



Co-funded by the
European Union

Pre- Clinical study – QbD application

mRNA Encapsulated in Lipid Nanoparticles



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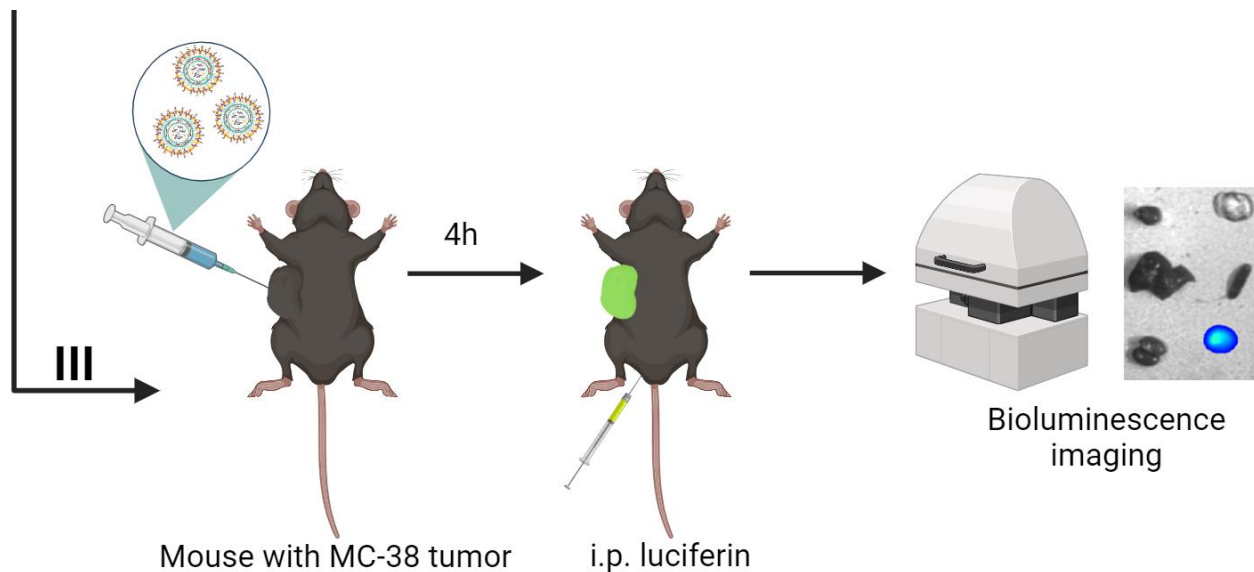
Module objectives

