WHAT’S T-REX?

• Control the synthesis of any protein of interest

• Silence the protein expression faster than using classic regulated promoters
This device is composed of two BioBricks:

- CIS – repressing
- TRANS – repressor
Transcription of the target gene yields a mRNA strand;

The mRNA with the CIS sequence at 5' end, is available for translation.
When the promoter controlling the TRANS coding sequence is active its transcript binds with the CIS mRNA.

This RNA duplex prevents ribosomes from binding to RBS, thus silencing protein synthesis.
BASER
Best Sequence Research by Andrea and Elisa

1) Maximal free energy in the secondary structure, reducing the probability of its intra-molecular annealing;

2) Minimal unwanted interactions with genomic mRNA;

3) Minimal probability of partial/shifted hybridization with complementary strands.
HOW BASER WORKS?

Starting from a randomly generated sequence (current sequence);

Conformity test:
- a) more than 5 adjacent repeats of the same nucleotide;
- b) restriction sites;
- c) RBS sequences;

NO

YES
Add RBS at 3’ end

Evaluate score of current sequence;

BASER replace 5 nucleotides randomly (generation of new sequence);

Evaluate score of new sequence;

Score of new sequence is better than score of current sequence?

YES
New sequence is preserved

NO
Current sequence is preserved
How BASER calculates the score?

• BASER calculates a score for the current sequence:

1) the self score;

2) the genomic score;

3) the shifted score;
Choose of a CIS sequence

AACACAAACTATCACTTTAACAACACATTACATATACATTAAAATATTACAAAGAGGAGAAA
(RBS in italic)
Choose of the TRANS sequences

**CCTCTTTGTAATATTTTAATGTATATGTAATGTGTTGTTAAAGTGATAGTTTGTGTT**
with a 7b-long RBS cover in **green**

**CTTTGTAATATTTTAATGTATATGTAATGTGTTGTTAAAGTGATAGTTTGTGTT**
with a 4b-long RBS cover in **green underlined**
Morphology:
- Eccentricity [0,1];
- Area [min,max];

Focus:
- Clustering;
  - High fluorescence;
  - High cell number;

Output: for each bacterium the area in pixels and the fluorescence
Part Characterization

- Promoter Strengths
- Plasmid copy numbers
- Influence of O2 operator
- Interaction between Lacl and O2 operator

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Promoter Strengths

**BBa_J23118 (1429)** vs **BBa_J23100 (2547)**

**BBa_K079031 on pSB1A2**

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Promoter Strengths

- **Methods**
  - DH5α cells
  - M9 medium
  - 37° overnight
- **Imaging Analysis**
  - VIFluoR
- **Fluorimeter Analysis**
  - Tecan M200
Promoter Strengths

- OD/Fluorescence overtime analysis from OD=0.1au
  - Growth Curve
  - Fluorescence
  - Fluorescence/OD ratio
Plasmid Copy Numbers

**pSB1A2**
(high copy)

**VS**

**pSB3K3**
(low/medium copy)

**BBa_K201003 on pSB1A2**

**BBa_K201003 on pSB3K3**
Plasmid Copy Numbers

- **Methods**
  - DH5α cells
  - M9 medium
  - 37° overnight
- **Imaging Analysis**
  - VIFluoR
- **Fluorimeter Analysis**
  - Tecan M200

### Plasmid Copy Numbers Table

<table>
<thead>
<tr>
<th></th>
<th>pSB1A2</th>
<th>pSB3K3</th>
<th>RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IMAGING ANALYSIS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN ± STD N. BACTERIA</td>
<td>188.3 ± 38.9</td>
<td>40 ± 6.4</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td><strong>FLUORIMETER ANALYSIS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN ± STD N. BACTERIA</td>
<td>294 × 10^5 ± 7 × 10^5</td>
<td>96 × 10^3 ± 1 × 10^3</td>
<td>3.1 ± 0.1</td>
</tr>
</tbody>
</table>

