ReciBio Pharm

Screening T7 Polymerases to Optimize xRNA Yield and Quality

RNA therapeutics and vaccines are revolutionizing medicine, with *in vitro* transcription (IVT) using T7 polymerase at the heart of their production. While wild-type T7 polymerases offer reliability, engineered variants are redefining performance with higher yields and lower impurities. ReciBioPharm is advancing the field by combining a robust knowledge library and advanced screening techniques to identify the optimal polymerase for each xRNA construct. Through this innovative process development approach, ReciBioPharm is accelerating development timelines, reducing costs, and setting new benchmarks for efficiency and quality in RNA manufacturing.

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Critical Role of IVT in xRNA Manufacturing

The production of xRNA modalities — such as messenger RNA (mRNA), circular RNA, and self-amplifying RNA — is most commonly powered by *in vitro* transcription (IVT) of DNA templates using RNA polymerases derived from bacteriophages. These templates are designed to encode the mRNA sequence, along with a 3′ poly-(A) tail that enhances stability, and a 5′ cap, which can be incorporated either during or after the IVT process. Together, these features are essential for both the stability and efficacy of xRNA therapeutics.

Although IVT bypasses cell-based systems, it is far from straightforward. The process involves more than a dozen interacting components, including nucleotides, buffers, and stabilizing agents. To achieve optimal yields and product quality, precise adjustments of key factors — such as the plasmid template, RNA polymerase, and capping agent, along with reaction conditions — must be tailored to the specific requirements of each xRNA construct.

The Engine of Innovation: T7 Polymerase in RNA Therapeutics

T7 polymerase, a DNA-dependent RNA polymerase derived from bacteriophage T7, is critical for nearly all xRNA manufacturing processes due to its ability to efficiently and specifically transcribe RNA from a DNA template, playing a pivotal role in the production of approved RNA therapeutics and vaccines. Available in both wild-type and engineered forms, T7 polymerases support research-scale and GMP-grade IVT reactions, making them indispensable in the development and production of RNA therapeutics.

Choosing the right T7 polymerase for a specific xRNA process involves multiple considerations. Foremost is the enzyme's performance, particularly its impact on xRNA yield and its ability to minimize double-stranded RNA (dsRNA) impurities — a critical quality parameter. Equally important are supply chain logistics, including the cost, availability, and consistency of GMP-grade materials, which can influence both project timelines and overall manufacturing costs.

Despite their reliability, wild-type T7 polymerases have inherent limitations, particularly in managing dsRNA impurities. This challenge has fueled the development of engineered T7 polymerases designed to address these deficiencies. Many engineered variants feature structural modifications that reduce the folding events responsible for dsRNA formation. Additionally, some engineered T7 polymerases improve the efficiency of capping reagent usage, a significant advantage given the high cost and proprietary nature of widely used capping reagents.

By balancing performance and supply considerations, the field continues to evolve toward more cost-effective and efficient T7 polymerase solutions, with engineered variants offering promising enhancements for next-generation xRNA manufacturing.

Data-Driven Selection of T7 Polymerases

To facilitate the selection of optimal T7 polymerases for different xRNA sequences, ReciBioPharm has built a knowledge library of commercially available T7 polymerases, DNA templates, and capping strategies, key raw materials used in the IVT process. The T7 library encompasses both wild-type (4 variants) and mutated (17 variants) T7 polymerases, each evaluated for their performance in the IVT process. To identify the most effective polymerases, ReciBioPharm employs an automatic high-throughput screening platform built on design-of-experiment (DoE) principles, utilizing 96-well plates and an advanced profiling system.

Each DoE screening tests ~40 IVT conditions — encompassing variables such as enzyme charge, capping reagent charge, buffer composition, pH, and reaction time. For every polymerase, full DOE experimental runs are conducted (two replicates per condition), ensuring robust data collection. Following this, the top conditions are selected and tested further, with multiple T7 polymerases evaluated per plate to minimize plate-to-plate variability. Key metrics — including yield, purity, double-stranded RNA (dsRNA) impurities, and potency — are measured using tools such as Nanodrop spectrophotometers, fragment analyzers, enzyme-linked immunosorbent assay (ELISA) analysis, and *in vitro* firefly luciferase (FLuc) expression tests (respectively).

This rigorous two-stage process is currently performed for each new xRNA construct. The initial screening examines a wide range of process conditions, while a secondary study narrows the focus to the top-performing polymerases and their optimal conditions, refining the process further. As the knowledge library expands, ReciBioPharm aims to streamline the workflow by eliminating the need for initial screening, significantly reducing both the time and cost required to develop optimal IVT processes for client constructs.

