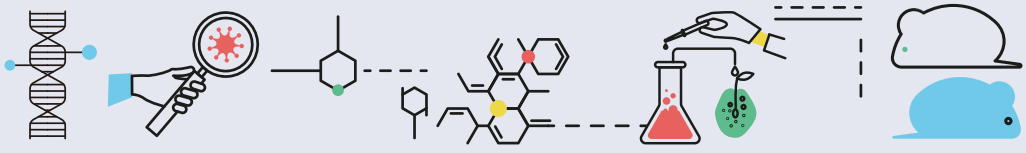
SYNTHGENE

THE STORY OF

mRNA

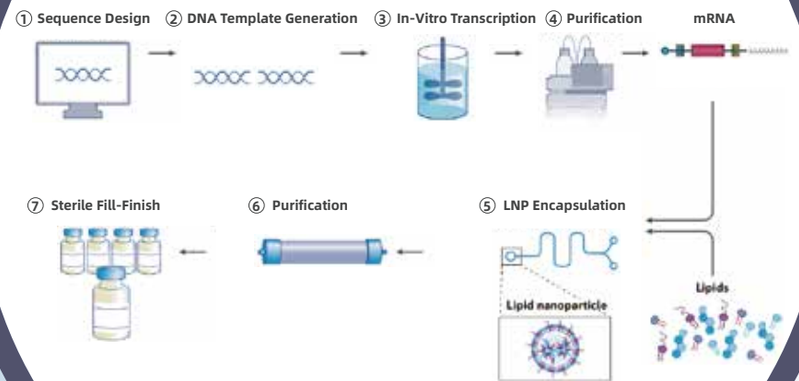
GMP CHEMICAL PLASMID SEQUENCE IVT LNP QUALITY CMC BIOLOGY



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 Synthgene Can Do

Products

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

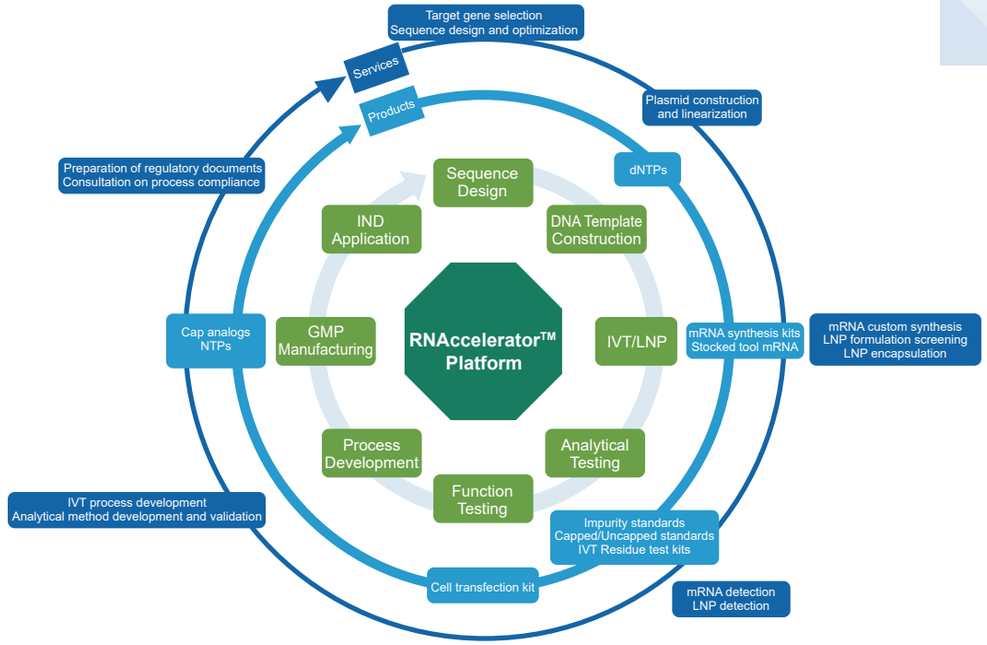
- mRNA Synthesis Kits
- Capped/Uncapped mRNA Standards
- Residual dsRNA Detection Kits
- Impurity Standards
- Stocked Tool mRNAs
- LNP Screening Plate

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 testing
mRNA stability testing
Assay development and transfer
(e.g. cleavage probe design)

6 Function Testing

Products: Cell transfection kit

7 Process Development

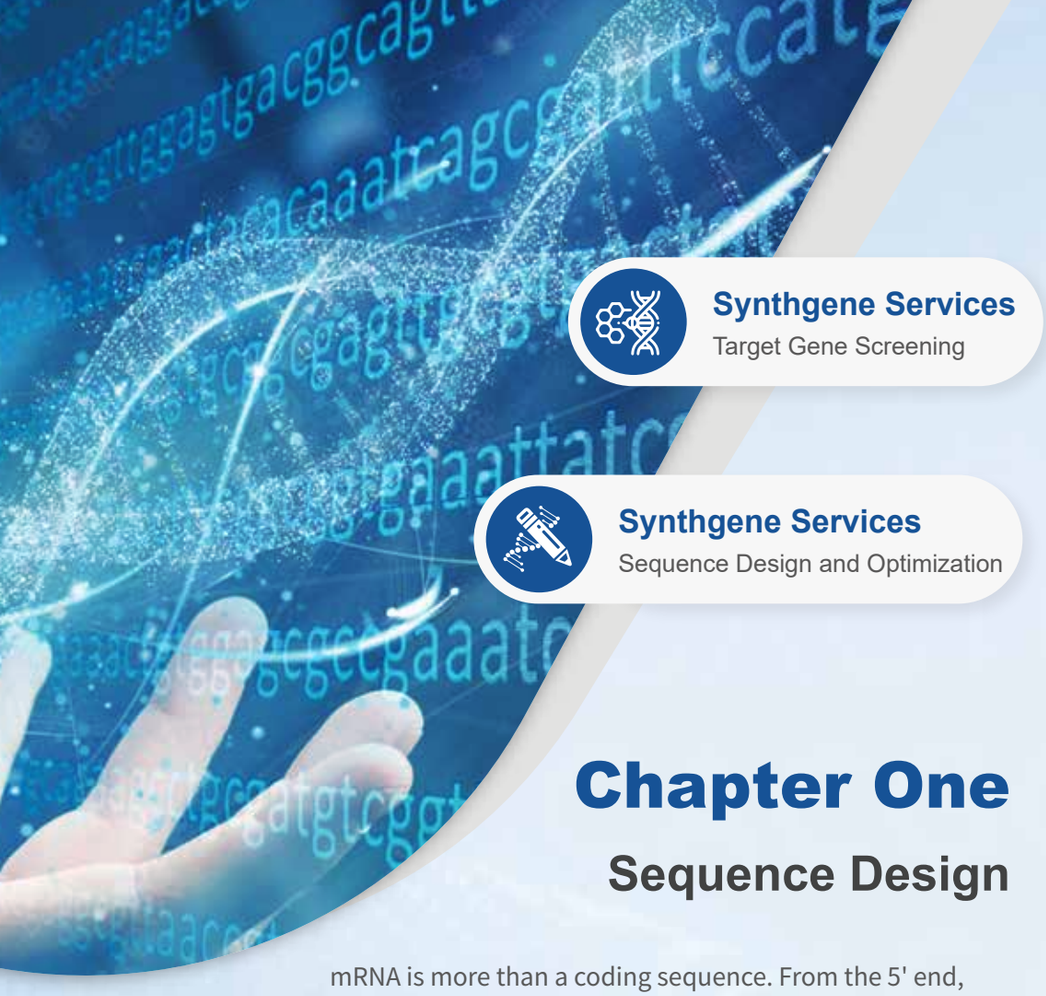
Services: IVT Process development
Analytical method development and validation

