

White Paper | PlasmidFactory

Minicircle DNA for LNP-Based Delivery: How Cargo Architecture Influences LNP Delivery



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Minicircle DNA for LNP-Based Delivery: How Cargo Architecture Influences LNP Delivery

Introduction

Lipid nanoparticles (LNPs) are one of the most promising routes toward scalable, virus-free delivery. But the next bottleneck in LNP delivery approaches might not be the particle itself, but the cargo it carries.

LNPs have already demonstrated their clinical relevance in nucleic acid delivery, most prominently through mRNA-based therapeutics and vaccines [1]. At the same time, LNP platforms are increasingly being explored beyond classical mRNA delivery, including broader use cases for RNA, DNA, proteins, peptides, small molecules, and other therapeutic payloads [2].

For cell and gene therapy, this creates a strategic question: **Which cargo architecture best fits the biological goal?** RNA is highly attractive when rapid, cytoplasmic expression is desired. DNA becomes interesting when expression should be more sustained, transcriptionally controlled, or suited for more complex genetic payload designs. However, conventional plasmid DNA brings specific challenges for LNP-based delivery. Plasmids are relatively large, contain bacterial backbone sequences, and can present challenges related to formulation, intracellular delivery, and transgene expression [3, 4].

Minicircle DNA offers a different DNA architecture.

Minicircle DNA is compact, supercoiled, and free of functional bacterial backbone sequences such as antibiotic resistance genes and bacterial origins of replication. This makes Minicircle DNA one of the most compelling DNA cargo formats for LNP-based delivery, especially where compact size, reduced bacterial backbone burden, and robust expression are desired.

Recent PlasmidFactory and partner data show that Minicircle DNA can be packaged into LNPs, delivered into cells, and support favorable particle characteristics and improved expression performance compared with plasmid DNA under tested conditions [5, 6].

Key Findings

1. LNPs Enable Scalable Virus-Free Delivery

Cell and gene therapy increasingly requires scalable, flexible delivery systems independent of viral vectors to reduce regulatory burden, production costs, and timelines while maximizing safety.

LNPs are attractive because they can protect therapeutic cargo, support cellular uptake, and enable intracellular delivery. Their success in RNA delivery has accelerated interest in LNPs as broader non-viral delivery platforms [1, 2].

For next-generation therapies, LNP-based delivery may become relevant for:

- non-viral cell engineering
- ex vivo and in vivo gene delivery approaches
- transient or sustained expression strategies

LNPs are therefore becoming more than a delivery technology and increasingly represent a broader platform concept whose success depends not only on lipid composition, particle size, and formulation conditions, but also on the cargo itself.

2. DNA Cargo Offers Capabilities Beyond mRNA

mRNA has become a highly effective cargo for LNP-based delivery because it can be translated directly in the cytoplasm and therefore enables rapid, transient protein expression without the need for nuclear entry.

DNA cargo offers a different biological opportunity. Once delivered to the nucleus, DNA can serve as a transcriptional template and support promoter-driven expression. Depending on construct design and application, this can enable more sustained expression profiles, larger or more complex genetic payload designs, and, when combined with suitable integrating systems such as transposons or genome-editing approaches, stable genetic modification strategies [3, 7].

This makes DNA interesting for applications where:

- a more sustained expression profile compared to mRNA is desired
- promoter-driven expression is required
- the payload requires DNA-based architecture
- transcriptional control is important

However, DNA delivery also comes with additional biological and technical requirements. Unlike mRNA, DNA must become available in the nucleus to drive expression, making nuclear delivery and intracellular trafficking important barriers [8].

The challenge is therefore not only to formulate DNA into LNPs, but to ensure that the DNA cargo architecture is compatible with efficient LNP-mediated delivery. Compared with mRNA, DNA differs in molecular size, structure, rigidity, and intracellular requirements. These factors can influence formulation behavior, cellular delivery, nuclear availability, and ultimately expression performance.