Precision Analytics in T7 Polymerase Development

The ongoing innovation in T7 polymerase development highlights the relative infancy of the xRNA manufacturing sector. Both the production processes and the analytics required for monitoring and product release are evolving rapidly, presenting opportunities and challenges alike.

One significant hurdle is the accurate analysis of dsRNA, a critical impurity that must be minimized for therapeutic xRNA products. However, the variability among analytical methods complicates this task. ReciBioPharm currently employs both commercial ELISA assays and an internal dot blot assay to measure dsRNA levels. Each method provides distinct results, driven by differences in format and antibody selection. While trends are consistent across methods, dot blot assays often yield values several-fold higher than those from ELISA, and their results can vary based on factors such as technician and timing.

To address these inconsistencies, the United States Pharmacopeia (USP) recommends ELISA assays for their operational consistency and suitability for quality control. However, the most critical factor is correlating assay results with *in vivo* data to set meaningful specifications. This approach ensures that impurity levels — regardless of the method used — align with the safety and efficacy requirements for clinical applications.

ReciBioPharm's flexibility in offering multiple assay options reflects its commitment to accommodating client preferences while adhering to best practices for dsRNA analysis, helping to advance the broader field of xRNA therapeutics.

The Economics and Efficiency of Wild-Type T7 Polymerases

ReciBioPharm has established robust supply chains for four wild-type T7 polymerases, available in both research and GMP grades. While these polymerases are theoretically identical in sequence, their performance in IVT reactions varies, likely due to differences in formulation and quality. As demonstrated in Figure 1, the polymerase from Supplier B delivers significantly higher yield compared with others, whereas the enzyme from Supplier D exhibits markedly higher levels of dsRNA impurities. Despite these differences, overall purity remains consistent across the suppliers. It is noted Supplier B offers T7 polymerase with a different enzyme activity unit compared to others.

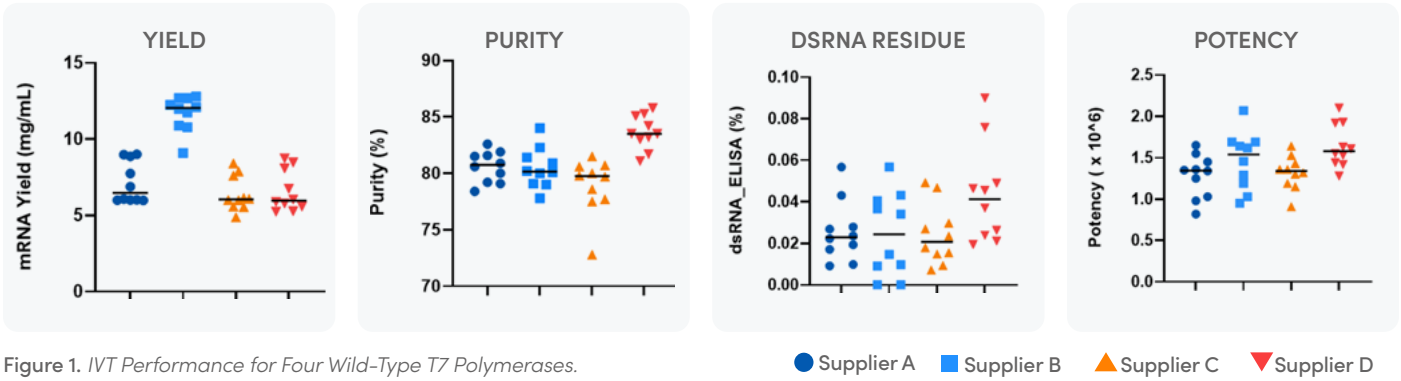

Figure 1. *IVT Performance for Four Wild-Type T7 Polymerases.*

To ensure reliability, the performance of these four polymerases was assessed across three GMP lots for each T7 polymerase under previously optimized IVT conditions designed to maximize yield, minimize dsRNA impurities, or enhance potency. As shown in Figure 2, these studies revealed consistent lot-to-lot performance for each enzyme, with only minor differences observed between suppliers in terms of yields and dsRNA levels.

