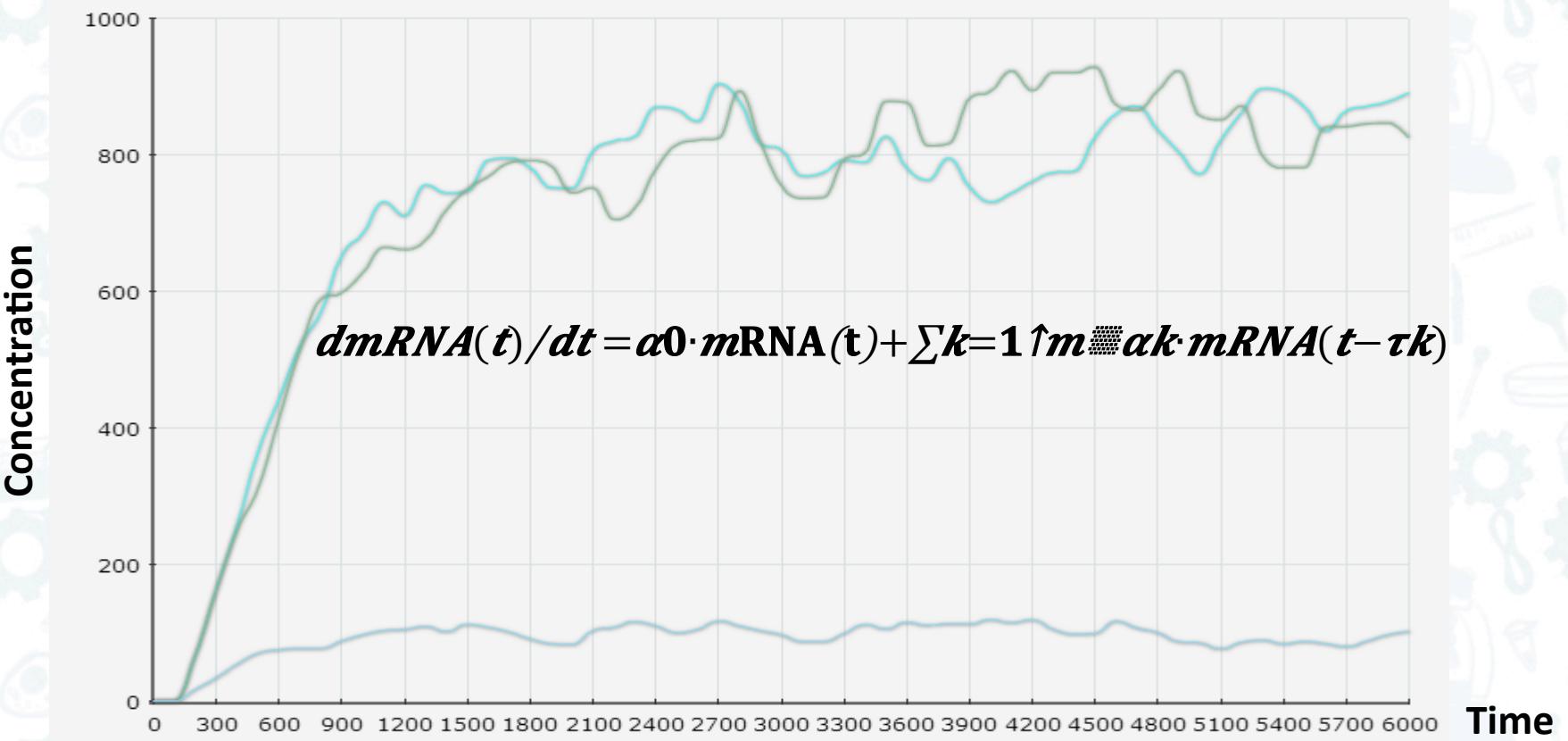


Interface

Challenge III

The fluctuations of the circuits

Solutions: stochastic and time delay simulations



Challenge IV

Inconsistent Parameters for Simulation

Solutions: develop the data standardization process

Output: Repository with standardized PoPS and RIPS

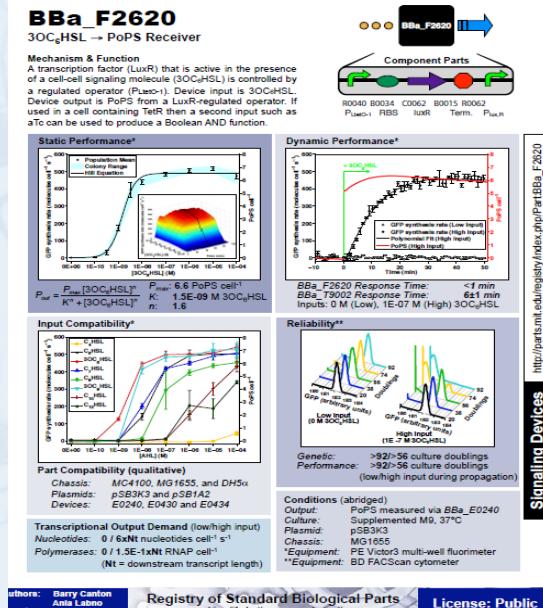
Registry
Papers
Experimental results



Data Source

More than
100 sources!!

