



Co-funded by the
European Union

Pre- Clinical study – QbD application

mRNA Encapsulated in Lipid Nanoparticles

cybernano

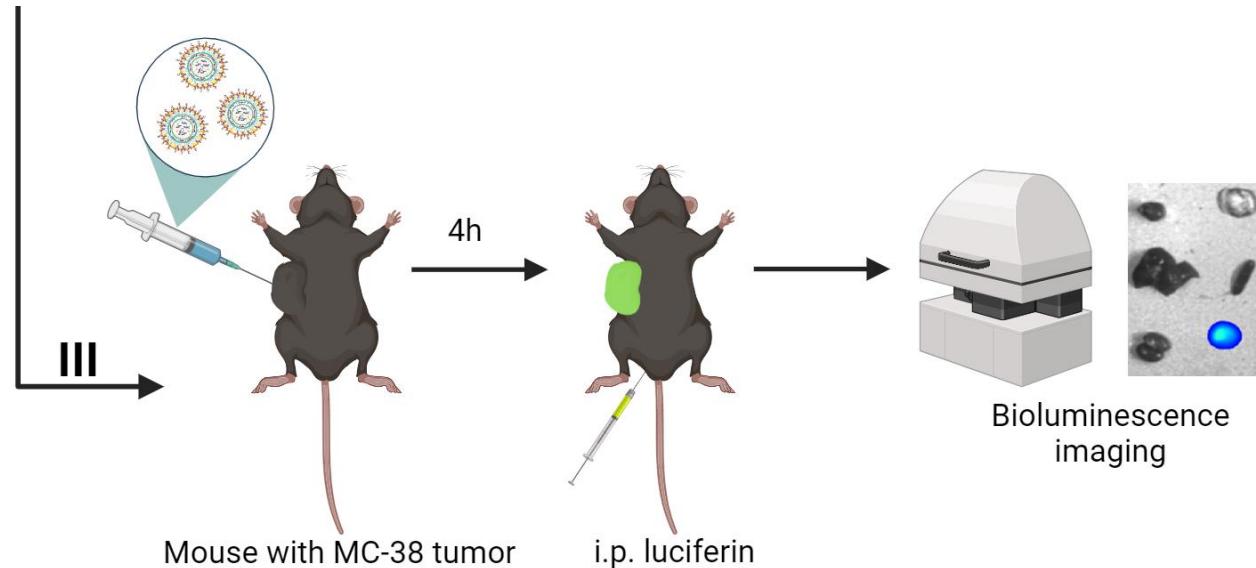
Module objectives

- ✓ **Understand the end-to-end manufacturing flow**
- ✓ **Identify key product attributes** at the preclinical stage
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Explain how **process choices influence the quality** of the final mRNA-LNP drug product

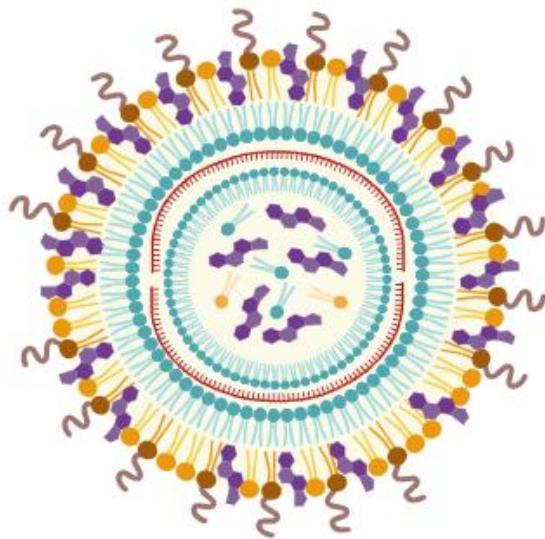


Role of the encapsulated mRNA in LNP

The mRNA–LNP used in this study is a *research-grade* product designed to deliver a reporter mRNA directly into tumor tissue.



