RNA 101:
IVT Workflows for GMP Manufacturing

Presenter:

Kenneth Warrington, Ph.D.
Executive Director of Strategic BD

Moderator:

Mingjuan Liu, Ph.D.
Director of Marketing

Logistics:

- You will be put on mute during the webinar
- We welcome you to ask questions using the “Q&A” on the top right corner of the platform
- Your questions will be addressed after the presentation

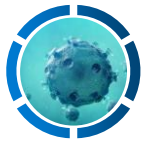
uBriGene's Global Manufacturing Platforms

Plasmids

- Plasmid construction
- Strain banking
- Plasmid PD & manufacturing

Cell therapy

- CAR-T
- CAR-NK
- MSC, iPSC, cell banking



Viral Vectors

- Adenovirus
- AAV
- LVV
- OV

Gene Editing

- IVT-sgRNA
- Nuclease
- RNP PD

RNA-LNP

- mRNA
- circRNA, saRNA
- sgRNA

QC release testing

- Strength
- Safety
- Identity
- Potency

Global GMP Sites Overview

Jinan, China GMP Facility

- ✓ >50,000 sqft
- ✓ Plasmid production lines (5L-200L)
- ✓ Mammalian culture production lines (10L-200L)

Suzhou, China GMP Facility

- ✓ >90,000 sqft
- ✓ Plasmid production lines (5L-200L)
- ✓ Mammalian culture production lines (10L-200L, 2000L for AAV)

Guangzhou, China GMP Facility

- ✓ >50,000 sqft
- ✓ Cell therapy excellence center (B+A)
- ✓ Automated process
- ✓ Robust Assay dev and QC capabilities

Maryland, USA, GMP Facility

- ✓ >10,000 sqft
- ✓ Four GMP cleanrooms to support cell therapy and viral vector enabling technologies programs.
- ✓ Comprehensive QA and QC capabilities including full-scope testing of final products.

- ✓ Grade C+A / B+A cleanroom standards
- ✓ Independent HVAC systems for each suite
- ✓ Monitoring room with BMS and EMS system

- ✓ Total of **6** plasmid, **9** viral vector, and **16** cell production lines (including cell banks).

RNA technologies under development

sgRNA

- A short RNA molecule guides a Cas9 enzyme to a specific DNA sequence.
- Consists of a 20-nucleotide sequence specific guide sequence and a scaffold sequence.
- The scaffold sequence interacts with the Cas9 enzyme, activating it.
- The activated Cas9 enzyme cuts the DNA at the target site.
- Precisely modifies genes.

mRNA

- Synthetic mRNA is designed to encode a specific protein.
- Delivered to cells using a delivery system (e.g., lipid nanoparticles).
- Can target a wide range of diseases.
- mRNA-based therapies can be produced quickly.
- Well-tolerated with a low risk of long-term side effects.

circRNA

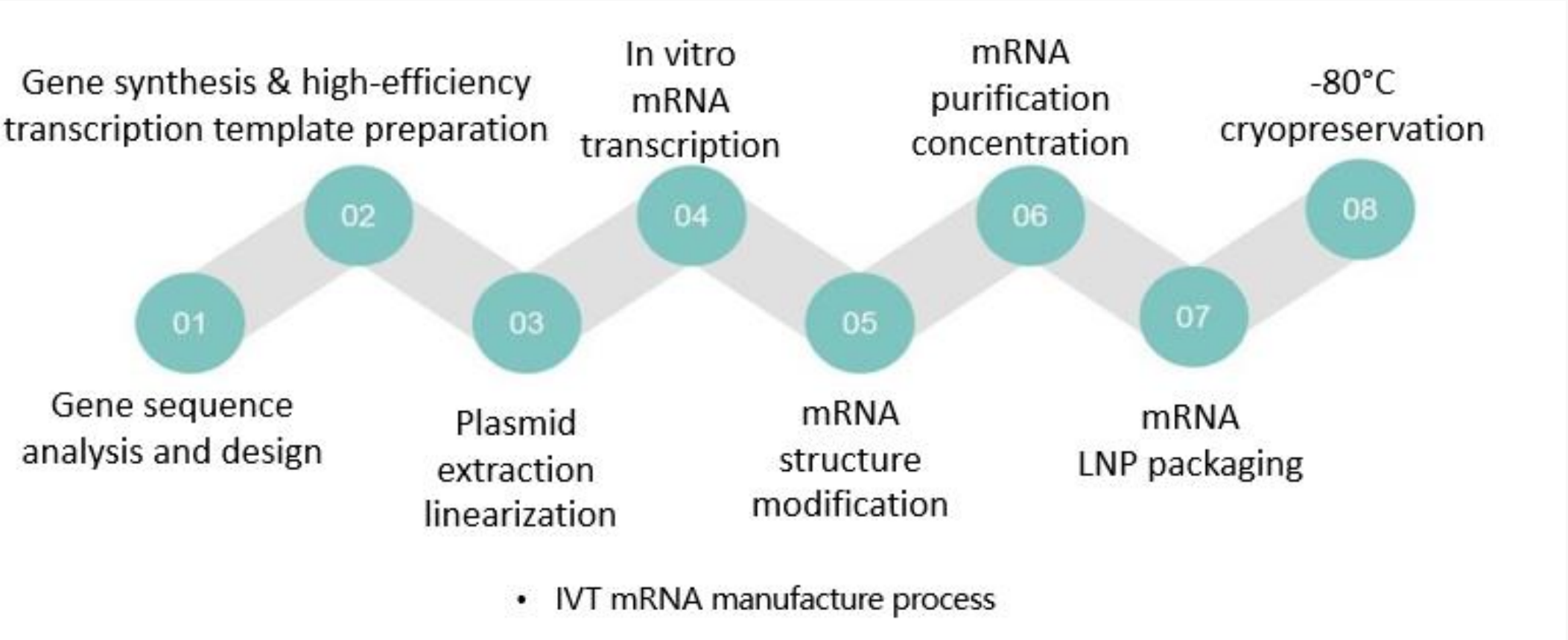
- circRNA is a class of RNA molecules that form a circular loop, lacking free 5' and 3' ends.
- Generated through alternative splicing, where mRNA is circularized before degradation.
- Acts as miRNA sponges to sequester and inhibit miRNAs.
- Serves as a scaffold platform for protein interactions.
- Modulates gene transcription and translation.
- Identifies circRNAs associated with specific diseases.
- Uses circRNAs as delivery vehicles for therapeutic molecules.

saRNA

- saRNA contains a self-replicating element that allows it to replicate within cells, significantly increasing its abundance.
- Amplified saRNA serves as a template for producing multiple copies of the desired protein.
- Delivers therapeutic genes to cells for treating genetic disorders.
- Produces larger quantities of therapeutic proteins and vaccine antigens.
- saRNA can persist within cells for a longer duration.

