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Gram-Scale mRNA Production Using a 250-mL Single-Use Bioreactor

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Optimization of IVT reaction using PrimaS analytics

The cost of mRNA production is driven by IVT reagents, particularly the co-transcriptional capping reagents. Optimization of mRNA yield is therefore crucial for lowering the cost of mRNA production. To monitor the IVT reaction over time, we implemented a rapid at-line HPLC monitoring of consumption of NTPs and production of mRNA, with a sub-3 min read-out. Use of CIMac PrimaS analytical (Fig. 1) column allowed us to determine and adjust key IVT components that influence the kinetics of mRNA production and are critical for optimization of continuous addition of reagents, i.e. fed-batch IVT.

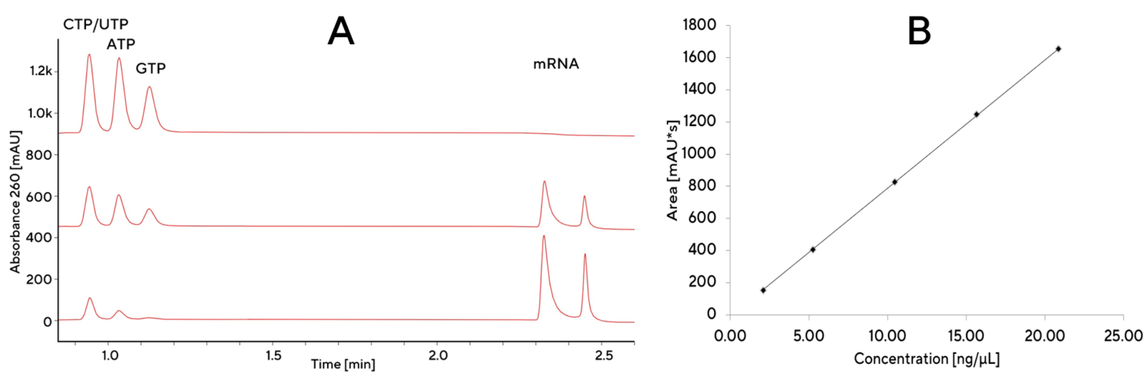

Figure 1: A) Representative CIMac PrimaS chromatograms from t=0, mid-point, end-point of IVT reaction. B) Calibration curve for eGFP mRNA concentration.

With this approach we found out that gradual addition (fed-batch), rather than high starting concentration of NTPs (batch), would be beneficial for increasing the productivity of the IVT. Fed-batch approach was also tested in the presence and absence of Mg^{2+} ions (Fig. 2). Reaction with addition of NTPs did not increase mRNA yield, while addition of NTP-Mg^{2+} resulted in significantly higher mRNA concentration, suggesting that Mg^{2+} was the limiting factor for the progression of mRNA production. With optimization of the fed-batch procedure we were able to reached mRNA concentrations of up to 10 mg/mL in 3 hours, demonstrated with two constructs (eGFP and Cas9).

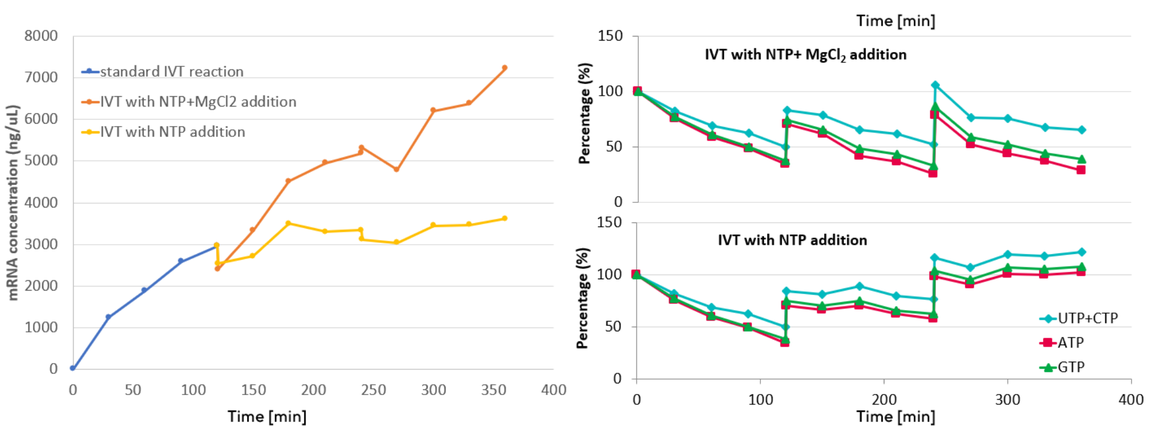

Figure 2: Effect of IVT components on the kinetics of mRNA production and nucleotide consumption. A) NTP-Mg^{2+} complex addition caused significantly higher mRNA conc. (B) in comparison to addition of NTPs alone that led to accumulation of NTPs (C).

Conclusion

- Rapid at-line HPLC analytics using CIMac PrimaS column enables an insight into kinetics of mRNA production.
- Fed-batch IVT can improve mRNA yield from 4-6 mg/mL to 10-12 mg/mL and reduce costs by a significant margin.