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

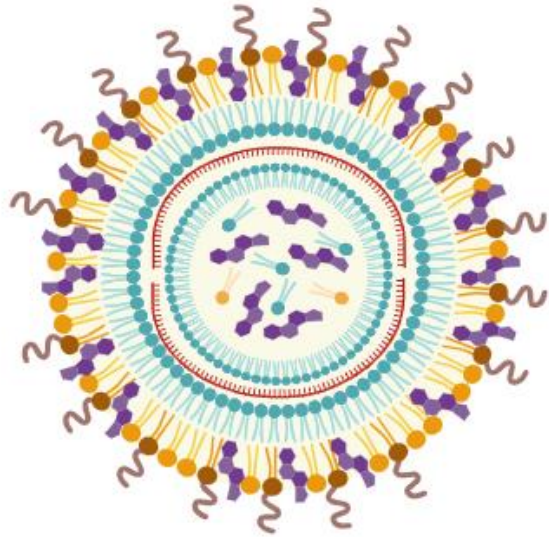


Role of the encapsulated mRNA in LNP

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

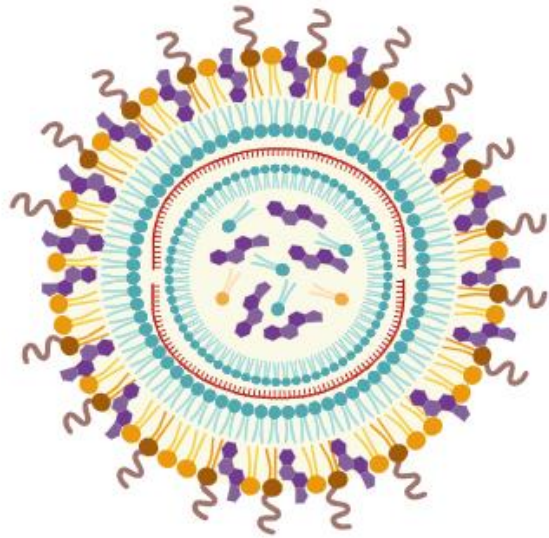
mRNA component:

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

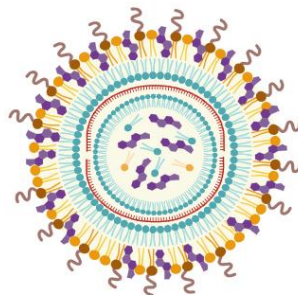
Full-length ratio
degraded fragments

Capping

Cap-1 efficiency

Residual DNA

Plasmid template



Lipid nanoparticle

