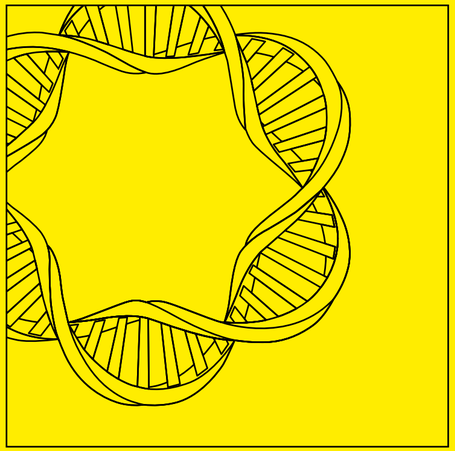
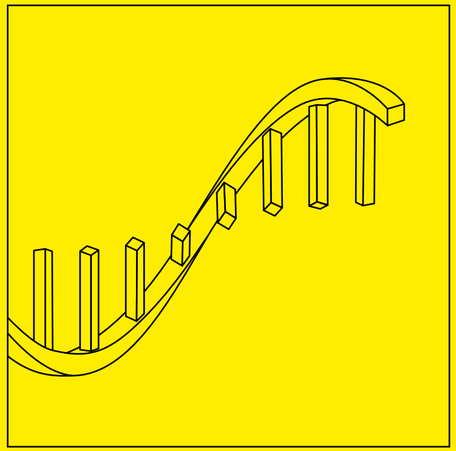
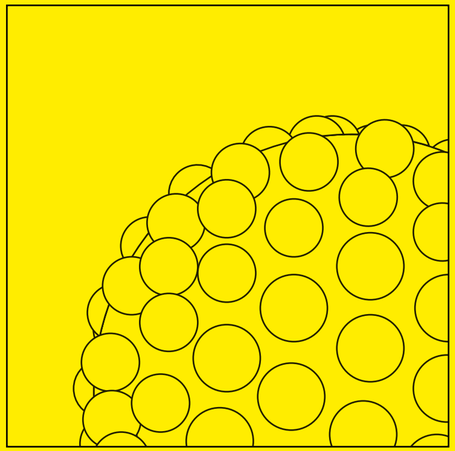
Define a Process for Your Unique mRNA: An Interactive Guide to pDNA, mRNA & LNP



pDNA
Production



mRNA
Production



LNP
Production

Find out more:
www.sartorius.com/mrna

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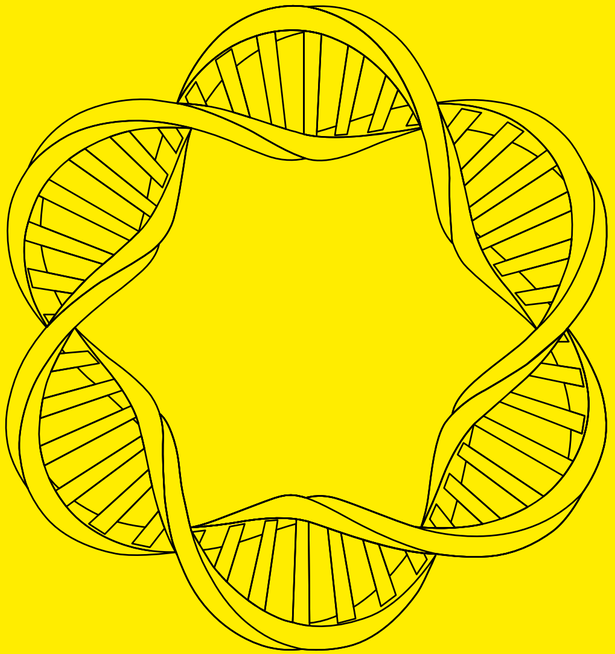
The pDNA Production Workflow

A linearized Plasmid DNA (pDNA), coding for the gene of interest, is typically used as a template for mRNA production during the In Vitro Transcription (IVT). A quality supply of pDNA is essential, as this critial raw material can impact IVT yield and mRNA quality.



Fermentation › Cell Harvest › Alkaline Lysis › Clarification › UF | DF › Capture and Polish › UF | DF › Sterile Filtration »

Production Workflow | Optimize Your Process

Did You Know?

