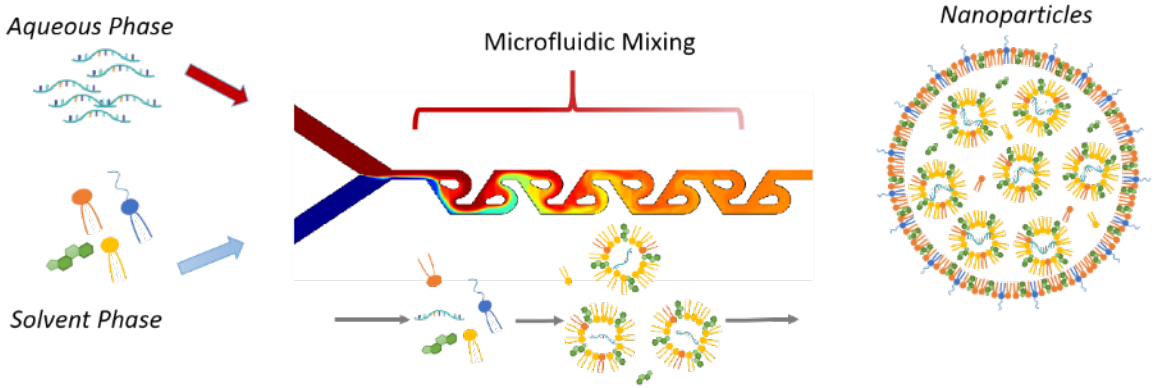
PreciGenome

NanoGenerator®
Flex-S Plus:

High throughput Formulation Automated Screening Platform for Nucleic Acid Encapsulated LNPs

V1.4-20250821

Components of Nucleic Acid Encapsulated LNPs



Lipid Components

- Cationic/ionizable lipid
- Helper lipid
- Cholesterol
- PEGylated lipid

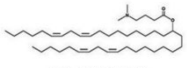
Genetic Materials

- mRNA
- DNA Plasmid
- SiRNA
- Cas9mRNA + sgRNA

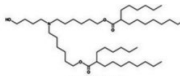
Lipid Components and Functions

Cationic/Ionizable Lipids

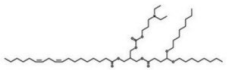
- Increases nucleic acid encapsulation rate
- Critical for endosomal escape
- Increases transfection efficiency



DLin-MC3-DMA
Alnylam (Onpattro)



ALC-0315
Pfizer/BioNTech (Comirnaty)

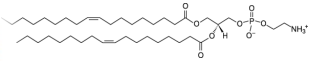


LP-01
Intellia (NTLA-2001)

Structural Lipids

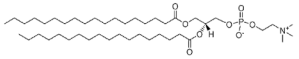
DOPE:

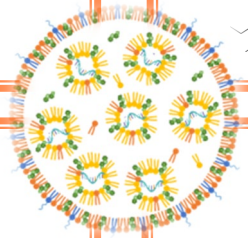
- Facilitates fusion between LNP membranes and cell membranes.
- Increases protein expression level.



DSPC:

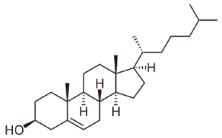
- Stabilizes lipid membrane structure
- Enhances nucleic acid encapsulation efficiency





Cholesterol

- Enhances membrane fluidity
- Increases LNP stability



PEGylated Lipid

- 0.5-2.5% molar ratio
- Increases LNP stability
- Extends circulation time
- Reduces clearance by blood proteins and macrophages
- Provides targeting function
- Triggers immune responses (anti-PEG antibody)
- Reduces cellular uptake, hinders the escape of nanoparticles from endosomes

Lipid nanoparticle (LNP) mediated mRNA delivery in cardiovascular diseases: Advances in genome editing and CAR T cell therapy Setareh Soroudi, Mahmoud Reza Jaafari, Leila Arabi, *Journal of Controlled Release*, **2024** *372*, 113-140, https://doi.org/10.1016/j.jconrel.2024.06.023

