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Simplifying Progress

mRNA/saRNA Process Development With PATfix Analytical Platform

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1. Introduction

Recent years have witnessed an unprecedented increase in development of mRNA-based vaccines and other mRNA-based therapeutics. The process of mRNA production is compared to production of traditional vaccines relatively simple and allows to produce large amount of therapeutic within a few days. Process usually consists of *in vitro* transcription reaction (IVT), followed by chromatographic purification of mRNA molecule (Fig. 1). Process needs to be coulpled with effective process analytical technology (PAT) at each step in order to achieve the highest product purity.

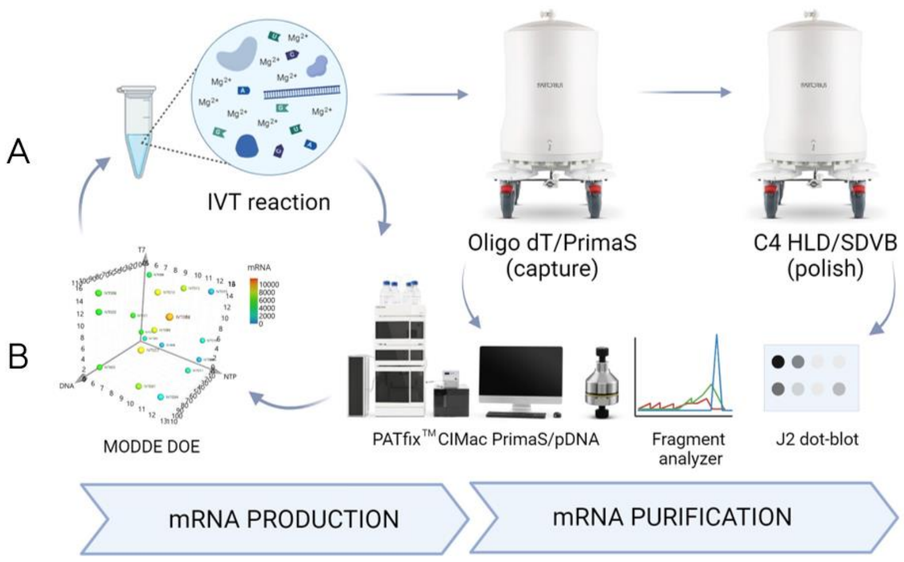

Figure 1: Schematic overview of mRNA production and purification workflow, consisting of mRNA production and purification (A), combined with various analytical tools (B) – PATfix PrimaS for IVT monitoring and detection of impurities e.g. NTPs, PATfix pDNA for detection of residual pDNA template, dot-blot for dsRNA detection and fragment analyzer for mRNA integrity determination.

2. Optimization of IVT reaction using PrimaS analytics

The cost of mRNA production is primarily driven by IVT reagents, therefore optimization of mRNA yield in IVT reaction is crucial for lowering cost-of-goods. To monitor the IVT reaction in near-real time, we implemented a rapid at-line monitoring of consumption of NTPs and production of mRNA (1, 2), with a 7 min read-out using PATfix® analytical platform, which separates all NTPs (wild-type and modified), mRNA and pDNA (Fig. 2).

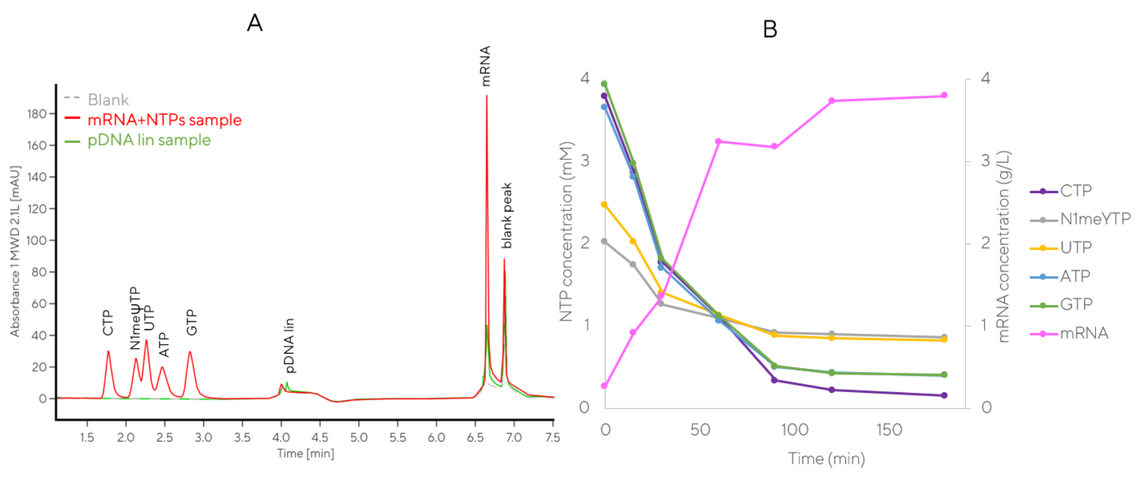

Figure 2: A) CIMac PrimaS separation of IVT components. CTP, UTP, ATP, GTP as well as modified nucleotide (N1meUTP) are baseline separated. B) IVT monitoring consumption of NTPs and production of mRNA.

