cytiva

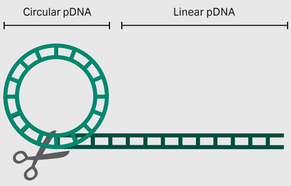
mRNA manufacturing workflow

Plasmid linearization

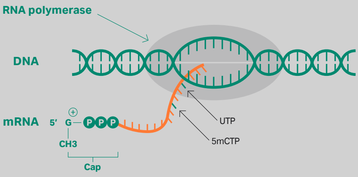
Objective: Produce DNA template

Considerations
- Restriction enzyme digest of plasmid DNA (pDNA) to create a linear template
- Quality of ingoing pDNA to control isomeric form

Strategies
- Choose appropriate scale and vessel with controlled environment
- Select plasmid source and use analytics with quality control in mind



In vitro transcription (IVT)



Objective: Enzymatic synthesis and capping of mRNA

Considerations
- Choice of enzymes, dNTPs and nucleosides
- Whether to cap before or during transcription
- DNase treatment and removal of residual DNA and RNA isoforms
- Scale and use of organic solvents

Strategies
- Identify enzymes and nucleotides specific to target mRNA; replace uridine and/or cytosine with modified nucleosides
- Precipitation and wash step to remove residual impurities
- Optimize reaction conditions for capping (e.g., temperature)

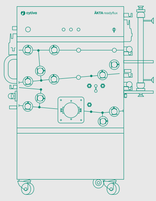
Buffer exchange

Objective: Prepare feed for purification step

Considerations
- Avoiding loss of titer for best product recovery
- Mitigating risk of failure in multistep process
- Avoiding filter fouling, clogging, and contamination

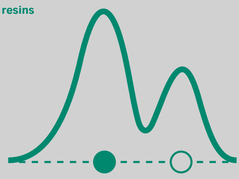
Strategies
- Select the right filter type and sizing for scale-up
- Use a scalable, automated TFF system
- Consider single-use, closed systems

ÄKTA readyflux™ system with ReadyToProcess™ hollow fiber cartridges



Capture

Oligo(dT) resins



Objective: mRNA purification

Considerations
- Molecule size – mRNA is ~10× larger than proteins
- Selecting resin that purifies full-length mRNA + removes contaminants
- Elution conditions for maximum recovery

Strategies
- Use resins with specific ligand for faster throughput (promising option: fiber-based chromatography)
- Control bioburden + speed changeover with prepacked resin formats
- Use scalable column platform from PD to full-scale manufacturing

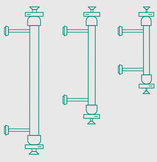
Concentration

Objective: Volume reduction and change to stable salt conditions

Considerations
- Avoiding loss of titer for best product recovery

Strategies
- Choose a scalable, automated, single-use, closed TFF system
- Tailor buffer conditions to control secondary mRNA structures
- Optimize concentration with filter cut-off

ÄKTA readyflux™ system with ReadyToProcess™ hollow fiber cartridges



LNP formation

Objective: Lipid nanoparticle (LNP) formation as an mRNA drug delivery vehicle

Considerations
- Optimizing mix of mRNA with lipids to generate desired LNP size
- Optimizing ratio of empty vs filled LNPs
- Avoiding excess lipid monomer formation

Strategies
- Monitor particle size, polydispersity index, LNP formation efficiency
- Validate chemistry, molecular weight cut-off/pore size of filters
- Validate parameters (e.g., flow rate, path length, shear rate, shear stress)
- Ensure scalability – optimized parameters from bench to process scale

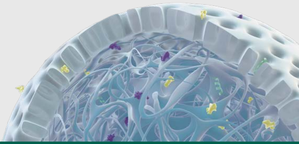
Polishing

Objective: Remove product and process impurities

Considerations
- Optimal balance – recovery of encapsulated mRNA and removal of free lipids and non encapsulated mRNA
- Matching polishing solution to impurity type

Strategies
- Choose core beads to scavenge impurities and meet regulatory demand
- Tangential flow filtration with appropriate cut off for optimal recovery and efficient impurity removal

ReadyToProcess™ hollow fiber cartridges
Capto™ Core 700 chromatography resin
ÄKTA ready™ single-use chromatography system



Drug product

SA25 Aseptic Filling Workcell

Objective: Drug product for delivery to patient

Considerations
- Which path? Personalized or platform mRNA drug product
- Long-term storage conditions
- Visual inspection of translucent drug product

Strategies
- Scale out to add capacity quickly, especially in personalized
- Co-locate drug product with drug substance to streamline production and reduce supply chain risk
- Options to transition from vial to syringe for launch

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Learn more about mRNA manufacturing **here.**

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