

Enabling cost-effective, rapid access to RNA vaccines and therapeutics with enzymatically generated z-dbDNA



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Synthetic dbDNA™ from Touchlight offers transformative advantages over fermentation-derived plasmid DNA (pDNA), including streamlined workflows, reduced manufacturing footprint, greater purity, and enhanced scalability. By enabling higher RNA yields with less template favourable quality profiles, z-dbDNA provides an efficient, cost-effective solution for producing mRNA, self-amplifying mRNA (saRNA), and other RNA-based therapeutics and vaccines, empowering rapid and reliable biopharmaceutical innovation.

RNA Therapeutics: A Growing Frontier

The global success of messenger RNA (mRNA) COVID-19 vaccines has sparked a surge of interest in RNA-based vaccines and therapeutics across the biopharmaceutical sector. This momentum is not limited to messenger RNA; saRNA and circular RNA are also making significant strides, progressing from preclinical stages to human clinical trials. These RNA modalities are being actively explored for diverse applications, including gene replacement therapy, gene editing, personalised cancer vaccines, and vaccines for a broad spectrum of diseases.

RNA-based therapeutics represent a transformative shift in medicine, offering novel approaches to targets previously considered to be unsuitable for nucleic acid — based modalities. The flexibility of RNA technology allows for rapid adaptation and development, enabling applications in both common and rare diseases. According to *MarketsAndMarkets*, the global RNA therapeutics market, encompassing mRNA, siRNA, and antisense oligonucleotides, is projected to grow from \$13.7 billion in 2023 to \$18.0 billion by 2028, with a compound annual growth rate of 5.6%.¹

Adding to this rapid expansion, RNA's potential has extended beyond traditional vaccine development.

It is now being employed as a transient delivery mechanism for gene editing tools, such as CRISPR nucleases and transposases, due to RNA's ability to express proteins quickly and degrade naturally after completing its function. This adaptability, combined with the urgent demand for scalable manufacturing solutions, underscores RNA's critical role in the future of medicine.

Synthetic DNA Advances: Cleaner, Faster, Smarter

Synthetic DNA manufactured using enzymatic processes in a cell-free manner is generally obtained in high yields using simpler processes with smaller footprints than those involved with bacterial fermentation to produce pDNA. It also does not contain bacterial or antibiotic resistance genes. These attributes translate into substantial time and cost savings, making it an attractive alternative for the production of RNA therapeutics and other advanced therapies. In addition, use of recombinant enzymes enables the production of complex and homopolymeric sequences that are incompatible with production in *Escherichia coli*.

Importantly, modern synthetic DNA technologies utilise engineered enzymes that achieve exceptionally high fidelity. Touchlight's proprietary dbDNA™ (doggybone™ DNA) exemplifies this advancement, delivering high yields while maintaining fidelity across complex DNA sequences up to 20 kilobases. By integrating such high-fidelity processes with advanced analytical methods, synthetic DNA producers can further ensure consistent quality and robust performance across applications.

dbDNA and z-dbdNA: Breaking Barriers in RNA Manufacturing

Touchlight's dbDNA and z-dbdNA redefine DNA manufacturing with its streamlined, cell-free production process. This DNA is produced via rolling circle amplification from a small amount of plasmid starting material and is completely free of bacterial sequences. By employing amplification and processing enzymes, Touchlight achieves rapid, high-quality DNA synthesis within a compact manufacturing footprint and supports the incorporation of genes of interest up to 20 kilobases in length, accommodating far greater complexity than traditional pDNA fermentation.

A standout feature of Touchlight's enzymatic DNA is its efficiency at scale. Production at under 10-litre scale can yield as much high-fidelity material as bacterial fermentation processes requiring 200 to 400 litres. With over 1,500 batches (as of Q2 2024) successfully produced, ranging from 600 base pairs to 20 kilobases across various modalities, Touchlight's expertise ensures unparalleled quality and adaptability.

Touchlight's z-dbdNA, derived from highly pure and well-characterised dbDNA, is tailored for in vitro transcription (IVT) through the removal of the 3' cap at the end of the poly(A) tail. z-dbdNA is available in diverse grades —Research, Smart-GMP, and GMP — enabling applications from small-scale experimentation to full-scale commercial programs.