3. Limitations Of Conventional Plasmid DNA

Conventional plasmids are powerful and widely used DNA tools. However, most established and commercially available LNP systems have been optimized primarily for other nucleic acid cargos, particularly mRNA, rather than for the delivery of large plasmid DNA molecules. The latter contain the expression cassette of interest, but also bacterial backbone elements required for propagation and selection in bacteria, which increase overall molecule size without contributing to the intended therapeutic payload.

For LNP delivery, this matters because plasmid size and architecture can influence:

- particle formation
- particle size
- polydispersity
- formulation stability
- cellular uptake
- nuclear availability
- expression performance

In addition, bacterial backbone sequences have been associated with reduced or silenced transgene expression in several non-viral DNA delivery contexts [4, 9].

The key question is not only whether DNA can be delivered by LNPs, but which DNA format is best suited as LNP cargo.

4. Minicircle DNA And The Role Of Backbone-Free Design

The Minicircle is a small, supercoiled and monomeric DNA molecule reduced to the expression cassette of interest. Unlike conventional plasmids, Minicircle DNA is free of functional bacterial backbone sequences such as antibiotic resistance genes and bacterial origins of replication.

Minicircle DNA offers several advantages for LNP-based delivery concepts:

- potentially lower immunogenicity due to reduced bacterial sequence burden
- reduced risk of bacterial-backbone-associated transgene silencing
- smaller size compared with the corresponding plasmid
- higher transfection efficiency and stronger expression in relevant non-viral delivery settings
- potentially improved LNP formulation behavior

Several studies have shown that Minicircle DNA can support stronger or more persistent transgene expression compared with conventional plasmid DNA in non-viral gene delivery settings [4, 9, 10].

PlasmidFactory has already supplied Minicircle DNA for multiple clinical studies involving genetically modified cells, demonstrating the translational relevance of this DNA format beyond early-stage research. Importantly, Minicircle DNA is available from Research Grade up to GMP Grade, enabling developers to evaluate the technology in exploratory studies and subsequently advance the same DNA format toward clinical and commercial applications. In the following chapter, we will present recent research data comparing Minicircle DNA with conventional plasmid DNA in LNP-based delivery settings. These findings provide further evidence for the potential advantages of Minicircle DNA as an LNP cargo format, particularly with regard to its compact size, reduced bacterial sequence burden, and transgene expression performance.

In short: **Minicircle DNA provides a compact, backbone-free DNA architecture that is particularly well suited for further evaluation as cargo in LNP-based delivery applications.**

5. Proof-of-Concept Data: Minicircle DNA As LNP Cargo

Recent PlasmidFactory and partner data support the relevance of Minicircle DNA as a compact, bacterial backbone-free DNA cargo for LNP-based delivery [5, 6].

Two proof-of-concept data sets are particularly relevant:

- PROGEN / ZentriForce / NanoFCM & PlasmidFactory partner study: characterization of ready-to-use LNP delivery kits for intracellular delivery of nucleic acids. This data set evaluated LNP particle characteristics and reporter gene expression after delivery of plasmid DNA and Minicircle DNA constructs [5].
- Bielefeld University & PlasmidFactory partner study: evaluation of SM-102 LNP-mediated delivery of Minicircle DNA into HEK cells, including time-course analysis of reporter gene expression compared with plasmid DNA and mRNA [6].

Together, these data address three central questions:

1. Does DNA cargo architecture influence LNP particle characteristics?
2. Does Minicircle DNA support functional expression after LNP-mediated delivery?
3. Can Minicircle DNA support a more sustained expression profile after LNP delivery?

The results indicate that Minicircle DNA can be packaged into LNPs, delivered into cells, and support favorable particle characteristics and reporter expression compared with conventional plasmid DNA under the tested conditions [5, 6].

5.1. Cargo Architecture Affects LNP Particle Characteristics

The PROGEN / ZentriForce / NanoFCM & PlasmidFactory partner study used the PROGEN mRNA LNP Delivery Kit to evaluate whether LNPs formulated for mRNA delivery could also be used for DNA cargo delivery [5].

