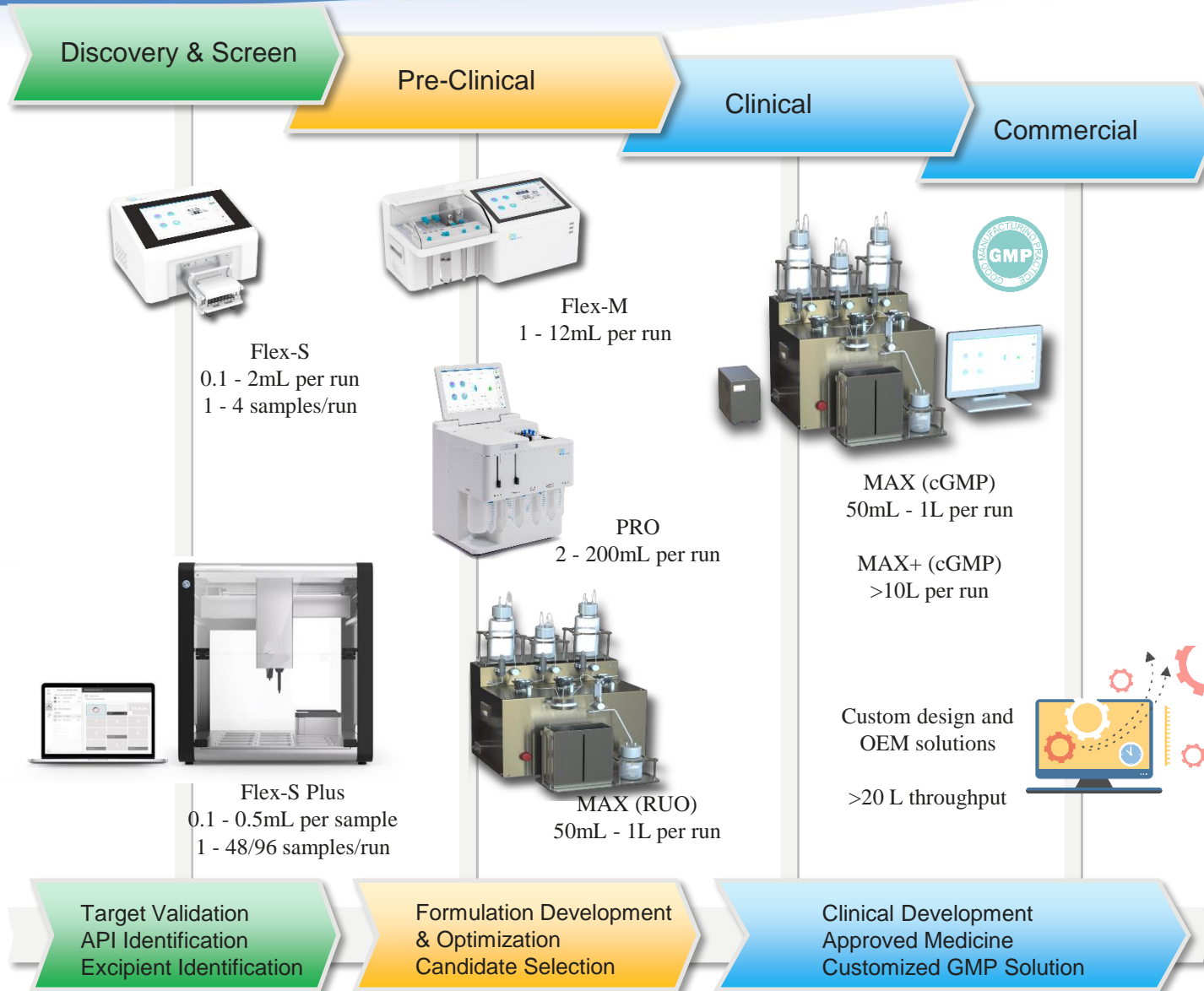
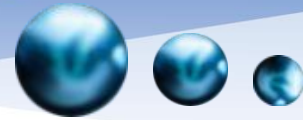


# NanoGenerator™ NanoParticle Synthesis System and Lipid-Flex™ Formulation

PreciGenome

Jan 2024

# NanoGenerator™ - Nanoparticle Synthesis System

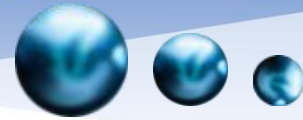


Target Validation  
API Identification  
Excipient Identification

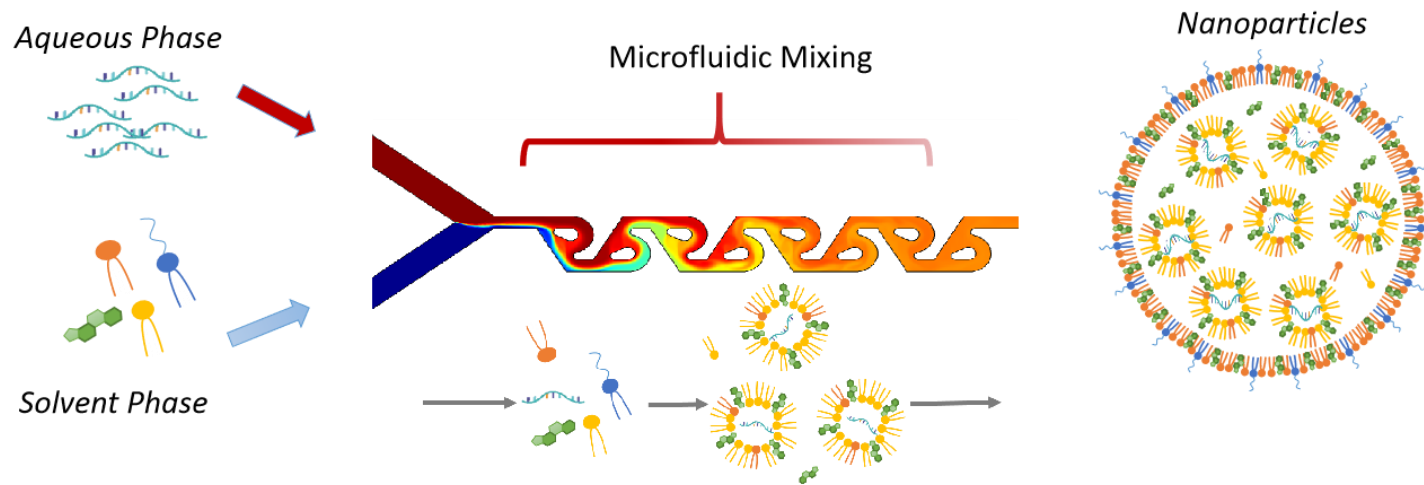
Formulation Development  
& Optimization  
Candidate Selection






Clinical Development  
Approved Medicine  
Customized GMP Solution

# What is Lipid Nanoparticle?

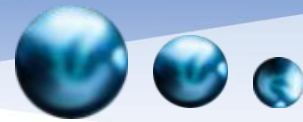


Lipid nanoparticles (LNP) are self-assembling structures of natural or synthetic lipids in aqueous environment.



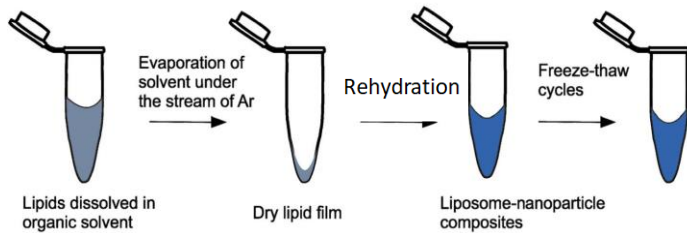
-  Cationic/ionizable lipid
-  Structural lipid
-  Cholesterol
-  PEGylated lipid
-  Nucleic acid payload

# Lipid Nanoparticle Synthesis Methods



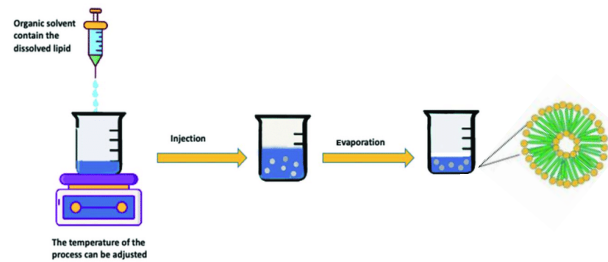
## Conventional Methods

### A Film hydration



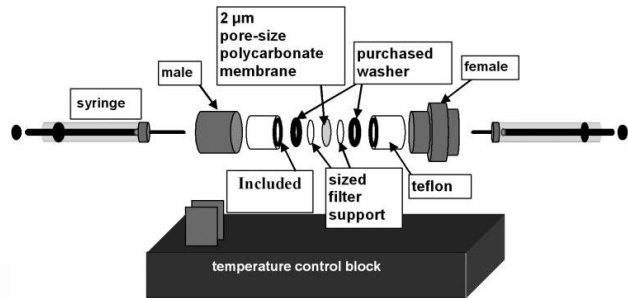
- Established method
- Understood method
- High consuming of the organic solvent
- High PDI
- Lack of reproducibility
- Need for additional downsizing step
- Difficulties in scaling-up

### B Solvent injection



- Simple and fast
- Scaling-up possibility
- Exposing to organic solvent
- High PDI
- Stability problem