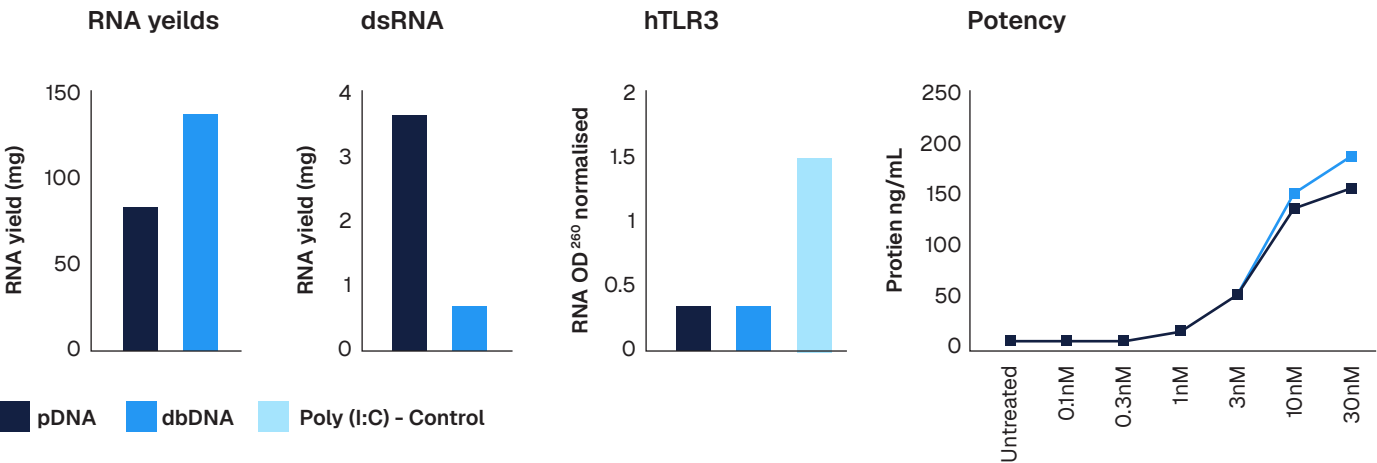
Overcoming RNA Manufacturing Challenges with z-dbdNA

Creating pDNA templates for RNA production often presents significant challenges, particularly when long poly(A) tails (80–120 units) are required for mRNA or when tackling the substantial length of saRNA. Touchlight's z-dbdNA provides an innovative solution, enabling the robust production of mRNA, saRNA, and circular RNA. Using comprehensive analytical techniques, Touchlight ensures control over critical elements like poly(A) tails, from gene synthesis and template production through the z-dbdNA production process. This precision delivers the consistency needed to support advanced RNA therapeutics and vaccines.

z-dbdNA offers several additional benefits for RNA synthesis. Its high purity stems from using the pure and well-characterised dbDNA as a starting point, benefitting from the robustness of the downstream purification employed for dbDNA. This foundational quality leads to fewer impurities, enhancing the overall RNA product. Additionally, the manufacturing process for dbDNA and z-dbdNA requires a much smaller footprint than traditional pDNA production, reducing costs and enabling fast and agile scale up to meet the target requirements. The use of z-dbdNA in RNA manufacturing is characterised by reduced template concentrations and improved yields. These efficiencies make scaling up z-dbdNA production significantly easier than scaling up fermentation based pDNA processes.

For many clients, Touchlight's z-dbdNA represents a transformative option, particularly when facing obstacles in pDNA-based manufacturing. Common challenges include quality issues, reproducibility problems, or limited access to GMP manufacturing capacity. Touchlight's z-dbdNA production method, as compared to other synthetic DNA methods, avoids the need to ligate poly(A) tails, greatly simplifying the DNA template workflow, enabling greater scalability and resulting in fewer impurities. Touchlight's ability to deliver high-quality z-dbdNA templates quickly and reliably helps clients overcome these barriers, ensuring their drug development programs stay on track. Beyond solving immediate issues, Touchlight also supports clients seeking to optimise their processes, offering cost and quality improvements that can reshape long-term production strategies.

Figure 1. Comparison of mRNA yields, dsRNA, immunogenicity, and potency between pDNA and dbDNA.



A Game-Changer for RNA Production

Touchlight's z-dbDNA demonstrates significant advantages as a template for mRNA production. Its suitability as an IVT template was demonstrated in a client study (**Figure 1**). Using a customised IVT protocol, the client observed a twofold increase in mRNA yield with z-dbDNA compared with pDNA, using copy-matched template amounts. In addition to these higher yields, RNA produced with z-dbDNA exhibited reduced levels of dsRNA (as confirmed by Sandwich ELISA) and with a favourable profile from a TLR3 immunogenicity assay. The study also highlighted the comparable potency of z-dbDNA derived RNA, with HEK293 transfection assays confirming similar levels of secreted protein across various RNA concentrations.