8 GMP Manufacturing

Products: Cap analogs
Cap analog intermediate
Modified nucleotides
Nucleotides
Enzymes

9 IND Support

Services: Preparation of regulatory documents
Consultation on process compliance



Synthgene Services
Target Gene Screening

Synthgene Services
Sequence Design and Optimization

Chapter One

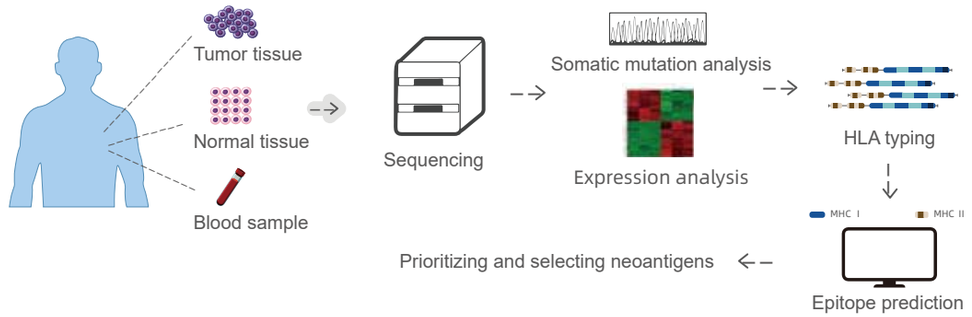
Sequence Design

mRNA is more than a coding sequence. From the 5' end, there is a cap structure that binds eIF4E to initiate protein translation, then a 5' untranslated region (UTR) for ribosome scanning, followed by the coding sequence, then a 3' UTR that regulates protein expression and stability, and a 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 a strong and durable immune response.

Target Gene Identification

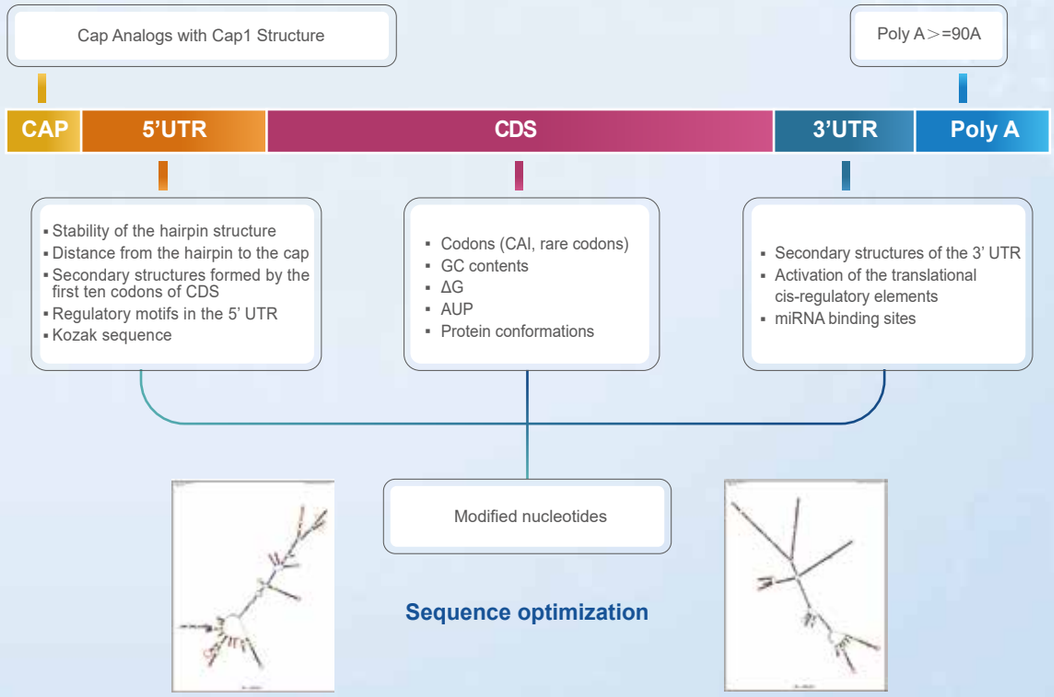
Target gene identification is the starting point for an mRNA drug or vaccine and can be a complex process. Poor choice of target gene can lead to failed drugs and wasted resources. The diagram below is an example of tumor neoantigen selection process. Synthgene can help customers to screen and identify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

