The Legendary Story of Speedy - How SpeedyBac saved the World

2010 iGEM NYMU - Taípeí
1. **Who** are we?

2. **What** are we trying to do?

3. **Why** do we want to do that?
   - The **Demand** and The **Significance**

4. **How** do we do it?
   - **Design** and **Engineering**

5. **What** have we done so far?
   - **Experimental Results**

6. **Our** Conclusions and **Summary**
1. **Who** are we?
2. What are we trying to do?
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4. How do we do it?
   - Design and Engineering
5. What have we done so far?
   - Experimental Results
6. Our Conclusions and Summary
The Story Begins,...

A group of iGEMers.
They lived on...

National Yang Ming University
1. Who are we?

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Our Project:

**SpeedyBac**

- **Our Goal:**
  Provide a faster assay system for exploring the design rules for synthetic biology.
Outline

1. Who are we?

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■ All the iGEMers are trying to:
  - Develop New Application
  - Generate Food/Energy
  - Make Foundational Advance
  - Improve Health/Medicine
  - Remedy Environment
  - Do Manufacturing
  - Information Processing
  - Write Software Tools

(The iGEM Tracks)

■ However, they realized a terrible truth,

■ that is, in order for synthetic biology to work, we need both of Biological Parts and Design Rules.
We can NOT save the world!

- We already have a Registry of Biological Parts, but **where is our Registry of Design Rules?**
- Nowadays, most iGEMers around the world are still doing their Black Box design.

**How to design a black box?**

**Is this black box “black” enough?**
Major Need of Synthetic Biology: Design RULES!

Combinations need Design RULES!!

But the lack of rules causes limitations in the development of synthetic biology.

Parts Circuits

http://blogs.smarter.com/blogs/Lego%20Brick.jpg
Finding design rules needs **time**!!

- Combining biobricks needs more knowledge of biological systems.
- Understanding these biological design rules needs **time**!!

It will take many years to find the Design Rules for Synthetic Biology.

You need our **SpeedyBac**!
Our focus is on Spatial-Temporal Control

- Conventionally → iGEM mostly uses transcriptional control
- Spatial-temporal control is actually also needed, but still remains largely unexplored!!

Restrict the development of synthetic circuits!!!

The focus of our SpeedyBac work!
Outline

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# Advantages of using our SpeedyBac for synthetic biology

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<tr>
<th></th>
<th>Conventional</th>
<th>With Our SpeedyBac</th>
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<tr>
<td><strong>Transcription / Translation</strong></td>
<td>Continuously</td>
<td>Can be separated by Speedy switch!</td>
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<td><strong>Gene regulation process</strong></td>
<td>Emphasis on results</td>
<td>Temporal dynamics</td>
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Aims of SpeedyBac
(for this summer)

1. Quantitative description of gene expression in both space and time
2. Specific insight into the flow of genetic information
3. Speedy ways to report and stop the gene expression

The precise measurements of these gene regulations will reveal more ‘rules’!!
Design of SpeedyBac

There are three devices in our SpeedyBac system.

- Your Promoter
- RNA Aptamer
- Riboswitch
- Your Protein
- Peptide Adaptor
- Terminator
Design of SpeedyBac

- RNA/protein Localization & Quantity
- Promoter Testing

1. Speedy RNA reporter

1. Speedy protein reporter

Your Promoter

RNA Aptamer

Riboswitch

Your Protein

Peptite Adaptor

Terminator
Design of SpeedyBac

- Tunable Protein Expression
- The Switch between RNA Assay & Protein Assay

2. Speedy switch

Your Promoter

RNA Aptamer

Riboswitch

Your Protein

Your Protein

Peptide Adaptor

Terminator
Design of SpeedyBac

- Output Removal
- Transient Gene Expression

3. Speedy degrader

Your Protein

RNA Aptamer

Riboswitch

Your Protein

Peptite Adaptor

Terminator
Design of SpeedyBac

Introduction

Your Protein

Your Promoter

RNA Aptamer

Riboswitch

Your Protein

Peptite Adaptor

Terminator
1. Speedy Reporter

mRNA reporter

Protein reporter

Your Protein

Peptide Adaptor

Terminator

Your Promoter

RNA Aptamer

Riboswitch
Speedy reporter

- Purpose in SpeedyBac system:
  - Detect the target’s location and measure its quantity

- What is Speedy reporter?
  - Makes the fluorescent protein express on the target
  - Changes fluorescence intensity immediately

Inducible promoter

GFP
Design of Speedy reporter

- **Our Design:**
  - A split fluorescent protein
  - A protein – aptamer binding pair

- **Two split fusion proteins are used. Each half contains:**
  - Half of the Fluorescent protein
  - A Peptide linker
  - Half of the aptamer binding protein
How speedy reporter works

- Use **split protein** method:
  - the formation of chromophore is the **rate limiting step** for fluorescence

- The split proteins are **constitutively** expressed

- When the aptamer is expressed:
  - The two split fluorescent proteins recombine and **light up immediately**
Speedy reporter: reporting RNA and proteins

RNA reporter

Your Promoter
RNA Aptamer
Riboswitch
Your Protein
Peptide Adaptor
Terminator

Protein reporter

Your Protein
RNA reporting part

- Split EGFP (between 157-158 aa)
- Split aptamer binding protein: eIF4A (between 215-216 aa)

- eIF4A: *dumbbell* structure, a nice protein for splitting.
- eIF4A aptamer binding pair: strong affinity with each other.
1. Reporter