A further examination of template quantities in the IVT reaction was done in-house using commercially available IVT kits (HiScribe (NEB) and MEGAscript (Thermo Fisher Scientific)). Reactions contained template DNA ranging from 2.5 to 50 ng/ μ L (50– 1000 ng total dbDNA or pDNA encoding the firefly luciferase gene) and were incubated for 2 hours at 37 °C. Following RNA purification using Monarch (NEB) spin columns, RNA yields were assessed via UV spectrometry, and double-stranded RNA (dsRNA) content was determined using a J2 monoclonal antibody-based Sandwich ELISA assay. Results consistently showed that dsRNA levels were low for both pDNA and z-dbDNA templates while RNA yields were significantly higher at lower template amounts when using z-dbDNA (**Figure 2**).

Figure 2. Comparison of dbDNA vs. pDNA templates in IVT reactions using standard kit

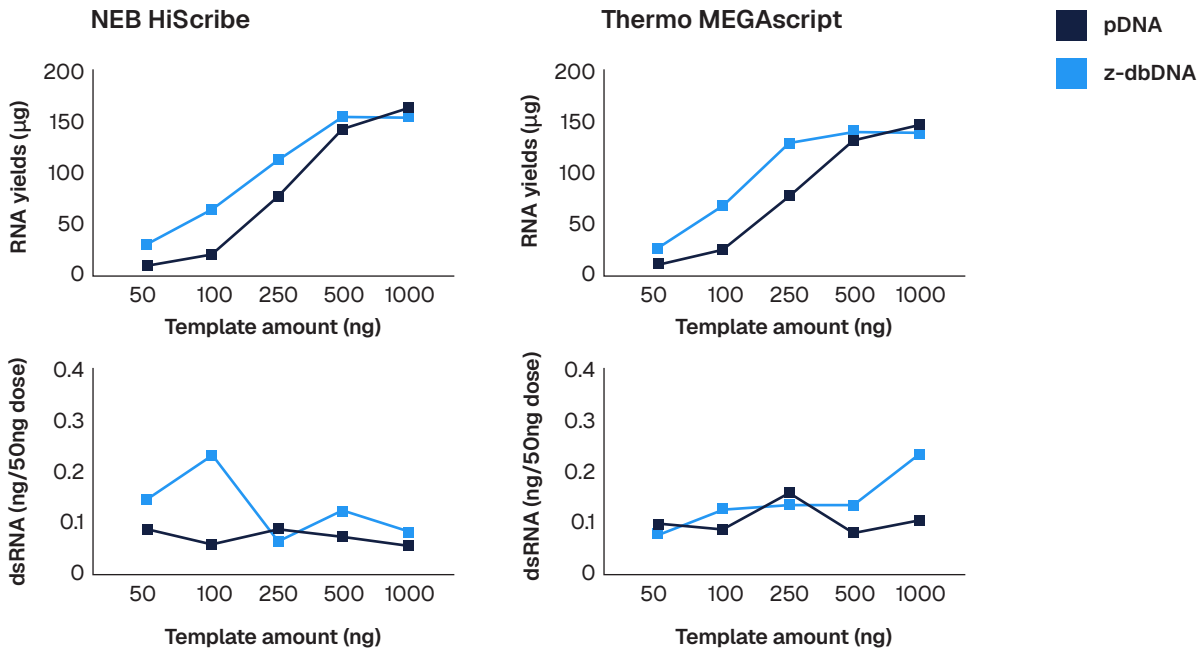
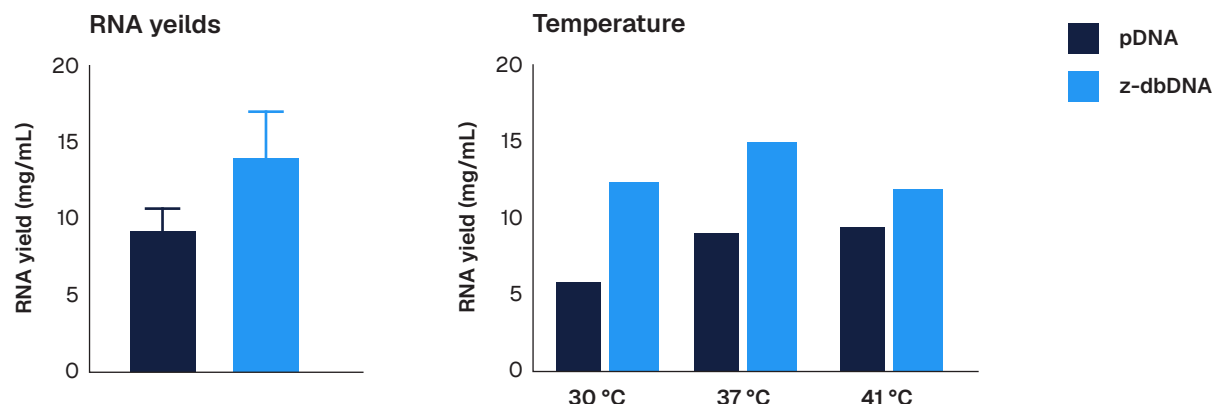


