Microfluidic Optimization of DNA-Lipid Nanoparticles Targeting HPV18 E7: Impact on Physicochemical Properties and HeLa Cell Response

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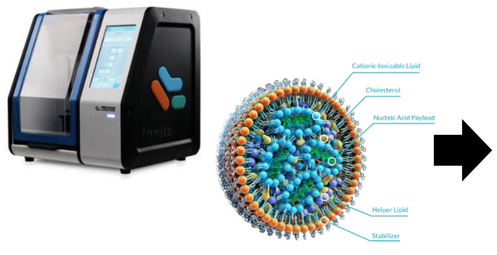
OVERVIEW

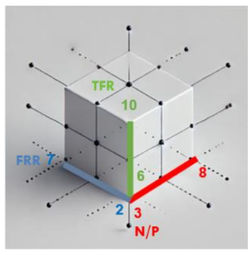
This work focused on optimizing the fabrication parameters of DNA lipid nanoparticles (DNA-LNPs) carrying an antiproliferative Polypurine Reverse Hoogsteen (PPRH) DNA sequence against the oncogenic protein E7 of Human papillomavirus type 18 (HPV18). Using a microfluidic system and a Central Composite Design (CCD), the effects of Flow Rate Ratio (FRR), Total Flow Rate (TFR), and N/P ratio (molar ratio between ionizable lipid nitrogens and DNA phosphates) were evaluated on particle size, zeta potential, polydispersity index (PDI), encapsulation efficiency, and cell proliferation. Experimental confirmation supported the model trends, with stronger predictability for physicochemical CQAs than for the biological response. The results demonstrate the value of DoE in developing efficient and reproducible DNA-LNPs for therapeutic use.

Microfluidics LNPs formulation with Ignite from Precision Nanosystems



Formulation optimization by Central Composite Design

Factors	Responses
	Size (nm)
FRR	PDI
TFR	%EE
N/P	%cell survival



INTRODUCTION

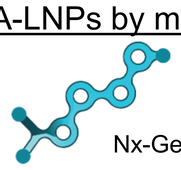
Human papillomavirus type 18 (HPV18) expresses the E7 oncoprotein, a key driver of cervical carcinogenesis through cell-cycle dysregulation and pRb inactivation. Microfluidic manufacturing can improve DNA–LNP reproducibility, but linking process parameters to critical quality attributes and biological performance remains challenging, especially for DNA cargos. Here, response-surface DoE was used to evaluate the impact of FRR, TFR and N:P ratio on size, PDI, encapsulation efficiency and HeLa cell viability in anti-HPV18 E7 PPRH-loaded DNA–LNPs, enabling identification of key factors and an optimized operating window.

METHODS

1. Formulation of DNA-LNPs by microfluidics

Aqueous phase (DNA in acidic buffer)

Organic phase (Lipids in ethanol)



DNA-LNPs

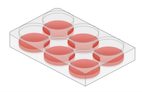
Nx-Gen

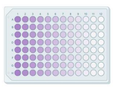
2. DNA-LNP morphology by TEM
3. Central Composite Design (CCD) to study:
   - Flow Rate Ratio (FRR)
   - Total Flow Rate (TFR)
   - N/P ratio (nitrogen-to-phosphate ratio)
4. Responses Measured
   - Particle size
   - Polydispersity index (PDI)

   - Encapsulation efficiency (%EE)

Fluorometry assay

   - Cell survival (MTT assay)



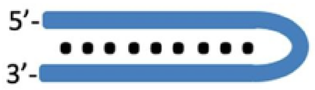


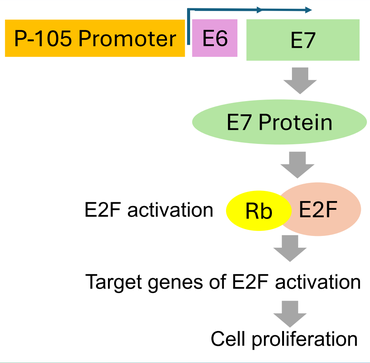
HeLa cell line

RESULTS

PPRH DNA sequence:

PolyPurine Reverse Hoogsteen hairpin, (PPRHs) against the protein E7 of the HPV18





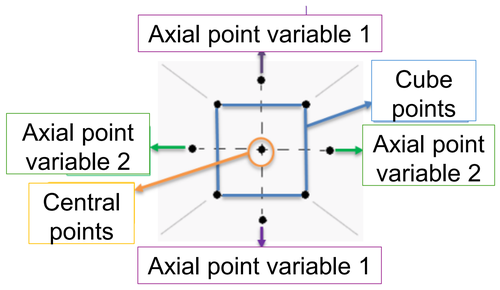
Central Composite Design (CCD)

Factors:

	Low	High
A: FRR	2:1	7:1
B: TFR	6	10
C: N/P	3	8

Cube points:	16
Center points in cube:	8
Axial points:	12
Center points in axial:	4

Runs: 20 | Replicates: 2 | α = 1.633 | Full factorial



Model results:

Responses	Significant parameters	R^2	Pred R^2
%EE	FRR×N/P, N/P, FRR²	60%	9%
Size (nm)	(N/P)², FRR², FRR, TFR²	75%	52%
PDI	N/P	44%	0%
Z-Pot (mV)	None	19%	0%

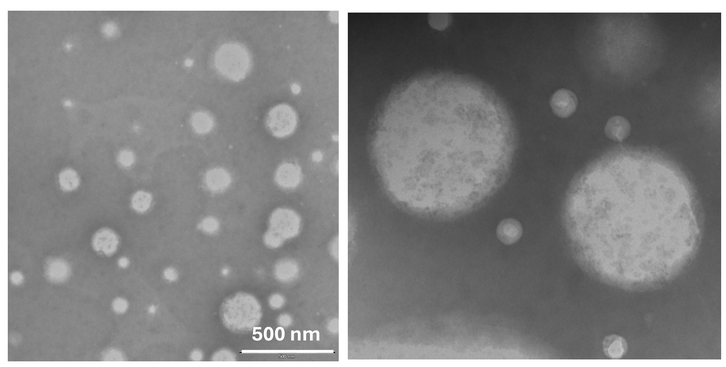
Optimization

Parameters	Response	Goal
	%cell survivol	Minimize
	Z-Pot (mV)	-2 to +2
	PDI	Minimize
	Size (nm)	60 to 85
Solution	%EE	Maximize

FRR	TFR	N/P	Composite desirability
4.21	4.73	4.80	0.245

	%cell survivol	Z-Pot (mV)	PDI	Size (nm)	%EE
Pred	63.2	-0.47	0.067	81.8	92.3
Exp	16.2	0.45	0.065	72.6	98.5
Bias	74.4%	4.3%	3.0%	11.2%	6.7%

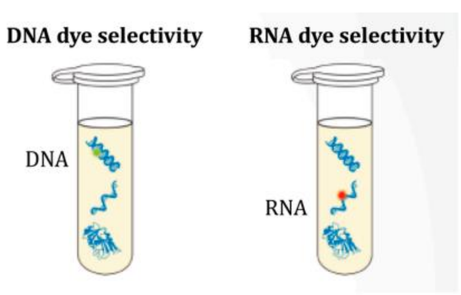
TEM:



CWA 18386: 2026 Determination of nucleic acid encapsulation efficiency in Lipid Nanoparticles using fluorometry

This method uses a two-step fluorometric approach to distinguish free from encapsulated nucleic acids. First, non-encapsulated nucleic acid is quantified in intact LNPs using a membrane-impermeable fluorescent dye. Then, total nucleic acid is measured after detergent-mediated LNP lysis, allowing %EE calculation as:

$$\%\,EE = \frac{\text{Total NA - Free NA}}{\text{Total NA}} \cdot 100\,\%$$



CONCLUSIONS

This study identified N/P ratio and FRR as key parameters influencing both the encapsulation efficiency and particle size—two crucial metrics in the development of gene delivery systems. While predictive accuracy varied across responses, the DoE approach proved effective for guiding formulation decisions. The combination of microfluidic fabrication and statistical modeling provides a robust framework for the rational design of therapeutic LNPs.

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