FLAME

FRAMEWORK-BASED LAYOUT AND METACIRCUIT DESIGN ENGINE

2014/11/1
Complex Systems or Networks
Design

Experiment

Unpredictable Circuitry
Our Goal

- Characterize and standardize circuits to organize standard parts correctly
- Apply framework-based design principle to simplify the design procedures.
- Design specific mathematical models for different framework to improve prediction accuracy
- Direct wet-lab experiments
Outline

Software introduction

Policy & Practice

Framework-base method

Wet-lab validation

Simulation & model
Software Introduction...
Software introduction

Our Work Flow
Design Module
First Glance
Select Input
Select Output
Select Design Frame
Assistive Design with Truth Table
Design Frame Recommendation
Display Module
More Complicated
Parts Viewer
FLAME Software introduction

pSB1C3(2070bp)

Vecto
DNA Sequence for Each Part

FLAME Software introduction
Simulation Module
Static Performance

Software introduction
Dynamic Performance
Changing the Input Concentration
Smart choice of RBS
EXPERIMENT MODULE
Obtain plasmid DNA

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.

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.

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 µg = 200 µL). For gels ≥2% agarose, double the volume of Buffer NT.

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

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.
Framework-Based Method
Framework-Based Method

- Matched Component Group (Input-Receptor-Promoter Relationships)
- Structure Framework
Input-Promoter-Receptor Tend to Cooperate with Each Other
Matched Component Group

• Input-Receptor-Output Relationships: Specificity
Framework-based method
Structure Framework
Framework: Abstraction from Published Synthetic Circuits
Structure Framework

Advantages

✓ Simplify design procedure
✓ Improve the accuracy of simulation
✓ Improve the reliability of design

Framework Simplified
1. Static and dynamic performance
2. Adjustment-simulation interactions
3. Automatic substitution of RBS

Simulation & Model
1. Static and dynamic performance
2. Adjustment-simulation interactions
3. Automatic substitution of RBS

Simulation Interface
Challenges in Modelling

2. Inconsistent Modelling Format in Practice.
4. Gap between Simulation and Wet-lab.

- Models of Process *versus* Models of Structures?

For Transcription
\[
\frac{d[\text{mRNA}]}{dt} = CN \cdot TS - \text{DeRNA} \cdot [\text{mRNA}]
\]

For Translation
\[
\frac{d[\text{Protein}]}{dt} = TE \cdot \text{TerE} \cdot [\text{mRNA}] - \text{DePr} \cdot [\text{Protein}]
\]

- Models of Process *versus* Models of Structures?
- Using studied circuits
- Basic Functional Units
- Standardization & Plug-and-play fashion
- Sustainability
2. Inconsistent Modelling Format in Practice

• Unifying functions into single format and unit: Consistency

• Homogeneous solutions from similar formats

\[
\frac{d[P]}{dt} = F([P],[R],\alpha, \beta, \gamma, K, n) = \frac{\alpha}{1+([R]/K)^n} - \beta[P] + \gamma
\]

\[
\frac{d[A]}{dt} = G([P],[A],\alpha, \beta, \gamma, K, n) = \frac{\alpha([A]/K)^n}{1+([A]/K)^n} - \beta[P] + \gamma
\]

• Efficiency and High-compatibility!
Different Models for Frameworks

\[
\frac{d[A]}{dt} = F([A],[C],\alpha_1,\beta_1,\gamma_1,K_1,n_1) = \frac{\alpha_1}{1 + ([C]/K_1)^{n_1}} - \beta_1[A] + \gamma_1
\]

\[
\frac{d[B]}{dt} = F([B],[A],\alpha_2,\beta_2,\gamma_2,K_2,n_2) = \frac{\alpha_2}{1 + ([A]/K_2)^{n_2}} - \beta_2[B] + \gamma_2
\]

\[
\frac{d[C]}{dt} = F([C],[B],\alpha_3,\beta_3,\gamma_3,K_3,n_3) = \frac{\alpha_3}{1 + ([B]/K_3)^{n_3}} - \beta_3[C] + \gamma_3
\]

BBa_C0040: TetR
BBa_K346001: MerR
BBa_K588000: TrpR
AND GATE as a Dual System

Dual-Activation system:

\[ H_{a,a}(P, S_1, S_2, \alpha, \beta, \gamma, n_1, K_1, n_2, K_2) = \alpha \frac{(S_1/K_1)^{n_1}}{1+(S_1/K_1)^{n_1}} \frac{(S_2/K_2)^{n_2}}{1+(S_2/K_2)^{n_2}} - \beta P + \gamma \]