Study case : mRNA encapsulated in Lipid nanoparticles (LNPs)

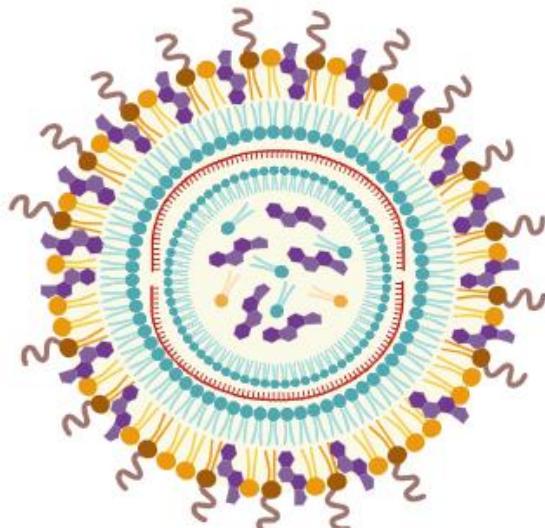


Lipid nanoparticle

mRNA component:

Firefly luciferase mRNA
Poly(A) tail
5' cap structure

Study case : mRNA encapsulated in Lipid nanoparticles (LNPs)



Lipid nanoparticle

mRNA component:

Firefly luciferase mRNA
Poly(A) tail
5' cap structure

Lipid components:

- Ionizable lipid: S-Ac7-DOG
- Helper phospholipid: DSPC
- Cholesterol: structural stabilizer
- PEG-lipid: DMG-PEG2000

Study case : mRNA encapsulated in Lipid nanoparticles (LNPs)

Common product quality attributes
for mRNA encapsulated in lipid nanoparticles

Integrity

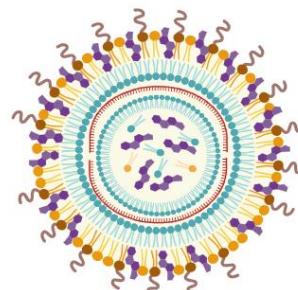
Full-length ratio
degraded fragments

Capping

Cap-1 efficiency

Residual DNA

Plasmid template



Lipid nanoparticle

Poly (A)

Length
Distribution

dsRNA

Innate immune activation

Residual proteins

T7, Dnase, capping
enzymes

Particule size / PDI

Size distribution, Polydispersity
index, LNP uniformity

Residual solvents & salts

Ethenol, salts

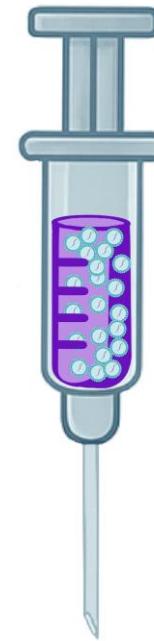
Quality attributes for final mRNA-LNP

Formulation : mRNA 0.125 mg/mL • Tris buffer saline (20mM Tris + 0.9% NaCl, pH 7.4) + 8% sucrose (the sucrose is for cryopreservation reasons) • Lipids: S-Ac7-DOG • DSPC • Cholesterol • PEG-lipid

Container: Type I borosilicate vial, 2 mL (1 mL LNP suspension) • Sterile chlorobutyl stopper

Stability: 12 months at -70°C • 3 months at -20°C • Short-term: 48 h at 2–8 $^{\circ}\text{C}$

Release Specifications: Particle size 70–100 nm • PDI < 0.2 • Encapsulation $\geq 85\%$ • mRNA integrity $\geq 90\%$ • Endotoxins < 5 EU/mL



