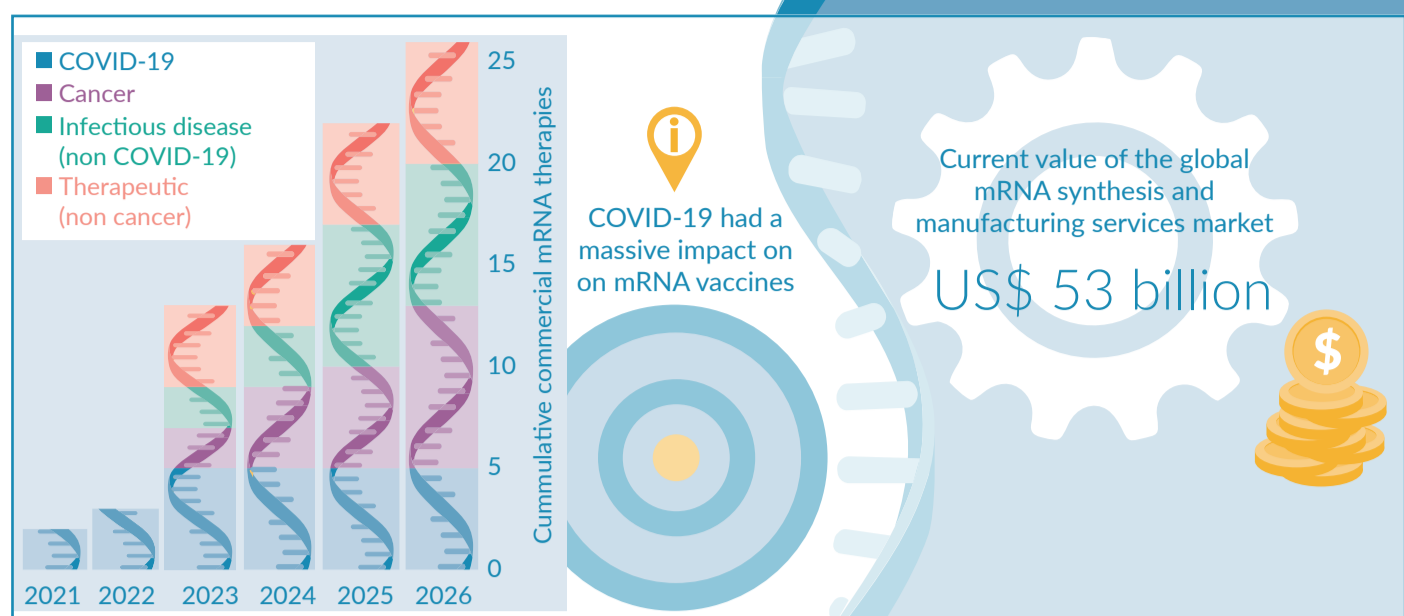


mRNA manufacturing and analytics

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.



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

- TARGET GENE DISCOVERY.** Target genes are discovered using techniques such as next-generation sequencing. (~4 weeks)
- PLASMID CREATION.** Once a gene of interest has been identified, the target sequence can be integrated into a plasmid.
- pDNA AMPLIFICATION.** Plasmid DNA (pDNA) is amplified in host bacteria, typically *E. coli*, which grows in a single-use fermenter. (12-16 h)

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.