Generic Material Optimization

mRNA material

- Synthetic cap analogues and capping enzymes
- Regulatory elements in the 5‘-untranslated region (UTR) and the 3‘-UTR
- Poly(A) tail stabilizes mRNA and increases protein translation
- Modified nucleosides decrease innate immune activation and increase translation
- Sequence and/or codon optimization increases translation

mRNA vaccines — a new era in vaccinology.
Pardi, N., Hogan, M., Porter, F. *et al. Nat Rev Drug Discov* **2018** *17*, 261–279
https://doi.org/10.1038/nrd.2017.243

siRNA materials

- 2’-Ribose modification
  - 2’-Ome, 2’-F
  - Increases metabolic stability and reduces degradation
- Phosphorothioate (PS)
  - Terminal backbone stabilization
- RISC loading, 5’ phosphate modification
  - Prolonged durability on target silencing
- GNA glycol nucleic acid reduces off-targeting
- 3’ End backbone increases stability

RNAi-based drug design: considerations and future directions
Tang, Q., Khvorova, A *Nat Rev Drug Discov* **2024** *23*, 341–36.
https://doi.org/10.1038/s41573-024-00912-9

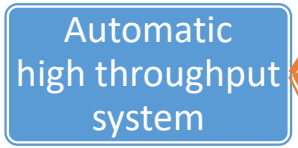
DNA materials

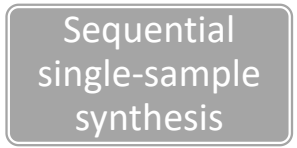
- Sequence optimization
  - Enhances transgene expression
  - Reduces autoimmunity
  - Promotes expression
- Codon optimization
  - Increases protein expression level
  - Codon preference
  - Secondary structure of resulting mRNA
  - Avoids restriction enzyme sites
  - GC ~40-60%

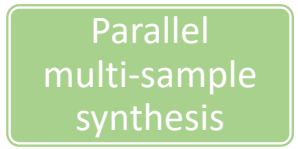
DNA-Based Nonviral Gene Therapy–Challenging but Promising
Xiaocai Guan, Yufeng Pei, and Jie Song
Molecular Pharmaceutics **2024** *21* (2), 427-453
DOI: 10.1021/acs.molpharmaceut.3c00907

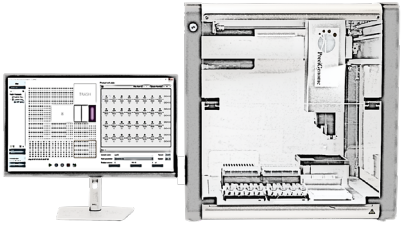
PreciGenome

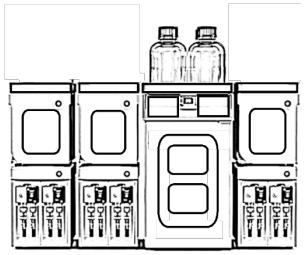
High throughput system for LNP preparation

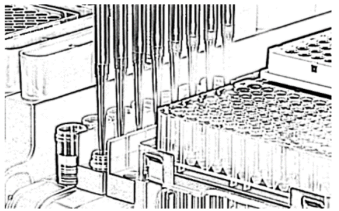












High throughput system for LNP preparation

	PreciGenome NanoGenerator® Flex-S Plus	Sequential microfluidic single-sample mixing	Robotic Liquid Handler
Mixing Methods	Microfluidic mixing	Microfluidic mixing	Pipette mixing
Synthesis Mode	Multi-sample	Single-sample	Multi-sample
Washing Needed	No	Yes	No
Run Time for 96 samples	90 mins	> 4 hours	40min
Sample volume	100 – 500 µL	400 µL – 2 mL	200 µL
Sample conc. range	Flexible	Flexible	Only low lipid concentration (1-2mM lipid)
LNP size difference compared to scale up production	Similar	Similar	20-25% larger
LNP PDI compared to scale up production	Similar	Similar	20-25% larger
EE% compared to scale up production	Similar	Similar	20-25% less
Protocol optimization	Well developed	Well developed	Intense (ratio, speed, concentration, tip choice, etc.)

NanoGenerator® Flex-S Plus

	NanoGenerator ® Flex-S/Flex-S Plus	Syringe Pump Systems	Tubing Connection Systems
Dead volume per sample	< 20 µl	0.5 mL	0.5 - 1 mL
Source of dead volume	Micro-channel in the mixing Chip	Syringe, connector, and/or mixing chip	Tubing, connector, and mixing chip
Typical production volume	100 - 500 µL	1 – 10 mL	1 – 10 mL
Minimum input volume (Aqueous :Lipid = 3:1)	Aqueous : 75ul Lipid: 25ul	Aqueous: 1 mL Lipid: 0.5 mL	Aqueous: 1 mL Lipid: 0.5 mL
Estimated minimum mRNA cost	$50	$660	$660

NanoGenerator® Flex-S

NanoGenerator® Flex-S Plus

Features of Flex-S Plus

- The Flex-S Plus System facilitates the **rapid screening** of nanoparticle formulations and early-stage payload candidates.

