Simplifying Progress

Optimization of mRNA Production and Purification: Characterization and Controlling of Post-Transcriptional Polyadenylation

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Introduction

While mRNA therapeutics developed in recent years show tremendous potential, there are still technical obstacles to overcome, two of the most significant being mRNA's intrinsic instability and its immunogenicity. In order to address these challenges, several different approaches can be used, including optimization of 3' poly(A) tail length.
mRNA is commonly polyadenylated co-transcriptionally, by transcription from DNA template encoding the tail. However, long (>120 nt) stretches of thymidines in DNA sequence can lead to cloning difficulties, plasmid instability during culture and formation of multimeric plasmid structures. Enzymatic post-transcriptional addition of poly(A) tail is thus an attractive alternative. Very limited knowledge base is available on post-transcriptional polyadenylation (PA), therefore our main focus was to improving the understanding of *in vitro* PA reaction.

Optimization of post-transcriptional polyadenylation

In order to control the addition of adenosine triphosphate (ATP) to the 3' end of mRNA, we applied rapid at-line analytics with CIMac PrimaS analytical column to monitor ATP consumption with sub-1.2 min analytical read-out time. A calibration curve for ATP (Figure 1A) was applied to quantify ATP levels in PA reaction at different time-points (Figure 1B).

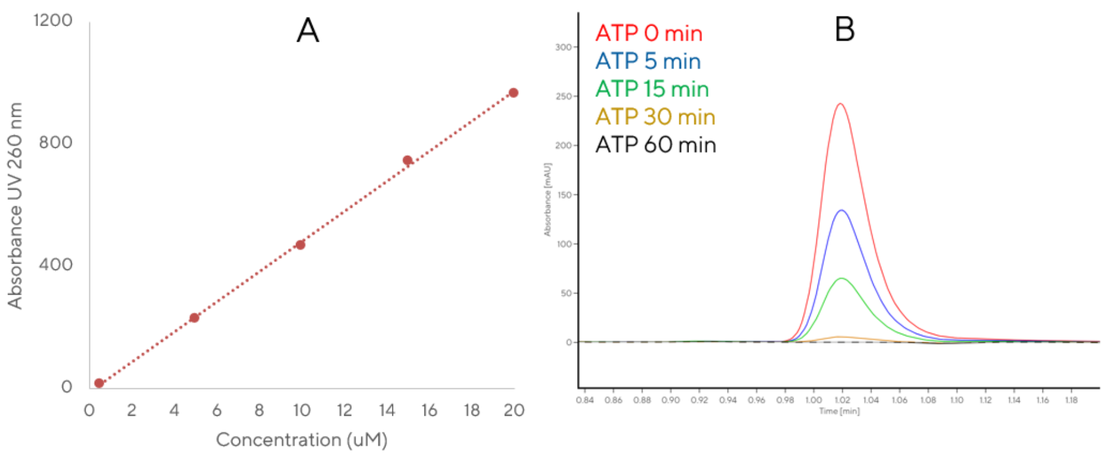

Figure 1: CIMac PrimaS HPLC analytics. A) Calibration curve for ATP concentration. B) Monitoring of ATP consumption during polyadenylation reaction with CIMac PrimaS.

First, the effect of poly(A) polymerase (PAP) concentration on ATP consumption was tested (Figure 2A). Higher PAP concentrations resulted in faster ATP consumption. Increasing mRNA concentration at fixed PAP concentration showed similar effect (Figure 2B). Next, poly(A) length was calculated from the consumption of ATP (assuming normal distribution of poly(A) lengths), showing that mRNA concentration has no observable effect on the kinetics of PAP reaction (defined as poly(A) length/min) (Figure 2C).

