Scalable Manufacture of mRNA Lipid Nanoparticles using a Novel Microfluidic Mixing Architecture

Jagbir Singh, Gemma Ryan, Ariel Zhang, James Ko, Andre Wild, Aleksei Angell, Robert Young, Hui Yee Chua, Kevin Ou, Lloyd Jeffs, and Euan Ramsay

Precision NanoSystems Inc., Vancouver, BC, Canada

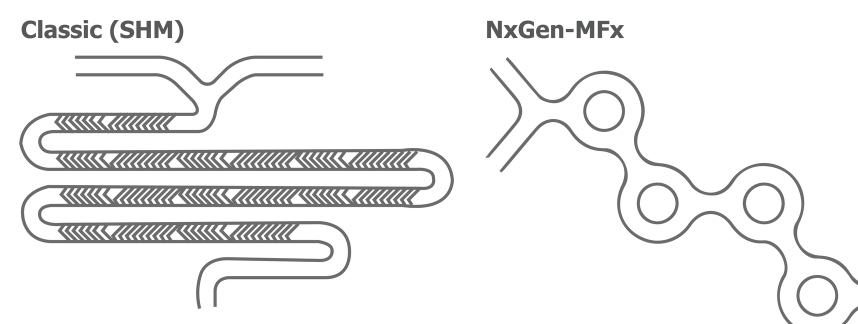
Purpose and Objectives

- The Staggered Herringbone Mixer (SHM, referred to here as "Classic") has successfully produced homogenous liposome and lipid nanoparticles (LNP) with defined particle sizes, narrowsize distributions, and high nucleic acid encapsulation efficiencies.
- PNI's new high capacity microfluidic architecture, the next generation mixer (demonstrated in 2 sizes: NxGen-MF60 and NxGen-MF80) will allow for higher throughput on a single chip, while preserving the precise and time invariant properties of the "classic" (SHM) mixer.
- We compare liposome characteristics between those formulated on the Classic, NxGen-MF60 and NxGenMF80 mixers using the Benchtop, Blaze or GMP systems.
- Classic and NxGen-MFx mixers were also compared for LNP formulations encapsulating either plamid or mRNA using size, PDI and encapsulation efficiency as metrics
- In vivo activity of mRNA LNPs prepared with classic and NxGen-MFx mixers were also compared

