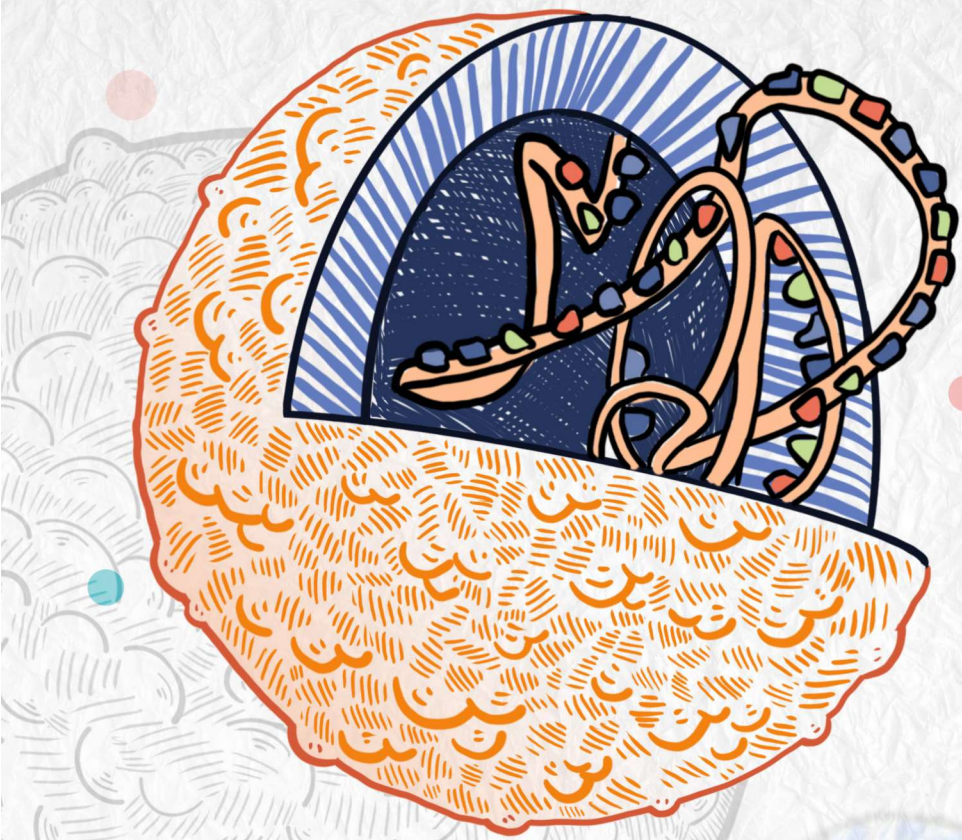
VectorBuilder

Revolutionize Gene Delivery

RNA

from Design to Therapy



VectorBuilder

Table of Contents

About VectorBuilder 1

Our mRNA Manufacturing Capabilities 2

mRNA CRO Services 3

- IVT Vector Design & Cloning 3
- IVT mRNA and LNP Manufacturing 4
- Quality Control 5
- Functional Validation 6

Off-the-Shelf IVT mRNA and LNP Products 7

mRNA CDMO Services 8

Technical Information 10

Additional Offerings 16

About VectorBuilder

As a global leader in gene delivery technologies, VectorBuilder offers a full spectrum of gene delivery solutions covering virtually all research and clinical needs from basic research to therapy. We have supported thousands of laboratories and biotech/pharma companies across the globe along their entire drug-discovery pipelines, going from research-grade vectors for early discovery, to GMP-like vectors for preclinical testing, to full GMP-grade vectors for clinical trials. Our 4 major business segments include research vectors & viruses, gene delivery CRO services, CDMO services, and IP out-licensing.

Research vectors & viruses.

- Vector design
- Vector cloning
- Virus packaging
- Plasmid DNA preparation

Gene delivery CRO services

- Library construction/screening
- mRNA therapeutics development
- AAV capsid evolution in NHP
- AAV biodistribution/PK/PD in NHP
- Promoter engineering
- BAC recombineering
- Stable cell line generation
- Mutagenesis

CDMO services

- Plasmid GMP manufacturing
- Virus GMP manufacturing (AAV,LV,MMLV, etc.)
- mRNA GMP manufacturing
- Process development & analytical development
- Cell banking
- Fill/finish
- Regulatory support & tech transfer

IPout-licensing.

- Novel AAV capsids
- Novel tissue-specific promoters
- Experimentally codon-optimized genes
- Virus producer cell lines

VectorBuilder has developed extensive in-house capabilities in method development and analytical testing to ensure the highest quality of IVT mRNA, which allows us to consistently exceed customer expectations. Our rigorous documentation practices empower the production of mRNA drugs that align with regulatory standards. By partnering with our GMP experts, you can seamlessly transition from discovery and development to clinical mRNA drug manufacturing.

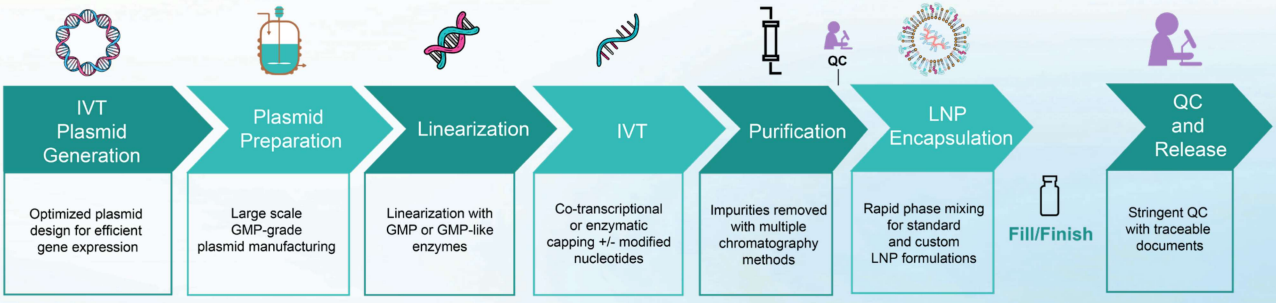
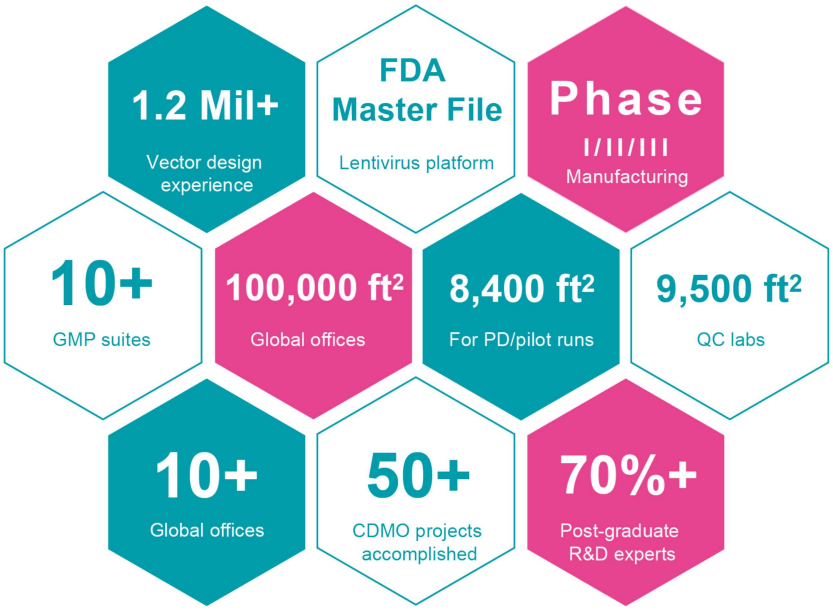


Figure 1. Workflow of IVT mRNA manufacturing at VectorBuilder.

VectorBuilder

Our mRNA Manufacturing Capabilities

Within our current infrastructure, we facilitate process development, custom IVT mRNA synthesis, and GMP-like mRNA manufacturing. Our suite of analytical services ensures comprehensive mRNA characterization, encompassing assessments of identity, integrity, purity, safety, potency, and functionality. With our commitment to innovation, we continuously refine our processes to stay at the forefront of mRNA technology.