Poly (A)

Length
Distribution

dsRNA

Innate immune activation

Particle size / PDI

Size distribution, Polydispersity
index, LNP uniformity

Residual proteins

T7, Dnase, capping
enzymes

Residual solvents & salts

Ethenol, salts

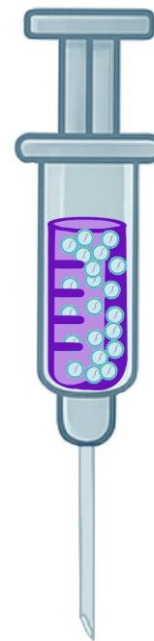
Quality attributes for final mRNA-LNP

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

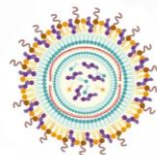
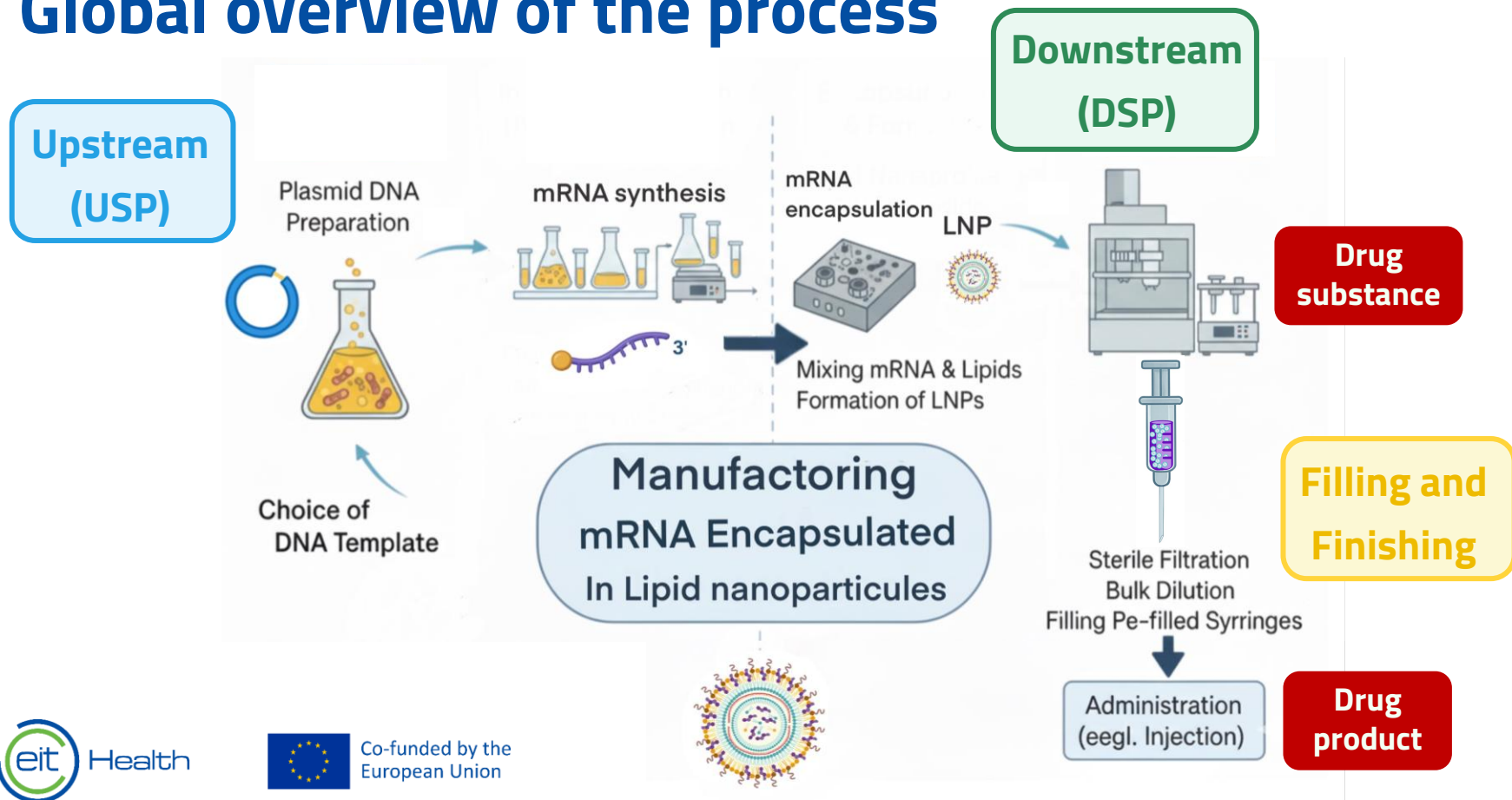


