Biotherm map
iGEM-2015

William (Zixu) Wang
James (Wenbo) Xu
Jodie (Zhe) Yang
Edward (Tianhua) Zhai

XJTLU-China
Sep-26-2015
• Ribothermometer

• Plux, LuxR & LuxI

• LVA/AAV tags
Key components

• Fast degraded chromoproteins
Color presenting strategy
Key components

- Fast degraded chromoproteins
- Lux system
Key components

- Fast degraded chromoproteins
- Lux system
- Temperature sensors
RNA thermometers

**Figure. 1**
Inland

Figure 2
Figure 3
Experiment session

Modularity

• What?
  1. Promoter + operator/ RBS/Ribothermometer
  2. Coding sequence + terminator

• Why?
  1. Time limitation
  2. Easy to combine different parts

• How?
  Gibson assembly
Main components

- **RNA thermometer**

- **Chromoproteins**

Figure 6
RNA thermometer

- First attempt: screening useful thermometer
  
<table>
<thead>
<tr>
<th>RNA T</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>BIT-China</td>
</tr>
<tr>
<td>A2</td>
<td>BIT-China</td>
</tr>
<tr>
<td>A3</td>
<td>BIT-China</td>
</tr>
<tr>
<td>U6</td>
<td>Literature</td>
</tr>
<tr>
<td>U9</td>
<td>Literature</td>
</tr>
<tr>
<td>U10</td>
<td>Literature</td>
</tr>
<tr>
<td>BBa_K115017</td>
<td>iGEM08_TUDelft</td>
</tr>
<tr>
<td>BBa_K115002</td>
<td>iGEM08_TUDelft</td>
</tr>
</tbody>
</table>

- Second attempt: screening the best combination of promoter and thermometer
RNA thermometers - Second attempt

Figure 7

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>J23119+A1</td>
<td>W4</td>
<td>pBAD+A1</td>
<td>W7</td>
<td>T7+A1</td>
</tr>
<tr>
<td>W2</td>
<td>J23119+4U</td>
<td>W5</td>
<td>pBAD+4U</td>
<td>W8</td>
<td>T7+4U</td>
</tr>
<tr>
<td>W3</td>
<td>J23119+U6</td>
<td>W6</td>
<td>pBAD+U6</td>
<td>W9</td>
<td>T7+U6</td>
</tr>
</tbody>
</table>

Table 2
RNA thermometers- Second attempt

Figure 8
RNA thermometers- Second trial

![A1 ribothermometer with J23119 promoter](image)

Figure. 9
Chromoproteins

1. Attach lva/aav tags to chromoproteins

2. Yield green: combination of two chromoproteins
Chromoproteins

1. Attach lva/aav tags to chromoproteins

![Diagram of Chromoproteins]

- **T7 Promoter**
- **Lac operator**
- **RBS**
- **AeBlue**
- **AAV tag**
- **LVA tag**

**Figure. 10**
**Chromoproteins**

1. Attach **lva** / **aav** tags to chromoproteins

![Table 3: Absorbance value divided by OD value](image)

