# Synthesizing Lipid Nanoparticle-RNA complex and validating the biological function.

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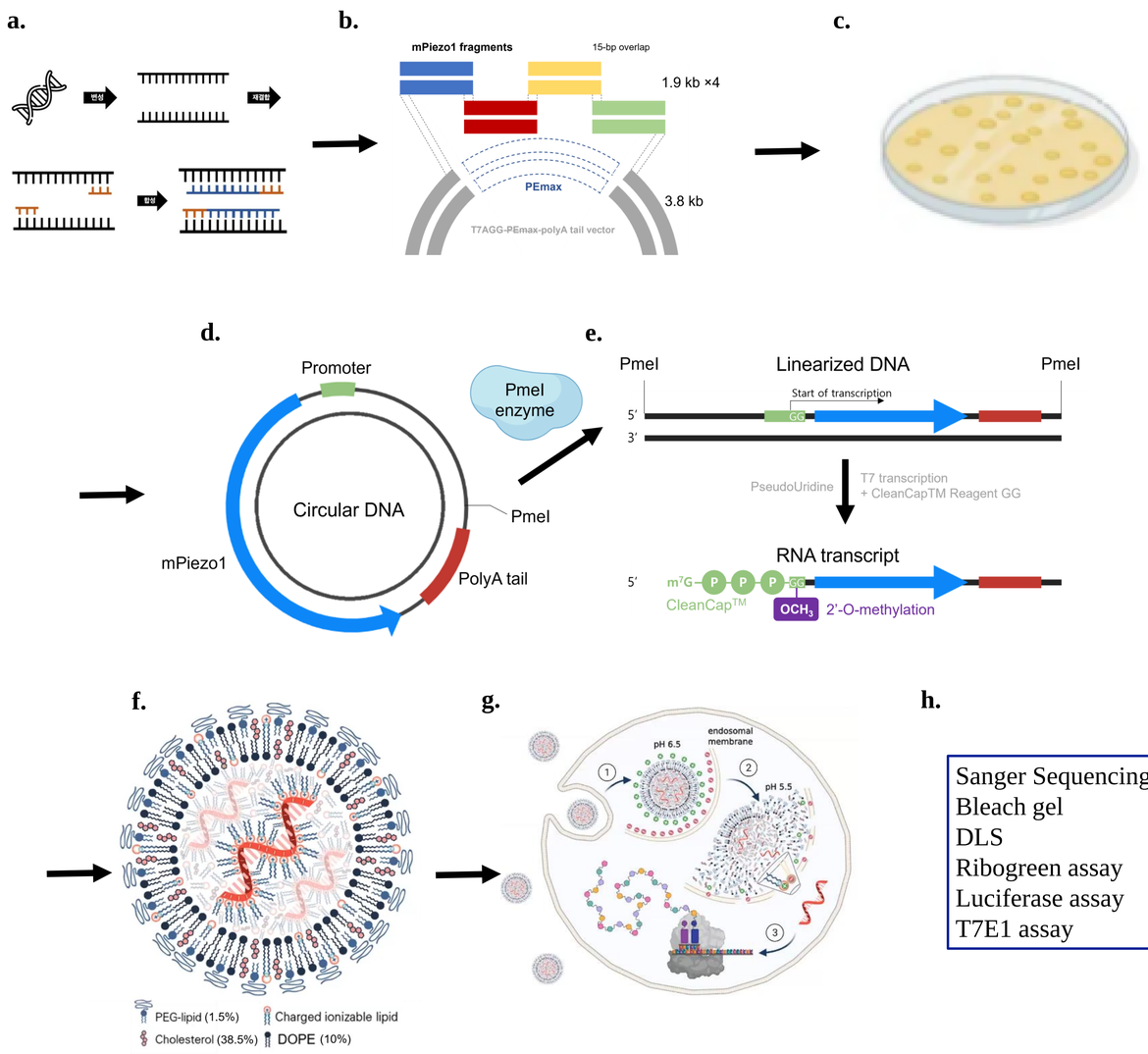
## Introduction