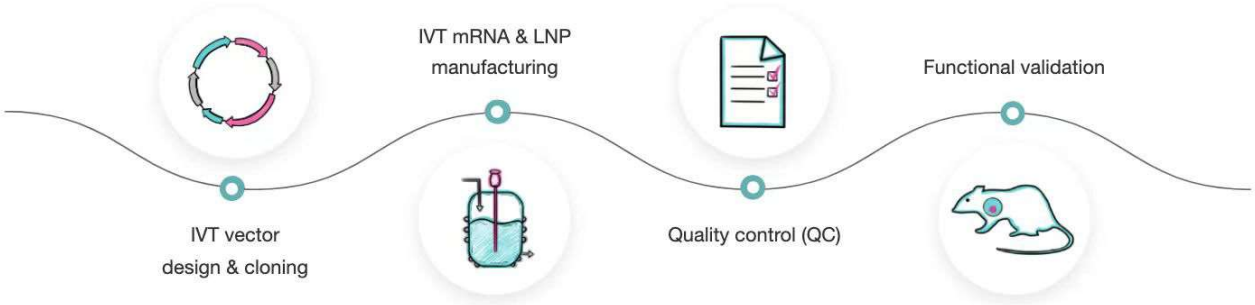
VectorBuilder currently has ~100,000 ft² of modern GMP facilities with advanced designs and state-of-the-art equipment. Anticipated to be launched in the fourth quarter of 2023, our new mRNA focused GMP facility has been meticulously designed to facilitate the seamless progression of mRNA from its discovery phase to clinical drug manufacturing. The design of our facilities prioritizes flexibility and adaptability to cater to custom project requirements.

Our comprehensive facilities include:

- Over 10 GMP manufacturing suites
- Fill/finish suites
- Quality control laboratories
- Analytical development suites
- Process development suites
- GMP warehouse

mRNA CRO Services

VectorBuilder provides a one-stop solution for the development of mRNA-based therapeutics, such as vaccines, gene editing, CAR immune therapy, and protein expression in cells or embryos. Based on extensive design and production experience, our team can support researchers for IVT vector design & cloning, IVT mRNA and LNP manufacturing, quality control (QC) assays, and in vitro/in vivo functional validation to accelerate the development of mRNA-based vaccines and gene therapy.



IVT Vector Design & Cloning

- Royalty-free IVT backbone with no IP constrains for commercial use
- Sequence optimization for achieving high expression
  - o 5' and 3' UTR
  - o Coding sequence
  - o Kozak

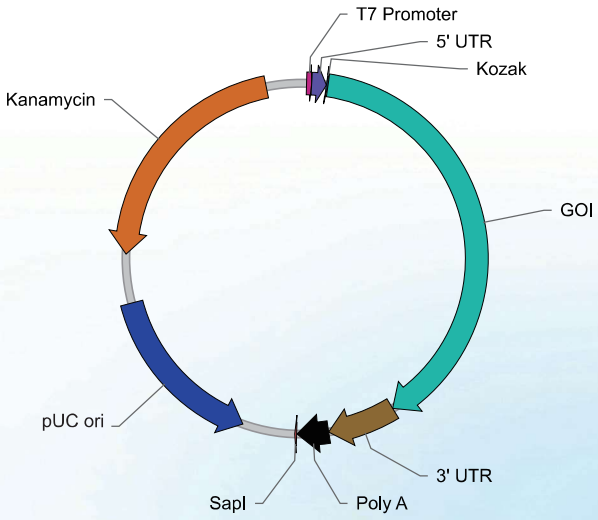


Figure 2. Design of IVT vector. Diagram of VectorBuilder's T7-based IVT vector including 5' & 3' UTRs and a template derived polyA tail.

VectorBuilder

IVT mRNA and LNP Manufacturing

- From vector cloning to LNP encapsulation in as fast as 5 weeks
- Synthesis of mRNA and self-amplifying RNA (saRNA) of up to 10,000 nt from ug to gram scale
- High capping efficiency (up to 99%) by co-transcriptional or enzymatic capping
- Modified nucleotides for reducing immune response and achieving robust expression in vivo
    - o m1Ψ
    - o m5C
    - o 5moU

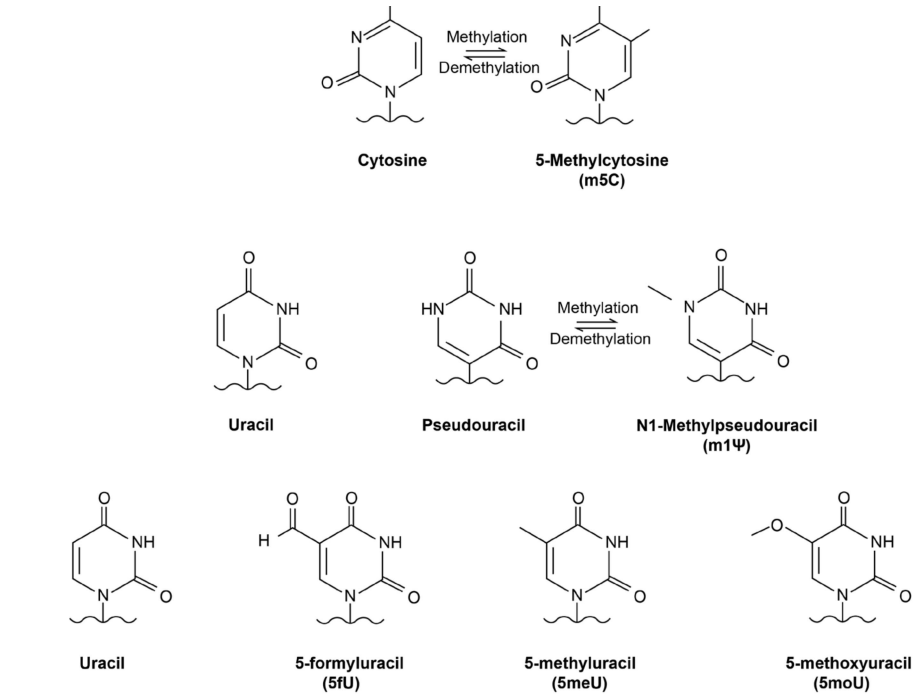


Figure 3. Structure of canonical and modified nucleobases.

- High-quality LNP with conventional and custom formulation:
    - o High encapsulation efficiency
    - o Low polydispersity index (PDI)
    - o Improved stability
    - o Increased delivery efficiency
    - o Antibody-conjugation compatibility

- Proprietary purification technology to rapidly and efficiently remove impurities include:
    - o IVT DNA template
    - o Partially transcribed mRNA products
    - o Residual protein
    - o Double-stranded RNA (dsRNA)

Quality Control

VectorBuilder offers an extensive variety of QC methods for IVT mRNA and LNP encapsulation. Default QC items (marked with √) are always performed while optional QC items are performed depending on individual project needs.

IVT mRNA

Attribute		QC Assay	Research-grade	GMP-like
Identity	mRNA sequence	Sanger sequencing	√	√
	mRNA length	Denaturing agarose gele electrophoresis	√	√
		Capillary gel electrophoresis (CGE)	Optional	√
General/physical property	mRNA concentration	UV spectrophotometry	√	√
		RiboGreen assay	Optional	√
	Appearance	Visual inspection	Optional	√
Potency	Gene expression	In vitro translation followed by Western blot	Optional	Optional
		Cell transfection	Optional	Optional
Safety	Sterility	Bioburden test	Optional	√
	Mycoplasma	Culture method	Optional	√
		qPCR	Optional	Optional
	Endotoxin	Kinetic chromogenic assay (KCA)	Optional	√
Purity	mRNA integrity	Denaturing agarose gel electrophoresis	√	√
		Capillary gel electrophoresis (CGE)	Optional	√
	A260/280	UV spectrophotometry	√	√
	Capping efficiency	LC-MS	Optional	√
	PolyA analysis	LC-MS	Optional	√
	Residual dsRNA	Dot blot assay	Optional	√
	Residual plasmid DNA	qPCR	Optional	√
	Residual protein	NanoOrange assay	Optional	√
	Residual solvents	Gaschromatography	Optional	Optional

LNP encapsulation

Attribute	QC Assay	Research-grade	GMP-like
Encapsulation efficiency	RiboGreen assay	√	√
Particle size, PDI	Dynamic light scattering (Zetasizer)	√	√
Surface charge	Dynamic light scattering (Zetasizer)	√	√

Note: The default testing listed reflects the standards at the time of release of this brochure. The default testing used for any given sample is based on the current standards at the time of order placement.