Data processing



Registry of Standard Biological Parts

Anderson promoter collection

Identifier	Sequence ^a	Measured Strength ^b
BBa_J2319	tttacacgttagtcctaggtaatgtctagc	n/a
BBa_J2310	tttacacgttagtcctaggtaatgtctagc	1
BBa_J2311	tttacacgttagtcctaggtaatgtctagc	0.70
BBa_J2312	tttacacgttagtcctaggtaatgtctagc	0.86
BBa_J2313	ctggtagtcctaggtaatgtctagc	0.01
BBa_J2314	tttacacgttagtcctaggtaatgtctagc	0.72
BBa_J2315	tttacacgttagtcctaggtaatgtctagc	0.24
BBa_J2316	tttacacgttagtcctaggtaatgtctagc	0.47
BBa_J2317	tttacacgttagtcctaggtaatgtctagc	0.36
BBa_J2318	tttacacgttagtcctaggtaatgtctagc	0.51
BBa_J2319	tttacacgttagtcctaggtaatgtctagc	0.04
BBa_J2310	tttacacgttagtcctaggtaatgtctagc	0.33
BBa_J2311	tttacacgttagtcctaggtaatgtctagc	0.58
BBa_J2312	ctggtagtcctaggtaatgtctagc	0.00
BBa_J2313	tttacacgttagtcctaggtaatgtctagc	0.01
BBa_J2314	tttacacgttagtcctaggtaatgtctagc	0.10
BBa_J2315	tttacacgttagtcctaggtaatgtctagc	0.16
BBa_J2316	tttacacgttagtcctaggtaatgtctagc	0.16
BBa_J2317	tttacacgttagtcctaggtaatgtctagc	0.06
BBa_J2318	tttacacgttagtcctaggtaatgtctagc	0.56

Table 1 Nominal values and meaning of the kinetic parameters for the model of the synthetic sensor construct.

Basal transcription rate	$k_1 = 0.02 \text{ sec}^{-1}$
Active-promoter transcription rate	$k_2 = 0.4 \text{ sec}^{-1}$
mRNA degradation rate	$k_3 = 0.3 \text{ sec}^{-1}$
Protein translation rate	$k_4 = 3 \text{ (nMsec)}^{-1}$
Dimerization rate	$k_5 = 0.1 \text{ (nMsec)}^{-1}$
Dimer dissociation rate	$k_6 = 0.001 \text{ sec}^{-1}$
Inhibitor binding rate	$k_7 = 0.011 \text{ (nMsec)}^{-1}$
Inhibitor unbinding rate	$k_8 = 0.2 \text{ sec}^{-1}$
Dimer-promoter binding rate	$k_9 = 0.21 \text{ (nMsec)}^{-1}$
Dimer-promoter unbinding rate	$k_{10} = 0.2 \text{ sec}^{-1}$
Protein degradation rate	$k_{11} = 0.2 \text{ sec}^{-1}$

Values are based on [19] and slightly adapted to obtain a desired threshold behavior.