Global overview of the process

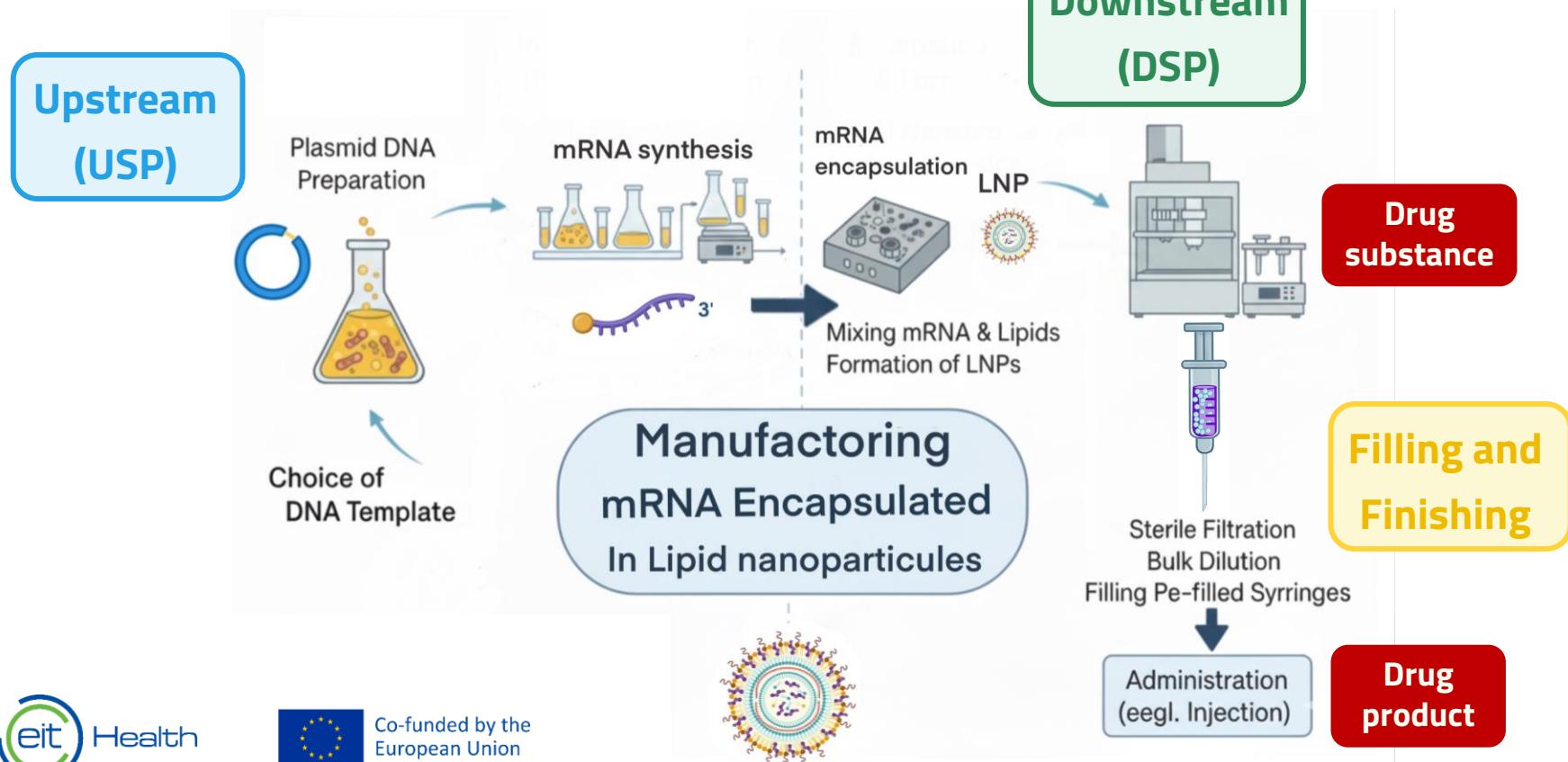


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Activity 1

Upstream process

Activity 2

Downstream process

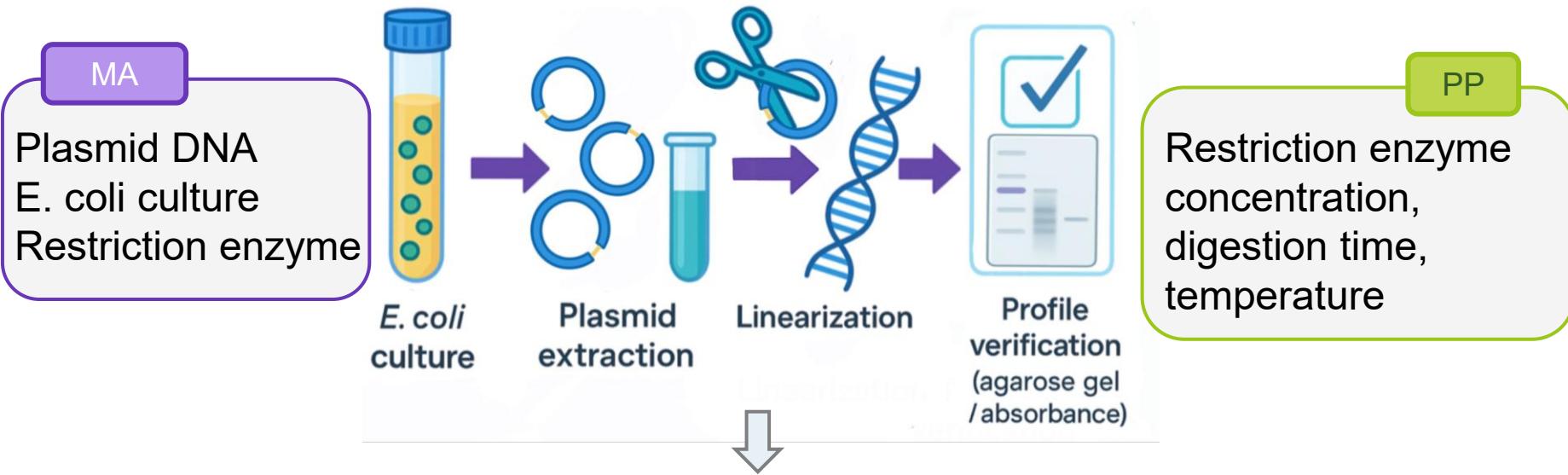
Drug
substance

Activity 3

Fill and finish

Drug
product

