WHITE PAPER

The current and future value of mRNA vaccines and therapeutics

With experience in both chemistry and biologics, Curia is uniquely positioned to provide mRNA drug development solutions spanning discovery and engineering, mRNA drug substance formulation, and fill-finish and manufacturing of lipids and nucleosides.

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The unprecedented and rapid global effort to develop vaccines for SARS-CoV-2 catapulted mRNA into the mainstream. It almost seemed as if mRNA-based therapeutics materialized out of nowhere, but they are based on technology that has been in development since 1990.

It was countless experiments over those decades that allowed companies — like BioNTech/Pfizer and Moderna — to leverage their internal research programs to develop the two mRNA vaccines that did much to reframe the effects of the COVID-19 pandemic.

These events catalyzed a significant increase in the implementation of mRNA-based drug development programs within the biopharma industry. The number of clinical trials featuring RNA-based drug products has increased dramatically since 2020. Infectious disease and cancer are the main targets (83%) for these clinical trials and, while some use antisense oligos (ASOs), small interfering RNA (siRNA) and other RNA modalities, the vast majority of these use mRNA.[1] Given this interest, it is no surprise that the mRNA cancer vaccines and therapeutics market was estimated to be worth about $47 billion in 2021 and is projected to grow to $109 billion by 2028.[2]

DIFFERENT TYPES OF RNA MODALITIES

Short non-coding RNAs

- **Aptamers**
  These are designed to bind specific therapeutic targets with high affinity and specificity. Pegaptanib received FDA approval in 2014 to treat macular degeneration.
- **Micro RNA**
  Promote degradation of multiple targets.

Antisense oligos (ASOs)
Bind to target genes or mRNAs and inhibit expression. There are four ASOs that have received either FDA or EMA approval.

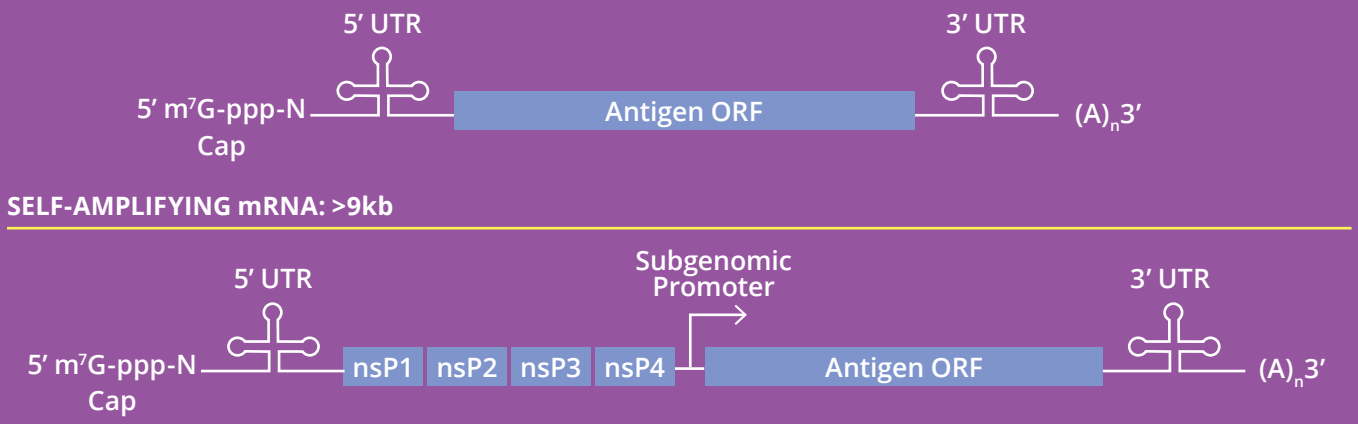
- **Small interfering RNA (siRNA)**
  Promote degradation of a single target. Four siRNA drugs have received FDA approval.[3]

Conventional and self-amplifying mRNA

Conventional mRNAs are usually 1–6 kb long. Typically >9 kb in size, self-amplifying RNA (saRNA) encodes an RNA-dependent RNA polymerase that replicates the mRNA inside the host cell of the cytoplasm using the host cell's translation machinery to generate the immunogen response. This allows smaller doses of mRNA to be used than with conventional mRNA.