### C Extrusion

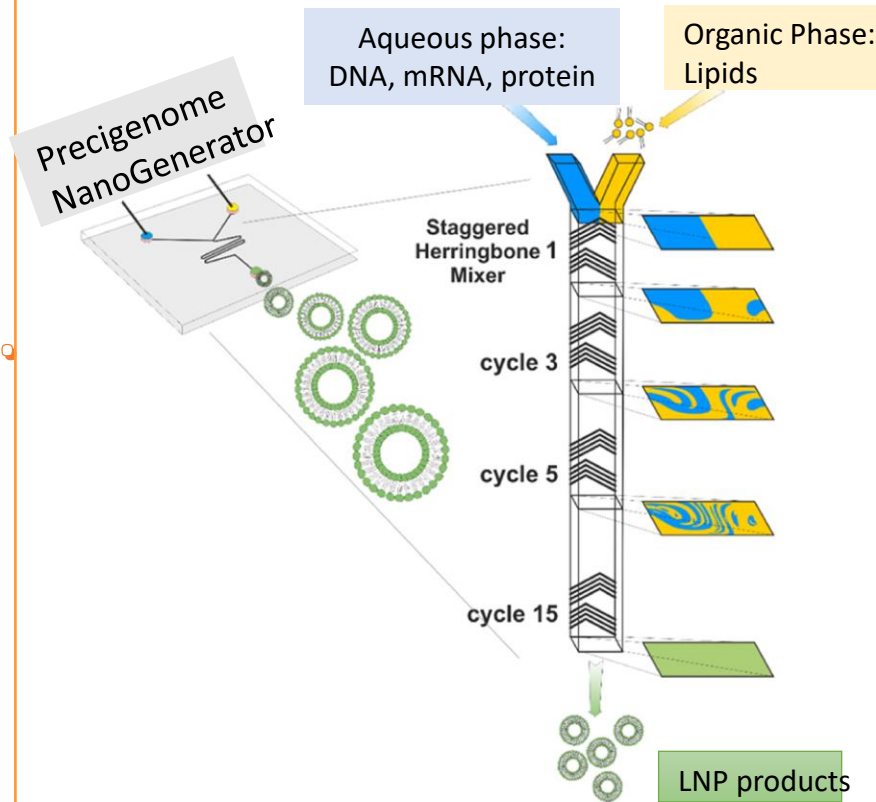


- Uniform and homogenous formulation
- Possible clogging of the membrane pores
- Difficulties in scaling up

Nanomaterials, Volume 11, 2021, 3440

## Microfluidic Mixer

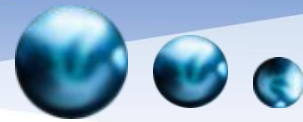
VS.



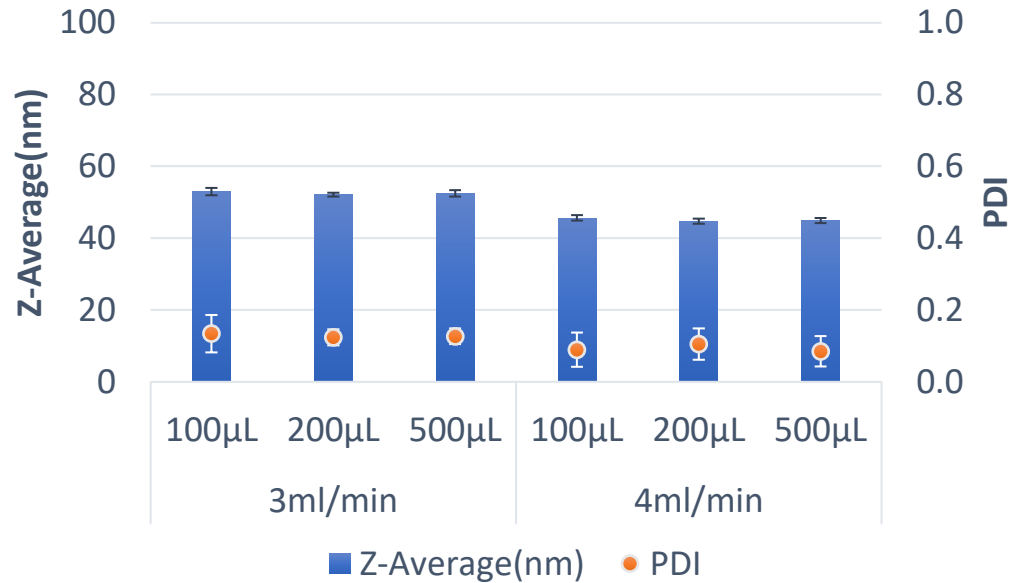
Reference: Scientific Reports volume 10, Article number: 5595 (2020)



BASIC FEATURES	Flex-S	Flex-M	PRO	MAX	MAX (cGMP)
Product Model Number	PG-SYN-FS	PG-SYN-FM	PG-SYN-P	PG-SYN-M	PG-SYN-G
R&D Stage	Screening & Discovery	Screening & Discovery	Preclinical Studies & Development	Preclinical Studies & Development	Clinical Development
Throughput	0.1 to 2mL	1 to 12mL	2 to 200mL	50mL to 1L	50mL to 1L
Multiple Samples Per Run	✓	✗	✗	✗	✗
Total Flow Rate	3 mL/min, 4 mL/min	1 to 5 mL/min	4 to 20 mL/min	1.2 to 4.8L/h	1.2 to 4.8L/h
Flow Rate Ratio	3:1	2:1 to 5:1	2:1 to 5:1	2:1 to 9:1	2:1 to 9:1
Tunable Flow Rate	✓	✓	✓	✓	✓
Intuitive & Easy To Use	✗	✓	✓	✓	✓
Compact Design	✓	✓	✓	✓	✓
Consumable Cost Per Run	\$	\$	\$	\$\$	\$\$\$



Flexible Synthesis Parameters

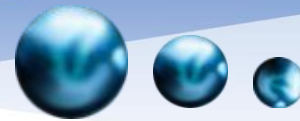


Model	Flex S
Aqueous phase	Sodium acetate buffer, 100mM, pH5.2
Solvent phase	Lipidflex, 15mM in ethanol

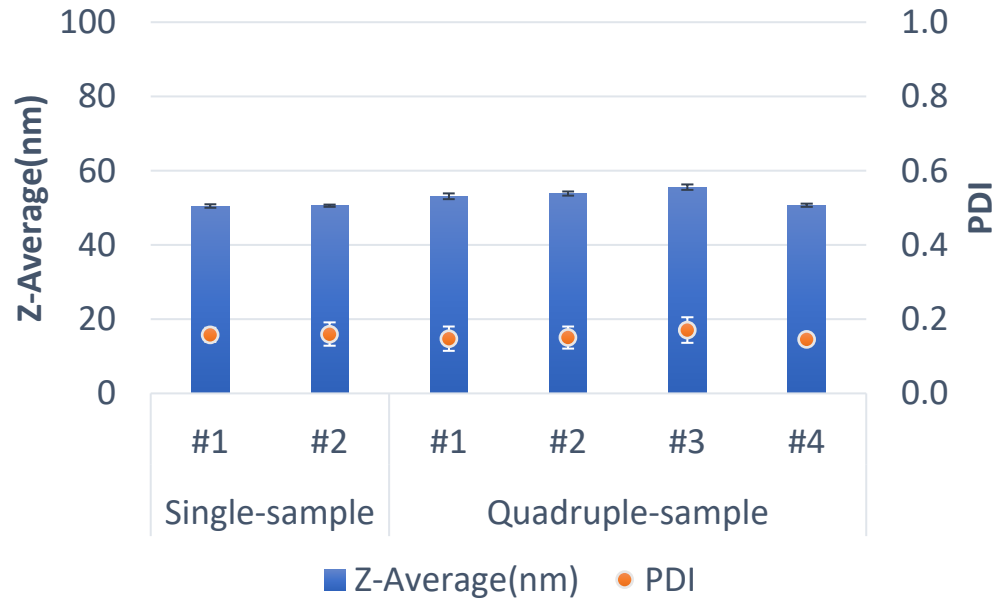


NanoGenerator Flex (S)

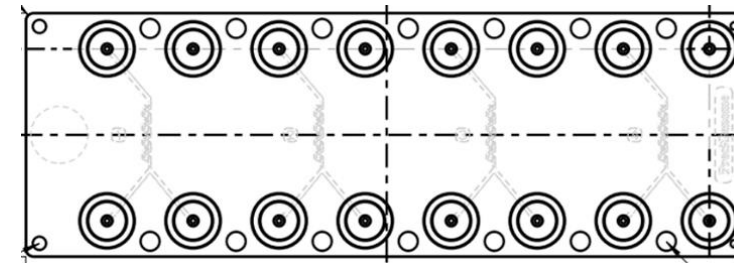
- **More total flow rate setting options.**
  - Users can choose **3ml/min** or **4ml/min** to conduct LNP synthesis.
  - Higher flow rate setting generates LNPs of smaller particle size.
- **Low synthesis volume limit (100-500 µL) per sample**
  - Minimum aqueous sample input volume: **75 µL**
  - Minimum Lipid formulation input volume: **25 µL**
- **Excellent batch-to-batch consistency**



