ARETERNA
A Synthgene Company

Areterna LLC

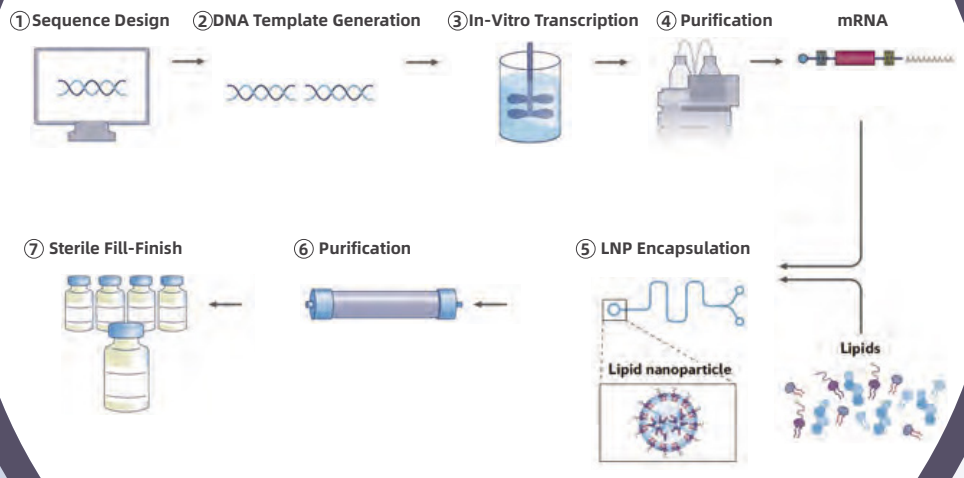
THE STORY OF

GMP CHEMICAL PLASMID SEQUENCE IVT LNP QUALITY CMC BIOLOGY

Background

The success of Pfizer and Modena COVID vaccines has catapulted mRNA to the forefront of drug development. The discovery of messenger RNA (mRNA) dated back to 1961, however, the molecule is not a viable therapeutic modality until the discovery of modified uridine and lipid nanoparticle (LNP) encapsulation. Both modified uridine and Cap1 structure help mRNA evade cell's innate immune system and thereby improve mRNA's stability. LNP protects mRNA in circulation and aids its cellular uptake and endosomal escape. Compared to other biologics, mRNA offers the benefits of simple manufacturing, quick design and no risk of genome integration. Unlike AAV or antibody drugs, the manufacturing of mRNA does not involve any cells. It starts with in-vitro transcription of a DNA template, followed by purification, LNP encapsulation and sterile fill-finish. As a new therapeutic modality, mRNA offers great potential to treat and prevent diseases, from vaccine, to cancer treatment, to protein replacement, gene editing, cell therapy and many more.

Manufacturing Process of mRNA Vaccine



What Areterna Can Do

Products

- Cap Analogs
- NTPs and Modified NTPs
- GMP and Non-GMP Grade
- Custom Options

- mRNA Synthesis Kits
- Capped/Uncapped mRNA Standards
- Residual dsRNA Detection Kits
- Impurity Standards
- Stocked Tool mRNAs
- LNP Screening Plate

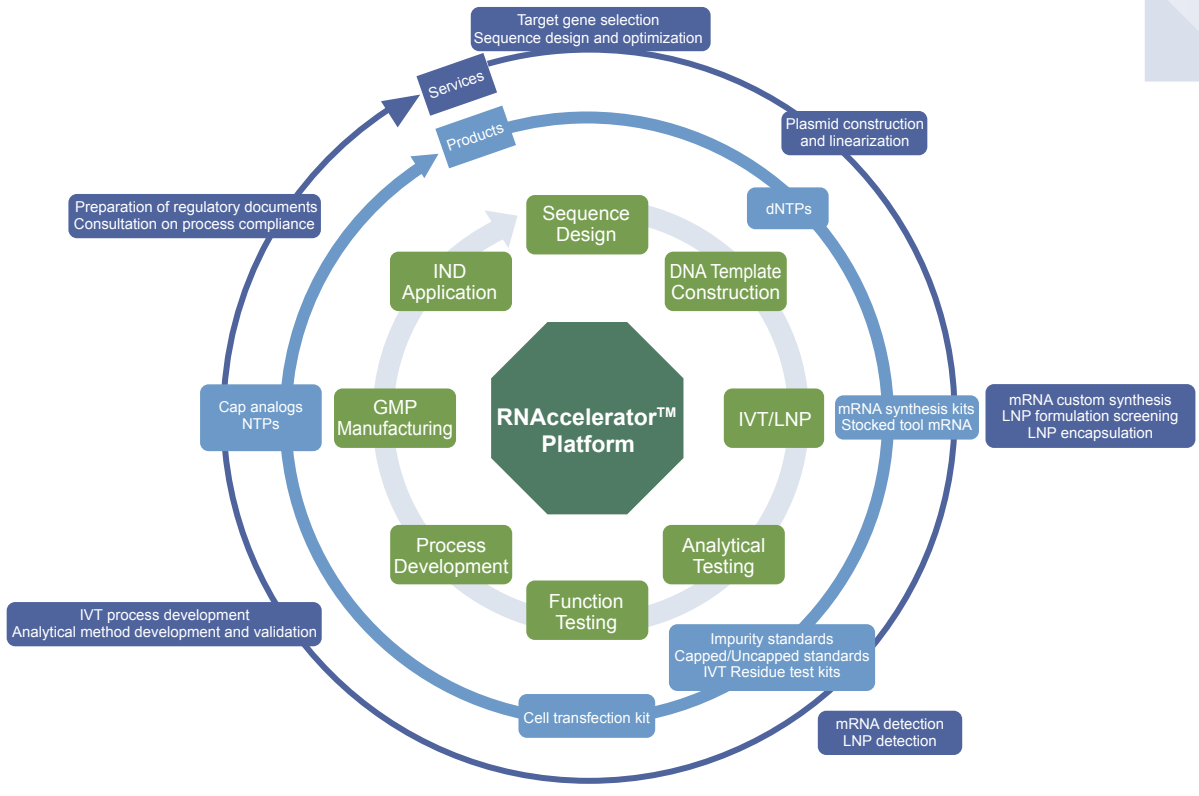
ARETERNA
A Synthgene Company

Services

- mRNA Sequence Design
- mRNA Custom Synthesis
- IVT Process Development
- mRNA Testing
- Analytical Development
- LNP Formulation Screening
- LNP Encapsulation
- IND Filing Support

Technology Platforms

- Sequence Design Platform
- Nucleotide Chemistry Discovery Platform
- IVT Manufacturing Platform
- LNP Encapsulation Platform



1 Sequence Design

Services: Target gene screening
Sequence design and optimization

2 DNA Template Construction

Products: dNTPS

Services: Plasmid construction and linearization

3 In-vitro Transcription

Products: Co-transcription kits

Services: Custom mRNA synthesis

4 LNP Encapsulation

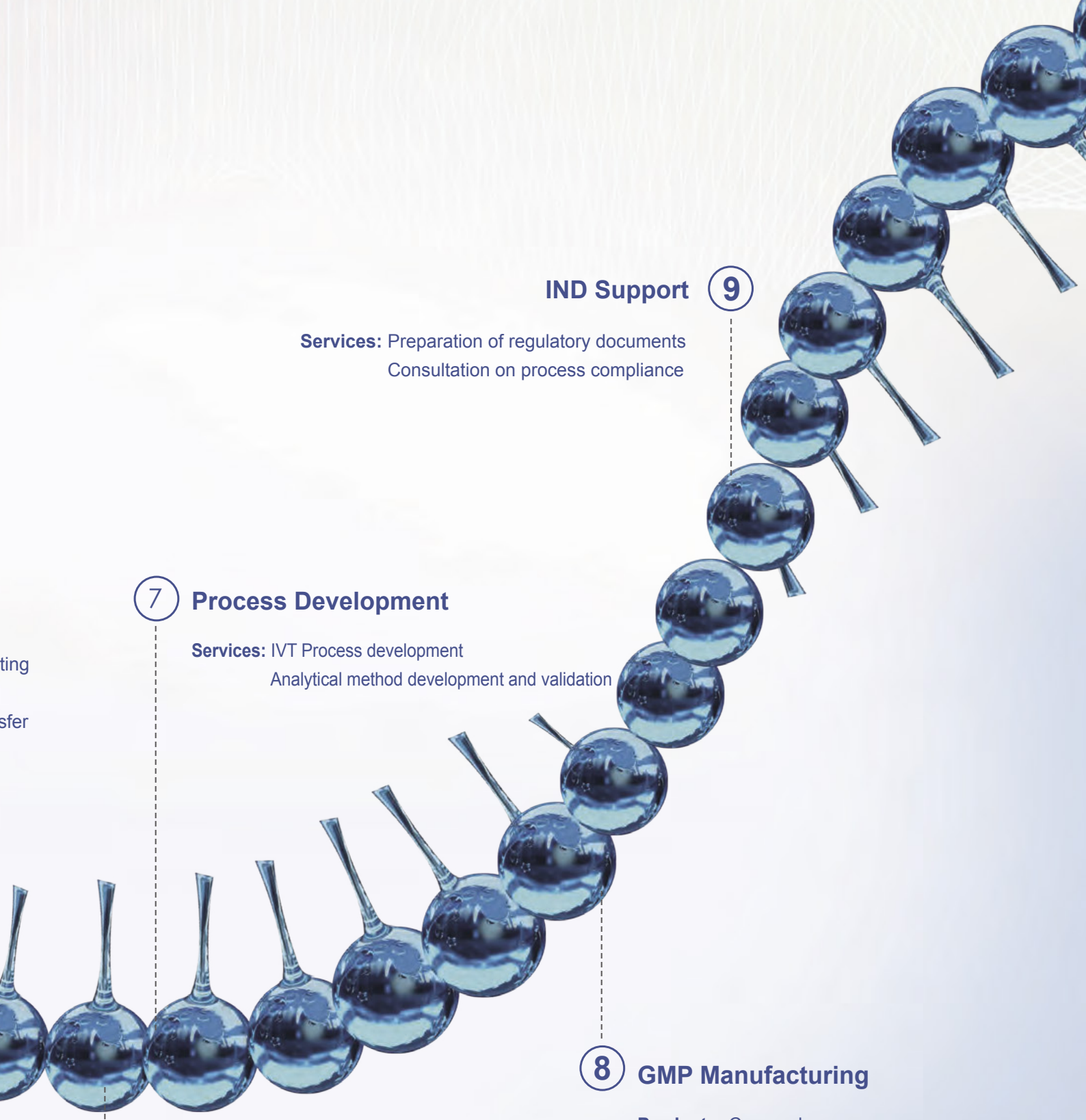
Products: Stocked Tool mRNAs

Services: LNP formulation screening
LNP encapsulation service

5 Analytical Testing

Products:
Impurity standards
Cap1&Uncap standards
Residue test kits

Services: mRNA release tes
mRNA stability testing
Assay development and trans
(e.g. cleavage probe design)