Manipulation

Statistical evaluation

Data trade-off

Unit conversion and integration

Calculation

$$RIPS = \frac{TE}{DeRNA}$$

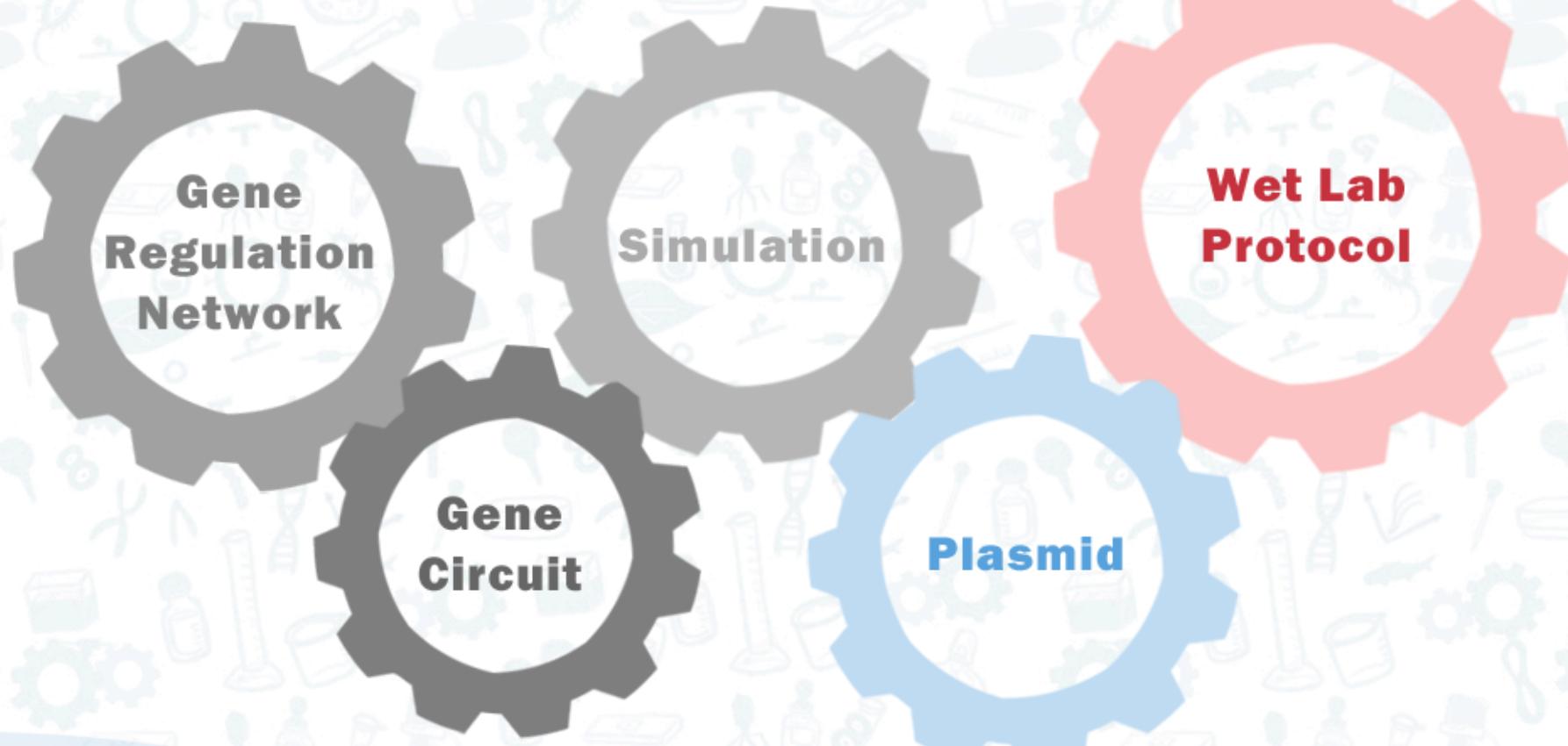
$$PoPS1 = \frac{(TS - LR)}{\frac{[R]}{1 + \left\{ \frac{[I]}{K2} \right\}^{n2}}} + LR$$

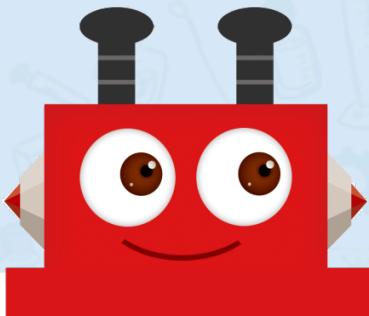
$$K1 \left[1 + \left(\frac{[I]}{K2} \right)^{n2} \right]^{n1}$$