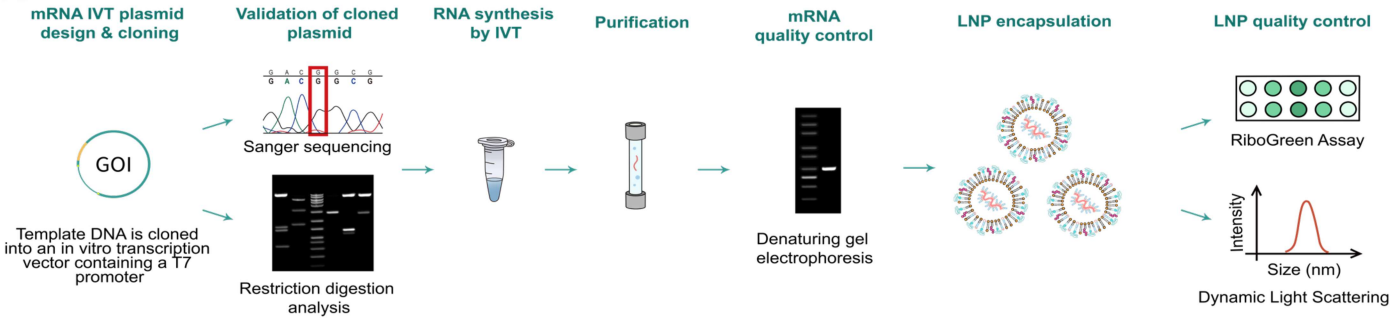


Figure 4. Typical workflow of mRNA manufacturing and QC.

As shown in Figure 4, our typical mRNA production workflow starts with designing and synthesis of the template DNA sequence with the consideration of preferred codons, GC content, and thermodynamic stability of RNA secondary structures, followed by its cloning into an in vitro transcription vector. The plasmid DNA is then purified, validated, and linearized before being subsequently subjected to the in vitro transcription reaction which results in the generation of the desired transcript. Modified nucleotides, like m1Ψ, m5C and 5moU, can be incorporated into the in vitro transcription reaction to improve in vivo translation and decrease immunogenicity. Highly efficient capping (>99%) can be achieved either using co-transcriptional or enzymatic approaches. The mRNA is then purified by mRNA-capture beads as the default purification process or other purification methods (i.e., oligo dT and ion-pair reversed-phase chromatography) upon request. Next, mRNA can be further encapsulated in LNP in a microfluidic mixer. The encapsulation efficiency and nanoparticle profiling are then processed for QC analysis.

Functional Validation

- Assess the effects of different sequence optimizations (UTRs, coding sequence, polyA, Kozak, etc.) on GOI expression in parallel, using our high-throughput cloning, synthesis, and testing platforms.
- Established functional validation platforms for various applications, such as antigen presentation, antibody expression, CAR expression, and CRISPR.
- Assess LNP-mRNA gene delivery efficacy and safety using animal models including rodents and non-human primates (NHPs).

Off-the-Shelf IVT mRNA and LNP Products

VectorBuilder offers off-the-shelf and ready-to-use IVT mRNA and LNP-mRNA products for in vitro and in vivo experiments. Their quality and efficacy have been fully validated in both cell culture and animal models, thus, they can be used to assess the efficiency of mRNA delivery and expression or used as control for your mRNA experiment.

Catagory	Catalog #	Products	Nucleotide	Scale
IVT mRNA	NR1010-0100	EGFP IVT mRNA	unmodified	100 ug
	NR1010-1000	EGFP IVT mRNA	unmodified	1 mg
	NR1011-0100	EGFP IVT mRNA	m1Ψ substitution	100 ug
	NR1011-1000	EGFP IVT mRNA	m1Ψ substitution	1 mg
	NR1020-0100	HiExpress™ Firefly Luciferase IVT mRNA	unmodified	100 ug
	NR1020-1000	HiExpress™ Firefly Luciferase IVT mRNA	unmodified	1 mg
	NR1021-0100	HiExpress™ Firefly Luciferase IVT mRNA	m1Ψ substitution	100 ug
	NR1021-1000	HiExpress™ Firefly Luciferase IVT mRNA	m1Ψ substitution	1 mg
	NR1030-0100	mCherry IVT mRNA	unmodified	100 ug
	NR1030-1000	mCherry IVT mRNA	unmodified	1 mg
	NR1031-0100	mCherry IVT mRNA	m1Ψ substitution	100 ug
	NR1031-1000	mCherry IVT mRNA	m1Ψ substitution	1 mg
	NR1040-0100	hSpCas9 IVT mRNA	unmodified	100 ug
	NR1040-1000	hSpCas9 IVT mRNA	unmodified	1 mg
	NR1041-0100	hSpCas9 IVT mRNA	m1Ψ substitution	100 ug
	NR1041-1000	hSpCas9 IVT mRNA	m1Ψ substitution	1 mg
	NR1050-0100	HiExpress™ Gaussia Luciferase IVT mRNA	unmodified	100 ug
	NR1050-1000	HiExpress™ Gaussia Luciferase IVT mRNA	unmodified	1 mg
	NR1051-0100	HiExpress™ Gaussia Luciferase IVT mRNA	m1Ψ substitution	100 ug
	NR1051-1000	HiExpress™ Gaussia Luciferase IVT mRNA	m1Ψ substitution	1 mg
	NR1070-0100	Zebrafish EGFP IVT mRNA	unmodified	10 ug
LNP-mRNA	NL1010-0100	EGFP LNP-mRNA	unmodified	100 ug
	NL1010-1000	EGFP LNP-mRNA	unmodified	1 mg
	NL1011-0100	EGFP LNP-mRNA	m1Ψ substitution	100 ug
	NL1011-1000	EGFP LNP-mRNA	m1Ψ substitution	1 mg
	NL1020-0100	HiExpress™ Firefly Luciferase LNP-mRNA	unmodified	100 ug
	NL1020-1000	HiExpress™ Firefly Luciferase LNP-mRNA	unmodified	1 mg
	NL1021-0100	HiExpress™ Firefly Luciferase LNP-mRNA	m1Ψ substitution	100 ug
	NL1021-1000	HiExpress™ Firefly Luciferase LNP-mRNA	m1Ψ substitution	1 mg

VectorBuilder

mRNA CDMO Services

VectorBuilder offers a full range of CRO and CDMO services for in vitro transcription (IVT) mRNA manufacturing and lipid nanoparticle (LNP) therapeutic development. Relying on our revolutionary vector design platform and extensive experience, we can provide optimal in vitro transcription vector designs, large-scale IVT mRNA manufacturing, and LNP encapsulation followed by thorough quality control tailored to a wide range of research and clinical needs. We offer several grades that cover different downstream needs including drug discovery research and pre-clinical studies.

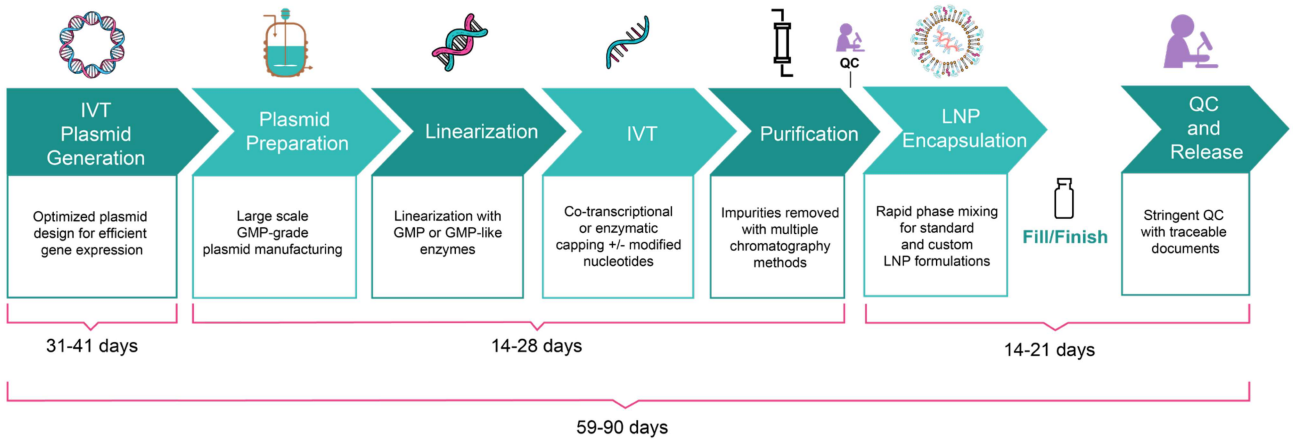


Figure 5. Typical workflow of mRNA CDMO services.