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Activity 1

Upstream process

Activity 2

Downstream process

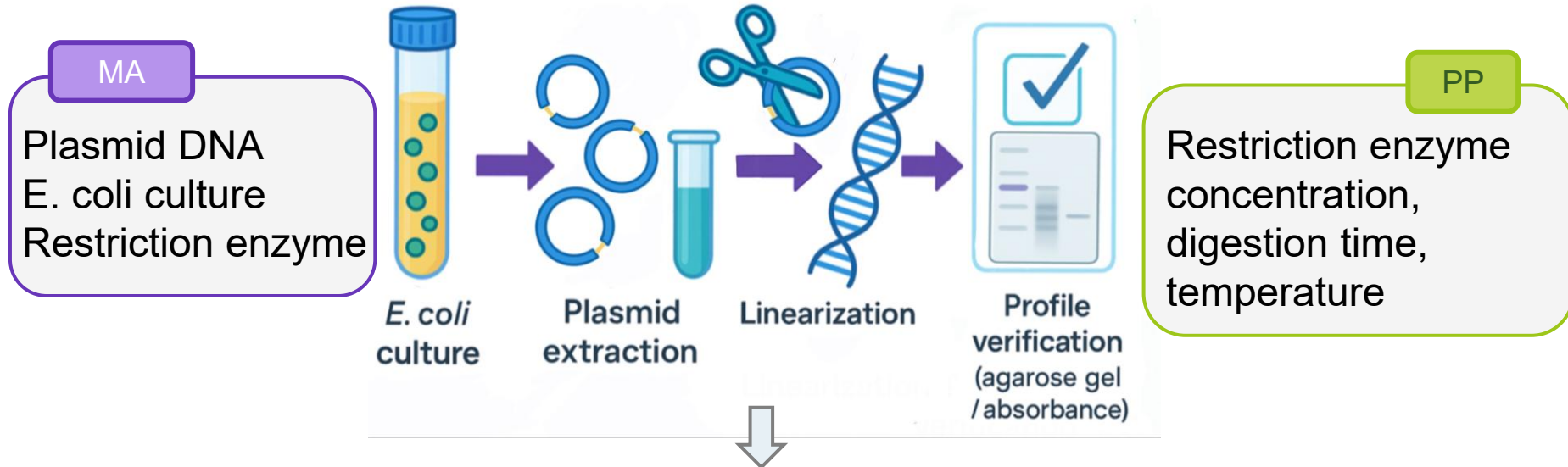
**Drug
substance**

Activity 3

Fill and finish

**Drug
product**

Preparation of the DNA Template



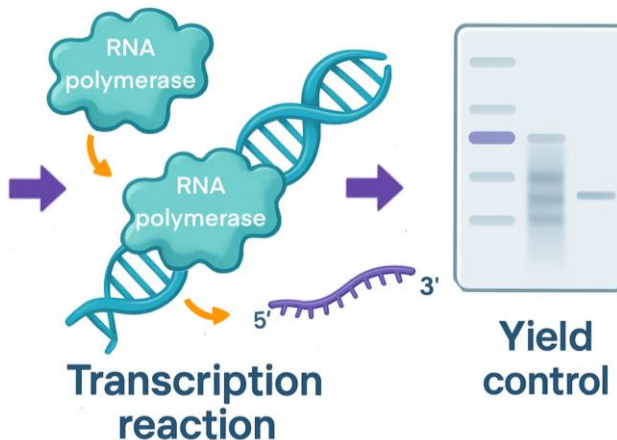
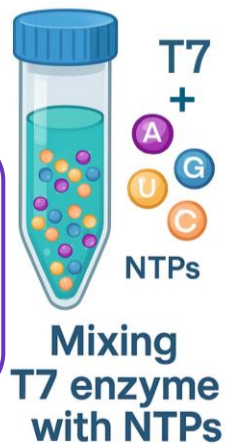
Output : Purified and linearized DNA plasmid

IPCs : concentration, purity, integrity (agarose gel)