Workflow





Satisfied Simulation Result

C.A.S.T designer
BY SYSU-SOFTWARE

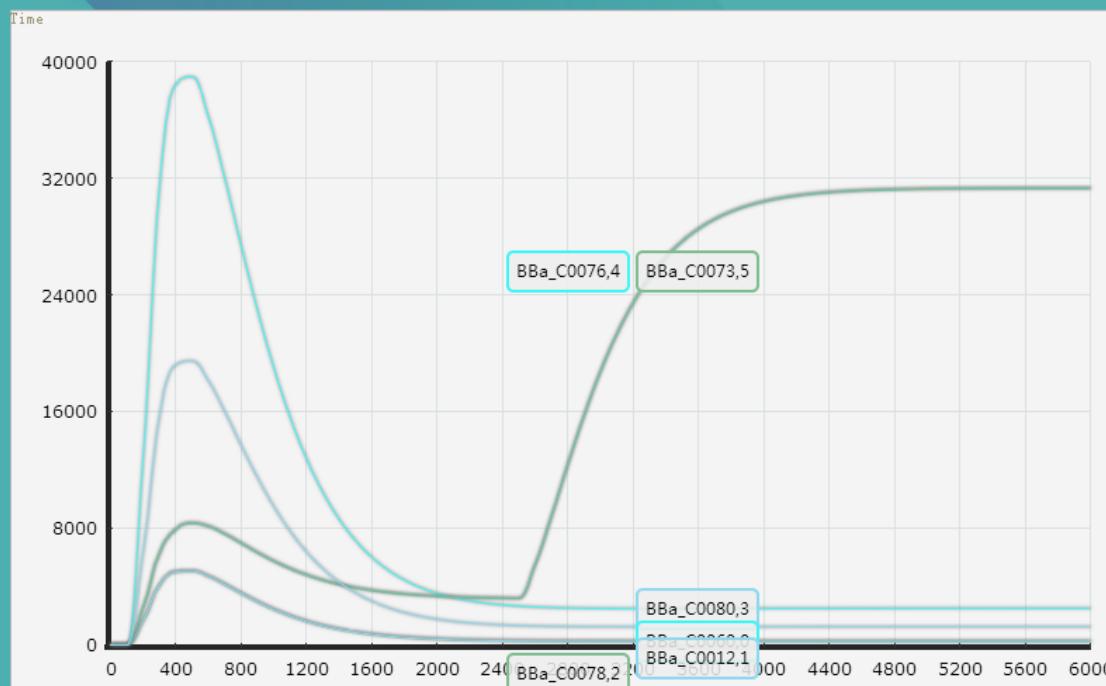
Regulation network

Gene Circuit

Simulation

Plasmid

Protocol



Curve

<input checked="" type="checkbox"/> BBa_C0076,4	<input checked="" type="checkbox"/> BBa_C0078,2	<input checked="" type="checkbox"/> BBa_C0080,3
<input checked="" type="checkbox"/> BBa_C0060,0	<input checked="" type="checkbox"/> BBa_C0073,5	<input checked="" type="checkbox"/> BBa_C0012,1

Option

stochastic

time delay

Inducer

Ind_Atc

back

Save Graph

Plasmid

Regulation x 

127.0.0.1:5000/genecircuit

C.A.S.T designer BY SYSU-SOFTWARE

Regulation network Gene Circuit Plasmid Protocol Simulation

Regulation network

Simulation

200000
160000
120000
80000
40000
0

0 1200 2400 3600 4800 6000

View more

BBa_K108001 BBa_K112006

before regulated before regulated
repress rate repress rate
induce rate induce rate

PoPS RIPS copy PoPS RIPS copy

K1 K1

concen concen

0.06 0.11 73 0.06 0.11 73

Regulation network

plasmid-0

BBa_J712074 BBa_J61104 BBa_K112000 BBa_B0013 cis

plasmid-1

BBa_K137032 BBa_J61104 BBa_K112002 BBa_J61104 BBa_K142003 BBa_B0013 cis

New plasmid

Undo Redo



Protocol



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Regulation network

Gene Circuit

Plasmid

Protocol

Simulation

Overview

1. Obtain plasmid DNA



2. Enzyme Digestion



3. Gel Electrophoresis



4. Gel Purification

5. Ligation

6. Transformation

7. Colony PCR

8. Conclusive Test

9. Glycerol Stock



Protocol

C.A.S.T designer

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Regulation network

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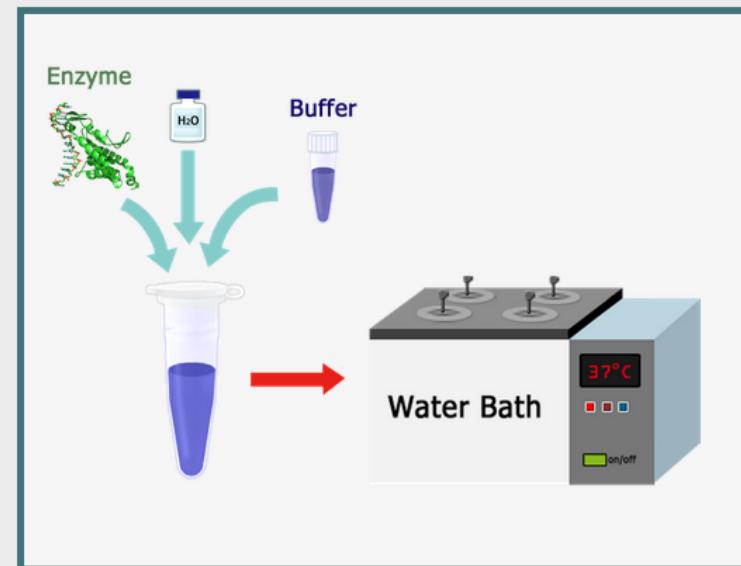
7. Colony PCR

8. Conclusive Test

9. Glycerol Stock

Digest the plasmid DNA for the two parts with the appropriate enzymes for the desired ligation.

2.1 If you are digesting with EcoRI and PstI to verify the size of an insert, use 12 μ L of miniprep DNA, or at least 400 ng. If digesting to make a vector or an insert, the amount of DNA to digest depends how much DNA you need for your ligation.



2.2 Mix the DNA with 2 μ L of the appropriate buffer (see Table 1) in a 500- μ L microfuge tube. Add 1 μ L of each enzyme (total enzyme volume cannot exceed 10% of reaction volume). Increase the volume to 20 μ L with dH₂O. Salt conditions for double digestion. Useful enzyme combinations for digesting BioBrick parts with their optimal buffer and what the digestion produces. All five reactions are optimal at 37°C



Protocol

C.A.S.T designer

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Regulation network

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Place the gel under UV light at an intensity just high enough to visualize the bands. Cut out the bands containing the insert and the vector with a razor and purify the DNA.