Neoantigen Screening for Cancer Vaccine



Sequence Design and Optimization

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



Synthgene Products
dNTPs

Synthgene Services
Plasmid Construction And Linearization

Chapter Two

DNA Template Construction

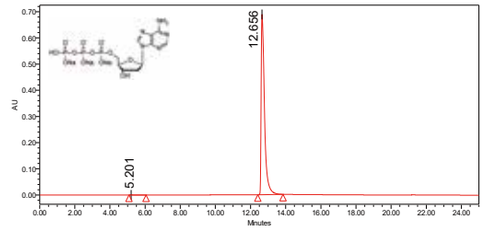
To synthesize mRNA, a DNA template is needed. It can be a linearized plasmid or a PCR product. For small-scale synthesis, PCR products are often used. However, PCR is not scalable, so plasmids are usually the choice for large-scale GMP manufacturing. Regardless of DNA sources, the template should contain a T7 polymerase promoter sequence in addition to the five parts of mRNA. One of the confusions for people who are new to mRNA 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 a linearized plasmid is used, be careful to avoid the linearization site in the sequence.

dNTPs

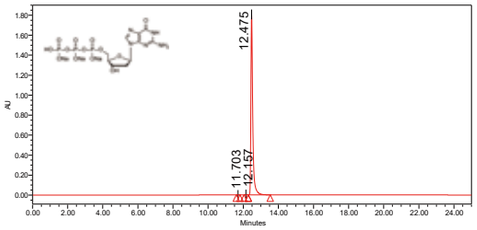
Synthgene offers high quality dNTPs.

✓	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

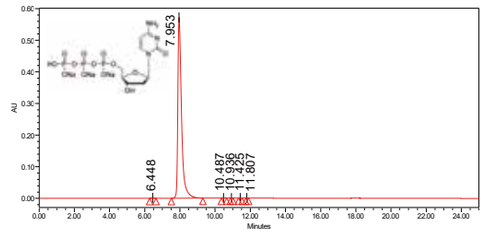
dATP | Purity : 99.95%, HPLC



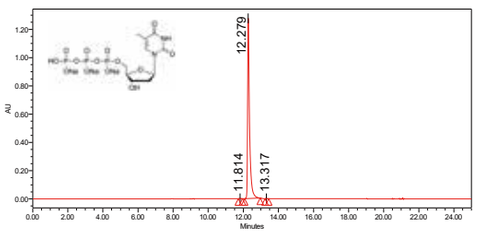
dGTP | Purity : 99.81%, HPLC



dCTP | Purity : 99.71%, HPLC



dTTP | Purity : 99.82%, HPLC



dNTPs Product List

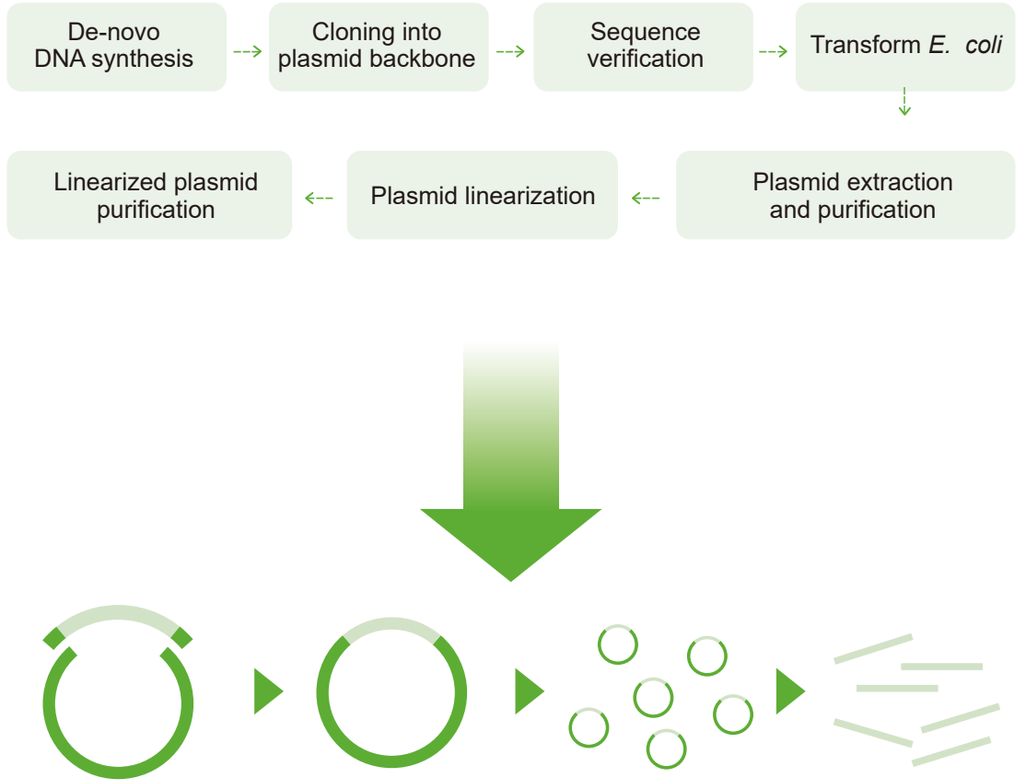
Cat. No.	Product Name
dNTP001-0.1、dNTP001-1、dNTP001-10、dNTP001-100、dNTP001-1K	dATP 100mM Sodium Solution
dNTP002-0.1、dNTP002-1、dNTP002-10、dNTP002-100、dNTP002-1K	dGTP 100mM Sodium Solution
dNTP003-0.1、dNTP003-1、dNTP003-10、dNTP003-100、dNTP003-1K	dCTP 100mM Sodium Solution
dNTP004-0.1、dNTP004-1、dNTP004-10、dNTP004-100、dNTP004-1K	dTTP 100mM Sodium Solution
dNTP005-0.1、dNTP005-1、dNTP005-10、dNTP005-100、dNTP005-1K	dUTP 100mM Sodium Solution

For more information, please visit Synthgene's website: www.synthgene-bio.com

Plasmid Construction and Linearization

To help customers with their mRNA drug development, Synthgene offers a plasmid synthesis service from microgram (μg) to milligram (mg) quantities. The linearized plasmid will be sequence-verified and ready to go into the in-vitro transcription (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. The cap can be added enzymatically post-IVT or in a one-pot co-capping reaction. The enzymatic capping is a four-step process using a vaccinia capping enzyme and 2'-O-methyl transferase, where the capping efficiency can be very high if the process is optimized. One-pot co-capping requires fewer steps and fewer unit operations. 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. The GpppAG cap analog and its variations, such as Synthgene's GAG (UNA) and GAG (ENE), offer high capping efficiency and a Cap1 structure, therefore becoming 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.