Comparison of different grades of mRNA

	Research-grade	GMP-like
Applications	Basic research, drug discovery, and preclinical studies	Preclinical studies such as animal testing of drug safety and metabolism
Production scales	mRNA: 0.1-10 mg LNP: 0.1-3 mg	mRNA: 0.01-5 g LNP: 3-20 mg
Turnaround time	49-71 days • Vector design & cloning: 26-36 days • Plasmid production & linearization: 14-21 days • IVT mRNA production: 14-21 days • LNP encapsulation: 9-14 days	59-90 days • Vector design & cloning: 31-41 days • Plasmid production & linearization: 14-28 days • IVT mRNA production: 14-28 days • LNP encapsulation: 14-21 days
Quality system	ISO9001	ISO9001 while adopting key features of GMP system
Production facility	In parallel production in shared laboratory space	Productions done in segregated suites
Document control and traceability	No	Yes
QC and release	Standard QC	Performed on a case-by-case basis depending on individual project needs
Aseptic fill/finish	N/A	Available upon request
Storage of retention sample	Available upon request	Available upon request
Other deliverable	COA	1. COA 2. Manufacturing summary 3. TSE/BSE statement upon request

- **Research-grade**

Research-grade mRNA is intended for basic research and drug discovery studies. It is made under standard laboratory conditions with stringent QC to ensure high quality suitable for all downstream research needs.

- **GMP-like**

GMP-like mRNA is intended for pre-clinical studies such as animal testing of drug safety and metabolism. It is produced in a manner that adopts key features of GMP guidelines, including comparable production process and similar quality attributes. Production is performed in segregated production suites with document control and traceability. GMP-like grade can thus be viewed as a small-scale mimic of the final GMP product, but with much lower cost and faster timeline. Where appropriate, GMP-like mRNA can be produced under RNase-free fermentation and purification conditions. A certificate of analysis (COA) is provided at the product release. TSE/BSE statement is available upon request.

- **GMP-grade** *Coming soon*

GMP-grade mRNA is produced in our certified GMP suite with strict adherence to GMP guidelines. A comprehensive quality assurance system is implemented throughout the production process. A wide range of in-process and release QC assays are performed to ensure that the mRNA meets or exceeds the desired quality and safety standards. A batch release report fully documenting the production process and a COA are provided at product release. Other documentation is available upon request.

QC assays

Standard QC assays include Sanger sequencing, denaturing agarose gel electrophoresis, UV-Vis spectrometry for IVT mRNA and encapsulation efficiency, diameter, PDI and Zeta potential for LNP-mRNA. For individual projects with personalized QC demands, QC assays are performed on a case-by-case basis (see below).

Product	Attribute	Analytical Assay
IVT DNA template	Concentration	Spectrometry
	Identity	Gel electrophoresis, Sanger sequencing
	Linearization	Capillary gel electrophoresis
	Residual host E.coli DNA	qPCR
mRNA	Concentration	UV-Vis spectrometry
	Identity	Capillary gele electrophoresis, reverse transciption followed by Sanger sequencing
	Capping efficiency	LC-MS, Cappillary gel electrophoresis
	PolyA tail integrity	LC-MS, Cappillary gel electrophoresis
	Residual protein	NanoOrange assay
	Residual plasmid	qPCR
	dsRNA	Dot blot
	Endotoxin	Kinectic chromogenic assay (KCA)
LNP	Endotoxin	Kinectic chromogenic assay (KCA)
	Encapsulation efficiency	RiboGreen assay
	Diameter, PDI, and Zeta potential	Zetasizer

IVT Vector Sequence Optimization

UTR

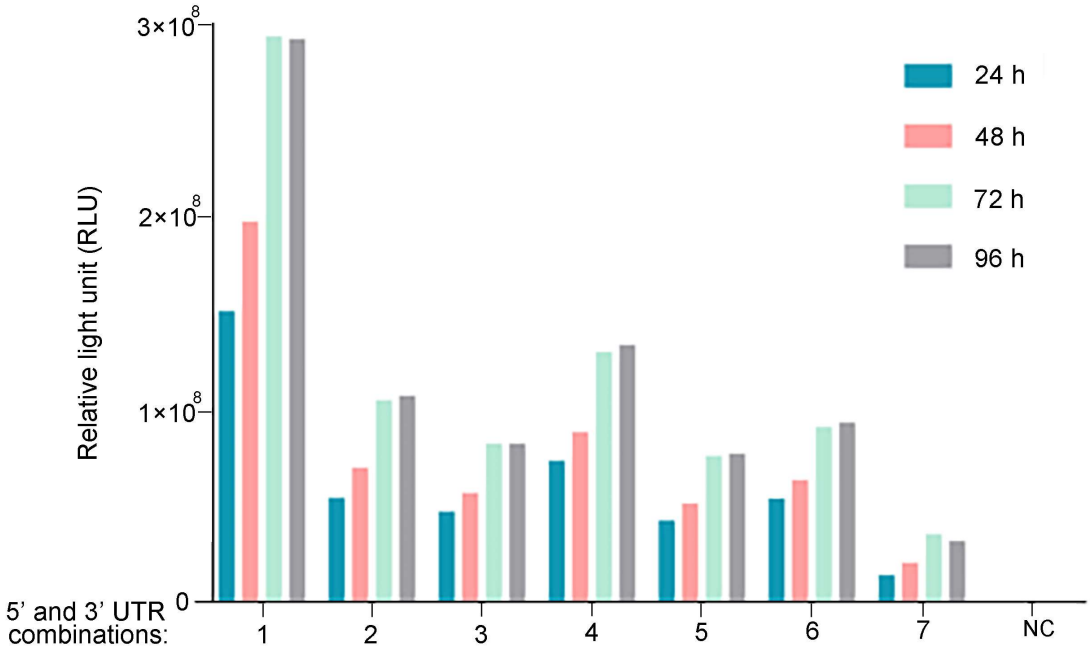


Figure 6. UTR optimization for improved mRNA expression. Different 5' and 3' UTR combinations were tested for regulating Guassia luciferase expression in vitro. 293T cells were seeded on 12-well plates at a density of $2.3x10^5$ cells per well. Cells were transfected with 1 ug of mRNA per well. At 6 h, 24 h, 48 h, 72 h, and 96 h post-transfection, Gaussia luciferase activities were measured from cell culture medium.