IND Support 9

Services: Preparation of regulatory documents
Consultation on process compliance

7 Process Development

Services: IVT Process development
Analytical method development and validation

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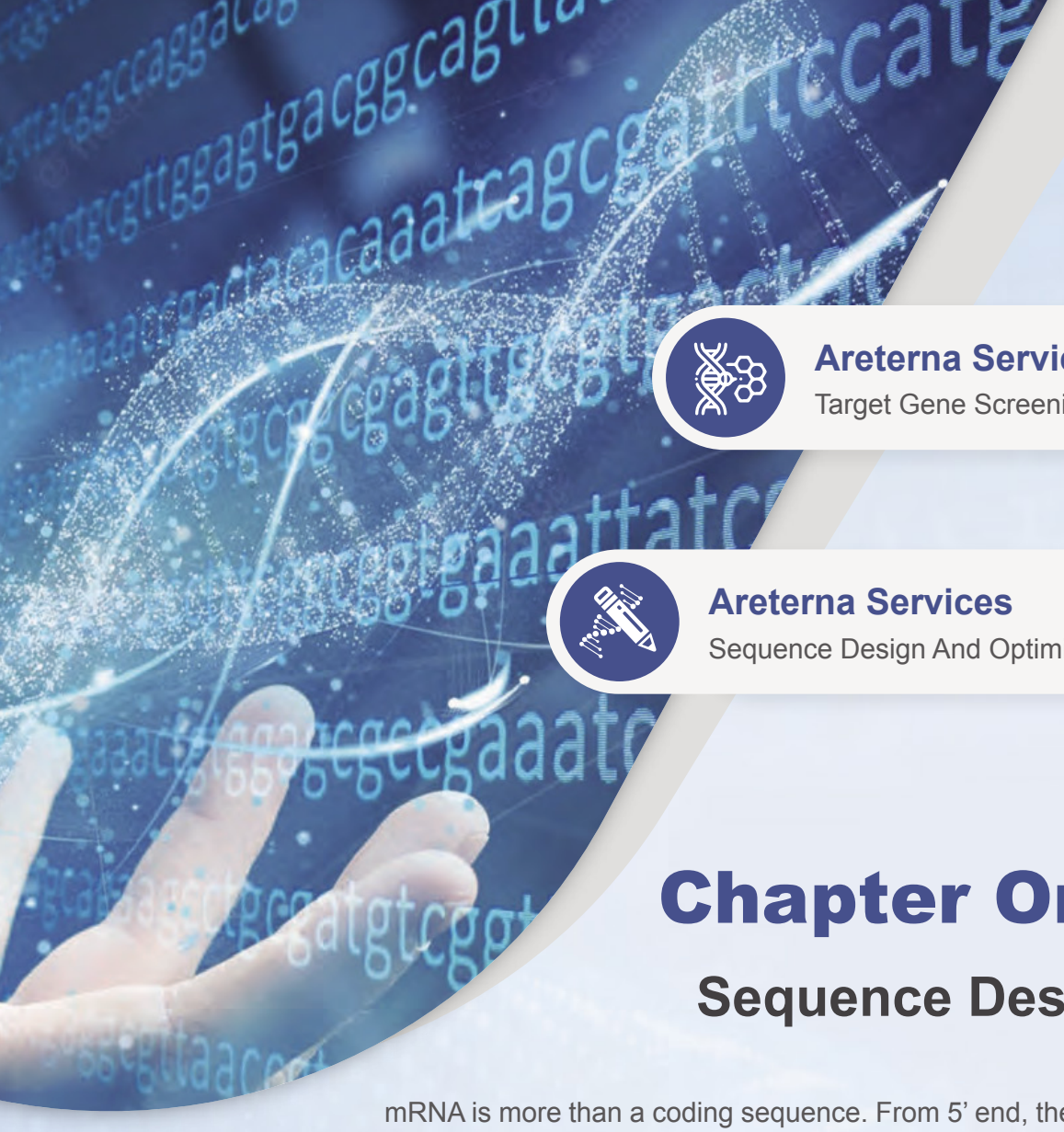
8 GMP Manufacturing

Products: Cap analogs
Cap analog intermediate
Modified nucleotides
Nucleotides
Enzymes

6 Function Testing

Products: Cell transfection kit



Areterna Services
Target Gene Screening

Areterna Services
Sequence Design And Optimization

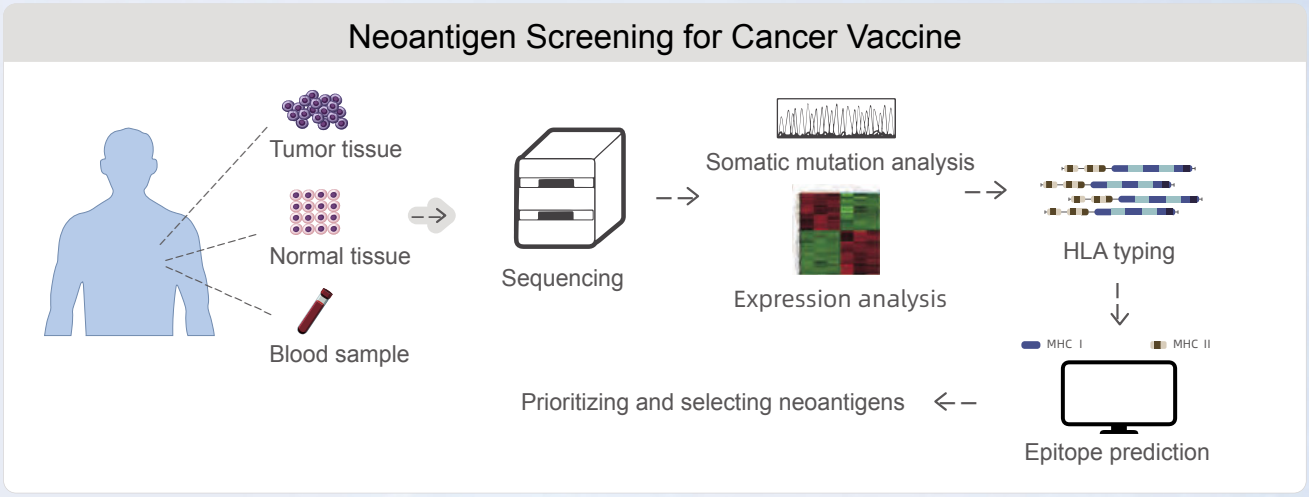
Chapter One

Sequence Design

mRNA is more than a coding sequence. From 5' end, there is a cap structure that binds eIF4E to initiate protein translation, then 5' UTR for ribosome scanning, followed by coding sequence, then 3' UTR that regulates protein expression, and poly-A tail to stabilize mRNA. Each segment can be optimized for optimal protein expression, reduced immunogenicity and better manufacturability. Selecting the right gene or protein to modulate is also critical. One of the challenges in cancer immunotherapy is to identify the appropriate antigen that elicits strong and durable immune response.

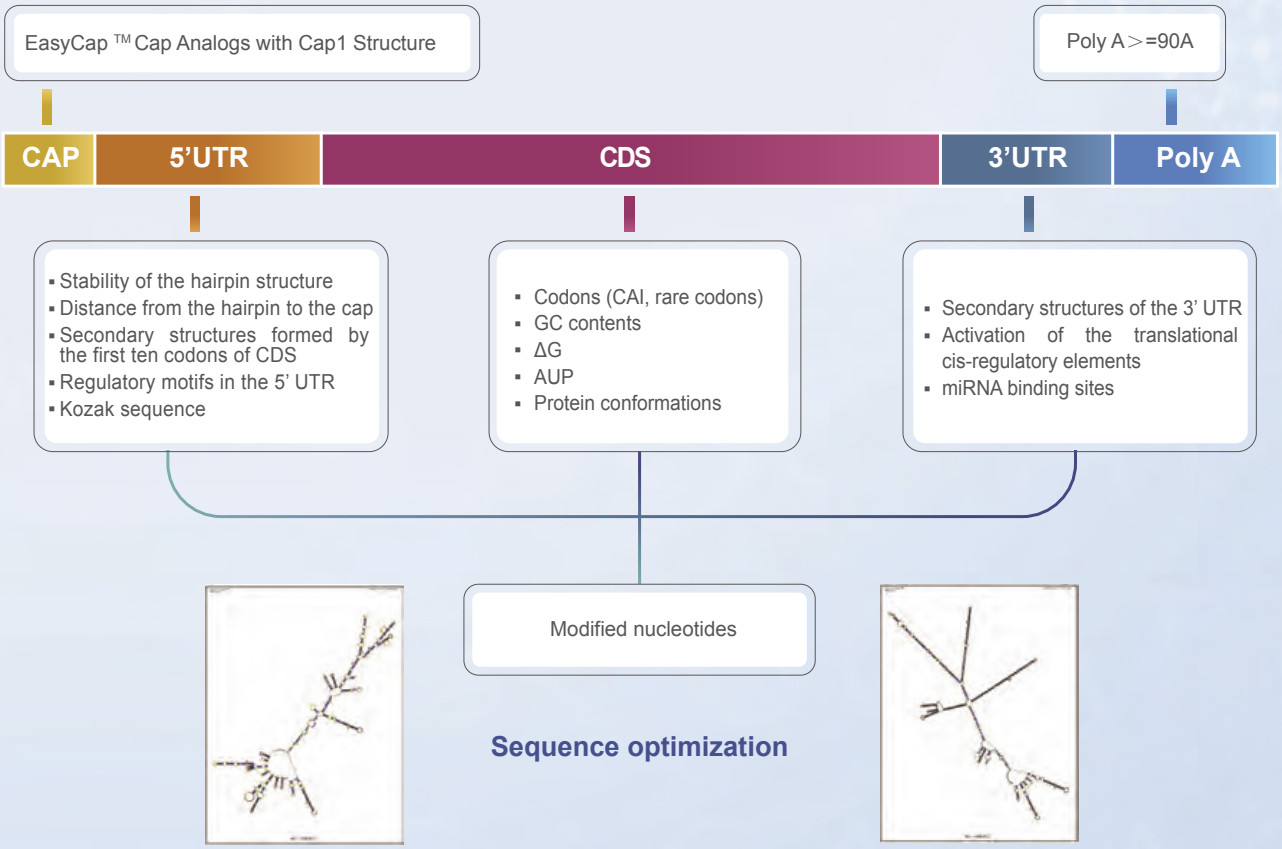
Target Gene Identification

Target gene identification is the starting point for an mRNA drug or vaccine and can be a complex process. Poor choice of target gene can lead to failed drugs and wasted resources. The diagram below is an example of tumor neoantigen selection process. Areterna can help customers to screen and identify the right targets.