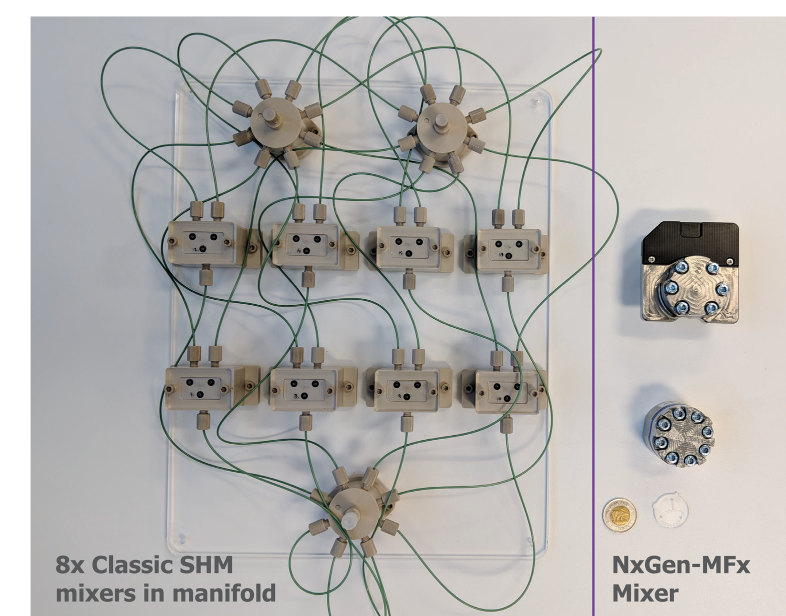
Methods Overview

NanoAssemblr® Systems

Mixer Geometries



SHM and Next Gen Mixer



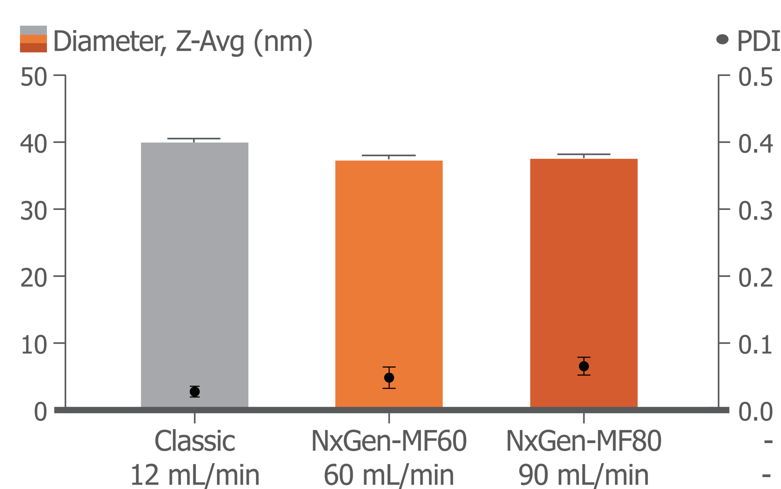
- Liposomes assembled from phosphatidylcholine lipid and cholesterol were formulated using Classic and NxGen-MFx Mixers using Benchtop Blaze and GMP Systems
- mRNA encoding luciferase was encapsulated in LNPs containing ionizable cationic lipid, helper lipids, cholesterol, and PEG-lipid that assemble into low-density lipoprotein-like particles that are taken up by receptor mediated endocytosis were formulated using Classic and NxGen-MFx Mixers across all systems.
- Liposomes and mRNA-LNPs across all systems were characterized by dynamic light scattering (DLS), Cryo-TEM, and Ribogreen or Picogreen Assay to determine physical characteristics, morphology, and nucleic acid encapsulation.

NxGen Mixer Achieves Similar Liposome Characteristics at 16x Throughput of Classic Mixer

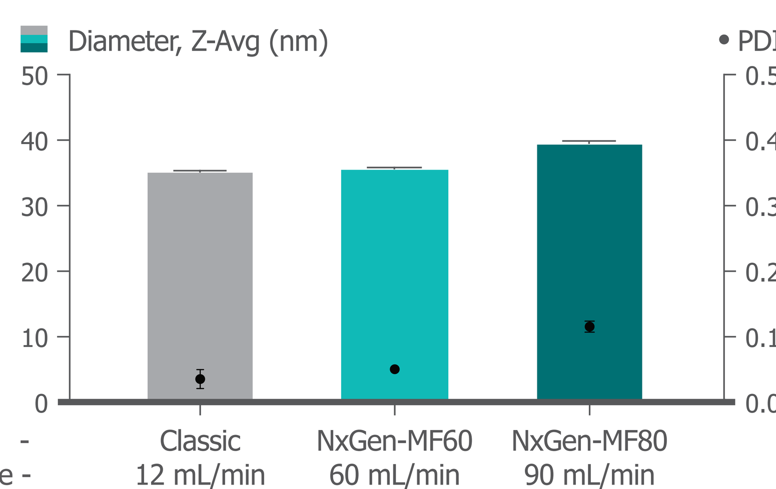
Dynamic Light Scattering (DLS) Comparing Size and PDI of Liposomes Made With Classic and NxGen Mixers