For this purpose, plasmid DNA and corresponding Minicircle DNA (mcDNA) constructs encoding reporter genes were loaded into LNPs and characterized using Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS) based particle analysis.

The central question was whether the DNA cargo format affects LNP particle properties.

As shown in Tables 1 and 2 and Figures 1 and 2, the data presented showed a clear difference between plasmid DNA-loaded and Minicircle DNA-loaded LNPs. Plasmid DNA-loaded LNPs showed substantially larger particle diameters and a higher polydispersity index, while Minicircle DNA-loaded LNPs showed particle characteristics closer to empty LNPs.

NTA Analysis of DNA-loaded LNPs

Table 1

Sample*	Particle Concentration (1/ml)	Diameter (nm)	D10 (nm)	D50 (nm)	D90 (nm)
Empty LNPs	1.7E+10 ± 2.4E+9	81 ± 4	50 ± 2	75 ± 2	121 ± 13
pDNA LNPs	6.3E+9 ± 8.2E+8	240 ± 8	141 ± 13	238 ± 1	342 ± 10
mcDNA LNPs	1.3E+10 ± 5.8E+08	96 ± 6	62 ± 2	89 ± 2	133 ± 5

Figure 1

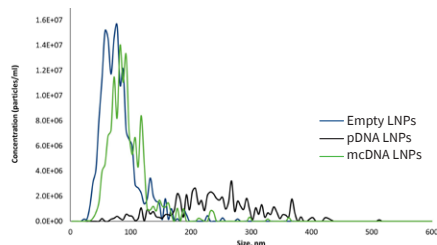


Table 1.

Characterization of empty and DNA-loaded LNPs. D10, D50, D90 describe percentile-based distribution.

Figure 1.

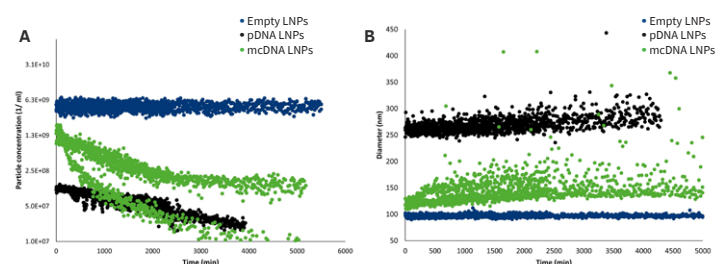
Particle concentration (particles/ml) vs. particle size (nm) for empty and DNA-loaded LNPs. pDNA LNPs showed a larger particle diameter and polydispersity, whereas empty and Minicircle DNA (mcDNA) filled LNPs showed comparable distribution.

DLS Analysis of DNA-loaded LNPs

Table 2

Sample	Diameter (nm)	Normalized Intensity (kCnt/s)	PD Index	Particle Concentration (1/ml)
Empty LNPs	94 ± 2	4.99E+04 ± 5.15E+03	0.23 ± 0.03	5.6E+09 ± 1.2E+09
pDNA LNPs	304 ± 10	1.37E+05 ± 9.58E+03	0.39 ± 0.05	1.0E+08 ± 6.8E+06
mcDNA LNPs	113 ± 4	4.41E+04 ± 2.58E+03	0.21 ± 0.01	1.8E+09 ± 4.5E+08

Figure 2



März M. et al., ASGCT Annual Meeting 2026, Abstract 3083 [5]

Table 2.

DLS/SLS characterization of empty and DNA-loaded LNPs.

Figure 2.

(A) Particle concentration over time for empty and DNA-loaded LNPs, measured by DLS/SLS. Empty LNPs showed constant concentration. LNPs filled with pDNA and mcDNA showed decreasing concentration indicating reduced stability over time. (B) Diameter over time for empty and DNA-loaded LNPs, measured by DLS/SLS. All LNPs showed stable diameters over time.

Key Takeaway

Under the tested formulation conditions, Minicircle DNA-loaded LNPs showed particle sizes and polydispersity closer to empty LNPs than plasmid DNA-loaded LNPs.