Conventional mRNA and saRNA share common features, such as the 5′ cap, the 5′ and 3′ UTR and a poly-A tail (Figure 1).

Figure 1. Structure of mRNAs



Adapted from: Blakney AK, Ip S, Geall AJ. An Update on Self-Amplifying mRNA Vaccine Development. *Vaccines (Basel).* 2021;9(2):97. https://www.mdpi.com/2076-393X/9/2/97. © 2021 by the authors; Licensee MDPI, Basel, Switzerland. CC BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).

Application of mRNA-based drugs

In addition to vaccines for infectious diseases and cancer, mRNA-based drugs have many other applications. In cancer immunotherapy, cells of the immune system are engineerezd *ex vivo* and even *in vivo* using mRNA-based drugs to elicit an effect. For example, CAR-T cells can be engineered to target a specific tumor. Protein replacement therapy is used to correct the deficiency of a particular protein, such as an enzyme. Regenerative medicine using mRNA drugs is designed to stimulate stem cells to differentiate into a variety of cell types.

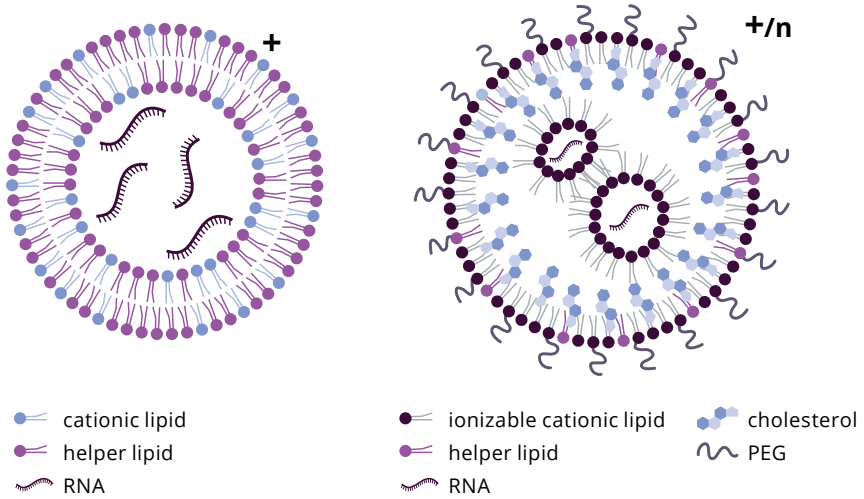
Finally, gene therapy, including gene editing, takes advantage of mRNA that's engineered to encode gene editing enzymes such as Cas9, as part of CRISPR.

Benefits and challenges of mRNA-based therapeutics	
Benefits	**Challenges**
Avoids insertional mutagenesis • Unlike DNA-based therapeutics, mRNA cannot integrate into the genome. **Cell-free mRNA synthesis poses no danger of infection** • As with an attenuated virus vaccine. **Rapid development timeline** • Much faster than traditional biologics that use cell lines. **Modularity** • mRNA drug substance can be engineered to encode any protein by altering its sequence. **Shorter manufacturing timelines** • *In vitro* enzymatic synthesis is significantly faster than traditional biologics manufacturing. • Cheaper and easier for mass production compared to conventional vaccines.	**Instability of mRNA** • mRNA is sensitive to RNase degradation; naked mRNA is rapidly degraded in the cell due to its hydrophilic nature. **Short duration of expression** • For example, replacement therapy expression is typically on the order of a few days. **Limited tissue targeting** • Targeting mRNA-based drugs to organs other than the liver is difficult.

Lipid nanoparticles

One of the main challenges of mRNA-based therapeutics is the instability of mRNA and the need to get drug product into cells. Delivery vehicles — like the lipid nanoparticles (LNPs) used to package COVID-19 vaccine mRNA — are needed to solve these problems. LNPs encapsulate mRNA in a mixture of lipids (Figure 2), protecting it from enzymatic degradation and facilitating endocytosis by binding to cell receptors. Methodologies used to make LNPs include high-pressure homogenization, extrusion, microfluidic mixing, sonication and freeze/thawing.

