Effective Quality Control is Crucial Throughout mRNA Product Development

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Abstract

Introduction: At the forefront of therapeutic delivery is an array of vectors and targeting methods that hold immense promise for precision medicine in cell and gene therapies. Lipid nanoparticle (LNP) encapsulated RNA and circular RNA (circRNA) are emerging as a preferred method for new therapies, offering faster development and production with high specificity, transient expression and reduced long-term risk, as it does not integrate in the host genome. As a result, mRNA therapeutics have the potential to quickly and effectively treat a wide range of diseases.

Methods: Here we present an end-to-end RNA production workflow engineered for quality and scalability through novel methods for minimizing synthetic risks and validation by sequencing. Critical to successful production is maintaining the integrity of complex polyA regions. Using proprietary methods for synthesis and plasmid preparation, monodispersity of polyA tails are conserved within DNA templates.

Results: Examination of the final mRNA transcript is performed utilizing a new library generation method for PacBio long-read Single-Molecule, Real-Time (SMRT) technology, capable of full-length sequencing of products up to and beyond 10kb without limitations or bias due to repetitive sequence or C/G content. Crucially, this method preserves the entire length of the polyA tail enabling sensitive and specific counting of the polyA tail length, an important parameter when confirming overall sample purity, including intermediate and alternative products present within the sample.

Conclusion: Both circRNA and linear mRNA products are amenable to the above workflow, providing a robust solution for any RNA research program. The workflow results in an accelerated mRNA development program with higher quality templates and rigorous qualification post transcription.

Conclusions

- This end-to-end workflow for codon-optimized high-quality gene synthesis and in vitro transcription enables rapid and efficient production of functional custom mRNA.
- For quality control throughout the development process, plasmids should be verified by Sanger and/or whole plasmid sequencing at each step to ensure clonal and intact sequence is maintained.
- Final product is verified with next-generation sequencing to detect primary, intermediate, and alternative products present within a sample and determine the proportion and full sequence identity of each mRNA, including polyA tail length.

Optimized mRNA Synthesis Solutions

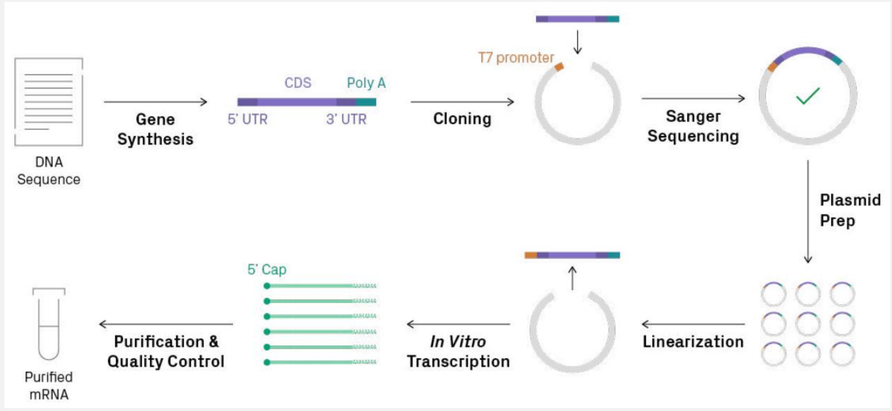


Figure 1. In vitro transcription is a powerful technique to generate messenger RNA (mRNA) that can be used to express proteins. A critical component of the mRNA molecule is the poly(A) tail, which regulates mRNA stability and promotes translational initiation. Longer poly(A) tails generally confer greater stability with an optimal length of 100-140 bases. Adding a poly(A) tail of >100 bases can be challenging to incorporate. Here we evaluate a novel synthetic approach for adding a poly(A) sequence up to 140 bases. A mega-scale plasmid preparation was performed for each culture and DNA yield was measured.

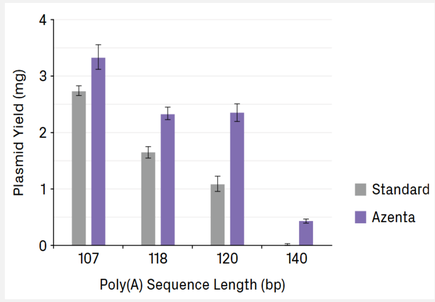


Figure 2. The protocol from Azenta generated plasmid preps with significantly higher yields compared to the standard protocol for all poly(A) lengths tested. Yield from mega-preps of plasmids with long poly(A) sequences using a standard protocol and the proprietary protocol from Azenta. Mean ± SEM

