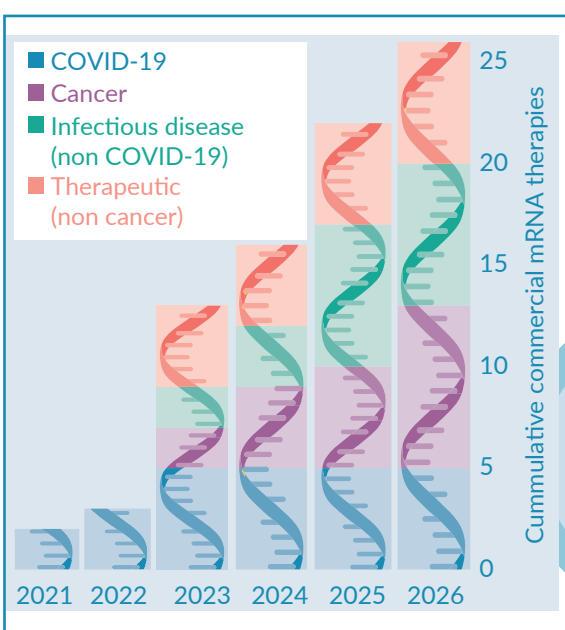
mRNA manufacturing and analytics

With the recent surge in use of mRNA as a vaccine and therapeutic modality, optimizing and understanding the development and manufacturing of mRNA for biotherapeutics has never been of greater importance.



COVID-19 had a massive impact on on mRNA vaccines

Current value of the global mRNA synthesis and manufacturing services market

US$ 53 billion

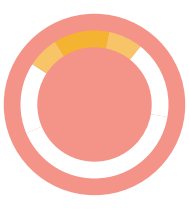
Use this infographic to guide you through the upstream and downstream steps in mRNA manufacture, along with the associated analytics

DNA TEMPLATE PREPARATION

Template design & plasmid production

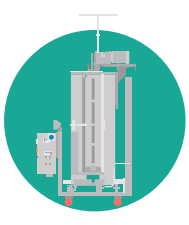
~4 weeks

TARGET GENE DISCOVERY. Target genes are discovered using techniques such as next-generation sequencing.



PLASMID CREATION. Once a gene of interest has been identified, the target sequence can be integrated into a plasmid.

12–16 h

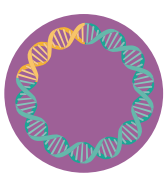


pDNA AMPLIFICATION. Plasmid DNA (pDNA) is amplified in host bacteria, typically *E. coli*, which grows in a single-use fermenter.

Plasmid purification

PURIFICATION

To achieve a high level of supercoiled plasmid.

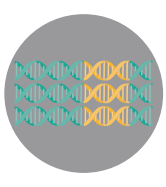


LINEARIZATION

With restriction enzymes that cleave DNA at specific sequences.

PURIFICATION

Recovery of the linearized plasmid.



ANALYTICS

Key technologies used to identify critical quality attributes and impurities are listed below.

Draft guidance is in process and therefore this is subject to change. Other technologies can be used.

Agarose gel electrophoresis: establish plasmid quality level & confirm linearity after restriction enzyme digest

Sequencing: plasmid identification

UV absorbance: pDNA quantification

Process-related impurity quantitation

qPCR and RT-qPCR: quantify residual host cell genomic DNA and RNA

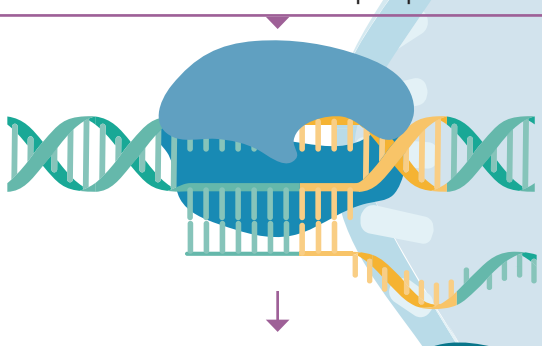
RP-HPLC: residual host protein and RNA detection

mRNA SYNTHESIS

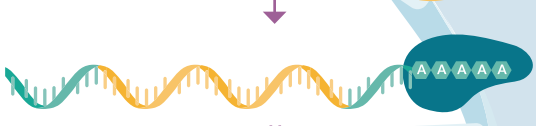
mRNA *in vitro* transcription and capping

mRNA is synthesized through the process of *in vitro* transcription (IVT) using linearized pDNA template

