



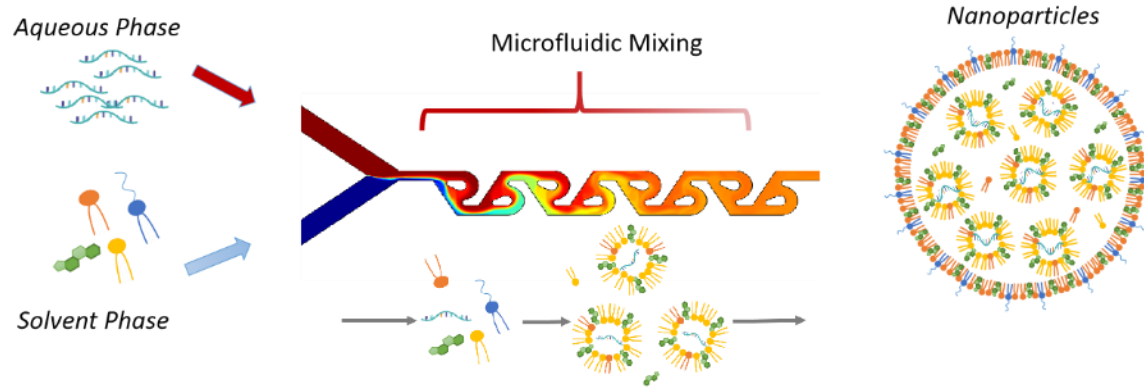
NanoGenerator® Flex-S Plus:

High throughput
Formulation Screening
platform
for Nucleic Acid
Encapsulated LNPs

V1.0-20240917



Components of Nucleic Acid Encapsulated LNPs



Lipid Components



Cationic/ionizable lipid



Helper lipid



Cholesterol



PEGylated lipid

Genetic Materials



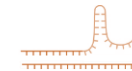
mRNA



SiRNA



DNA Plasmid



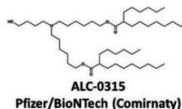
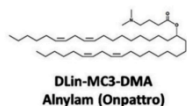
Cas9mRNA + sgRNA

Lipid Components and Functions



Cationic/Ionizable Lipids

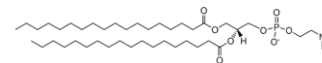
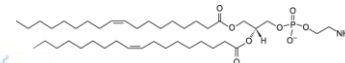
- Increase nucleic acid encapsulation rate
- Critical for endosomal escape
- Increase transfection efficiency



Helper Lipids

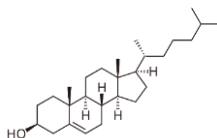
DOPE –facilitate fusion between LNP membranes and cell membranes.
Higher protein expression level.

DSPC – stabilizing lipid membrane structure, enhance nucleic acid encapsulation efficiency



Cholesterol

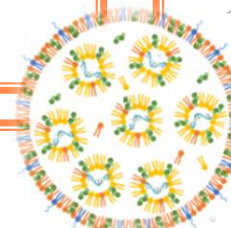
- Enhancing membrane fluidity
- Increasing LNP stability



PEGylated Lipids

0.5-2.5% molar ratio • Targeting function

- Increase LNP stability • immune responses (anti-PEG antibody)
- extend circulation time • reduce cellular uptake and hinder the escape of nanoparticles from endosomes
- reducing clearance by blood proteins and macrophages



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 increase 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
 - Increase metabolic stability and reduce degradation
- Phosphorothioate (PS)
 - Terminal backbone stabilization
- RISC loading, 5' phosphate modification
 - Prolonged durability on target silencing
- GNA glycol nucleic acid, reducing off-targeting
- 3' End backbone extra stabilization

