Gene editing in mammalian cells via Lipid nanoparticle gene delivery

In Gyung Choi[1*], Yuna Jin[1*], JunSuk Lee[1,2], Christian Macks[1,2], Changho Sohn[1,2**]

[1] Center for Nanomedicine(CNM), Institute for Basic Science (IBS), Seoul, Republic of Korea
[2] Graduate Program of Nano Biomedical Engineering (NanoBME), Yonsei Advanced Science Institute, Yonsei University, Seoul, Republic of Korea

*These authors contributed equally to this work

Introduction

CRISPR-Cas9 is a powerful tool that enables efficient and effective genome editing in living cells and have potential for gene therapy applications. However, in order for CRISPR-Cas9 to be delivered to living cells and function within them, a cargo is essential. While viral vectors are commonly used, their limited load capacity and potential integration into host cells pose challenges. In contrast, Lipid Nanoparticles (LNPs) exhibit lower immunogenicity and relatively fewer load capacity constraints compared to viral vectors. LNPs can carry CRISPR-Cas9 in the form of plasmid DNA, mRNA, or ribonucleoprotein (RNP). In this study, I focused on mRNA encapsulated in LNPs. mRNA offers higher expression level than RNPs and quicker protein expression than plasmid DNA. Plasmid DNA was extracted from transfected E.coli, while mRNA was obtained by in vitro transcription (IVT). I compared IVT mRNA with and without pseudouridine and capping reagent. Without these, translation is poor due to the immunogenicity of exogenous mRNA.

Initially, I transfected EGFR reporter plasmid, Cas9 plasmid, and sgRNA plasmid into HEK293T (human embryonic kidney) cells using lipofectamine to observe GFP expression. Subsequently, I loaded Cas9 mRNA and SRD5A2 sgRNA into LNPs. After LNP characterization, HEK293T cells and NIH3T3 (mouse embryonic fibroblast) cells were transfected, and gene editing was confirmed by T7E1 assay. Human steroid 5α-reductase 2 (SRD5A2) is a membrane enzyme essential for steroid metabolism, catalyzing the reduction of testosterone to dihydrotestosterone, which plays an essential role in male sexual development. SRD5A2 deficiency correlates with male pseudohermaphroditism and prostate cancer. Observation of intracellular gene editing through CRISPR-Cas9 LNP transfection would suggest that CRISPR-Cas9 has the potential to be expanded to in vivo gene therapy for various diseases.

Experimental Methods

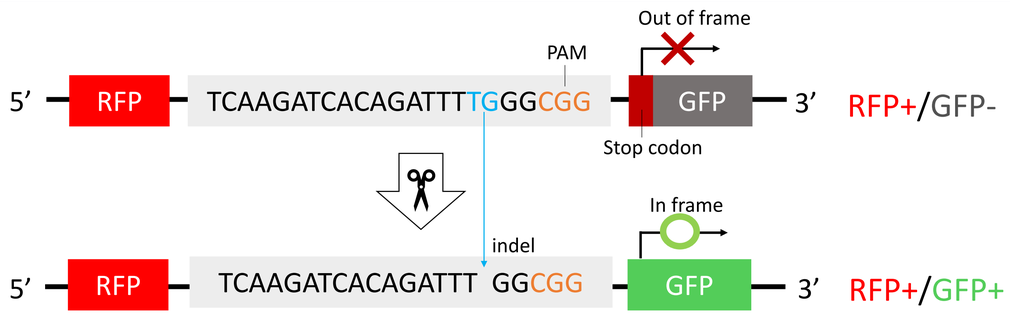


Fig1. EGFR reporter system
If gene editing occurs in the EGFR sequence by CRISPR-Cas9, co-expression of GFP alongside RFP is observed.