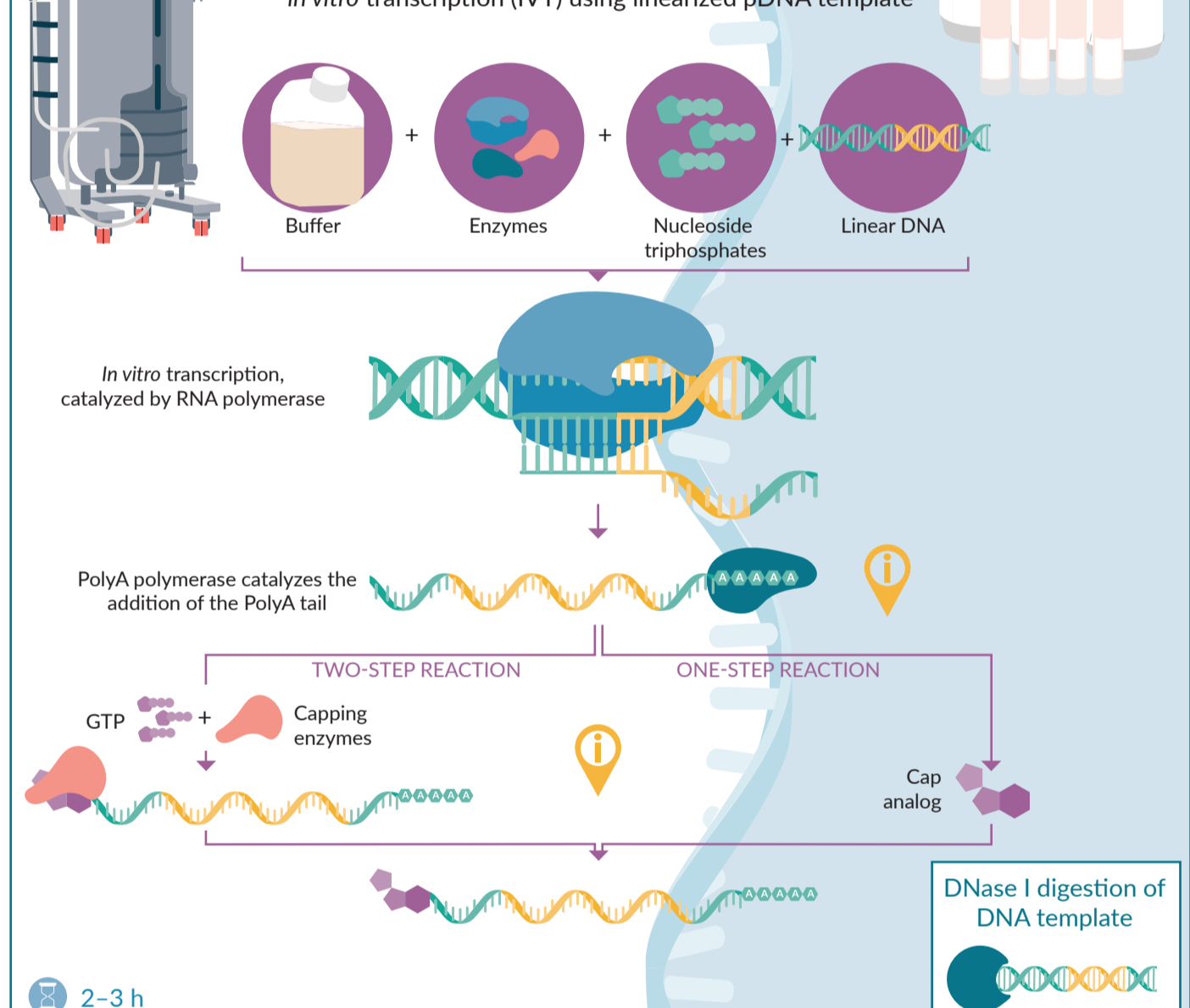
Draft guidance is in process and therefore this is subject to change. Other technologies can be used.

- 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



DOWNSTREAM

mRNA purification

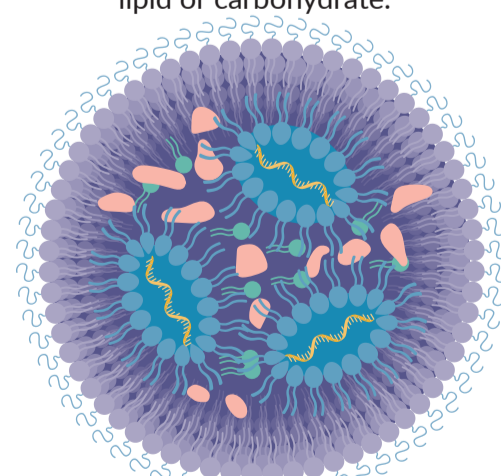
mRNA is produced in a cell-free system using non-animal-derived raw materials. This simplifies downstream purification. However, the reaction mixture contains impurities including enzymes, residual NTPs and DNA template, and aberrant mRNAs (dsRNA and truncated RNA) formed during the IVT.

- 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 μ m filtration

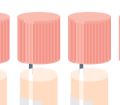
PURIFIED mRNA

Formulation, fill and finish

The purified mRNA is encapsulated in a drug delivery vehicle, such as a lipid or carbohydrate.



FINAL BUFFER EXCHANGE
FINAL FORMULATION & FILTRATION
Concentration adjustment and 0.2 μ m 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, fluorescence-based mRNA assay
- Lipid identity & impurities:** LC-MS, Fatty acid analysis: HPLC