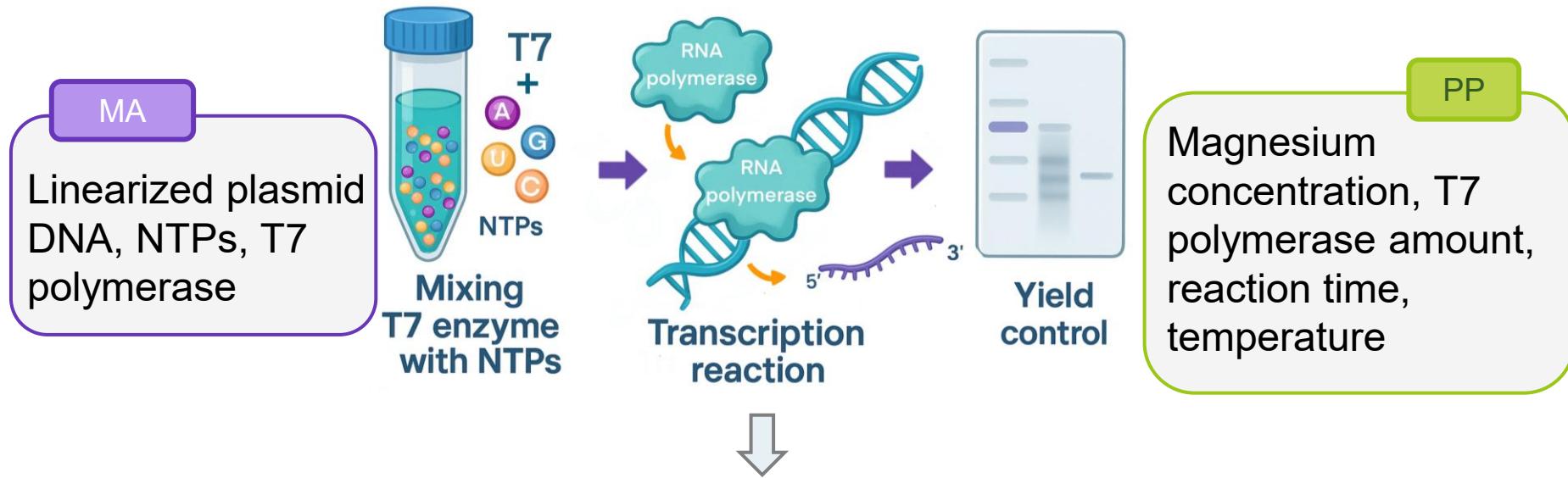
Preparation of the DNA Template



Output : Purified and linearized DNA plasmid

IPCs : concentration, purity, integrity (agarose gel)

In-vitro Transcription (IVT) of mRNA



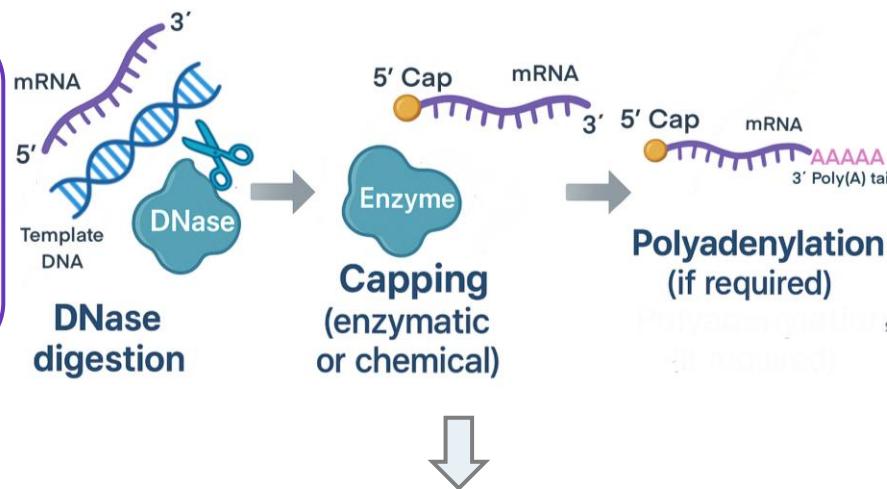
Outputs : Crude mRNA transcript