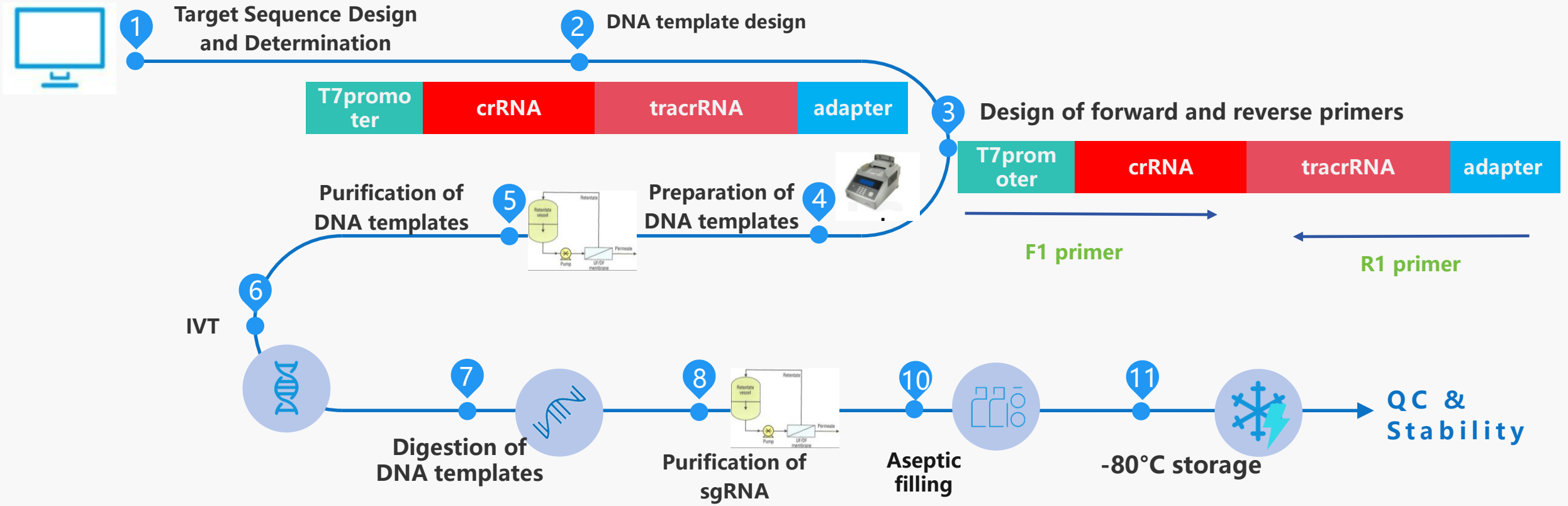
uBriGene IVT-based RNA platforms

We have established a comprehensive RNA process platform to provide one-stop service for RNA synthesis. This includes **gene sequence analysis and design, high-efficiency in vitro transcription template preparation, plasmid linearization, IVT RNA synthesis, purification, quality inspection and LNP packaging**, including mRNA for prefabricated and customized products, to promote the commercialization of mRNA drugs.



- IVT mRNA manufacture process

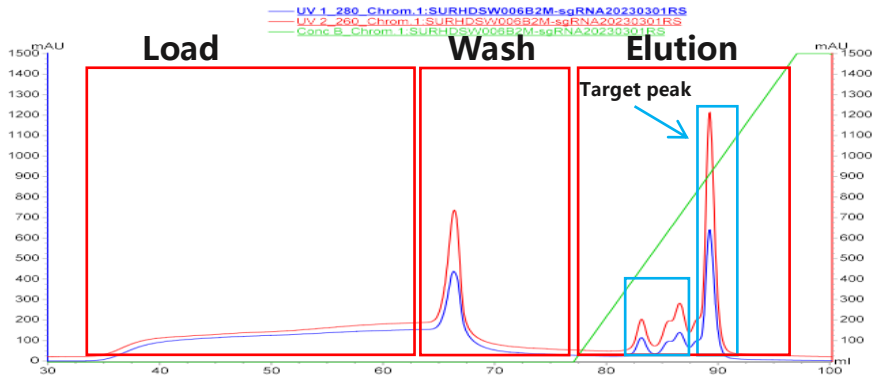
sgRNA IVT technology



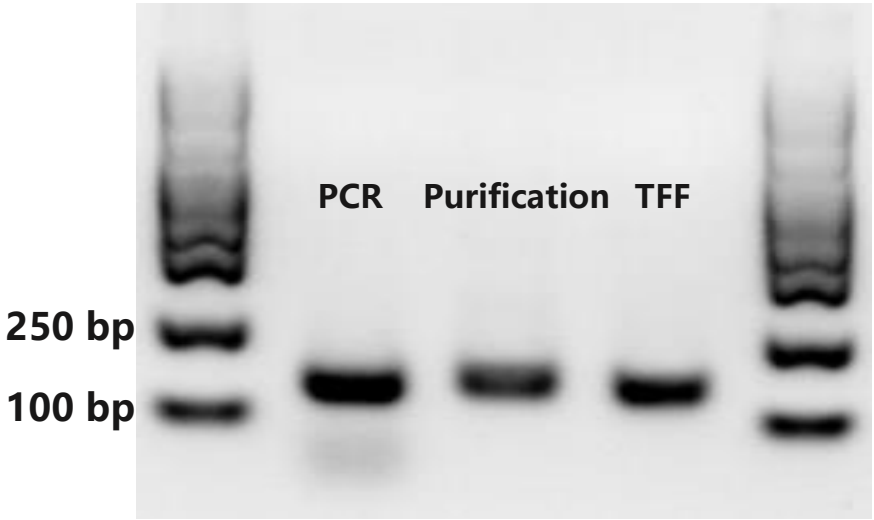
DNA template preparation

- The template DNA turnover time is only two days
- High purity of DNA template is critical for the yield and quality of IVT sgRNA

Name`	PCR	Purification	TFF
	Reaction volumes (μl)	Quantity (μg)	Quantity (μg)
DNA template	4000	304	272