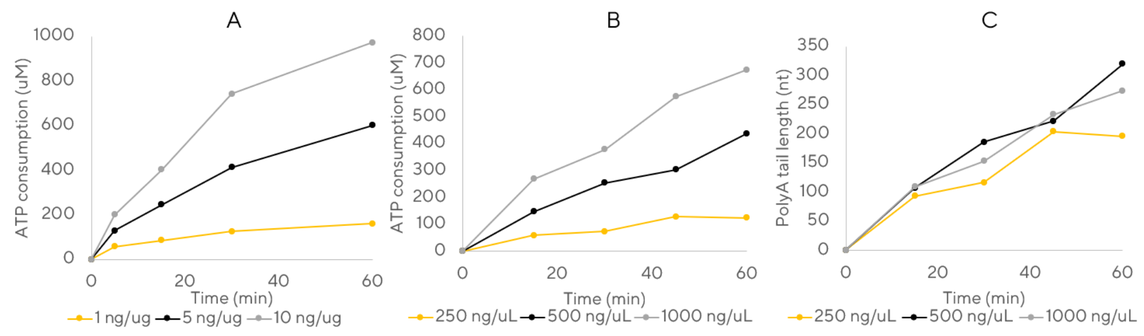

Figure 2: Optimization of PA reaction. A) ATP consumption in PA reaction at different PAP concentrations. B) ATP consumption in PA reaction at different mRNA concentrations. C) Poly(A) length calculated from consumption of ATP determined by CIMac PrimaS at different mRNA concentrations.

Selected PA reaction conditions (500 ng/uL mRNA; 1 mM ATP, 1 ng/ug PAP) were tested on three mRNA constructs – Decorator (1260 nt), GFP (915 nt) and Luciferase (1710 nt). Reactions were quenched at approximately 250 mM, 400 mM and 600 mM of ATP consumed (Figure 3A). Poly(A) tail length was calculated from consumption of ATP and kinetics of poly(A) addition to 3' mRNA end was comparable between all constructs (Figure 3 B).

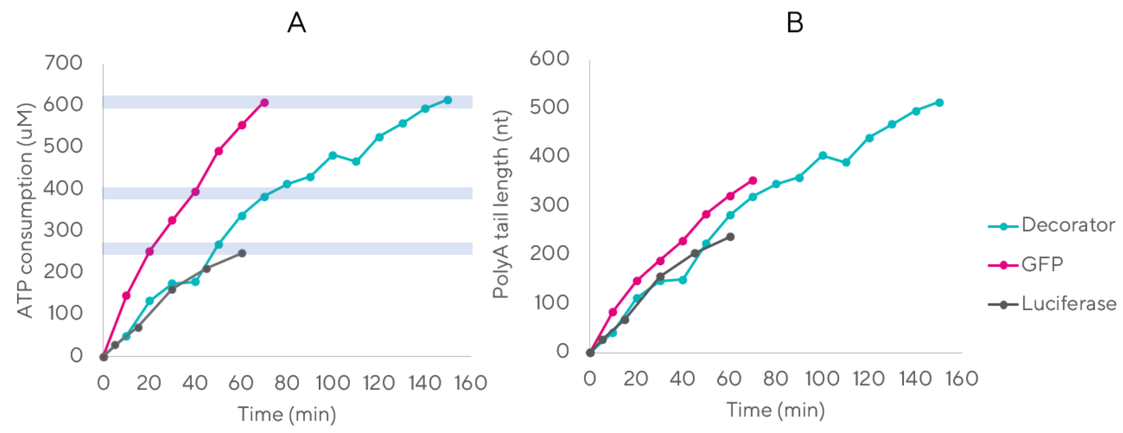

Figure 3: PA reaction with different mRNA constructs. A) Consumption of ATP. B) Poly(A) tail length determined by CIMac PrimaS.

Characterisation of poly(A) tail

For additional characterization of poly(A) tail lengths, mRNAs were digested with Rnase T1, which cleaves ssRNA at G-residues and leaves poly(A) tail intact. After T1 digestion, poly(A) tails were purified with CIM Oligo dT affinity chromatography and analyzed with agarose gel electrophoresis and a newly developed IP-RP method for poly(A) tail length determination using CIMac SDVB analytical column (Figure 4). Poly(A) tail lengths were compared with theoretical calculations based on ATP consumption. Values were in close agreement, demonstrating the potential to use ATP consumption methodology to control poly(A) tail length in PA reaction.