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Influence of O2

**BBa_K079032** (O2 absent)  **VS**  **BBa_K201001** (O2 present)

*BBa_K079032 on pSB1A2*  

*BBa_K201001 on pSB1A2*
• Methods
  - DH5α cell
  - M9 medium
  - 37° overnight
• Fluorimeter Analysis
  - Victor 2

### Influence of O2

<table>
<thead>
<tr>
<th>FLUORIMETER ANALYSIS</th>
<th>O2 PRESENT</th>
<th>O2 ABSENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN ± STD N. WEELS</td>
<td>65 ( \cdot 10^3 \pm 22 \cdot 10^4 )</td>
<td>64 ( \cdot 10^3 \pm 14 \cdot 10^5 )</td>
</tr>
<tr>
<td>N. WEELS</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
Positive Control of Testing Circuit

BBa_K201002 on pSB3K3

BBa_K201001 on pSB1A2
IPTG induction: Static Response

- **Methods**
  - DH5α cells
  - M9 medium
  - 37° overnight
  - several IPTG levels

- **Imaging Analysis**
  - VIFluoR
  - several images
  - >60 bacteria/image
IPTG induction: Dynamic Response

- **Methods**
  - DH5α cell
  - M9 medium
  - 37° overnight
  - No IPTG

- **Growth Curve**
- **Fluorescence**

- **Fluorimeter Analysis**
  - Tecan M200
  - Dilution to OD=0.1
  - 1° sample: No IPTG
  - 2° sample: IPTG 100μM

![Graph showing fluorescence response to IPTG induction](image)
MATHEMATICAL MODEL

- Transcription and translation processes were considered similar to a second order kinetics, like an enzymatic reaction:

**TRANSCRIPTION**

\[
D_i + E_P \xrightarrow{a_{EpD_i}} C_{EpDi} \xrightarrow{s_{EpD_i}} M_i + D_i + E_P
\]

**mRNA DEGRADATION**

\[
M_i \xrightarrow{d_{Mi}}
\]

**EQUILIBRIUM CONSTANT**

\[
K_{EpDi} = \frac{b_{EpDi} + s_{EpDi}}{a_{EpDi}}
\]

**TRANSCRIPTION**

\[
\frac{dC_{EpDi}}{dt} = (b_{EpDi} + s_{EpDi})\left(\frac{D_iE_P}{K_{EpDi}} - C_{EpDi}\right)
\]

\[
\frac{dM_i}{dt} = s_{EpDi}C_{EpDi} - d_{Mi}M_i - \frac{dC_{EBM_i}}{dt}
\]

\( i = G \) (GFP) or \( R(\text{LacI repressor monomer}) \) or \( T(\text{Trans}) \)
MATHEMATICAL MODEL

TRANSLATION

\[ M_j + E_B \xrightarrow{a_{EMj}} C_{EMj} \xrightarrow{s_{EMj}} M_j + P_j + E_B \]

PROTEIN DEGRADATION

\[ dP_j \]

AFFINITY CONSTANT

\[ K_{EMj} = \frac{b_{EMj} + s_{EMj}}{a_{EMj}} \]

TRANSLATION

\[ \frac{dC_{EMj}}{dt} = (b_{EMj} + s_{EMj})(\frac{M_j E_B}{K_{EMj}} - C_{EMj}) \]

\[ \frac{dP_j}{dt} = s_{EMj} C_{EMj} - dP_j P_j \]

\[ j = G \text{ (GFP)} \text{ or } R \text{ (LacI Repressor monomer)} \]

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**LacI DIMERIZATION**

\[ 2P_{R_m} \xleftrightarrow{a_d} P_{R_d} \]

**LacI-OPERATOR SITE INTERACTION**

\[ P_{R_d}^{free} + D_G \xleftrightarrow{a_{Pr_d}D_G} C_{Pr_d}D_G \]