All samples were measured within ± 4 nm of 38.4 nm

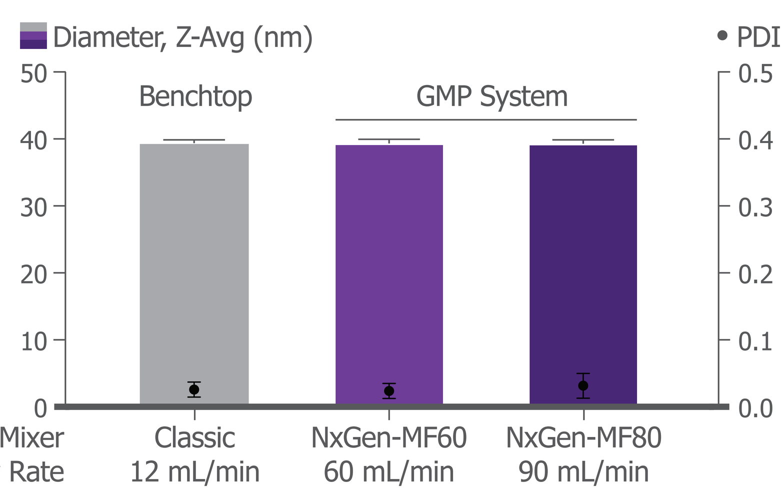
A **Formulated on Custom Bench-Scale Setup**



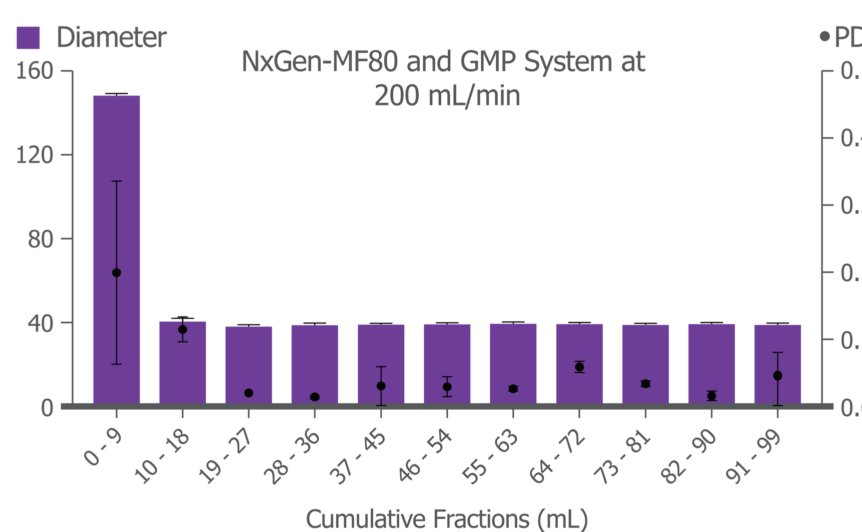
B **Formulated on Blaze (Continuous Flow)**



C **GMP System (Continuous Flow) vs Benchtop**



D **Consistent size and PDI rapidly achieved within 20 mL initial volume**



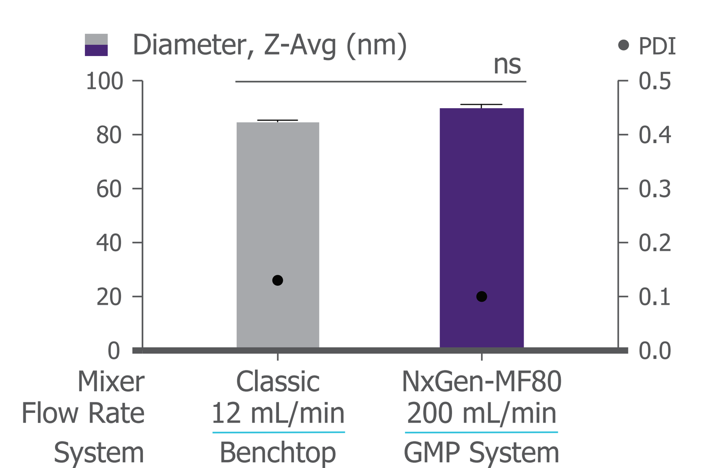
Further Details

Formulated POPC/Cholesterol liposomes were collected from Benchtop, Blaze, or GMP System then a 250 µL fraction was diluted four times in 1X PBS. DLS measurements were performed immediately after dilution. A & B) 2 measurements each of 3 samples. C) Data from Benchtop are mean of 2 measurements of one sample; GMP system data are the mean of 10 fractions collected between 19 mL and 99 mL of a batch, each measured twice. D) 2 measurements of a single sample per fraction.