Synthgene Products
mRNA Co-transcription Kits

Synthgene Services
mRNA Custom Synthesis

Co-transcription Kits

To help bench scientists quickly synthesize mRNA for research, Synthgene has 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 for the DNA template.

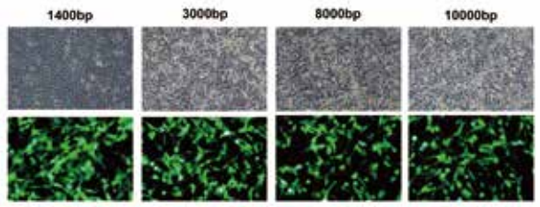
✓ Ease of use	Cap1 mRNA can be synthesized in one step
✓ High yield	up to 200 μg of mRNA can be generated from 1μg DNA template
✓ High capping efficiency	capping efficiency of > 95% can be achieved
✓ Reduced immunogenicity	by replacing wild-type UTP with N1-Me-pUTP

Recommended IVT Conditions

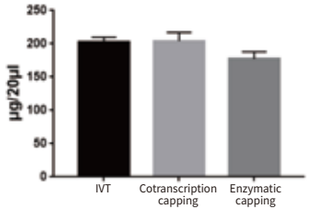
10*Reaction Buffer	2 μL
100mM ATP Solution	2 μL
100mM CTP Solution	2 μL
100mM GTP Solution	2 μL
Reagent A	2 μL
DNA Template	1 μg
Reagent B	2 μL
T7 RNA polymerase (250U/μL)	1 μL
Inorganic Pyrophosphatase (1U/μL)	0.04 μL
RNase inhibitor (40U/μL)	1 μL
RNase free ddH_2O	up to 20 μL

Co-transcription Kit Performance

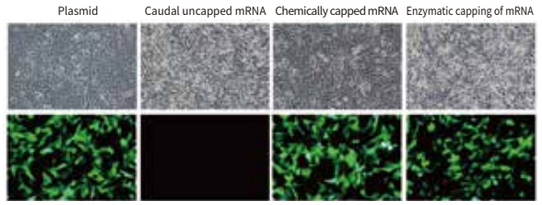
The T7 Co-transcription mRNA Synthesis Kit works with short and long DNA templates. We have seen high protein expression from mRNA constructs up to 10kb. Compared to the enzymatic capping method, the kit yields a similar amount of mRNA with higher activity, as shown in the data below.



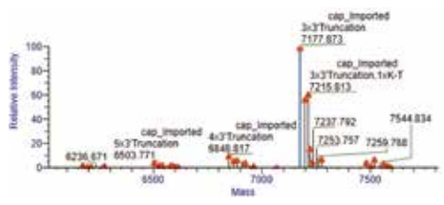
Accommodate templates with various sizes



Similar yield as enzymatic capping



High activity of mRNA products



Capping efficiency ≥95%

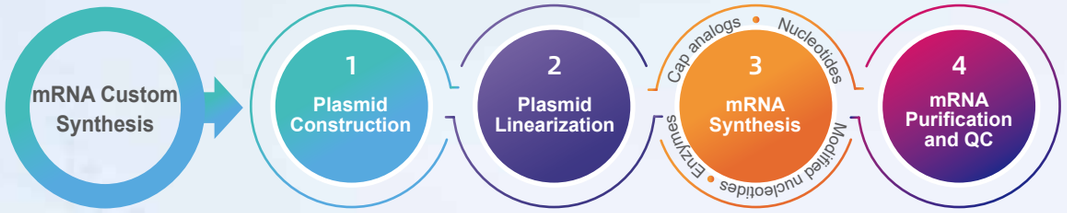
The IVT and Capping Kit Product List

Cat. No.	Product Name
10110U	T7 High Yield RNA Synthesis Kit (AGCU)
10110N	T7 High Yield RNA Synthesis Kit (AGCN)
10111-4011	T7 Co-transcription RNA Synthesis Kit (CAP4), 50 reactions
10111-5011	T7 Co-transcription RNA Synthesis Kit (CAP5), 50 reactions