4.1 Place the gel under UV light at an intensity just high enough to visualize the bands. Cut out the bands containing the insert and the vector to purify the DNA.

4.2 Place the gel slice in a 1.5-mL microfuge tube and weigh it. Add two volumes of Buffer NT to one volume of gel (100 mg = 200 µL). For gels >2% agarose, double the volume of Buffer NT.

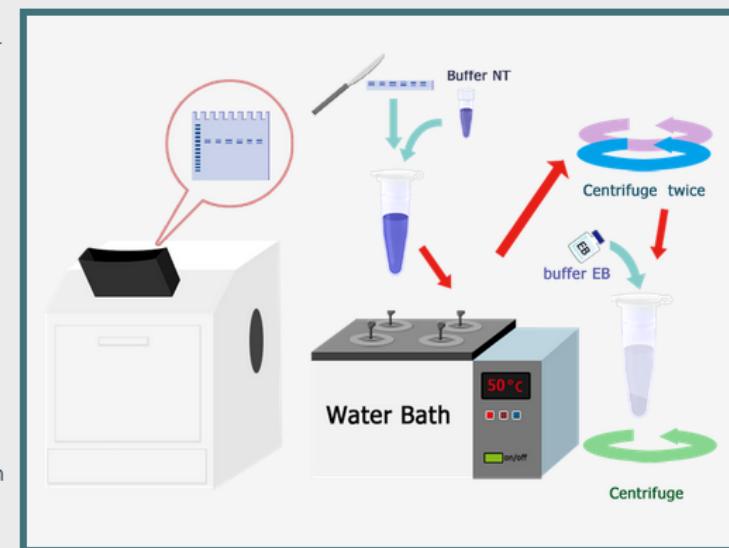
4.3 Incubate the gel at 50°C for 5–10 min until the gel slice is completely dissolved. Vortex the tube every 2–3 min to speed up the dissolving process.

4.4 Place a spin column in one of the provided 2-mL collection tubes.

4.5 Place a NucleoSpin® column into a collection tube. Pipette the DNA solution onto the column. Centrifuge the DNA solution at 13,000 g for 1 min. The maximum volume the column can hold is 800 µL, so repeat this step using the same column if the volume is larger than that.

4.6 Discard flow-through from the previous step and place the column back in the collection tube.

4.7 Wash the DNA in the column by applying 600 µL of buffer NT3. Centrifuge the column for 1 min at 13,000 g.



127.0.0.1:5000/protocol#0

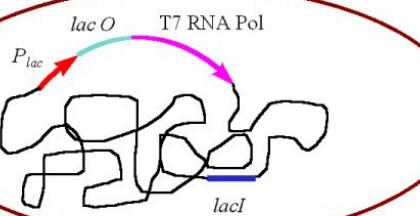


Wet-lab Validation

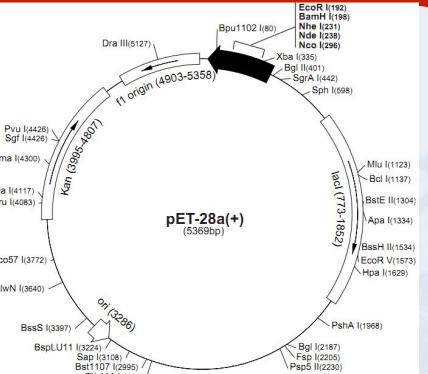
Design

Protocol

Experiment



BL(DE3) Host Chromosome



C.A.S.T designer

Protocol

1. Obtain plasmid DNA

2. Enzyme Digestion

3. Gel Electrophoresis

4. Gel Purification

5. Ligation

6. Transformation

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9. Glycerol Stock

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Ligation

1.1 Place 50 ng of digested vector, 5 μ l of ligation buffer, and 1 μ l of T4 ligase into a 500- μ l microfuge tube. The amount of insert to add is calculated from the following formula:

$$\text{ng of insert} = \frac{\text{size of plasmid} \times \text{size of insert}}{200}$$

Add water to bring the total volume to 50 μ l.

2.2 Keep both a positive ligation mixture that contains the digested vector and insert as well as a negative ligation mixture that contains only the digested vector. Add more water to the negative ligation mixture to make it roughly equal to the positive ligation mixture.