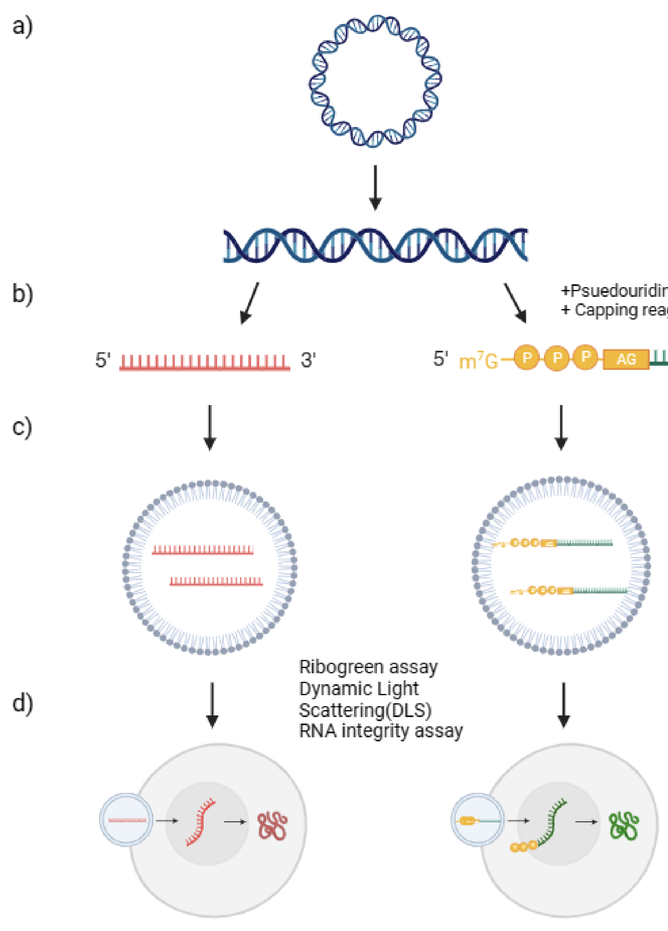


Fig2. Overall scheme of transfection using LNP

a) IVT template plasmid linearization
b) IVT without pseudouridine and capping reagent & with pseudouridine and capping reagent
c) LNP formulation & Check the LNP size via DLS and the encapsulation efficiency via Ribogreen assay. RNA integrity assay was also conducted.
d) Transfection of LNPs

Reference

1. Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature Communications*, 9(1). https://doi.org/10.1038/s41467-018-04252-2
2. Li, D., Ouyang, K., Xu, X., Xu, L., Wen, C., Zhou, X., Qin, Z., Zhang, X., Sun, W., & Liang, Y. (2021). Nanoparticle delivery of CRISPR/CAS9 for genome editing. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.673286
3. Kazemian, P., Yu, S., Thomson, S. B., Birkenshaw, A., Leavitt, B. R., & Ross, C. J. (2022). Lipid-Nanoparticle-Based delivery of CRISPR/CAS9 Genome-Editing components. *Molecular Pharmaceutics*, *19*(6), 1669–1686. https://doi.org/10.1021/acs.molpharmaceut.1c00916
4. Lin, Y., Wagner, E., & Lächelt, U. (2022). Non-viral delivery of the CRISPR/Cas system: DNAversusRNAversusRNP. *Biomaterials Science, 10*(5), 1166–1192. https://doi.org/10.1039/d1bm01658j
5. Xiao, Q., Wang, L., Supekar, S., Shen, T., Liu, H., Ye, F., Huang, J., Fan, H., Wei, Z., & Zhang, C. (2020). Structure of human steroid 5α-reductase 2 with the anti-androgen drug finasteride. *Nature Communications, 11*(1). https://doi.org/10.1038/s41467-020-19249-z
6. Nishina, K., Unno, T., Uno, Y., Kubodera, T., Kanouchi, T., Mizusawa, H., & Yokota, T. (2008). Efficient In Vivo Delivery of siRNA to the Liver by Conjugation of α-Tocopherol. *Molecular Therapy, 16*(4), 734–740. https://doi.org/10.1038/mt.2008.14
7. Bae, T., Kim, H., Kim, J. H., Kim, Y. J., Lee, S. H., Ham, B. J., & Hur, J. K. (2019). Specificity Assessment of CRISPR Genome Editing of Oncogenic EGFR Point Mutation with Single-Base Differences. *Molecules*, *25*(1), 52. https://doi.org/10.3390/molecules25010052
8. Created with BioRender.com