## Flex-S Multi-sample Synthesis Mode



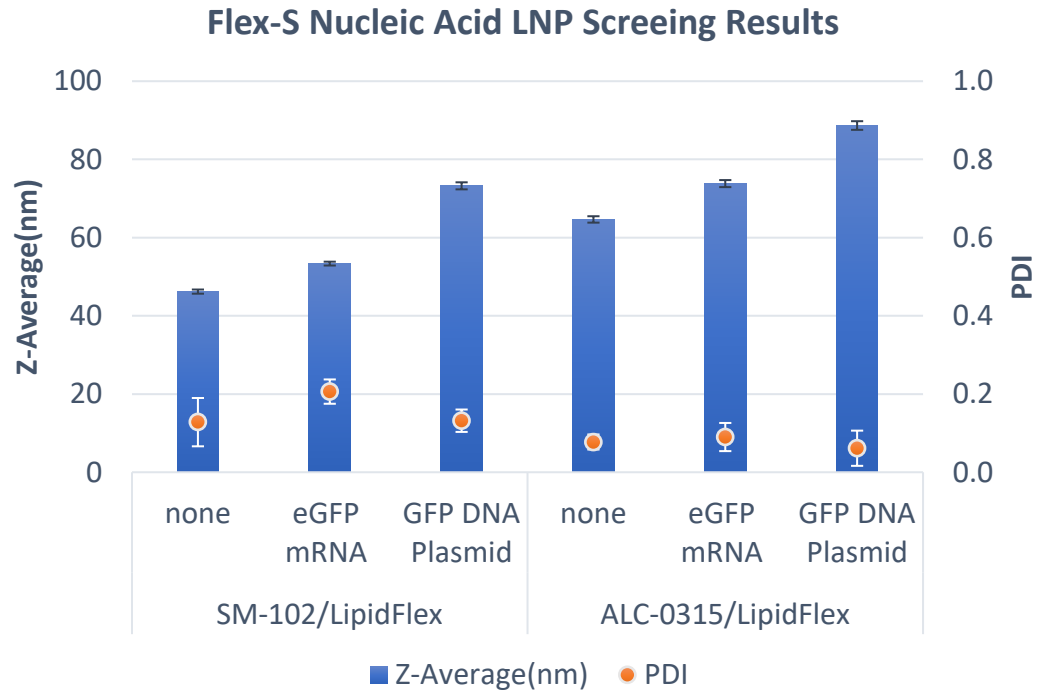
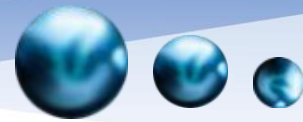
Model	Flex S
Aqueous phase	Sodium acetate buffer, 100mM, pH5.2
Solvent phase	Lipidflex, 15mM in ethanol



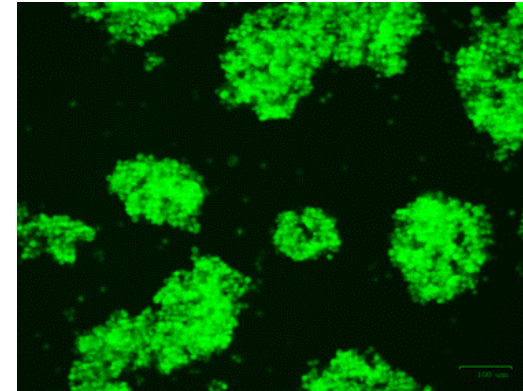
CHIP-MIX-4

### Multi-sample Synthesis by NanoGenerator™ Flex-S:

- **10 seconds, 4 samples!** Users can choose multi-sample synthesis mode to conduct formulation screening. The screening time is as low as 10 seconds
- **Reliable screening results.** Using PreciGenome's advanced air-flow control technology, users can obtain reliable LNP results on both single- or multi-sample synthesis modes.



eGFP mRNA LNP Delivery to Jurkat Cells



Jurkat Cells transfected with eGFP mRNA LNP. Green fluorescence image at 48 hours post transfection.

- **Robust Formulation Screening.** Using NanoGenerator™ Flex-S, users can conduct formulation screening using minimum reagent consumption, which saves lots of cost.
- LNP size and PDI depend on the payload and formulation choice.

Model	Flex S
Aqueous phase	100 µg/mL eGFP mRNA (CATUG) or GFP DNA (ALDEVRON) in sodium acetate buffer ( 100mM, pH5.2)
Solvent phase	Ionizable lipid/Lipidflex, 40/60, 12.5mM in ethanol

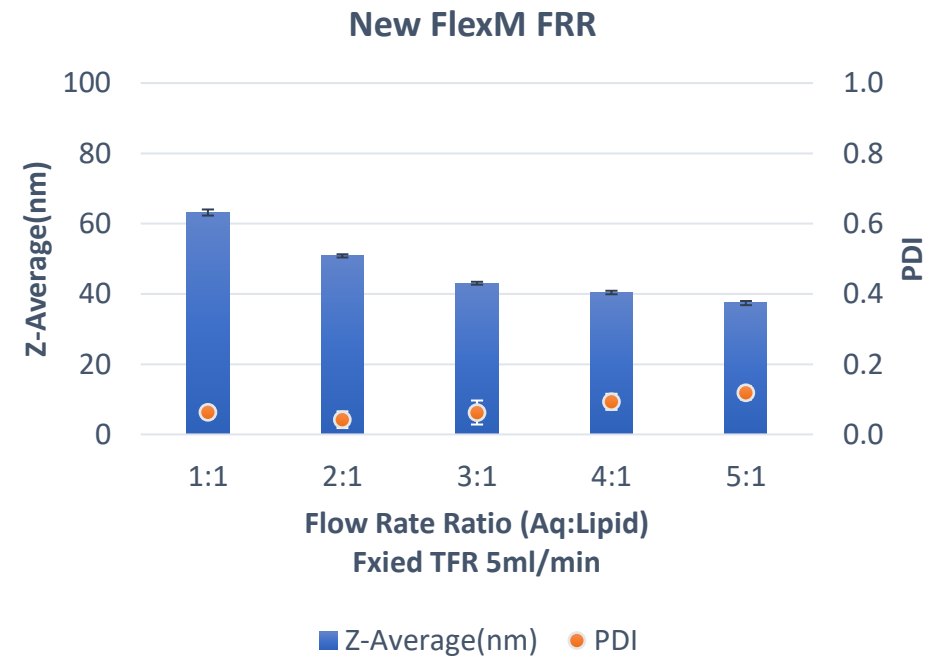
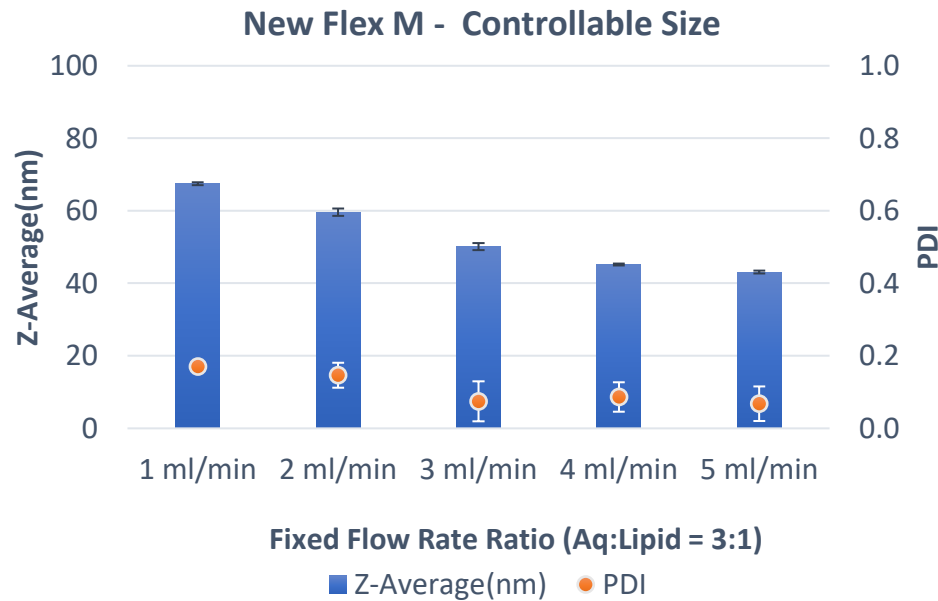


# NanoGenerator™ Flex M



NanoGenerator Flex (M)

- 1-12mL synthesis volume per batch
- Tunable total flow rate (TFR, 1-5 ml/min) and flow rate ratio (FRR, 2:1 to 5:1) in Flex-M



Model	Flex S/M
Aqueous phase	Sodium acetate buffer, 100mM, pH5.2
Solvent phase	Lipidflex, 15mM in ethanol

# Transferable results between Flex-S/-M

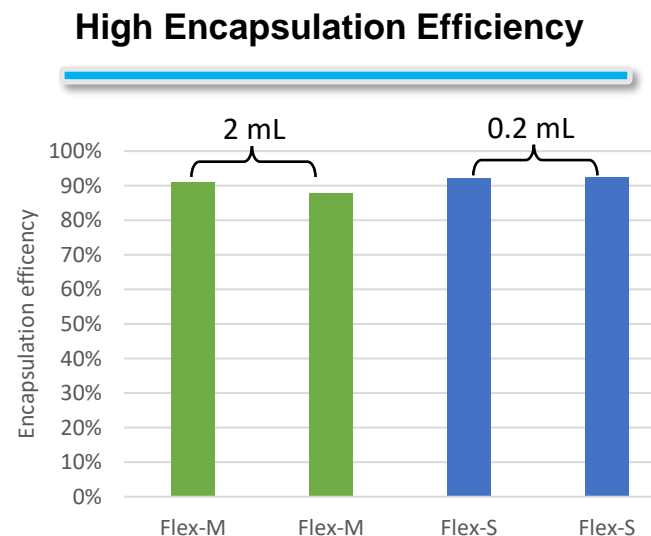
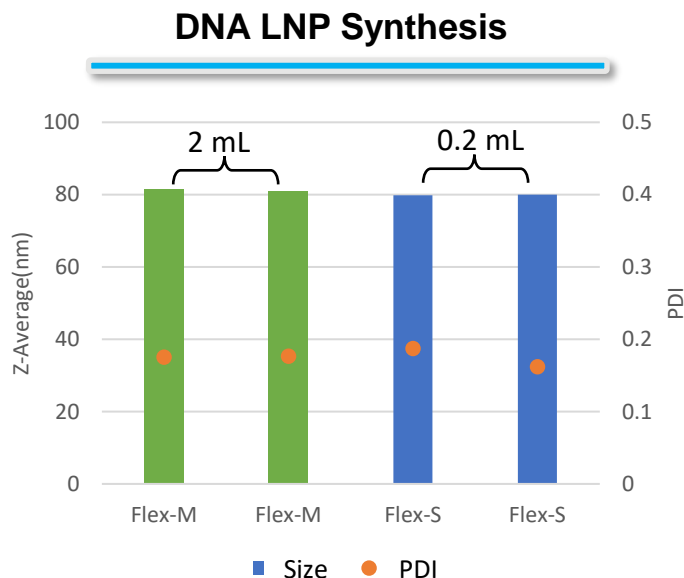
- The mixing chip (CHIP-MIX-4) is compatible for both Flex-S and Flex-M models.
- Customer can transfer their early screening results to later stage production seamlessly.