References

- Pregeljc, D. et al. "Increasing yield of IVT reaction with at-line HPLC monitoring." Biotechnology and Bioengineering (2022), 3, 737-747

Continuous feeding of IVT reaction with AMBR® bioreactor

Fed-batch reactions can also be performed by continuous feeding, requiring automated control system. We used Ambr® 250 bioreactor platform, demonstrating for the first time its potential for mRNA production. First we designed a fed-batch IVT reaction in a thermal shaker, sampled and analyzed at-line by CIMac PrimaS analytics. Based on NTP consumption kinetics, the Ambr® 250 protocol was then designed to feed a defined mixture of NTP-Mg^{2+} continuously (Fig. 3).

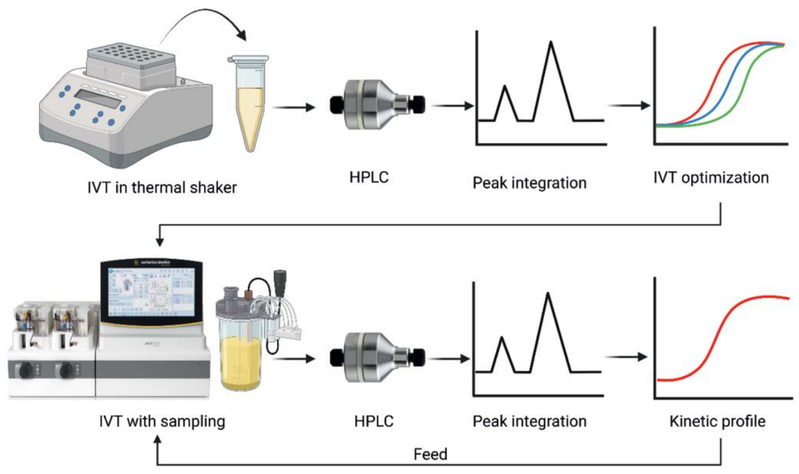

Figure 3: IVT method transfer from thermal shaker to Ambr® 250 Modular. IVT reaction in thermal shaker with manual bolus addition of NTP-Mg^{2+} feeds. Continuous feed addition in Ambr® 250 is based on kinetics determined in thermal shaker.

The Ambr® 250 bioreactor run was planned in four phases (start, feed 1, feed 2, feed 3) with gradual decrease of feed flow rates to match the NTPs kinetics observed in the thermal shaker. The lowest volume that allows impeller function in Ambr® 250 is 100 mL; this volume was utilized as a starting volume for the IVT reaction, thereby theoretically allowing 150 mL of feed to be added. Aliquots were removed manually every 15–30 min via septum cap and quenched with the same volume of 100mM EDTA before analysis on CIMac PrimaS column (Fig. 4). The reaction was arbitrarily quenched after 420 min, after 177 mL total volume was reached with a 12 mg/mL final concentration, equal to production of 2.12 g of mRNA.

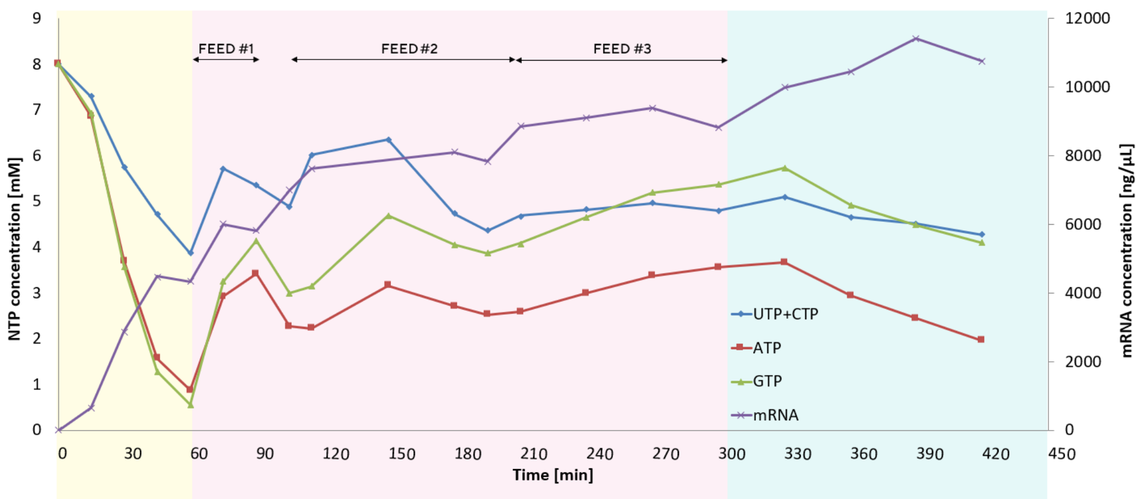

Figure 4: Ambr® 250 run info. Kinetics of mRNA production and NTP consumption as determined by CIMac PrimaS HPLC analytics.

Conclusion

- Standard fed-batch is highly comparable to Ambr® run, demonstrating scalability from 100 µL to 100 mL IVT reaction volume
- Ambr® 250 Modular is suitable for multi-gram synthesis of mRNA

References

- Skok, J., Megušar, P. et al. "Gram-Scale mRNA Production Using a 250-mL Single-Use Bioreactor." Chemie Ingenieur Technik (2022) 94 1928-1935