For more information, please visit Synthgene's website: www.synthgene-bio.com

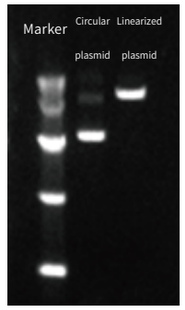
mRNA Custom Synthesis

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

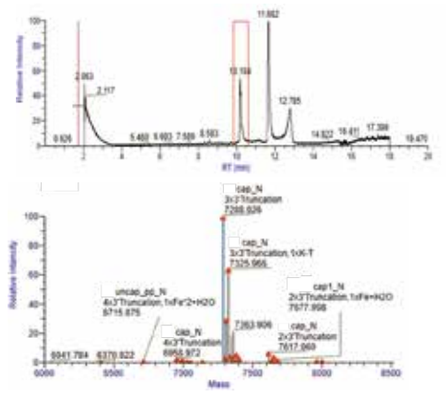


Examples of Custom Synthesized mRNA

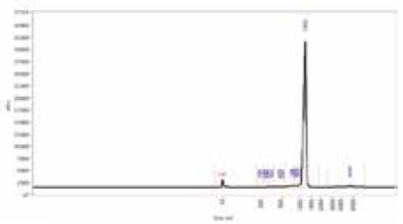
Quality of linearized plasmid



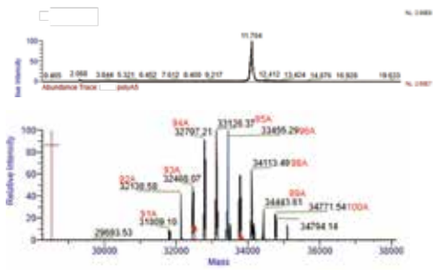
Capping efficiency by LC-MS



mRNA Integrity by fragment analyzer



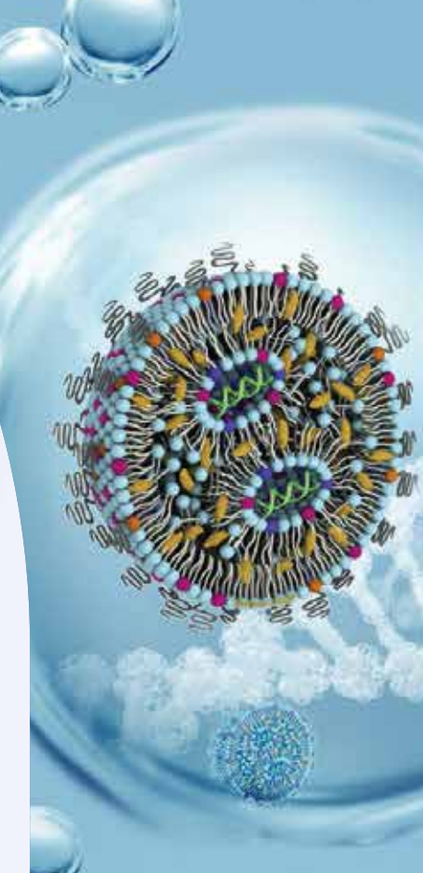
Poly A tail length and distribution by LC-MS



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 nanoparticles (LNPs) are 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 the endosome, which facilitates mRNA endosomal escape. PEG-lipids play an important role in extending the half-life of LNPs in circulation and preventing particle aggregation. Phospholipids and cholesterol contribute to the structural integrity of LNPs. The generation of LNPs relies on the rapid mixing of mRNA in the aqueous phase with lipids dissolved in ethanol.



Synthgene Products
Stocked Tool mRNAs

Synthgene Services
LNP Formulation Screening

Synthgene Services
LNP Encapsulation

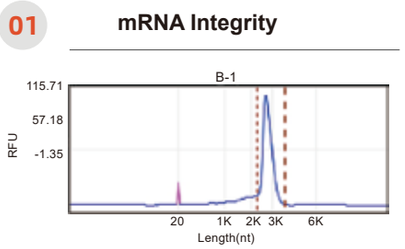
4 SYNTHGENE PRODUCTS

Stocked Tool mRNAs

Synthgene 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.

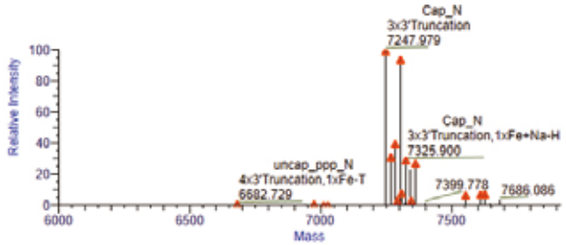
Fluc-eGFP mRNA Data

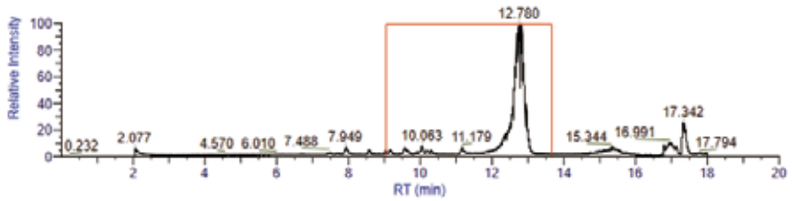
01 mRNA Integrity



Fluc-eGFP mRNA integrity > 90%
by Fragment Analyzer

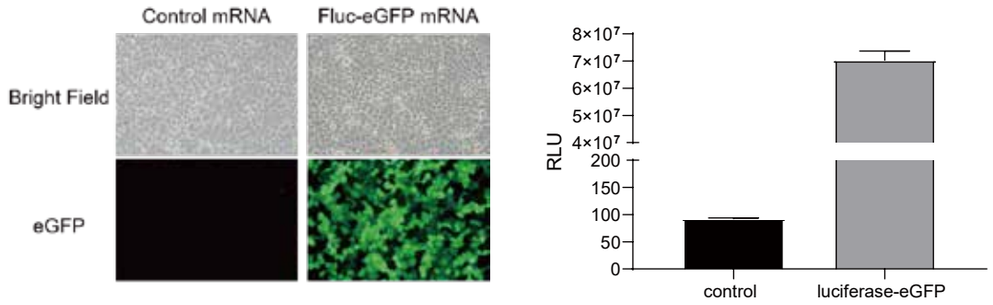
02 Capping Efficiency





Capping Efficiency > 95% by LC-MS

03 mRNA Activity



Cells were transfected with Fluc-eGFP mRNA. Both GFP and luciferase showed strong signals 24 hours after transfection

Stocked Tool mRNAs

Cat. No.	Product Name
11001-CAP-1、11001-CAP-5、11001-CAP-10	eGFP mRNA (5’ CAP)
11002-CAP-1、11002-CAP-5、11002-CAP-10	Fluc mRNA (5’ CAP)
11003-CAP-1、11003-CAP-5、11003-CAP-10	Fluc-eGFP mRNA(5’ CAP)
11004-CAP-1、11004-CAP-5、11004-CAP-10	mCherry mRNA (5’ CAP)
11006-CAP-1、11006-CAP-5、11006-CAP-10	OVA mRNA (5’ CAP)
11007-CAP-1、11007-CAP-5、11007-CAP-10	Cre mRNA (5’ CAP)

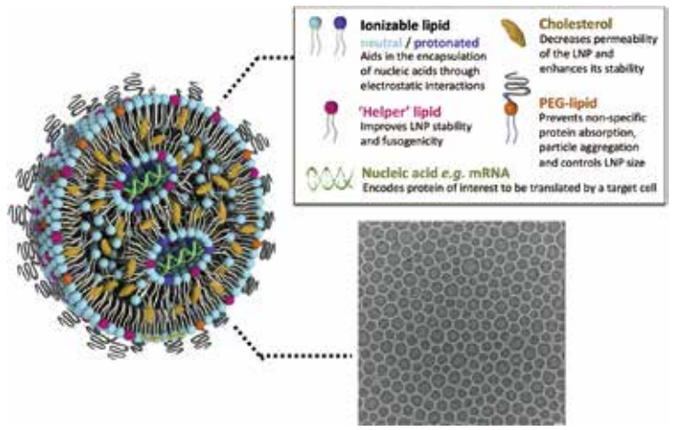
For more information, please visit Synthgene's website: www.synthgene-bio.com

LNP Formulation Screening
LNP Encapsulation Service

Synthgene has a library of ionizable lipids that it makes available to customers for screening. LNP formulation screening can be conducted at Synthgene or at the customer's site. To help customers with their mRNA research, Synthgene also offers LNP encapsulation services, should customers choose to use them.