Results

Fig1. HEK293T cells transfected with EGFR reporter plasmid

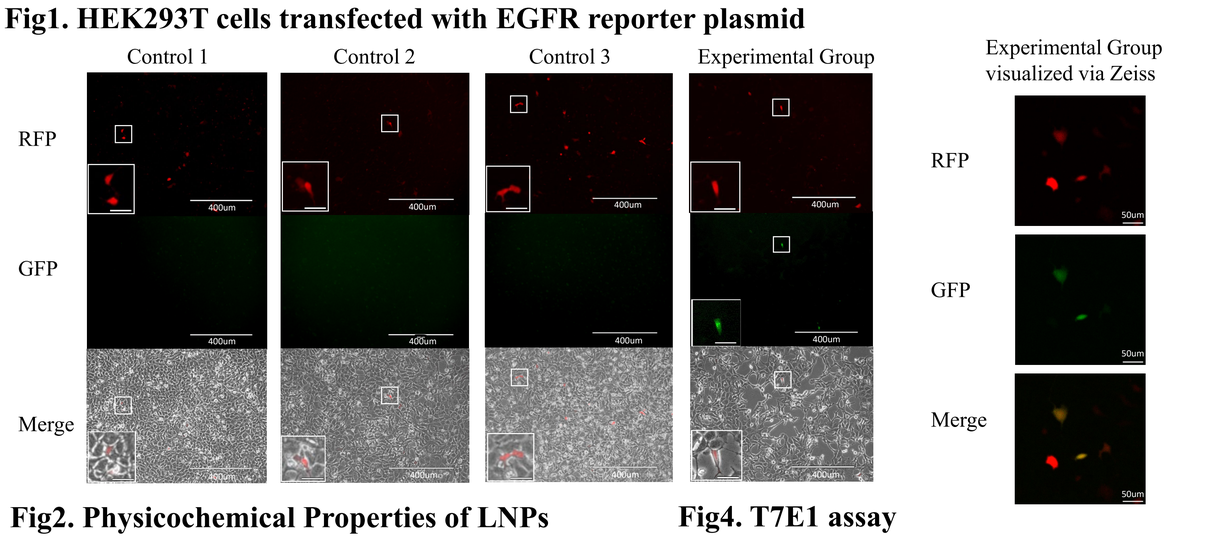
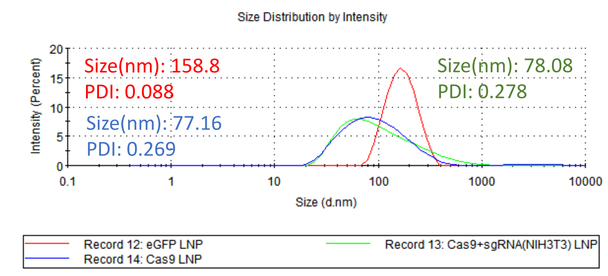