Figure 3. Improved RNA yield with less dbDNA at three temperatures



These results underscore the transformative potential of z-dbDNA in mRNA production. By providing increased RNA yields, and favourable immunogenicity and potency, z-dbDNA offers a superior alternative for clients aiming to enhance the scalability and performance of RNA-based therapeutics and vaccines.

saRNA Production Simplified with z-dbDNA

Touchlight's z-dbDNA technology offers distinct advantages for the production of large, complex saRNA molecules. By utilising z-dbDNA, RNA yields are significantly improved across various temperatures, all while requiring less template DNA compared with plasmid DNA (pDNA). Furthermore, saRNA generated from z-dbDNA demonstrates activity equal to or greater than that of pDNA-derived saRNA,

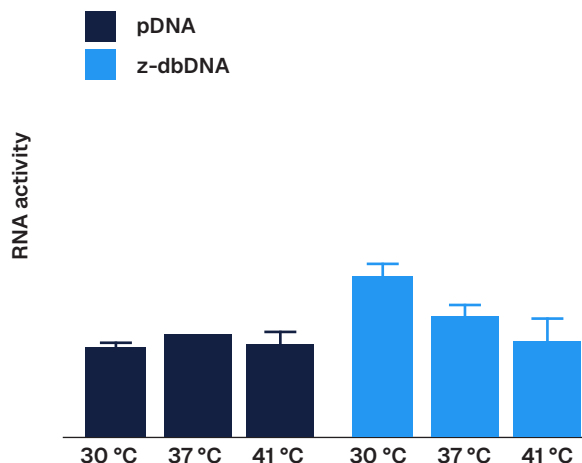
making it a highly efficient and cost-effective solution for scaling up saRNA production. Results from a client study with the CDMO Curia showcasing these benefits are illustrated in **Figures 3 and 4**.

In this study, optimised conditions were employed for IVT reactions using both pDNA and z-dbDNA templates. Five DNA template concentrations (25–125 mg/L) were evaluated across multiple temperatures (30°C, 37°C, and 41°C).

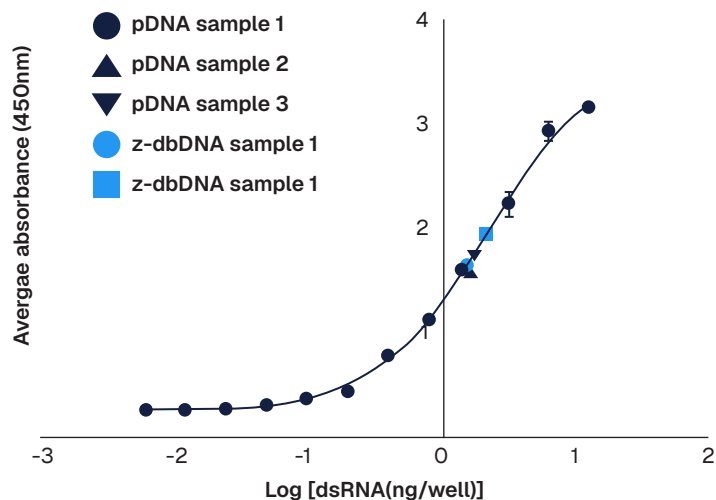
These data showed a 1.5-fold increase in saRNA yield was achieved using z-dbDNA compared with pDNA, using copy-matched z-dbDNA template input, a key advantage of the functionally smaller z-dbDNA. This improved efficiency, coupled with comparable levels of residual double-stranded RNA (dsRNA), underscores z-dbDNA's ability to deliver high-quality saRNA while reducing template usage.

Figure 4. z-dbDNA-derived saRNAs exhibit higher activity than pDNA-derived saRNAs

Cell expression assay



Residual dsRNA ELISA



In addition to enhanced yields, z-dbdNA derived saRNA exhibited significantly greater activity than saRNA produced using pDNA at the lowest temperature.

