Simplifying Progress

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

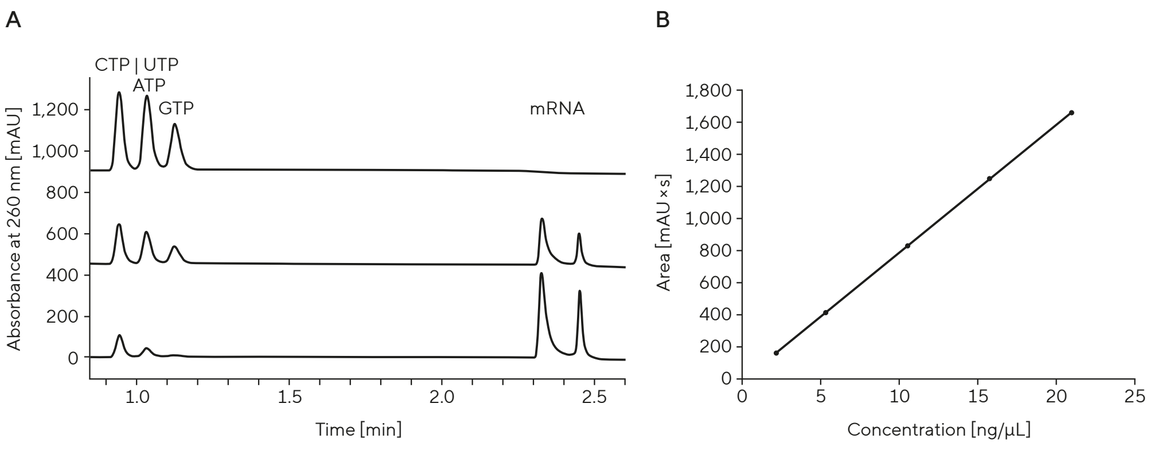
Janja Skok[1], Polona Megušar[1], Tina Vodopivec[1], Domen Pregeljc[1], Nina Mencin[1], Matevž Korenč[1], Andreja Krušič[1], Anže Martinčič Celjar[1], Nejc Pavlin[1], Jana Krušič[1], Matthias Mueller[2], Kevin McHugh[2], Aleš Štrancar[1], and Rok Sekirnik[1*]

[1] Sartorius BIA Separations d.o.o., Mirce 21, 5270 Ajdovščina, Slovenia
[2] Cell culture Technology, Sartorius Stedim Biotech GmbH, August Spindler-Straße 11, 37079 Göttingen, Germany
* Corresponding author: rok.sekirnik@biaseparations.com

Optimization of the In Vitro Transcription Reaction Using CIMac PrimaS® Analytic

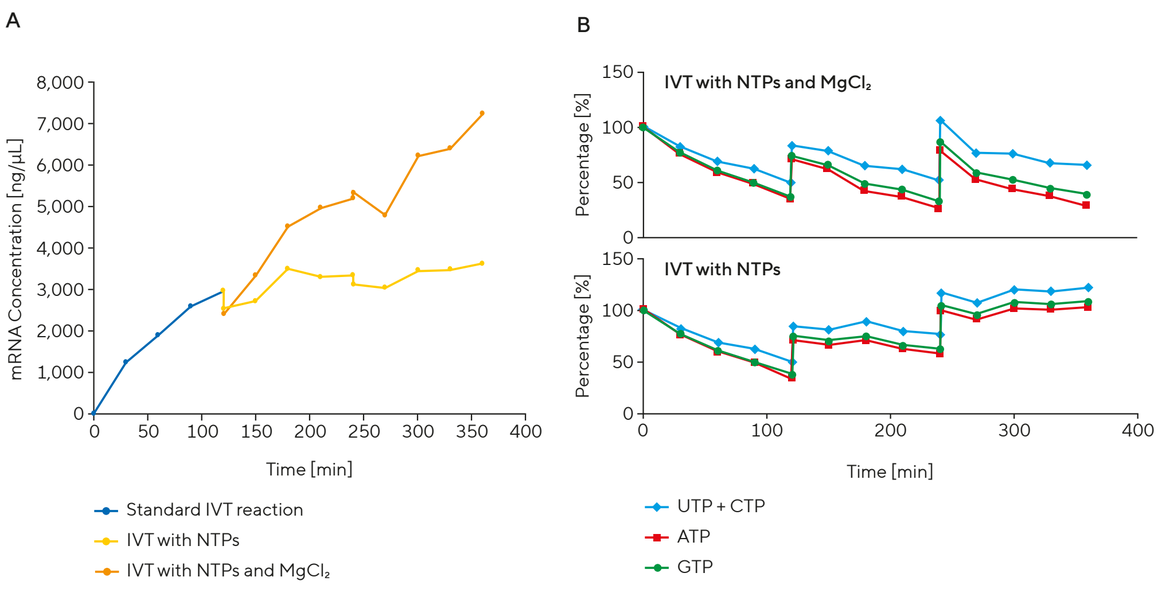
The cost of mRNA production is driven by in vitro transcription (IVT) reagents, particularly the co-transcriptional capping reagents. Therefore, optimizing mRNA yield is crucial for lowering production costs. To monitor the IVT reaction over time, we implemented rapid at-line HPLC monitoring of consumption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

Figure 1: ***(A)*** *Representative CIMac PrimaS® Chromatograms From t = 0, Mid-Point, and End-Point of IVT Reaction.* ***(B)*** *Calibration Curve for eGFP mRNA Concentration*



With this approach, we discovered that the gradual introduction of NTPs (i.e., a fed-batch process) rather than starting with a high concentration (i.e., a batch process) can enhance the productivity of the IVT. The fed-batch approach was also tested in the presence and absence of magnesium ions (Mg^{2+}) (Figure 2). The reaction with the addition of NTPs did not increase mRNA yield. In contrast, the 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. By optimizing the fed-batch procedure, we were able to reach 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 Nonsumption*



Note. **(A)** NTP-Mg^{2+} complex addition produced a significantly higher mRNA concentration. **(B)** The addition of NTPs alone led to the accumulation of NTPs.