This supports the central concept that DNA architecture can directly influence LNP particle characteristics. The compact, bacterial backbone-free Minicircle format may therefore be advantageous when particle size, homogeneity, and formulation behavior are important development parameters.

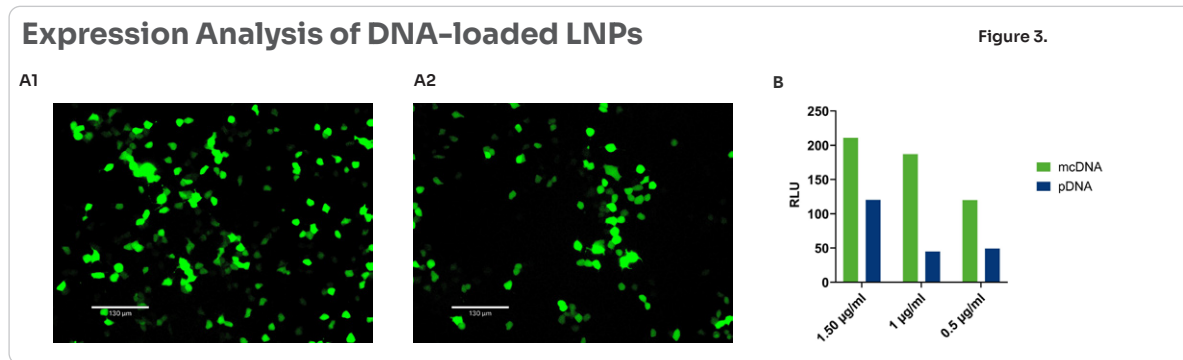
5.2. Minicircle DNA Supports Higher Reporter Expression Under Tested Conditions

The same PROGEN / ZentriForce / NanoFCM & PlasmidFactory partner study also evaluated whether DNA-loaded LNPs could support functional reporter gene expression after intracellular delivery.

Plasmid DNA and Minicircle DNA constructs encoding GFP or firefly luciferase were delivered into HEK293T cells using the PROGEN mRNA LNP Delivery Kit. Reporter expression was then assessed by fluorescence microscopy for GFP and by luciferase assay for firefly luciferase [5].

The data presented in Figure 3 showed that both plasmid DNA and Minicircle DNA constructs could be delivered by the LNP system and resulted in detectable reporter expression.

Importantly, Minicircle DNA-encoded luciferase constructs showed higher luciferase signals compared with the corresponding plasmid DNA constructs across the tested DNA concentrations.



März M. et al., ASGCT Annual Meeting 2026, Abstract 3083 [5]

Figure 3.

(A_{1/2}) GFP expression in HEK293 T cells after LNP transfection with (1) mcDNA-GFP and (2) pDNA-GFP.

(B) Quantification of luciferase expression showed higher RLU signal with Minicircle DNA constructs compared to plasmid DNA for three different DNA concentrations.

Key Takeaway

In this experimental setup, Minicircle DNA supported stronger reporter expression than the corresponding plasmid DNA after LNP-mediated delivery [5].

These data suggest that Minicircle DNA may provide functional advantages as DNA cargo for LNP-based delivery. The improved reporter signal may reflect better delivery, formulation behavior, intracellular availability, expression efficiency, or a combination of these factors.

These results provide a clear proof-of-concept that cargo architecture matters for LNP-mediated DNA delivery. While performance will depend on the specific formulation, construct, dose, and cell type, the data support Minicircle DNA as a highly compelling cargo format for further LNP-based delivery development.

5.3. Minicircle DNA Can Support Sustained Expression After LNP Delivery

A Bielefeld University & PlasmidFactory partner study presented at ASGCT 2026 investigated the delivery of Minicircle DNA using SM-102 lipid nanoparticles (LNPs) in HEK293 cells. LNPs originally developed for mRNA delivery were adapted to carry Minicircle DNA, and GFP reporter expression was monitored over time by fluorescence microscopy and flow cytometry.