In recent years, Lipid nanoparticles (LNP) have gained immense popularity as the promising delivery platform for various therapeutics. A representative example of this would be regarding its role within the COVID-19 mRNA vaccine as they are essential components for successfully preserving and delivering mRNA to cells. Other advantages of lipid nanoparticles are visible when comparing its features with viral vectors which are known to have limitations in its packaging capacity and immunogenicity. Therefore, considering these, we have decided to examine the ability of packaging various RNAs, unlike other studies which have attempted to package Ribonucleoproteins (RNP) and plasmid DNAs. One of the crucial reasons behind this would be due to the low copy number of RNP once transfected in cells unlike RNAs which have a high copy number as they are able to be translated within the cells. In our study, we are going to package eGFP mRNA, Cas9 mRNA, single guide fLuciferase RNA, single guide EGFR RNA and Piezo1 mRNA within the LNP and transfect the mammalian cells , HeLa and stable Luciferase-expressing HEK293T cells. Within the RNAs, Piezo1 mRNA is known to be large. Concerns arose regarding packaging large size RNA within LNPs, however, in most recent studies, large size self amplifying mRNAs were found to be successfully encapsulated within the LNP. Due to this, we decided to attempt to package the large size Piezo1 mRNA. In order to carry out the study, we first synthesized the RNAs via in vitro transcription. We then authenticated the ability of packaging biologically functional RNAs such as eGFP mRNA within the LNP and its successful delivery by first formulating the LNP and detecting the size and Zeta potential through DLS. After checking these features, we transfected the mammalian cells to assess the successful delivery of the LNP. Once we assessed the possibility of successful encapsulation of eGFP RNA and its delivery through LNP, we then proceeded with packaging the remaining RNAs mentioned above. For Piezo1 mRNA, we initially planned to validate its successful delivery through Luciferase assay however could not conduct this step as we were unable to successfully package it in the LNP. On the other hand, for the Cas9 and sgRNAs, we attempted to verify successful gene editing through studies such as Luciferase assay and T7E1 assay .

## Experimental Methods

**Fig. 1 | Overall Scheme**




Sanger Sequencing
Bleach gel
DLS
Ribogreen assay
Luciferase assay
T7E1 assay

**Fig. 1 Overall Scheme**
**a.** PCR **b.** Hifi cloning **c.** Transformation **d.** Vector linearization **e.** In vitro transcription **f.** LNP formulation **g.** Transfection **h.** Validation methods

## Results

**Fig. 2 | Piezo1 PCR**

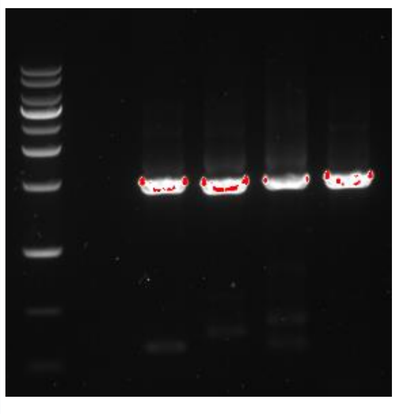


**Fig. 2** successful PCR result for 4 fragment of Piezo1

**Fig. 3 | Screening result via PmeI**

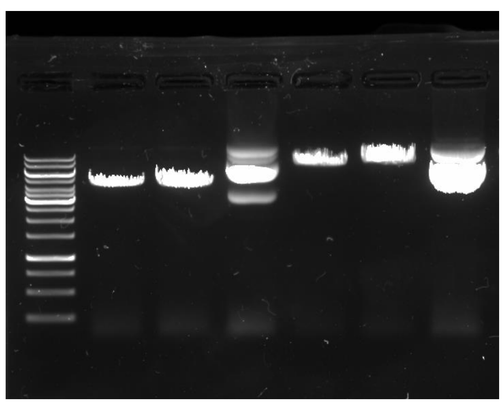


**Figure 3**. Vector linearization by PmeI for eGFP, Cas9, Piezo1 genes with T7AGG-polyA tail backbone. Each band was positioned at 5, 8, 11kb respectively.

**Fig. 4 | RNA integrity**

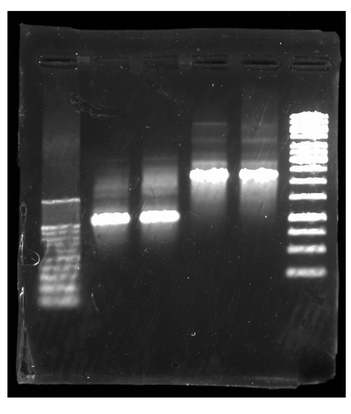
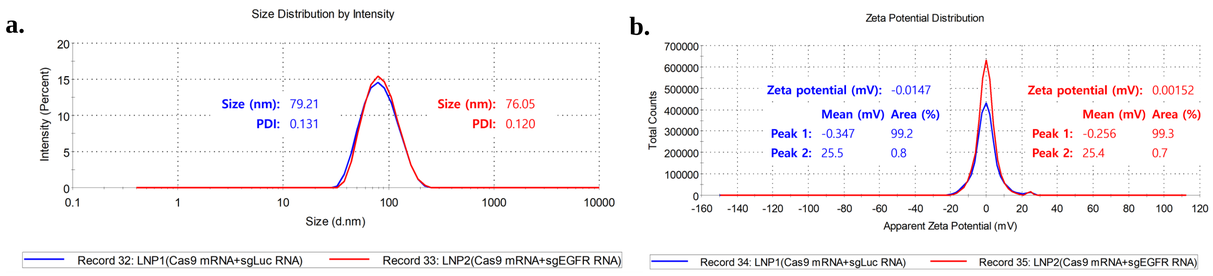