RNA reporting part

- Promoter Testing:
  Your promoter is inserted in front of the RNA aptamer.
- RNA localization tracking and quantity measurement

Split EGFP (between 157-158 aa)

Split aptamer binding protein: eIF4A (between 215-216 aa)
Protein expression tracking: Your protein (the target) is inserted in front of the peptide adaptor.
Protein reporting part

Protein expression is controlled by the Speedy switch.
2. Speedy Switch

Your Promoter

RNA Aptamer

Riboswitch

Your Protein

Peptite Adaptor

 Terminator
Aim: Specific insight into the flow of genetic information

Function in SpeedyBac system:
- Provides a critical switch between RNA and protein for controlling translation
Purpose in SpeedyBac system:
- Perform RNA assay & protein assay in a single cell
- Control the protein expression
2. Switch

### Speedy Switch - Riboswitch

- A part of mRNA
- Bind a specific inducer to change the structure of riboswitch, thereby regulating the gene expression.

**Specific inducer (Example: Theophylline)**

Guideline:

- The riboswitch and the specific inducer do not naturally exist or metabolize in the target organism.

Example: Theophylline Riboswitch
How to degrade the signal?

Your Promoter

RNA Aptamer

Riboswitch

Your Protein

Peptide Adaptor

Terminator

ssrA Tag

3. Degrader
3. Speed Degrader

Your Promoter

RNA Aptamer

Riboswitch

Your Protein

Peptide Adaptor

Terminator
What is SsrA tag?

- A short peptide which can accelerate protein degradation
- LVA tag is one of the most efficient ssrA tags

Purpose in SpeedyBac system

- Remove signal
- Detect transient gene expression
SspB captures the protein substrate for ClpXP protease to degrade it easily.

Degradation Time for Variants of SsrA Tag

LVA is the most efficient tag

Degradation curve of SsrA protein

Fluorescence Intensity

Time

Protein Production

Protein Degradation

Normal protein

Protein with ssrA tag

Stop producing protein
Degradation curve of SsrA protein

Fluorescence Intensity

Stop producing protein

Normal protein

Changeable degradation time

Time
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Experiment results

Your Promoter
mRNA Aptamer
Riboswitch
Your Protein
Protein Aptamer
Terminator

ssrA Tag

2010 NYMU
Concentration-dependent riboswitch

Under 4mM, the higher the concentrations of Theophylline, the higher fluorescent intensity.
Concentration-dependent fluorescence intensity

Under 4mM, the higher the concentrations of Theophylline, the higher fluorescent intensity.

Relative Fluorescence Intensity (units)

log of theophylline concentration (log uM)
The degradation of RFP & RFPLva
# Our Biobrick Parts

<table>
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<th>Type</th>
<th>Description</th>
<th>Designer</th>
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RNA Assay & Protein Assay in a Single Cell Assay: Location & Quantity Promoter Testing

→ Explore the System much Faster
1. Speedy RNA Reporter

- mRNA Location & Quantity
- Promoter Testing

- Constitutive Promotor
- GFP
- Linker
- eIF4A
- RBS
- Term

- Inducible Promotor
- eIF4A binding aptamer
- Riboswitch
- Target Protein
- Linker
- Tag Your Protein
- Peptide Adaptor
- Terminator
2. Speedy Switch

- Tunable Protein Expression
- The Switch between RNA Assay & Protein Assay

DNA ↔ RNA → Protein

Conditional Protein Expression
3. Speedy Protein Reporter

Protein Location & Quantity

- Constitutive Promotor
- Linker
- Binding Protein \(_A\)
- Linker
- Binding Protein \(_B\)
- RFP \(_B\)
- RFP \(_A\)
- Your Protein
- Peptide Adaptor
- Terminator

Your Protein
Peptide Adaptor
4. Speedy Degrader

- Reporter Degradation
- Detect transient Gene Expression

Protein Expression

Normal Condition

Adding ssrA Tag

Flexibility
SpeedyBac is a tool to look closely and quickly in each step of central dogma.

More Principles in the System of life

When we know more about the principles quickly, we can find more rules to design the circuit.
The more we know about the design rules of the System, the more we can do in Synthetic biology.
Our Achievements

- **NYMU-Taipei** has:
  - Successfully characterized the **Theophylline Riboswitch** (BBa_I752000 & BBa_K411001)
  - Successfully created and characterized YFP (BBa_K411232) and RFPLVA (BBa_K411238)
  - Submitted 34 biobricks to the Registry
  - Created new biobricks using novel genes

**Speedy Reporter**
- Nearly Finish Constructing

**Speedy Switch**
- BBa_I752000
- BBa_K411001
- Characterized

**Speedy Degrader**
- BBa_K411232 (YFP)
- BBa_K411238 (RFPLVA)
- Created and Characterized
From then on, all the iGEMers around the world can continue their works of saving the world.

And They Lived Happily Ever After...
Acknowledgement

President
Prof. Kung-Yee Liang

Prof. Chris Proud

Prof. Wailap Victor Ng
Prof. Ann-Ping Tsou
Prof. Ueng-Cheng Yang
THANKS FOR LISTENING !!

SpeedyBa

NYMU iGEM 2010
Rule a tool to
Rock your rule.

Our wiki::
http://2010.igem.org/Team:NYMU-Taipei
Main References:


More references available from our wiki page: http://2010.igem.org/Team:NYMU-Taipei