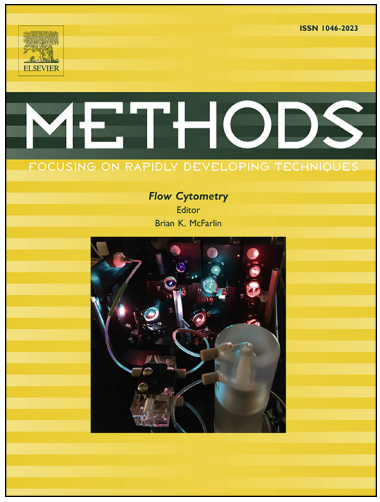

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Construction of synthetic T7 RNA polymerase expression systems

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Abstract:

T7 RNA polymerase (T7 RNAP) is one of the preferred workhorses for recombinant gene expression, owing in part to its high transcriptional activity and the fact that it has a small (17 base-pair), easily manipulated promoter. Furthermore, the fact that T7 RNAP is largely orthogonal to most hosts enables its use in a wide variety of contexts. However, the high activity of the enzyme also often leads to an increased fitness burden on the host, limiting the predictability of its interactions with and impact on physiology, and potentially leading to mutations to constructs. Here we use a synthetic biology approach to design and characterize a panel of T7 RNAP expression circuits with different modes of regulation that enable the reliable expression of downstream targets under a variety of conditions. First, we describe the construction of a minimal T7 RNAP expression system that is inducible by a small molecule anhydrotetracycline (aTc), and then characterize a self-limiting T7 RNAP expression circuit that provides better control over the amount of T7 RNAP produced upon induction. Finally, we characterize a so-called T7 RNAP homeostasis circuit that leads to constitutive, continuous, and sub-toxic levels of T7 RNAP. Coupled with previously characterized mutant T7 RNAP promoters *in vitro*, this modular framework can be used to achieve precise and predictable levels of output (sfGFP) *in vivo*. This new framework should now allow modeling and construction of T7 RNAP expression constructs that expand the utility of this enzyme for driving a variety of synthetic circuits and constructs.

Keywords

T7 RNA polymerase, synthetic circuits, feedback systems, gene expression