Figure 2. Composition of a lipid nanoparticle



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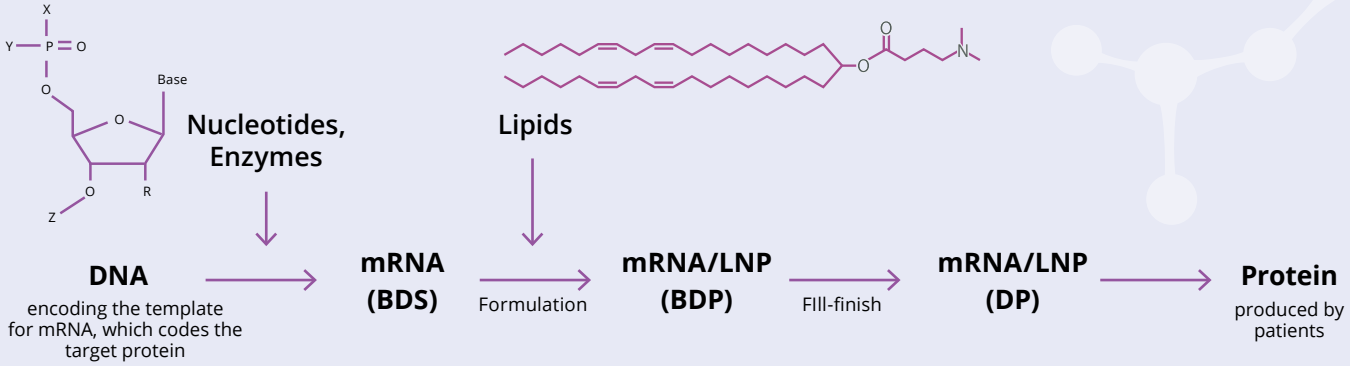
Curia's mRNA development and manufacturing services

Curia supports all aspects of clinical development and the manufacture of mRNA drug product for phase 1 and 2 clinical trials, the main steps of which are highlighted in Figure 3. Curia is capable of DNA template engineering and can manufacture a linearized DNA template that is used for cell-free enzymatic *in vitro* synthesis. This is a two-step process of *in vitro* transcription (IVT) followed by enzymatic capping, using the Vaccinia capping enzyme, and is free of animal-derived raw materials.

The next step is chromatography followed by diafiltration to produce the drug substance (mRNA). This mRNA bulk drug substance (BDS) is encapsulated, then filtered to form the LNP-mRNA bulk drug product (BDP), which is packaged as the final drug product (DP). Due to the temperature-sensitive nature of LNP-mRNA DP, the product should be stored at -70°C and repeated freeze/thaw cycles should be avoided.

All GMP-grade materials and reagents are produced per the ISO 13485:2016 Quality Management Standard and the principles defined in 21 CFR 210 and 211.

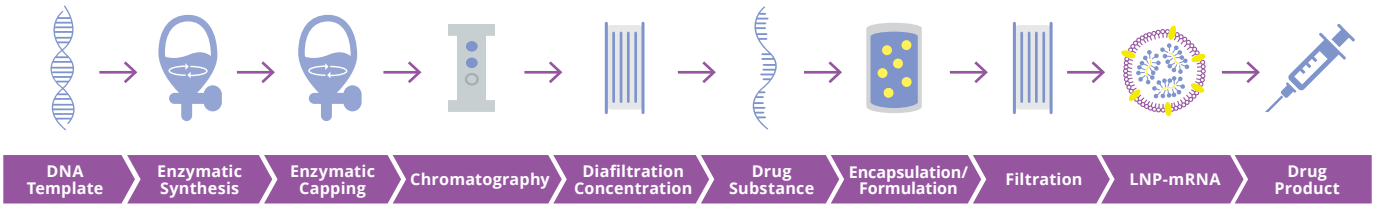
Figure 3. Major steps in making mRNA therapeutics



BDS = bulk drug substance; BDP = bulk drug product; DP = drug product

The manufacture of drug substance can be completed in one to two days using single-use consumables (Figure 4). Yields in the range of >5 g/L are typical, which translates to as many as 400,000–500,000 doses for saRNA, assuming a dose of 10 micrograms, or as many as 40,000–50,000 doses for conventional mRNAs, assuming a dose of 100 micrograms.