**Figure 4**. RNA integrity check for eGFP and Cas9 IVT product. RNAs were successfully collected via gel elution (bands 1,3) and PCR purification (bands 2,4) for each sample.

**Fig. 5 | Dynamic Light Scattering of LNP size and Zeta potential**




**c.**

| | sgLuc RNA + Cas9 mRNA | sgEGFR RNA + Cas9 mRNA |
|---|---|---|
| Encapsulation efficiency (%) | 97.5 | 97.4 |
| Encapsulated RNA concentration (ng/μl) | 22.2 | 23.4 |

**Fig. 5 measured and graphed by Dynamic Light Scattering**
**a.** Record 32 (blue) showing LNP size and PDI for LNP-Cas9 mRNA +sgLuc. Record 33 (red) showing LNP size and PDI for LNP-Cas9 mRNA + sgEGFR **b.** Record 34 (blue) showing average Zeta potential and mean zeta potential for each peak for LNP-Cas9 mRNA + sgLuc. Record 35 (red) showing average Zeta potential and mean zeta potential for each peak for LNP-Cas9 mRNA +sgEGFR **c.** Table showing encapsulated efficiency (%) and encapsulated RNA concentration (ng/ul) for LNP-Cas9 mRNA + sgLuc RNA and LNP-Cas9 mRNA + sgEGFR RNA

