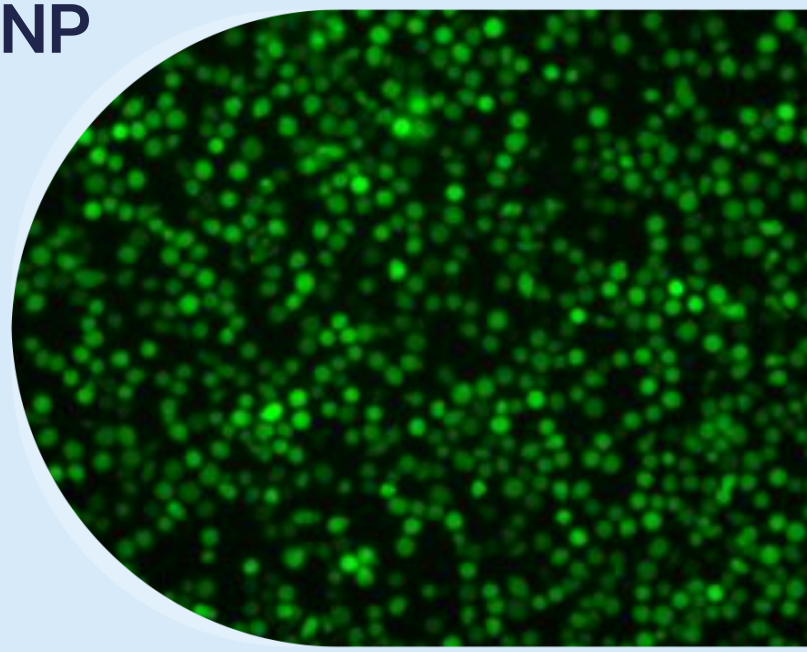
Application note Short summary

eGFP-RNA and CRISPR–Cas9 Delivery in Hematopoietic Stem Cells (HSCs) Using LNP



Efficient delivery of RNA and CRISPR–Cas9 components into hematopoietic stem cells (HSCs) is essential for advancing gene and cell therapies. HSCs hold strong therapeutic potential but remain difficult to transfect due to their quiescent state and sensitivity to stress.

Lipid nanoparticles (LNPs) have emerged as a gentle, scalable, and highly biocompatible non-viral delivery modality. Their tunable formulation properties and suitability for both invitro, ex-vivo and in-vivo applications make them well aligned with the requirements of modern genome-editing workflows.

This study provides a comprehensive evaluation RNA and CRISPR-Cas9 delivery efficiency in HSC using LNP formulated with TAMARA. eGFP mRNA delivery was first assessed across multiple doses, followed by functional CRISPR knockout targeting the B2M gene. A CRISPR knockout model targeting the B2M gene was then used to benchmark the genome-editing performance of LNP-mediated delivery against electroporation.

The results demonstrate that LNPs can achieve utmost RNA delivery efficiency and functional CRISPR knockout while maintaining near-complete cell viability in HSCs, positioning them as a gentle and scalable alternative to other delivery methods.

This work was carried out by Gregory Noel at the BIGRes team led by Prof. Michel Cognes, the university of Rennes

GFP mRNA-LNP delivery in HSC

HSCs were treated with LNPs carrying increasing doses of eGFP mRNA (0 to 0.5 µg)

Key findings

- → Near-complete transfection was achieved across all tested doses.
- → **eGFP expression increased in a dose-dependent manner, represented by rising MFI values.**
- → Cell viability remained exceptionally high, with near 0 cell death.

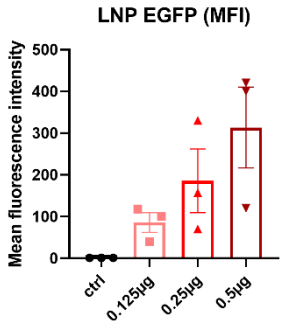


Figure 1: GFP expression in HSC cells after exposure to four eGFP doses (0 µg control, 0.125 µg, 0.25 µg, and 0.5 µg)

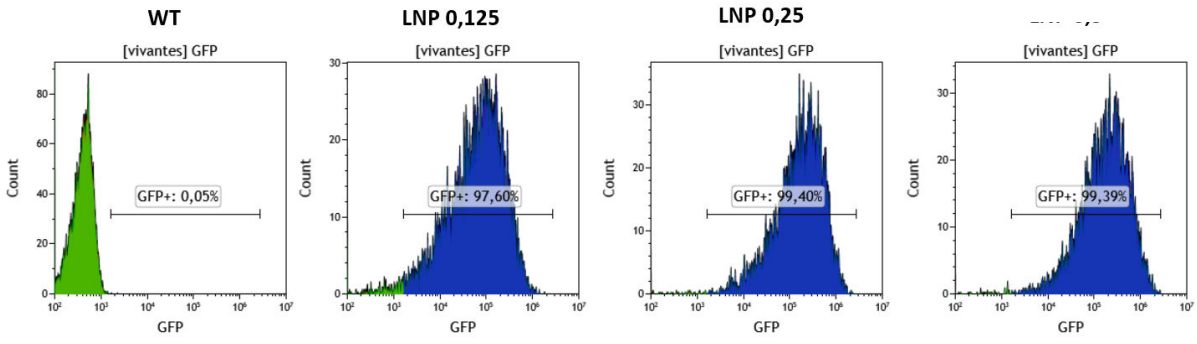
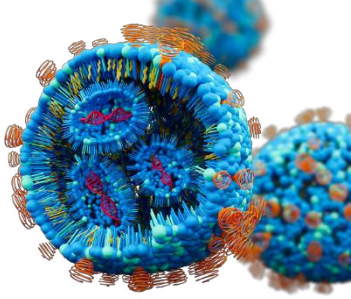