Figure 4 illustrates the distinct expression profiles of Minicircle DNA and mRNA following LNP delivery. While both formats produced strong GFP expression early after transfection, the mRNA-derived signal declined rapidly after 48 hours. In contrast, Minicircle DNA supported a more sustained expression profile, with GFP-positive cells remaining detectable up to 144 hours after transfection.

A separate experiment comparing mcDNA with pDNA further supported the prolonged expression profile of Minicircle DNA (data not shown here; see [6]). Compared with plasmid DNA, Minicircle DNA reached its peak GFP expression on day 3, with a 3.1-fold higher efficiency, and remained superior for up to seven days. Experiments using different amounts of Minicircle DNA and mRNA further confirmed their distinct expression kinetics over time. These results demonstrate that LNP platforms developed for mRNA can also be adapted for Minicircle DNA delivery and suggest Minicircles as an attractive option when transient expression with an extended duration of action is desired.

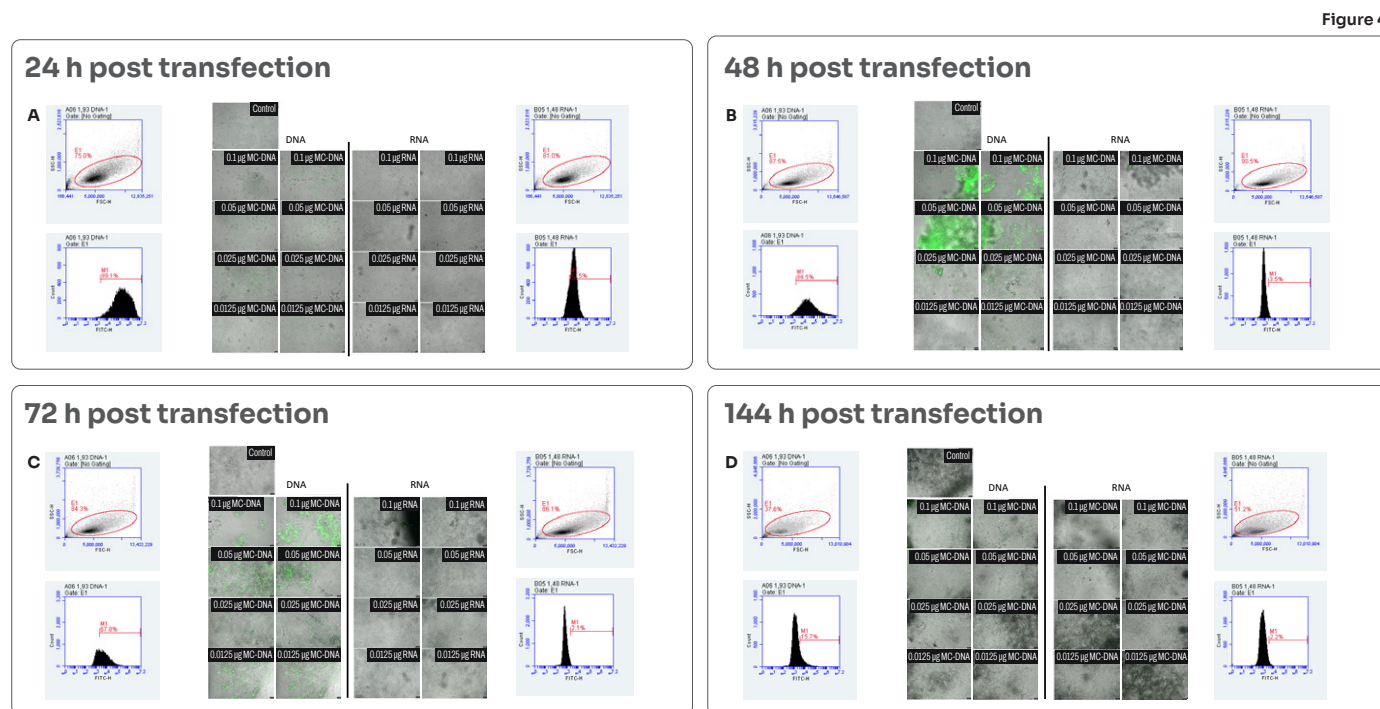


Figure 4 A-D.