Sequence Design and Optimization

Areterna has its own proprietary algorithm for sequence design and optimization. All we need is the amino acid sequence, we can add UTRs, cap and poly-A, and adapt the sequence to specific species if veterinary vaccine is the intended use. We use our proprietary cap analogs to introduce Cap1 structure. The approaches to optimizing UTRs and CDS are listed below-



Areterna Products
dNTPs

Areterna Services
Plasmid Construction And Linearization

Chapter Two

DNA Template Construction

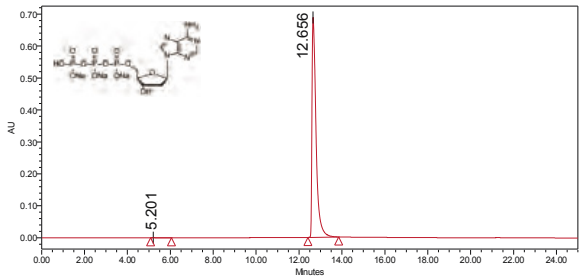
To synthesize mRNA, a DNA template is needed. It can be a linearized plasmid or a PCR product. For small scale synthesis, PCR products are often used. However, PCR is not scalable, so plasmid becomes the template of choice for large scale GMP manufacturing. Regardless of DNA source, the template should contain T7 Polymerase promoter sequence in addition to the 5 parts of mRNA. One confusing point for mRNA novices is that the cap is not included in the DNA template as the inverted G is added by the cap analog or enzymatic reaction. If linearized plasmid is used, care should be taken to avoid linearization site in the sequence.

dNTPs

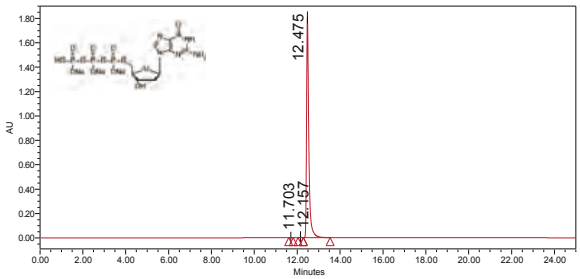
Areterna offers high quality dNTPs.

✓	High purity	Purity verified by HPLC, higher than 99%
✓	High quality	Ensuring batch stability and freeze-thaw stability
✓	High sensitivity	No DNase, RNase and cleavage enzyme residues
✓	High specificity	No bacterial or human genome contamination

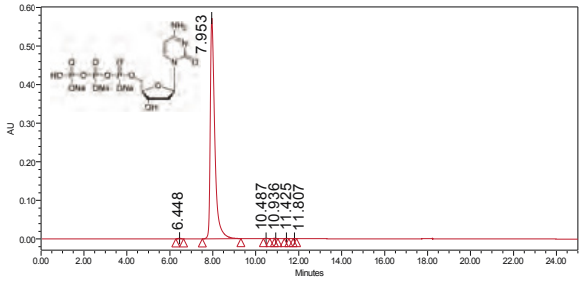
dATP | Purity 99.95%



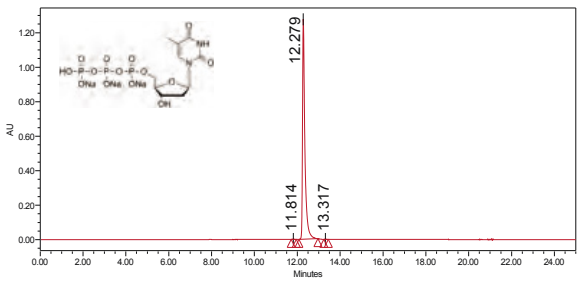
dGTP | Purity 99.81%



dCTP | Purity 99.71%



dTTP | Purity 99.82%



dNTPs Product List

Cat. No.	Product Name
dNTP001	HiPure® dATP
dNTP002	HiPure® dGTP
dNTP003	HiPure® dCTP
dNTP004	HiPure® dTTP
dNTP005	HiPure® dUTP
dNTP006	HiPure® dNTP Mix (25mM each)
dNTP007	HiPure® dNTP Mix (10mM each)
dNTP008	HiPure® dNTP Mix (2.5mM each)

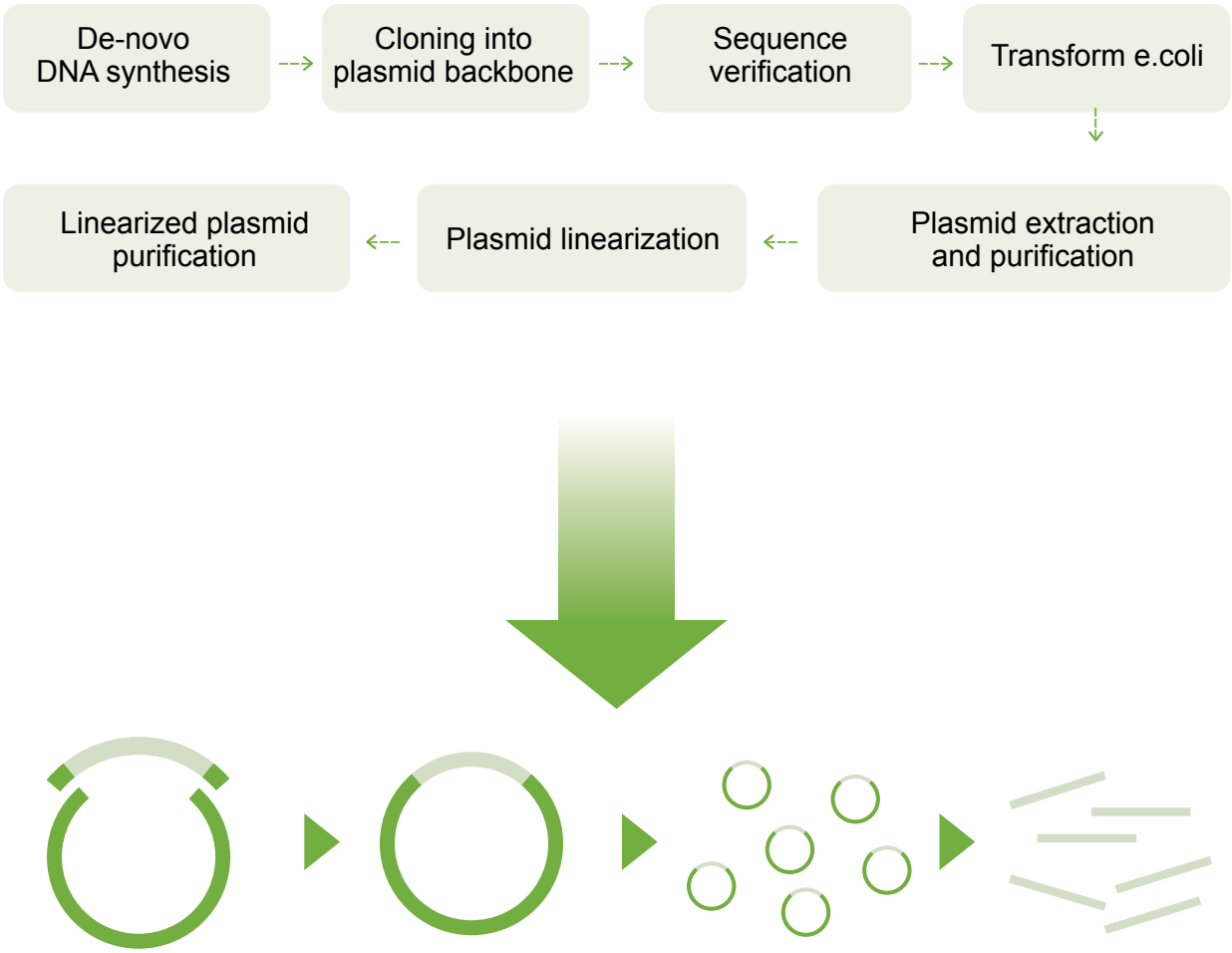
Cat. No.	Product Name
dNTP009	HiPure®Cy3-dUTP
dNTP010	HiPure®Biotin-11-dUTP
dNTP011	HiPure® 3'-ONH_2 -dATP
dNTP012	HiPure® 3'-ONH_2 -dGTP
dNTP013	HiPure® 3'-ONH_2 -dCTP
dNTP014	HiPure® 3'-ONH_2 -dTTP
dNTP015	HiPure® Fluorescein-12-dUTP

2 ARETERNA SERVICES

Plasmid Construction and Linearization

To help customers with their mRNA drug development, Areterna offers plasmid synthesis service from microgram (ug) to milligram (mg) quantities. The linearized plasmid will be sequence verified and ready to go into IVT reaction.

Flow Chart of Plasmid Construction and Linearization



Chapter Three

In Vitro Transcription

In-Vitro Transcription (IVT) is the core of mRNA synthesis. It utilizes T7 or SP6 RNA Polymerase to incorporate NTPs into the growing mRNA chain. Cap can be added enzymatically post-IVT or in a one-pot co-capping reaction. The enzymatic capping is a 4-step process using vaccina capping enzyme and 2'-O-methyl transferase, the capping efficiency can be very high if the process is optimized. One-pot co-capping requires less steps and less unit of operation. The capping efficiency varies depending on the cap analogs used. Anti-Reverse-Capping-Analog (ARCA) is the first-generation cap analog which yields a Cap0 structure with moderate capping efficiency. GpppAG cap analog and its variation such as Areterna's GAG (UNA) and GAG (ENE) offer high capping efficiency and Cap1 structure, therefore have become the cap analogs of choice for many applications. To reduce the immunogenicity of mRNA, modified uridine or cytidine can be used in the IVT reaction.

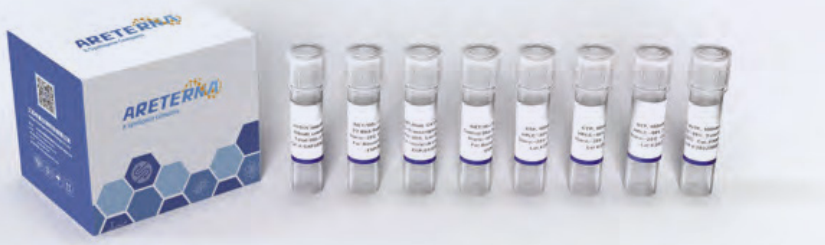
Areterna Products
Mrna Co-transcription Kits

Areterna Services
mRNA Custom Synthesis

3 ARETERNA PRODUCTS

Co-transcription Kits

In order to help bench scientists to quickly synthesize mRNA for research, Areterna introduced a T7 Co-transcription mRNA Synthesis Kit, with our novel Cap1 cap analogs and N1-methyl pseudo uridine. The kit contains all components needed for IVT except the DNA template.