Through the combination of higher yields, improved activity, and reduced template requirements, z-dbdNA positions itself as a pivotal technology for large-scale saRNA production, with significant implications for rapid pandemic response and the broader development of RNA-based therapies.

Enabling Rapid Pandemic Response

In the race to make vaccines widely available during a global pandemic, achieving a development and manufacturing timeline of 100 days or less is critical. Synthetic DNA technologies like Touchlight's z-dbdNA play an essential role in meeting this ambitious goal. The enzymatic production process provides unparalleled flexibility, offering simple workflows, rapid setup, and easy scalability. These features, combined with the smaller manufacturing footprint, enable RNA material to be produced in weeks rather than months — an essential advantage during health emergencies.

Beyond speed, the adaptability of z-dbdNA ensures it can be implemented across diverse geographic locations, facilitating in-country vaccine manufacturing. This capability enhances vaccine equity by reducing logistical barriers and ensuring rapid local supply, particularly in regions with limited traditional vaccine infrastructure. As part of a global mission to mitigate pandemic risks, z-dbdNA exemplifies how cutting-edge synthetic technologies are transforming vaccine development and ensuring equitable access worldwide.

Strategic Partnerships Powering Synthetic DNA Adoption

To accelerate the adoption of synthetic DNA for RNA therapeutics and vaccine production, Touchlight has established strategic partnerships with leading pharmaceutical companies and contract manufacturers, leveraging its innovative dbDNA and z-dbdNA technologies.

In 2022, Touchlight entered a landmark patent license agreement with Pfizer, enabling the use of z-dbdNA in the manufacture of mRNA-based therapeutics. This collaboration underscores the critical role of Touchlight's synthetic DNA technology in advancing cutting-edge medical solutions. Building on this momentum, GlaxoSmithKline (GSK) signed a similar license agreement in mid-2024 to incorporate z-dbdNA production technology for rapid DNA template generation to support mRNA manufacturing.

That same year, Touchlight signed agreements with established CDMOs Curia and Lonza, providing streamlined access to z-dbdNA for clients seeking improved manufacturing solutions. These partnerships not only expand Touchlight's footprint in the biopharmaceutical landscape but also reaffirm the versatility and value of its synthetic DNA technologies in a rapidly evolving industry.

Expanding Boundaries in Synthetic DNA and RNA Therapeutics

Touchlight is paving the way for a brighter future in synthetic DNA and RNA therapeutics through strategic initiatives and groundbreaking achievements. To ensure supply chain resilience, the company has established partnerships with GMP suppliers, implemented dual sourcing for critical raw materials, and secured immediate access to off-the-shelf consumables — eliminating the need for lengthy customisation lead times. In 2023, Touchlight tripled its production capacity for dbDNA to 8 kilograms per year, a milestone that underscores its readiness to meet increasing demand for advanced therapies.

Touchlight's journey is marked by several industry firsts: it was the first company to secure 100 granted patents in synthetic DNA, produce GMP-grade materials, scale production to multi-gram levels, be granted a Drug Master File (DMF) with the FDA, and enter human clinical studies, including the first pivotal trial expected in 2025. Today, hundreds of biotech and pharmaceutical companies rely on Touchlight's z-dbdNA as a critical starting material for manufacturing RNA, gene-editing templates and viral vectors (AAV and lentivirus). Additionally, dbDNA serves as an active pharmaceutical ingredient for DNA vaccines and non-viral gene therapy candidates.

These accomplishments represent just the beginning of dbDNA's transformative potential. Looking ahead, Touchlight anticipates even greater productivity and cost efficiencies for advanced therapies, particularly RNA-based modalities. With an unwavering commitment to innovation and patient-centric solutions, Touchlight is poised to unlock new possibilities for therapy developers, revolutionising the landscape of modern medicine.

References

RNA Therapeutics Market: Size, Share, and Growth Insights. *MarketsAndMarkets*. 9 Jun. 2024.

About the author



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Lisa has two decades of experience working in therapeutics discovery including developing the Touchlight platform across multiple application areas for the last 15 years, specialising in DNA API. She has managed internal and external multi-functional teams within academia and industry. She has BSc honours and PhD from the University of Edinburgh and following a post-doc, joined the vaccine development company, Emergent Biosolutions. She joined Touchlight in 2009, leveraging her experience in molecular biology and immunology to further the understanding of dbDNA use *in vivo*.

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