Schematic Diagram of Typical LNP Formula

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

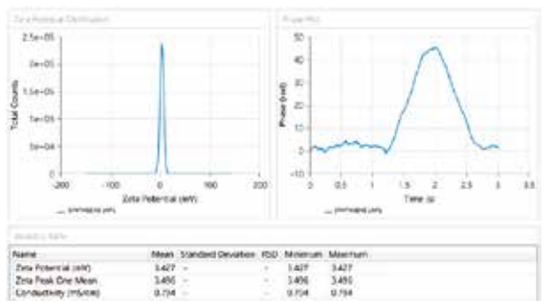


Reference：Acta Biomater. 2021 Sep 1;131:16-40. doi: 10.1016/j.actbio.2021.06.023.

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 LNPs, additional tests must be performed to measure particle size, polydispersity index, %encapsulation, identify, purity and content.

Synthgene Products
Impurity Standards

Synthgene Services
mRNA Analytical Testing

Synthgene Products
Cap1&uncap Standards

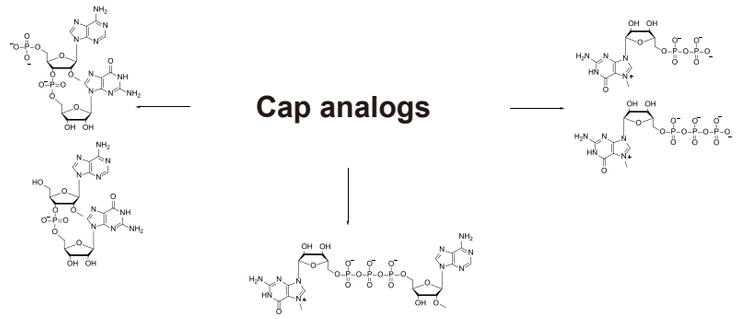
Synthgene Services
LNP Analytical Testing

Synthgene Products
Residue Test Kits

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, Synthgene offers a variety of impurity standards.

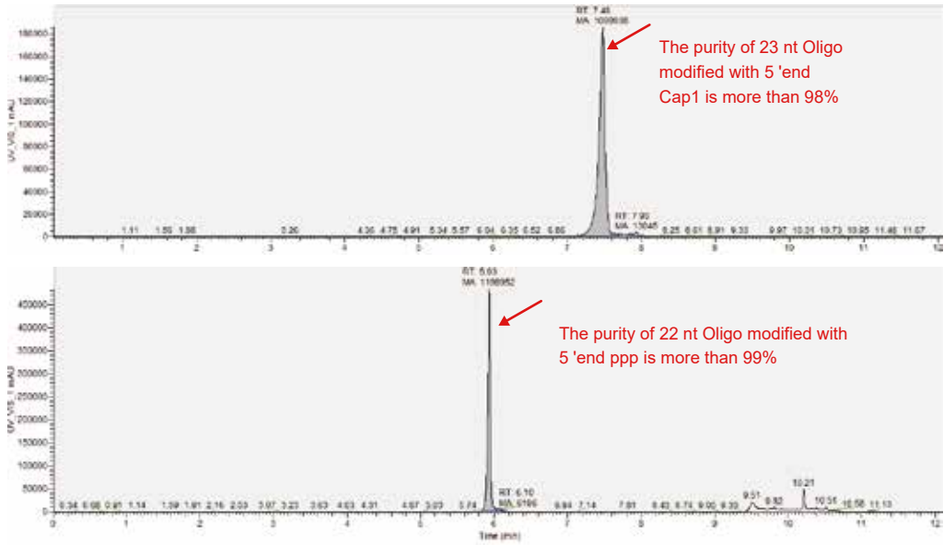
Cap Analog Impurity Standards



Paired Capped / Uncapped mRNA Standards

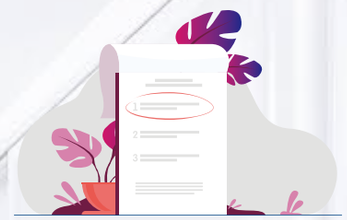
To assay capping efficiency, a cleavage probe is needed to bind to the 5'UTR of the mRNA and cleave off a short sequence so that the presence or absence of one guanosine can be resolved by LC-MS. Synthgene can design the cleavage probe for customers, or develop the capping assay and tech transfer the 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 a critical quality attribute (CQA) because it can trigger an innate immune response. Other IVT related impurities include T7 RNA Polymerase, DNase I, RNAase inhibitor, and inorganic pyrophosphatase. Synthgene offers ELISA kits to test these residues.



ELISA Kits for Detection
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.

IVT Process Impurity Detection Kits Product List

Cat. No.	Product Name
T70001	T7 Polymerase detection kit (ELISA), 96 reactions
DI0001	DNase I detection kit (ELISA), 96 reactions
RI0001	RNase Inhibitor detection kit (ELISA), 96 reactions
PI0001	Inorganic Pyrophosphatase detection kit (ELISA), 96 reactions
DS0001	dsRNA detection kit (ELISA), 96 reactions

For more information, please visit Synthgene's website: www.synthgene-bio.com

5

SYNTHGENE SERVICES

Analytical Method Development
mRNA Release Testing and Stability Testing

Synthgene 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.