Fluorescence microscopy and flow cytometry analysis following transfection with mRNA or Minicircle DNA using SM-102 lipid nanoparticles. Minicircle DNA showed sustained GFP reporter expression over several days, with the highest measured expression at the 24-hour time point and still detectable expression at 144 h under the tested conditions.

Shankar R. et al., ASGCT Annual Meeting 2026, Abstract 3097 [6]

Key Takeaway

SM-102 LNP-mediated Minicircle DNA delivery supported prolonged transient reporter expression in HEK cells, with expression still detectable at 144 h after transfection in the tested setup [6].

This approach combines the advantages of a DNA cargo, including sustained expression and the option to incorporate integrating systems such as Sleeping Beauty, with virus-free LNP delivery. Minicircle DNA is therefore a compelling cargo format for both in vivo and ex vivo applications that require efficient non-viral delivery, with the flexibility to support either non-integrating or integrating strategies.

6. Strategic Implications For Cell And Gene Therapy

The future of virus-free delivery will not be defined by the delivery particle alone, but also by the cargo. For LNP-based cell and gene therapy concepts, cargo architecture may influence whether a workflow is efficient, scalable, reproducible, and biologically useful. This is particularly relevant as the field moves toward delivery strategies that reduce reliance on viral vectors while still supporting robust and application-appropriate expression.

Minicircle DNA is highly relevant in this context because it combines three features that are particularly attractive for LNP-based delivery:

- compact DNA cargo architecture
- reduced bacterial backbone burden
- robust, application-dependent expression

This makes Minicircle DNA especially interesting for developers who aim to combine non-viral delivery with scalable development strategies and flexible DNA cargo design.

Potential application areas include ex vivo immune cell engineering and in vivo gene delivery, particularly for non-viral approaches to genetically modify therapeutic immune cells such as T cells, NK cells, or other immune cell formats.

For developers, this is not only a question of cargo performance, but also of development continuity: access to Minicircle DNA from Research Grade up to GMP Grade can help bridge early feasibility work, clinical translation, and later-stage manufacturing requirements.

Conclusion

Virus-free delivery strategies are becoming increasingly important in cell and gene therapy, and LNPs are emerging as an important delivery platform in this context. While mRNA has already demonstrated the power of LNP-based delivery, there are applications where DNA cargo is required, for example when promoter-driven expression, more sustained expression profiles, or DNA-based genetic modification strategies are desired.

For these applications, the DNA format matters. Conventional plasmid DNA remains an important research and development tool, but its bacterial backbone elements increase molecule size and can create limitations for LNP formulation, intracellular delivery, nuclear availability, and expression performance.

Minicircle DNA addresses these limitations through its small, monomeric, supercoiled, bacterial backbone-free architecture. The data summarized in this white paper show that Minicircle DNA can be packaged into LNPs, delivered into cells, and support favorable particle characteristics, stronger reporter expression, and more sustained expression profiles compared with plasmid DNA under the tested conditions [5, 6].

Taken together, these findings support Minicircle DNA as a preferred DNA cargo format for LNP-based delivery concepts, particularly where compact size, reduced bacterial sequence burden, and robust expression are important. With availability from Research Grade up to GMP Grade, Minicircle DNA can support a scalable development path from early feasibility studies toward clinical translation and commercial cell and gene therapy applications.

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Discover more details about the **two studies featured in this white paper**:



[Ready-to-use mRNA LNP kit for DNA delivery](#) [5]
(PROGEN, ZentriForce, NanoFCM, PlasmidFactory partner study)



[Prolonged transient expression with Minicircle DNA in LNP delivery](#) [6]
(PlasmidFactory/Bielefeld University partner study)