Improvement of self-amplifying RNA quality and potency with IVT optimization

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Abstract

Working with large/self-amplifying RNAs provides its own set of challenges. In this study we aimed to improve yields and quality of self-amplifying RNAs (saRNA) by optimizing conditions of the In-Vitro Transcription (IVT) reaction. Most pre-made IVT buffers are not optimized for saRNA. We explored incubation temperatures, magnesium source and content, and reaction buffer conditions. These efforts have allowed us to produce RNAs as large as 16kb at high yields, low residual dsRNA, and high potencies.

Introduction

Self-amplifying RNAs provide many benefits in the field of RNA therapeutics. This technology has the ability to encode for multiple proteins with a relatively low dose of payload.

Due to their large size, self-amplifying RNAs are more prone to hydrolyzation and degradation during processing. Along with a streamlined manufacturing process, carefully optimized IVT conditions can preserve the integrity of longer RNAs. Buffer composition and incubation conditions can have significant effects on both RNA yield and quality. In conjunction, residual levels (especially dsRNA) and cost-effectiveness should also be taken into account when altering these parameters. By considering the entire process, we can maximize the potential of saRNA as a novel therapeutic.

Methods

IVT reactions were incubated in a shaking thermomixer and purified by chromatographic methods. RNA yields were determined by A260 readings using a NanoDrop® spectrophotometer (Thermo Fisher Scientific). dsRNA was assessed by ELISA using the K1 antibody. RNA integrity was assayed by capillary electrophoresis on a PA800 Plus System (Sciex). Potency assay performed by Replicate Bioscience.

Results

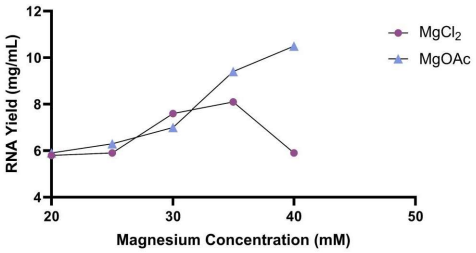

Figure 1. Magnesium source and concentration drive RNA yield
$MgCl_2$ and MgOAc were titrated along with total NTP concentration to maintain a 1:1 ratio. Stability of the IVT reaction was higher in reactions using MgOAc, allowing us to reach concentrations of >10mg/mL.

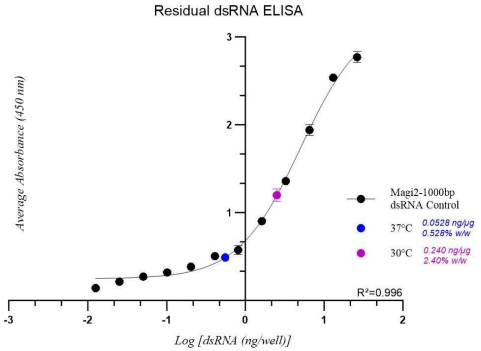

Figure 2. dsRNA values at different IVT incubation temperatures
Lower IVT incubation temperatures help preserve the integrity of longer RNAs, but also promotes the formation of dsRNA. IVT reactions were run at 30°C and 37°C and dsRNA was measured by ELISA. Lower IVT incubation temps are not the ideal way to improve saRNA integrity.

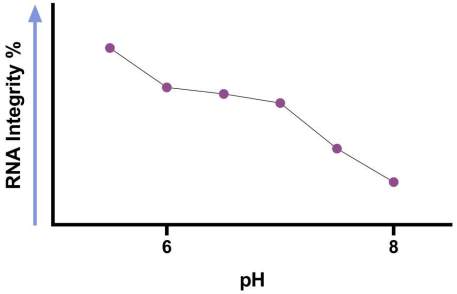

Figure 3. RNA Integrity greatly influenced by pH
IVT reactions were run using a construct >10kb at a range of pHs. No significant yield differences were observed between samples, but RNA integrity was greatly improved at lower pH.

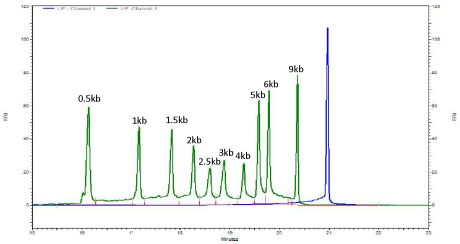

Figure 4. saRNA is larger than commercial standards
Integrity of saRNA can be difficult to interpret due to the lack of commercial standards >9kb. Extensive development of the CE method has allowed us to analyze RNA integrity of molecules as large as 16kb.

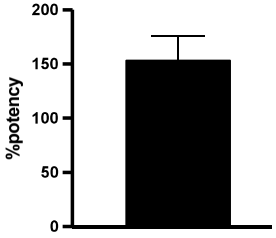

Figure 5. Increased RNA activity levels achieved with optimized conditions
By optimizing conditions to maximize capping efficiency and integrity, high potencies can be observed at very low doses with saRNA.

Conclusions

Self-amplifying RNA is more challenging to produce due to higher instability and lack of commercially-available assays. Process development was performed alongside analytics to improve metrics that ultimately lead to high RNA potency with low residuals. While RNA purification is also optimized for saRNA, we have found the parameters of the IVT reaction to be most important for producing high quality RNA.

References

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