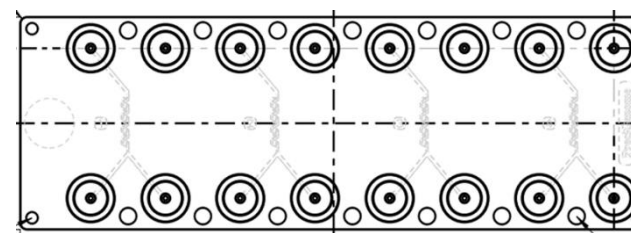
NanoGenerator Flex S



NanoGenerator Flex M



Model	Flex S/M
Aqueous phase	GFP DNA plasmid (100ug/mL) in sodium acetate buffer(100mM, pH5.2)
Solvent phase	SM102/Lipidflex (40/60 mol%, 12.5mM total lipid concentration) in ethanol
N/P ratio	6



CHIP-MIX-4

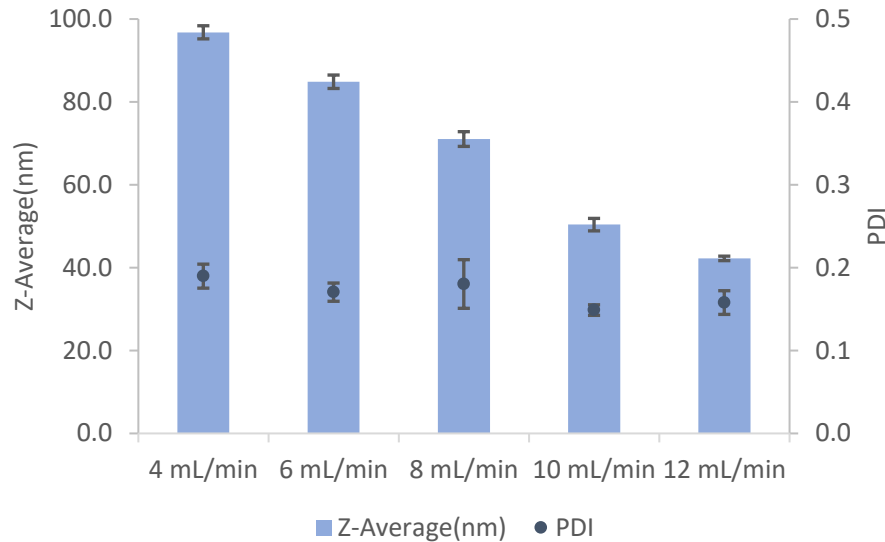
# NanoGenerator Pro

- More powerful pump, higher total flow rate.
- Mixing Chip: Mix-3
- Through put: 2-200 mL
- Total flow rate: 4-20 ml/min , Flow rate ratio (W:O): 2:1 to 5:1

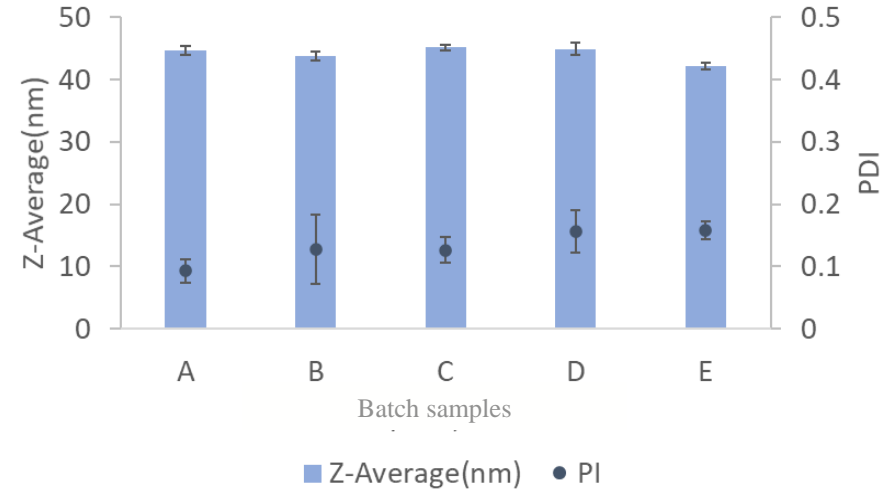


NanoGenerator Pro

## Tunable Size

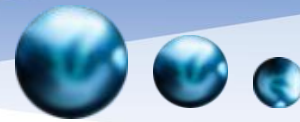


## Batch Consistency



Model	Flex M
Aqueous phase	PBS
Solvent phase	LipidDemo, 15mM in ethanol

# Simple Workflow



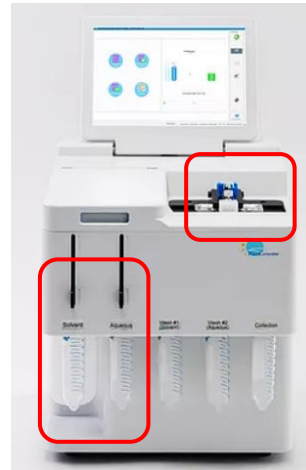
## Step 1: Preparation



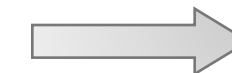
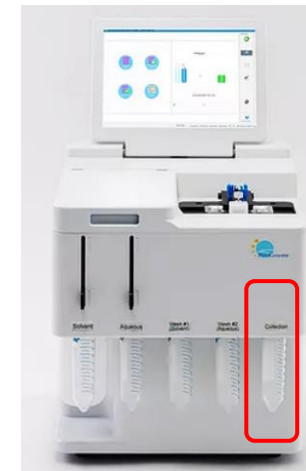
Aqueous: DNA, mRNA in buffer  
Solvent: lipid mix in ethanol  
(Lipid-Flex formulation)