Figure 4. Manufacturing mRNA-based vaccines and therapeutics

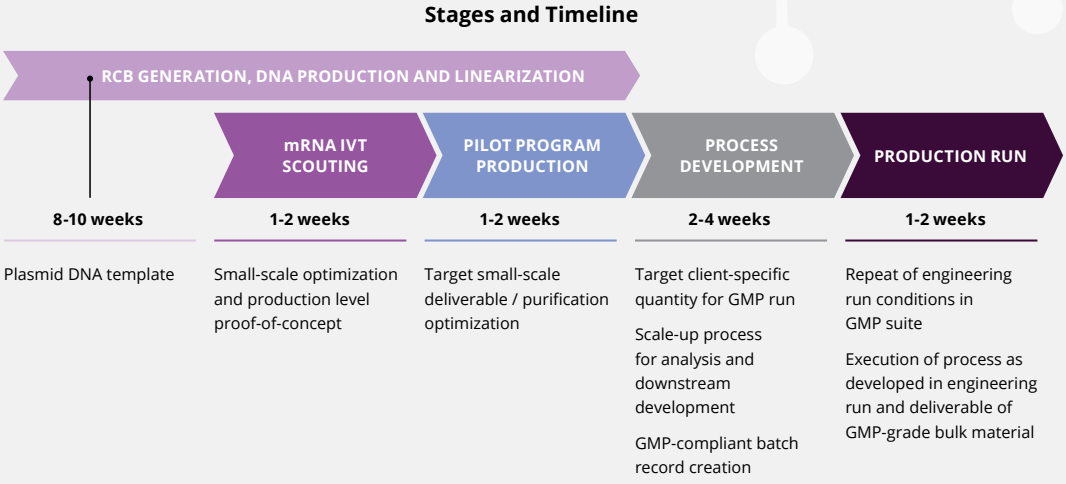


This process does not require complex cell culture, complex purification or novel equipment.

A complete timeline to produce drug product is shown in Figure 5.

Figure 5. Drug substance mRNA manufacturing timeline*

- GMP-compliant, single-use equipment
- Cell-free mRNA manufacturing
- Up to 8 L scale
- ISO_7 suite
- Expertise in self-amplifying mRNA production
- On-site analytics for in-process testing and batch release



*The timeline outlined here is a typical scenario for a well-behaved product. Curia takes a bespoke approach to each project and applies appropriate timelines based on the needs of each program.

ANALYTICS FOR DRUG SUBSTANCE RELEASE

Curia offers a variety of assays and analytics that includes compendial assays for safety and quality, but also RNA-specific assays that Curia has developed (Figure 6).

Figure 6. Assays

Test	Purpose	Assay
Appearance	Safety, quality	Visual Inspection
Bacterial Endotoxin	Safety, quality	Chromogenic LAL
Sterility (w/BnF)	Safety, quality	1 mL x 2 Direct Inoculation
pH	Safety, quality	pH
Osmolality	Safety, quality	Osmolality
RNA Concentration	Strength	UV A260
RNA Identity	Identity	CE-Based
Identity (as RNA)	Identity	Enzyme Degradation and CE
Identity (RNA sequence)	Identity	NGS
Residual Protein	Purity	BCA, PAGE, Fluorescent
Residual DNA	Purity	qPCR
Residual dsRNA	Purity	Dot Blot, ELISA
Residual DNA:RNA Hybrids	Purity	Dot Blot, ELISA
RNA Integrity	Quality	CE, HPLC
% Cap	Strength	LC-MS, CE
Functional Assay	Strength	Optional