- With a max throughput of **48 samples per run**, **96 samples in 90 mins**, the Flex-S Plus greatly streamlines screening processes. It offers comprehensive automation of complex protocols, enabling users to concentrate on other laboratory duties.

- The system also permits experimentation with **100 – 500 µl of samples** while providing control over collection volumes. This allows users to optimize the use of valuable materials.

- **Library preparation function** enables lipid/payload formulation prep from raw stock materials before the LNP synthesis

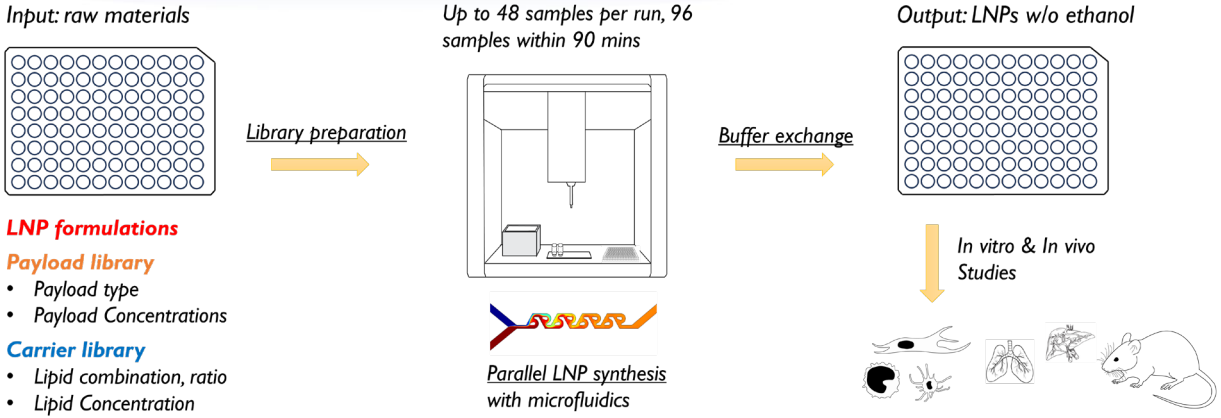
- **Buffer exchange function** enables removing of ethanol in the final products

NanoGenerator® Flex-S Plus

- Rapid screening of LNP formulations
- Rapid screening of payload
- 48 samples per run
- 96 samples in 90 mins
- Disposable consumables
- Optional functions: formulation library prep, buffer exchange, temperature control, HEPA filter, UV sterilization light

Model	Flex-S	Flex-S Plus
Multi-sample per run	1 – 4	(1 – 12) × 4 per run Up to 96 samples in 90 mins
Full automation	N/A	Yes
Library preparation	N/A	Optional
Buffer exchange	N/A	Optional
Throughput	0.1 – 0.5 ml per sample	0.1 – 0.5 ml per sample
Total flow rate	3 ml/min, 4 ml/min	3.5 & 5.0 ml/min
Flow rate ratio	3:1	3:1
Custom design flow rate	Yes	Yes
Size range	40 – 200 nm	40 – 200 nm
PDI	0.05 – 0.2	0.05 – 0.2
Encapsulation efficiency	Up to 99%	Up to 99%
Payload	DNA, mRNA, siRNA, Protein, small molecules, etc.	DNA, mRNA, siRNA, Protein, small molecules, etc.
HEPA filter/UV light	N/A	Optional
Dimension	320 mm × 400 mm × 210 mm	630 mm × 570 mm × 660 mm
Weight	8.1 kg	50 kg

NanoGenerator® Flex-S Plus workflow



NanoGenerator® Flex-S Plus

Workflow:

1. Load raw materials in 96 well plates
2. Seal the 96 well plates (optional)
3. Put consumables on the deck: chips, 96 well plates, pipette tips, and gaskets
4. Set parameters in the software and run the program (library prep & buffer exchange are optional)
5. Collect samples in 96 well plate
6. Discard/change consumables