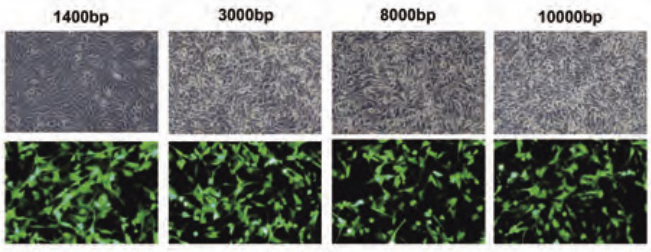
✓ Ease of use	Cap1 mRNA can be synthesized in one step
✓ High yield	up to 200 μg of mRNA can be generated from 1μg DNA template
✓ High capping efficiency	capping efficiency of > 95% can be achieved
✓ Reduced immunogenicity	by replacing wild-type UTP with N1-Me-pUTP

Recommended IVT Conditions

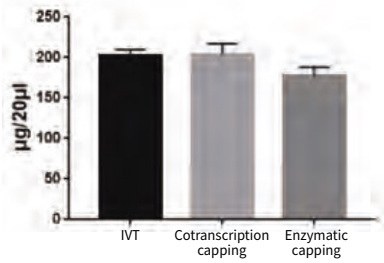
10*Reaction Buffer	2 μL
100mM ATP Solution	2 μL
100mM CTP Solution	2 μL
100mM GTP Solution	2 μL
Reagent A	2 μL
DNA Template	1 μg
Reagent B	2 μL
T7 RNA polymerase (250U/μL)	1 μL
Inorganic Pyrophosphatase (1U/μL)	0.04 μL
RNase inhibitor (40U/μL)	1 μL
RNase free ddH_2O	up to 20 μL

Co-transcription Kit Performance

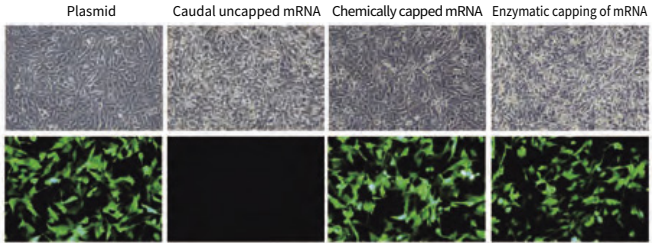
The HiSynth® T7 Co-transcription mRNA Synthesis Kit works with short and long DNA templates. We've seen high protein expression from mRNA constructs ranging from 1.4kk to 10kb. Compared to enzymatic capping method, the kit yields the similar amount of mRNA which shows higher activity in the data below.



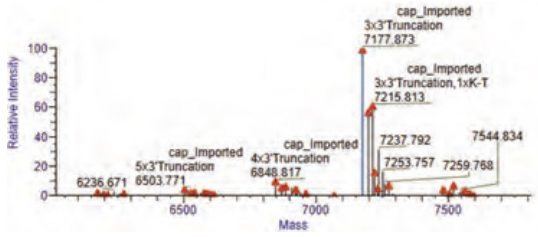
Accommodate Templates with various sizes



Similar Yiled as Enzymatic Capping



High activity of mRNA products



Capping efficiency ≥95%

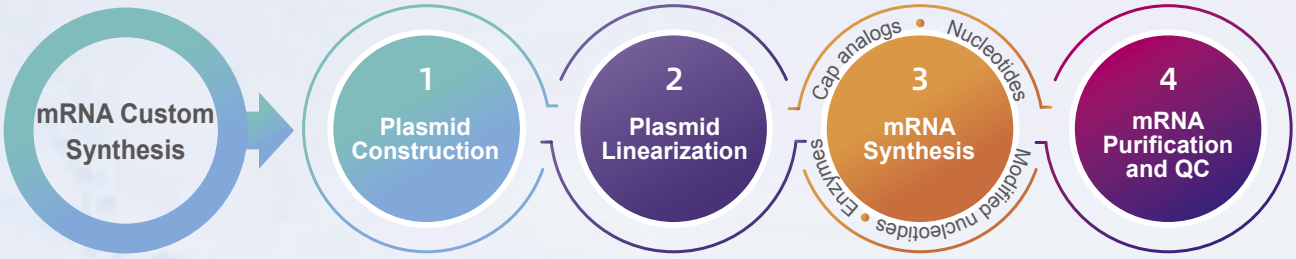
The IVT and Capping Kit Product List

Cat. No.	Product Name
10110U	HiSynth® T7 High Yield RNA Synthesis Kit (AGCU)
10110N	HiSynth® T7 High Yield RNA Synthesis Kit (AGCN)
10111-4011	HiSynth® T7 Co-transcription RNA Synthesis Kit(4011)
10111-5011	HiSynth® T7 Co-transcription RNA Synthesis Kit(5011)
10111-6011	HiSynth® T7 Co-transcription RNA Synthesis Kit(6011)
10111-7011	HiSynth® T7 Co-transcription RNA Synthesis Kit(7011)
10111-8011	HiSynth® T7 Co-transcription RNA Synthesis Kit(8011)
10111-9011	HiSynth® T7 Co-transcription RNA Synthesis Kit(9011)
10112	HiSynth® Cap 1 Capping System

3 ARETERNA SERVICES

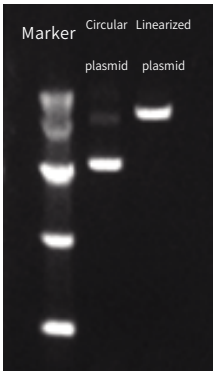
mRNA Custom Synthesis

For customers who do not have time or expertise to synthesize mRNA, Areterna offers custom synthesis service using our HiTrans® IVT platform. We can start with digital sequence and deliver purified mRNA in milligram (mg) and gram (g) quantities with accompanying QC data.

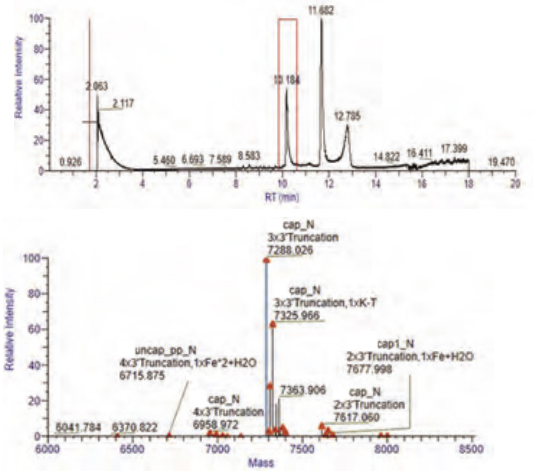


Examples of Custom Synthesized mRNA

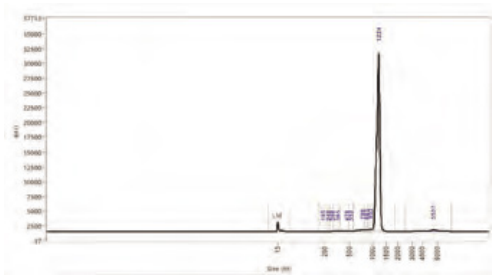
Quality of Linearized Plasmid



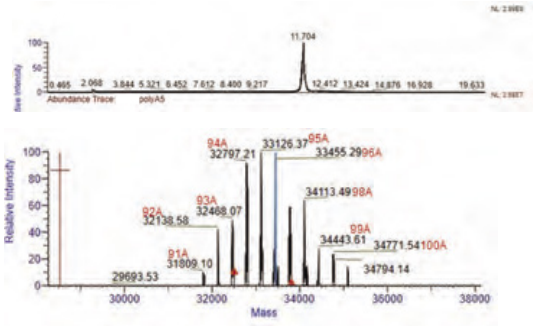
Capping Efficiency by LC-MS



mRNA Integrity by Fragment Analyzer

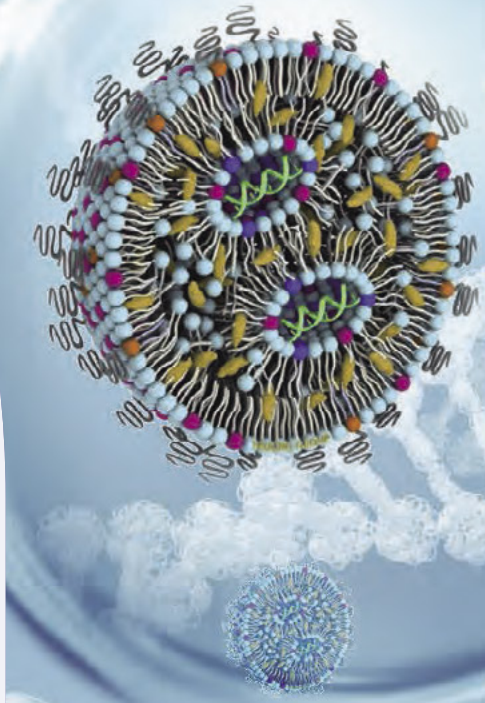


Poly A tail length and distribution by LC-MS



Chapter Four

LNP Encapsulation



Naked mRNA is vulnerable to digestion by circulating nucleases and has poor intracellular uptake properties due to its size and charge. Lipid nanoparticle (LNP) is used to protect mRNA and facilitate its delivery. LNPs are typically composed of four lipids - cationic or ionizable lipids, cholesterol, phospholipids, and PEG-lipids. Ionizable lipids are essential for mRNA complexation by forming a core around negatively charged mRNA. They remain neutral in circulation but become protonated in endosome which facilitate mRNA's endosomal escape. PEG-lipids play an important role in extending LNP's half-life in circulation and preventing particle aggregation. Phospholipids and cholesterol contribute to the structural integrity of the LNP. The generation of LNP relies on rapid mixing of mRNA in aqueous phase with lipids dissolved in ethanol.

Areterna Products
Stocked Tool mRNAs

Areterna Services
LNP Formulation Screening

Areterna Services
LNP Encapsulation

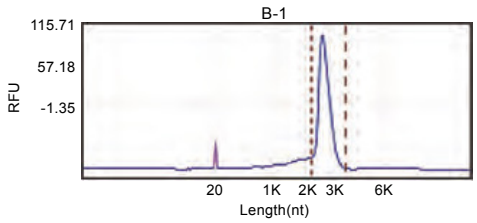
4 ARETERNA PRODUCTS

Stocked Tool mRNAs

Areterna stocks a list of “Tool” mRNAs for LNP encapsulation and delivery research, including eGFP mRNA, FLuc mRNA, OVA mRNA and Fluc-eGFP mRNA. The beauty of Fluc-eGFP mRNA is that it can be assayed both in cells (eGFP) and in mice (FLuc). The construct is longer than FLuc and can be a better surrogate for longer mRNAs.