Fig2. Physicochemical Properties of LNPs

a) Pseudouridine, capping x



b) Pseudouridine, capping o

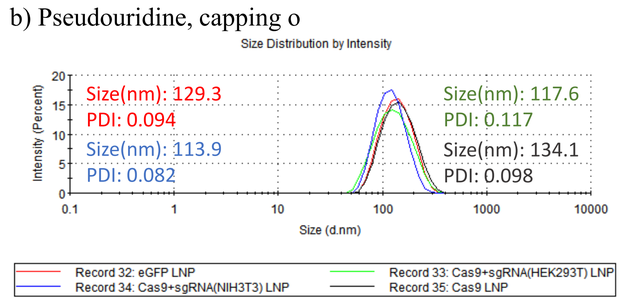


Fig4. T7E1 assay

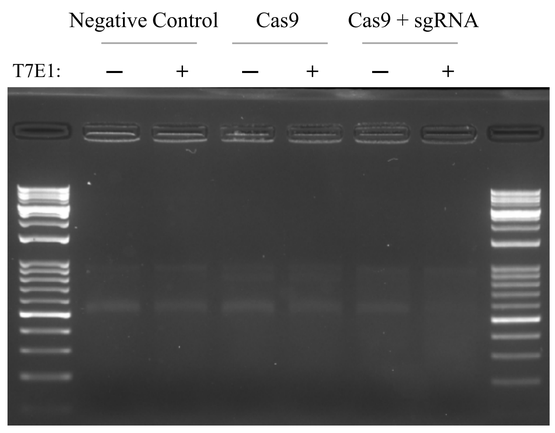
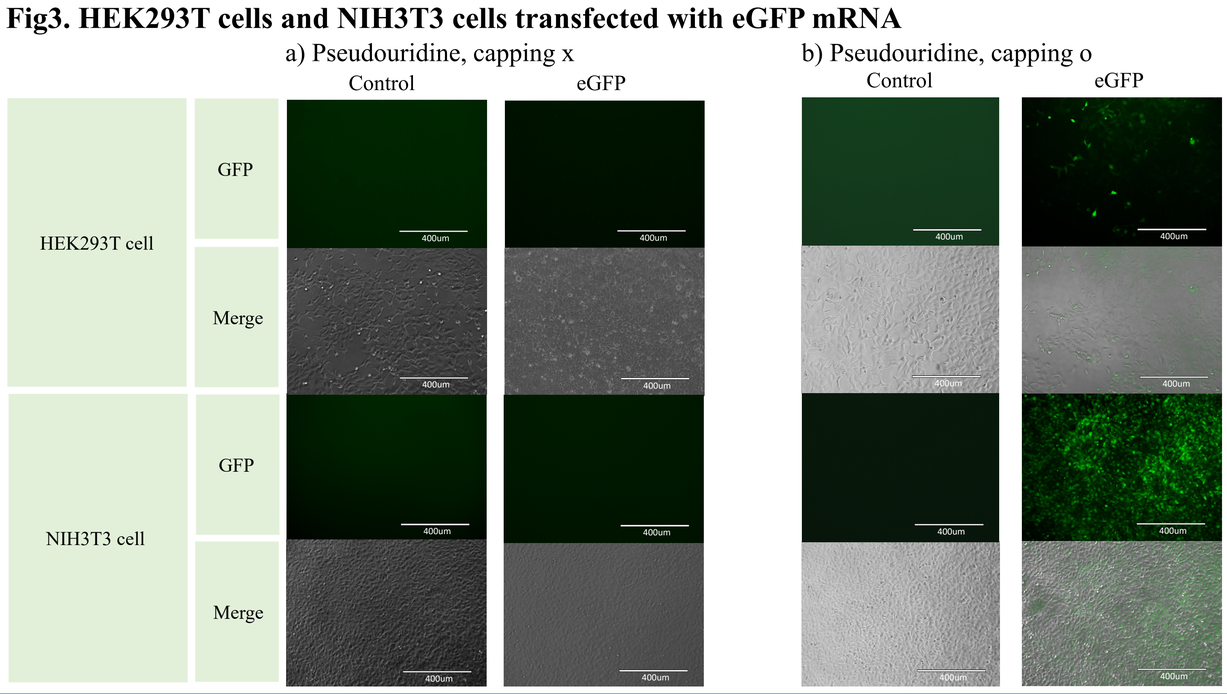


Fig3. HEK293T cells and NIH3T3 cells transfected with eGFP mRNA



Conclusion & Further Study

When I transfected the EGFR reporter plasmid, Cas9 plasmid, and sgRNA plasmid into HEK293T cells using Lipofectamine 3000, both GFP and RFP were expressed. This confirmed that the gene editing was accurate in the cells.

Transfection of LNPs encapsulating eGFP mRNA, synthesized without pseudouridine and capping reagents, showed no observable GFP expression. However, GFP expression was observed when the cells are transfected with LNPs encapsulating eGFP mRNA synthesized using these modifications. This indicates that appropriate chemical modification of the IVT mRNA is important to improve translation and in vivo stability.

After confirming the expression of eGFP, I conducted T7E1 assay on cells transfected with Cas9 and sgRNA LNPs. However, due to unsuccessful PCR amplification, precise conclusions could not be drawn. One contributing factor to the poor PCR performance was the enzyme inefficiency. Upon future confirmation of successful gene editing, CRISPR-Cas9 could demonstrate its potential as a novel therapeutic approach not only for SRD5A2 mutations but also for various genetic diseases.

In addition, comparing GFP expression in HEK293T and NIH3T3 cells revealed stronger GFP expression in NIH3T3 cells even though the same amount of LNPs were treated. The reason for this may be that transfection efficiency varies depending on the cell line due to various factors such as the type of mRNA transfected, chemical modification of mRNA, amount of mRNA, and transfection reagent. Further research may uncover the optimal chemical modifications and quantities of Cas9 mRNA for efficient transfection and expression within human cells.

These findings suggest that in the future, therapies using CRISPR-Cas-based technologies beyond genome editing, such as dCas9-methyltransferase, may be developed.