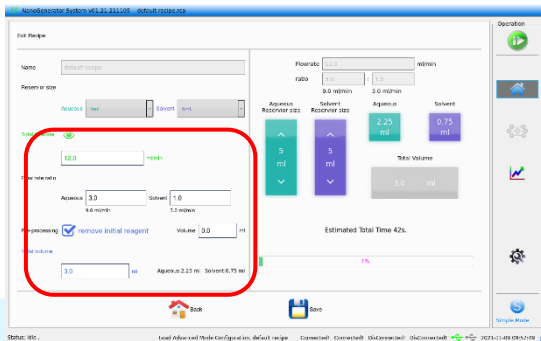
## Step 2: Load chip and sample tubes



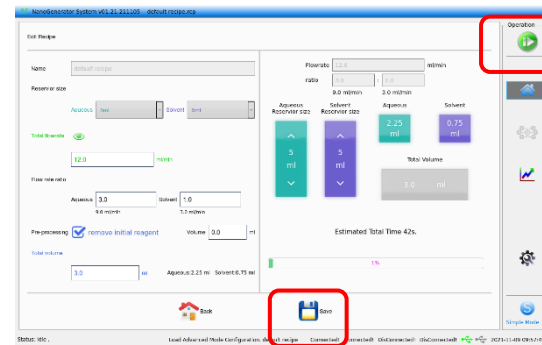
## Step 3: Load product tube



## Step 4: Set parameters or Load a saved recipe



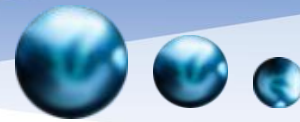
## Step 5: Run



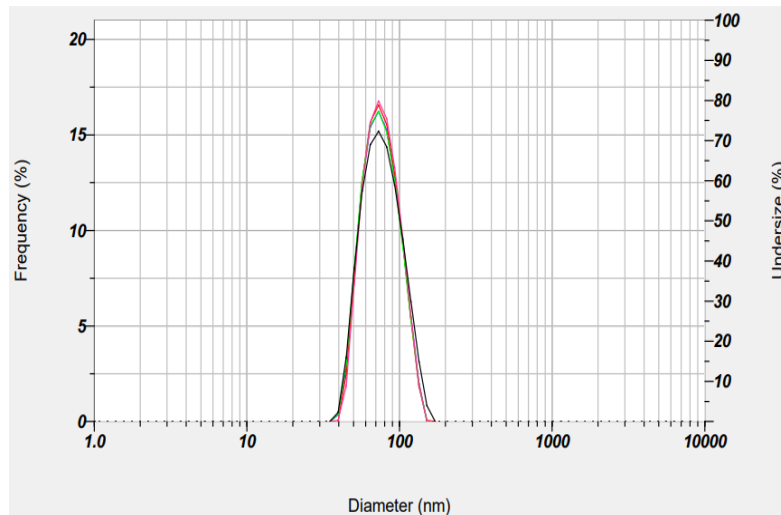
## Step 6: Collect LNPs in seconds



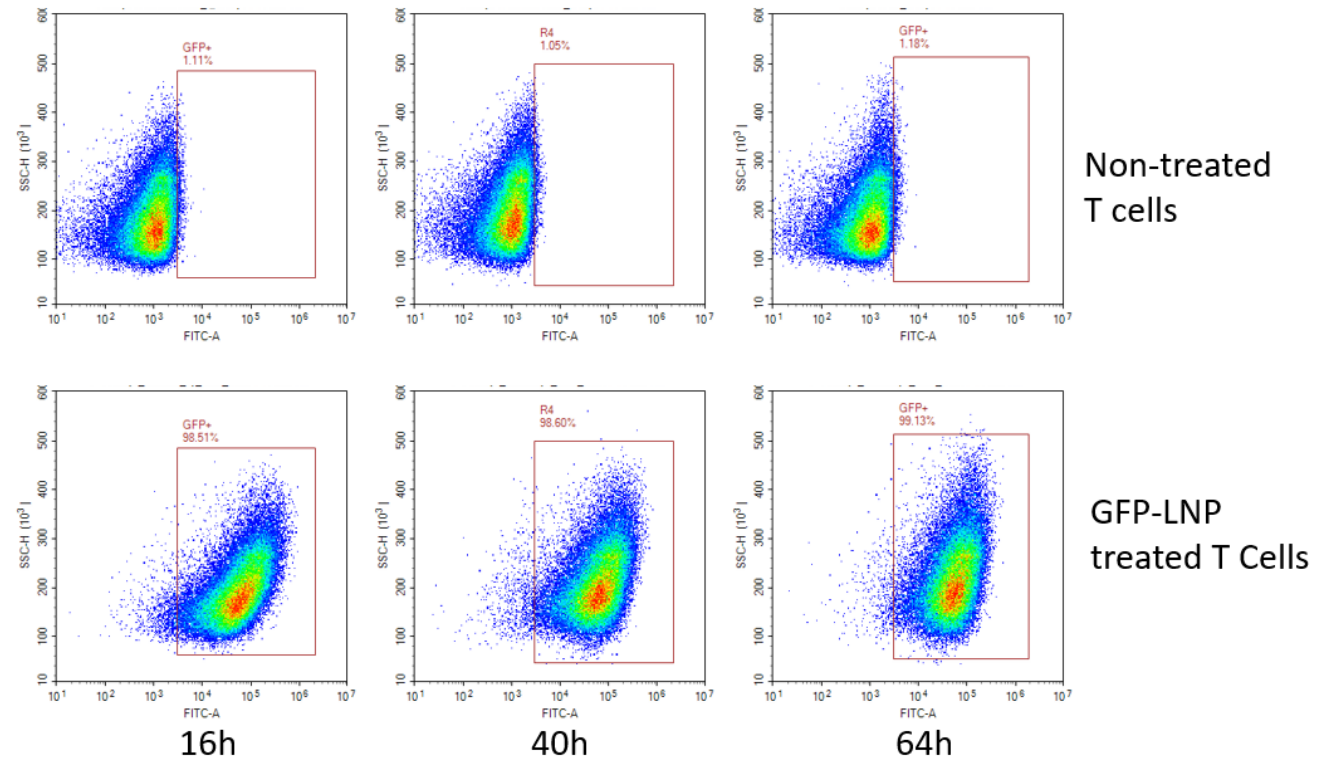
# Case Study: mRNA LNP for T cell Transfection



**eGFP mRNA Lipid Nanoparticles by Flex-S**  
Z-Average Diameter: 67.3 nm  
PDI: 0.106

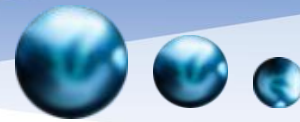


**Figure 1.** mRNA(eGFP)-LNP Synthesized by NanoGenerator Flex-S. Average diameter is 67.3 nm. PDI is 0.106. Encapsulation efficiency is 94.5% (Ribo Green RNA Quantification Kit).



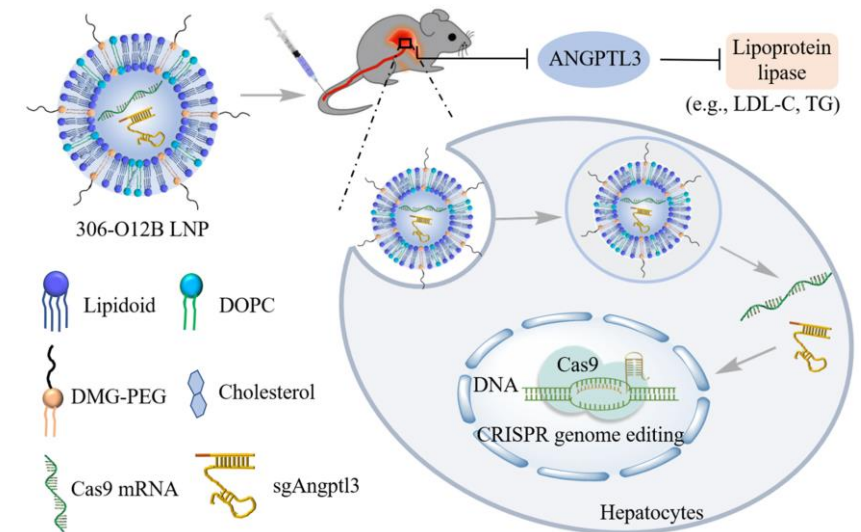
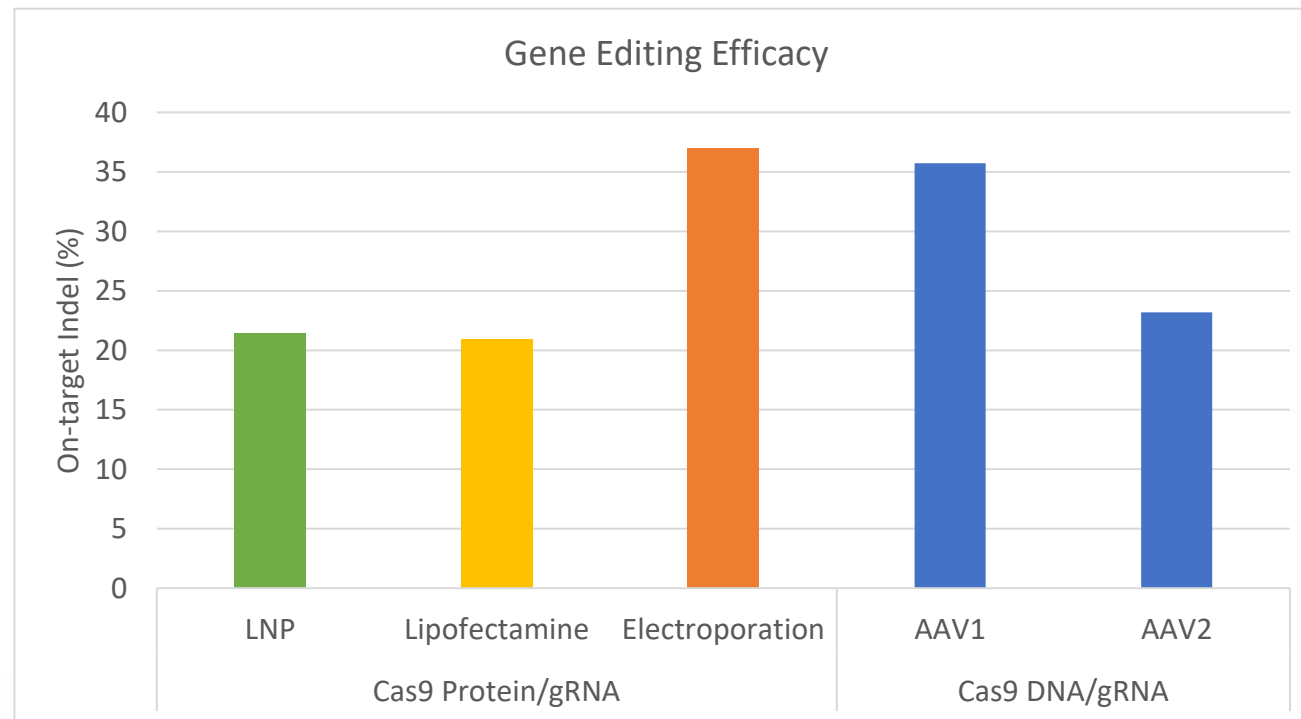
**Figure 2.** GFP(+) positive population of control (non-treat) and EGFP mRNA LNP treated primary T cells at 16, 40 and 64 hours. Cells were stained (1:50) using Biolegend 7-AAD Viability Staining for 10 minutes. Gating: First select for individual cells (excluding doublets). Then select for the healthy cell population. Then select for viable cells by excluding cells which are positive for 7-AAD. Gate for FitC-A channel (GFP)

# Case Study: LNP for Gene Editing



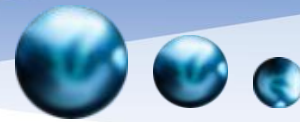
For *in-vitro* gene editing demonstration, Cas9 protein and guide RNA complex was encapsulated in lipid nanoparticle using NanoGenerator Flex-S. The size of resulted LNP was 135nm with a PDI 0.19.

HepG2 cells were treated using Cas9protein/gRNA LNP. Then the gene editing efficacy was determined through NGS.