**Fig. 6 | Bleach Gel Electrophoresis**

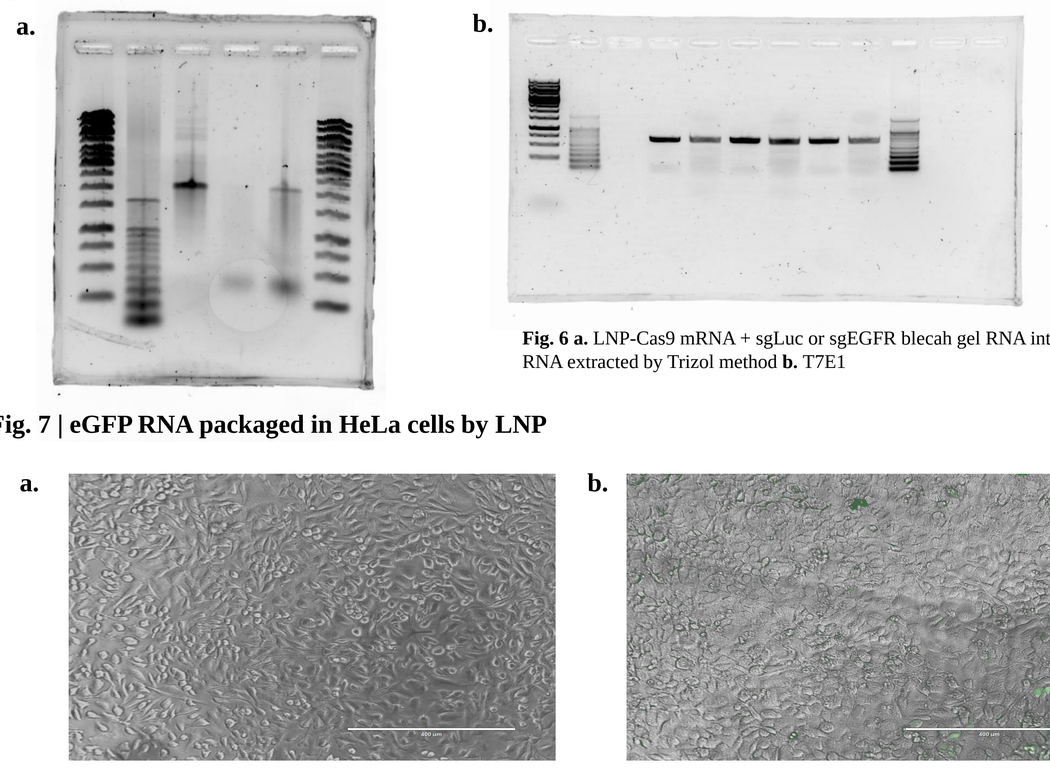



**Fig. 6 a.** LNP-Cas9 mRNA + sgLuc or sgEGFR blecah gel RNA integrity test RNA extracted by Trizol method **b.** T7E1

**Fig. 7 | eGFP RNA packaged in HeLa cells by LNP**

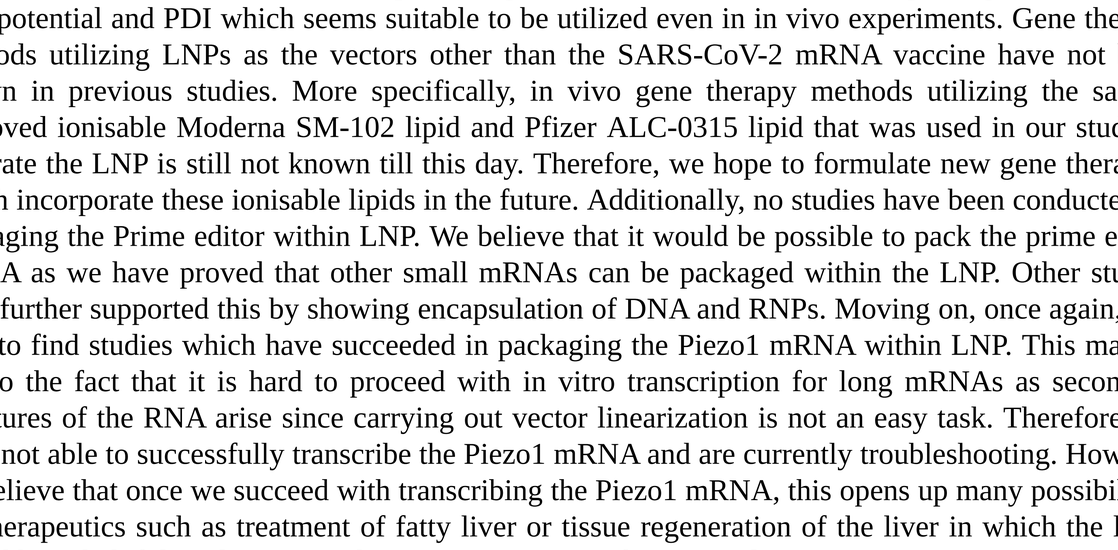



**Fig. 7** visualized via EVOS cell imaging system **a.** Negative **b.** RNA 100ng **c.** 200ng **d.** 500ng

## Conclusion & Further Study

In conclusion, we were able to successfully transcribe the modified eGFP mRNA, Cas9 mRNA and sgLuc and sgEGFR and encapsulate them in the LNP. We modified the RNAs by incorporating Pseudouridine and utilizing the CleanCap technology to introduce a 5' cap as it not only enhances the stability of the RNAs but also increases the translation rate and minimizes the toxicity when transfected within the cells. Another promising result that we achieved was regarding the LNP size, Zeta potential and PDI which seems suitable to be utilized even in in vivo experiments. Gene therapy methods utilizing LNPs as the vectors other than the SARS-CoV-2 mRNA vaccine have not been known in previous studies. More specifically, in vivo gene therapy methods utilizing the safety-approved ionisable Moderna SM-102 lipid and Pfizer ALC-0315 lipid that was used in our study to generate the LNP is still not known till this day. Therefore, we hope to formulate new gene therapies which incorporate these ionisable lipids in the future. Additionally, no studies have been conducted on packaging the Prime editor within LNP. We believe that it would be possible to pack the prime editor mRNA as we have proved that other small mRNAs can be packaged within the LNP. Other studies have further supported this by showing encapsulation of DNA and RNPs. Moving on, once again, it is hard to find studies which have succeeded in packaging the Piezo1 mRNA within LNP. This may be due to the fact that it is hard to proceed with in vitro transcription for long mRNAs as secondary structures of the RNA arise since carrying out vector linearization is not an easy task. Therefore, we were not able to successfully transcribe the Piezo1 mRNA and are currently troubleshooting. However, we believe that once we succeed with transcribing the Piezo1 mRNA, this opens up many possibilities for therapeutics such as treatment of fatty liver or tissue regeneration of the liver in which the latter would have helpful application in the recovery process after organ donation.

## Reference

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