invitrogen

Invivofectamine™ Reagents: Novel Lipid Nanoparticles (LNPs) for Therapeutic *In Vivo* mRNA delivery – Research and Therapeutic Use

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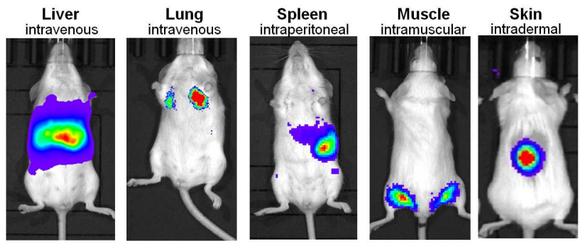
INTRODUCTION

The rapidly expanding utilization of mRNA as a therapeutic tool has presented the field with the task of innovating and optimizing delivery methods. We have leveraged over 25 years of delivery expertise and the power of Design of Experiment (DOE) to engineer LNPs that can deliver mRNA efficiently *in vivo*

The process of formulating these LNPs is simple, scalable, and results in uniform-sized LNPs with a narrow PDI. These LNPs efficiently encapsulate and protect the mRNA from degradation and facilitate cellular uptake which translates into efficient delivery and reduced toxicity *in vivo*. We have developed several potent organ specific LNPs for delivery of mRNA/siRNA. These novel LNPs are also well tolerated and do not exhibit systemic toxicity.

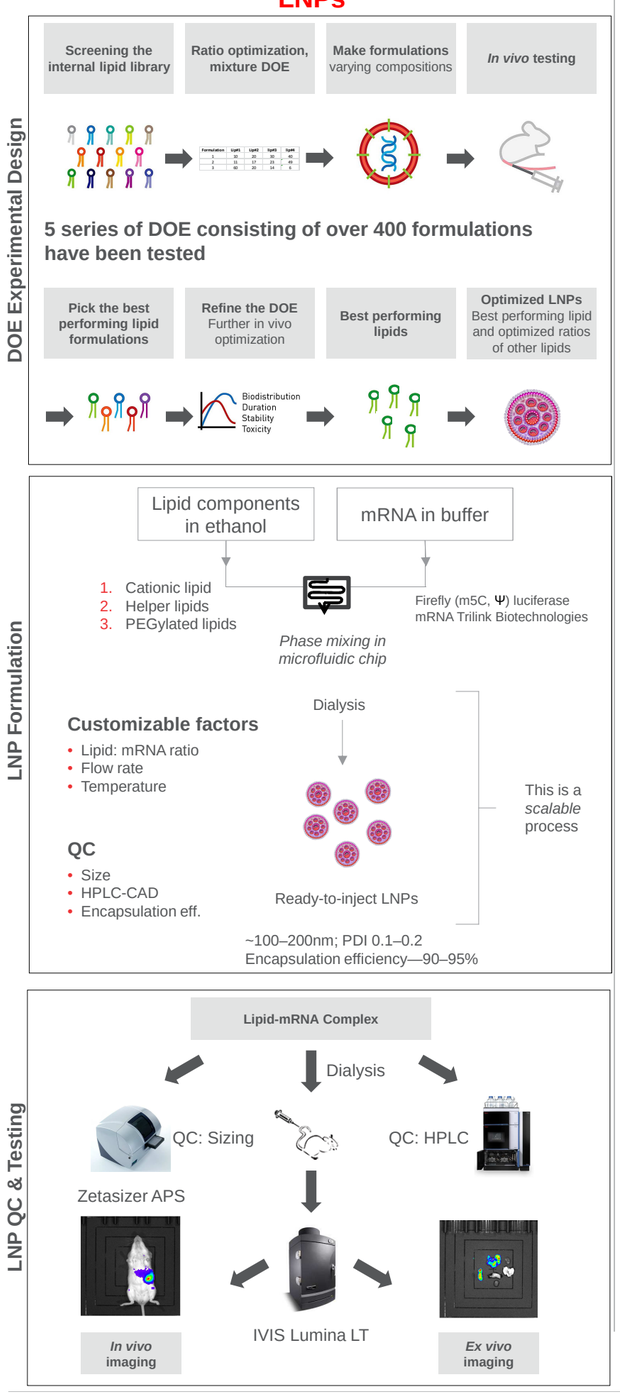


Luciferase mRNA delivery

Our primary aim is to enable our customers solve their nucleic acid delivery challenges by:

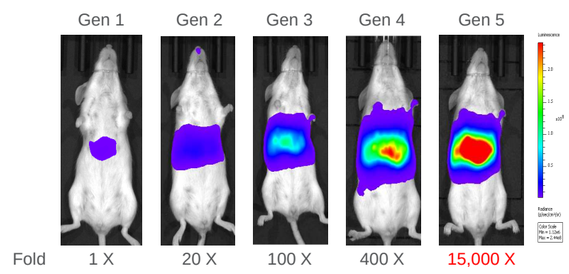
- Developing Invivofectamine™ reagents that will efficiently deliver mRNA, siRNA and other nucleic acids efficiently *in vivo*, thus, speeding up the translation from the bench to the clinic.
- Closely working with our academic and therapeutic customers in the form of R&D collaborations to understand their pain points for delivery and working towards developing tools to address those issues and enable their success.
- Licensing access to our library of screening reagents for development of application specific delivery.