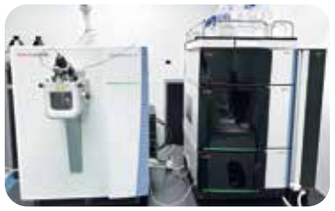
mRNA QC Panel

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

Synthgene Products
Cell Transfection Kit

Chapter Six

Functional Testing

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

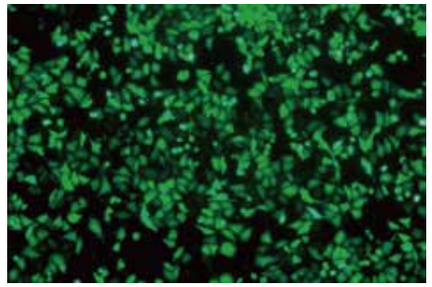
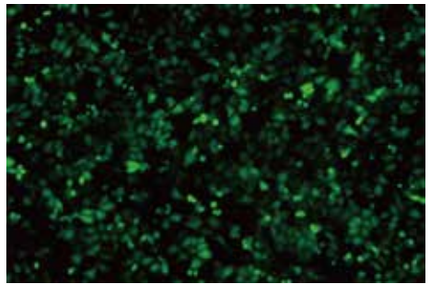
6 SYNTHGENE PRODUCTS

Cell Transfection Kit

The Easy Cell Transfection Kit is designed to transfect complex mRNA and deliver it into cells. The kit has been tested on many commonly used cell lines.

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

Performance Data

Transfection of eGFP mRNA using Easy Cell Transfection Kit	Transfection of eGFP mRNA using commercial transfection kit
	

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

Synthgene Services
IVT Process Development

Synthgene Services
Analytical Method Development and Validation

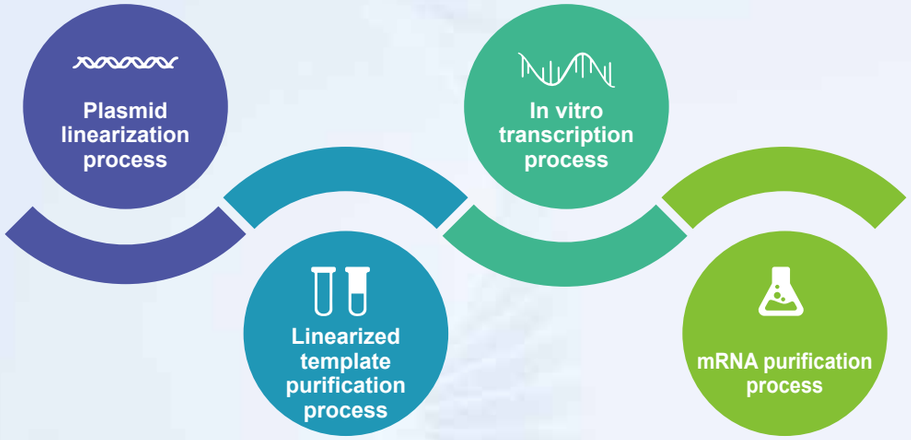
Chapter Seven

Process Development

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

IVT Process Development

There is no one-size-fits-all IVT process. Sometimes, trade-offs must be made to achieve the best purity, highest yield, or lowest dsRNA. Depending on the desired critical quality attributes, 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 a 20 µL reaction in microcentrifuge tubes to a 10 L reaction in bioreactors, one must also consider mixing, heating, adding reagents, etc. Synthgene can help customers scale-up their mRNA manufacturing process.



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. The capping efficiency assay is construct-specific and requires the design of a cleavage probe specific to the customer's mRNA. Synthgene can develop the analytical methods needed for mRNA development.

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, mRNA must be manufactured following cGMP practices to ensure the safety and effectiveness of the product. As mRNA is considered an advanced therapy medicinal product (ATMP), there is a phase-appropriate approach that reflects the increasing degree of control as the drug moves from Phase I to commercial launch. cGMP manufacturing means tighter control of raw materials and requires the raw material supplier to have a robust quality system to support high-grade products. Synthgene offers GMP-grade cap analogs, NTPs, and modified NTPs, with drug master files on a selected number of products.

Synthgene Products
Cap Analogs

Synthgene Products
Modified Nucleotides

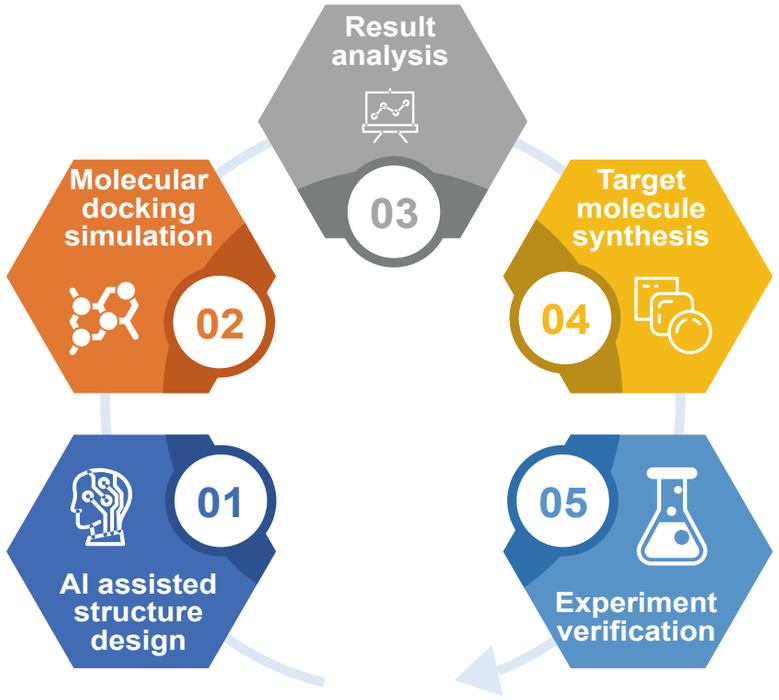
Synthgene Products
Nucleotides

Synthgene Products
Enzymes

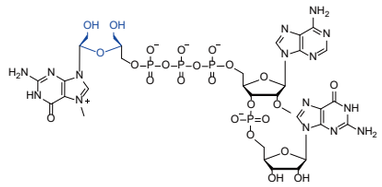
Cap Analogs

Cap analogs are a critical starting material for the synthesis of mRNA. In the cytosol, the cap structure binds to eIF4E to initiate translation. It also helps to protect mRNA from exonucleases and mediate mRNA turnover.

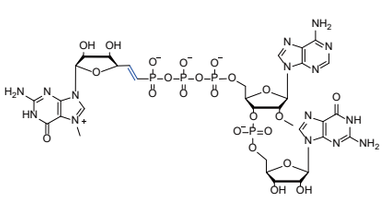
Using AI-assisted structure design and molecular docking simulation, Synthgene has developed several novel cap analogs that offer comparable capping efficiency and yield as GpppAG.



