Advancing mRNA Manufacturing and Characterization: Comprehensive Analytical Capabilities for mRNA Therapeutics

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About Exothera

Exothera is a trusted analytics partner, offering **flexible, scalable, and regulatory-compliant analytical services** across virus-based and RNA therapeutics. Whether part of a full CDMO project or as standalone services, our advanced analytical platforms support clients at every stage, ensuring product quality, safety, and efficacy.

The growing demand for high-quality mRNA therapeutics requires robust manufacturing and analytical solutions to ensure product integrity, efficacy, and regulatory compliance.

The Ntensify™ mRNA platform provides a scalable, GMP-compliant approach for mRNA production, while our portfolio of advanced analytical tools enables comprehensive mRNA characterization. Internalized analytics help to optimize mRNA production efficiency by reducing lead times and consolidating critical quality attribute data.

We offer a full-service mRNA release testing panel to ensure product quality, safety, and efficacy. This panel assesses key attributes such as identity, purity, potency, and residual impurities, adapting to different manufacturing stages—from starting materials to drug substance (DS) and drug product (DP). **By also integrating orthogonal approaches, we reinforce data reliability and provide a comprehensive quality control strategy for mRNA therapeutics.**

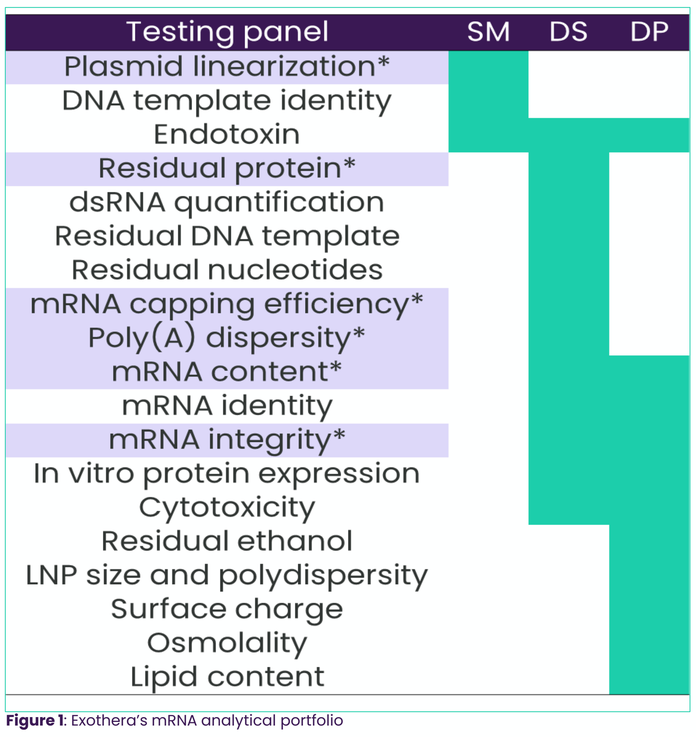


Figure 1: Exothera's mRNA analytical portfolio

Innate Immune Response Reporter Assay and dsRNA ELISA provide complementary strategies for dsRNA Detection

J2 Antibody-based ELISA

J2 monoclonal antibody is widely recognized as the gold standard for detecting dsRNA due to its high specificity and broad recognition of dsRNA structures.

Functional Readout of dsRNA Innate Immune Activation

- The Reporter assay enables the detection of dsRNA-mediated immune activation, providing insight into the pathway IFN-I.
- Cells stimulated with HMW poly I:C exhibit a strong dose-dependent activation, confirming the assay's ability to detect dsRNA-driven immune activation

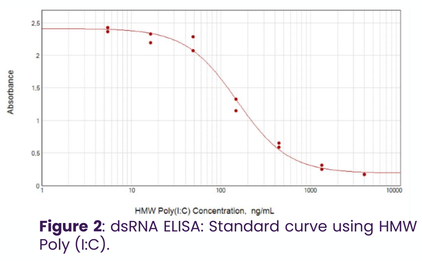


Figure 2: dsRNA ELISA: Standard curve using HMW Poly (I:C).

Same Standard Across Assays, ensuring consistency across methodologies

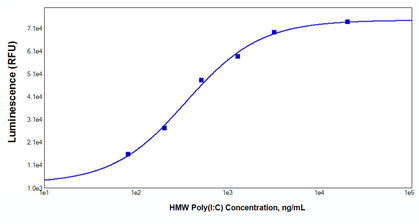


Figure 3: dsRNA Report Cell based Assay: Dose Response Validation with HMW Poly (I:C).

Key Message

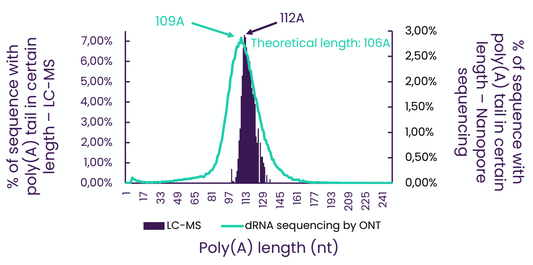
- dsRNA ELISA provides absolute quantification of dsRNA.
- Innate Immune Response Reporter Assay confirms whether dsRNA contaminants activate innate immunity.
- This offers a biological validation of immunostimulatory dsRNA contaminants.

