A library of synthetic transcriptional AND gates built with split T7 RNA polymerase mutants

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The construction of synthetic gene circuits relies on our ability to engineer regulatory architectures that are orthogonal to the host's native regulatory pathways. We modified T7 RNA polymerase (T7 RNAP) to create a library of transcriptional AND gates for use in Escherichia coli by first splitting the protein and then mutating the DNA recognition domain of the C-terminal fragment to alter its promoter specificity. This work demonstrates that mutant split T7 RNAP can be used as a transcriptional AND gate and introduces a new library of components for use in synthetic gene circuits.

***Keywords* — T7 RNA Polymerase, AND gates, Split Proteins**

I. Purpose

Synthetic gene circuits provide valuable insights into bio-physical phenomena by enabling the construction and characterization of genetic systems from the ground up [1]. The continued development of synthetic gene circuits necessitates the development of additional parts and component libraries with which to build them [2].

T7 RNAP is a single-subunit RNA polymerase that is a strong driver of transcription. It is functionally orthogonal to most hosts, acting only on its cognate promoter, P_{T7}. During the purification of T7 RNAP, the protein was sometimes found cleaved between amino acids 179 and 180 to create a 20 kD N-terminal fragment and a 80 kD C-terminal fragment [3]. Only when mixed together, however, would the two fragments drive transcription from P_{T7} *in vitro*. Further, the promoter specificity of T7 RNAP is determined by a specificity loop near the C-terminus that forms specific contacts with P_{T7} [4]. Point mutations in the specificity loop have been demonstrated to significantly alter the promoter specificity of full length T7 RNAP, targeting the mutants to versions of P_{T7} that have been mutated between base pairs -11 to -8 [4,5].

II. Results

A. *In vivo activity of split T7 RNAP*

We found split T7 RNAP to be active *in vivo* albeit with reduced activity. We also an expression system using split T7 RNAP seems to be more stable than an expression system using full length T7 RNAP

B. *Characterizing the promoter specificity of split T7 RNAP*

Point mutants found to alter the activity of split T7 RNAP *in vitro* were found to function *in vivo.* When applied to split T7 RNAP, we identified a set of three mutant T7 promoters and three T7 RNAP point mutants to be functionally orthogonal to one another.

C. *Characterizing the two-input function of split T7 RNAP*

We find independent and inducible expression of split T7 RNAP supports an AND two-input function where a gene is expressed from PT7 if and only if both fragments of split T7 are expressed. We further find modified versions of split T7 are capable of producing different levels of maximal gene expression.

III. Conclusion

As a two components transcriptional activator, split T7 RNAP creates opportunities to create novel gene transcription networks. Because this library of AND gates was derived from a single component, it increases the number of available parts with which to build synthetic gene circuits. This, in turn, will enable the construction larger and more complex gene circuits.

References

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