Conclusion

- Rapid at-line HPLC analytics using CIMac PrimaS® column delivers insights into the 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

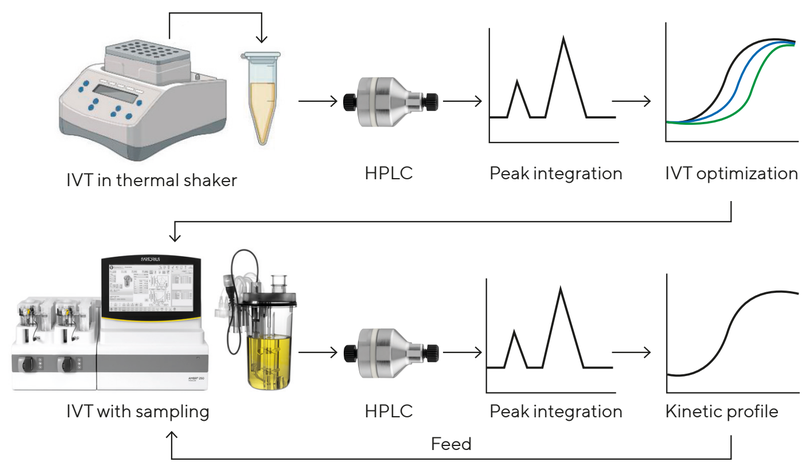
References

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

Continuous Feeding of the IVT Reaction With the Ambr® 250 Bioreactor

Fed-batch reactions can also be performed by continuous feeding, requiring an automated control system. We used the Ambr® 250 bioreactor platform, demonstrating for the first time its potential in mRNA production. First, we designed a fed-batch IVT reaction in a thermal shaker, sampled, and analyzed the reaction 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 (Figure 3).

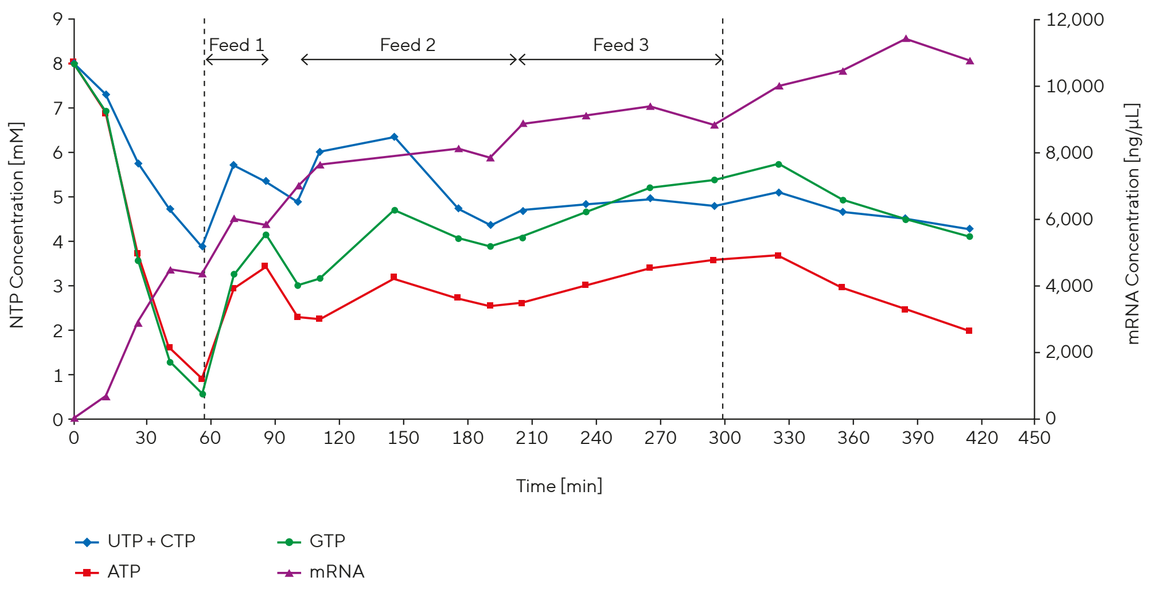
Figure 3: *IVT Method Transfer from Thermal Shaker to Ambr® 250 Modular*



Note. 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, and feed 3) with a gradual decrease of feed flow rates to match the NTP 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, theoretically allowing the addition of 150 mL of feed. Aliquots were removed manually every 15 – 30 min via septum cap and quenched with the same volume of 100 mM EDTA before analysis on a CIMac PrimaS® column (Figure 4). The reaction was quenched after 420 min, when 177 mL total volume was reached with a 12 mg/mL final concentration, equal to the production of 2.12 g of mRNA.

Figure 4: *Ambr® 250 Run Info*



Note. **(A)** Kinetics of mRNA production and NTP consumption as determined by CIMac PrimaS® HPLC analytics.

Conclusion

- The standard fed-batch reaction is highly comparable to the Ambr® 250 run, demonstrating scalability from 100 µL to 100 mL IVT reaction volume
- The 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