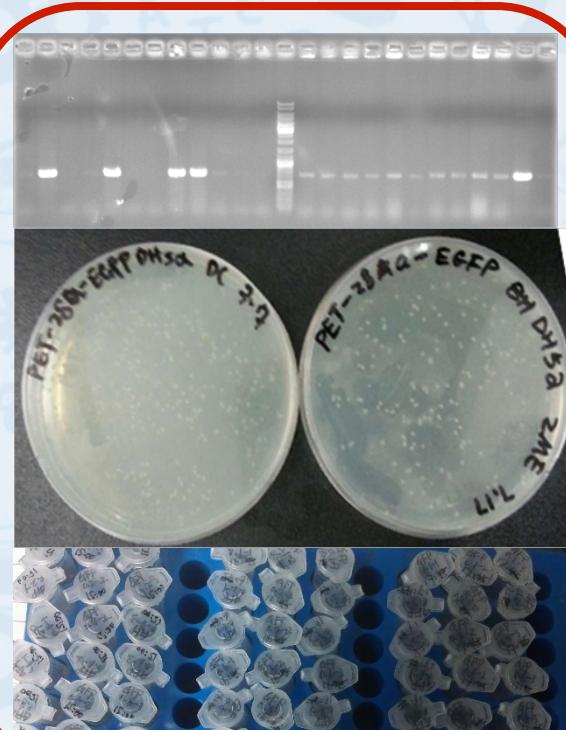
2.3 Leave the ligation mixture at room temperature for 5 min, and then use it directly for transformation of *E. coli* competent cells, or store it by freezing until transformation.

Transformation

When obtaining plasmid DNA for digestion and ligation, a good rule of thumb is to generate as much as possible. Also, it is better to purify more than one sample of each type of plasmid DNA every time the miniprep procedure is performed. This will reduce the chance of contamination during the cloning process, so minimize the number of times you perform minipreps. Another benefit of having a large volume of DNA is that you have the option of concentrating the DNA if needed.

2.1 Mix the DNA with 2 μ l of the appropriate buffer (see Table 3) in a 500- μ l microfuge tube. Add 1 μ l of each enzyme (total enzyme volume cannot exceed 10% of reaction volume), increase the volume to 20 μ l with deionized water, and incubate for 1 h at 16°C.

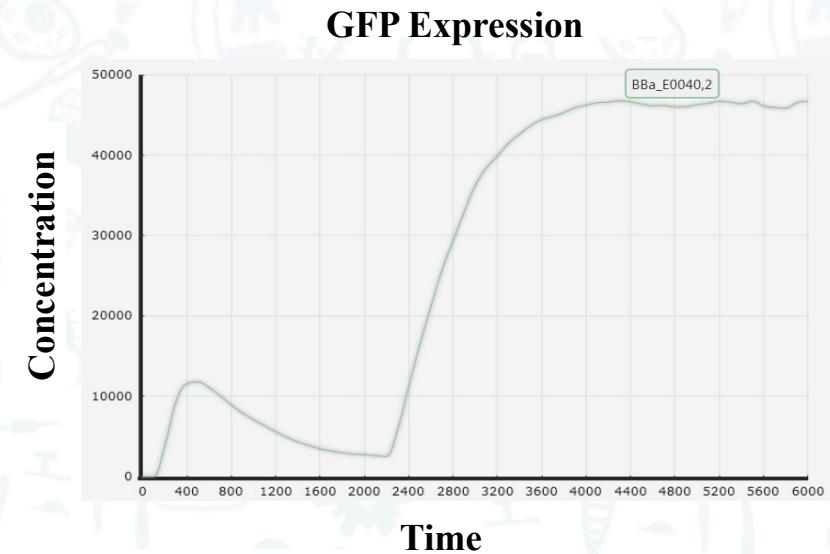
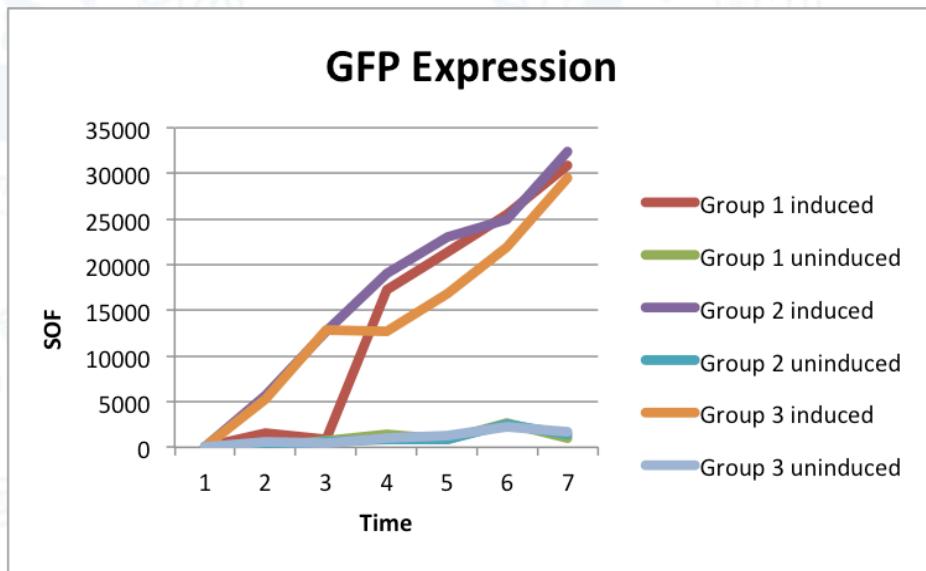
2.2 Mix the DNA with 2 μ l of the appropriate buffer (see Table 3) in a 500- μ l microfuge tube. Add 1 μ l of each enzyme (total enzyme volume cannot exceed 10% of reaction volume), increase the volume to 20 μ l with deionized water, and incubate for 1 h at 16°C.