General approach to formulate and screen LNPs



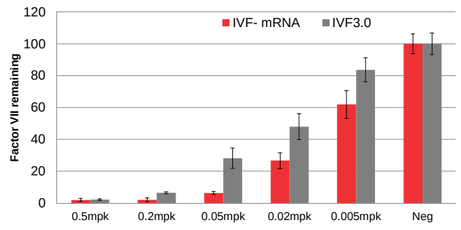
Liver delivery

The current generation *Invitrogen™ Invivofectamine™ reagent* (IVF-mRNA) is **15,000 X more potent** than its first generation predecessor in delivering **mRNA** to the Liver



Relative-fold Luciferase expression 4 hours post-IV injection from mRNA

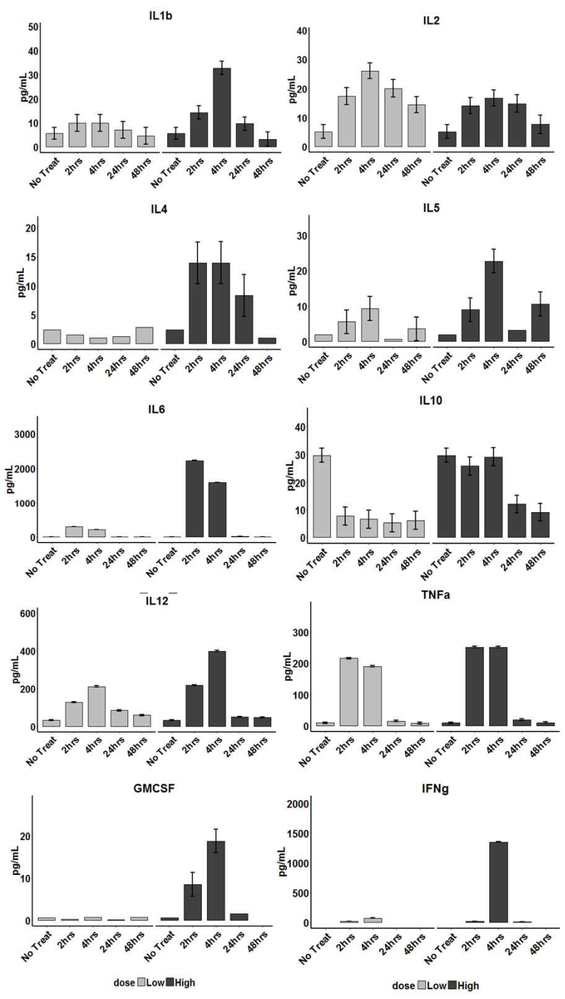
IVF-mRNA is **4-6 X more potent** than its predecessor IVF 3.0 in delivering **siRNA** to the Liver



FVII levels in blood 24 hours post-injection with siRNA against mouse FVII at different doses

Acute toxicity evaluation

Serum cytokines - Luminex 10-plex

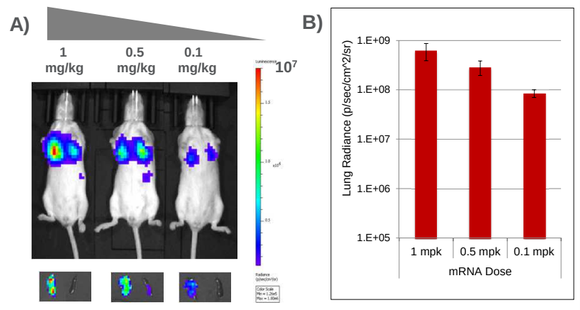


Mouse serum cytokines quantification using Luminex-200 system at 2 h, 4 h, 24 hrs and 48 hrs post-injection with IVF-mRNA reagent at low (1 mg/kg) and high (3 mg/kg) mRNA dose

Beyond the Liver

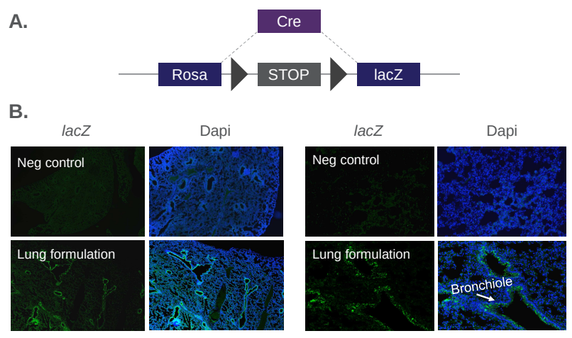
Lung delivery

mRNA delivery to the Lung using Invitrogen™ Invivofectamine™ Lung reagent (IVF-Lung)