Coding sequence

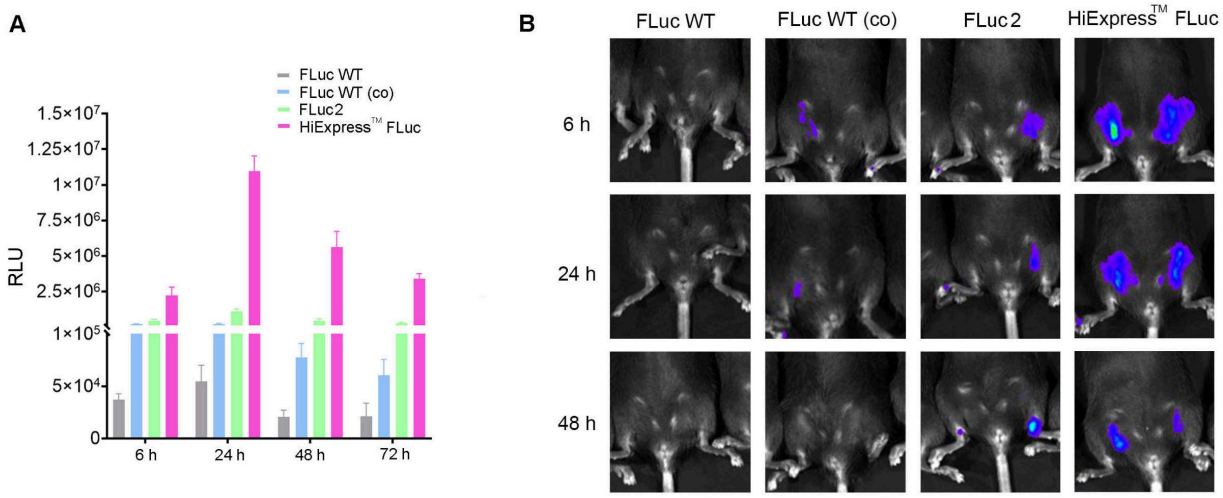


Figure 7. Codon optimization increases mRNA expression in vitro and in vivo. Expression of HiExpress™ Firefly Luciferase mRNA and other luciferase mRNA in HEK293T cells. (A) Cells grown on a 12-well plate were transfected with 0.5 ug of mRNA per well and luciferase activity was measured at 6 h, 24 h, 48 h, and 72 h post-transfection. (B) Luciferase activity measured in adult C57BL/6 mice injected intramuscularly with 30 ug of LNP encapsulated mRNA at 6 h, 24 h, and 48 h post-injection. FLuc WT indicates wild-type firefly luciferase. FLuc WT (co) indicates codon-optimized wild-type firefly luciferase. FLuc2 indicates Luc2 firefly luciferase.

Try our HiExpress™ Firefly Luciferase IVT mRNA
Try our HiExpress™ Firefly Luciferase LNP-mRNA

PolyA tail

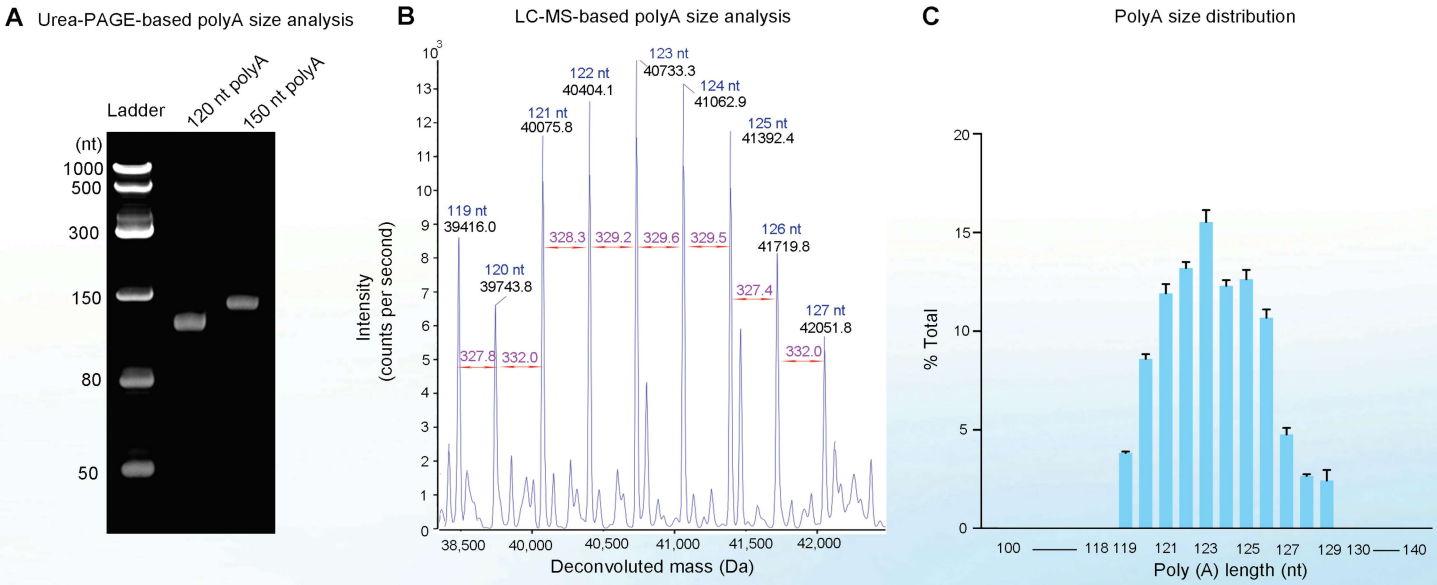


Figure 8. PolyA tail size analysis. PolyA tails were cleaved from IVT mRNA using ribonuclease T1 and isolated by oligo dT affinity chromatography. (A) Isolated polyA tails analyzed by Urea-PAGE gel electrophoresis. 60 ng of polyA tails with expected size of 120 nt and 150 nt were loaded on denaturing Urea-PAGE gel, respectively. (B) Isolated polyA tails analyzed by LC-MS. Deconvoluted spectrum was generated from a 120 pmol of polyA tails with an expected size of 120 nt. (C) Size distribution of the polyA tails with an expected size of 120 nt. The bar graph demonstrates the polyA tails have small heterogeneity. The error bars represent standard deviation from technical triplicates. The weighted average length is 123 nt.

Kozak sequence

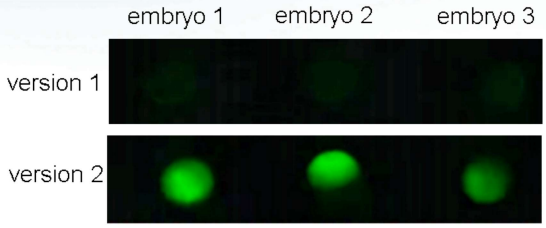


Figure 9. Kozak sequence optimization increased IVT EGFP mRNA expression in zebrafish. One-cell stage zebrafish embryos were measured for EGFP expression at 6 h post-microinjection of 250 pg of zebrafish EGFP IVT mRNA. Fluorescent images indicated optimized Kozak sequence (version 2) greatly improved EGFP expression in vivo.

Try our Zebrafish IVT mRNA

IVT mRNA Synthesis Optimization

IVT mRNA integrity

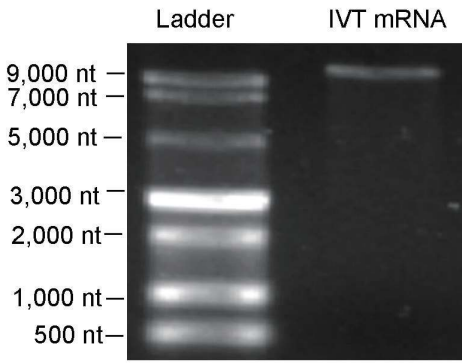


Figure 10. Denaturing agarose gel result indicated high integrity was achieved for >10,000 nt IVT mRNA.

Nucleotide modification

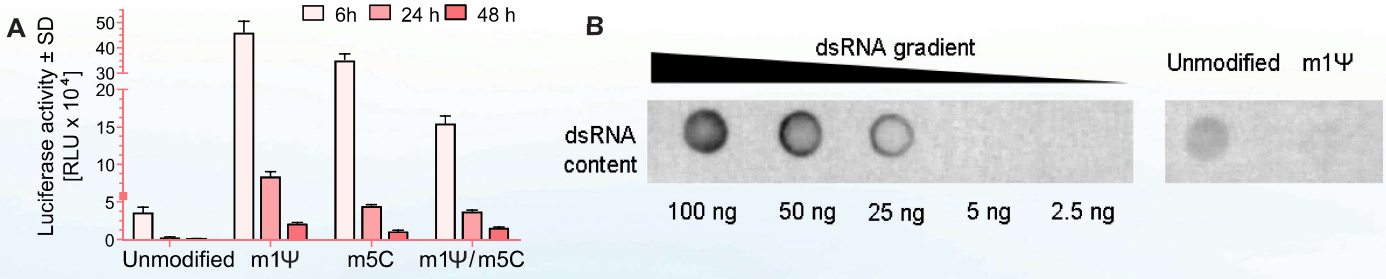


Figure 11. Modified nucleotides increased mRNA expression and decreased dsRNA impurities. (A) Expression of firefly luciferase in 293T cells. The mRNA was generated with or without modified nucleotides, N1-Methylpseudouridine (m1Ψ) and 5-Methylcytosine (m5C). Cells grown on the 12-well plates were transfected with 1 ug of mRNA per well. Luciferase activities in 293T cells at 6 h, 24 h, and 48 h post-transfection were measured. Error bars indicate standard deviations. (B) Equal amount (750 ng per dot) of magnetic bead-purified EGFP IVT mRNA with or without nucleotide modification (m1Ψ) was blotted and subsequently detected by dot blot assay for estimating the dsRNA impurity.

