Transcription Terminator Efficiency Calculator (TTEC)

SUSTC-Shenzhen-B
10/6/2012
1 Background & Motivation

A rho-independent terminator
A rho-independent terminator
Regulation of gene expression by terminator
Background & Motivation

**Prediction**
Predict the terminator efficiency

**Calculation**
Calculate the whole process of transcription

**Efficiency:**
- Promoter
- RBS
- CDS
- Terminator
Outline

1. Background & Motivation

2. Terminator Efficiency Calculator
   - Algorithm
   - Database
   - Experiment

3. Human Practice
**Algorithm**

1. **Determine the secondary structure**

2. **Calculate the d-score**

3. **Use our score-efficiency equation**

**Score** = \( nT \times 18.16 + \frac{\Delta G}{LH} \times 96.59 - 116.87 \)

\( = 0.4 \)

**Efficiency** = \( 1.449 \times \text{Score} + 39.142 \)

\( = 40\% \)
The Training Set Data

Score (From TTEC prediction)

Efficiency (From BioFab)

Efficiency = 1.449*score + 39.142

![Graph showing the relationship between Score (From TTEC prediction) and Efficiency (From BioFab)].

*Linear fitting $
R^2 = 0.51$

2

Score-Efficiency Relationship
### Database

#### 75 Terminators

**Complete description**

**Multiple sources**

**The largest So far**

<table>
<thead>
<tr>
<th>Name</th>
<th>Direction</th>
<th>Environment</th>
<th>Stem-Loop</th>
<th>A-Tail</th>
<th>T-Tail</th>
<th>Efficiency</th>
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</thead>
<tbody>
<tr>
<td>Bba_B1005</td>
<td>Forward</td>
<td>E. coli</td>
<td>CCCGCTTCGGCGGGG</td>
<td>TTCG</td>
<td>TTT</td>
<td>86%[CH]</td>
</tr>
<tr>
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<td>E. coli</td>
<td>CCCGCCCTTGACAGGGCGG</td>
<td>CAGTCA</td>
<td>TTTTTTTTT</td>
<td>99%[CH]</td>
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<tr>
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<td>CAGTCA</td>
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<td>83%[CH]</td>
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<tr>
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<td>TTTTTTTTT</td>
<td>95%[CH]</td>
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<td>E. coli</td>
<td>CCCGCCCTTGACAGGGCGG</td>
<td>CAGTCA</td>
<td>TTTTTTTTT</td>
<td>94%[CH]</td>
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<td>E. coli</td>
<td>CCCGCCCTTGACAGGGCGG</td>
<td>CAGTCA</td>
<td>TTTTTTTTT</td>
<td>95%[CH]</td>
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<td>trpC302</td>
<td>Forward</td>
<td>mutant</td>
<td>GTGCGCTGTTCGCGCGGC</td>
<td>GATC</td>
<td>TTT</td>
<td>8%</td>
</tr>
<tr>
<td>trp t</td>
<td>Forward</td>
<td>mutant</td>
<td>GCAGCAGTTCGCGCGGC</td>
<td>TTCG</td>
<td>TTT</td>
<td>25%</td>
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<td>E. coli</td>
<td>AGCCGCCCTTAATGACGCGGCT</td>
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<td>TTTGCAGGTT</td>
<td>3%</td>
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<td>E. coli</td>
<td>AGCCGCCCTTAATGACGCGGCT</td>
<td>CTAATGA</td>
<td>TTTGCAGGTT</td>
<td>3%</td>
</tr>
<tr>
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<td>E. coli</td>
<td>AGCCGCCCTTAATGACGCGGCT</td>
<td>CTAATGA</td>
<td>TTTGCAGGTT</td>
<td>3%</td>
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<td>rmmB t1</td>
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<td>TTTGCAGGTT</td>
<td>3%</td>
</tr>
<tr>
<td>tonB t</td>
<td>Forward</td>
<td>E. coli</td>
<td>AGCCGCCCTTAATGACGCGGCT</td>
<td>CTAATGA</td>
<td>TTTGCAGGTT</td>
<td>3%</td>
</tr>
<tr>
<td>trp a</td>
<td>Forward</td>
<td>E. coli</td>
<td>AGCCGCCCTTAATGACGCGGCT</td>
<td>CTAATGA</td>
<td>TTTGCAGGTT</td>
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<td>3%</td>
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<tr>
<td>T7Te</td>
<td>Forward</td>
<td>phage termi</td>
<td>GCCCTACCTTCGAGGTCGCC</td>
<td>TTCG</td>
<td>TTT</td>
<td>88%+2%</td>
</tr>
<tr>
<td>T3Te</td>
<td>Forward</td>
<td>phage termi</td>
<td>GCCCTACCTTCGAGGTCGCC</td>
<td>TTCG</td>
<td>TTT</td>
<td>88%+2%</td>
</tr>
<tr>
<td>tR2</td>
<td>Forward</td>
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<td>TTCG</td>
<td>TTT</td>
<td>88%+2%</td>
</tr>
<tr>
<td>tRNA I</td>
<td>Forward</td>
<td>phage termi</td>
<td>GCCCTACCTTCGAGGTCGCC</td>
<td>TTCG</td>
<td>TTT</td>
<td>88%+2%</td>
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<tr>
<td>ampl35A mutant</td>
<td>Forward</td>
<td>E. coli</td>
<td>AGGAGGACGGTATCGAGGCC</td>
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<td>TTTTTATGGG</td>
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<td>tR2-6</td>
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<td>88%+2%</td>
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<td>phage termi</td>
<td>GCCCTACCTTCGAGGTCGCC</td>
<td>TTCG</td>
<td>TTT</td>
<td>88%+2%</td>
</tr>
</tbody>
</table>
The Testing Data set