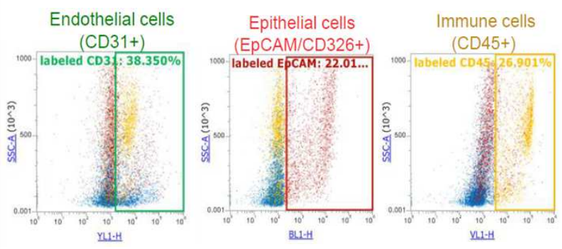
IVF-Lung reagent injected with luciferase mRNA. 4 hours post-injection A) *In vivo* and *ex vivo* imaging B) Luciferase quantification at different doses

Lung mRNA Delivery



Immunofluorescence staining in LacZ mice to study delivery localization in the Lung tissue. A) Schematic illustrating reporter mouse expression system. B) Immunofluorescent staining for detection of lacZ expression following delivery of Cre mRNA

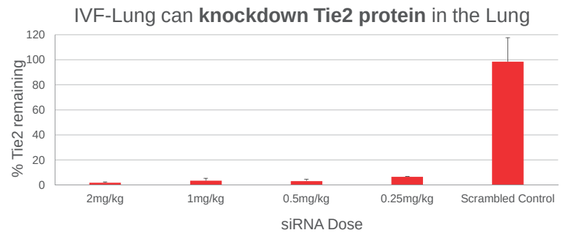
mRNA delivery into different lung cells



In vivo systemic delivery of LNPs complexed with Cy5 labeled mRNA
• Single cell isolation prepared from harvested lung tissue
• Cells labeled with fluorescent cell surface antibodies for classification.

Lung siRNA Delivery

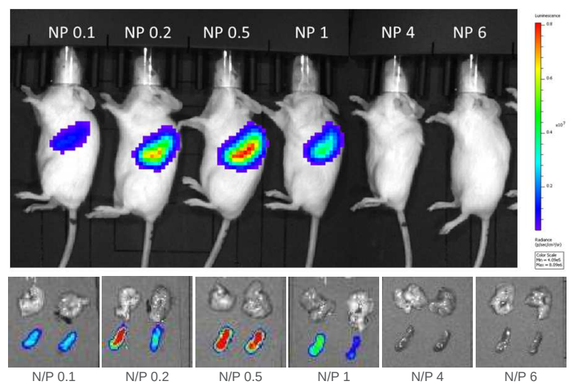
IVF-Lung can **knockdown Tie2 protein** in the Lung



siRNA targeting an endothelial specific receptor was complexed with IVF-Lung reagent. siRNA dose was varied from 0.25–2 mg/kg. >90% knockdown was observed at all doses administered, indicating high efficiency siRNA delivery in a cell type specific manner.

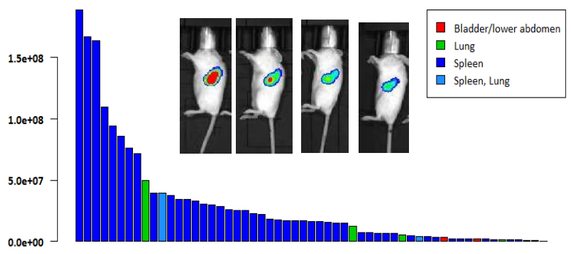
Spleen delivery

Varying the **N/P ratio** has a significant impact on the bio-distribution of the LNPs and this optimization allows exclusive delivery in the spleen



N/P ratio optimization for exclusive spleen delivery. Top-liver, bottom-spleen. TriLInk luciferase mRNA at 0.5 mg/kg by IV. Imaged at 4H post.

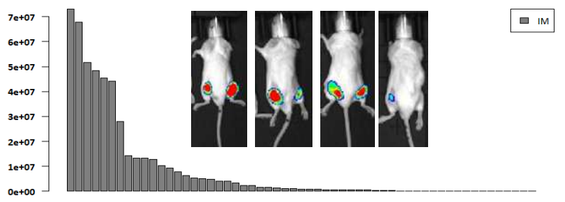
Lipid library screen for specific Spleen delivery using Luc mRNA



Luc mRNA was complexed with different lipids, injected in mice IV and *in vivo* spleen flux measured at 4h post-injection

Intramuscular (IM) delivery –

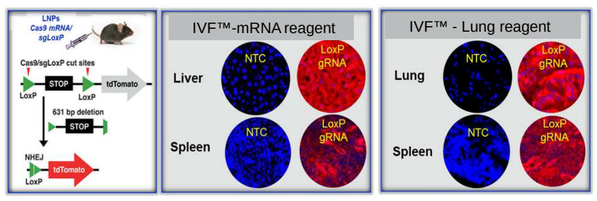
Lipid library screen for muscle delivery using Luc mRNA



Luc mRNA was complexed with different lipids, injected in mice IM and *in vivo muscle* flux measured at 4h post-injection

Gene editing

CRISPR/Cas9 gene editing in reporter mice



Co-delivering Cas9 mRNA/sgLoxP complex with IVF reagents into Td Tomato mice can induce Td Tomato protein expression in the Liver, Lung and Spleen

TRADEMARKS/LICENSING

For more information about licensing opportunities contact: outlicensing@thermofisher.com, or for collaborations contact: Xavier de Mollerat du Jeu, PhD - Director R&D, Life Science Group - xavier.demolleratdujeu@thermofisher.com

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