IPCs : reaction yield, residual DNA

Enzymatic Treatments (Capping and Cleanup)

MA

DNase enzyme,
Capping enzyme /
Chemical capping
reagent, Poly(A)
polymerase



PP

Enzyme
concentration,
incubation time,
temperature

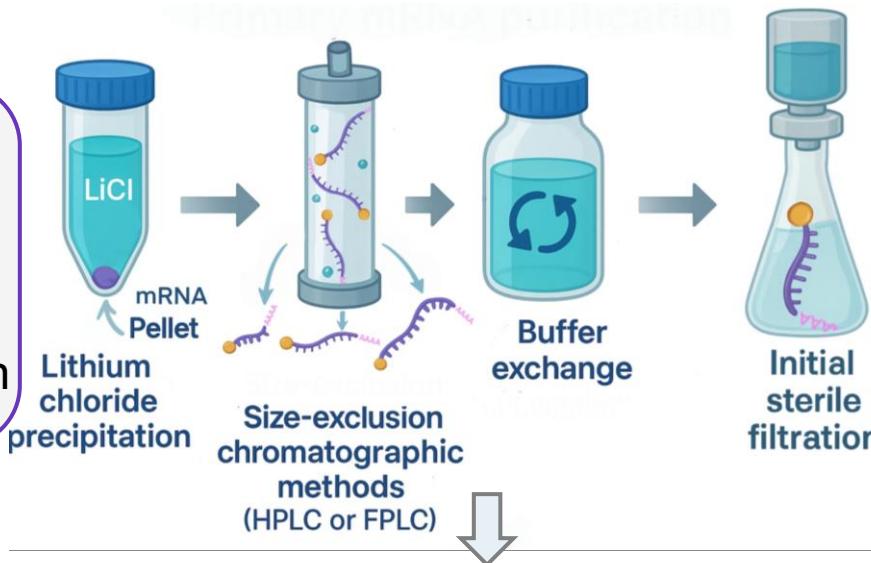
Outputs : Capped mRNA with defined 3' Poly(A) tail