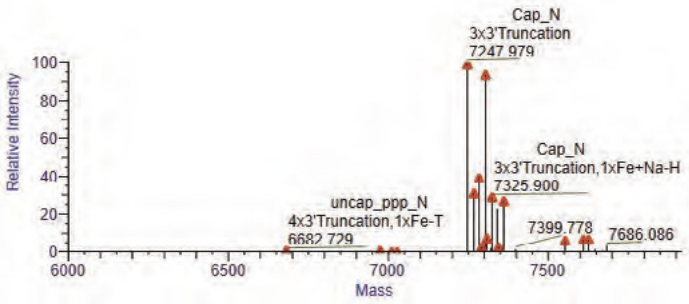
FLuc-eGFP mRNA Data

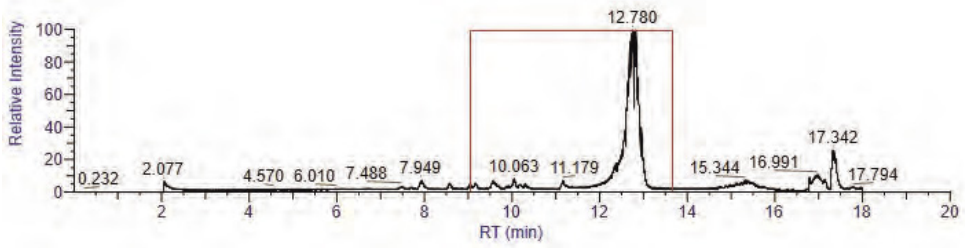
01 mRNA Integrity



Fluc-eGFP mRNA integrity > 90%
by Fragment Analyzer

Capping Efficiency 02

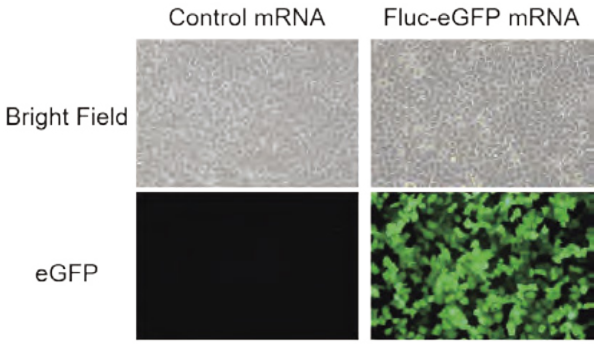


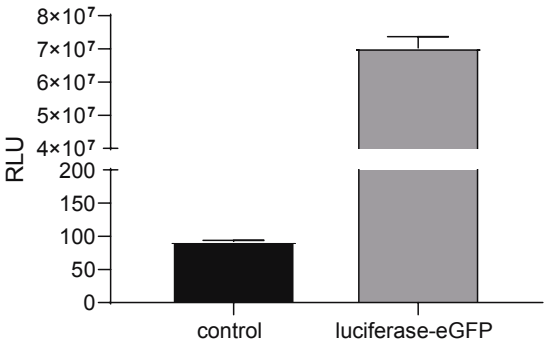


Capping Efficiency > 95% by LC-MS

03

mRNA Activity





Cells were transfected with Fluc-eGFP mRNA. Both GFP and luciferase show strong signal 24 hours post transfection.

Stocked Tool mRNAs

PRODUCT NAME
HiSignal® eGFP mRNA
HiSignal® Fluc mRNA
HiSignal® Fluc-eGFP mRNA
HiSignal® mCherry mRNA mRNA
HiSignal® OVA mRNA

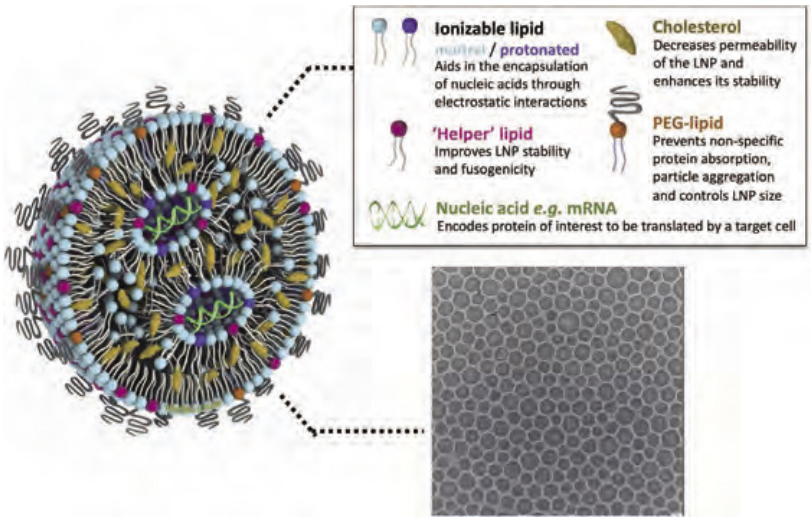
4 ARETERNA SERVICES

LNP Formulation Screening
LNP Encapsulation Service

Areterna has a library of ionizable lipids and makes it available to customers for screening. The LNP formulation screening can be conducted at Areterna or at customer's site. To help customers with mRNA research, Areterna also offers LNP encapsulation service should customers choose to do so.

Schematic Diagram of Typical LNP Formula

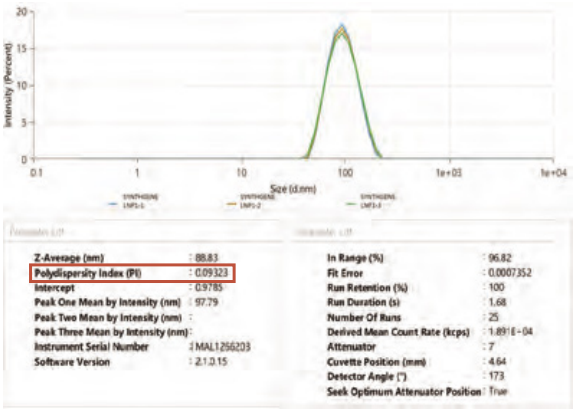
LNPs are typically composed of four lipids - cationic or ionizable lipids, cholesterol, phospholipids, and PEG-lipids.

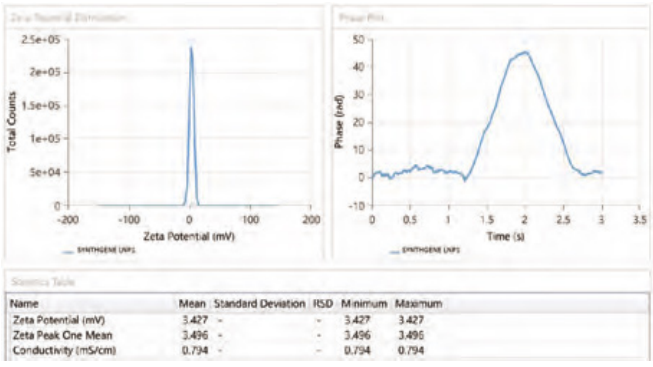


Reference: Acta Biomater. 2021 Sep 1;131:16-40. doi: 10.1016/j.actbio.2021.06.023.

LNP Encapsulation

Particle Size 60-150nm | PDI < 0.2 | Encapsulation Efficiency > 90%





Particle Size and PDI | Zeta Potential

Chapter Five

Analytical Testing

To release an mRNA drug substance or drug product, a set of testing needs to be performed to monitor critical quality attributes, process-related residuals and safety. Some tests are standard, such as appearance, pH, endotoxin, bioburden and sterility. Key attributes that are unique to mRNA include characterization of the 5' cap and poly-A tail to ensure mRNA translation in vivo, as well as assessment of dsRNA as a potential cause for immunogenicity. If the mRNA is encapsulated with LNP, additional tests need to be done to measure particle size, polydispersity index, %encapsulation, lipid identify, purity and content.

Areterna Products
Impurity Standards

Areterna Products
Cap1&uncap Standards

Areterna Products
Residue Test Kits

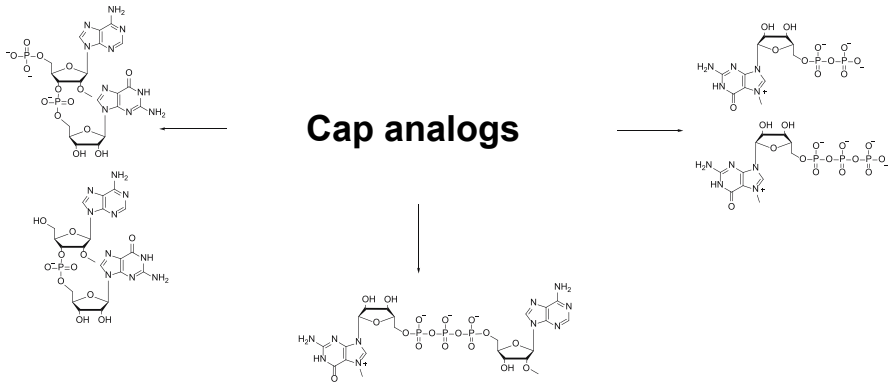
Areterna Services
mRNA Analytical Testing

Areterna Services
LNP Analytical Testing

Impurity Standards

As customers progress from early clinical phase to commercial phase, quality control of starting materials becomes important. Critical impurities need to be identified and tested. To help customers validate impurity testing methods, Areterna offers a variety of impurity standards.