In-vitro Transcription (IVT) of mRNA

MA

Linearized plasmid DNA, NTPs, T7 polymerase



Yield control

PP

Magnesium concentration, T7 polymerase amount, reaction time, temperature

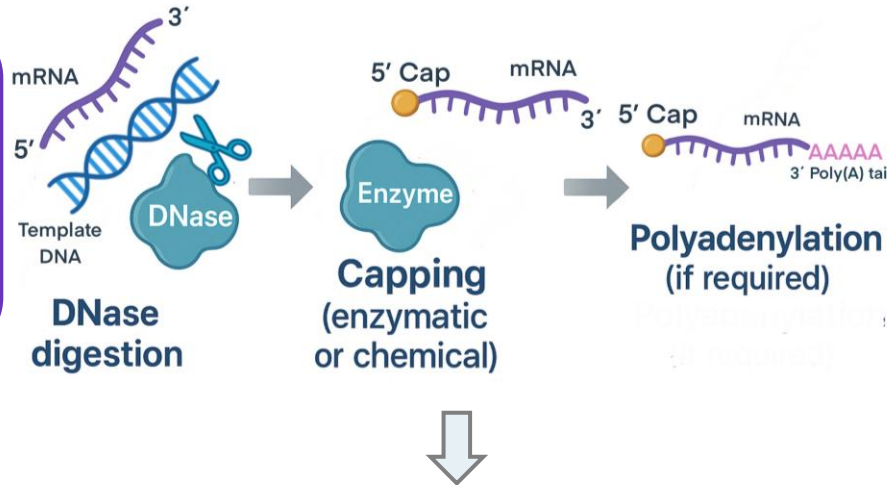
Outputs : Crude mRNA transcript

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

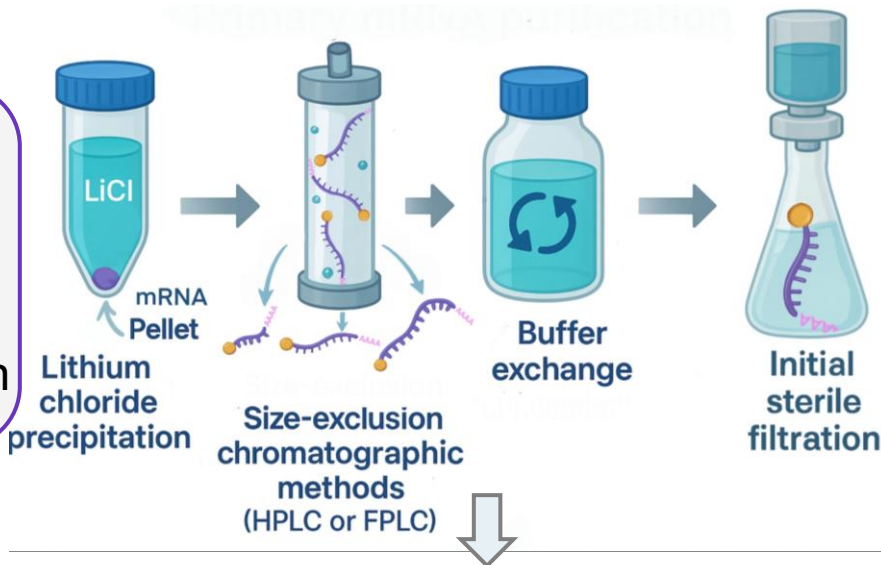
Outputs : Capped mRNA with defined 3' Poly(A) tail

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

Outputs : Drug Substance (Purified mRNA)

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

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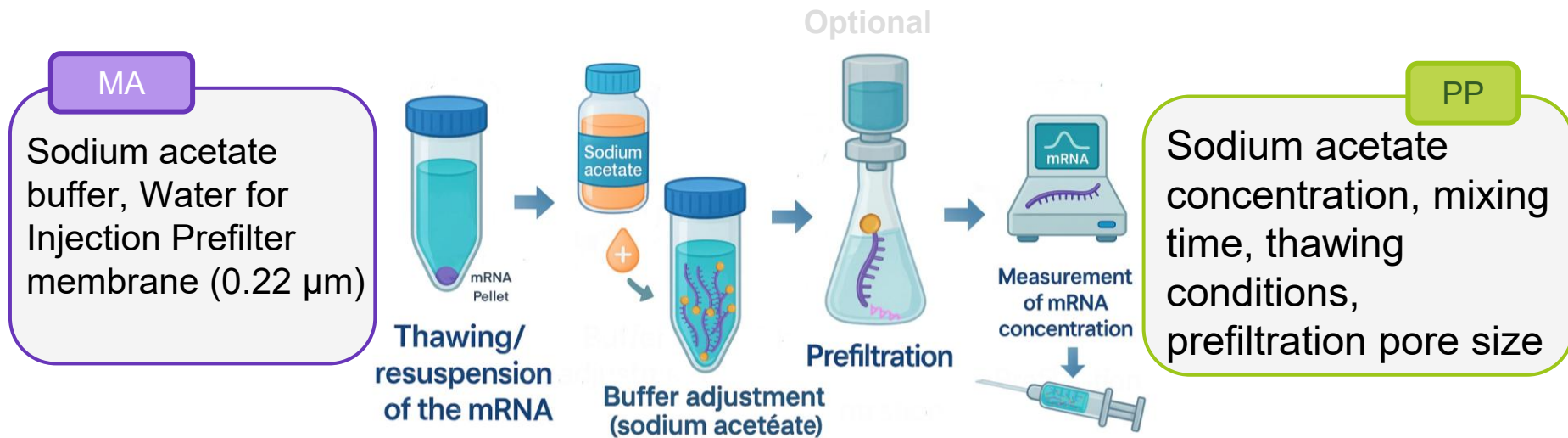
Drug
substance