PATfix coupled with CIMac PrimaS® supports both one-factor-at-a-time (OFAT) and Design-of-Experiment (DOE) optimization of IVT. With literature study we identified pDNA, T7 RNAP, Mg^{2+}, NTPs and Mg:NTP ratio to be important factors for mRNA production. A DOE was planned and analysed with MODDE® 13 in 3 iterations to achieve high model validity and good model fit (R^2 0.97), with maximum relative yield reached at 7 mM of NTP and MgNTP ratio of 1.2 while maximum total yield of 10 g/L was reached at 11 mM of NTP and MgNTP ratio of 1.0 (4).

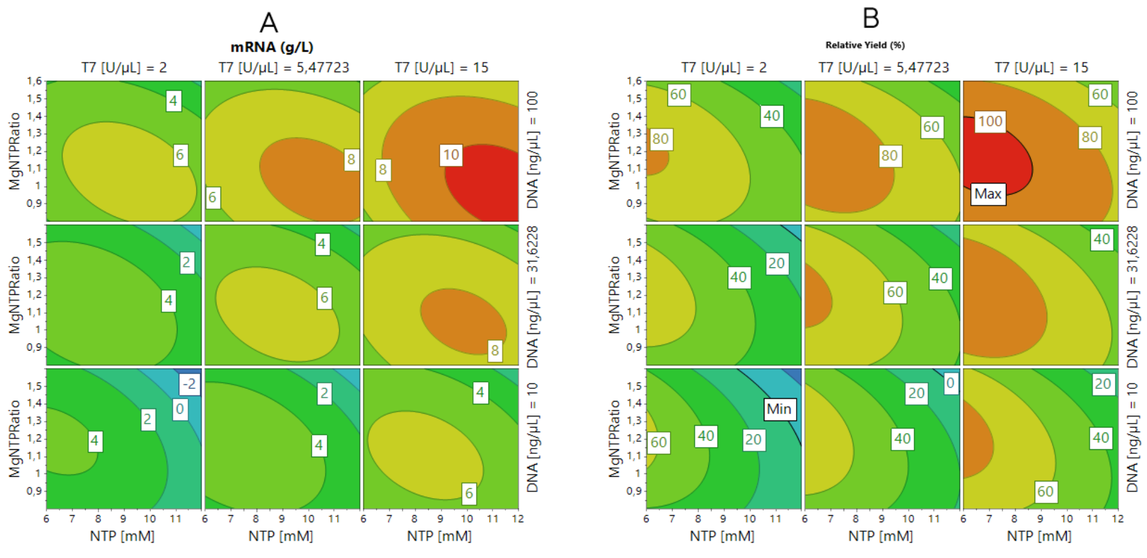

Figure 3: Response surfaces of DOE model. A) mRNA yield response. B) Relative yield response. Relative yield is defined as percentage of maximum possible theoretical yield.

3. Purification of IVT mRNA/saRNA

mRNA purification is usually two-step process, consisting of capture and polishing steps. For capture, affinity chromatography is typically used for polyadenylated mRNA (5). Multimodal chromatography such as CIM Swiper can be used for purifying RNA modalities without polyA tail (e. g. tRNA, circRNA) or larger RNAs (saRNA). CIM Swiper removes majority of process impurities (NTPs, pDNA, proteins) in flow-through (FT) and wash fractions. RNA is eluted in neutral pH at room temperature with high recovery (>80%, Fig. 4) (6).