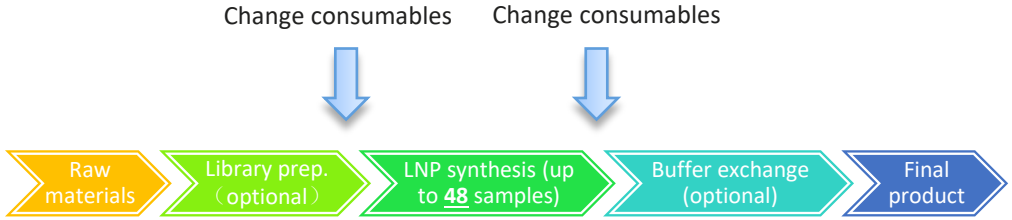
Demo video: Demo of NanoGenerator® Flex-S Plus Platform, Automated High-throughput LNP Preparation & formulation

NanoGenerator® Flex-S Plus

One instrument: two workflows

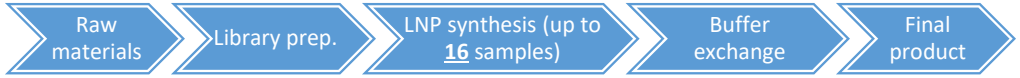
- High throughput workflow
- Automated workflow

- **High throughput workflow**

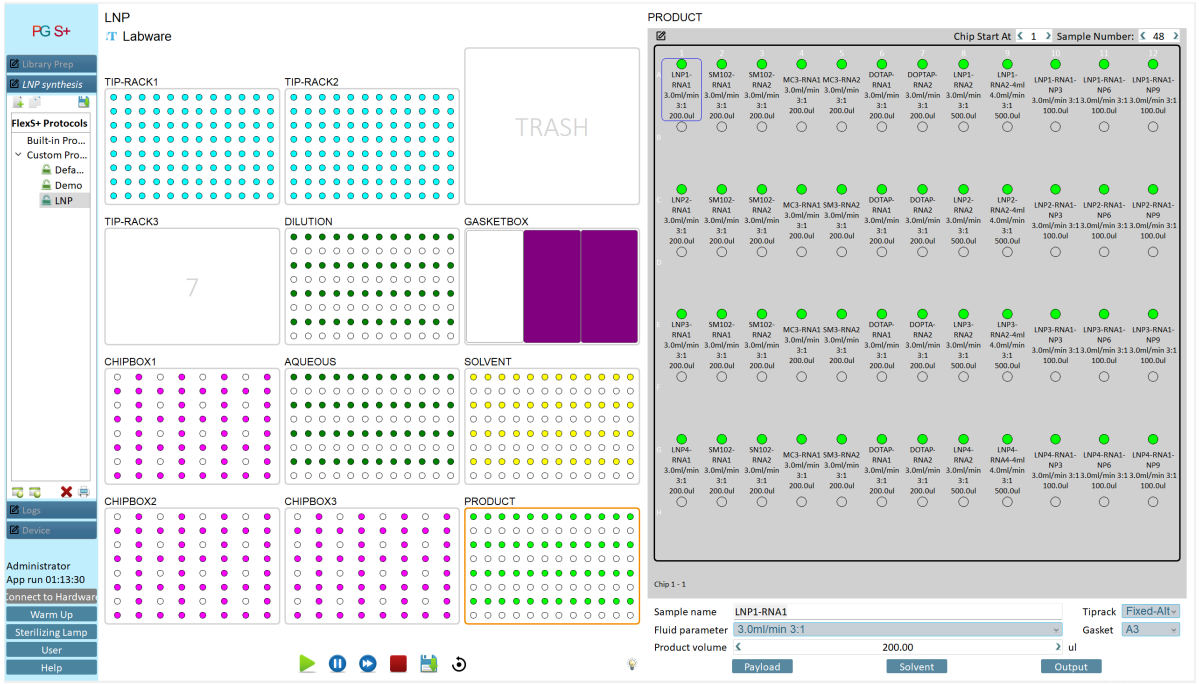


- **Automated workflow**

(no need to change consumable)



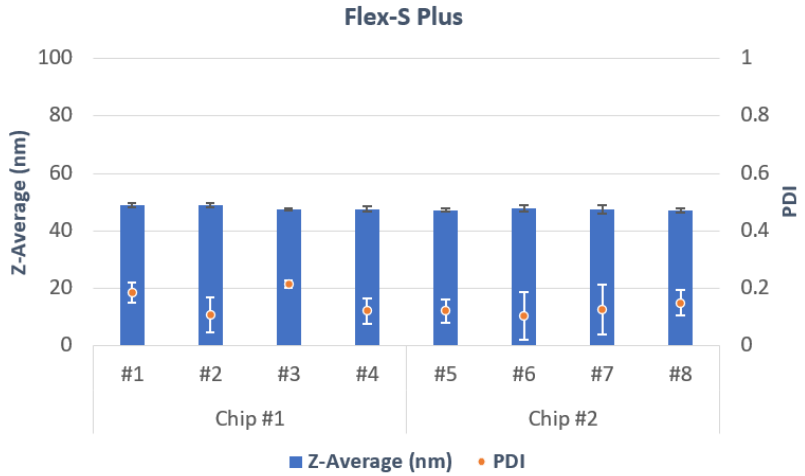
NanoGenerator® Flex-S Plus



User friendly UI:

1. Up to 48 samples (12 chips) per run;
2. Different chips can apply different fluid parameters or/and product volumes
3. In one chip, up to 4 different formulations can be synthesized;
4. Offer recipe exporting and importing. Setting can be done in other computers;
5. Offer printing function. Layout can be printed out for sample prep guidance;
6. Clear consumable layout to help set up consumables.

NanoGenerator® Flex-S Plus



- Robust multi-sample synthesis
- Reliable performance
- Consistent results

Model	Flex-S Plus
Aqueous phase	Sodium acetate buffer, 100mM, pH5.2
Solvent phase	LipidFlex, 15mM in ethanol
Parameters	3ml/min, FRR 3:1, 200µL

NanoGenerator® Flex-S Plus

Size (nm)

54.9	45.4	45.5	55.5
54.4	47.6	45	46.2
54.7	46.5	47.4	62.3
54.4	48.2	51.9	57.3
42.6	47.8	44.8	51.2
41.1	50	46.9	48.2
62.8	48.5	46.7	74
56.6	50.7	52.1	59.2

40 80

PDI

0.216	0.13	0.126	0.473
0.175	0.107	0.092	0.08
0.185	0.09	0.104	0.113
0.16	0.1	0.137	0.107
0.306	0.121	0.101	0.403
0.063	0.129	0.128	0.124
0.118	0.066	0.148	0.109
0.041	0.099	0.163	0.113

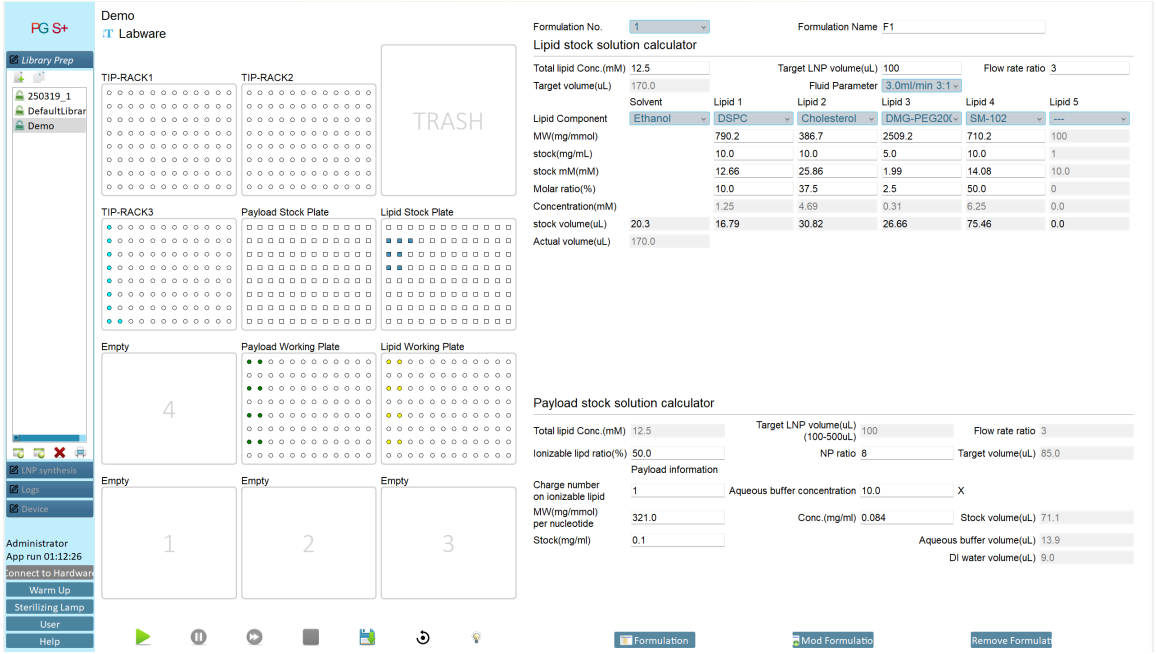
0.02 0.50

Example

- 32 sample screening (formulation & N:P ratio screening)
- 32 samples done in 30 mins
- 96-well Plate format

Model	Flex-S Plus
Aqueous phase	RNA in Sodium acetate buffer, 100mM, pH5.2
Solvent phase	Different lipid formulation

NanoGenerator® Flex-S Plus Library prep.