Another crucial consideration is cost (Figure 3). T7 polymerase contributes approximately 20–30% of the total IVT process cost, making it a key economic factor. Supplier A offers the lowest-cost option, closely followed by Supplier B. However, Supplier B exclusively provides one grade to cover both research and GMP needs, which helps to reduce any potential risk during tech transfer from development to manufacturing stages as well as to simplify supply chain management. This highlights the importance of balancing performance, cost, and logistical considerations when selecting a T7 polymerase for xRNA manufacturing.

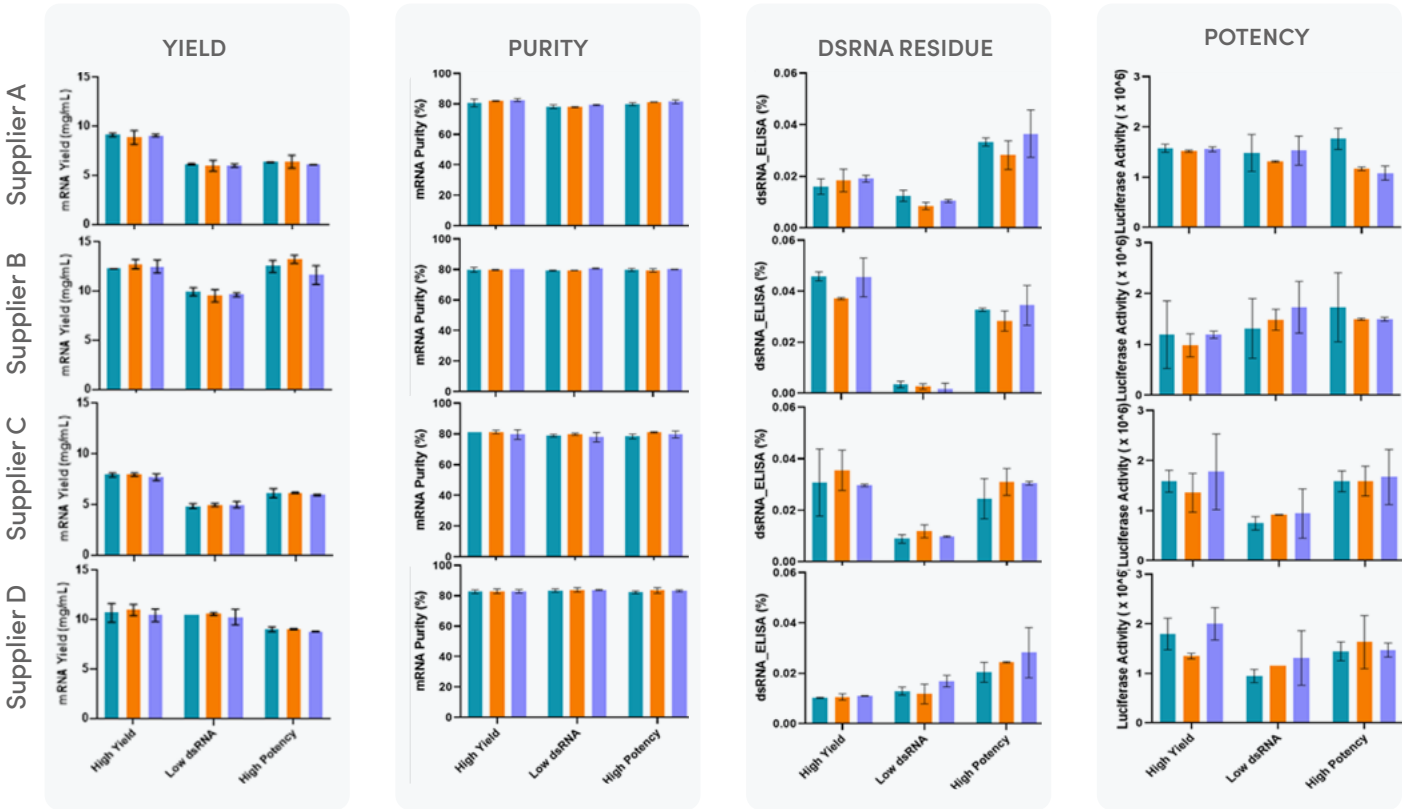

Figure 2. *Consistent Lot-to-Lot Performance for Wild-Type T7 Polymerases.*

The Economics and Efficiency of Wild-Type T7 Polymerases

Figure 3. *Cost Comparison for Four Wild-Type T7 Polymerases.*

Enhancing Yield and Purity with Engineered T7 Polymerases

To explore the potential of engineered T7 polymerases in enhancing xRNA manufacturing, ReciBioPharm conducted a comprehensive DoE study involving 18 wild-type and modified variants (3 modified T7 polymerase are eliminated from this study due their undesired performance form initial screening). These polymerases demonstrated noticeable variability in performance, but overall, they outperformed wild-type enzymes in several key metrics. As illustrated in Figure 4, modified T7 polymerases yielded slightly higher mRNA yields, significantly reduced dsRNA impurity levels, and modestly enhanced potency of the xRNA products. Most strikingly, the dsRNA impurity levels remained consistently lower across various reaction conditions, showcasing the robustness of these engineered enzymes.