**LacI-IPTG INTERACTION**

\[ 2I + P_{R_d}^{free} \xleftrightarrow{a_{Pr_d}I} C_{Pr_d}I \]

**CIS-TRANS INTERACTION**

\[ M_R + M_T^{free} \xleftrightarrow{a_{MrMT}} C_{MrMT} \]

**DISASSOCIATION CONSTANT**

\[ K_{Pr_d}D_G = \frac{b_{Pr_d}D_G}{a_{Pr_d}D_G} \]

\[ K_{Pr_d}I = \frac{b_{Pr_d}I}{a_{Pr_d}I} \]

\[ K_{MrMT} = \frac{b_{MrMT}}{a_{MrMT}} \]

**Lacl DIMERIZATION**

\[ \frac{dP_{R_d}}{dt} = a_dP_{R_m}^2 - (b_d + d_{Pr_d})P_{R_d}^{free} - d_{Pr_d}C_{Pr_d} \]

**CIS-TRANS INTERACTION**

\[ \frac{dC_{MrMT}}{dt} = b_{MrMT}(\frac{M_{RMT}}{K_{MrMT}} - C_{MrMT}) \]

**Lacl-OPERATOR SITE INTERACTION**

\[ \frac{dC_{Pr_d}D_G}{dt} = b_{Pr_d}D_G(\frac{P_{R_d}^{free}D_G}{K_{Pr_d}D_G} - C_{Pr_d}D_G) \]

**Lacl-IPTG INTERACTION**

\[ \frac{dC_{Pr_d}I}{dt} = b_{Pr_d}I(\frac{P_{R_d}^{free}I_i}{K_{Pr_d}I} - C_{Pr_d}I) \]

---

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PARAMETERS ASSIGNMENT

From Literature

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
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<tbody>
<tr>
<td>$d_M$</td>
<td>mRNA degradation rate</td>
<td>$1.89 \cdot 10^{-1}$</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>$d_p$</td>
<td>protein degradation rate</td>
<td>$1.73 \cdot 10^{-2}$</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>$s_{E_p D_i}$</td>
<td>mRNA synthesis rate</td>
<td>0.14</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>$s_{E_p M_i}$</td>
<td>Protein synthesis rate</td>
<td>2.5</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>$E_P_0$</td>
<td>number of RNA polymerase</td>
<td>1500</td>
<td>molecules</td>
</tr>
<tr>
<td>$E_{B0}$</td>
<td>number of Ribosome</td>
<td>6800</td>
<td>molecules</td>
</tr>
</tbody>
</table>
PARAMETERS ASSIGNMENT

From Experimental Measurement

PROMOTER RATIO = 1.2

PLASMID COPY NUMBER RATIO = 4.6

We simulated testing circuit when T-REX device is **idle** (Initial Trans-DNA = 0)
LacI SIGMOIDAL REPRESSION CURVE
We fitted experimental data in order to identify LacI-O2 dissociation constant and LacI-IPTG dissociation constant.

\[ K_{Pr_d} D_G = 1.7 \text{nM} \]
DYNAMIC IPTG INDUCTION

- Experimental Data (with IPTG)
- Model Response with Time Varying RNAP
- Model Response with Constant RNAP
- Experimental Data (without IPTG)
T-REX SIMULATION

\[
\frac{GFP - GFP_{K_{M_{MT}} = \infty}}{GFP_{\text{open}} - GFP_{K_{M_{MT}} = \infty}} \times 100\%
\]

CIS-TRANS Dissociation Constant $K_{M_{MT}}$
T-REX STORY

We didn’t manage to get the final circuit because we didn’t achieve the assemblying of the CIS and TRANS parts

Which were the problems?

1. Parts are only 100 bp in length: **Quantity problem, due to purification?**
   
   * P1010 death gene ligation protocol.