Chromatographic purification of DNA templates

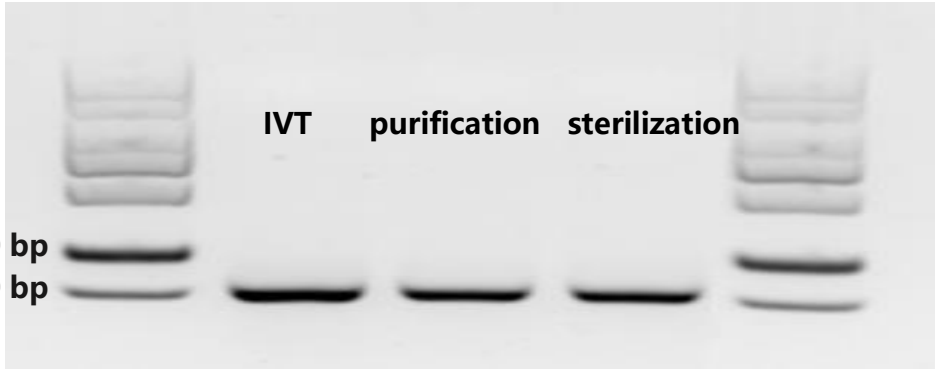


Detection of DNA templates purity by gel electrophoresis

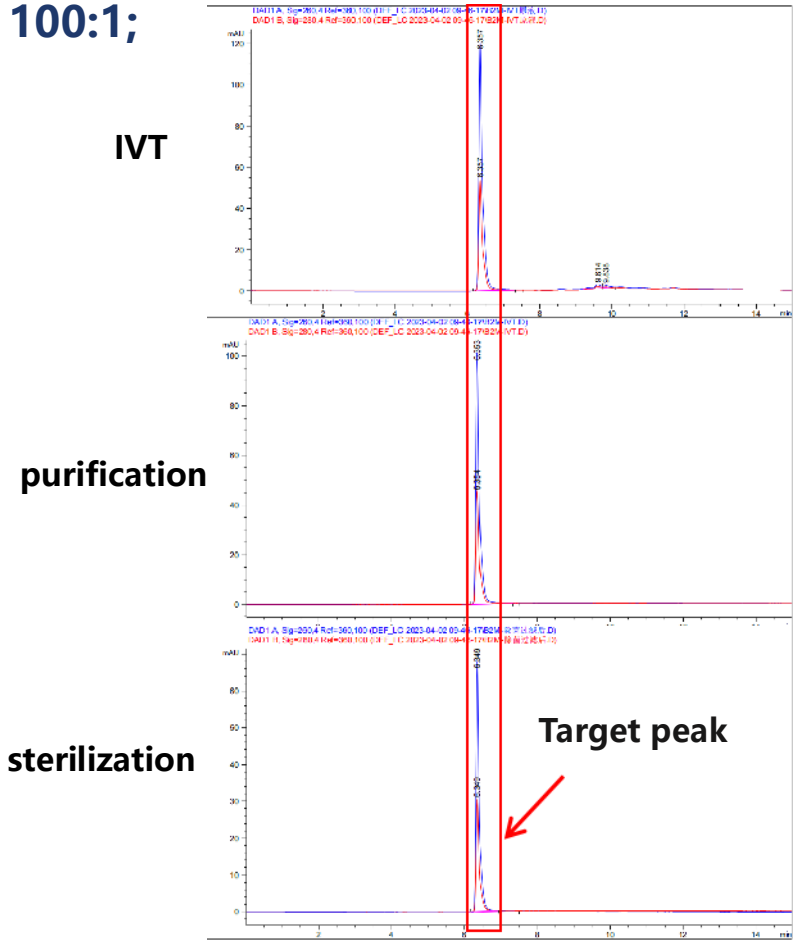
sgRNA IVT and purification

- The sgRNA:DNA mass ratio in the IVT reaction system reaches 100:1;
- sgRNA purity in DS is 98.8%

IVT	purification	sterilization
Reaction volume (μl)	Quality (μg)	Quality (μg)
2000	10880.26	9383.87

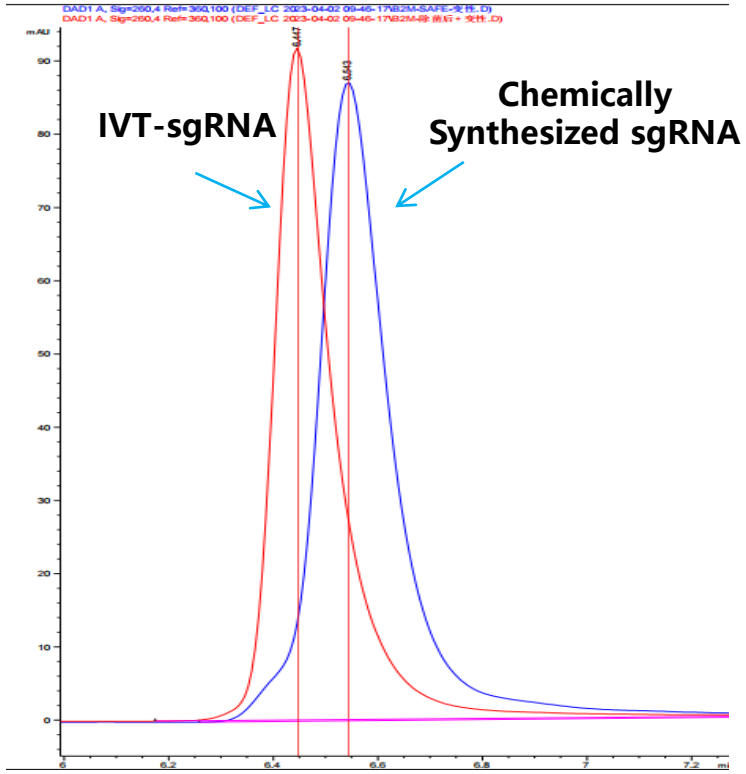


Detection of sgRNA purity by gel electrophoresis



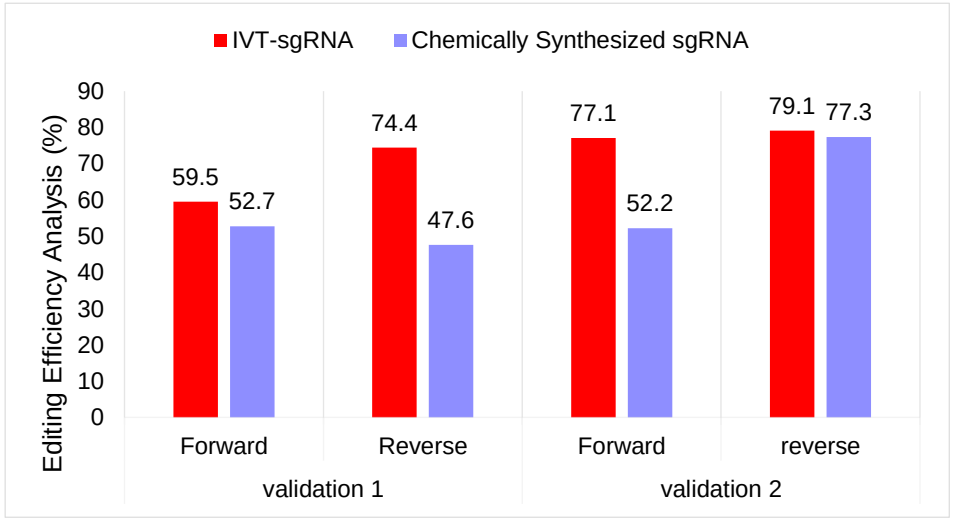
In process Purity testing of sgRNA by HPLC

Purity and potency comparison



Purity of sgRNA by HPLC

Group	sgRNA	IVT-sgRNA		Chemically Synthesized sgRNA	
Editing Efficiency Analysis Tide (%)	Sequencing Direction	Forward	Reverse	Forward	reverse
	validation 1	59.5	74.4	52.7	47.6
	validation 2	77.1	79.1	52.2	77.3