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>

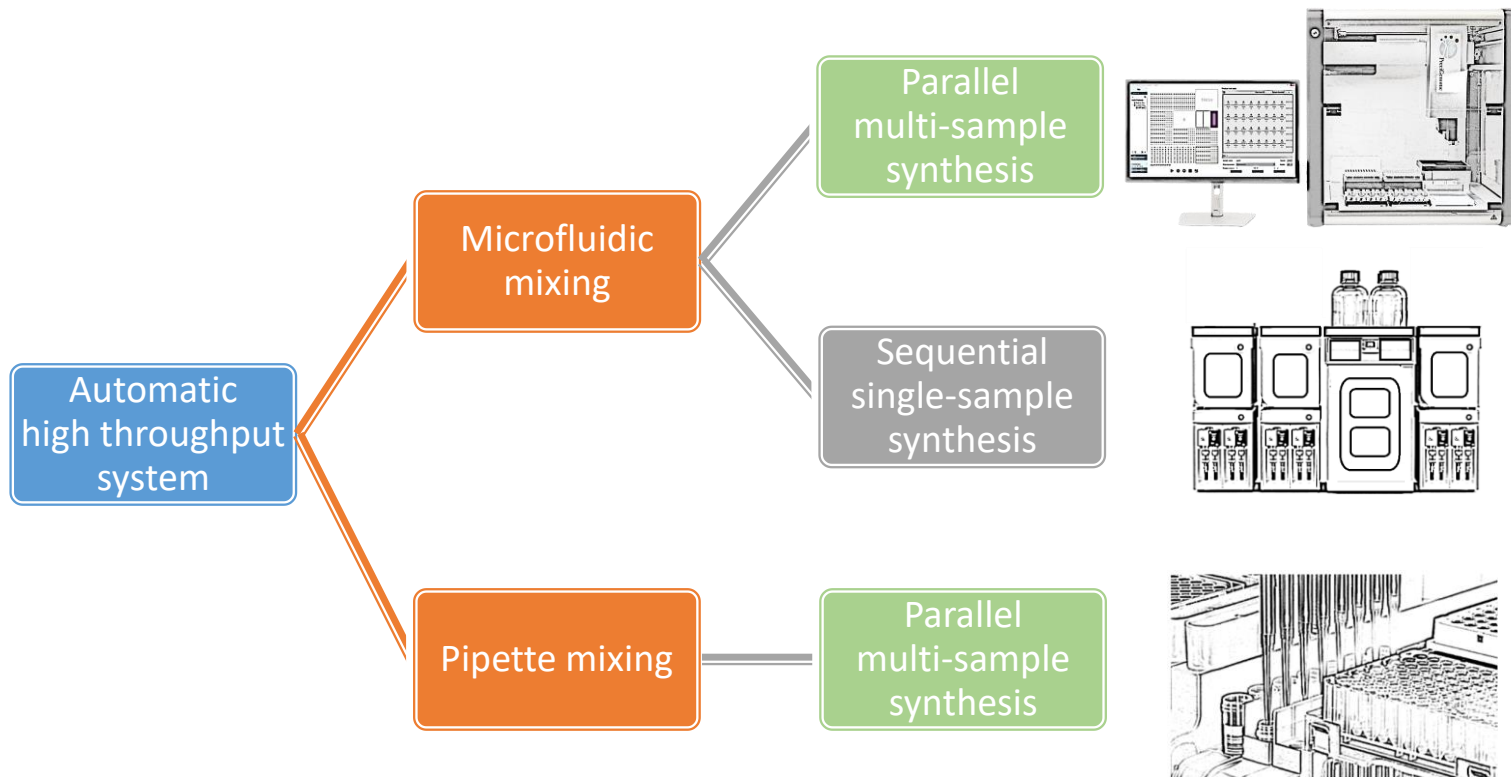


DNA materials

- Sequences optimization
 - Enhance transgene expression
 - Reduce autoimmunity
 - Strong promoter for expression
- Codon Optimization
 - Increase protein expression level
 - Codon preference
 - Secondary structure of resulted mRNA
 - Avoid 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](https://doi.org/10.1021/acs.molpharmaceut.3c00907)

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	1 hour	> 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.)

Features of Flex-S Plus



- The Flex-S Plus System facilitates the rapid screening of nanoparticle formulations and early-stage mRNA candidates, offering a substantial increase in project efficiency.
- With a max throughput of 32 samples per run, 96 samples per hour, 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 as little as 20 μl of payload reagent (e.g. mRNA) while providing control over collection volumes. This allows users to optimize the use of valuable materials.

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
Estimated minimum mRNA cost	\$50	\$660	\$660