- pDNA is usually produced in *E. Coli* (intracellular) and can reach 1–2 g/L
- pDNA are produced in 3 isoforms: supercoiled (sc), open circular (oc) and linear. Their ratio is process and plasmid dependant.
- pDNA size vary from 4 to 15 Kb.

Meet the Challenges

- Large plasmids are difficult to separate.
- Impurities to be removed are difficult to separate (hcDNA, RNA, endotoxins).

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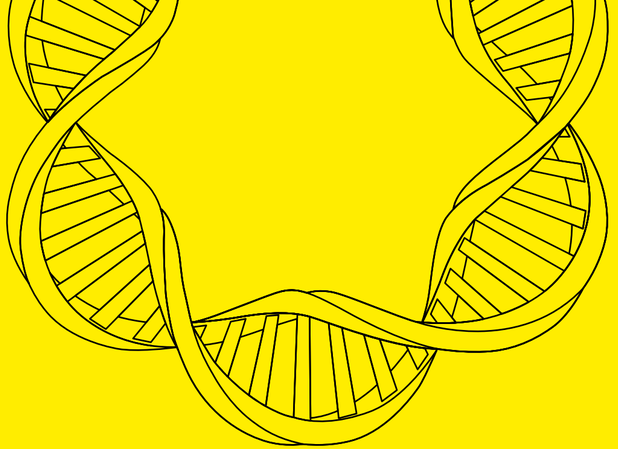

Master. Purify. Scale-up.

Overcome Challenges With Solutions That Help You Optimize for a Next-Gen pDNA Production Strategy.

Fermentation › **Cell Harvest** [1] › **Alkaline Lysis** › **Clarification** [2] › **UF | DF** [3] › **Capture and Polish** [4] › **UF | DF** [3] › **Sterile Filtration** [5] »

Hollow fiber technology is best suited for high content of processing suspended solids and large molecules.
Monolith technology used in CIMmultus® and CIMac™ columns for chromatography shows low shear and works even for large plasmids.

Production Workflow | **Optimize Your Process**

[1] TFF: **Hollow Fiber Modules** 750 KD – 0.1 µm

[2] TFF: **Hollow Fiber Modules** 500 – 750 KD

[3] TFF: **Hollow Fiber Modules** 100 – 300 KD

[4] **CIMmultus® HiP² Plasmid Process Pack™** include CIMmultus® columns and process instructions to get pure sc pDNA.

[5] **Sartopore® 2 XLG** suitable for large molecule containing a 0.8 µm prefilter. Use **SartoScale 25** (4.5 cm²) for process development.

Analytical Solution to Get Process Insights

MODDE®
Design effective experiments to increase productivity, yield and quality.

SIMCA®
With an advanced data analytics solution you can model complex systems and gain deep process understanding.

PATfix® HPLC
Along with CIMac™ pDNA to separate sc | oc | linear

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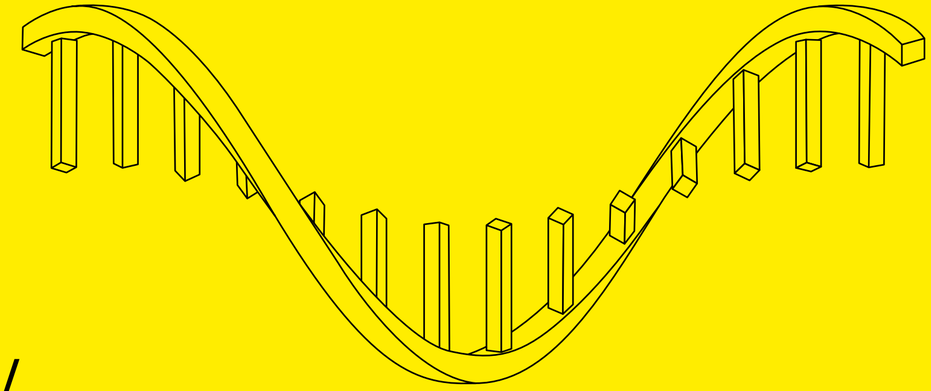
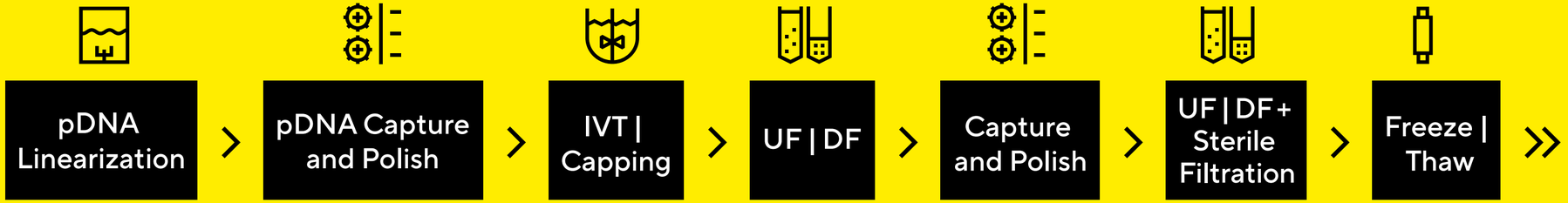