Comparison of intracellular gene editing efficiency

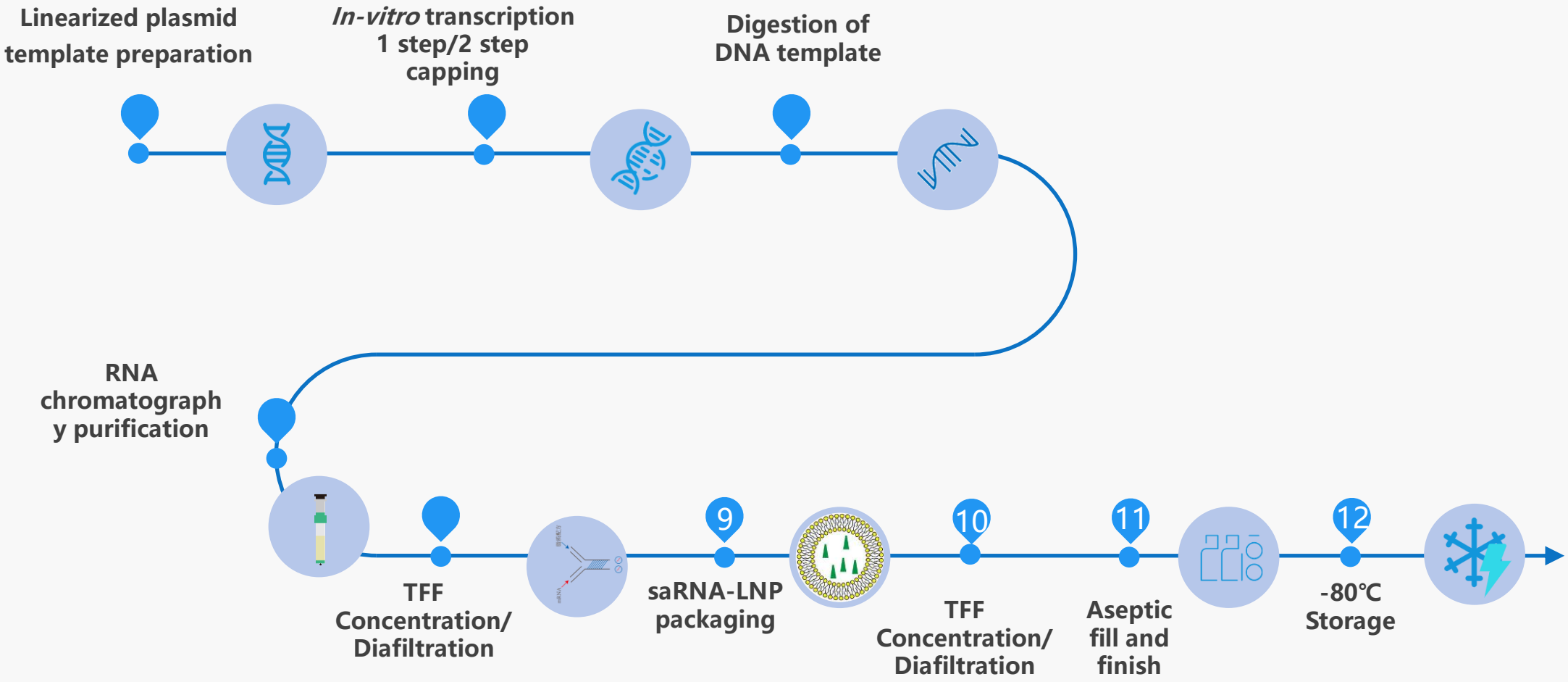
Gene editing summary:

- **IVT-sgRNA and chemically synthesized sgRNA have high purity;**
- **IVT-sgRNA has higher gene editing efficiency than chemically synthesized sgRNA.**

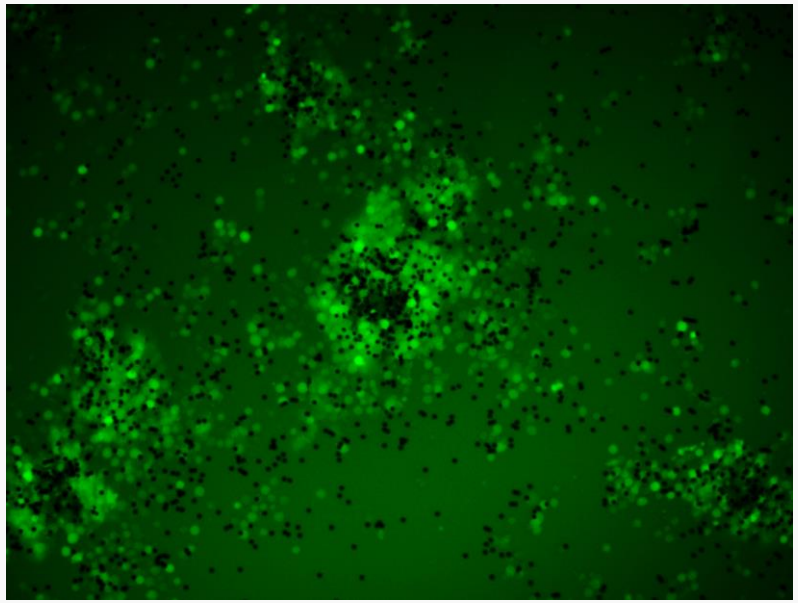
QC release testing for sgRNA

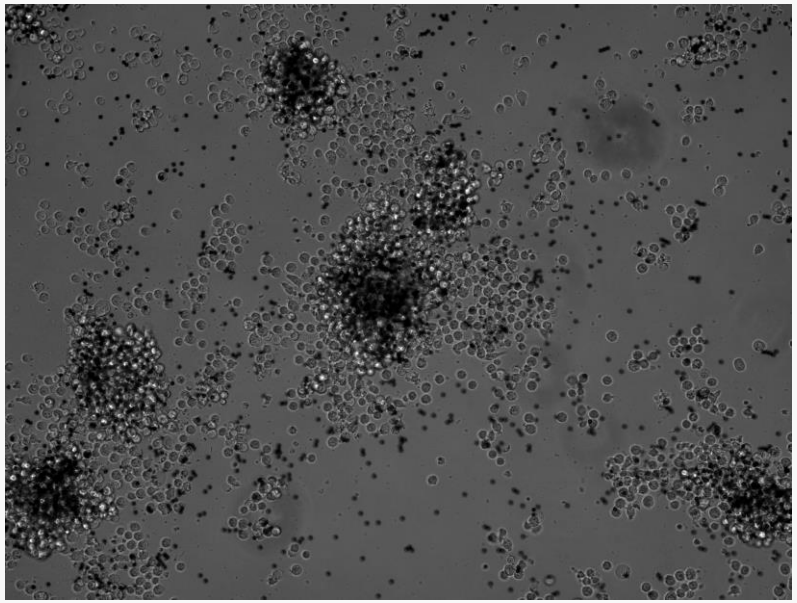
Testing items	Test Methods
Appearance	Visual method
RNA Concentration	UV absorbance
Purity	HPLC
Purity	A260/A280
Molecular Weight	Mass spectrometry
Identifying Sequence	High—hroughput sequencing
Endotoxin Detection	BDBU GEL-CLOT Method
Mycoplasma	Colorimetric assay
Sterility	Culture method
Total Protein Residue	Qubit / MicroBCA
pH	Multi-Parameter Tester
Template Residue	qPCR
dsRNA	Elisa