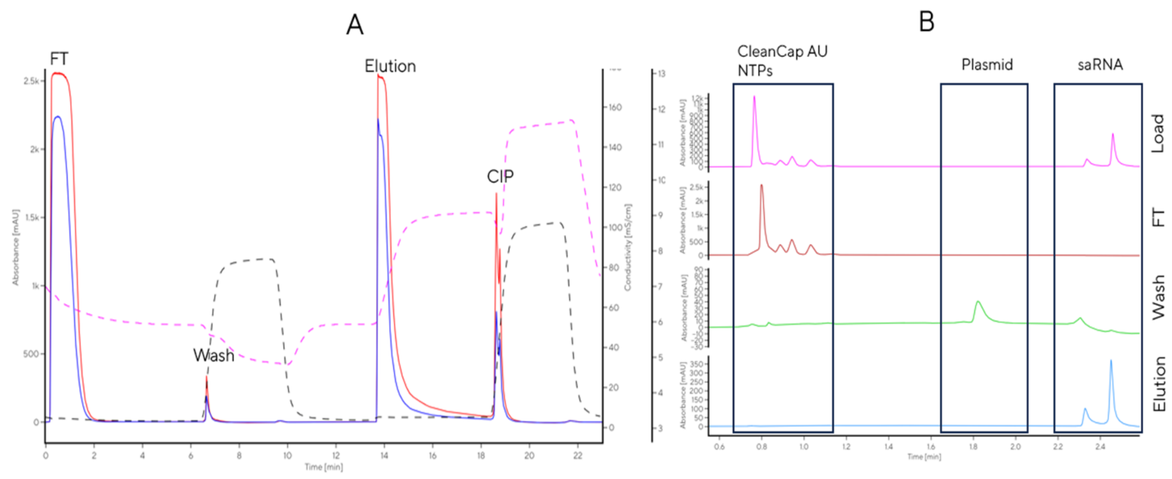

Figure 4: Purification of saRNA with multimodal chromatography using CIM Swiper column. A) CIM Swiper preparative chromatogram. B) CIMac PrimaS/PATfix analytics of CIM Swiper elution fractions showing removal of NTPs in flow through (FT), of residual template DNA in wash and high purity of saRNA in elution fraction.

After capture, polishing of mRNA/saRNA with ion-pair reverse phase chromatography (e.g. CIM SDVB) at room temperature or mildly elevated temperatures (37-50°C) achieves size-based, denaturing separation of RNA species. SDVB removes dsRNA, shorter RNA molecules (fragments) as well as residual pDNA template (Fig 5). High purity of even the most labile RNAs (e.g. saRNA) is achieved with high yield (7,8).

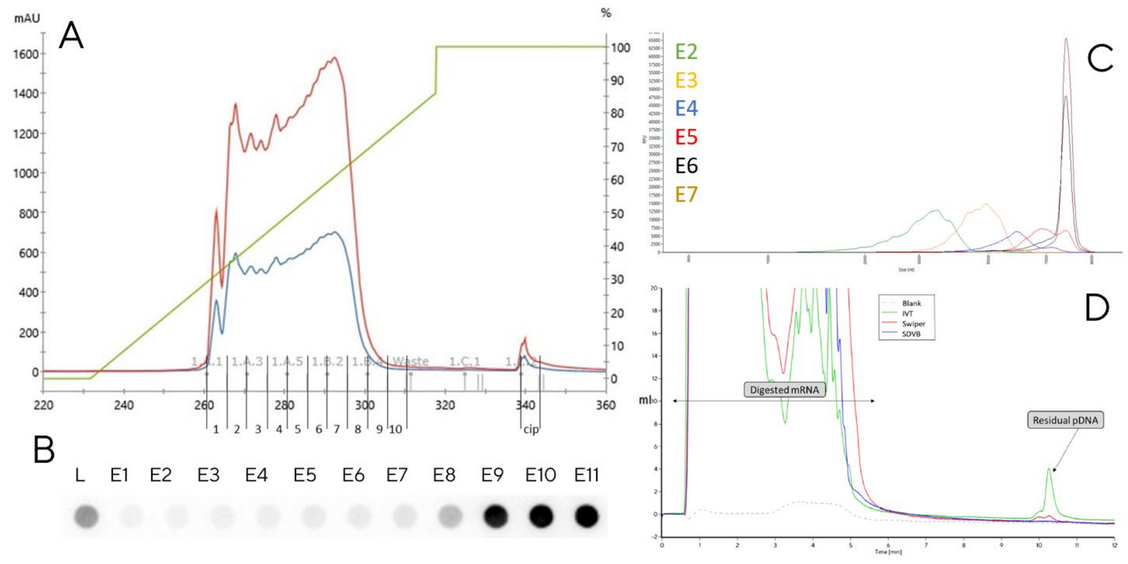

Figure 5: saRNA polishing with reverse-phase chromatography using CIM SDVB column. A) saRNA was purified with CIM SDVB operated in linear gradient from 7.5% - 18% CAN pH 7.0 with 50 mM TEAA. Fragmented RNA is removed in fractions E1-5 and dsRNA is removed in late elution fractions (E8-E11). B) J2 dot-blot analytics of elution fractions. C) Fragment Analyzer purity profile of elution fractions. D) CIMac pDNA analytics of residual pDNA shows complete removal of pDNA with SDVB chromatography (method described in reference 7).

4. Conclusions

- Rapid at-line HPLC analytics using CIMac PrimaS column on PATfix system allows separation of all NTPs (WT and modified).
- DOE is a preferable approach to optimization of multiple-parameter reaction, such as IVT supported by MODDE®13 software.
- Two step purification of mRNA (Swiper and SDVB) removes process impurities (pDNA, NTPs, proteins) as well as sample impurities (fragmentation, dsRNA).

5. References

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