Activity 3

Fill and finish

Drug
product

Preparation of the mRNA Aqueous Phase



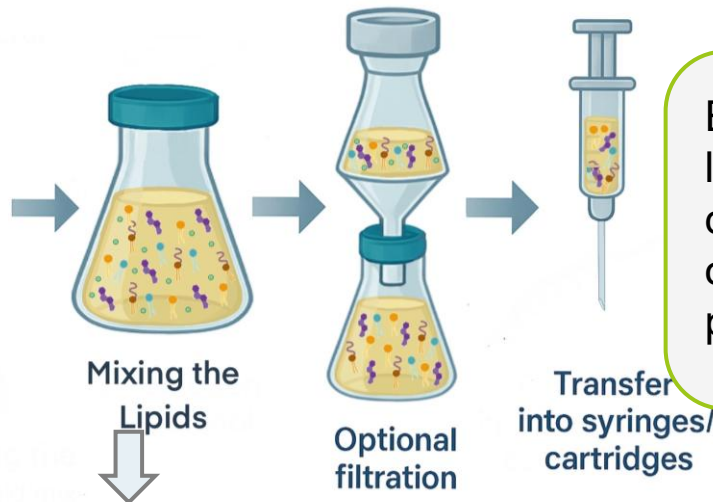
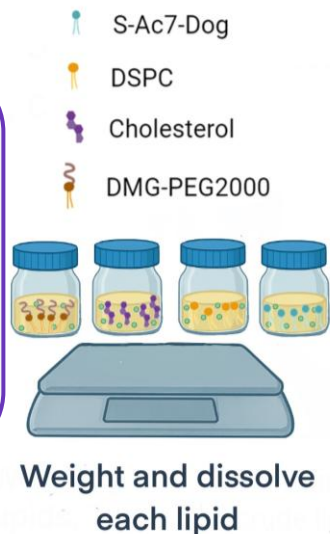
Outputs : mRNA aqueous solution at the correct pH and ionic strength