NxGen Mixer Achieves Similar LNP Characteristics at 16x Throughput of Classic Mixer

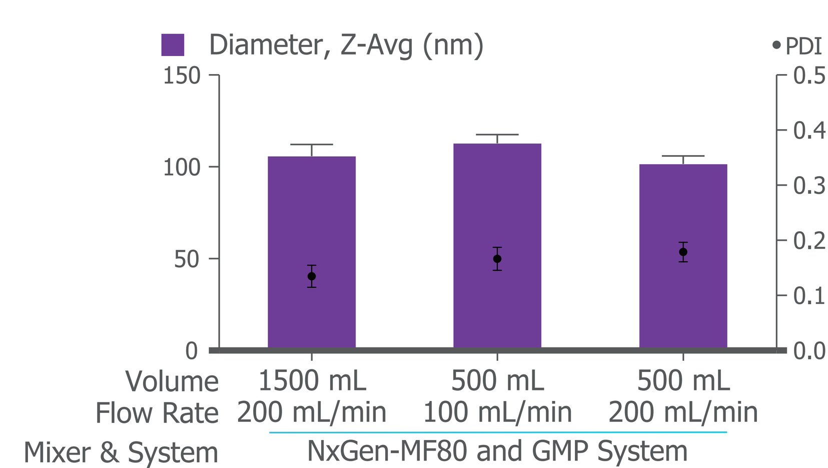
Dynamic Light Scattering of mRNA-LNPs Prepared with Classic and NxGen-MF80 Mixers

Mean hydrodynamic diameters were statistically equivalent



Dynamic Light Scatteringof pDNA-LNPs Indicate Robust Results With Various Flow Rates and Batch Volumes

Mean hydrodynamic diameters were within ±6 nm of 106.8 nm



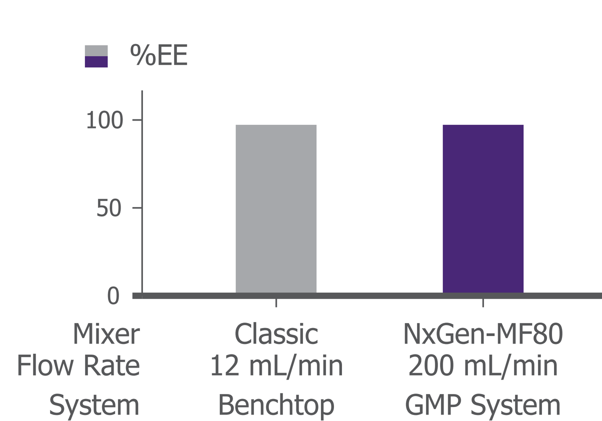
Further Details

mRNA data are the mean of 2 measurements of 1 sample. Significance ($p<0.05$) determined by student's t-test. pDNA data are the mean of 10 fractions from 19 to 99 mL each measured twice. Error bars are Standard Deviation

Encapsulation Efficiency of LNPs

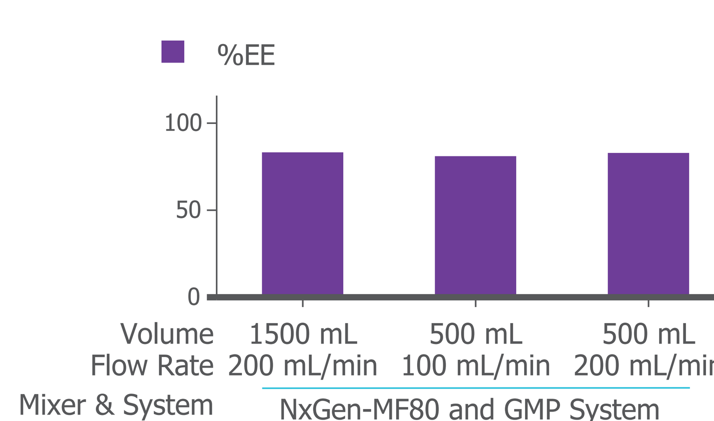
mRNA-LNPs

Encapsulation efficiency (>90%) was maintained across formulations on different mixers