Activation-repression coexist system:

\[ H_{a,r}(P, S_1, S_2, \alpha, \beta, \gamma, n_1, K_1, n_2, K_2) = \alpha \frac{(S_1/K_1)^{n_1}}{1+(S_1/K_1)^{n_1}} \frac{1}{1+(S_2/K_2)^{n_2}} - \beta P + \gamma \]

Dual-repression system:

\[ H_{r,r}(P, S_1, S_2, \alpha, \beta, \gamma, n_1, K_1, n_2, K_2) = \alpha \frac{1}{1+(S_1/K_1)^{n_1}} \frac{1}{1+(S_2/K_2)^{n_2}} - \beta P + \gamma \]
3. Evaluation of Circuit Performance

- Sensibility
- Demand
- Reliability
- Accessibility
- Specificity
4. Gap between Simulation and Wet-lab

HOW WE OBTAIN SIMULATION RESULT:

two basic types of interactions in our models:

\[
\frac{d[P]}{dt} = F([P],[R],\alpha,\beta,\gamma,K,n) = \frac{\alpha}{1+([R]/K)^n} - \beta[P] + \gamma
\]

\[
\frac{d[P]}{dt} = G([P],[A],\alpha,\beta,\gamma,K,n) = \frac{\alpha([A]/K)^n}{1+([A]/K)^n} - \beta[P] + \gamma
\]
Wet-lab validation
Wetlab Validation

- *E. coli* strain: BL21(DE3) as host cell

- When IPTG is added, the repressor from the lac operator is displaced thus T7 polymerase present and the transcription of GFP started
We use standard biobricks provided by iGEM Distribution to construct the plasmids.
Wetlab Validation

Protocol generated by FLAME

Wet-lab Experiment
The results above indicate that our models accord well with experimental data.

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*Time lag due to T7 RNA Polymerase synthesis.
Conclusion

Framework-based Circuit Design
- Simplification of design procedure
- Potential of automatic design of larger circuits
- Hierarchical Design: Device, Parts, DNA, Vector

New Simulation Module
- New and efficient models
- Dynamic Performance, Static Performance

Wet-lab validation
- Successfully validated our model with a self practice wet-lab experiment
Policy & practice
Biobrick Blast Online

• OpenSource online BLAST tool for Biobrick searching.

Have trouble finding biobricks containing or related to a known sequence? Biobrick Blast Online can certainly help you!

Made by YSU-Software and YSU-China, Biobrick Blast Online is an online Biobrick alignment and search toolkit. Enter only your sequence, and you will soon get all Biobricks that contain or are related to your sequence, as well as the overall information on those Biobricks.

This toolkit not only contains the latest complete iGEM part registry data but also helps you search Biobricks in 2013 or 2014 IGEM DNA Distribution. So move on and have a try!

If you need any help or have any suggestion, please don't hesitate to contact us!
Practices in High School

- Promoted iGEM and Synbio to high school students around April.
- Helped build the first iGEM HS team, SKLBC-China, in Guangzhou, and they won the Best Software Tool award!
iGEM China Community

- **First** to propose the idea of setting up *iGEM China Community*, an online & offline platform for information sharing.

- **Offline activities** were held and informed via this platform, for instance,
  - NCTU-Formosa & the Taiwan meetup,
  - USTC-Software & the Hefei meetup.
Future work

1. Update and technological support of online version.

Requirements

Bronze

The following 4 goals must be achieved:

- 1. Register the team, have a great summer, and have fun attending the Jamboree in Boston.
- 2. Create and share a description of the team's project via the iGEM wiki.
- 3. Present a Poster and Talk at the Regional Jamboree and World Championship Jamboree.
- 4. Develop and make available via The Registry of Software Tools.

Silver

In addition to the Bronze Medal requirements, the following 4 goals must be achieved:

- 1. Demonstrate the relevance of your development for Synthetic Biology based on standard Parts.
- 2. Provide a comprehensive and well-designed User Guide.
- 3. Provide detailed API documentation, preferably, automatically built from source code documentation.
- 4. Demonstrate that you followed best practises in software development so that other developers can modify, use and reuse your code.

Gold

In addition to the Bronze and Silver Medal requirements, two additional goals must be achieved:

- 1. Provide a convincing validation, testing the performance of the development -- experimentally (can be outsourced) or by other teams and users.
- 2. And the second goal can be any one of the following:
- 3. Make your software interact / interface with the Registry.
- 4. Re-use and further develop previous iGEM software projects.
- 5. Develop a well-documented library or API for other developers.
- 6. Support and use the SBOL and/or SBOLv standard.
- 7. iGEM projects involve important questions beyond the bench.

We fulfilled all of these requirements!
Team
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Sponsor

Instructors
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