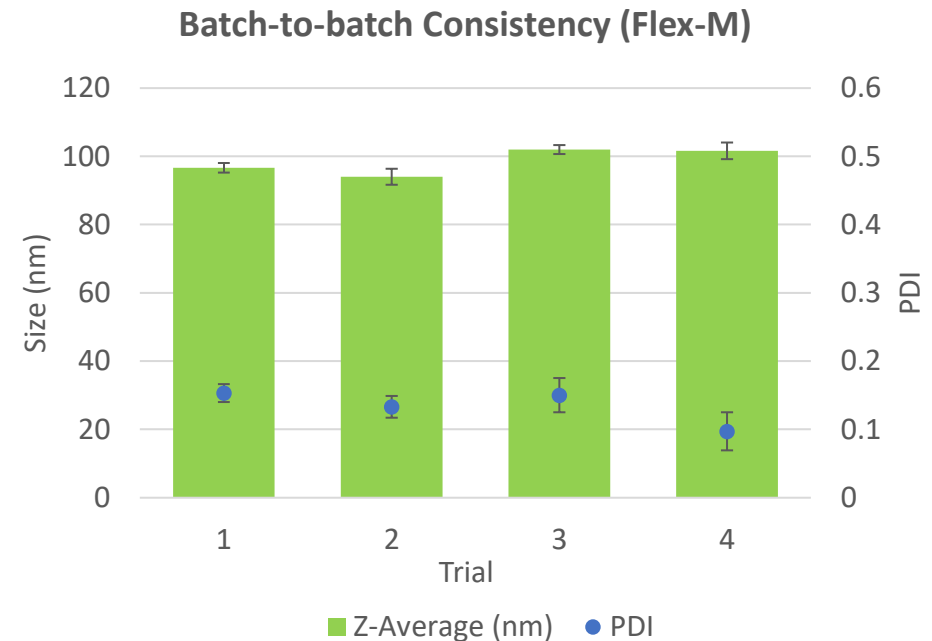
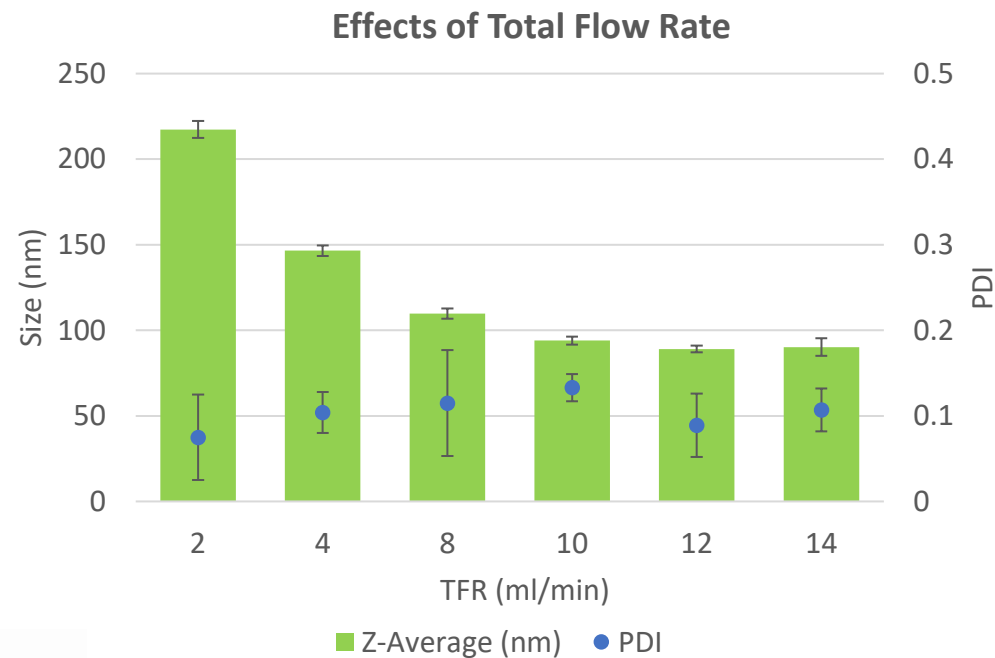


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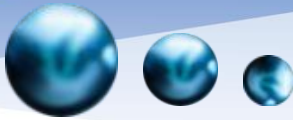
# Case Study: PLGA Nanoparticle Synthesis



- PreciGenome's NanoGenerator™ is used for the synthesis of a variety of nanoparticles, including PLGA (poly(lactic-co-glycolic acid)) nanoparticles.
- PLGA NP size tuning is controlled by the formulation parameters, the total flow rate and the flow rate ratio.



# Scalable LNP Production



NanoGenerator Flex-S



Early  
Screening

0.1 – 2 mL

NanoGenerator Flex-M



Small  
Production

1 – 12 mL

NanoGenerator Pro



Medium  
Production

2 - 200 mL

NanoGenerator Max



Commercial  
Production

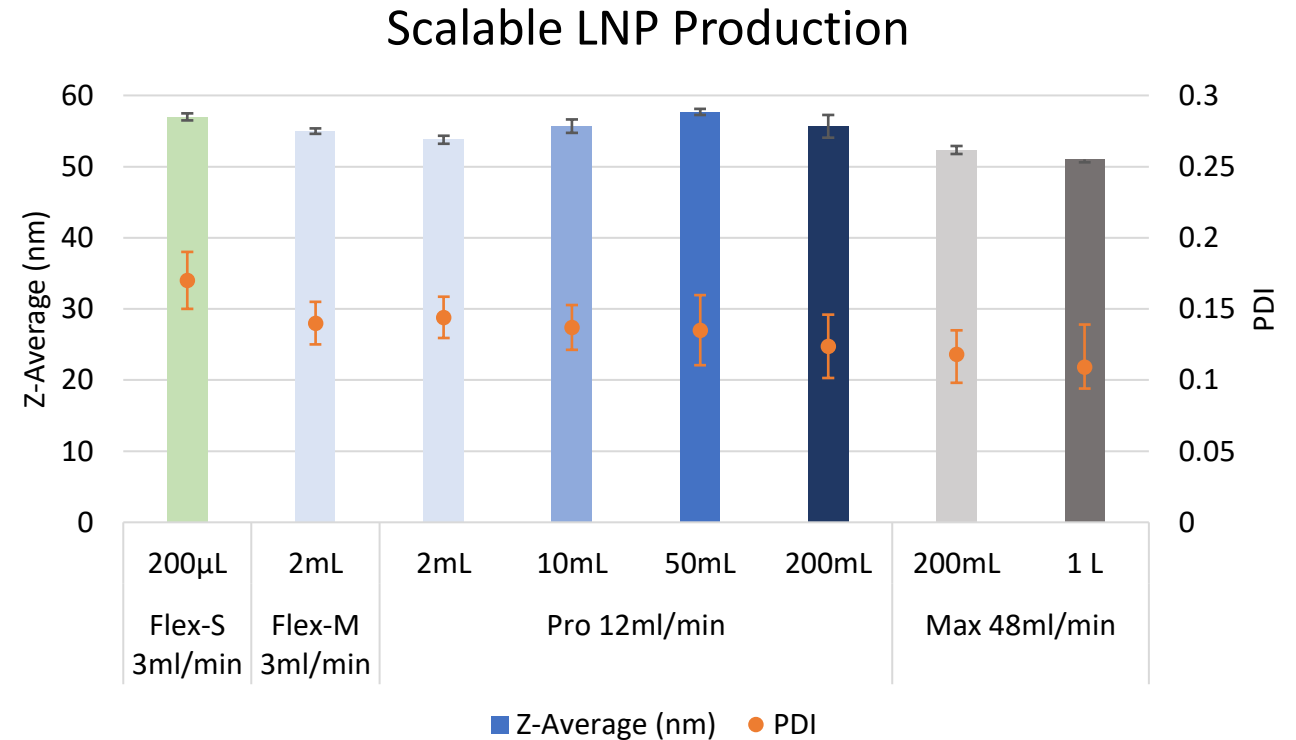
1 L



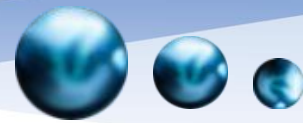


# Scalable LNP Production

- Transferable results from early screening (Flex-S, 0.2mL) to pre-clinical development (Pro, 200mL), then commercial production (Max, 1L)



Reagents	
Aqueous phase	Sodium acetate buffer (100mM, pH5.2)
Solvent phase	LipidFlex, 15mM in ethanol



## LipidFlex™

### Flexible Lipid Nanoparticle Formulation

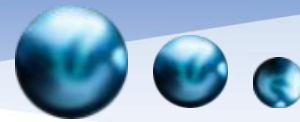
LipidFlex™ is a 3-component lipid nanoparticle formulation that compatible with various cationic/ionizable lipids for nucleic acid encapsulation and cell transfection. LipidFlex™ Pack kit includes ionizable lipid (SM102).