pDNA-LNPs

Encapsulation efficiency was 83.2 ± 1.2% regardless of flow rate or volume



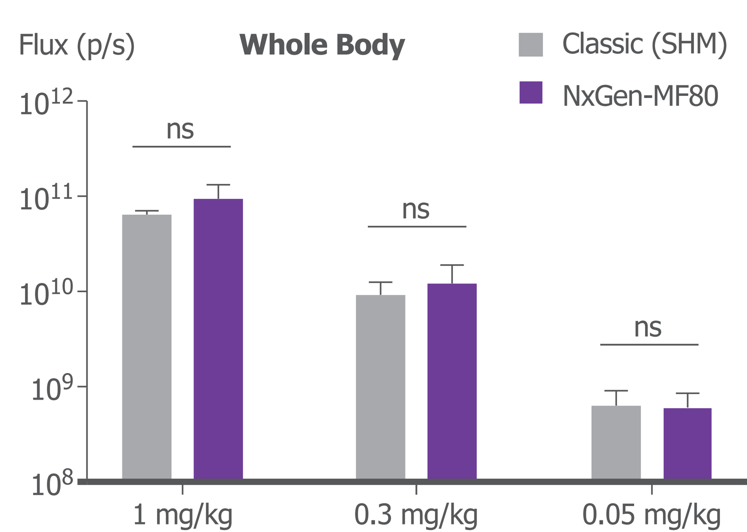
Further Details

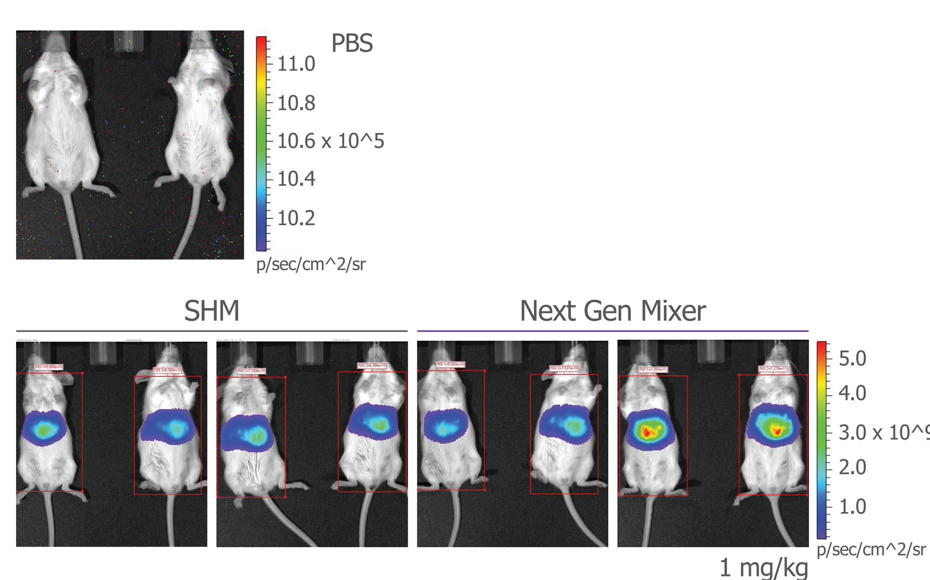
Encapsulation efficiency of mRNA and pDNA determined by modified Ribogreen and PicoGreen assay respectively. Data are mean of 2 measurements ± SD