IPCs : capping efficiency, dsRNA content

Primary mRNA Purification

MA

Lithium chloride solution (LiCl),
Chromatography buffers, Buffer
suitable for injection



PP

Chromatography flow rate, buffer composition, LiCl concentration

IPCs : purity, RNA integrity, residual salts, endotoxins, sterile filtrability

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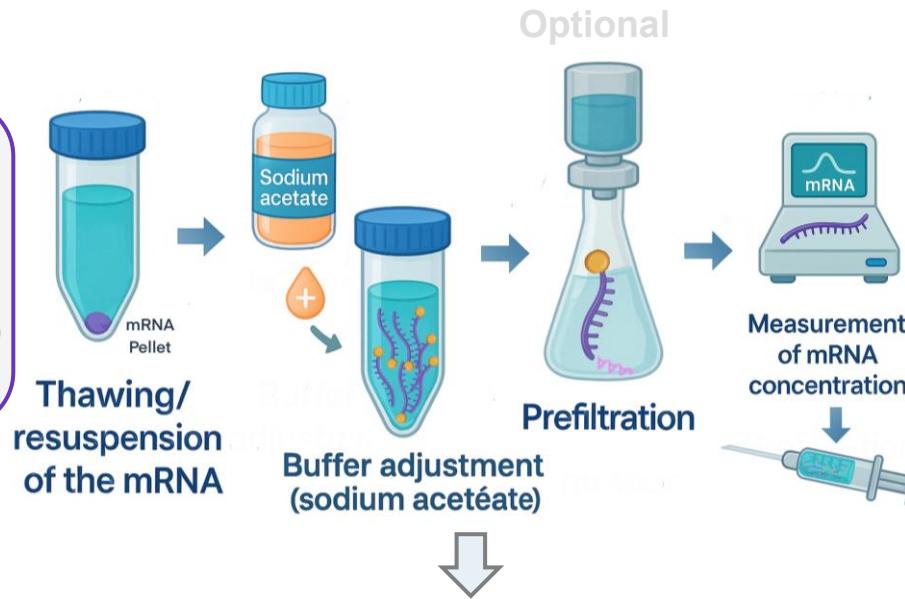
Fill and finish

Drug
product

Preparation of the mRNA Aqueous Phase

MA

Sodium acetate buffer, Water for Injection Prefilter membrane (0.22 µm)



PP

Sodium acetate concentration, mixing time, thawing conditions, prefiltration pore size

Outputs : mRNA aqueous solution at the correct pH and ionic strength

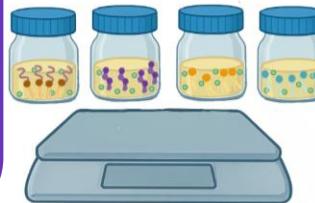
IPCs : mRNA concentration, pH, osmolality, particulate level (pre-filtration)

Preparation of the Lipid Phase (Organic)