Efficiency
(From TTEC prediction)

Efficiency (From Our Collected Database)
Outline

1. Background & Motivation
2. Terminator Efficiency Calculator
   - Algorithm
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   - Experiment
3. Human Practice
Experiment Design

Experiment schematic diagram

The Experimental Group

The Control Group
Experiment Procedure

1. Sequence Synthesis
2. Plasmid Construction
3. Terminator Insertion
4. Fluorescence Measurement
5. Efficiency Analysis
Experiment in good agreement with software prediction. Efficiency from TTEC prediction vs. Efficiency from Experiment Data. The expression of RFP and GFP is in good agreement with the software prediction. Efficiency (From TTEC prediction) vs. Efficiency (From Experimental measured) with a correlation coefficient of 0.93.

The expression of RFP is well predicted by the software, with an efficiency that is consistent with the experimental data.
BBF RFC 90:
The Measurement of rho-independent Transcription Terminator Efficiency.

We hope all the terminator efficiency will be measured in our standard way.
Demo Time!
In the future

1. Measure more than 100 terminators;
   Get a more accurate equation;
   Create a new model.

Plan 1

Plan 2
Outline

1. Background & Motivation
2. Terminator Efficiency Calculator
   - Algorithm
   - Database
   - Experiment
3. Human Practice
Lecture 1
Introduction to Synthetic Biology

Lecture 2
Interesting Stories

Lecture 3
Central Dogma

Lecture 4
BioBrick and Basic Idea

Lecture 5
Lab Work
Audiences Rapidly Increase

Comments

“This lecture is very interesting and very easy to understand.”

“I’m not in this field and know nothing about synthetic biology, but now I’m quite interested in it!”
Thanks to:

Prof. Huang Wei (Hong Kong University)
Dr. Ying-Ja Chen (Synthetic Biology Center, MIT)
2012 iGEM team: Shenzhen
2012 iGEM team: Shenzhen-SUSTC-A
2012 iGEM team: OUC