How to leverage existing analytical tools for RNA vaccines applications

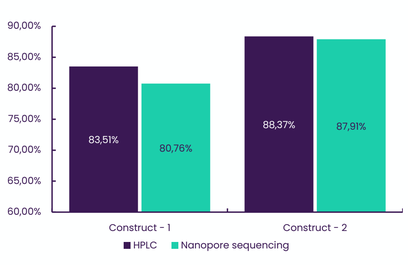
Development of a Nanopore sequencing platform to replace multiple existing methods with a single tool

Beside the confirmation of nucleotide sequence, Nanopore workflows offers a transformative solution, with the capability to streamline analytical workflows by replacing multiple existing methods with a single, real-time, high-resolution tool.

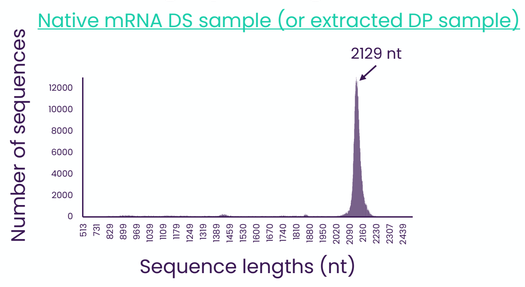
A. Poly(A) tail dispersity by Nanopore and LC-MS



B. 5'-capping efficiency by nanopore and HPLC



C. mRNA integrity testing



D. saRNA integrity testing

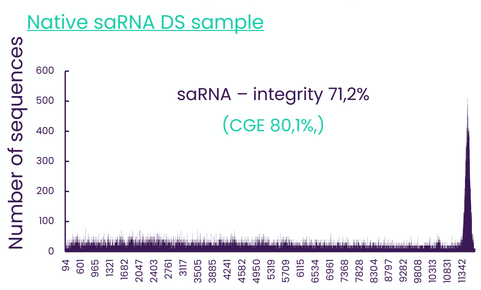


Figure 4: Non-exhaustive examples of possible read-outs derived from Nanopore sequencing experiments using mRNA or saRNA samples. A. Orthogonal comparison of Nanopore sequencing and LC-MS analysis of mRNA polyA tail length distribution suggesting normal distribution. B. Orthogonal comparison of Nanopore and HPLC for the analysis of mRNA capping efficiency. C . And D. Integrity evaluation using Nanopore sequencing data for mRNA and saRNA samples.

Automated capillary gel electrophoresis: Repurpose for growing applications

Automated CGE for plasmids

- Linearization of plasmid DNA is the very first step for an *In Vitro* Transcription (IVT) process. The percentage of plasmid DNA linearization is a critical release criterium for this Starting Material (SM).
- Agarose gel electrophoresis is the reference method for this evaluation, despite its limited sizing accuracy and sensitivity.
- At Exothera, we repurpose kits initially dedicated for the sizing and quantification of dsDNA smears and fragments by automated capillary electrophoresis (CGE), to determine plasmid linearization efficiency.
- Automated CGE improves relative quantification and sizing accuracy of the plasmid dsDNA fragments that are obtained after the linearization process.

Automated CGE for RNA mRNA release: identity testing

- Self-amplifying RNA (saRNA) vaccines allow an enhanced antigen expression at lower doses compared to conventional mRNA.
- However, saRNAs are relatively large (9000 to 15,000 nt) molecules.
- At Exothera, we customize kits initially dedicated for the sizing of total RNA of at most 6,000 nt in length by automated capillary electrophoresis (CGE), to profile saRNA.

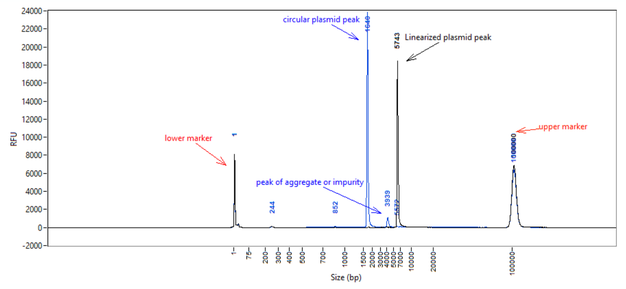


Figure 5 Overlay of two electropherograms corresponding to a linearized plasmid (black peak) and control circular plasmid (blue peaks). This overlay allows to identify the contribution of non-linearized plasmid in the linearized sample. The shown example corresponds to a linearization efficiency of 100 %. This experiment was performed with an Agilent Fragment Analyzer system.