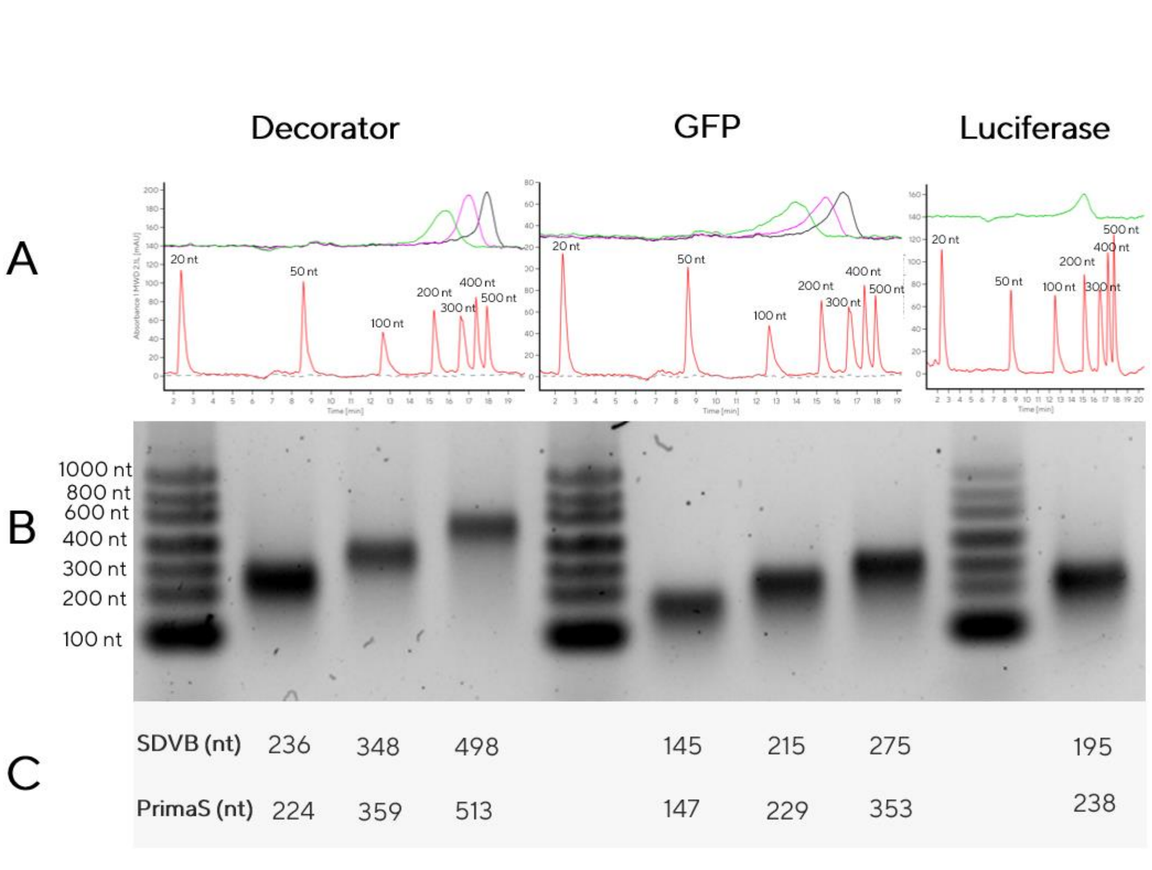

Figure 4: Analytics of poly(A) tail lengths for different mRNA constructs. A) CIMac SDVB analytics of min (green), mid (pink) and max (black) poly(A) length compared to RNA Ladder (red). B) Agarose gel electrophoresis of different poly(A) tails. C) Comparison between CIMac SDVB and PrimaS determined poly(A) lengths.

Conclusions

- Rapid HPLC analytics using CIMac PrimaS column enables monitoring of poly(A) length near real time.
- Post-transcriptional polyadenylation reaction can be sampled at high frequency and analyzed at-line
- CIMac SDVB analytics in combination with enzymatic digestion can be used for characterization and poly(A) length determination.