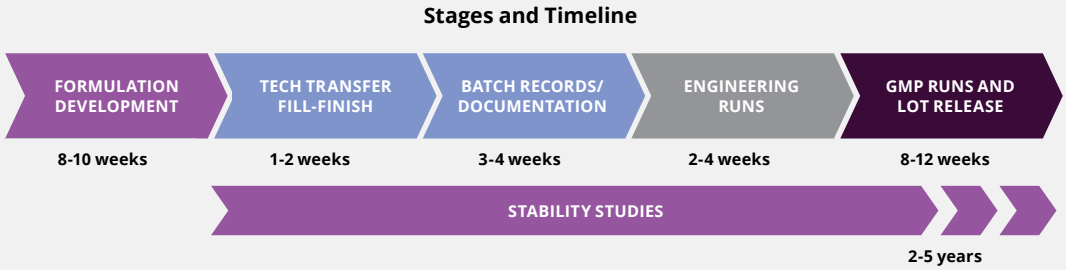
DRUG PRODUCT FORMULATION DEVELOPMENT AND MANUFACTURING

Following the successful production of drug substance, Curia moves to formulation, fill-finish and lyophilization process development. This can involve tech transfers, batch record documentation, engineering runs and GMP runs and lot release (Figure 7).

Curia also offers stability studies, which can extend up to several years. Curia works with vial sizes from 2–30 mL; fill volumes of 0.2–32 mL and batch sizes of 20,000 units for liquid and 1,200 to 5,000 units for lyophilized.

Figure 7. Drug product formulation development and manufacturing timeline*

- EU Grade A Environment
- Vial Size: 2–30 cc
- Fill Volume: 0.2–32 ml
- Max batch size:
  - Liquid: 20,000 units
  - Lyophilized: 1,200 – 5,000 (2–20 cc)
- On-site analytics for in-process testing and batch release



*The timeline outlined here is a typical scenario for a well-behaved product. Curia takes a bespoke approach to each project and applies appropriate timelines based on the needs of each program.

LIPID MANUFACTURING

Curia is able to manufacture up to 10 kg of lipids under non-GMP and GMP conditions. In February 2021, Curia was selected as a manufacturer of lipid excipients for a COVID-19 vaccine.

Figure 8. Lipid manufacturing

DEVELOPMENT

- International plants focused on process development and analytical services
- Scale-up to 10 kg (non-GMP and GMP)
- MF-UF-NF, chromatography development (1–500L)
- Focus on custom ionizable, PEG and phospholipids
- Extensive analytical capabilities

COMMERCIAL MANUFACTURING

- Multiple lines for manufacturing
- Multiple chromatographic columns in various sizes (semi-automated). 14–500 L each for normal phase, reverse phase and ionic bed
- Cold storage for lipids and packaging configurations to accommodate various volume split needs

FLEXIBLE OPTIONS

- Site selection based on the unique process, analytical and chromatographic needs of each program
- Multiple teams and parallel activities are planned to compress timelines when appropriate
- Expedition of any regulatory needs
- Established RM supply chain

Ionizable	Cationic	PEG-conjugates	Phospholipids	Steroid analogs

NUCLEOSIDE AND NUCLEOTIDE CHEMISTRY AND MANUFACTURING CAPABILITIES

Curia cGMP-manufactures nucleosides and nucleotides at kilogram scale, including novel nucleotides, guanosine caps and a variety of different types of nucleotides.

- Monomer and oligomer manufacturing
- Kilogram scale with ion exchange chromatography and TFF purification
- Expertise in development and manufacturing of modified nucleosides
- Guanosine caps and novel nucleotides

CASE STUDY

mRNA DRUG SUBSTANCE

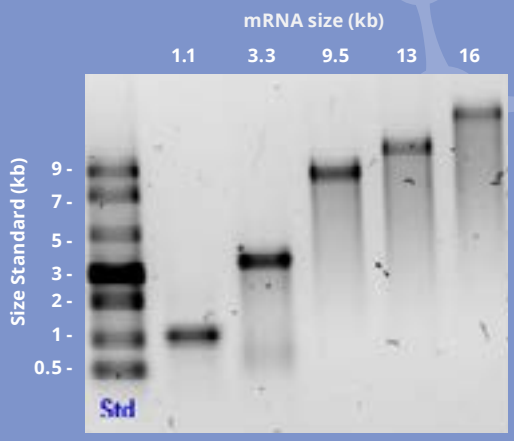
Curia has manufactured self-amplifying RNA (saRNA) at 9.5 kb as well as RNAs as long as 16 kb. Here we describe the purification and analysis of a 9.5 kb saRNA.