We successfully validated our model with wet-lab experiment



Experiment VS. Simulation



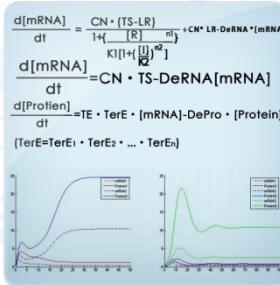
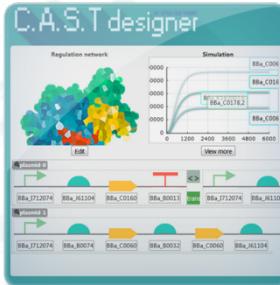
SOF\Time	0h	1h	2h	3h	4h	5h	6h
Group 1 induced	0	1540	814	17203	21411	25558	30901.7
Group 1 uninduced	0	48	771	1453	880	2618	1018.7
Group 2 induced	0	5737	12652	19068	23012	24989.2	32363.2
Group 2 uninduced	0	27	599	916	875	2581.2	1387.5
Group 3 induced	0	5236	12900	12714	16805	21862.2	29452.7
Group 3 uninduced	0	654	513	1034	1230	2240.2	1633.5

We successfully validated our model with wet-lab experiment



Conclusion & Human Practice

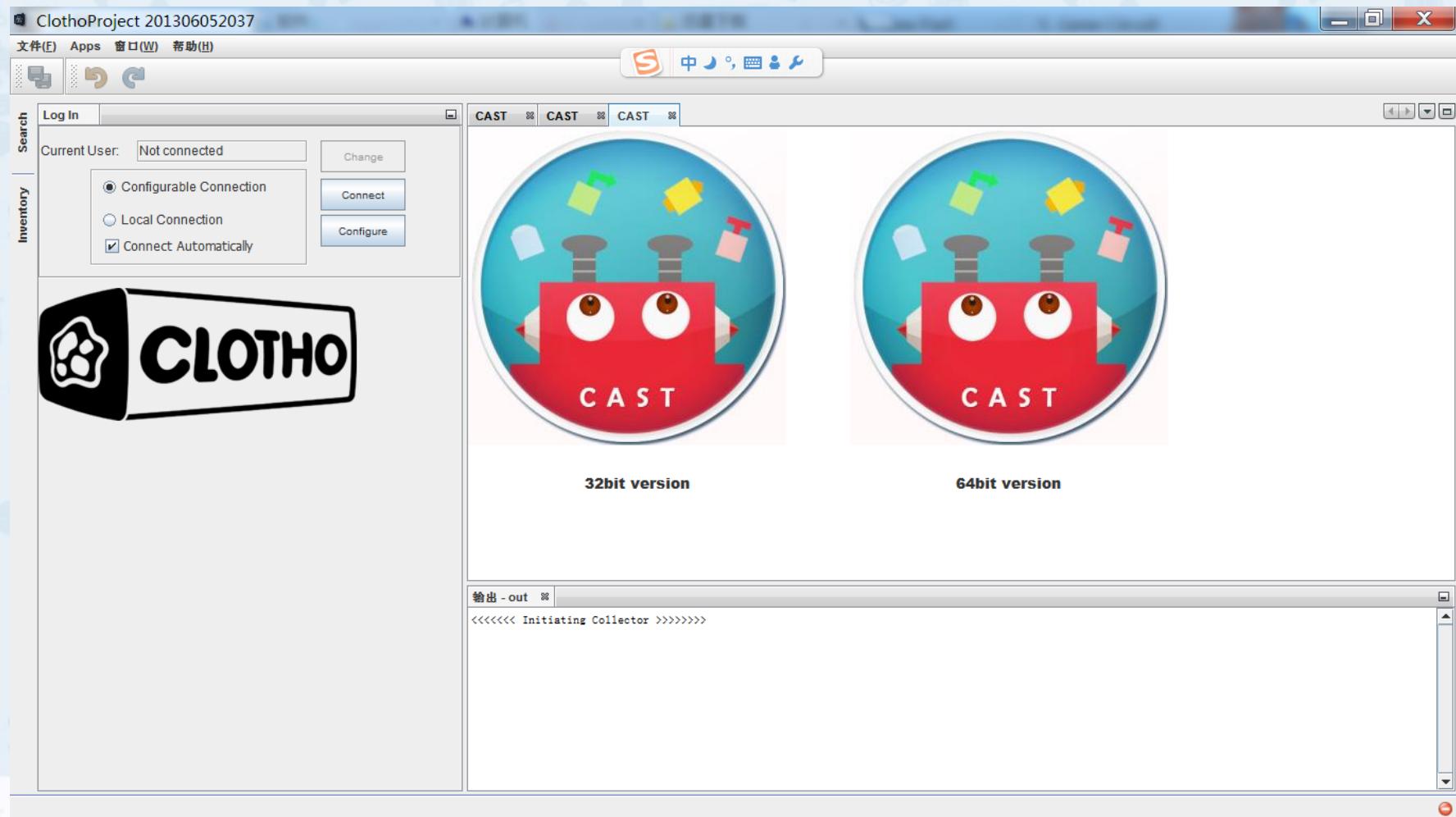
Distinctive Features



- Automatic Design
 - Construct new software system to convert primary idea into complex gene circuit.
- Innovative models and algorithms
 - New Iteration Algorithms
 - Improved simulation models for multi-level regulation and metabolic network.
- Wet lab experiments
 - Successfully validate our model with a self practice wet lab experiment.



Clotho App



Integrated into Clotho platform.



New Standard

```
1 ▼ DnaComponent [
2   uri: http://sbol.org/
3   displayId: test
4   name: BBa_I0011
5   description: made by CAST designer
6   MPPromoter: 0.44
7   LeakageRate: 0.2
8   PoPS: 0.018
9   Source: Paper
10  K1: None
11  Type: Negative
12
13 ▼ annotations: [
14   SequenceAnnot
15   bioEnd: 90
16   bioStart: 2
17   strand: +
18   uri: http://
19   subComponen
20   DnaCompon
21   descrip
22   display
23   name: B
24   type: R
25   uri: ht
26
27   ]
28
29
30   ]
31 ▼ DnaSequence [
32   uri: http://s
33   nucleotides:
34   ]
35 ]
```

The screenshot shows the C.A.S.T. designer software interface. At the top, there's a navigation bar with tabs: Regulation network, Gene Circuit (which is selected), Plasmid, Protocol, and Simulation. Below the tabs, there are three main sections: 'Regulation network' (a 3D molecular model of a complex regulatory network), 'Simulation' (a line graph showing protein concentration over time for two conditions, with one reaching a plateau around 180,000 and the other around 20,000), and 'Gene Circuit' (two detailed views of plasmids. Plasmid 0 contains genes BBA_J712074, BBA_J61104, BBA_K112000, BBA_B0013, cis, BBA_R0085, BBA_J61104, BBA_K112002, BBA_J61104, BBA_K142003, BBA_B0013, and cis. Plasmid 1 contains genes BBA_J45993, BBA_J61104, BBA_K108001, BBA_J61104, BBA_K112006, BBA_J61104, BBA_S04301, BBA_B0013, and cis. Each view includes parameters like PoPS, RIPS copy, K1, and concen, with numerical values and sliders. At the bottom, there are 'Undo' and 'Redo' buttons.

Improved SBOL assembled with dynamic parameters.

Human Practice