mRNA/saRNA Production and LNP Packaging Workflow



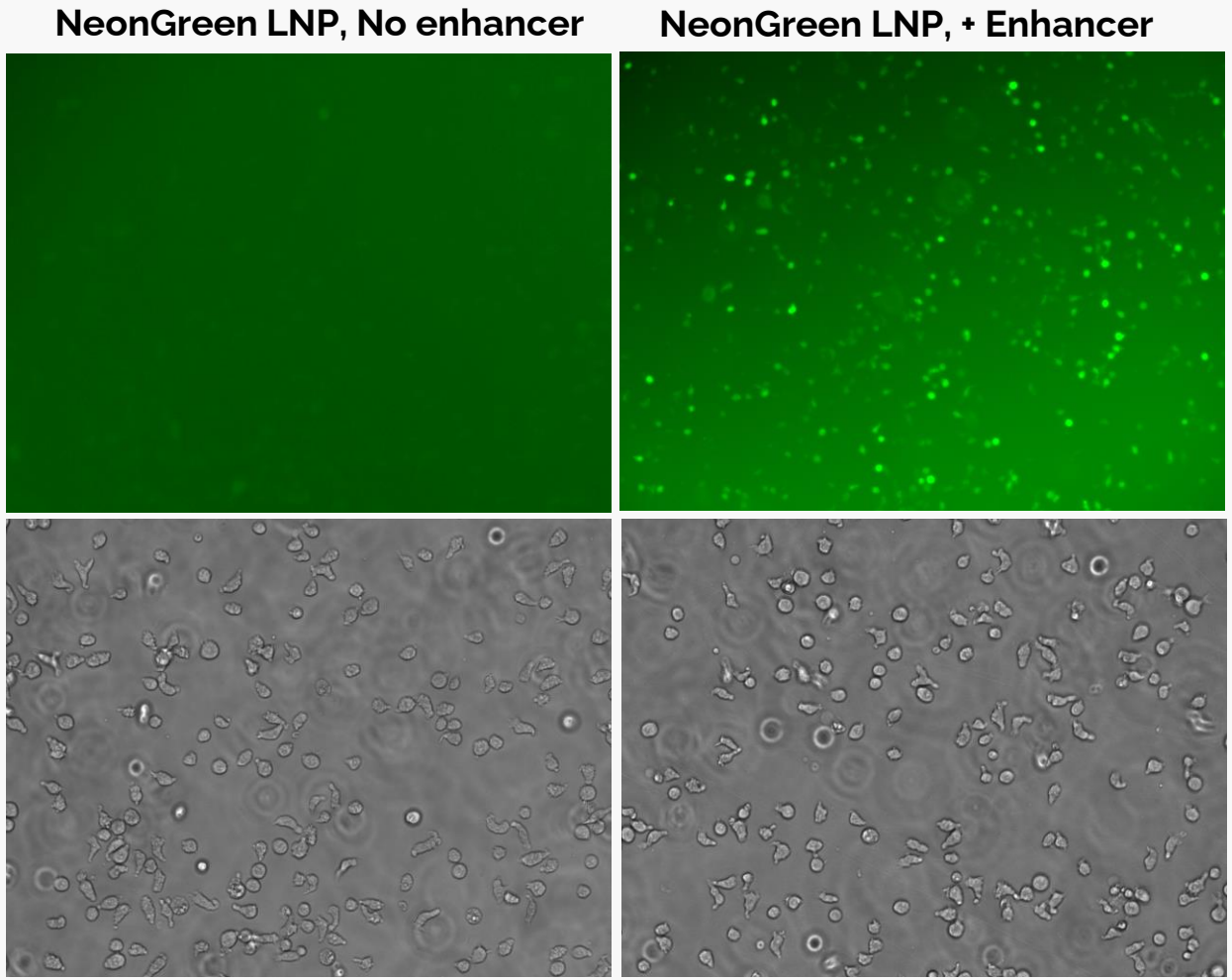
mRNA-LNP Efficient Delivery into Primary T cells





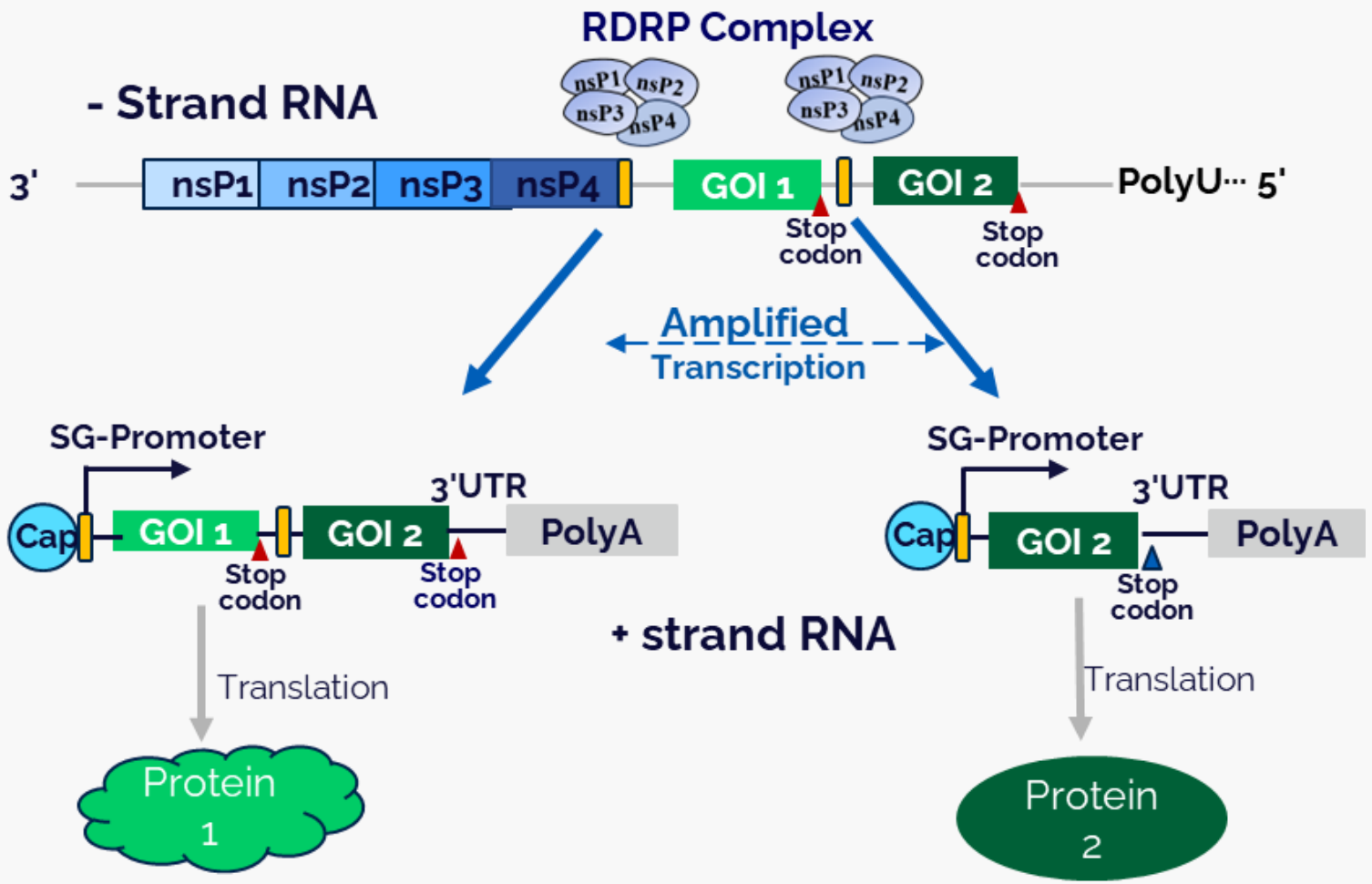
NeonGreen mRNA lipid nanoparticles were added to activated human primary T cells, in the presence of 1X serum-free enhancer A (uBriGene). Fluorescence images were captured 24 hours after treatment. The left image shows fluorescence, while the right image shows the phase contrast.

