Manufacturing Platform - From pDNA and mRNA to LNP With Multiple Nucleic Acids Loads

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Introduction

The typical mRNA production process is a complex sequence of four key steps, each crucial for ensuring the quality and efficacy of the final product. The process begins with the expression of plasmid DNA (pDNA) in *E. coli*, followed by linearization and purification of the pDNA. This is succeeded by the in-vitro transcription (IVT) reaction, where mRNA is synthesized. The third step involves the purification of mRNA, ensuring its purity and quality. Finally, the nucleic acids are encapsulated into lipid nanoparticles (LNPs), which are essential for the *in vivo* delivery of mRNA vaccines and other nucleic acid-based therapies.

Throughout these steps, various analytical methods need to be employed to monitor yields, recoveries and critical quality attributes. A multi step manufacturing process of complex biopharmaceuticals like these requires multiple orthogonal methods for a comprehensive understanding and control of the manufacturing. High purity of raw materials and meticulous in-process control during the IVT phase are critical for achieving high transcription yields, purer mRNA, and reduced consumption of costly reagents. The PATfix analytical platform for pDNA and mRNA enables such accurate and fast orthogonal chromatographic analytics

The encapsulation and subsequent downstream processing of RNA-LNPs is the last step in the manufacturing process, that is formulating the final drug product. LNPs are the most advanced non-viral delivery vehicles in nucleic acid delivery technologies, significantly advancing the possibilities of nucleic acid delivery. However, several challenges persist in their manufacturing and characterization. These include ensuring the correct formation and integrity of the drug product, achieving adequate recovery during purification while maintaining functionality, and producing a thoroughly purified and characterized final product. To address these challenges, purification using CIM monolithic columns has been developed, enabling fine-tuned separation and the production of a highly uniform and functional product.

1. End-to-End manufacturing process controlled by PATfix

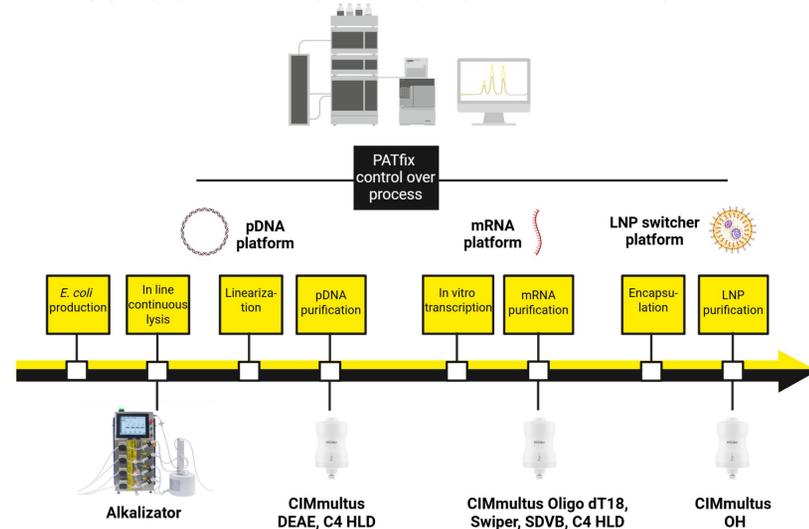Scheme below (Figure 1) represent intensified process from pDNA production in *E.coli* to encapsulated RNA-LNP.

Figure 1: Schematic diagram of end-to-end manufacturing process from pDNA production to LNP purification.

2. pDNA: from in-line lysis to purified pDNA

Alkalizator is a fully automated in-line lysis system designed for pDNA production after *E. coli* expression, equipped with a closed single-use loop and advanced mixing control. It efficiently processes up to 10–50 kg of resuspended cells daily in GMP environments, minimizing pDNA degradation risks associated with uncontrolled alkaline lysis and mechanical stress. Purification is achieved with a platform process which employs chromatography and tangential flow filtration to achieve high purity and formulation of the desired form of plasmid DNA (e.g. supercoiled pDNA for transfection or linear pDNA for in vitro transcription). Chromatography consists of two steps: anion exchange chromatography (CIM DEAE) removes process related impurities and hydrophobic chromatography (CIM C4 HLD) polishes the sample to remove residual contaminants and, most critically, endotoxins, and undesired plasmid DNA isoforms.
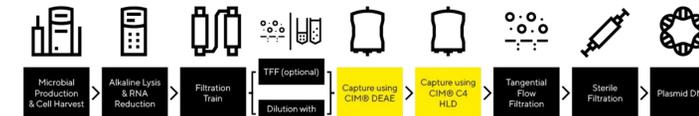

Figure 2: pDNA production and purification scheme.

3. mRNA: IVT and preparation of purified payloads

Upon obtaining purified linear pDNA, template undergoes in vitro transcription (IVT) to produce mRNA. mRNA is captured using Oligo dT18 (affinity column, capture polyadenylated mRNA, elution in water) or Swiper column (multimodal column, capture all RNA, elution in pH gradient). Polishing is achieved with SDVB column (ion-pair RP, dsRNA removal), or C4 HLD column (hydrophobic, aqueous conditions). The toolbox can fit different RNA modalities: from mRNA, saRNA (self-amplifying RNA), to emerging RNA modalities, such as circular and long non-coding RNA.
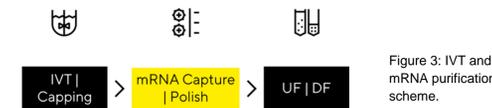

Figure 3: IVT and mRNA purification scheme.

