Title:

A Nanopore-Based Assay for High-Throughput Evaluation of Transcriptional Orthogonality in DNA Storage Systems

Abstract:

In living systems, DNA serves as a stable genomic repository, while RNA provides dynamic, environment-responsive outputs generated by transcription. A similar principle can be applied to DNA-based data storage: DNA acts as the immutable master archive, and RNA molecules serve as user-defined readouts triggered by specific queries. In this framework, transcription becomes the interface that selectively converts stored information into accessible formats.

Selective access critically depends on the orthogonality of RNA polymerase (RNAP)–promoter pairs. When each RNAP exclusively recognizes its cognate promoter, multiple independent information layers can coexist on the same DNA substrate. Distinct RNAP inputs thus operate as molecular queries, retrieving or activating different subsets of data without crosstalk. This enables parallel operations, hierarchical organization, and selective addressing within a single molecular storage pool.

Quantitative evaluation of RNAP–promoter orthogonality is therefore essential: it determines how reliably one channel can be accessed without interference and how many independent channels can be multiplexed. Beyond characterization, rational design or directed evolution of novel orthogonal RNAP–promoter pairs would expand the molecular “instruction set” available for DNA storage. These advances could transform DNA from a passive archival medium into an active, multi-layered storage and computation platform, where programmable transcriptional queries enable precise information retrieval.

Here, we present a nanopore-based assay to quantify RNAP–promoter orthogonality. Transcribed RNAs carry unique molecular identifiers (UMIs) that watermark their promoter of origin, allowing direct, scalable readout by nanopore sequencing. Unlike fluorescence-based assays constrained by spectral overlap, barcode-driven multiplexing supports simultaneous evaluation of tens to hundreds—or even thousands—of RNAP–promoter combinations. We will discuss ongoing results from this high-throughput framework and its potential to accelerate the development of orthogonal transcriptional channels for DNA-based data storage.