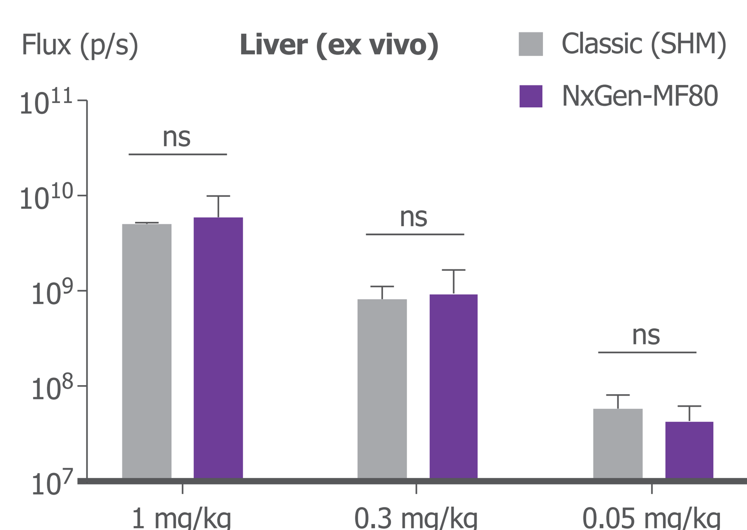
In Vivo Performance of mRNA-LNPs are Equivalent When Made with Classic or NxGen Mixers

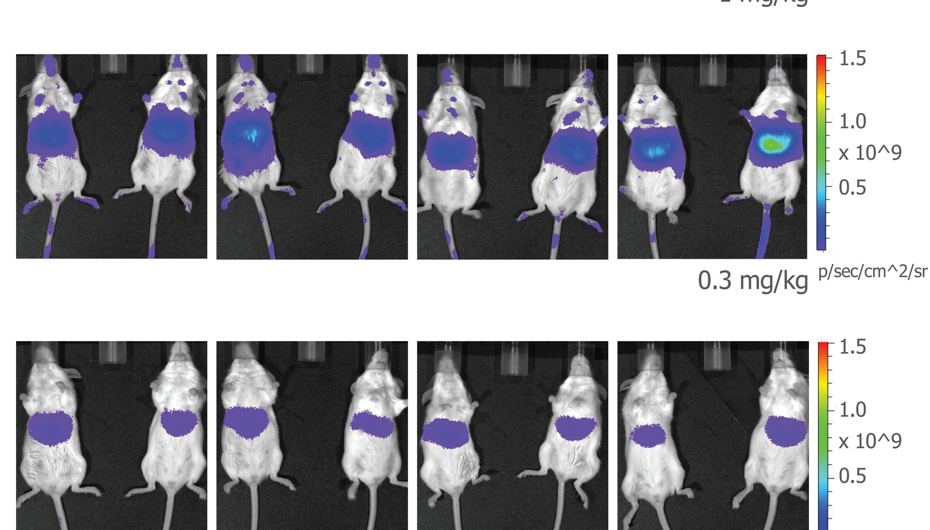
Luciferase Bioluminescence

No significant difference observed in gene expression from mRNA-LNPs formulated on SHM or Next Gen Mixers for three different doses