OPTIMIZATION OF *IN VITRO* TRANSCRIPTION AND CAPPING

Optimizing reaction conditions maximizes the yield and quality of saRNA. This includes fine-tuning DNA template concentration reaction time and temperature, magnesium concentration, polymerase and capping enzyme concentrations, and rNTP concentrations and chromatography to each client molecule.

Figure 9 shows variant sizes of mRNAs ranging from 1.1–16 kb, using agarose gel electrophoresis. Post-purification yields range >5 g/L with recovery of 80–90%.

Figure 9. Purification of mRNAs



Analytical assays

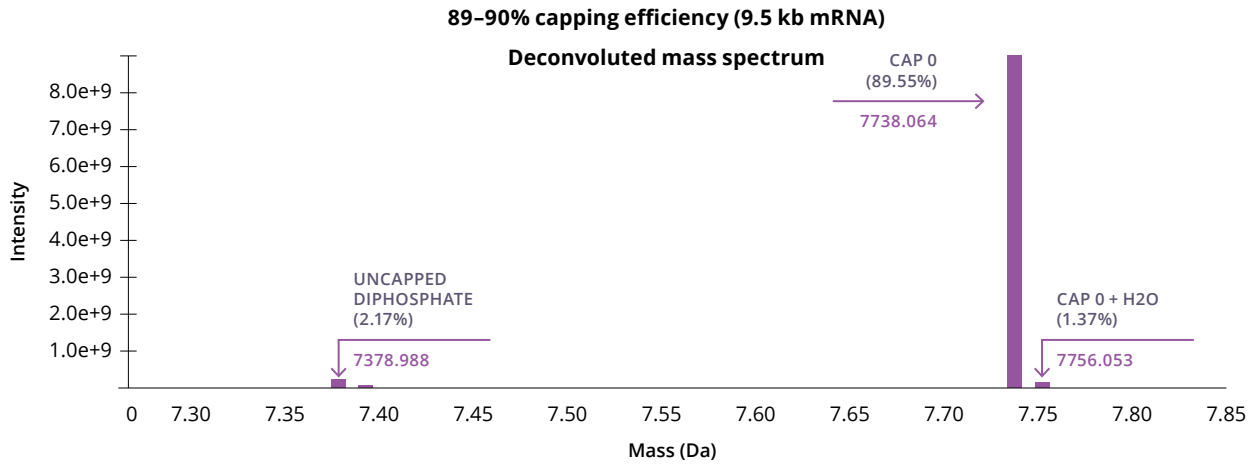
Critical quality attributes (CQAs) are being developed to perform a range of analytical tests for the purified 9.5 kb drug substance. These CQAs include testing for identity, residual protein, residual DNA, residual double-stranded RNA, RNA integrity and percent capping.

Identity as RNA was tested using RNase digestion in combination with agarose gel electrophoresis. The integrity of the RNA was measured with capillary electrophoresis using a PA 800 Plus system.

MEASUREMENT OF CAPPING EFFICIENCY

An LC-MS-based assay measured capping of a fragment of the mRNA.[4] This used an RNA-DNA hybrid probe that was biotinylated. The probe was complementary to the 5' end of the synthesized mRNA. The DNA-RNA duplexes were incubated with RNase H, which cleaved RNA-DNA duplexes. The biotinylated material was affinity purified to remove RNA and enzymes, then the cleaved fragment was dissociated. This left about a 30–40 nucleotide fragment with the cap on the end. This material was injected into the LCMS or analyzed by LCMS. In the example shown here (Figure 10), we achieved 80–90% capping efficiency. The purple lines show peaks of DNA.