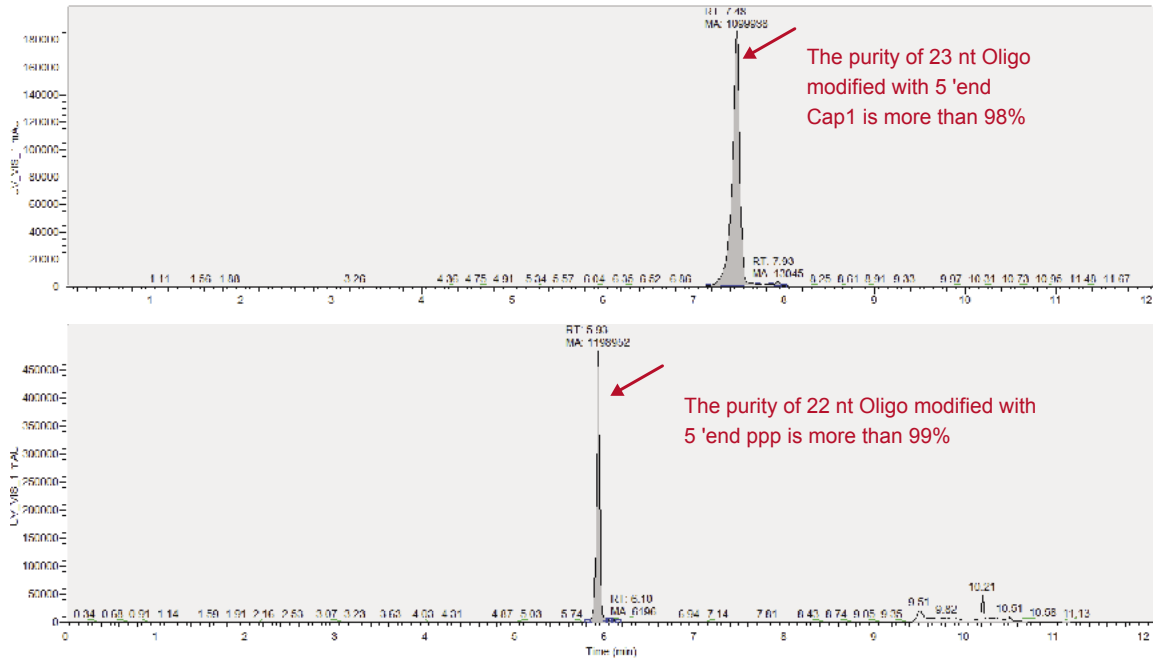
Cap Analog Impurity Standards



Paired Capped /Uncapped mRNA Standards

To assay capping efficiency, a cleavage probe is needed to bind to the 5'UTR of the mRNA and cleave off a short sequence so that the presence or absence of one guanosine can be resolved by LC-MS. Areterna can design the cleavage probe for customers, or develop the capping assay and tech transfer the method, or even run the capping assay for customers. We also custom synthesize paired capped & uncapped mRNA standards for customers to validate their capping assays.

Paired Capped /Uncapped mRNA Standards



ELISA Kits for Detecting IVT Process Residues

Process-related impurities need to be monitored, particularly dsRNA which is considered as a CQA as it can trigger innate immune response. Other IVT related impurities include T7 RNA Polymerase, DNase Ⅰ, RNAase Inhibitor, Inorganic Pyrophosphatase. Areterna offers ELISA kits to test these residues.

ELISA Kits for Detecting
IVT Process Residues

Product Name	Intended Use
T7 RNA Polymerase Test Kit	Quantitative detection of T7 RNA Polymerase content in mRNA stock solution.
DNase I Test Kit	Quantitative detection of DNase I content in mRNA stock solution.
RNase Inhibitor Test Kit	Quantitative detection of RNase Inhibitor content in mRNA stock solution.
Pyrophosphatase Inorganic Test Kit	Quantitative detection of Pyrophosphatase Inorganic content in mRNA stock solution.
dsRNA Test Kit	Quantitative detection of dsRNA content in mRNA stock solution.

mRNA Vaccines/Drugs Enzyme Residue Test Product List

Cat. No.	Product Name
T70001	T7 RNA Polymerase Test Kit
DI0001	DNase I Test Kit
RI0001	RNase Inhibitor Test Kit
PI0001	Pyrophosphatase Inorganic Test Kit
DS0001	dsRNA Test Kit

5 ARETERNA SERVICES

Analytical Method Development
mRNA Release Testing and Stability Testing

Areterna provides full panel release testing and stability testing for mRNA and mRNA/LNP. We can also develop analytical methods such as capping and tailing assays, and tech transfer to customers.

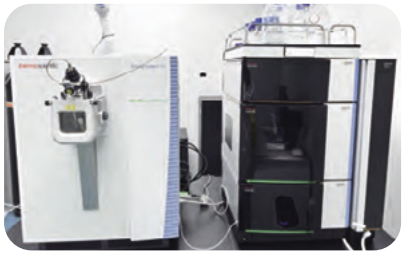
mRNA QC Panel

Test	Method
Appearance	Visual inspection
pH	USP <791>
Bioburden	USP <61>
Endotoxin	USP <85>
Concentration	UV Spectrophotometry (A260)
Identity	Sequencing (Outsource)
Purity & Integrity	CGE /HPLC
Capping Efficiency	LC-MS
PolyA Tail Length	LC-MS
Residual pDNA	qPCR
Residual Protein	NanoOrange /ELISA
Residual NTPs and Cap Analog	HPLC
dsRNA	ELISA

mRNA/LNP QC Panel

Test	Method
Appearance	Visual inspection
Osmolality	USP <785>
pH	USP <791>
Particulate Matter	USP <788>
Endotoxin	USP <85>
Lipid Identity & Content	HPLC- CAD
RNA Content and % Encapsulation)	RiboGreen Assay
Polydispersity Index & Particle Size	Zetasizer
Residual Solvents	GC (FID)
Biological Activity	Flow /ELISA
Sterility	USP <71>

mRNA/LNP Analytical Platforms



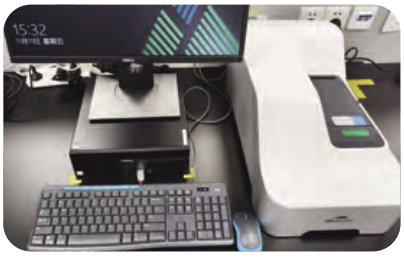
LC-MS

FA5200

HPLC

LC-MS

Tecan Spark



Zetasizer Lab

Areterna Products
Cell Transfection Kit

Chapter Six

Function Testing

During the process of mRNA drug product development, cell based assays are often performed to quickly verify the protein expression from mRNA. Different mRNA constructs will be tested in cells for their activity, potency and toxicity before advancing the final candidates to animal testing.

6

ARETERNA PRODUCTS

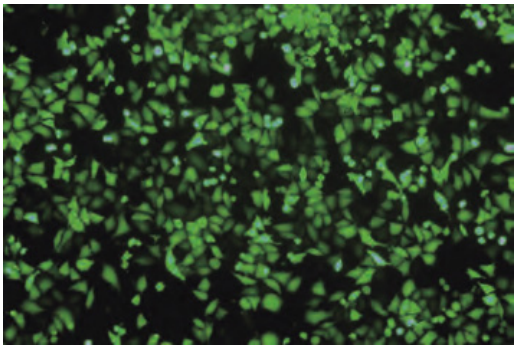
Cell Transfection Kit

HiTrans® mRNA Easy cell transfection kit relies on LNP to complex mRNA and deliver it into cells. The kit has been tested on many commonly used cell lines and was found to offer-

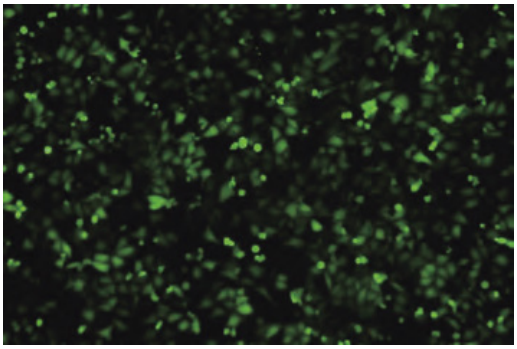
- ✓ High transfection efficiency
- ✓ Low toxicity
- ✓ Good repeatability
- ✓ Cost-effectiveness

Performance Data

Transfection of eGFP mRNA using HiTrans® mRNA Easy kit



Transfection of eGFP mRNA using commercial transfection kit



Hela cells on a 48-well plate were transfected with the HiTrans® mRNA Easy kit or a commercial transfection kit. The eGFP expression was analyzed 24h after transfection. Cells transfected with HiTrans® mRNA Easy kit much stronger eGFP expression.

Areterna Services
IVT Process Development

Areterna Services
Analytical Method Development And Validation

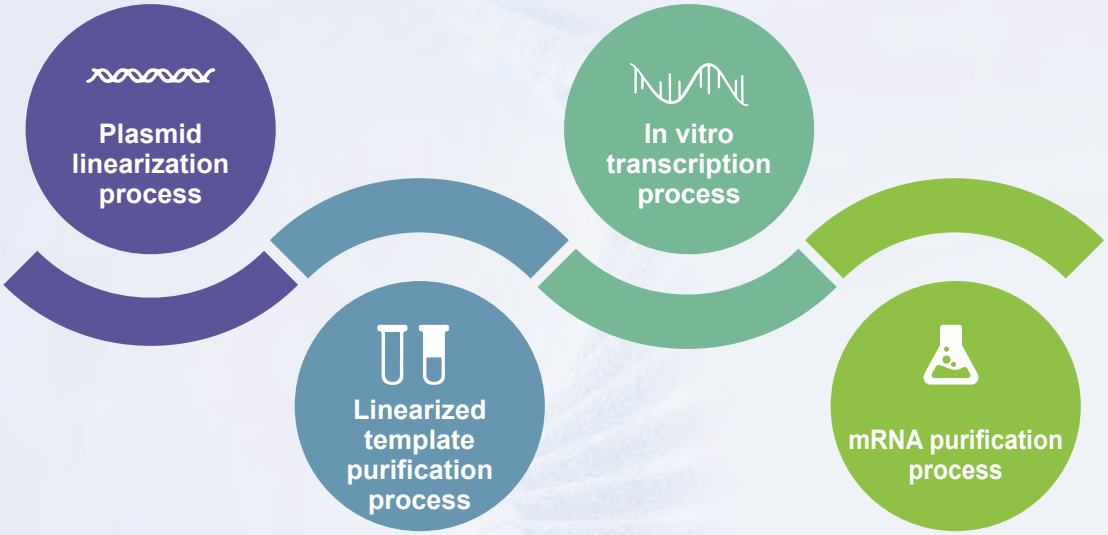
Chapter Seven

Process Development

To advance the research-lab-made mRNA to the clinical stage, one needs to consider the manufacturability of the construct, batch-to-batch consistency and scalability of the process. Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) need to be identified, and the non-compendial analytical methods need to be qualified and validated. Sometimes the construct may need to be redesigned to fit the cGMP manufacturing. Regardless, the IVT process and down-stream purification process need to be investigated and optimized to deliver consistent CQAs.

IVT Process Development

There is no IVT process that fits all. Sometimes trade-offs need to be made to achieve the best purity, highest yield or the lowest dsRNA. Depending on the CQAs desired, many process parameters can be modified, such as [Mg2+], [NTP], reaction temperature, amount of DNA template, and amount of T7 polymerase. To scale up from 20ul reaction in microcentrifuge tubes to 10L reaction in bioreactors, one also needs to consider mixing, heating, and reagent addition, etc. Areterna can help customers with process development and scale up.



Analytical Method Development and Validation

There are a few assays that are unique to mRNA, such as capping efficiency, poly-A tail length and dsRNA content. Capping efficiency assay is construct specific and requires designing of a cleavage probe specific to customer's mRNA. Areterna can develop the analytical methods and transfer to customers.