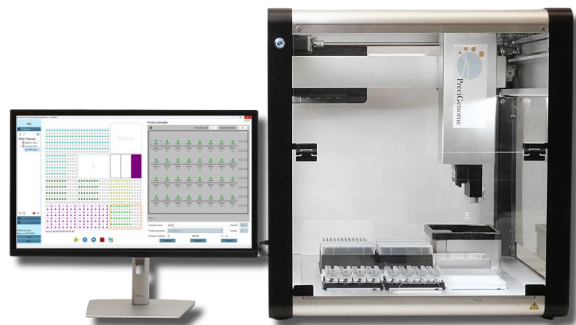


NanoGenerator® Flex-S



NanoGenerator® Flex-S Plus

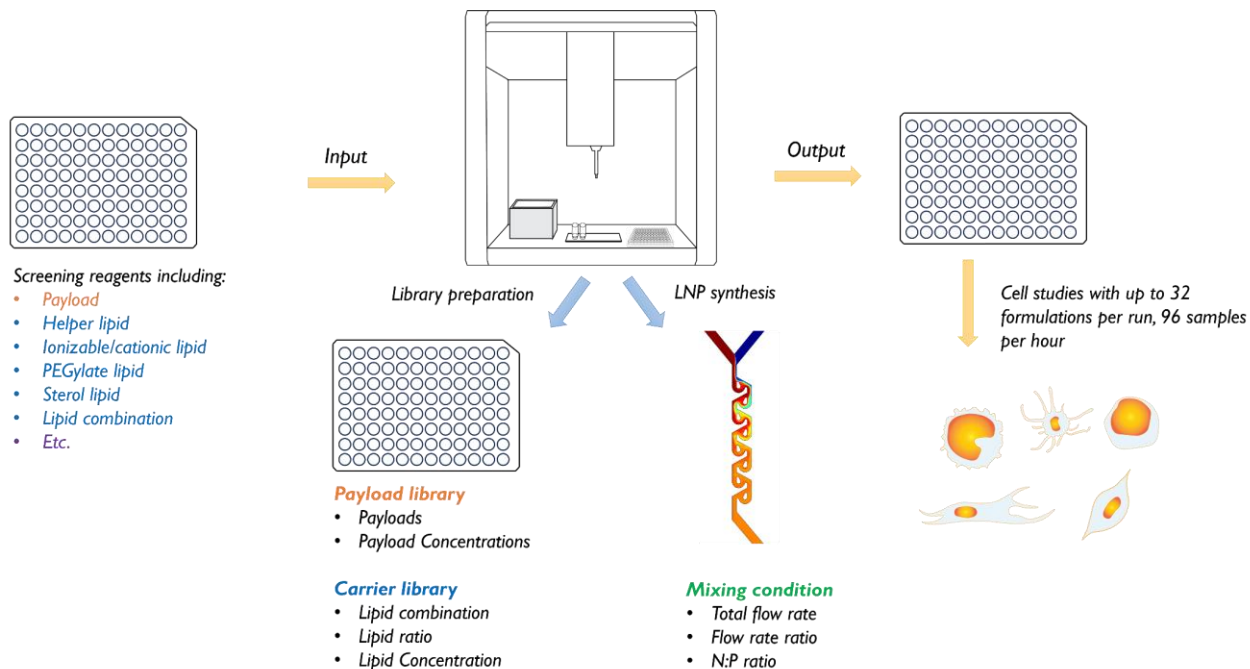
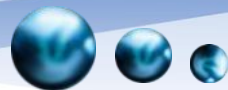
NanoGenerator® Flex-S Plus



- Rapid screening of LNP formulations
- Rapid screening of mRNA/siRNA
- 32 samples per run
- 96 samples within one hour
- Disposable consumables

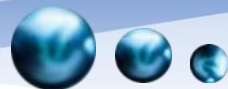
Model	Flex-S	Flex-S Plus
Multi-sample per run	1 – 4	(1 – 8) × 4 per run Up to 96 samples per hour
Full automation	N/A	Yes
Library preparation	N/A	Optional
Throughput	0.1 – 0.5 ml per sample	0.1 – 0.5 ml per sample
Total flow rate	3 ml/min, 4 ml/min	3 ml/min, 4 ml/min
Flow rate ratio	3:1, 4:1	3:1, 4:1
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 mol ecules, etc.	DNA, mRNA, siRNA, Protein, small mol ecules, etc.
Dimension	320 mm × 400 mm × 210 mm	630 mm × 570 mm × 660 mm
Weight	8.1 kg	50 kg

NanoGenerator® Flex-S Plus for screening



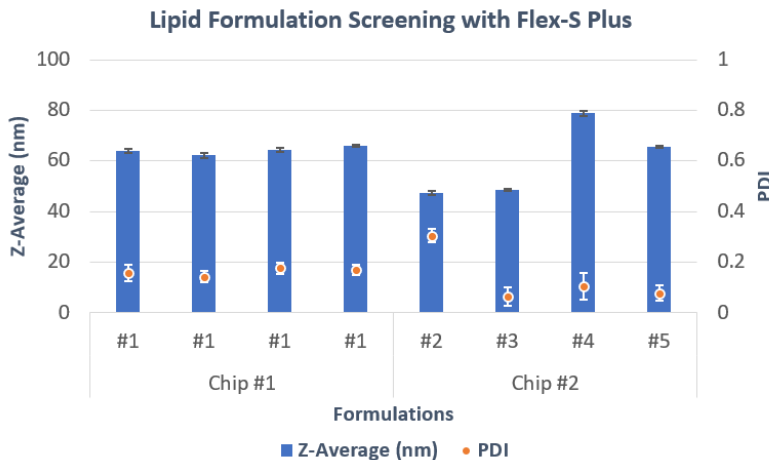
Sample Workflow:

1. Load samples in 96 well plates;
2. Seal the 96 well plate (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;
5. Collect samples in 96 well plate;
6. Discard/Change consumable.