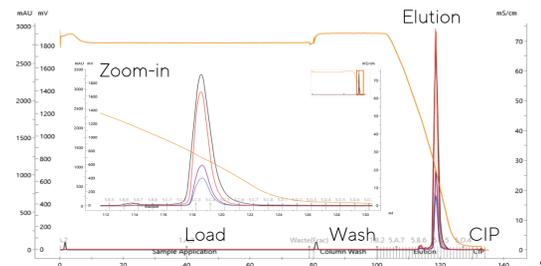## 4. LNP: High recovery process that yields improved particles

Figure 4: Chromatogram of an example of LNP purification. The sample is loaded onto the column, eluted in Elution step and the column is cleaned in CIP step. UV signal is used for detection at 260 nm, 314 nm and 350 nm and MALS detector at 90°.

Purified RNA than undergoes encapsulation into LNP. The purification of the encapsulated RNA-LNP product is currently streamlined at large scales with standard tangential flow filtration (TFF), which removes ethanol used for formulation, exchanges the buffer and concentrates the particles, while at small scales dialysis or ultrafiltration is used. To offer an alternative improved method, chromatographic purification for the RNA-LNP product was developed using CIM monolithic OH columns. Mobile phases were fine-tuned to achieve high recovery and product stability. Under optimized conditions, LNPs were loaded onto CIMmultus columns immediately after encapsulation and subsequent neutralization (Figure 4). The purification process effectively removed ethanol and unencapsulated RNA. Compared to standard purification process, the results demonstrate higher recoveries (>90 %), more uniform particles due to lower size distribution and elevated activity in terms of protein production, assessed by cell-based assay measuring luciferase reporter protein, indicating less active LNP populations were removed during chromatography step (pre-peak, peak tail). Total mRNA recovery and LNP activity were significantly improved using this novel process compared to existing filtration methods (Figure 5).
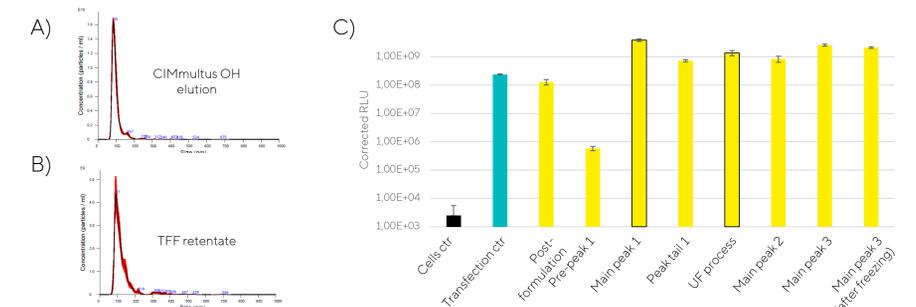

Figure 5: LNP product size measurement and distribution using Nanoparticle Tracking Analysis (NTA) for A) OH column elution and B) TFF retentate. C) Luciferase reporter protein expression using HEK-293 cell assay for OH column elution (3 different processes) and control ultrafiltration (UF) process.

5. PATfix LNP Switcher platform: analysis of multiple payloads

For RNA-LNP characterization, the PATfix LNP Switcher Platform was employed to assess product quality, including encapsulation efficiency, encapsulated RNA quantity and purity, and the presence of RNA-lipid adducts.
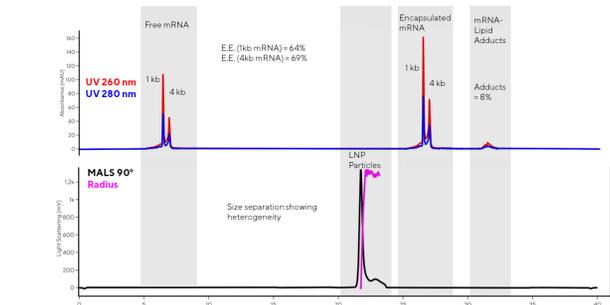

Figure 6: An LNP Switcher chromatogram showing analysis of an LNP with co-encapsulated cargo of 2 different mRNAs.

The platform enables the analysis of encapsulated multiple cargos within a single formulation, crucial for proper quality control of drug products for CAR-T, CRISPR and combination vaccine applications.

6. Conclusion

Presented end-to-end manufacturing process from pDNA, mRNA to LNP, showcases different strategies that are exploited for each step during the preparation and especially purification of the products during manufacturing. CIMmultus chromatographic columns are ideal for this applications. Throughout this intricate process, it is imperative to rigorously monitor and uphold critical checkpoints to the highest standards, utilizing the PATfix system for real-time process analytics and control. This approach results in

- enhanced product quality and process performance, significantly improving purification efficiency
- development of safe and effective RNA-LNP-based therapies, offering promising solutions for multi nucleic acid delivery technologies.

7. References

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