The game interface features a central grid of 12x12 slots. The first column contains blue rectangular blocks labeled "Promoter". The second column contains orange leaf-like blocks. Subsequent columns contain various combinations of green, purple, and blue shapes, some with red "Terminator" blocks. A red-bordered box highlights a cluster of purple dots in the fourth column, fifth row. To the right of the grid is a summary panel:

- LEVEL.1**
- SPro**
- Cells: $2^{\wedge} 1.70$**
- Split Remain Time: 10**

Below the grid, resource counts are displayed: 6, 4, 8, 5, 0, and No. The background is a light blue with white sea creature illustrations like fish, shells, and coral.

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Any questions or suggestions please visit LiuXiangyu.net

Fun and safe: the importance of biosafety.



Human Practice

TEDxSYSU
x = independently organized TED event



We spread the iGEM idea with Sun Yat-sen University students.
Discussed innovative biosafety measure.





Bronze Medal

Register the team, have a great summer, and have fun attending the Regional Jamboree and World Championship Jamboree.

- Create and share a description of the team's project via the iGEM wiki.
- Present a Poster and Talk at the Regional Jamboree and World Championship Jamboree.
- Develop and make available via the [The Registry of Software Tools](#) an open source software tool that supports synthetic biology based on BioBrick standard biological parts (remember, the iGEM judges will be looking for substantial team-based software projects).

Silver Medal

- Provide a detailed, draft specification for the next version of your software tool
- Provide a second, distinct (yet complementary) software tools project.

In addition the team must:

- Provide a demonstration of their software either as a textual or video tutorial made available on their wiki. This tutorial should explain all the features of the tool as well as provide sample input and output as appropriate.

Gold Medal

To earn a Gold Medal, in addition to the Silver Medal requirements, a team must:

- 1. Have another team utilize the software developed by your team. You must clearly show how your software was used and the results that were obtained.
- 2b. In place of requirement two, a team may instead use [SBOL](#) in your software documentation.
- 3a. Develop and document a new technical standard that supports one of the following:
The analysis, modeling, and simulation of BioBrick Parts or Devices
The sharing of BioBrick Parts or Devices, either via physical DNA or as information via the internet.



Acknowledgment



Sponsors



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