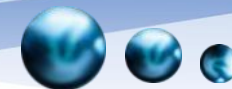
- Robust multi-sample synthesis
- Reliable performance
- Consistent results

Model	Flex-S Plus
Aqueous phase	Sodium acetate buffer, 100mM, pH5.2
Solvent phase	Lipidflex, 15mM in ethanol
Parameters	3.3ml/min, FRR 3:1, 200µL



- Lipid formulation screening
- 96 samples < 1hour
- 96-well Plate format

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



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

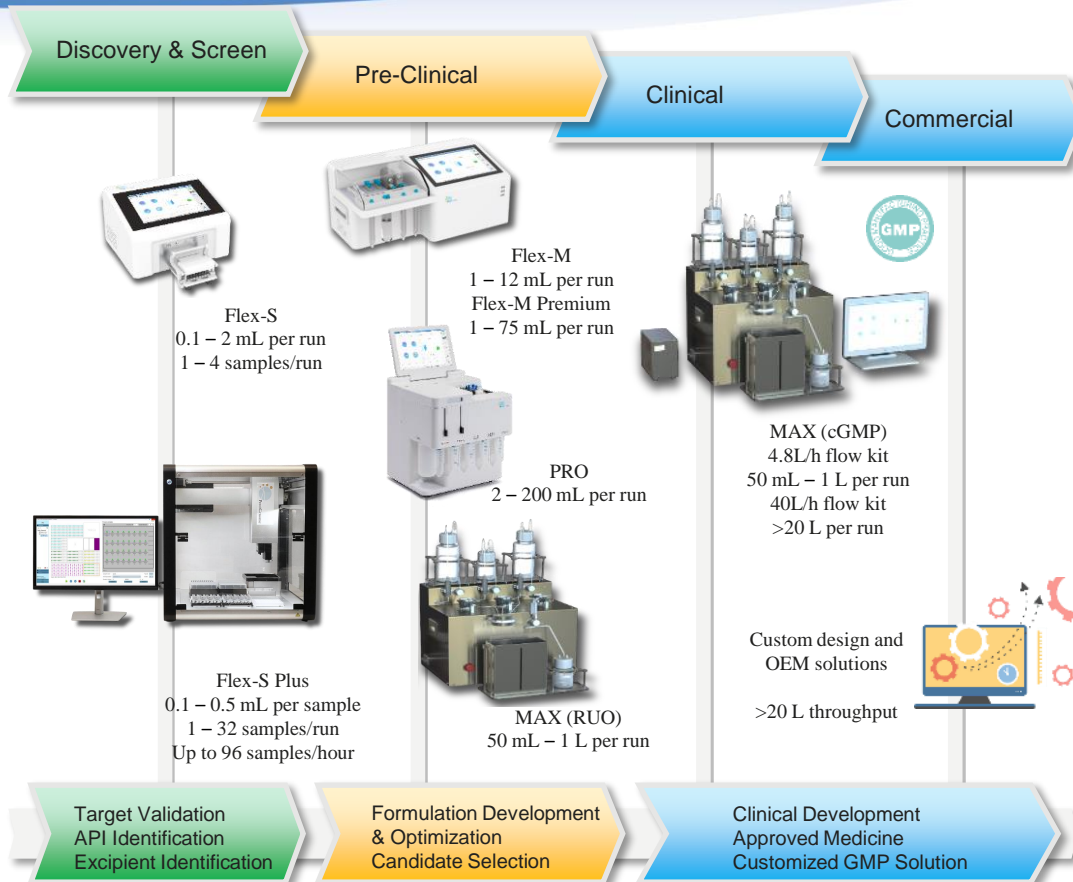
0.02

0.50

- 32 sample screening (formulation & N:P ratio screening)
- 96 samples < 1hour
- 96-well Plate format

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

NanoGenerator® - Nanoparticle Synthesis System



PreciGenome (Confidential)

NanoGenerator® Scaling Up



- Transferable results from early screening (Flex-S/Flex-S Plus, 0.1mL) to pre-clinical development (Pro, 200mL), then commercial production (Max: 1L, MAX 40L/H: >20L)