Figure 10. Measurement of capping efficiency

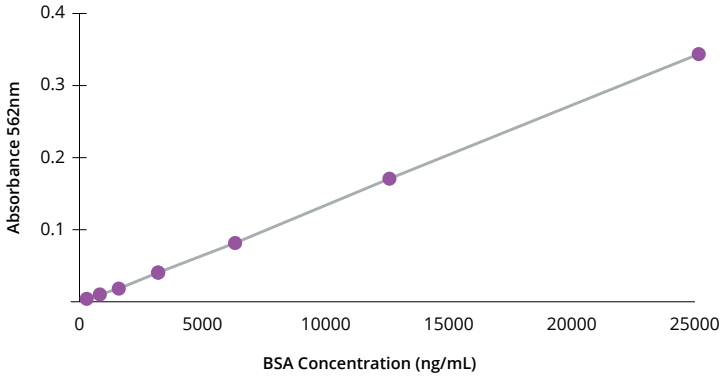


IMPURITY ANALYSIS

Residual protein

A BCA assay, a colorimetric assay measuring absorbance at 562 nm, measured residual enzymes used in the reactions (Figure 11). In the figure, the total *in vitro* transcription reaction is represented on a weight-weight basis (126% protein). This was reduced to 0.59% after purification, which is a significant reduction in total protein.

Figure 11. Measurement of protein impurities by BCA assay



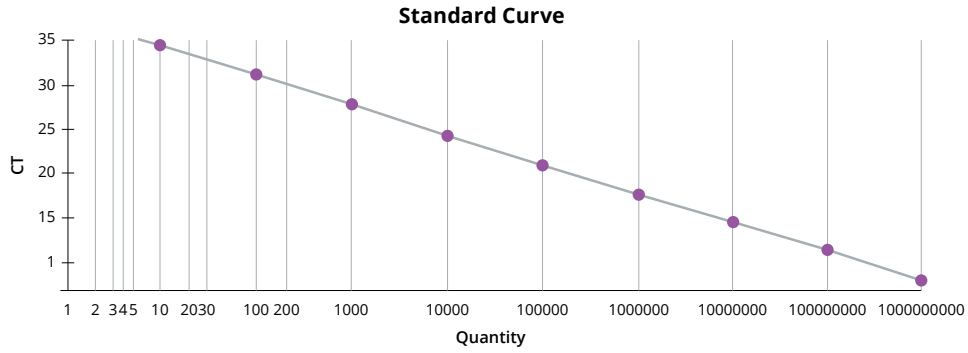
Sample	mRNA conc. (mg/mL)	Protein conc. (ng/mL)	Final residual protein (ng/mL)	W/W % protein/ RNA
Total reaction	0.50	630839	1,261,679	126.17%
Purified RNA	0.50	2957	5,914	0.59%

Disclaimer: The downstream purification process was not fully optimized at the time of publication and Curia expects to achieve further reduction to acceptable limits.

Residual plasmid DNA

The TaqMan qPCR was used to measure residual plasmid DNA. Examples shown here (Figure 12) detect kanamycin (Kan), the selectable marker in the plasmid. A standard curve was generated using our linearized plasmid DNA and plotted the CT versus quantity of DNA to assess the concentration of residual DNA remaining. The results were consistent between the batches and also between analysts.