In vitro transcription, catalyzed by RNA polymerase

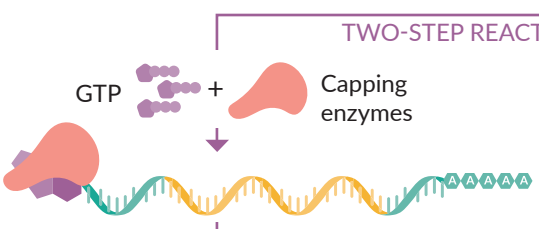


PolyA polymerase catalyzes the addition of the PolyA tail



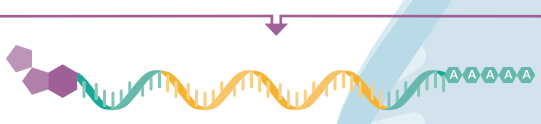
TWO-STEP REACTION

GTP + Capping enzymes



ONE-STEP REACTION

Cap analog



DNase I digestion of DNA template

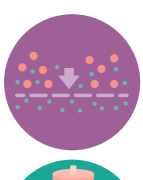
2–3 h

DOWNSTREAM

mRNA purification

mRNA is produced in a cell-free system using non-animal-derived raw materials. This simplifies downstream purification.

However, the reaction mixture contains impurities including enzymes, residual NTPs and DNA template, and aberrant mRNAs (dsRNA and truncated RNA) formed during the IVT.



ULTRAFILTRATION & BUFFER EXCHANGE

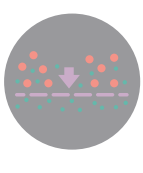
Reduce volume and remove small impurities

AFFINITY CHROMATOGRAPHY

Process related components such as truncated mRNA, DNA template, buffer components and NTPs

POLISH

Reduce dsRNA and uncapped RNA products from the final product



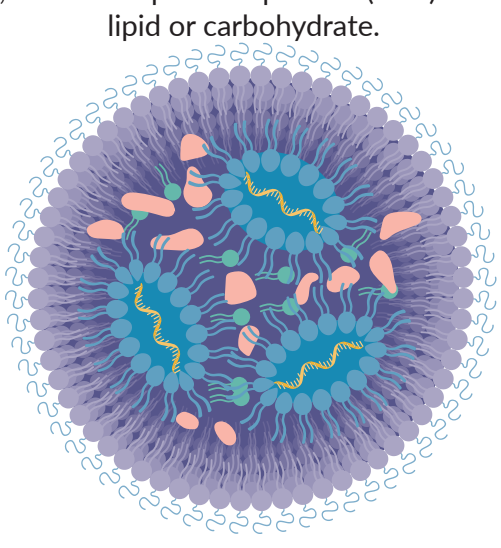
ULTRAFILTRATION & BUFFER EXCHANGE

Reduce volume and final 0.2 µm filtration

PURIFIED mRNA

Formulation, fill and finish

The purified mRNA is encapsulated in a drug delivery vehicle, such as a lipid nanoparticle (LNP) or another lipid or carbohydrate.



FINAL BUFFER EXCHANGE

FINAL FORMULATION & FILTRATION

Concentration adjustment and 0.2 µm sterile filtration

FILLING

Closed methods for aseptic filling of mRNA-based therapeutics reduce risk of contamination.

Packaging

The filled packages undergo final stage quality control and are stored in ultra-low temperature (below -80°C) freezers, ready for delivery to patients.

Impurities from IVT can reduce translation efficiency and cause unwanted immune responses.

Characterization & critical quality attribute testing: purified mRNA drug substance

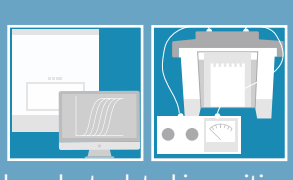
IDENTITY

Sequence confirmation: sequencing, RT-PCR

RNA CONTENT

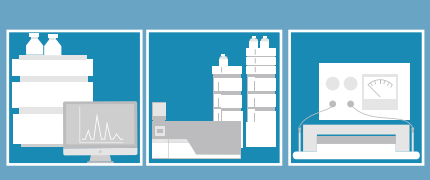
RT-qPCR, RT-dPCR, UV absorbance, fluorescence-based RNA-specific assays

PURITY



Process and product related impurities
Residual DNA template: qPCR
Protein & dsRNA: immunoblot

INTEGRITY



% intact & fragment mRNA: capillary gel electrophoresis
% 5′ capped: UPLC, RP-HPLC and LC/MS
% 3′ polyA: RP-HPLC
mRNA integrity: Gel electrophoresis

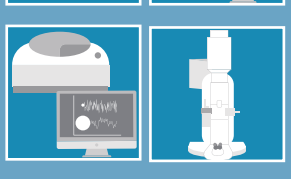
SAFETY & OTHER

Endotoxin, bioburden sterility, appearance

Characterization & critical quality attribute testing: mRNA–LNP drug product

Lipid content: LC/MS, HPLC

Particle size: Dynamic light scattering, electron microscopy



% RNA encapsulation: RiboGreen RNA assay, fluoresence-based mRNA assay

Lipid identity & impurities: LC-MS
Fatty acid analysis: HPLC