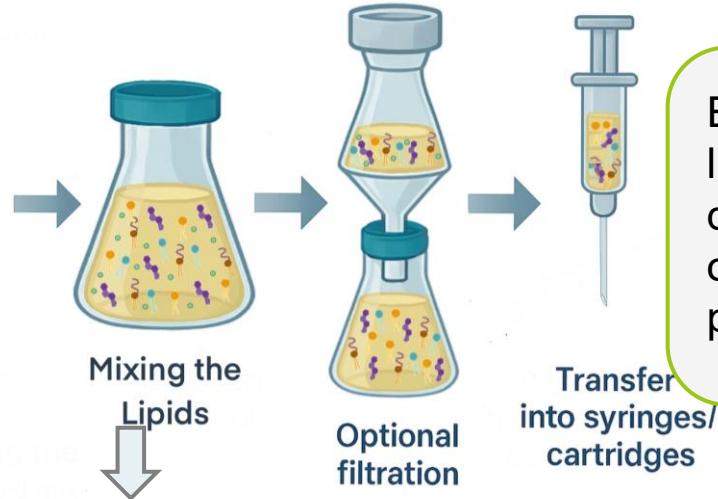
MA

Lipid components:
 S-Ac7-DOG
 (ionizable lipid),
 DSPC,
 Cholesterol, DMG-
 PEG2000, Ethanol

-  S-Ac7-Dog
-  DSPC
-  Cholesterol
-  DMG-PEG2000



Weight and dissolve
 each lipid



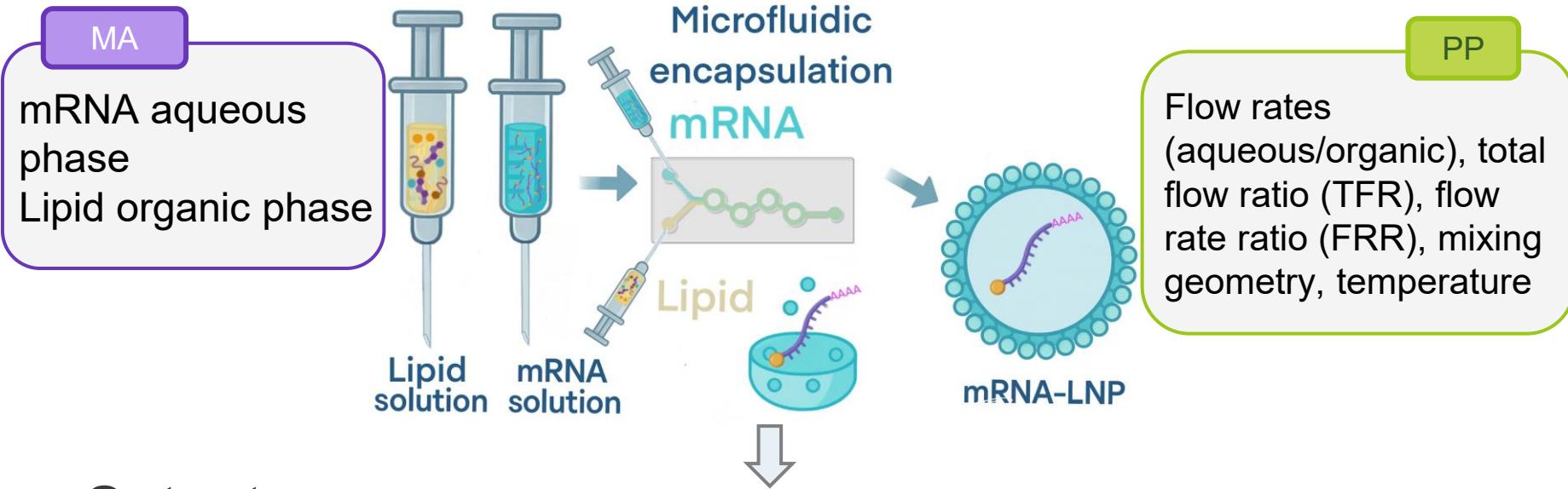
PP

Ethanol purity,
 lipid proportions,
 dissolution time,
 optional filtration
 parameters

Outputs : Homogeneous lipid–ethanol solution

IPCs : lipid ratio accuracy, lipid solubility, ethanol concentration, clarity

Microfluidic Encapsulation (LNP Formation)



