Promoter Design, Characterization and Consequences

2009 Virginia Commonwealth University iGEM Team

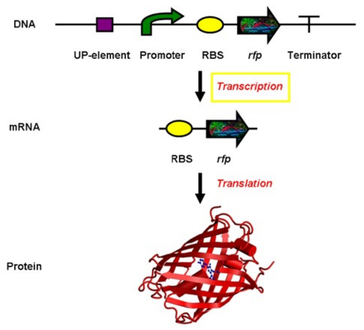
Craig Alberg, Adam Bower, Kevin Bussing, Richard C. Crenwelge IV, Maria McClintock and Afton Trent

Department of Chemical and Life Science Engineering, Virginia Commonwealth University, Richmond, VA

INTRODUCTION

The generation of well-characterized genetic parts is a prerequisite for the rational design and construction of reliable genetically-encoded devices and systems. However, most publicly available parts remain largely uncharacterized. Therefore, we propose a minimal measurement standard for the quantitative characterization of one of the most frequently used parts, promoters. This approach uses both mRNA and protein measurements to provide a tractable and universal analysis of relevant promoter characteristics.

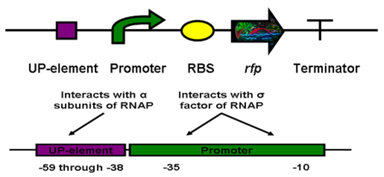
In an effort to elucidate promoter design principles, we have also designed and characterized new promoter and enhancer sequences. Our goal is to contribute to the advancement of fundamental synthetic biology by evaluating the performance of new and existing promoters and enhancers, which may serve as a model for describing other basic parts such as ribosome-binding sites and transcriptional terminators.



DESIGN

We designed a variety of UP-element BioBrick parts to serve as transcriptional enhancers and characterized them with our proposed minimal measurement standard. In addition to designing and characterizing UP elements, we were also able to further characterize existing constitutive promoters in the registry.

1



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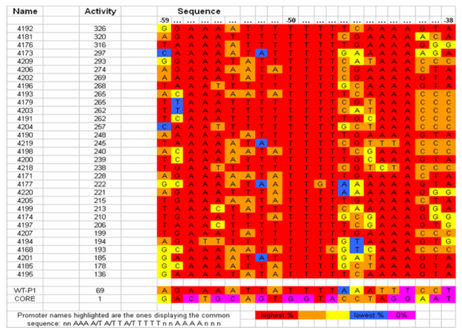


Fig.1 (top) - Up-element in context

Fig.2 (bottom) – Up-element as an enhancer for transcription initiation

Fig.3 (right) – Nucleotide sequences of different UP-elements. The color indicates the nucleotide frequency at that position.

UP-elements are known to significantly increase the RNA polymerase-recruiting power of promoters by interacting with the alpha subunit of RNA polymerase. Work has been done to identify a consensus sequence for this transcriptional enhancer based on 31 natural *E. coli* promoters (Estrem ST, Gaal T, Ross W, Gourse RL, Identification of an UP element consensus sequence for bacterial promoters, *PNAS*, (1998), 95, 9761-9766).

Our designs are based on the hypothesis that the variation of strength of the UP-element is due to different combinations of nucleotides in the non-conserved regions of the UP-element, and that there is some correlation between UP-element strength and the frequency of bases in the sequence. Therefore the strength of the UP-element can be tuned by mutating individual nucleotides of the consensus UP-element sequence using the statistical frequency of occurrence.