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



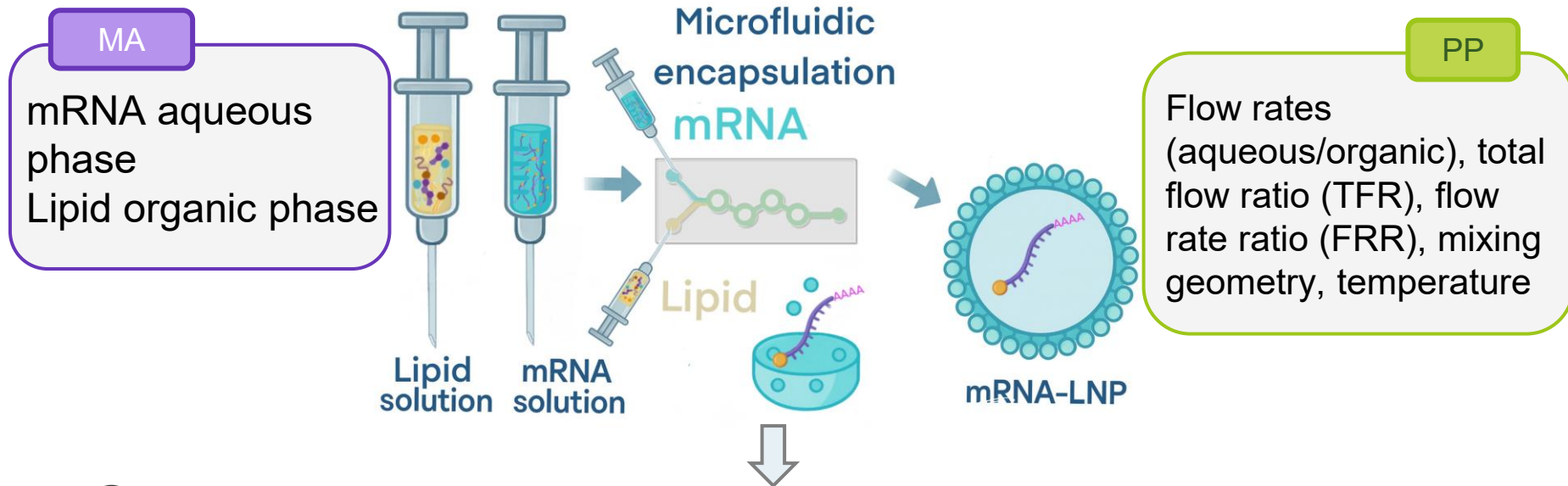
PP

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

Outputs : Homogeneous lipid–ethanol solution

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

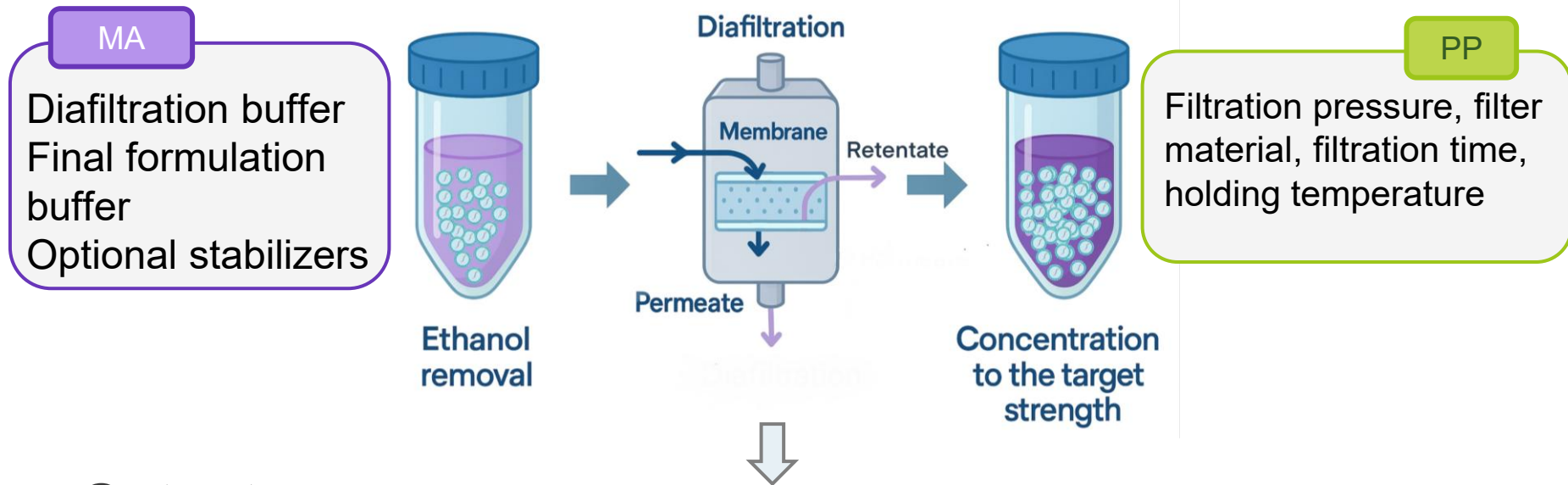
Microfluidic Encapsulation (LNP Formation)