mRNA-LNP Efficient Delivery to HSC cells

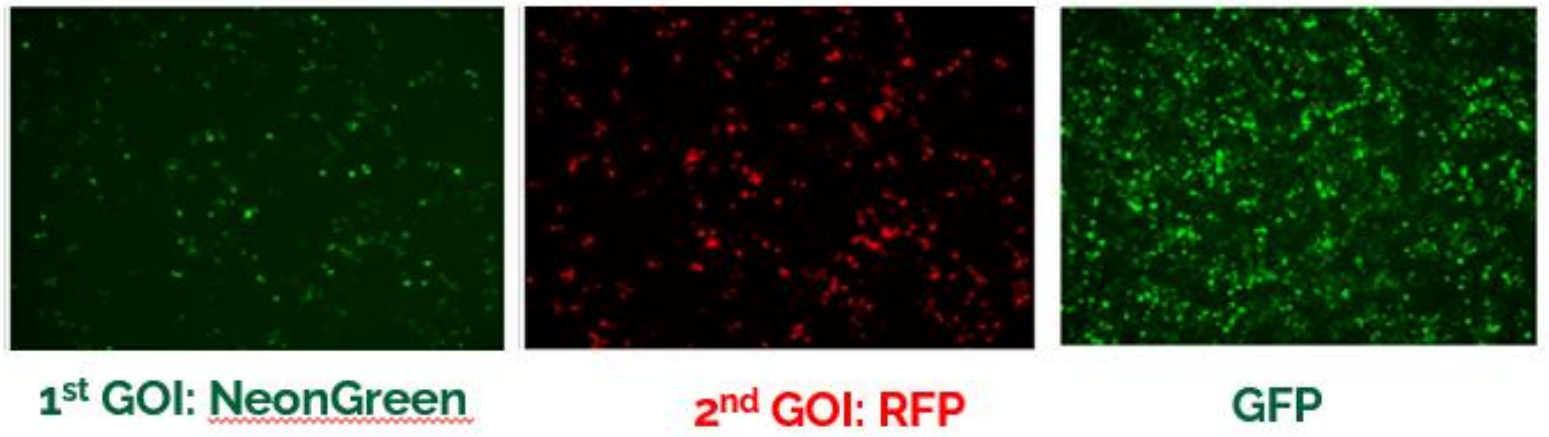


NeonGreen mRNA lipid nanoparticles manufactured by uBriGene were added to HSC cells in the absence (left) or presence (right) of 1X serum-free enhancer A. Fluorescence images (top) were captured 24 hours post-treatment, with phase contrast images shown in the lower panels.

Polycistronic saRNA Design



High Protein Expression from Polycistronic saRNA

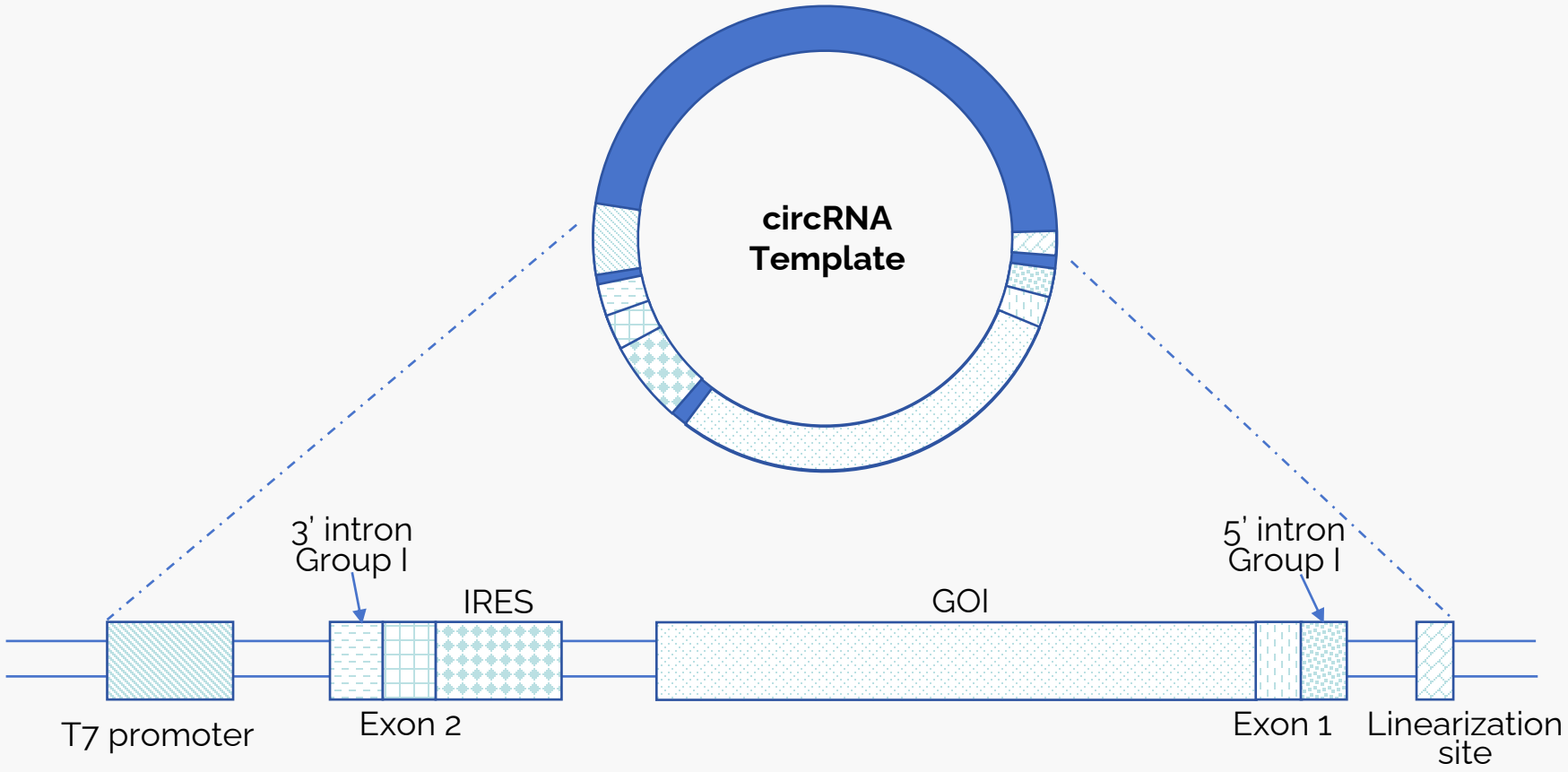


Note: The same amount of saRNA and mRNA were used in this experiment. Due to its larger size, the molar concentration of saRNA is significantly lower compared to linear mRNA.