The mRNA Production Workflow

mRNA is synthesized in a few hours, during an enzymatic reaction called In Vitro Transcription (IVT) using a linearized pDNA. Downstream steps are implemented to purify the mRNA and remove the IVT reagents (templates, enzymes…) and contaminants created such as double-stranded RNA.



Production Workflow | Optimize Your Process

Did You Know?

- High mRNA content vary depending on applications (15 mg for therapies vs 30 µg for COVID vaccine)
- saRNA, circRNA are also a new type of RNA
- mRNA construct have various structure and length (500 – 12,000 nt)
- mRNA is a large molecule (up to 30 times size of a mAb)

Meet the Challenges

- RNA is sensitive to hydrolysis, oxydation and RNase
- Impurity removal (truncated RNA, dsRNA, pDNA, enzymes…)
- No platform yet as there is a large variation in RNA construct

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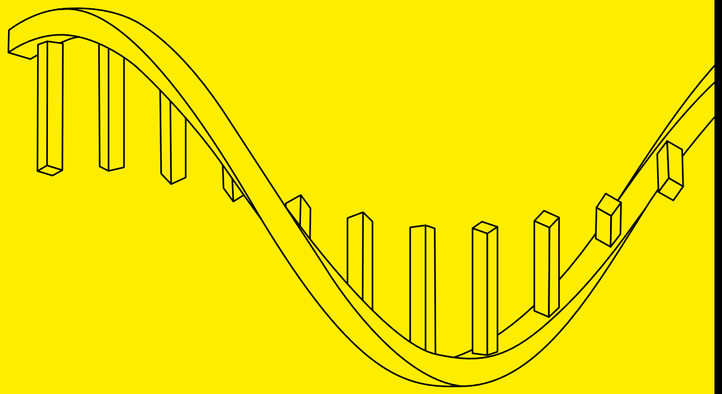

Master. Purify. Scale-up.

Overcome Challenges With Solutions That Help You Optimize for a Next-Gen mRNA Production Strategy.

pDNA Linearization	pDNA Capture and Polish	IVT | Capping	UF | DF	Capture and Polish	UF | DF + Sterile Filtration	Freeze | Thaw
	1	2	3	4	3 5	6

Monolith technology used in CIMmultus® and CIMac™ columns for chromatography shows low shear and works even for large plasmids.
Hydrosart® SU TFF cassette is a highly hydrophilic membrane for low mRNA absorption.

Production Workflow | Optimize Your Process

1 **CIMmultus® C4-HLD** to purify the linearized plasmid.

2 **Ambr® 15 and 250:** multiple bioreactors for parallel IVT reaction to fasten process development.

3 **Hydrosart®** cut off of 30, 100 and 300 KDa depending on your mRNA.

Sartocon® Slice 50 Hydrosart® (50 cm^2) used with **Ambr® Crossflow** for process development.

4 **CIMmultus® Toolbox**
OligodT (affinity), PrimaS® (AEX), C4HLD (HIC) SDVB (reverse phase) to fit any process | RNA construct. Use 96 well plate for process development.

5 **Sartopore® 2 XLG** suitable for large molecule with a 0.8 µm prefilter. Use **SartoScale 25** (4.5 cm^2) for process development.

6 **Celsius® family** for a stable storage and shipment to LNP encapsulation facility. Starting from 30 mL bag for your process development.

