Introduction

Transcription terminator is an essential part of biobrick circuits, but is not well characterized. We studied the rho-independent transcription terminators using both theoretical modeling and experimental analysis. We first developed a theoretical model to predict the secondary structure of terminators. From the secondary structure, we developed an algorithm that can calculate terminator efficiency. In the aspect of experiment, we constructed 100 terminators and measured the terminator efficiency by quantifying the GFP and RFP which are placed before and after terminators. The efficiency calculated from theoretical model fit quite well with experimental results. We also created a software Transcription Terminator Efficiency Calculator (TTEC) and a web server for people to calculate their terminators and built a database of terminator efficiency which we believe to be the largest database of such kind.

Algorithm

Step 1: Take direct input or open SBOL format file.
Step 2: Use RNA folding algorithm and terminator features to predict the secondary structure of terminator and recognize A tail, stemloop and T tail.
Step 3: D score (D) calculated based on the free energy of hairpin (H), T tail score (T) and the length of hairpin (L).

\[
D = T \times 18.16 \times H \times 96.59 \times L^{-116.87} \times \sum_{i=1}^{n} (0.9 \times x_{i-1} + 0.6 \times x_{i})
\]

Step 4: Predict terminator efficiency from D score.
(D) – efficiency (E) relationship: \( E = 1.449 \times D + 39.142 \)

Database

We built a database of terminators whose efficiency had been measured. Not only terminator efficiency, other information such as direction, category and structure are also included. Some of the data were from partsregistry and Biofab Terminator Library, others were from paper. So far, the database contains 75 terminators which we believe is best terminator database, and it will continue to be updated so it will be more useful for people.

Software

TTEC is the first published software to predict terminator efficiency. It takes DNA sequences as input and returns the terminator efficiency value. Both online tool and offline tool are available now.

Experimental Design

As the picture Figure 8 shows, Terminator to be characterized is flanked by two fluorescent proteins, RFP and GFP. A control group without terminator is shown at Figure 9. If the terminator is 0% efficient, then the GFP will have a high-level expression, just as the control group does. On the contrary, no GFP protein will be observed. We used flow cytometer to measure the fluorescence strength. By comparing the expression of GFP between experimental group and control group, we can calculate the terminator efficiency as \( E = \frac{S - S'}{S} \). Here S and S’ represent the fluorescence strength of GFP with and without terminator.

Results

We have tested 9 terminator sequences. Fluorescence microscope image (Figure 10) confirmed the GFP is expressed. We have achieved a good agreement between experimental efficiency and TTEC predicted efficiency. (Figure 11)

Conclusion

1. We have published first terminator efficiency calculator.
2. We characterized TTEC.
3. So far, we have measured efficiency of 9 terminators.
4. We built a comprehensive terminator database.

Human Practice

1. We have recorded 5 open classes and uploaded to the web aiming at giving people a whole picture of synthetic biology.
2. We have written an article to help people distinguish synthetic biology from genetic engineering.
3. We have held 3 speeches in high schools to motivate high school students to study synthetic biology and guide them to participate in IGEM High School Division.
4. We have made 8 posters to let people who attend our lectures have a further understanding of our project and synthetic biology.

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