Figure 12. Measurement of residual plasmid DNA

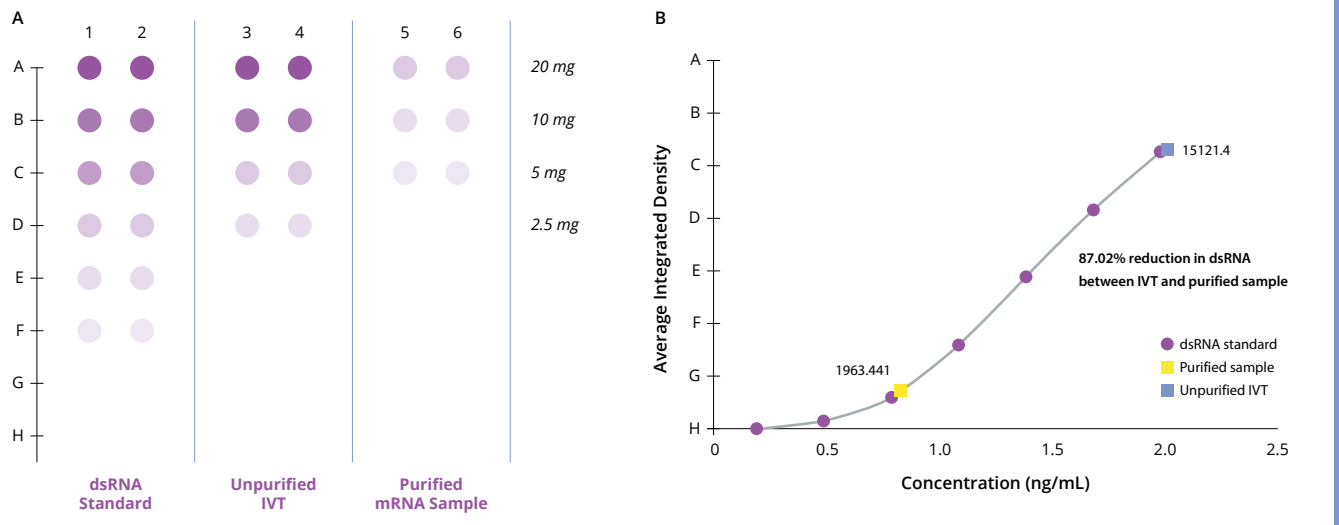


Measurement	Target	Residual DNA (ng/mg)
1	Kan	26.85
2	Kan	22.12
3	Kan	21.70

Presence of dsRNA

To measure double-stranded RNA, Curia used a dot blot method (Figure 13). A double-stranded RNA standard, total IVT reactions and purified RNA were diluted and spotted onto the membrane. A primary antibody that detects double-stranded RNA was used. We observed an 87% reduction in double-stranded RNA from the *in vitro* transcription reaction to purified product.

Figure 13. Measurement of dsRNA



A. Dot blot comparing an mRNA sample before and after chromatography and TFF. dsRNA was used as a standard.
B. Interpolated residual dsRNA between unpurified and purified samples using a dsRNA as the standard curve. Average integrated densities of each sample are depicted in blue and yellow next to their respective sample.

POTENCY

An *in vitro* cell-based assay, testing two batches, showed that the potency was greater than or equal to 90%.

SUMMARY

This case study shows that Curia is well-positioned to provide mRNA drug discovery, development and manufacturing solutions, as well as components required for the manufacturing process that include lipids and nucleosides or nucleotides. Curia has successfully produced high-quality mRNAs up to 16 kb and has extensive analytical capabilities in-house and a robust quality management system.

Future directions

The future continues to look bright for mRNA therapeutics. Because of this, Curia continues to develop our processes.

Curia is increasing our drug substance manufacturing capacity to the 50-L scale in 2023. We are also developing our full LNP-mRNA formulation development services. On the analytics front, Curia is adapting the double-stranded RNA assay to the ELISA format to produce a more robust and sensitive assay; is developing NGS to assess mRNA identity and integrity; is adapting a CE-based capping assay; and is developing HPLC assays to measure impurity and integrity of RNA.

With the acquisition of LakePharma and Integrity Bio in September 2021, Curia moved fully into the biologics space with state-of-the-art manufacturing and testing facilities and a robust quality management system. Our integrated solutions for biologics, with expertise in antibodies, proteins and mRNA, span discovery, engineering, development and clinical manufacturing.

Curia is well-positioned to provide mRNA drug discovery, development and manufacturing solutions

Learn about our full-scale biologics capabilities at curiaglobal.com/biologics.

ABOUT CURIA

Curia is a global contract research, development, and manufacturing organization (CDMO) with over 30 years of experience. With an integrated network of 20+ facilities worldwide and a team of 3,000+ dedicated professionals, we specialize in partnering with biopharmaceutical customers to bring life changing therapies to market. Our offerings in small molecules, generic APIs, and biologics span discovery through commercialization, with integrated regulatory, analytical, and sterile fill-finish capabilities. Our scientific and process experts, along with our regulatory-compliant facilities, deliver a best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we are your trusted ally in accelerating life-changing therapeutics. Learn more at curiaglobal.com

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