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MODDE®
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SIMCA®
With an advanced data analytics solution you can model complex systems and gain deep process understanding.

PATfix® HPLC
Along with CIMac™, pDNA can separate linear from other isoforms. Along with CIMac™ PrimaS®, Oligo dT or SDVB you will be able to quantify mRNA and IVT components, verify mRNA size, separate dsRNA | mRNA.

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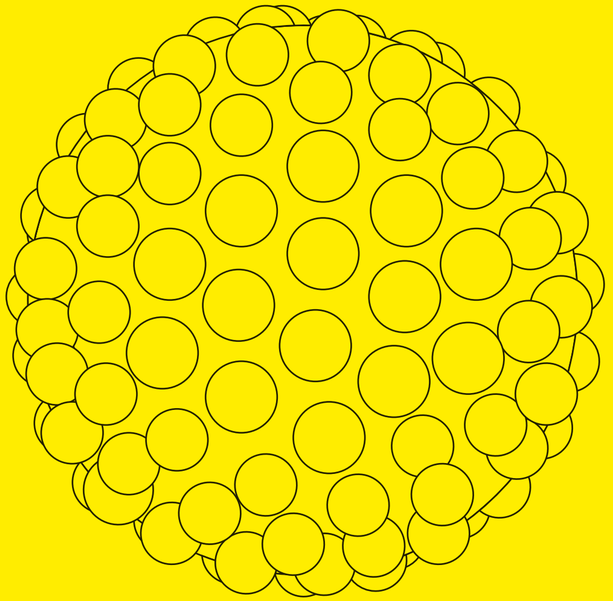
The LNP Production Workflow

mRNA is encapsulated into Lipo-NanoParticles (LNPs) to protect it against degradation and facilitate entrance into the patient's cells. The LNPs are purified before form and finish.



Formulation > **UF | DF** > **Sterile Filtration** > **Fill & Finish**

Production Workflow | Optimize Your Process

Did You Know?

- Lipids composition of LNP may vary to optimize cellular uptake, endosomal escape, mRNA payload...
- LNP size varies from 50 – 150 nm

Meet the Challenges

- Aggregation and Stability
- Control of lipid type, source and quality

Ready for Larger Production?

Build your mRNA facility of the future! Conceptual design services can take you from idea to concept design in 10 weeks.

Consult Our Experts

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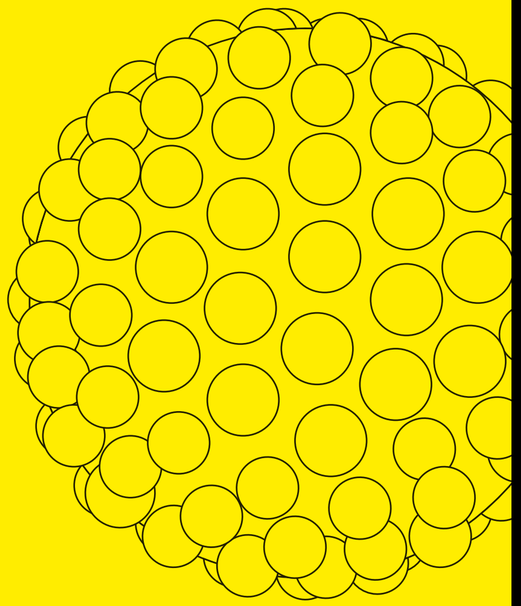

Formulation > **UF | DF** [1] > **Sterile Filtration** [2] > **Fill & Finish** [3]

Hydrosart® SU TFF cassette is a highly hydrophilic membrane for low mRNA absorption.

Production Workflow | Optimize Your Process

[1] **Hydrosart®** cut off of 30, 100 and 300 KDa depending on your LNP size. **Sartocon® Slice 50 Hydrosart®** (50 cm^2) used with **Ambr® Crossflow** for your process development.

Or **Hollow Fiber Modules** cut off of 100 or 300KDa for very gentle ultrafiltration.

[2] **Sartopore® 2 XLG** is suitable for large molecule with a 0.8 µm prefilter. Use **SartoScale 25** (4.5 cm^2) for process development.

[3] **Celsius® family** for a stable storage and shipment to the filling line. Starting from 30 mL bag for your process development.

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