Try our HiExpress™ Firefly Luciferase IVT mRNA

Co-transcriptional and enzymatic capping

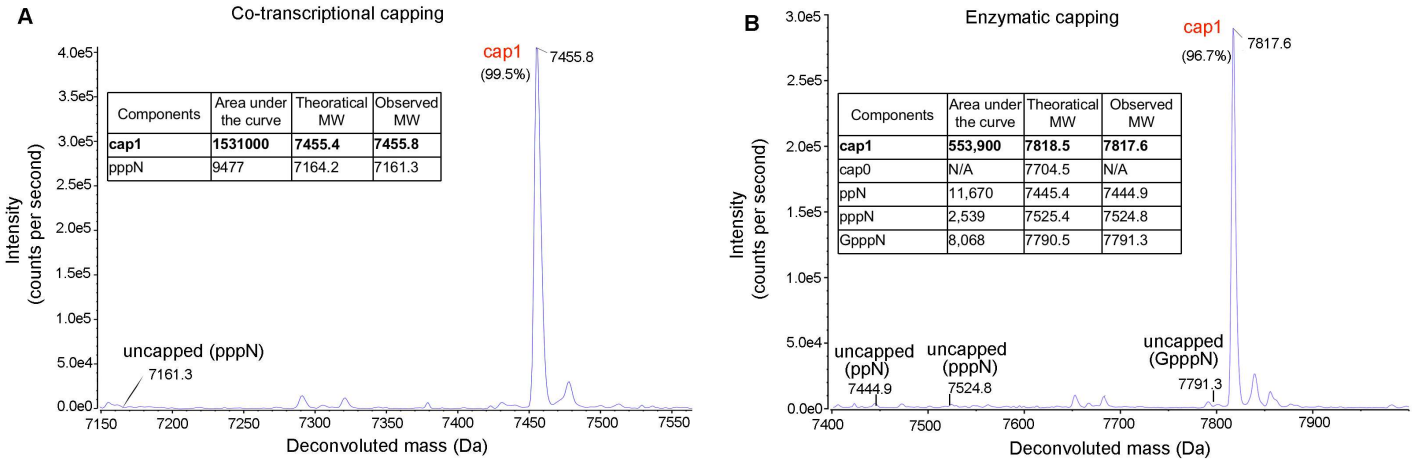


Figure 12. IVT mRNA capping efficiency analyzed by LC-MS. Highly efficient capping (>99%) can be achieved either using (A) co-transcriptional or (B) enzymatic approaches.

dsRNA removal

As a major trigger of undesired immunogenicity, double-stranded RNA (dsRNA) is a by-product of IVT. Dot blot result demonstrates extra purification steps (e.g. IP-PR) may be necessary for achieving an ultra-purification scale with very low levels of dsRNA.

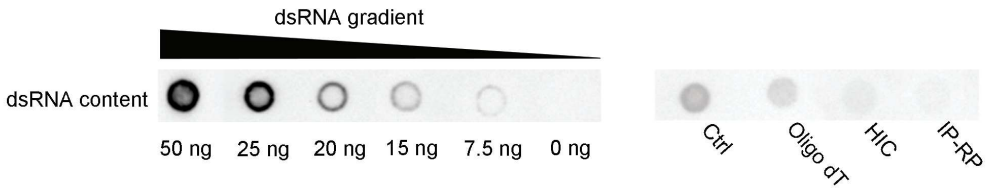


Figure 13. dsRNA removal efficiency of different purification processes. Equal amounts (1500 ng per dot) of hSpCas9 IVT mRNA purified by different processes were blotted and subsequently detected by dot blot assay for estimating the dsRNA impurity. Abbreviations: HIC, Hydrophobic interaction chromatography; IP-RP, Ion-pair reversed-phase liquid chromatography.

Try our hSpCas9 IVT mRNA

LNP-mRNA QC Data

TEM

Transmission electron microscopy (TEM) results show good structural integrity and size homogeneity of our LNP-mRNA.

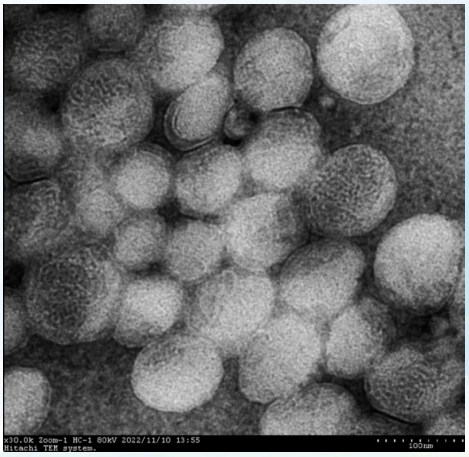


Figure 14. Negative staining TEM of LNP-mRNA. Scale bar=100 nm.

PDI and zeta potential

Dynamic light scattering (DLS) analysis shows our LNP-mRNA products can reach a very low PDI (as low as PDI<0.1).

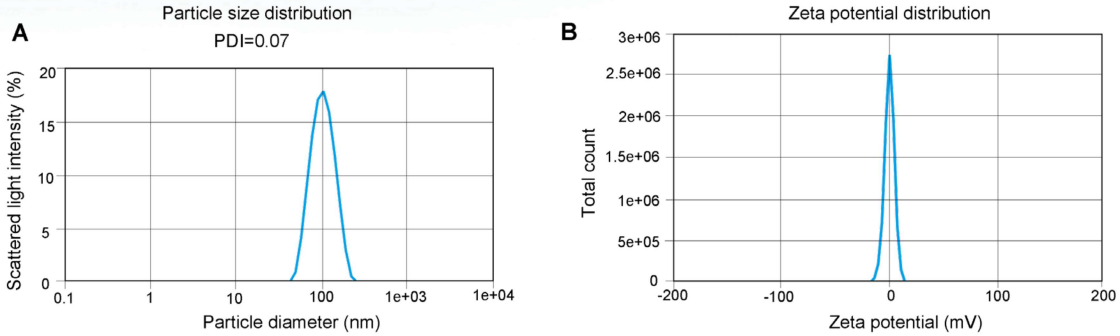


Figure 15. Particle size and Zeta potential distribution analysis. PDI (A) and Zeta potential (B) was determined by DLS which measures the intensity differences of fluctuated light due to motion of particles. The Zeta potential of the sample is between -1.872 mV and +1.872 mV.