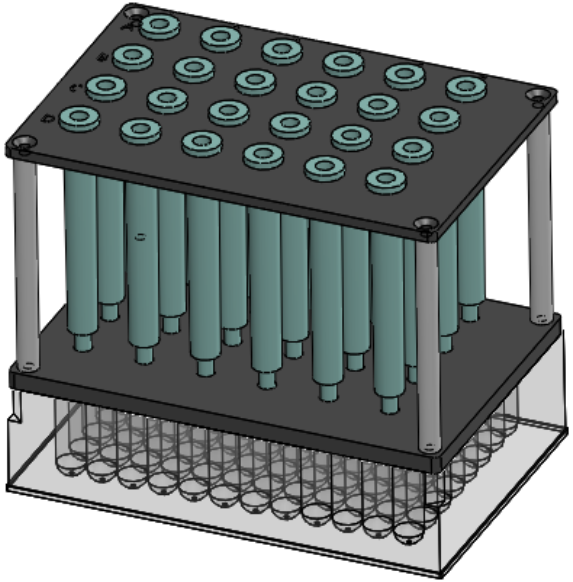
Library prep.

- Optional functions to help library prep. form raw materials;
- Up to 12 lipid formulations (4 replicates) per run;
- Automatically generated the volume of raw material need to be prepared;
- Payload stock solution calculator helps prepare payload stocks based on different N/P ratio;
- Automatically generate LNP synthesis recipe based on the library prep setting.

NanoGenerator® Flex-S Plus buffer exchange

Buffer exchange

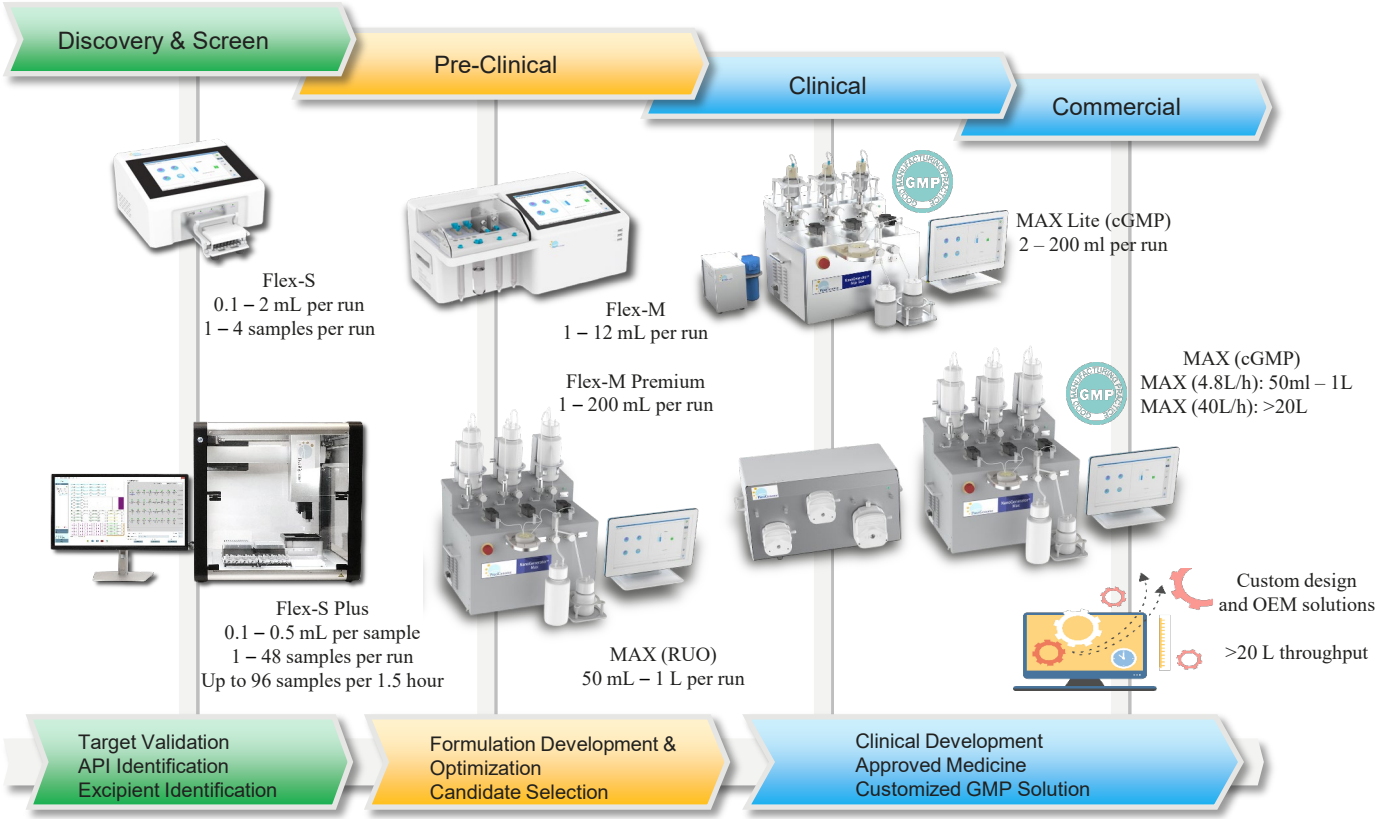
- Optional functions to help removing ethanol in the LNP products
- Buffer exchange column arrays with different sizes;
- Ethanol residue < 0.005%;
- RNA recovery yield > 80 – 90%;
- LNP size change < 5%;
- Up to 48 samples in the high throughput workflow;
- Up to 16 samples in the automation workflow.