2. Enzyme efficiency is lower with short flanking sequences: **Were our digestions effective?**
   
   * We order longer PCR primers and doubled the digestion time.
CONCLUSIONS

Enter information detailing at least one new standard BioBrick Part or Device in the Registry of Standard Parts and demonstrate that works as expected;

Submit DNA for at least one new BioBrick Part or Device to the Registry of Parts.

<table>
<thead>
<tr>
<th>No.</th>
<th>NAME</th>
<th>TYPE</th>
<th>DESCRIPTION</th>
<th>PLASMID</th>
<th>LENGTH (bp)</th>
<th>SEQUENCED</th>
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<tbody>
<tr>
<td>1</td>
<td>BBa_K201000</td>
<td>Generator</td>
<td>Lac repressed GFP generator with BBa_K201000</td>
<td>BBa_PSB1A2</td>
<td>986</td>
<td>-</td>
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<tr>
<td>2</td>
<td>BBa_K201001</td>
<td>Generator</td>
<td>Lac repressed GFP generator with BBa_K201001</td>
<td>BBa_PSB1A2</td>
<td>986</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>BBa_K201002</td>
<td>Generator</td>
<td>Lac repressor constitutive supplier</td>
<td>BBa_PSB1A2</td>
<td>1351</td>
<td>Yes</td>
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<tr>
<td>4</td>
<td>BBa_K201004</td>
<td>Generator</td>
<td>Lac repressor constitutive supplier</td>
<td>BBa_PSB3K3</td>
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<td>-</td>
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<tr>
<td>5</td>
<td>BBa_K201003</td>
<td>Generator</td>
<td>GFP generator</td>
<td>BBa_PSB1A2</td>
<td>918</td>
<td>Yes</td>
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<tr>
<td>6</td>
<td>BBa_K201005</td>
<td>Generator</td>
<td>GFP generator</td>
<td>BBa_PSB3K3</td>
<td>918</td>
<td>-</td>
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<tr>
<td>7</td>
<td>BBa_K201006</td>
<td>Regulatory</td>
<td>Lac natural operator Q2 with RFP</td>
<td>BBa_PSB1A2</td>
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<td>8</td>
<td>BBa_K201007</td>
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<td>BBa_K201008</td>
<td>Regulatory</td>
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<td>Yes</td>
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<td>BBa_K201009</td>
<td>Regulatory</td>
<td>Lac repressed promoter BBa_J23118</td>
<td>BBa_PSB1A2</td>
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<tr>
<td>11</td>
<td>BBa_K201006</td>
<td>Regulatory</td>
<td>Lac repressed promoter BBa_J23100</td>
<td>BBa_PSB1A2</td>
<td>776</td>
<td>-</td>
</tr>
</tbody>
</table>
CONCLUSIONS

Characterize or improve an existing BioBrick Part or Device and enter this information back on the Registry.

<table>
<thead>
<tr>
<th>NO</th>
<th>NAME</th>
<th>TYPE</th>
<th>DESCRIPTION</th>
<th>LENGTH (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BBa_K079031</td>
<td>Reporter</td>
<td>GFP reporter protein under the control of the BBa_J23118 promoter</td>
<td>957</td>
</tr>
<tr>
<td>2</td>
<td>BBa_K079052</td>
<td>Reporter</td>
<td>GFP reporter protein under the control of the BBa_J23100 constitutive promoter</td>
<td>957</td>
</tr>
</tbody>
</table>

Help another iGEM team:
Cloned and sent the BioBrick BBa_K201002 to the UNIPV-Pavia iGEM team.
HUMAN PRACTICE- SHARING

We realized:

• An Information Booklet

• An Online Survey
ON-LINE SURVEY RESULTS

Total respondents: 484

- General lack of knowledge about Synthetic Biology

- Most people expressed curiosity about Synthetic Biology and iGEM

- After reading the booklet, great part of the respondent recognized the importance of a responsible and conscious use of Synthetic Biology
Acknowledgements

- University of Bologna
- Ser.In.Ar Cesena
- Cultural Association San Sebastiano

Instructors:
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Thanks!