Try our EGFP LNP-mRNA

LNP-mRNA Functional Validation

LNP-mRNA expression in vitro

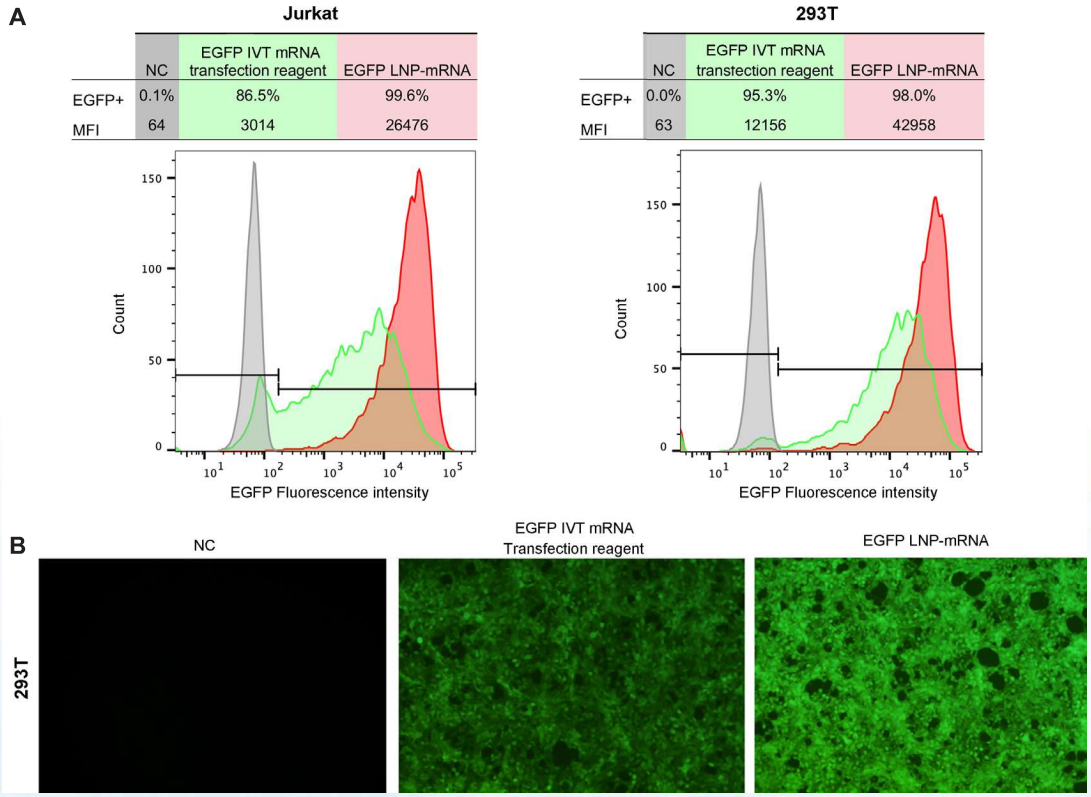


Figure 16. Efficient mRNA delivery and expression using LNP in vitro. Cells were transfected with LNP encapsulated EGFP mRNA or EGFP mRNA mixed with commercial transfection reagent. (A) Flow cytometry analysis of EGFP expression in Jurkat and 293T cells and (B) Fluorescent imaging of 293T cells at 24 hours post-transfection. MFI: median fluorescence intensity.

Try our EGFP LNP-mRNA

LNP-mRNA expression in vivo

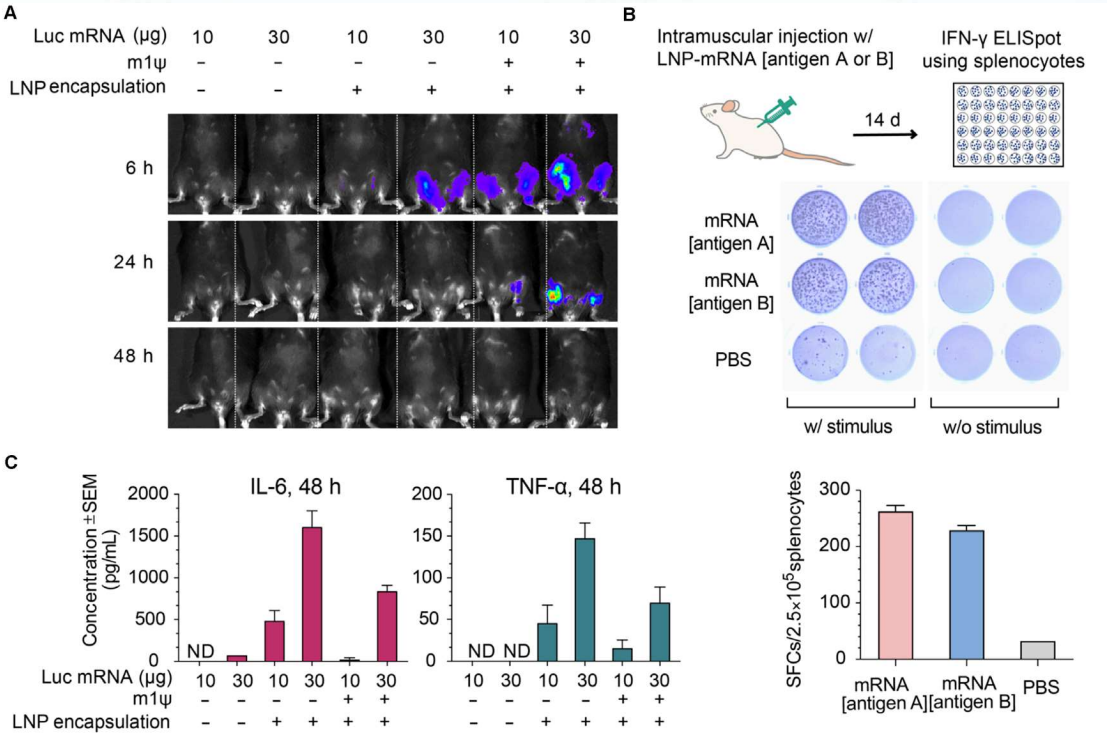


Figure 17. Expression of luciferase (Luc) mRNA and mRNA induced immune response in mice. (A) Luciferase activity visualized by live imaging at 6 h, 24 h, and 48 h post-injection. (B) Two pro-inflammatory cytokines, IL-6 and TNF-α, were quantified in the serum at 48 h post-injection. Error bars represent standard errors. Mice strain: C57BL/6J; mice age: 8 weeks; injection method: intramuscular injection. (C) IFN-γ ELISpot assay of splenocytes derived from Balb/C mice 14 days post intramuscular injection of 30 ug LNP-encapsulated mRNA coding for viral antigen A, viral antigen B, or control PBS.

Try our HiExpress™ Firefly Luciferase LNP-mRNA

Antibody-conjugated LNP-mRNA

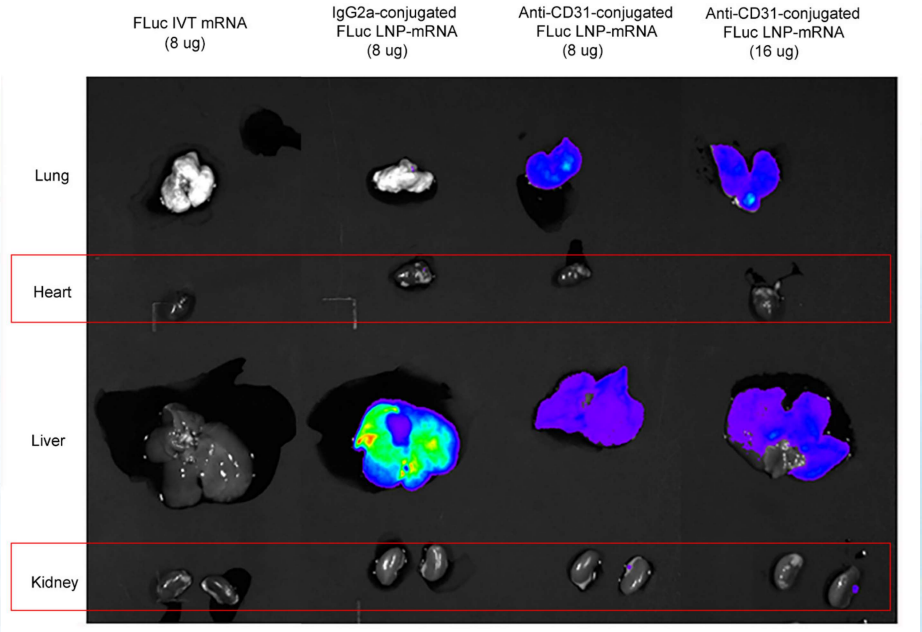


Figure 18. Anti-CD31 conjugated firefly luciferase (FLuc) LNP-mRNA showed improved luciferase expression in lung. Mice strain: C57BC/6J; mice age: 6-8 weeks; mice gender: female; administration route: tail vein. Negative controls: IgG2a-conjugated FLuc LNP-mRNA and naked FLuc mRNA.