Quality control testing for mRNA/saRNA

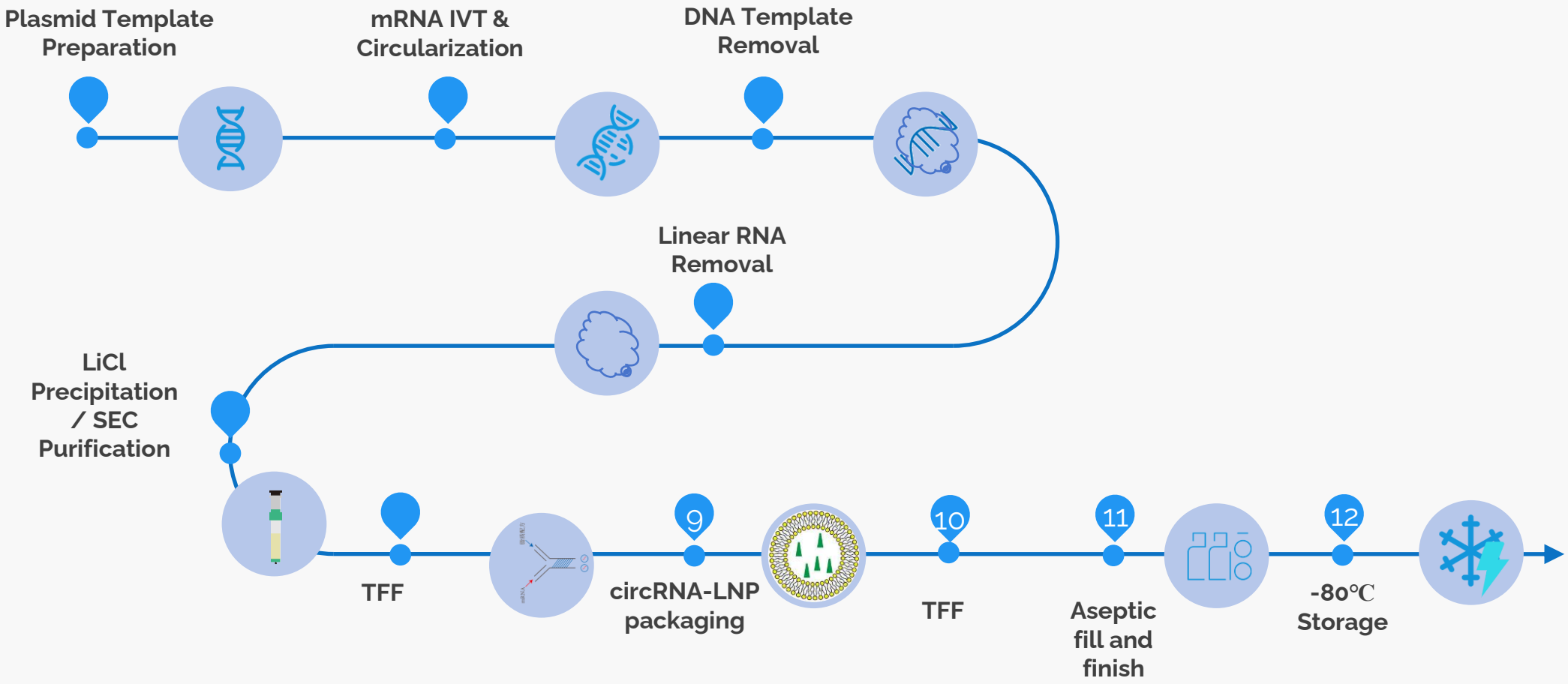
Classification	Test Item	Method
Identity	mRNA sequence identity	RT-PCR+Sanger Sequencing
Content	Concentration	UV absorbance
Purity	5’ capping efficiency	CE/LC-MS
	3’ polyA tail length	CE/LC-MS
	A260/A280	UV absorbance
	RNA integrity	HPLC
	mRNA fragments	
	Aggregate quantitation	
	Residual protein	Qubit / MicroBCA
	dsRNA	ELISA
	Residual DNA template	qPCR
	Residual solvents	Residual ethanol（GC-MS）
Potency	Expression of target protein	Cell-based assay
Safety	Sterility	Culture method
	Endotoxin	GEL-CLOT Method
Physical/chemical Properties	Appearance	Visual method
	pH	pH

circRNA Design Based on PIE Method

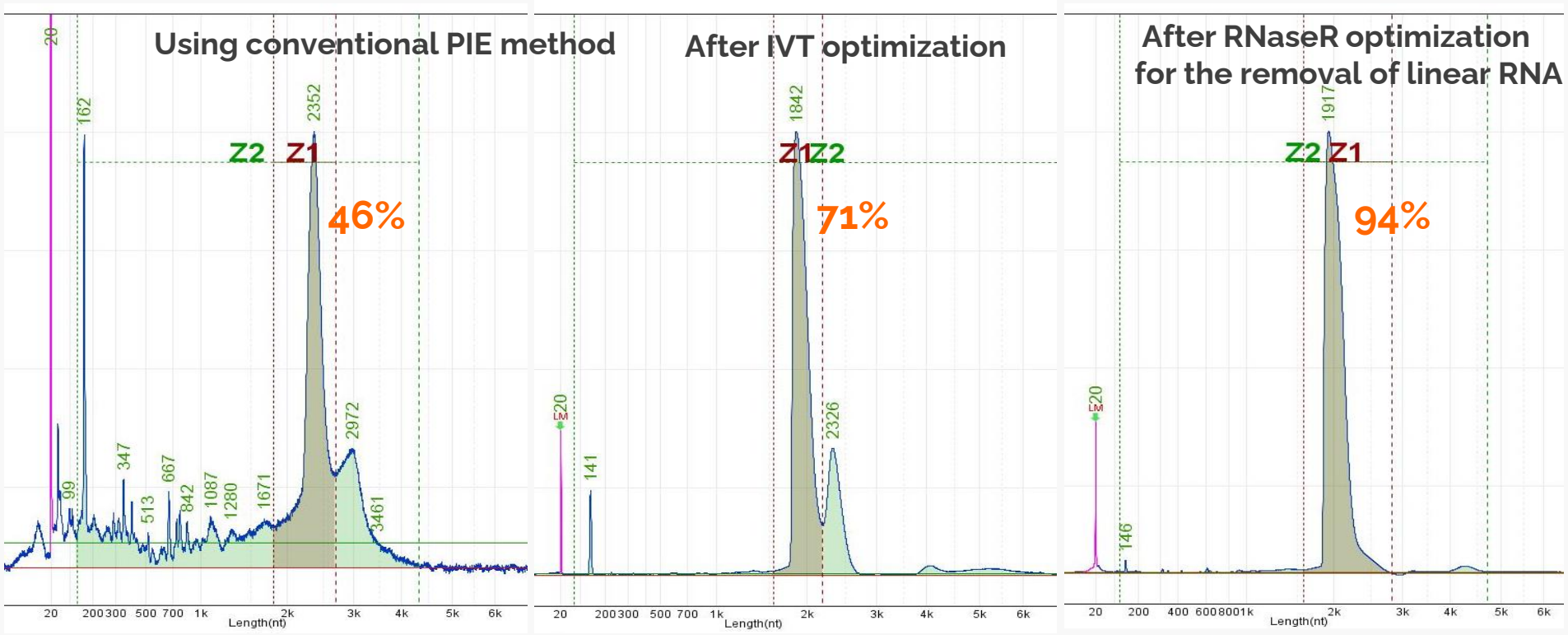


circRNA design based on PIE method (Group I intron)

