Lighting up transcription: a real-time assay breaks new ground

FAYETTEVILLE, GA, UNITED STATES, July 8, 2025 /EINPresswire.com/ -- A breakthrough assay is advancing how researchers observe gene expression in real time. Named QRIVTA (quantitative real-time in vitro transcription assay), this technique provides a sensitive, reproducible method for quantifying RNA production dynamics. By integrating fluorescence-based detection with a new kinetic model, QRIVTA delivers high-resolution insight into transcriptional regulation processes. Researchers demonstrated its precision by profiling how the transcriptional repressor H-NS regulates gene expression under various DNA topologies and temperatures. The platform's ease of use and compatibility with standard lab equipment open new avenues for both basic research and drug discovery.

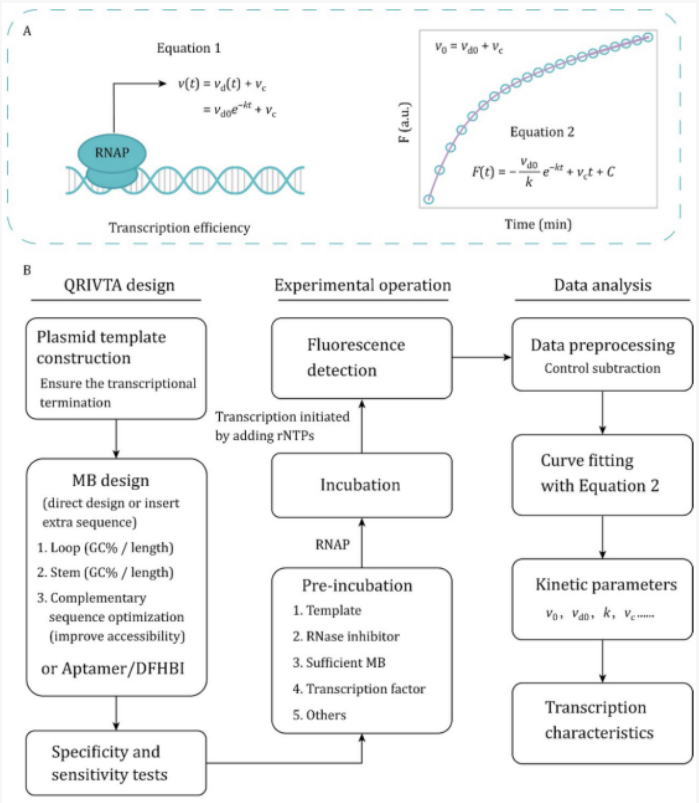

Illustration of the concept and workflow of QRIVTA.

Understanding how genes are turned on and off lies at the heart of molecular biology and disease research. In vitro transcription (IVT) assays have long served as essential tools for exploring these processes. However, conventional methods often rely on radioactive labeling, cumbersome protocols, and yield only qualitative or semi-quantitative results. Recent efforts to modernize IVT through fluorescence-based detection—such as using RNA aptamers or molecular beacons—have shown promise but lack standardized workflows and reproducible data analysis frameworks. Due to these challenges, there is an urgent need to develop a robust, standardized, and quantitative platform to advance transcriptional regulation research.

In a letter-style study (DOI: 10.1093/procel/pwae054) published on October 10, 2024, in Protein & Cell, researchers from Peking University and Soochow University introduced QRIVTA (quantitative real-time in vitro transcription assay)—a fluorescence-based assay designed to quantify transcription in real time. By optimizing detection strategies, plasmid architecture, and data

modeling, QRIVTA offers a unified, scalable workflow for analyzing gene expression. The team validated the platform using a model bacterial promoter (LEE5p) and explored how transcription is dynamically repressed by H-NS, a global regulatory protein, under physiological conditions. This work sets a new benchmark for IVT assays in molecular biology.

The core innovation of QRIVTA lies in its ability to capture the nuanced dynamics of transcription using a standard PCR machine. Researchers fine-tuned molecular beacon and RNA aptamer systems, improving signal specificity and sensitivity. They observed that fluorescence signals from supercoiled plasmids attenuate over time—not due to reagent depletion, but because of transcription-induced topological stress (e.g., positive supercoiling ahead of the RNA polymerase (RNAP) elongation complex). To quantify this, they proposed a new kinetic model (Equation 2) that fits the entire fluorescence curve with high accuracy, enabling extraction of key parameters like the initial transcription rate.

Using this platform, the team investigated how H-NS represses the LEE5 promoter from pathogenic E. coli. They found that repression increases steeply with H-NS concentration and is significantly stronger in supercoiled versus linear DNA (e.g., 74% versus 10% repression at 2 μmol/L H-NS)—highlighting the biological relevance of DNA topology. Additional experiments showed that the repressive effect of H-NS strengthens at lower temperatures (e.g., 19% at 30°C versus 9% at 37°C with 0.5 μmol/L H-NS), aligning with H-NS's temperature-dependent oligomerization. QRIVTA reliably captured these dynamics across multiple detection methods (MB1s, iSpinach, MB2), validating its robustness. The study also resolved technical issues such as nonspecific fluorescence and lag phases, offering best-practice guidelines for future users.

QRIVTA offers a long-needed solution for quantitative, real-time transcription analysis, said Prof. Bin Xia, one of the corresponding authors of the study. What makes it powerful is not just the assay itself, but the comprehensive framework—from plasmid design to data modeling—that ensures high sensitivity, specificity, and reproducibility. By using readily available lab instruments, QRIVTA enables labs of all sizes to dive deeper into the dynamics of gene regulation. The authors believe it will play a critical role in both academic and applied bioscience.

The implications of QRIVTA extend far beyond the lab bench. With its high-throughput capability and standardized workflow, the assay is quite suitable for screening transcriptional modulators, evaluating RNA-based therapeutics, and designing synthetic gene circuits. Its ability to simulate physiologically relevant supercoiled DNA structures makes it especially valuable for studies requiring precise transcription modeling. As transcriptional regulation emerges as a key focus in fields like synthetic biology, oncology, and virology, QRIVTA provides a timely and practical solution to meet growing analytical demands. Its integration into mainstream molecular biology could accelerate discoveries and innovations across a wide range of disciplines.

References
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Lucy Wang
BioDesign Research
email us here