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

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

TFF Purification & Buffer Exchange



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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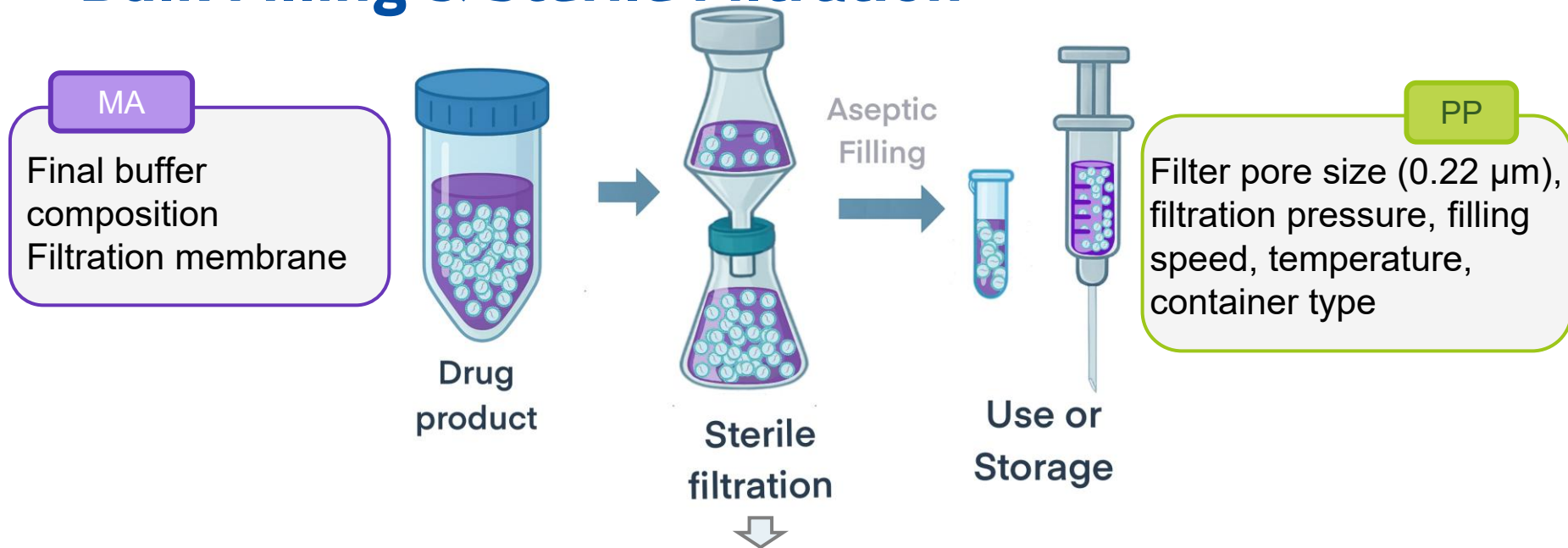
**Drug
substance**

Activity 3

Fill and finish

**Drug
product**

Bulk Filling & Sterile Filtration

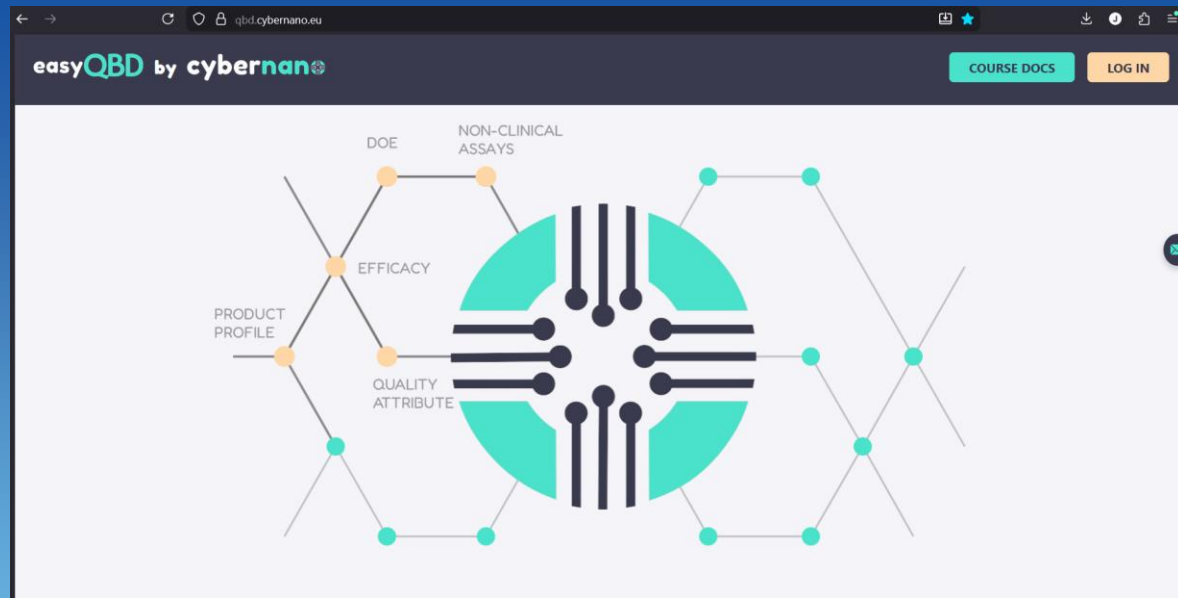


Outputs : Sterile, filled bulk Drug Product

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



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