3

Position	-59	-58	-57	-56	-55	-54	-53	-52	-51	-50	-49	-48	-47	-46	-45	-44	-43	-42	-41	-40	-39	-38
UP Element																						
K264000 (From ref.)	G	G	A	A	A	A	T	T	T	T	T	T	T	T	C	A	A	A	A	G	T	A
K264001	T	A	A	A	A	T	T	T	T	T	T	T	T	T	G	A	A	A	A	G	T	A
K264002	T	G	A	A	A	T	T	T	T	T	T	T	T	T	G	A	A	A	A	G	T	A
K264003	A	A	A	A	A	T	T	T	T	T	T	T	T	T	G	A	A	A	A	G	T	A
K264004	A	G	A	A	A	T	T	T	T	T	T	T	T	T	G	A	A	A	A	G	T	A
K264005	G	A	A	A	A	T	T	T	T	T	T	T	T	T	G	A	A	A	A	G	T	A
K264006	G	G	A	A	A	T	T	T	T	T	T	T	T	T	G	A	A	A	A	G	T	A
highest %																						
lowest %																						

Fig. 4 - The table above shows the consensus UP-element as well as our designed UP-elements, respectively. The color indicates the mutations made to the consensus sequence.

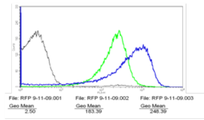
[1] Estrem ST, Gaal T, Ross W, Gourse RL, Identification of an UP element consensus sequence for bacterial promoters, PNAS, (1998), 95, 9761-9766.

[2] Ross W, Aiyar SE, Salomon J, Gourse RL, *Escherichia coli* Promoters with UP Elements of Different Strengths: Modular Structure of Bacterial Promoters, J Bacteriol, (1998), 180:20 p5375-5383

CHARACTERIZATION

Minimal measurement standard for part characterization

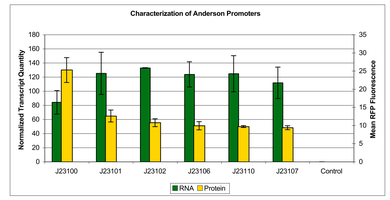
Real-time PCR ⟷ Flow Cytometry



References

Approach:

Existing BioBrick constitutive promoters were assembled and mRNA transcripts and RFP fluorescence were measured.



Result:

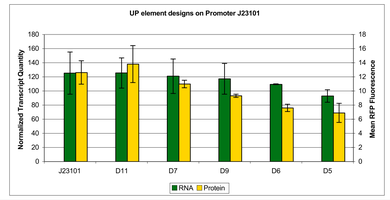
RFP measurements were consistent with previously reported results. mRNA transcript levels were inversely related to fluorescence levels.

Test Case I

Design Strategy/Hypothesis:

Strongest expression and fluorescence will occur in UP-element sequences containing bases with highest naturally occurring frequency

Strong D5 > D6 > D7 > D9 > D11 weak



Result:

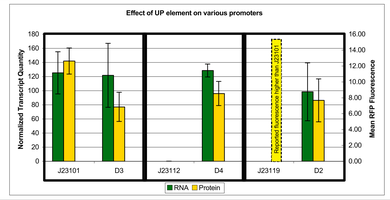
Strength of mRNA expression and RFP fluorescence were NOT directly correlated to highest naturally occurring frequency

Test Case II

Design Strategy/Hypothesis:

Adding the strongest predicted UP-element would increase the mRNA expression of promoters of different strength

Strong J23119 > J23101 > J23112 weak



Result:

Addition of an UP-element attenuated all of the mRNA and fluorescence levels to the same amount regardless of promoter strength.

CONCLUSIONS

Our project focused on design, construction, and characterization of biological components to achieve finer control over transcription of mRNA molecules. We focused on more detailed characterization of existing constitutive promoters and developed a new category of BioBrick parts (UP-elements) in attempt to achieve finer transcriptional control.

Registry Contributions		Conclusions from Results		Open Questions/Issues	
New BioBrick category created and parts deposited: UP-elements	New minimal standard for characterization of transcription: mRNA level and protein expression	UP-element appears to have a stronger effect on transcription than promoter strength	UP-element strength does not appear to correlate with DNA sequences with highest naturally-occurring frequency	Fluorescent protein expression (i.e. RFP) has a significant time delay (8-16 hours) which can affect the accuracy of measurements	Optimal UP-element spacing to promoter is 6 bases. BioBrick 3A assembly produces an 8 base scar. A new method of assembly may be needed if spacing is a concern.

Acknowledgements

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