Consumables for Flex-S Plus

Catalogue number or brand	Items	Description
KIT-SP-MIX4G-Q96	Chip & gasket package	Each package contains 24 chips and 24 gasket sheets; For 96 samples
CHP-MIX4-Q24	Chip for Flex-S plus	Qty: 96 samples pack, including mixing chips and reagent reservoirs, 24pcs (4 samples/pc).
PG-GSK-SP-Q24	Gasket sheets for Flex-S plus	Qty: 96 samples pack, including gasket 24pcs (4 samples/pc).
PG-PTIP200-Q960	200 µl pipette tip with filter	Boxes of pipette tips with filter for transferring solutions. 960 tips, 10 racks per pack
NEST® Wuxi NEST Biotechnology	Full skirt 100 µl 96-well PCR plate	For dilution buffer, aqueous samples, solvent samples or collection samples
Eppendorf® Eppendorf SE	Semi-skirt 200 µl 96-well PCR plate	For dilution buffer, aqueous samples, solvent samples or collection samples; Need to be used with 96-well plate holders
Any brand	Full skirt 300 µl 96-well cell culture plate	For collection samples
NEST® Wuxi NEST Biotechnology	Deep well 1 ml 96-well plate	For dilution buffer, aqueous samples, or collection samples
Nunc™ 96-Well	Deep well 1300 µl 96-well plate	For dilution buffer, aqueous samples, or collection samples
PG-P96-FOIL-Q100	Aluminum foil	To seal the 96-well plate
PG-SSEAL-Q10	Slit seal	96-well plate seal, allowing muti-times insertion and withdrawal of pipette tips
PG-SSEAL-STZ-Q10	Slit seal (Sterilized)	96-well plate seal, allowing muti-times insertion and withdrawal of pipette tips

NanoGenerator® - Nanoparticle Synthesis System



Scalable LNP Production

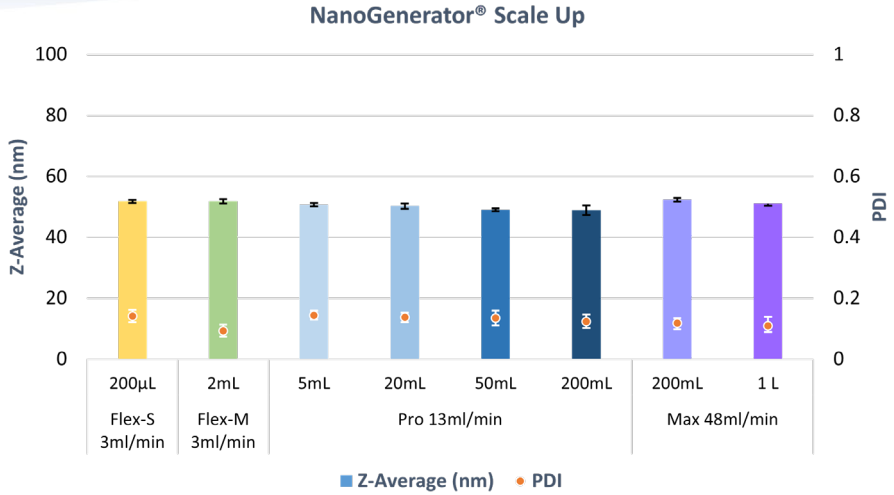
Transferable results from early screening (Flex-S, 0.1mL) to pre-clinical development (Flex-M/M Premium, 12ml/200mL), then clinical studies and production (Max Lite: 200ml Max: 1L, MAX 40L/h: >20L)