**Table 3: Absorbance value divided by OD value**

<table>
<thead>
<tr>
<th>Time (after induced)</th>
<th>blueA</th>
<th>blueB</th>
<th>Blue Average</th>
<th>blueaav A</th>
<th>blueaav B</th>
<th>Blue AAV Average</th>
<th>blueva A</th>
<th>blueva B</th>
<th>Blue LVA Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>300min</td>
<td>0.206</td>
<td>0.140</td>
<td>0.173</td>
<td>0.088</td>
<td>0.075</td>
<td>0.082</td>
<td>0.045</td>
<td>0.049</td>
<td>0.047</td>
</tr>
<tr>
<td>330min</td>
<td>0.161</td>
<td>0.185</td>
<td>0.173</td>
<td>0.105</td>
<td>0.103</td>
<td>0.104</td>
<td>0.073</td>
<td>0.069</td>
<td>0.071</td>
</tr>
<tr>
<td>360min</td>
<td>0.185</td>
<td>0.199</td>
<td>0.192</td>
<td>0.107</td>
<td>0.112</td>
<td>0.109</td>
<td>0.069</td>
<td>0.067</td>
<td>0.068</td>
</tr>
<tr>
<td>390min</td>
<td>0.274</td>
<td>0.215</td>
<td>0.245</td>
<td>0.156</td>
<td>0.125</td>
<td>0.141</td>
<td>0.064</td>
<td>0.075</td>
<td>0.069</td>
</tr>
<tr>
<td>420min</td>
<td>0.349</td>
<td>0.287</td>
<td>0.318</td>
<td>0.160</td>
<td>0.174</td>
<td>0.167</td>
<td>0.108</td>
<td>0.108</td>
<td>0.108</td>
</tr>
<tr>
<td>450min</td>
<td>0.259</td>
<td>0.208</td>
<td>0.234</td>
<td>0.112</td>
<td>0.119</td>
<td>0.116</td>
<td>0.079</td>
<td>0.076</td>
<td>0.078</td>
</tr>
<tr>
<td>480min</td>
<td>0.330</td>
<td>0.184</td>
<td>0.257</td>
<td>0.102</td>
<td>0.132</td>
<td>0.117</td>
<td>0.079</td>
<td>0.093</td>
<td>0.086</td>
</tr>
<tr>
<td>510min</td>
<td>0.258</td>
<td>0.200</td>
<td>0.229</td>
<td>0.130</td>
<td>0.122</td>
<td>0.126</td>
<td>0.084</td>
<td>0.071</td>
<td>0.077</td>
</tr>
<tr>
<td>540min</td>
<td>0.241</td>
<td>0.215</td>
<td>0.228</td>
<td>0.127</td>
<td>0.120</td>
<td>0.123</td>
<td>0.098</td>
<td>0.056</td>
<td>0.077</td>
</tr>
<tr>
<td>570min</td>
<td>0.268</td>
<td>0.212</td>
<td>0.240</td>
<td>0.105</td>
<td>0.132</td>
<td>0.118</td>
<td>0.074</td>
<td>0.080</td>
<td>0.077</td>
</tr>
<tr>
<td>600min</td>
<td>0.252</td>
<td>0.194</td>
<td>0.223</td>
<td>0.114</td>
<td>0.116</td>
<td>0.115</td>
<td>0.070</td>
<td>0.076</td>
<td>0.073</td>
</tr>
<tr>
<td>660min</td>
<td>0.267</td>
<td>0.213</td>
<td>0.240</td>
<td>0.141</td>
<td>0.168</td>
<td>0.155</td>
<td>0.105</td>
<td>0.094</td>
<td>0.100</td>
</tr>
<tr>
<td>720min</td>
<td>0.274</td>
<td>0.203</td>
<td>0.239</td>
<td>0.134</td>
<td>0.094</td>
<td>0.114</td>
<td>0.081</td>
<td>0.056</td>
<td>0.069</td>
</tr>
</tbody>
</table>
Chromoproteins

1. Attach **lva** / **aav** tags to chromoproteins
Chromoproteins

2. Yield green-combination of two chromoproteins

① Fw Yellow chromoprotein

Ae blue chromoprotein

Figure. 13
Chromoprotein

2. Yield green------combination of two chromoproteins

Figure. 14
2. Yield green—-combination of two chromoproteins

Figure. 15
Chromoproteins

2. Yield green----combination of two chromoproteins
Conclusion

- Modularity: 31 parts
  - 4 parts validated

- Perfect thermometer (A1) with promoter J23119
  - Proof of concept

- Color change with useful tag - LVA tag & AAV tag
  - Proof of concept

- Obtain combination of green color
  - Validated
NO support!
NO $$$$$$$!
NO laboratory!

MISSION IMPOSSIBLE

NEVER. GIVE. UP.
Outside activities: Public Speeches
Outside activities: Negotiating with Synbio Tech
Establishing XJTLU-Synbio Tech iGEM Studio
Collaboration: Consulting other team instructors

Help from BIT-CHINA and desirable results obtained

Figure 1. Analysis of temperature dependence of RNA and protein accumulation in E. coli strains harboring EGFP constructs under the control of RNA thermometers.
(A) An illustration of LacO-random sequence-A1 secondary structure. The melting temperature of A1 is 42 degrees and the 50th base is the starting point of RNAT.
(B) A quick test of RNA thermometer A1 constructs by temperature-controlled EGFP (Enhanced green fluorescent protein) expression in E. coli. The efficient synthetic thermometer A1 was compared with the control groups. The three rows (from above to below) indicated that trials were treated in 30, 37, 42 degrees respectively and six columns (from left to right) represented control groups, experimental groups alternating.
Collaboration: Workshop in Shanghai

Communicating with other iGEM teams and Giving suggestions on projects
Collaboration: Supporting NYU-Shanghai

Plasmids provided for NYU-Shanghai team

E. coli samples made by NYU-Shanghai team by using our donations
Public Education: LEGO Robot

LEGO video: Protein translation
Summary

• Tested 8 ribothermometers, A1 was the best.

• Tested the efficiency of LVA/AAV tags, both of them can help the degradation process of chromoproteins.

• Successfully combined the blue and yellow chromoprotein and got the green color.

• Held public speeches of iGEM in our university and biotech companies.

• Actively took part in the work shop at Shanghai Science and Technology Museum.

• Assembled a LEGO robot to show the translation process to the public.
Sponsors

- Xi'an Jiaotong-Liverpool University
- Synbio Tech
- Agilent Technologies
- IDT: Integrated DNA Technologies
- SnapGene: Software for molecular biology