Figure 2 : Transfection efficiency in HSC cells at four eGFP doses (0 µg control, 0.125 µg, 0.25 µg, and 0.5 µg), delivered via LNPs and quantified by flow cytometry.



CRISPR-Cas9 Knockout via LNP delivery

Cas9 mRNA and sgRNA targeting the B2M gene were co-delivered using LNPs across a range of RNA doses.

Key findings

- → Knockout efficiency increased proportionally with dose.
- → **The highest dose (1 µg) achieved near-complete B2M knockout.**
- → Viability remained above 99% across all conditions.

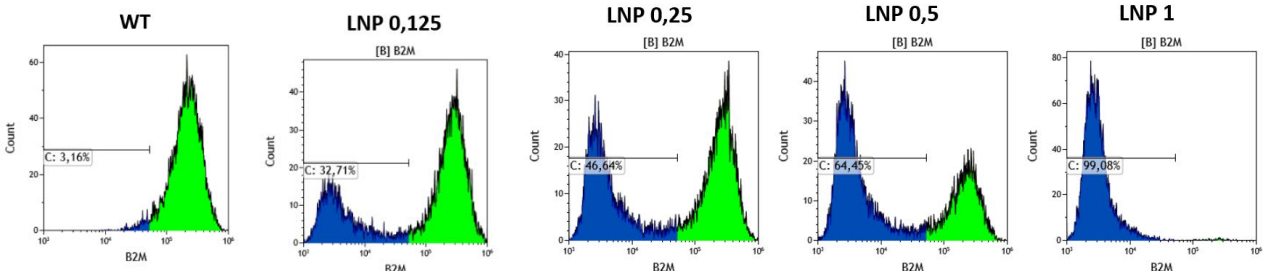


Figure 3:: B2M knockout efficiency in HSC cells at five gRNA doses (0 µg control, 0.125 µg, 0.25 µg, 0.5 µg and 1µg) delivered via LNPs and quantified by flow cytometry.

The data confirm that LNP-mediated RNA delivery enables functional CRISPR editing in HSCs with minimal cytotoxicity.

LNP Delivery Compared with Electroporation

Electroporation using the same CRISPR reagents was performed as a benchmark

Key findings

- → **At their optimal doses, both approaches reached similar knockout efficiencies.**
- → LNP delivery maintained near-zero cytotoxicity, when cell death in electroporation remained important (20%)
- → Electroporation required a 10x higher sgRNA dose for similar results

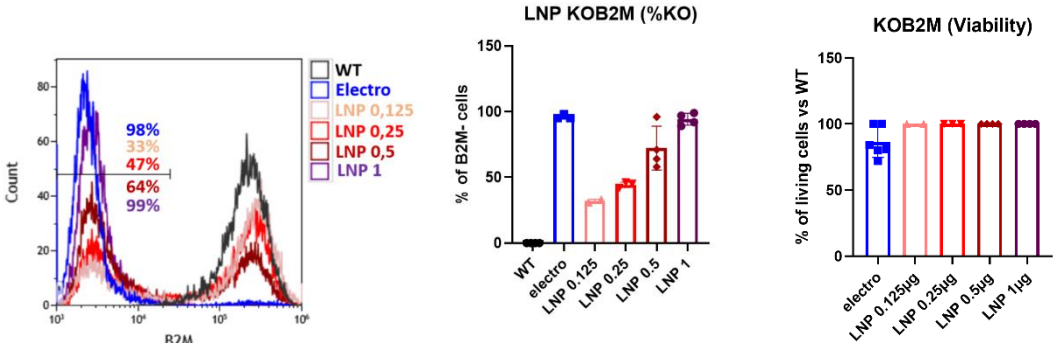


Figure 4 : Comparison of B2M knockout efficiency in HSC cells between LNP-delivered samples across four doses and electroporated benchmark.

LNPs therefore represent a more gentle and potentially more scalable option for genome-editing applications where maintaining cell health is essential.

Conclusion

This application note highlights the strong performance of LNP-mediated RNA and CRISPR–Cas9 delivery in **hematopoietic stem cells**. LNPs formulated using SM-102 based formulation and the TAMARA microfluidic system:

- → **Delivered GFP mRNA and Cas9 mRNA/sgRNA efficiently into HSCs.**
- → **Achieved** dose-dependent gene expression and **genome-editing activity**
- → **Preserved** nearly complete **cell viability.**
- → **Matched the editing performance of electroporation** while avoiding associated cytotoxicity.

LNPs therefore represent a more gentle and more translatable option for genome-editing applications, while offering in-vivo capabilities.

Read the full Application Note on https://insidetx.com

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