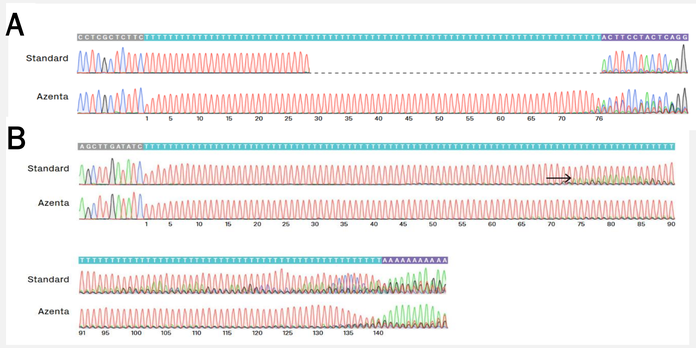


Figure 3. Poly(A) integrity was analyzed using Sanger sequencing. In plasmids with poly(A) lengths from 70 to 140 bp, truncations were observed more frequently with standard plasmid preparation than with the optimized protocol from Azenta. Clonal truncations and mixed populations were detected. Sequencing of representative plasmid preparations using a standard protocol and the proprietary protocol from Azenta. (A) Plasmid with a 76-bp poly(A) sequence. A deletion is observed with the standard protocol, whereas the protocol from GENEWIZ produced the expected results. (B) Plasmid with a 140-bp poly(A) sequence. The chromatogram of the standard protocol shows high background (arrow) starting at position 74 of the poly(A) region, indicating a heterogeneous mixture of plasmids. The background signal is consistent with a truncated poly(A) sequence. The chromatogram of the protocol from GENEWIZ shows an intact poly(A) sequence that is highly homogeneous.

Novel Sequencing Solutions

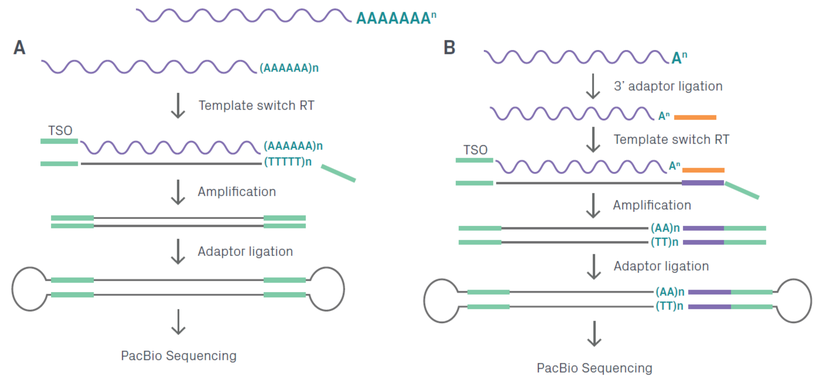
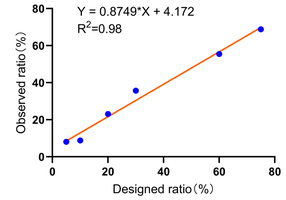


Figure 4. Validate and confirm RNA therapeutic product. Full-length sequencing of an RNA molecule determines the exact length of the polyA tail by deep sequencing to validate therapeutic purity and accuracy. This method also allows for sequencing of intermediate and alternative products.



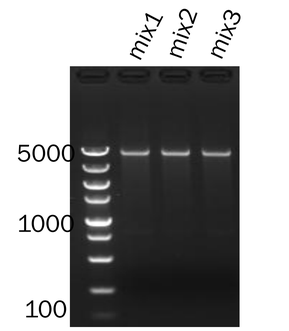
SAMPLE	OBSERVED	EXPECTED
10A	8.8%	10%
30A	8.1%	5%
50A	35.7%	30%
70A	23.1%	20%
100A	55.5%	60%
120A	68.8%	75%

Figure 5. Accurate quantification of polyA lengths in an RNA mixture. The synthetic RNA molecules shown in Figure 2 were mixed in the ratios indicated in the table. The observed percentage of each template closely matches with the expected (R^2= 0.98).

MIX 1	CCS	OBSERVED	EXPECTED
Spike A	10,728	33%	33.3%
Spike B	10,837	33%	33.3%
Spike C	11,339	34%	33.3%

MIX 2	CCS	OBSERVED	EXPECTED
Spike A	7,471	10%	10%
Spike B	22,141	30%	30%
Spike C	43,757	60%	60%

MIX 3	CCS	OBSERVED	EXPECTED
Spike A	23,144	24%	25%
Spike B	24,464	25%	25%
Spike C	49,212	51%	50%



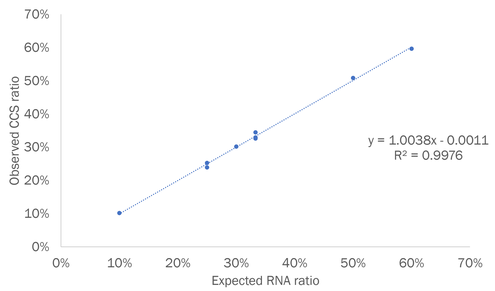


Figure 6. Accurate detection of proportion of each mRNA in multivalent mix. Using 3 admixtures, observed frequency of CCS reads was consistent with expected frequency in a multivalent mRNA vaccine mixture.