- Flexible cationic/ionizable lipid ratio
- Flexible with various N/P ratio
- High nucleic acid encapsulation efficiency
- High mammalian cell transfection rate

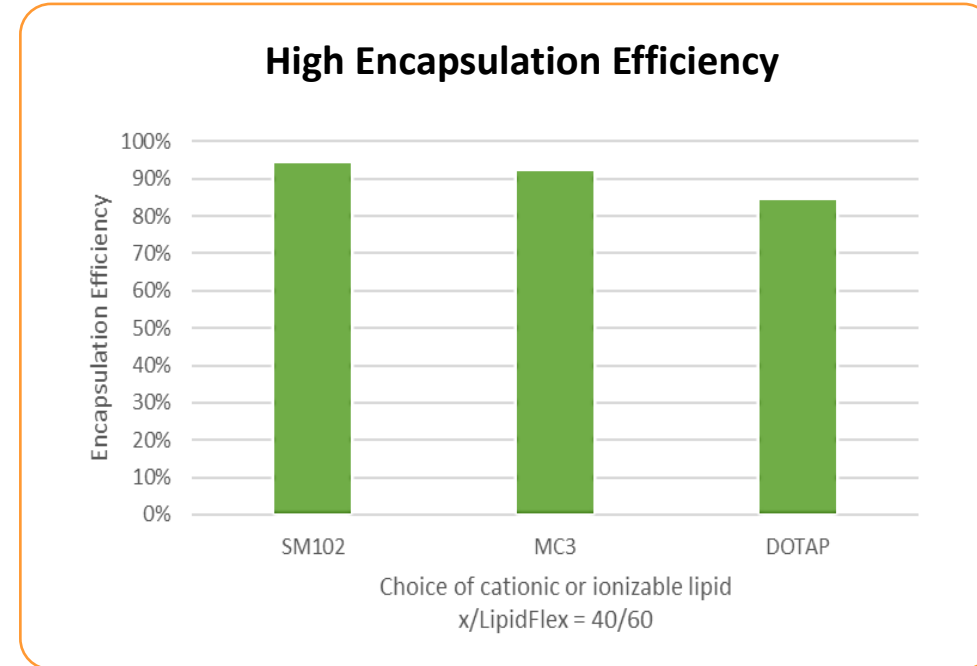
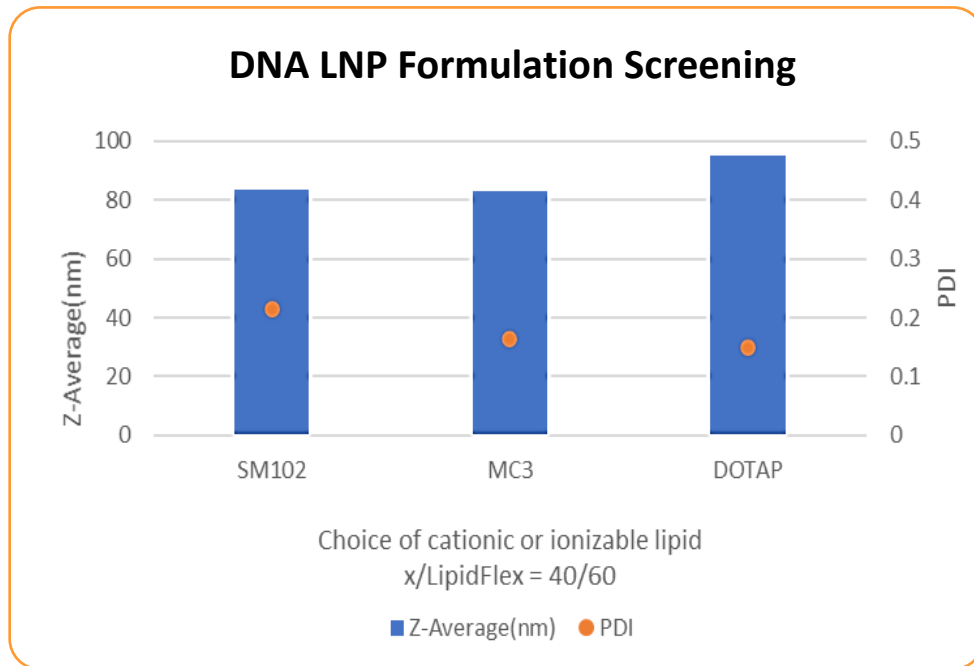


Model	LipidFlex™
Catalog #	PG-SYN-LF1ML
Components	Structural Lipid/ Cholesterol/Stabilizer
Product size	1000 µL
LipidFlex Conc.	30 mM
Ionizable lipid	NA

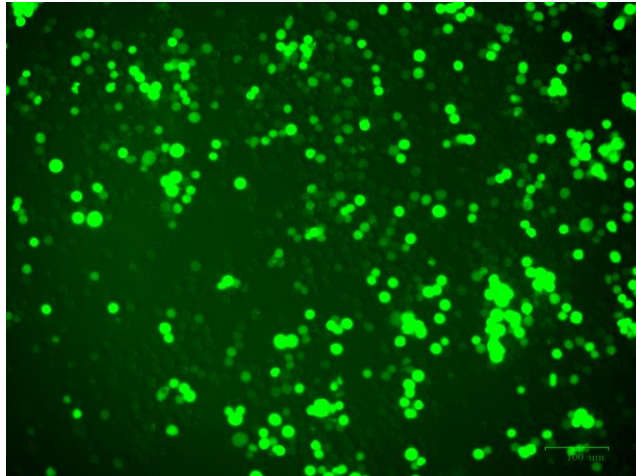
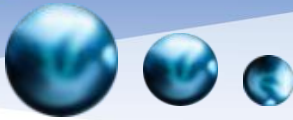
# LipidFlex – Flexible Starting Kit



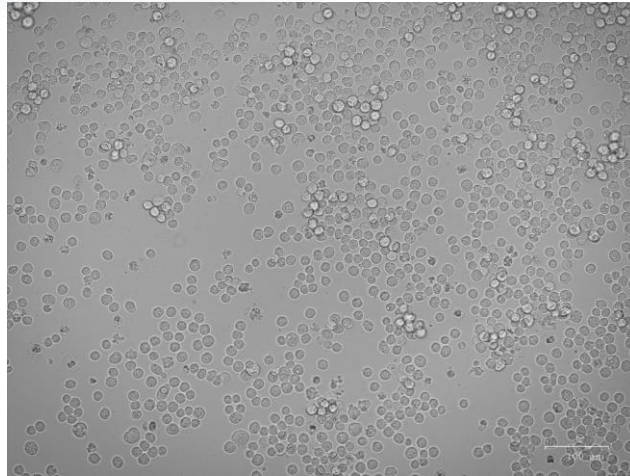
- PreciGenome provides a general LipidFlex formulation for quick formulation screening.
- By adding cationic/ionizable lipid into LipidFlex, customer can prepare nucleic acid LNP with high encapsulation efficiency.



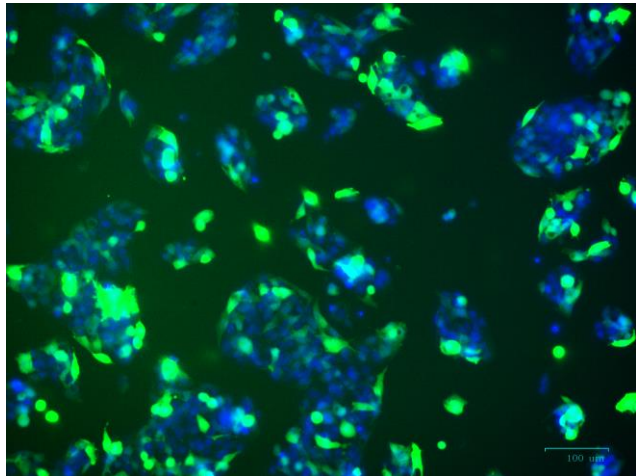
# LipidFlex LNP – Cell Transfection to Different Cell Lines



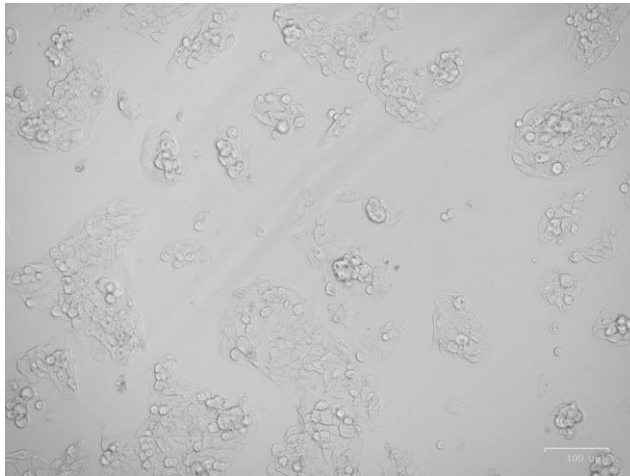
K562 – Green Fluorescence Field



K562 –Bright Field



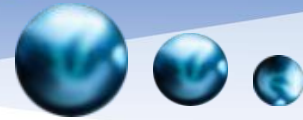
HepG2 – Green and Blue field overlay



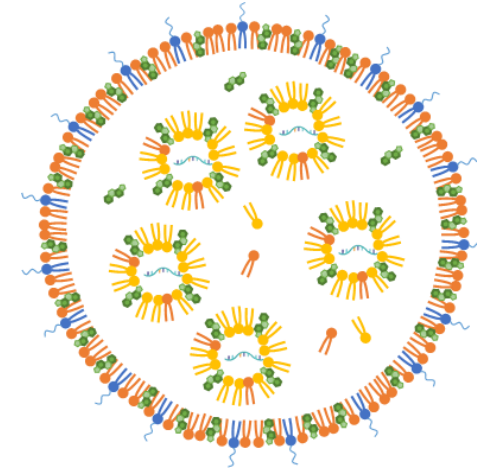
HepG2 –Bright Field

- DNA lipid nanoparticle (gWiz GFP plasmid, Aldervon) was generated using SM102/PG-LipidFlex (40/60 mol%) formulation by PreciGenome NanoGenerator.
- HepG2 and K562 Cell lines are successfully transfected by GFP DNA LNP. 48 hours post transfection, HepG2 Cell nucleuses are stained with Hoechst 33342 dye (blue color) before imaging.

# LipidFlex™ T Cell Kit

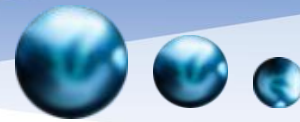


LipidFlex™ T cell kit is a highly efficient lipid formulation to synthesize mRNA lipid nanoparticles (LNP) for primary human T cell gene delivery. Using NanoGenerator™ Flex-S system and CHIP-MIX-4 cartridge, customers can prepare potent mRNA LNP in a convenient and efficient way.