Additional Offerings

In addition to mRNA, VectorBuilder offers IVT Cas9 mRNA and sgRNA for CRISPR gene editing as well as self-amplifying mRNA (saRNA). LNP formulations can be used to encapsulate IVT mRNA molecules, siRNA, and plasmid DNA allowing for multiple methods of gene delivery.

saRNA

Self-amplifying mRNA (saRNA) is a novel technology in the development of drugs and vaccines. While maintaining advantages of conventional IVT mRNA, such as cell-free synthesis, low risk of mutagenesis and fast reprogramming efficiency, IVT saRNA has the major advantage of self-amplification. After entering host cells, saRNA can self-replicate like viruses, improving the expression efficiency of IVT saRNA. saRNA molecules share the same basic elements with conventional mRNA, including a 5' cap, 5' & 3' UTRs and a poly(A) tail, however, saRNA contains a large open reading frame (ORF) at the 5' end that encodes for the non-structrual proteins (nsP1-4) and a subgenomic promoter derived from the genome of the alphavirus. Hence, saRNA are considerably larger (9k-12k nt) than non-amplifying mRNAs. nsP1-4 encodes a replicase which is responsible for rapidly amplifying a gene-of-interest (GOI) driven by the internal subgenomic promoter.

saRNA has many beneficial features, which include low administration dose and the ability to easily modify the antigenic domain, increasing the potential for translation into human use. Compared to conventional mRNA vaccines, saRNA upregulates the expression of a target gene with a much smaller dosage. For example, the administration dose (5 ug) of COVID-19 saRNA vaccine ARCT-154, which is in phase 3 clinical trial, is 6 times smaller compared with the dose of mRNA in the Pfizer-BioNTech vaccine (30 ug) and 20 times smaller than that of Moderna vaccine (100 ug). As saRNA requires a smaller dose for similar efficacy, this reduces overall costs of manufacturing and decreases the potential side effects when receiving the vaccine. These properties make saRNA an attractive therapeutic modality.

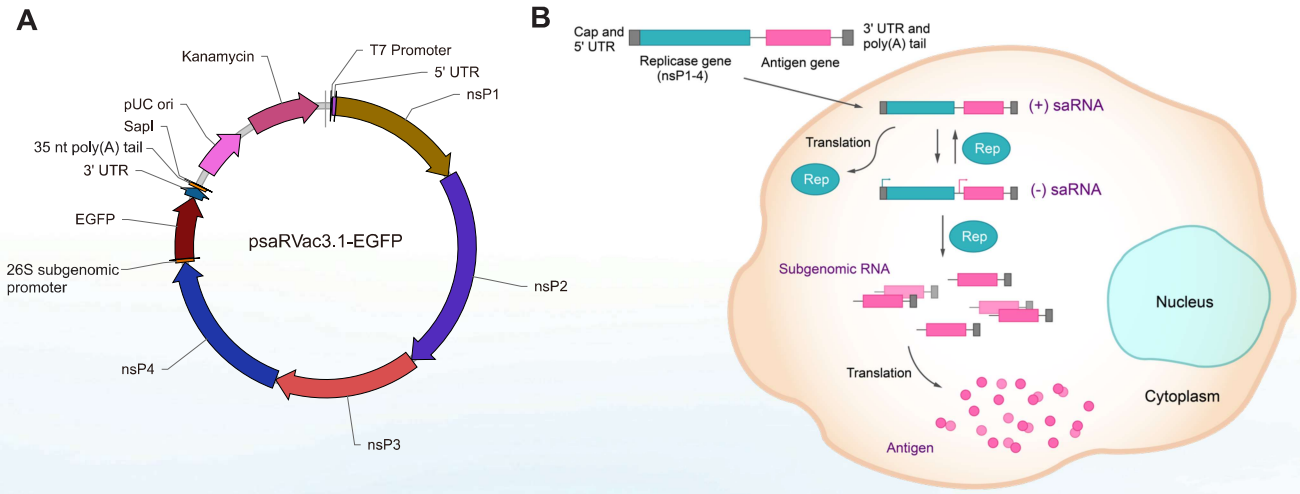


Figure 19. Diagram of saRNA. (A) Example of saRNA backbone design. (B) Mechanism of saRNA. Upon entering into the cytoplasm, the positive-strand saRNA is first translated into nsP1-4 that form the replicase (shown as Rep in the figure) by the host ribosome. nsP1-4 all have their own unique functions. For example, nsP4 is a RNA-dependent polymerase that uses the positive-strand RNA as a template to synthesize the negative-strand saRNA. The negative-strand saRNA then acts as a template for the replicase which recognizes a subgenomic promoter and initiates the synthesis of subgenomic RNA. The subgenomic RNA accumulates in host cells, approaching more than 10^6 copies, which can then be translated into more copies of the antigen.

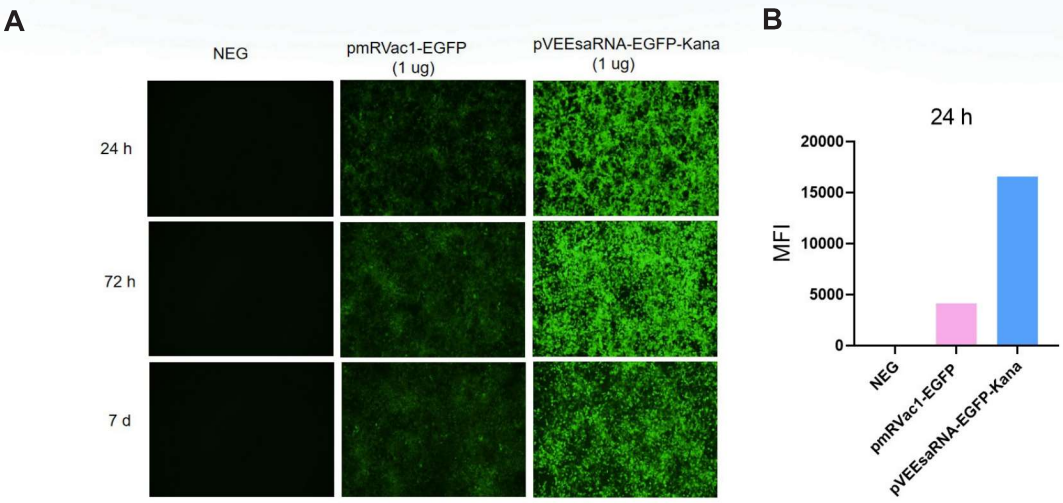
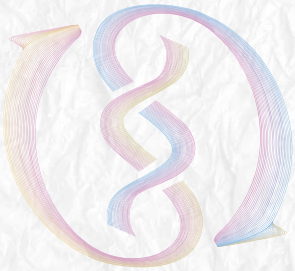


Figure 20. In vitro validation of saRNA. HEK 293T cells grown on a 12-well plate were transfected with 1 ug of EGFP-mRNA or EGFP-saRNA per well. (A) Representative images of EGFP expression at 24 h, 72 h, and 7d post-transfection. (B) MFIs were quantified in HEK 293T cells 24 h post-transfection.



US Headquarters

VectorBuilder Inc.
1010 W 35th Street, Suite 515
Chicago, IL 60609, USA
Tel: 800-517-2189 (9:00 am to 5:30 pm CST)
+1 773-475-7751 (international)
Fax: 408-649-5280
Email: service@vectorbuilder.com (for general inquiry)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)

Japan

VectorBuilder Japan, Inc.
2-12-16, Shin-Yokohama, Kouhoku-ku,
Yokohama, Kanagawa, 222-0033, Japan
Tel: +81-45-628-9207
Email: service-jp@vectorbuilder.com
(for all inquiries including CDMO and CRO services)

UK Contact

VectorBuilder Ltd.
Suite F1 Bush House, Edinburgh Technopole
Penicuik, EH26 0PH, United Kindom
Tel: +44 (0) 7448341295 (9:30 am - 5:30 pm BST)
Email: service@vectorbuilder.com (for general inquiry)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)

Australia Contact

Tel: 1800-719-779 (9:00 am - 5:30 pm AEDT)
Email: service@vectorbuilder.com (for general inquiry)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)

Europe

VectorBuilder GmbH
Martin-Behaim-Str. 15
63263 Neu-Isenburg, Germany
Tel: +49 (0) 6102-2486890 (9:00 am - 5:00 pm CEST)
Fax: +49 (0) 6102-2486891
Email: service@vectorbuilder.com (for general inquiry)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)

China (Mainland)

VectorBuilder China
Building D, 3rd Floor, 3 Juquan Road, Science City
Guangzhou, 510663, China
Tel: +86 20-28069042 (8:45 am - 5:45 pm China Time)
Email: service@vectorbuilder.cn (for general inquiry, Chinese)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)

Korea Contact

Tel: +82-10-8381-9361 (9:00 am to 5:30 pm Korea time)
Email: service-kr@vectorbuilder.com
(for general inquiry, Korean)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)

Israel Contact

Tel: + 34631 821 941 (10:00 am - 6:00 pm IDT)
Email: service@vectorbuilder.com (for general inquiry)
cdmo@vectorbuilder.com (for CDMO inquiry)
cro@vectorbuilder.com (for CRO inquiry)