Flex-S/Flex-S Plus: 0.1 – 0.5 ml per sample



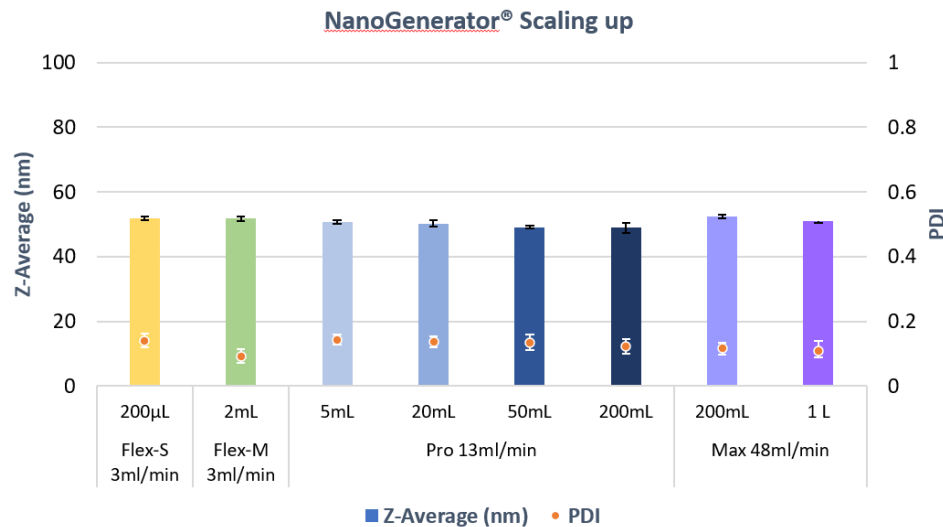
Flex-M: 1 – 12 ml
Flex-M Premium: 1 – 75ml



Pro: 2 – 200 ml

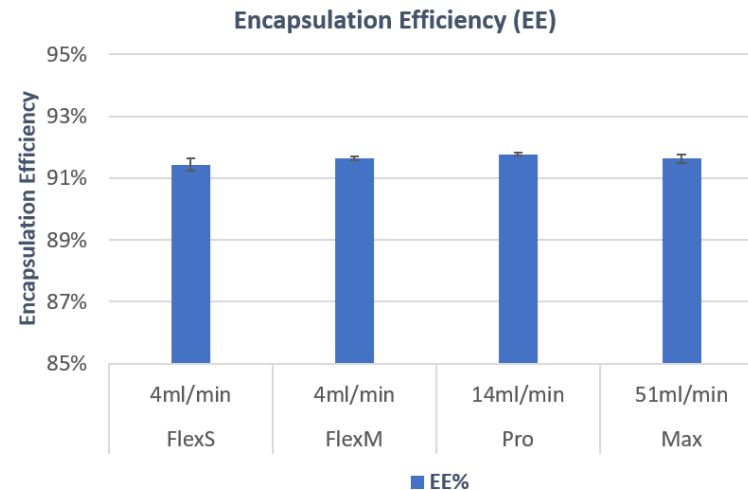
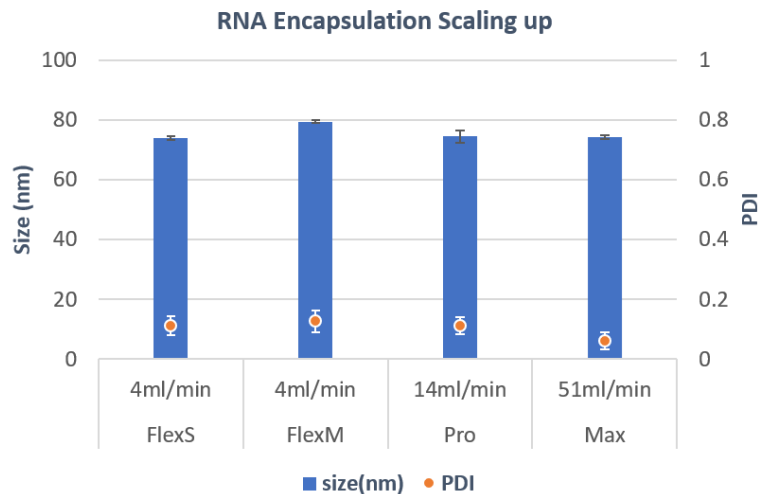


MAX : 50ml – 1L
MAX (40L/H): >20L



Reagents	
Aqueous phase	Sodium acetate buffer (100mM, pH5.2)
Solvent phase	LipidFlex, 15mM 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 (1/4/32) per run.
- Runtime <5 min for 4 samples, 48/96 samples per hour.

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 50 μ l) for each sample.
- Save up to 80% of RNA/lipid cost

Custom Design & Service



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