Building on these promising findings, a second round of screening is planned to refine the selection of top-performing modified T7 polymerases. This next phase will focus on more challenging mRNA constructs known to exhibit low yields or high dsRNA levels when processed with wild-type enzymes. Additionally, capping efficiency — a critical factor in xRNA stability and efficacy — will be incorporated into the evaluation matrix, further enriching the screening process.

This iterative approach exemplifies ReciBioPharm's commitment to advancing xRNA manufacturing technologies, ensuring that both performance and cost-efficiency are optimized for a diverse range of therapeutic applications.

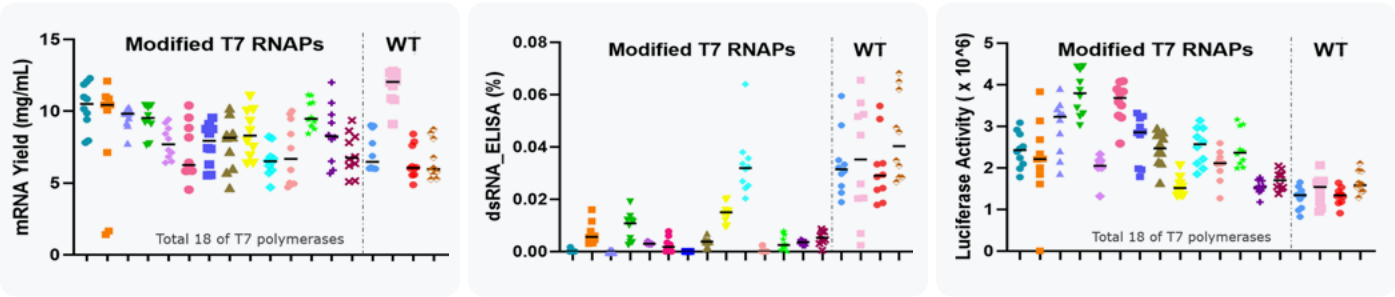

Figure 4. *Initial screening results for selected modified T& polymerases.*

Why Wild-Type T7 Polymerases Still Lead the Pack

Despite the growing appeal of modified T7 polymerases, wild-type variants remain the preferred choice for many xRNA manufacturing processes, primarily due to their well-established supply chains and logistical reliability. These polymerases, while not delivering the highest yields or the lowest dsRNA impurities, provide a dependable supply of GMP-grade material, ensuring process stability and scalability. In contrast, the newer modified polymerases, though promising in performance, pose higher supply risks due to their limited market availability.

ReciBioPharm's ongoing DoE studies with modified T7 polymerases are yielding invaluable data, enabling the company to expand its knowledge library and refine its screening process. Moving forward, the company plans to use wild-type polymerases for initial screenings. If specific challenges are identified, a secondary screening will be performed with the top three or four selected modified polymerases, based on insights from advanced studies.

This strategic approach aims to streamline and accelerate the polymerase selection process, reducing the evaluation timeline from several months to just one to two weeks. By balancing the reliability of wild-type polymerases with the enhanced performance potential of modified variants, ReciBioPharm continues to advance the field of xRNA manufacturing with a focus on efficiency and innovation.

About us

ReciBioPharm, a division of Recipharm, is a contract development and manufacturing organization (CDMO) specifically established to focus on serving companies seeking to develop and commercialize advanced therapy medicinal products (ATMPs). ReciBioPharm's specialized CDMO capabilities include pre-clinical to clinical and commercial development and manufacture for new biological modalities encompassing technologies based on live viruses and viral vectors, live-microbial biopharmaceutical products, nucleic acid-based mRNA and plasmid DNA production. Led by a management team and technical experts with a proven track record in both process development and contract manufacturing, ReciBioPharm offers the knowledge and resources necessary to help customers develop and manufacture promising new therapies to meet the needs of patients across the world.

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