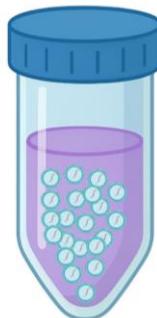
Outputs : mRNA–LNP Drug Product Intermediate (pre-purification)

IPCs : Flow rate, Cartridge temperature, Syringe/cartridge integrity, Collected volume

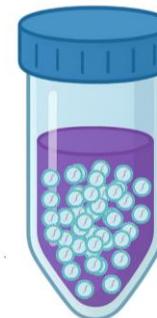
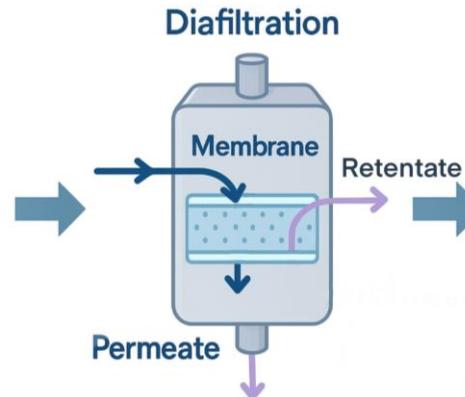
TFF Purification & Buffer Exchange

MA

Diafiltration buffer
Final formulation
buffer
Optional stabilizers



Ethanol
removal



Concentration
to the target
strength

PP

Filtration pressure, filter
material, filtration time,
holding temperature

Outputs : Sterile mRNA–LNP formulation ready for filling & finishing

IPCs : Sterile filtrability, Visual inspection (no particles), pH, Osmolality, Final particle size / PDI, Endotoxin level

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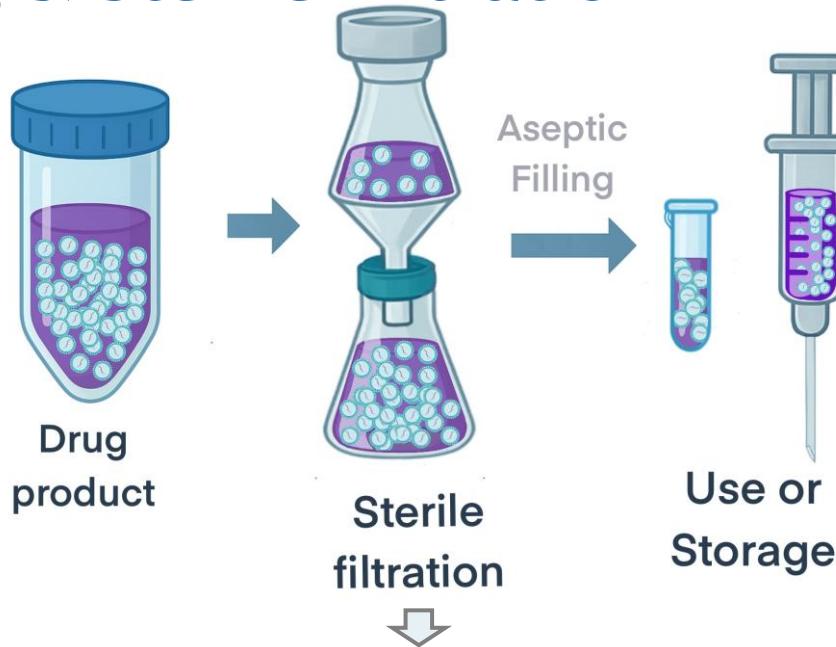
Activity 3

Fill and finish

Drug
product

Bulk Filling & Sterile Filtration

MA
Final buffer composition
Filtration membrane



PP
Filter pore size (0.22 μ m),
filtration pressure, filling
speed, temperature,
container type

Outputs : Sterile, filled bulk Drug Product

IPCs : Sterile filtrability, Final particle size, Osmolality, pH



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COURSE DOCS LOG IN

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