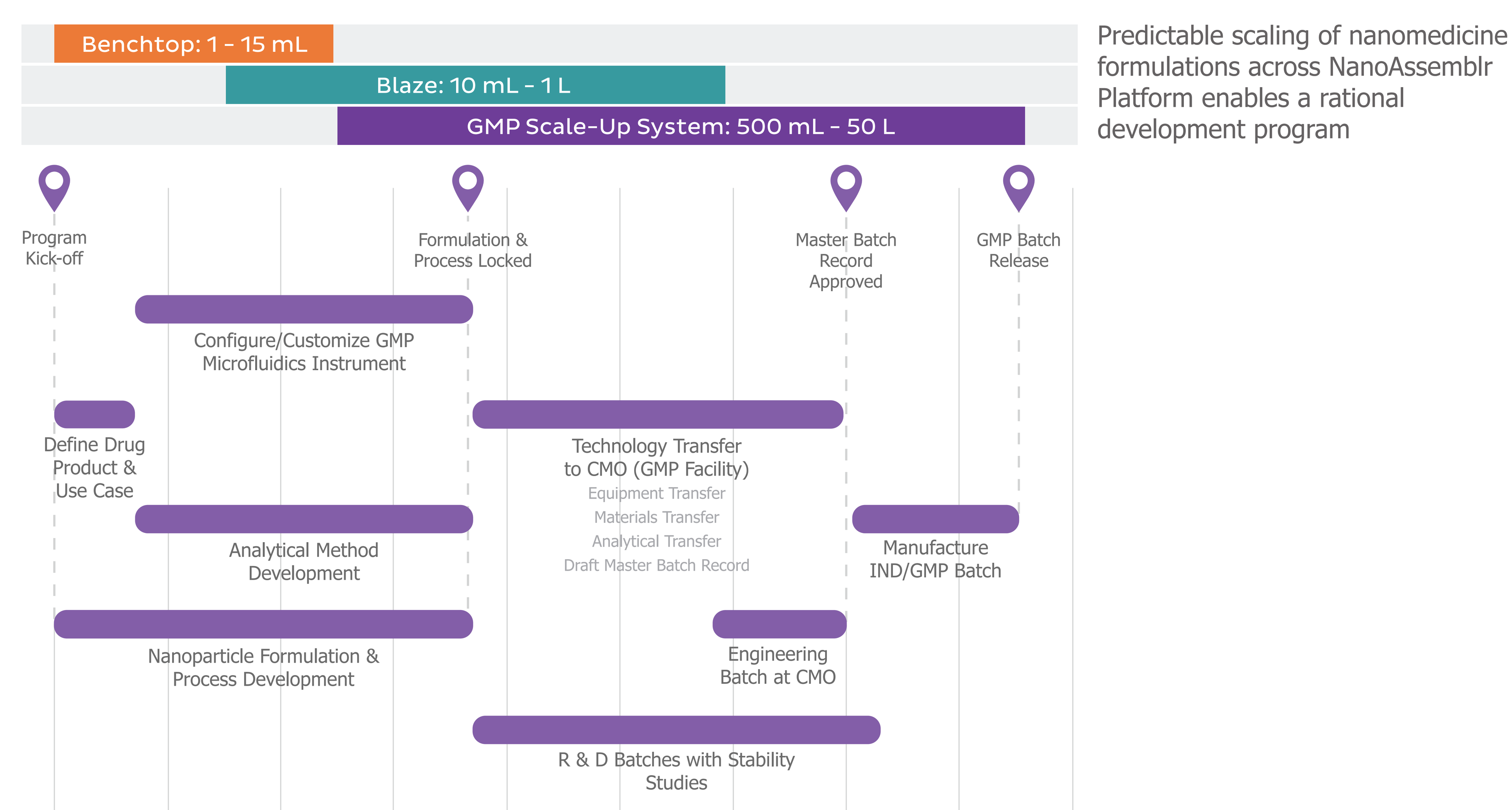




Further Details

LNPs loaded with luciferase mRNA were formulated using Classic or NxGen Mixers. Mice were injected IV with one of 3 doses (1 mg/kg, 0.3 mg/kg, 0.05 mg/kg) and 6 hours later, were injected IP with luciferin. Mice were imaged for bioluminescence as a reporter for gene expression. Significance ($p<0.05$) determined by unpaired t-test.

NanoAssemblr® Systems: Path to the Clinic



Predictable scaling of nanomedicine formulations across NanoAssemblr Platform enables a rational development program

Conclusions

- Sizing data shows consistent physical properties for liposomes formulated on SHM and Next Gen Mixer across the NanoAssemblr platforms as confirmed by DLS and Cryo-TEM (Available with Layar App).
- Sizing data shows consistent physical properties for pDNA-LNPs and mRNA-LNPs formulated on SHM or Next Gen Mixer as confirmed from DLS and Cryo-TEM (Available with Layar App).
- Encapsulation efficiency (>80%) was achieved for pDNA and mRNA and maintained across Classic and NxGen Mixer formulations as confirmed by Picogreen and Ribogreen assays.
- Statistically Equivalent gene expression of luciferase mRNA encapsulated in LNPs formulated on Classic and NxGen Mixers is demonstrated
- Overall, these results demonstrate the equivalence and seamless transfer of manufacturing liposomes and nucleic acid LNPs from Classic and NxGen Mixers, using multiple NanoAssemblr platforms Systems.

www.precisionnanosystems.com info@precision-nano.com /company/precision-nanosystems-inc @PrecisionNano

PRECISION NANOSYSTEMS
Create Transformative Medicines™