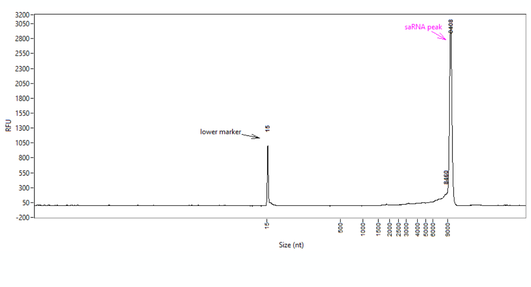


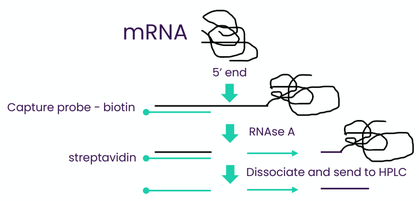
Figure 6 Electropherogram corresponding to a 9,000 nt saRNA. This experiment is an adaptation of an Agilent application note and was performed with an Agilent Fragment Analyzer system.

HPLC for mRNA Characterization: Capping, Integrity, Aggregation, and Impurities

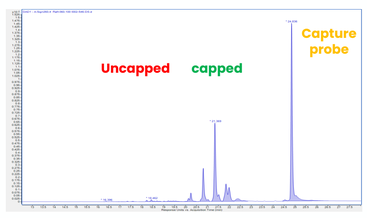
HPLC represents another versatile analytical platform. Ion-exchange, ion-pairing reverse phase, size exclusion, affinity chromatography workflows allow to characterize many properties of mRNA products: capping efficiency, aggregation, integrity, impurities profiling (residual nucleotides, residual proteins).

A. Capping efficiency by RP-IP-HPLC-UV

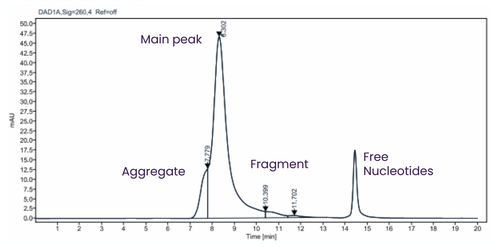
5' fragments isolation strategy



5' fragments HPLC separation



B. mRNA aggregation by SEC-HPLC-UV



C. mRNA integrity by RP-HPLC-UV

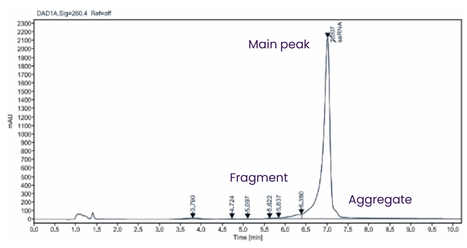


Figure 7: A. Capping efficiency assay strategy (adapted from Beverly et al.) and representative capping profile of mRNA DS sample. | B. Size-exclusion profile of mRNA DS | C. Integrity profile of mRNA by RP-HPLC

Cell Based Assays: *In vitro* Expression Potency Assay (IVEPA)

IVEPA are essential for evaluating mRNA functionality, confirming mRNA integrity, translation efficiency, and potency to ensure proper protein expression in a biologically relevant system.

Adapting IVEPA for Different LNP Formulations (DP)

A study was conducted comparing LNPs from Formulation A and Formulation B, that encapsulated the same mRNA sequence.

- For Formulation A, LNPs were directly applied to the cell line in regular cell growth medium.
- For Formulation B, transfection conditions and cell lines required optimization, including the supplementation of transfection medium with specific proteins that enhance LNP uptake by target cells.

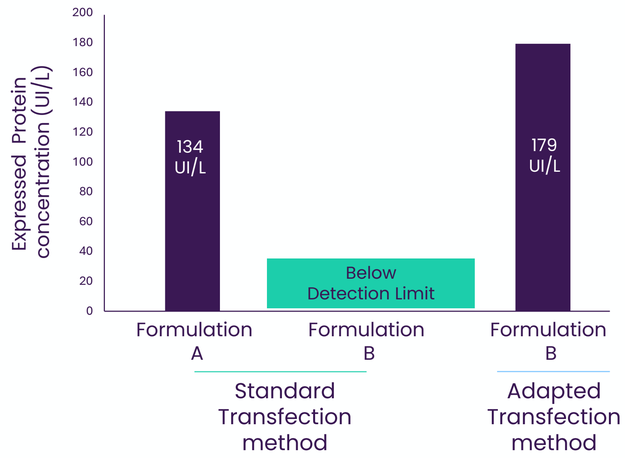


Figure 8: Concentration levels of an antigen of interest measured in the cell lysates of transfected host cells. Formulations A and B encapsulated the same mRNA construct.

Key Message

- This study highlights that IVEPA is a complex process dependent on the biologically relevant attributes of DPs and must be optimized for each LNP formulation.
- By optimizing cell models, transfection conditions, and assay sensitivity, we ensure robust, reproducible *in vitro* expression analysis.

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Conclusion

Our integrated manufacturing and analytical capabilities ensure a streamlined workflow from mRNA production to final release, meeting the stringent quality requirements for clinical and commercial applications. By utilizing the Ntensify™ mRNA platform and advanced analytical tools, we maintain the integrity, efficacy, and compliance of mRNA therapeutics at every stage. Our comprehensive testing panel, combined with extensive orthogonal approaches, reinforces data reliability and provides a robust quality control strategy, supporting the successful development and delivery of RNA vaccines.