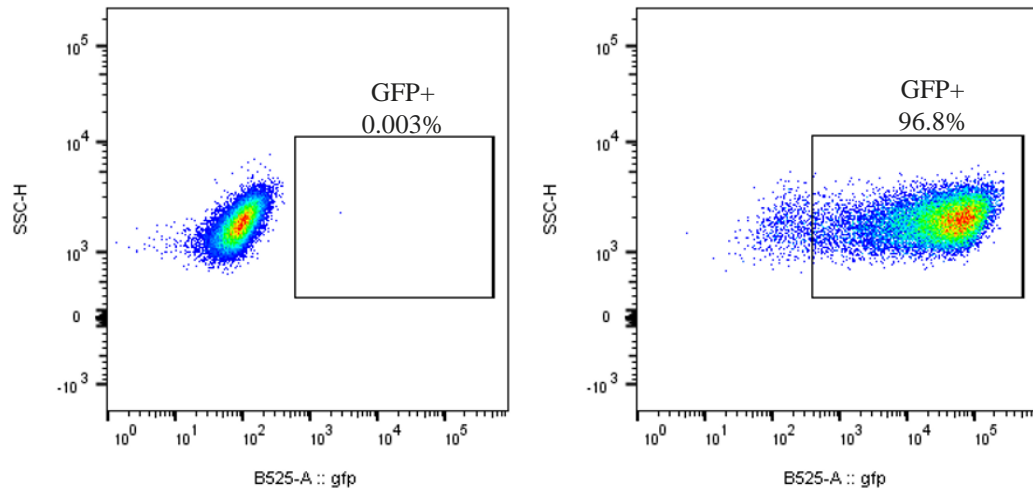


- Over 90% mRNA encapsulation efficiency
- High transfection efficiency
- High protein expression level
- High cell viability
- Time efficient synthesis process

Component	Size	Storage
LipidFlex T cell Lipid mix	125 µL	-80 °C
Formulation Buffer 1 (10x)	60 µL	4 - 8 °C
Formulation Buffer 2	600 µL	4 - 8 °C

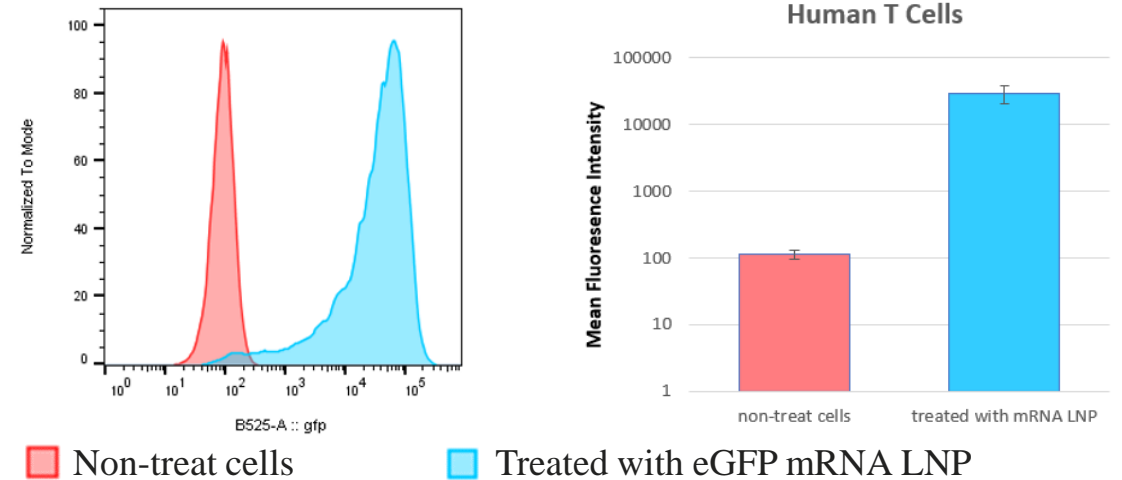


## High Human T Cell Transfection Efficiency

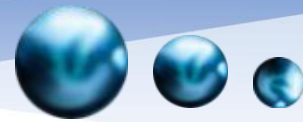


\* 24 hours post-treatment Human T cells (eGFP mRNA from Trilink)

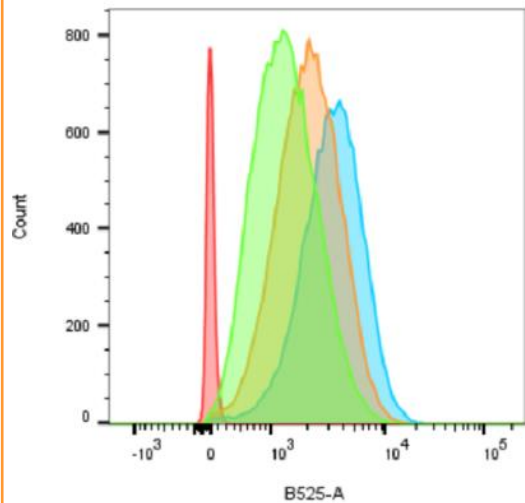
## High Protein Expression Level



\* 24 hours post-treatment Human T cells (eGFP mRNA from Trilink)

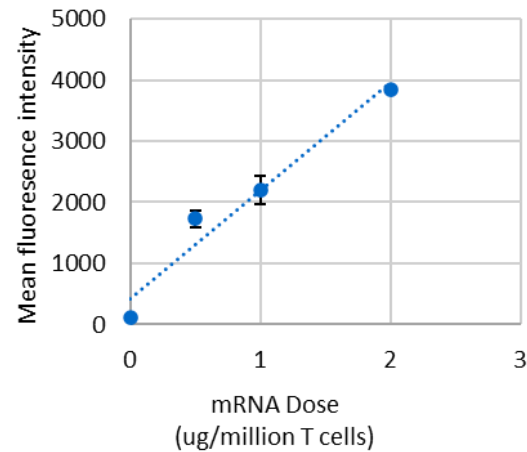


## mRNA LNP Dose Dependency

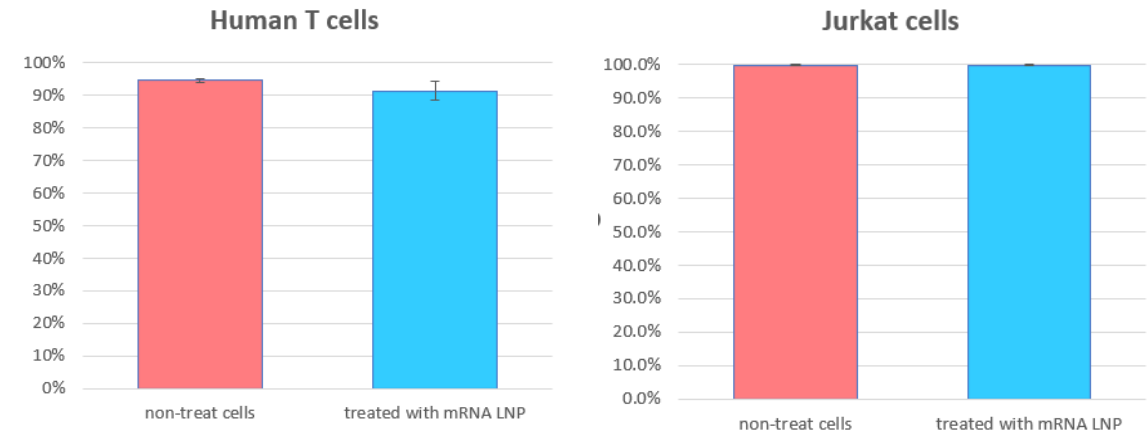


\* 24 hours post-treatment Jurkat cells (eGFP mRNA from ProMab)

## Dose Dependent of mRNA LNP



## High Cell Viability



\* 24 hours post-treatment Human T cells and Jurkat cells

# Why PreciGenome?



NanoGenerator Flex (S)



NanoGenerator Flex (M)



NanoGenerator Pro

## High Performance & Efficiency



- Tunable size (40-200nm)
- Low PDI (0.05-0.2)
- High encapsulation efficiency

## Open Platform



- Upgradable system
- Transferable microfluidic chips

## Scalable Throughput



- Low volume for screening (Flex-S)
- Medium volume production (Flex-M)
- High volume production (Pro, GMP-Max)

## Simple Operation



- Simple setup
- Compact size
- Intuitive UI w/ touchscreen

## Cost Effective



- Affordable configuration
- Lower cost per run

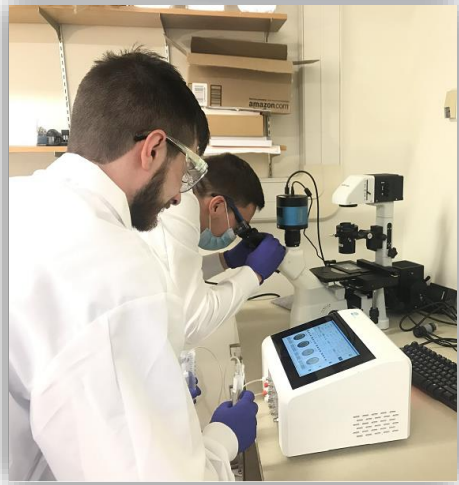
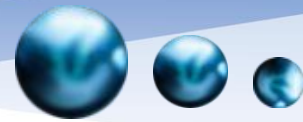
## Custom Support



- Demo, Training and Support
- Extended Warranty
- Hot swap option
- Local US company



# Some of Our Customers



## PreciGenome LLC

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