GMP circRNA-LNP Workflow

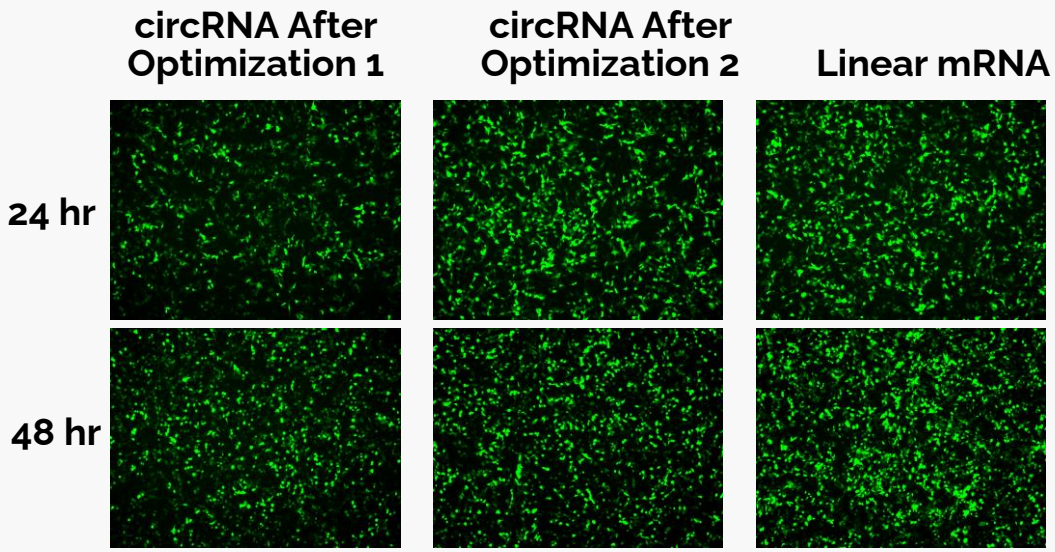


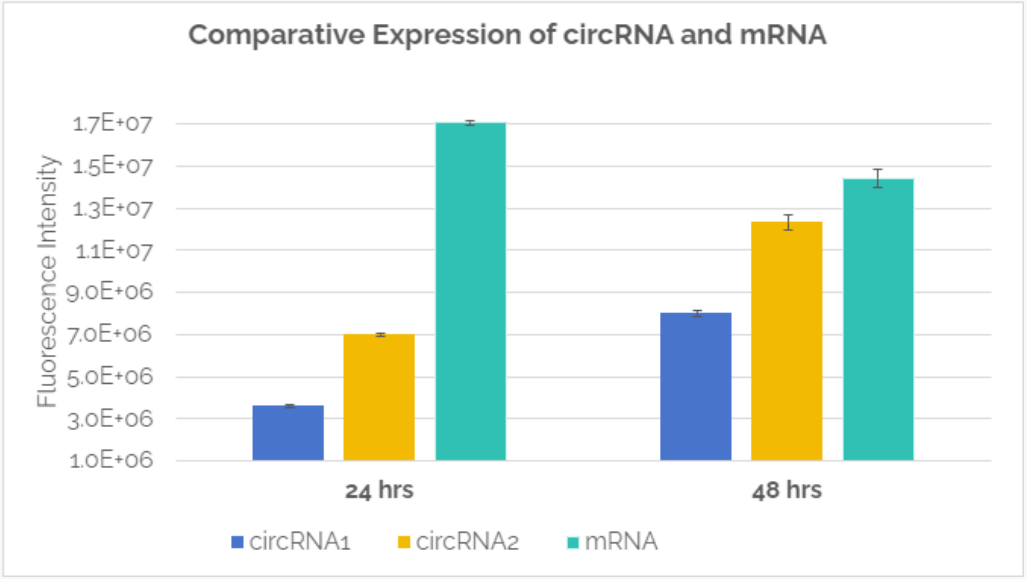
circRNA Purity up to 94%



Using the conventional PIE strategy, circRNA purity is 46%. After optimizing mRNA in vitro transcription and RNaseR efficiency to remove linear RNA, circRNA purity exceeds 90%.

Comparative Expression of circRNA and mRNA



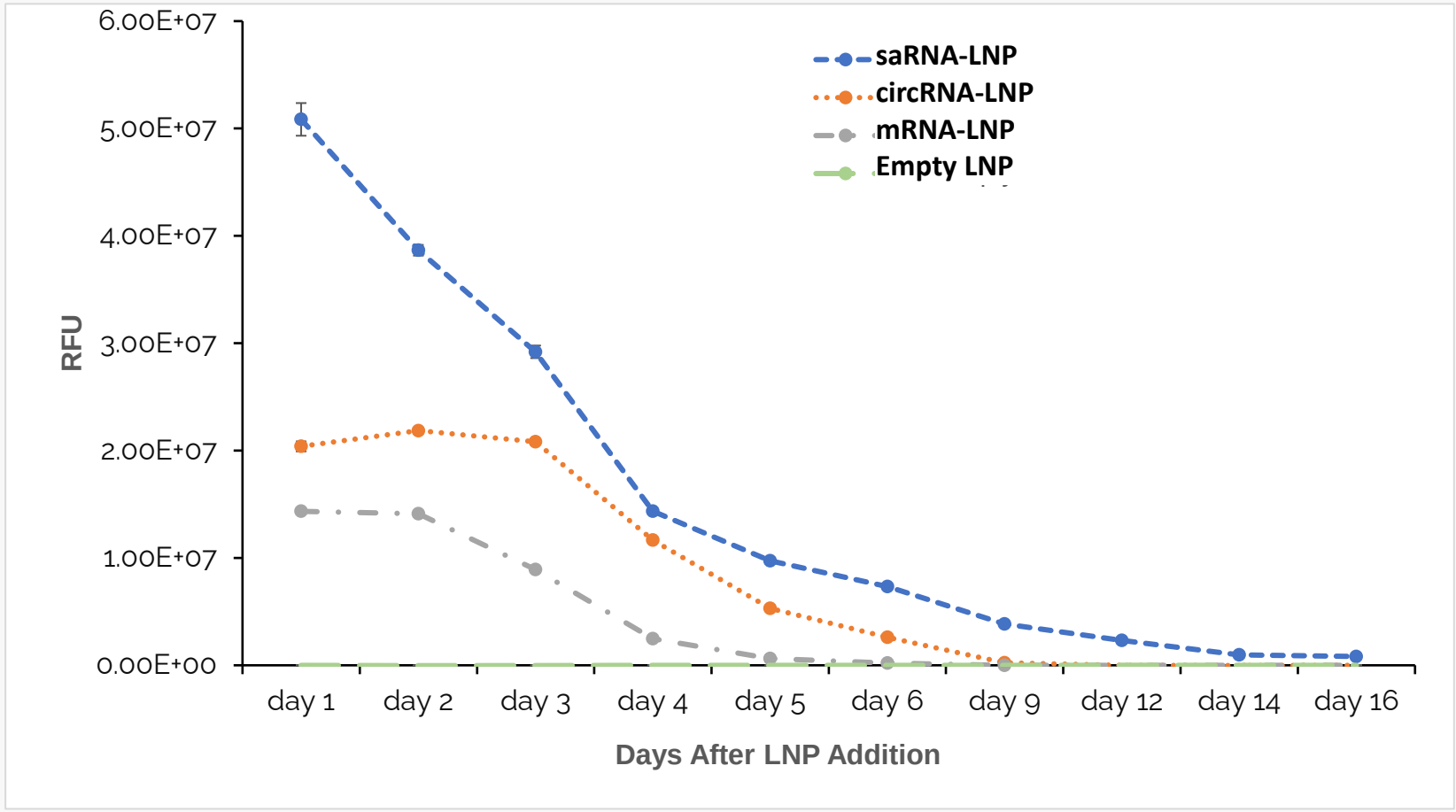


Same amount of RNA-LNPs (circRNA-LNP, mRNA-LNP respectively) were added to HEK293 cells. Fluorescent images were taken 24 hours and 48 hours post the treatment.

Quality control testing for circRNA

Test Item		Method
Identity	mRNA sequence identity	NGS/ NanoporeSequencing
Content	Concentration	UV absorbance
Purity	A260/A280	UV absorbance
	RNA circularization efficiency	HPLC/ CE
	Residual RNase R	Fluorescent probe method
	dsRNA	ELISA
	Residual DNA template	qPCR
	Residual solvents	Residual ethanol（GC-MS）
Potency	Expression of target protein	Cell-based assay
Safety	Sterility	Culture method
	Endotoxin	GEL-CLOT Method
Physical/Chemical Properties	Appearance	Visual method
	pH	pH

Protein Expression over Time for mRNA, circRNA and saRNA



293 Cells

uBriGene Biosciences Inc.

20400 Century Blvd, Suite 125
Germantown, MD 20874

www.ubrigene.com
contact@ubrigene.com
Phone: 800 663 2528