Test	Method
Residue DNA	qPCR
Residue Protein	Nano Orange
Capping Efficiency	HPLC-MS
mRNA purity	HPLC/CGE
dsRNA content	ELISA
polyA Tail Length	LC-MS
Identity by Oligo Mapping	LC-MS

Chapter Eight

GMP Manufacturing

For clinical trials and commercial use, the mRNA needs to be manufactured following cGMP practice to ensure safety and effectiveness of the product. As mRNA is considered as an Advanced Therapy Medicinal Product (ATMP), there is a phase appropriate approach reflecting the increasing degree of control as the drug moves from Phase I to commercial launch. cGMP manufacturing means tighter control of raw materials and requirement for the raw material supplier to have a robust quality system to support high grade products.Areterna offers GMP grade cap analogs, NTPs and modified NTPs, with Drug Master Files on a selected number of products.

Areterna Products
Cap Analogs

Areterna Products
Modified Nucleotides

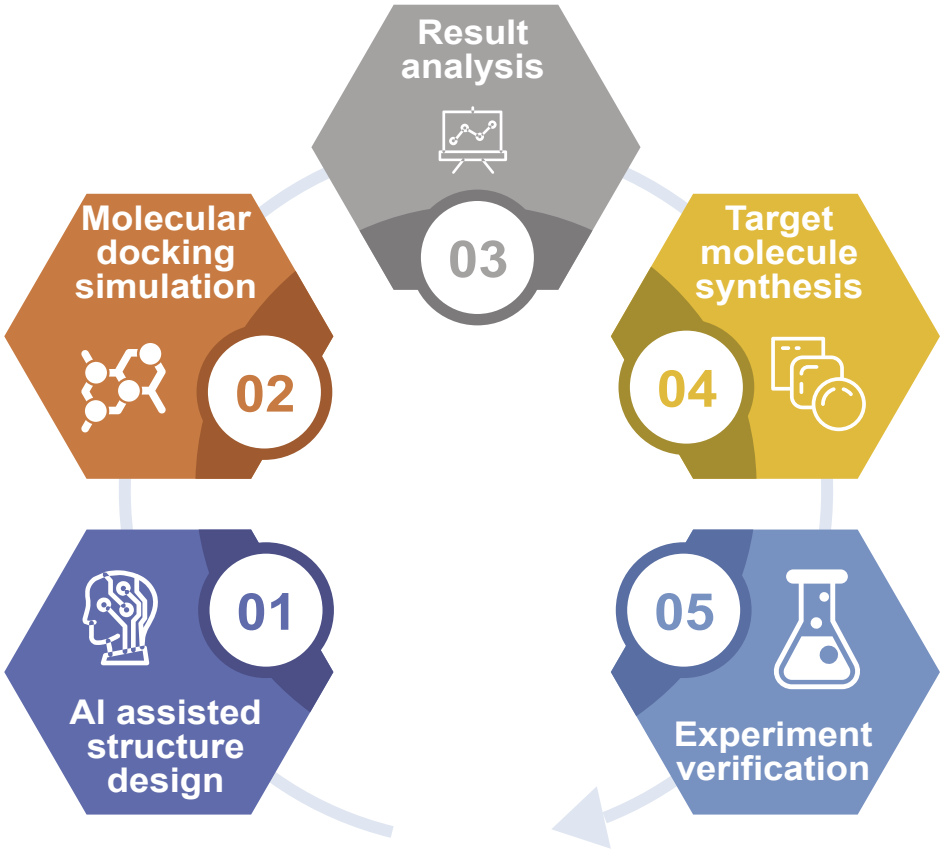
Areterna Products
Nucleotides

Areterna Products
Enzymes

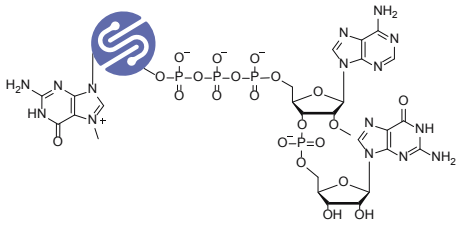
Cap Analogs

Cap analog is considered as a critical stating material for the synthesis of mRNA. In cytosol, the cap structure binds to eIF4E to initiate translation. It also helps to protect mRNA from exonuclease and mediate the turnover of mRNA.

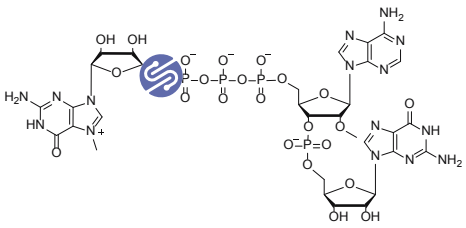
Using AI-assisted structure design and molecular docking simulation, Areterna has developed several novel cap analogs that offer comparable capping efficiency and yield as GpppAG.



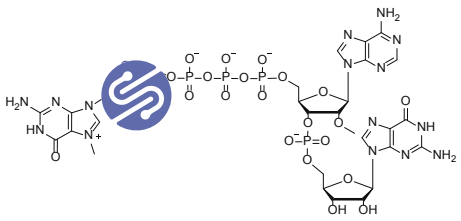
Areterna’s Cap Analogs



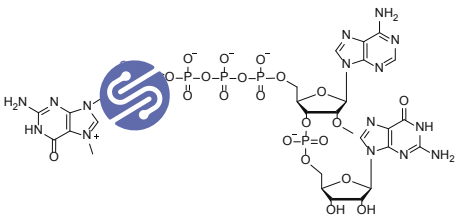
CAP 4



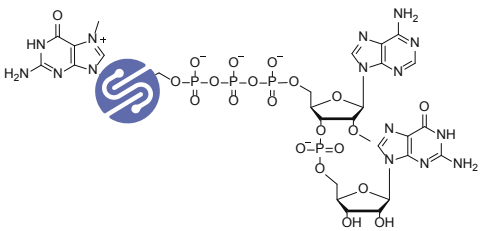
CAP 5



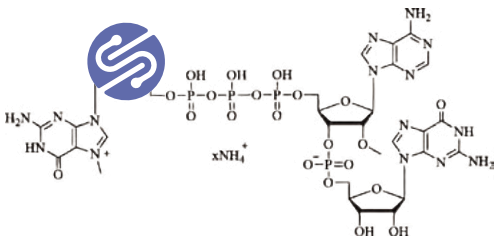
CAP 6



CAP 7



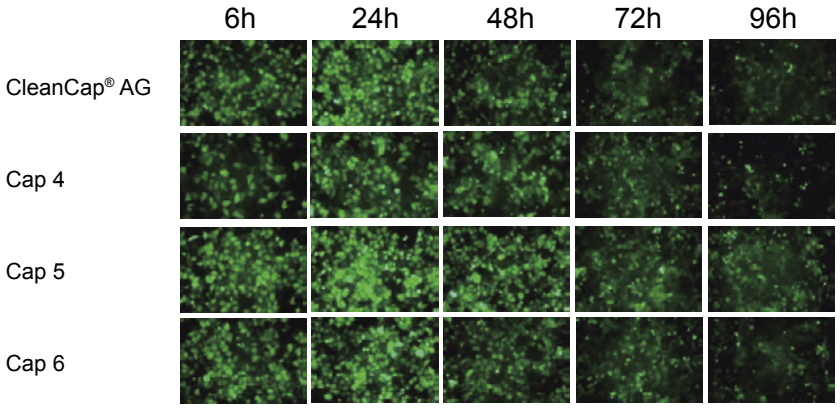
CAP 8



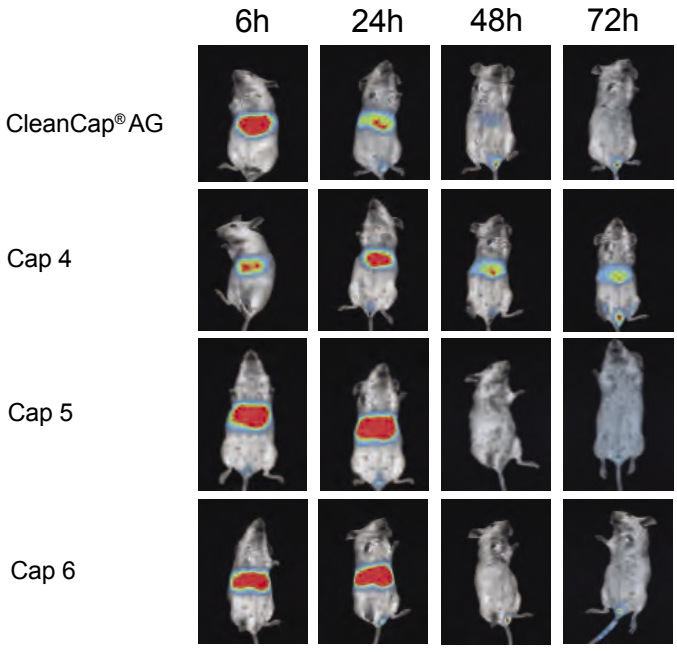
CAP 9

Cap Analogs

Of the new cap analogs, CAP 4 and CAP 5 have shown prolonged protein expres-sion in cells and mice.



eGFP mRNA



luciferase mRNA

Cap Analogs Product List

Cat. No.	Product Name
CAP3130	ARCA
CAP4011	CAP 4
CAP5011	CAP 5
CAP6011	CAP 6

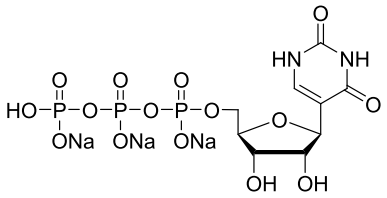
Cat. No.	Product Name
CAP7011	CAP 7
CAP8011	CAP 8
CAP9011	CAP 9

Modified Nucleotides

The commercial success of mRNA vaccine is largely attributable to the discovery of modified uridine by Dr. Kariko and Dr. Weissman. The uridine-rich RNA is recognized by toll-like-receptors as virus and triggers immune response. Replacing wild type uridine with pseudo-uridine or N1-methly pseudo uridine will improve the stability and translational capacity of mRNA. Areterna offers GMP grade pseudo-UTP and N1-Me pUTP*.