- Flex-S: 0.1 – 2 mL
- Flex-S Plus: 0.1 – 0.5 mL

- Flex-M: 1 – 12 mL
- Flex-M Premium: 1 – 200 mL

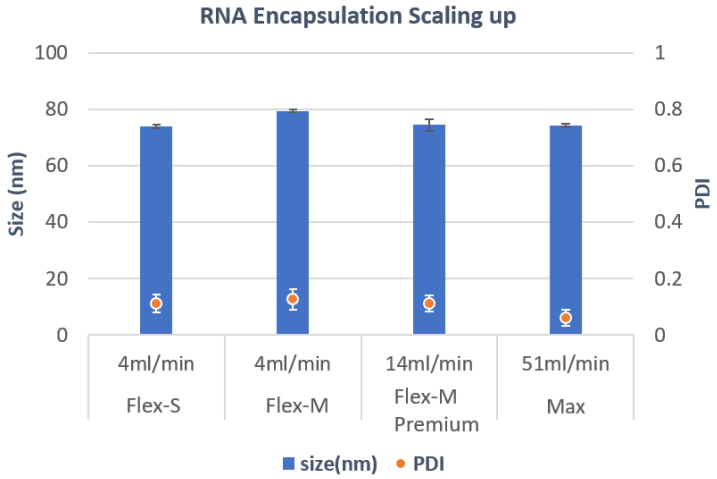
- MAX Lite cGMP : 2 – 200 mL

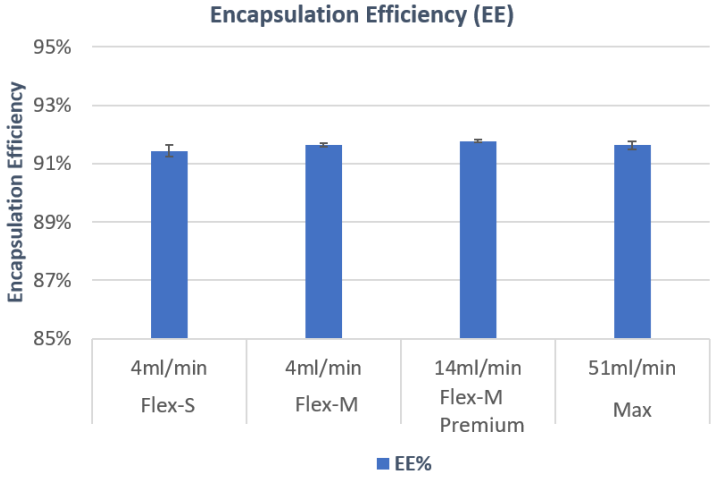
- MAX RUO & cGMP (4.8L/h) : 50 mL – 1 L
- MAX cGMP (40L/h): > 20 L



Reagents	
Aqueous phase	Sodium acetate buffer (100 mM, pH 5.2)
Solvent phase	LipidFlex, 15 mM in ethanol

NanoGenerator® — Scale Up





	Reagents
Aqueous phase	Sodium acetate buffer (100mM, pH5.2)
Payload	RNA (~600 nt)
Solvent phase	LipidFlex RNA-LNP kit

System Benefits

High Throughput & Efficiency

- Multiple sample (1/4/48) per run.
- Up to 48 samples per run, 96 samples in 90 mins.

Regulatory Compliance

- Intuitive software (21 CFR Part 11 compliant)
- Single-use mixing cartridge

Scalable & Reproducible

- Direct transfer from discovery to clinical manufacturing
- Reproducible manufacturing

Automation

- Automated workflow
- Real-time data monitoring & recording
- Electronic batch records

High Yield

- Small reagent volume (minimum 100 µl) for each sample.
- Save up to 80% of RNA/lipid cost

Custom Design & Service

- On-site installation & 3Q qualification
- Custom design & OEM