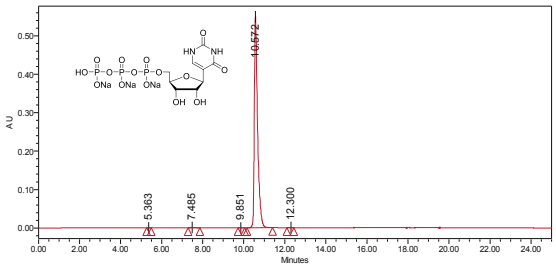
Product Recommendation

Pseudo-UTP



Cat. No.: PUTP001

Purity 99.54%



✓	High purity	>99% by HPLC
✓	Low immunogenicity	Low immunogenicity
✓	High quality	GMP grade/non-GMP grade
✓	High manufacturing capacity	Available in kilogram quantities

Modified Nucleotides Product List

Cat. No.	Product Name
PUTP001	HiPure® Pseudo-UTP 100mM Sodium solution
PUTP001T	HiPure® Pseudo-UTP 100mM Tris solution
PUTP002T	HiPure® Pseudo-UTP 200mM Tris solution
*NMPUTP001	HiPure® N1-Me-pUTP 100mM Sodium solution
*NMPUTP001T	HiPure® N1-Me-pUTP 100mM Tris solution
*NMPUTP002T	HiPure® N1-Me-pUTP 200mM Tris solution
N7MGTP001	HiPure®N7-Me-GTP
5MCTP001	HiPure® 5-Me-CTP
M6ATP001	HiPure® m6A-ATP
5OMUTP001	HiPure® 5-OMe-UTP
CY5UTP001	HiPure® Cy5-UTP

Cat. No.	Product Name
F12UTP001	HiPure® Fluorescein-12-UTP
CY3UTP001	HiPure® Cy3-UTP
2NHATP001	HiPure®2-NH2-ATP
MOEATP001	HiPure® 2'-MOE-ATP
3M5OMUTP001	HiPure® 3-Me-5-OMe-UTP
DM6ATP001	HiPure® 6-N,N-Dimethyl-ATP
SATP001	HiPure® ATP(αS)
DSATP001	HiPure® SPS ATP
5AUTP001	HiPure® 5-Aminpropargyl UTP
3OAATP001	HiPure® 3'O-Allyl-ATP
3OAGTP001	HiPure® 3'O-Allyl-GTP
3OACTP001	HiPure® 3'O-Allyl-CTP
3OAUTP001	HiPure® 3'O-Allyl-UTP

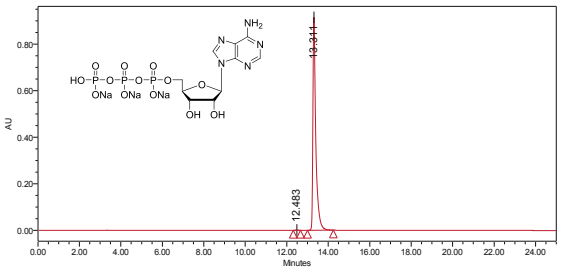
*N1-Me-pUTP has DMF filed at FDA

Nucleotides

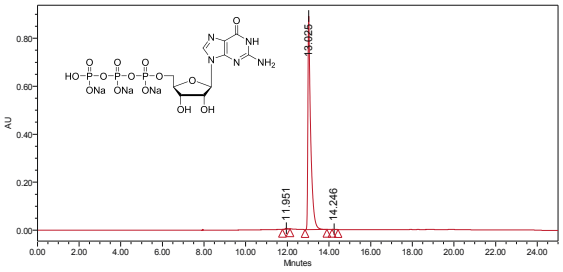
Areterna offers GMP grade NTPs in sodium salt or Tris solution, at 100mM or 200mM.

High Purity Nucleotides≥99%

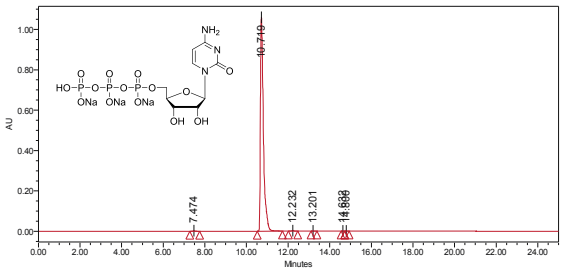
ATP | Purity 99.89%



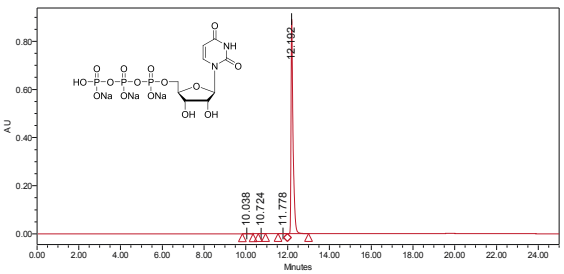
GTP | Purity 99.62%



CTP | Purity 99.53%



UTP | Purity 99.60%



✓	High purity	>99% by HPLC
✓	High quality	GMP grade/ non-GMP grade
✓	High manufacturing capacity	Available in kilogram quantities

Nucleotides Product List

Cat. No.	Product Name
ATP001	HiPure® ATP 100mM Sodium solution
GTP001	HiPure® GTP 100mM Sodium solution
CTP001	HiPure® CTP 100mM Sodium solution
UTP001	HiPure® UTP 100mM Sodium solution
ATP001T	HiPure® ATP 100mM Tris solution
GTP001T	HiPure® GTP 100mM Tris solution

Cat. No.	Product Name
CTP001T	HiPure® CTP 100mM Tris solution
UTP001T	HiPure® UTP 100mM Tris solution
ATP002T	HiPure® ATP 200mM Tris solution
GTP002T	HiPure® GTP 200mM Tris solution
CTP002T	HiPure® CTP 200mM Tris solution
UTP002T	HiPure® UTP 200mM Tris solution

*ATP has DMF filed at FDA

* CTP has DMF filed at FDA

* GTP has DMF filed at FDA

Enzymes

To provide one-stop shop for customers, Areterna offers enzymes required for mRNA synthesis, from linearization enzymes, Dnase I, to T7 RNA Polyermase and vaccina capping enzyme.

Enzymes Product List

Cat. No.	Product Name
10201-0.5K 10201-2.5K 10201-10K	HiTrans® BspQI
10202	HiTrans® BsaI
10203	HiTrans® XbaI
10204	HiTrans® XhoI
10301-10K 10301-100K 10301-250K	HiTrans® T7 RNA polymerase
10302A-0.5 10302A-1 10302A-5	10×Transcription Buffer
10302B-0.5 10302B-1 10302B-5	10×Transcription Buffer
10303-10 10303-100 10303-1K 10303-40K	HiTrans® Inorganic Pyrophosphatase
10304-10K 10304-20K 10304-40K 10304-100K 10304-1M	HiTrans® Murine RNase Inhibitor
10211-0.5K 10211-2K 10211-10K	HiTrans® DNase I

Cat. No.	Product Name
1030M-0.1 1030M-1 1030M-10	HiTrans® T7 RNA polymerase mix
10221-001 10221-010 10221-025 10221-500	HiPure® Proteinase K
10511-005 10511-040 10511-450	HiPure® RNA Isolation beads
10501-005 10501-060 10501-450	HiPure® DNA Isolation beads
10401-2K 10401-10K 10401-100K 10401-5M	HiTrans® Vaccinia capping Enzyme
10402-001 10402-010 10402-025 10402-500	10×Capping buffer
10403-10K 10403-50K 10403-250K 10403-20M	HiTrans® 2-O'-Methyltransferase

Areterna Services
Preparation Of Regulatory Documents

Areterna Services
Consultation On Process Compliance

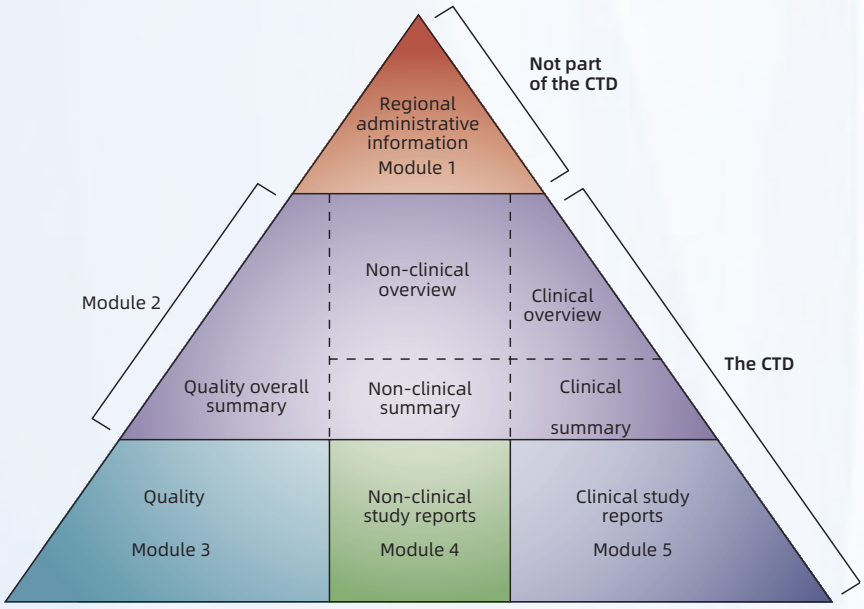
Chapter Nine

IND Support

IND submissions must be filed with regulatory agencies before human clinical trials can start. The submission includes detailed information on the drug's chemical composition, proposed clinical trial design and safety and efficacy data from preclinical studies. One challenge in IND submissions is the requirement for extensive documentation, such as Chemistry, manufacturing and control (CMC) data.

Preparation of Regulatory Documents ARETERNA SERVICES

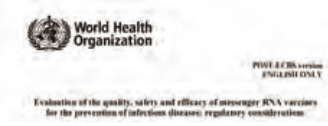
Areterna has worked with many customers in China and assisted them in their IND filing. We can provide data on our raw materials and help to write certain sections for Module 2.3 and Module 3.



PICTURE SOURCE：WWW.ICH.ORG/PAGE/CTD

Consultation on Process Compliance

Regulations and Guidelines for mRNA Vaccines and Drugs



In October 2021, WHO issued guidance on "Evaluation of the Quality, Safety and Efficacy of messenger RNA Vaccines for the Prevention of Infectious Diseases: Regulatory Considerations" which outlined the regulatory considerations for the qualiry, safety and efficacy of mRNA vaccines.

In April 2023, the US Pharmacopoeia (USP) published the second edition of "Analytical Procedures for mRNA Vaccine Quality". The draft guideline proposed testing panels for plasmid DNA, mRNA drug substance and mRNA drug product with both release assays and characterization assays.

In August 2020, CDE released "Technical Guidelines for Pharmaceutical Research of mRNA Vaccines for Prevention of Novel Coronavirus (Trial Implementation)" where CMC requirements for mRNA drug substance and drug product are outlined, covering manufacturing process, product characterization, specification and stability testing among others.

In May 2022, CDE published "Technical Guidelines for Pharmaceutical Research of ex-Vivo Gene Editing (Trial Implementation)" . The guideline covers mRNA as one of the non-viral gene editing methods and provides guidance on mRNA manufacturing, quality attributes and testing for activity and safety.

Materials and Services to Enable mRNA Vaccines and Therapeutics

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Version 2
202309

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