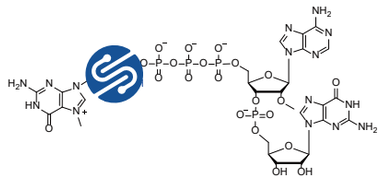
Synthgene's Cap Analogs



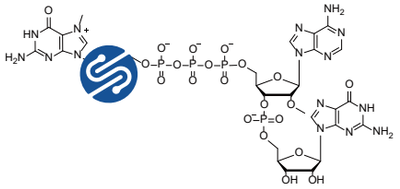
CAP 4



CAP 5



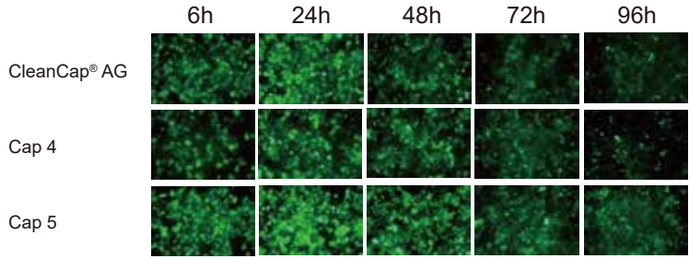
CAP 7



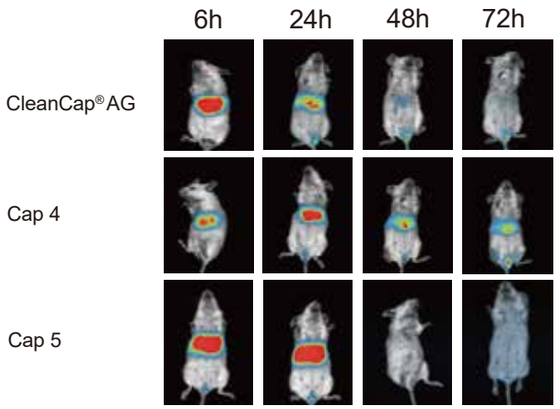
CAP 8

Cap Analogs

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



eGFP mRNA



luciferase mRNA

Cap Analogs Product List

Cat. No.	Product Name
CAP4011-0.1、CAP4011-1、CAP4011-10、CAP4011-100、CAP4011-500	CAP 4 m7G(UNA)(5')ppp(5')(2'OMeA)pG 100mM Ammonium Solution
CAP5011-0.1、CAP5011-1、CAP5011-10、CAP5011-100、CAP5011-500	CAP 5 m7G(5')vppp(5')(2'OMeA)pG 100mM Ammonium Solution
CAP7011-0.1、CAP7011-1、CAP7011-10、CAP7011-100、CAP7011-500	CAP 7 m7G(HNA)(5')ppp(5')(2'OMeA)pG 100mM Ammonium Solution
CAP8011-0.1、CAP8011-1、CAP8011-10、CAP8011-100、CAP8011-500	CAP 8 m7G(PMO)(5')ppp(5')(2'OMeA)pG 100mM Ammonium Solution

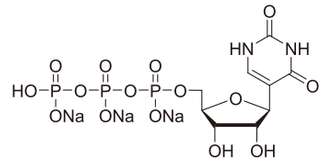
For more information, please visit Synthgene's website: www.synthgene-bio.com

Modified Nucleotides

The commercial success of mRNA vaccines is largely attributable to the discovery of modified uridine by Dr. Kariko and Dr. Weissman. Uridine-rich RNA is recognized by toll-like receptors as viral and triggers an immune response. Replacing wild-type uridine with pseudo-uridine or N1-methyl pseudo-uridine improves mRNA stability and translational capacity. Synthgene offers GMP-grade pseudouridine triphosphate (UTP) and N1-methyl pseudo-uridine triphosphate (pUTP).

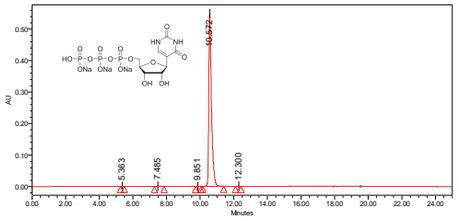
Product Recommendation

Pseudo-UTP



Cat. No.: PUTP001

Purity : 99.54%, HPLC



✓	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
GMP-PUTP001-0.1、GMP-PUTP001-1、GMP-PUTP001-10、GMP-PUTP001-100、GMP-PUTP001-500	pUTP 100mM Sodium Solution, GMP grade
GMP-NMPUTP001-0.1、GMP-NMPUTP001-1、GMP-NMPUTP001-10、GMP-NMPUTP001-100、GMP-NMPUTP001-500	N1-Me-pUTP 100mM Sodium Solution, GMP grade
PUTP001T-0.1、PUTP001T-1、PUTP001T-10、PUTP001T-100、PUTP001T-500	pUTP 100mM Tris Solution
PUTP002T-0.1、PUTP002T-1、PUTP002T-10、PUTP002T-100、PUTP002T-500	pUTP 200mM Tris Solution
NMPUTP001T-0.1、NMPUTP001T-1、NMPUTP001T-10、NMPUTP001T-100、NMPUTP001T-500	N1-Me-pUTP 100mM Tris Solution
GMP-NMPUTP002T-0.1、GMP-NMPUTP002T-1、GMP-NMPUTP002T-10、GMP-NMPUTP002T-100、GMP-NMPUTP002T-500	N1-Me-pUTP 200mM Tris Solution, GMP grade
5MCTP001-0.1、5MCTP001-1、5MCTP001-10	5-Me-CTP 100mM Sodium Solution
GMP-5OMUTP001-0.1、GMP-5OMUTP001-1、GMP-5OMUTP001-10	5-OMe-UTP 100mM Sodium Solution, GMP grade

For more information, please visit Synthgene's website: www.synthgene-bio.com

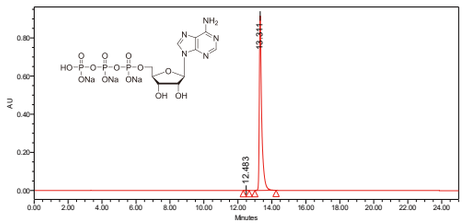
*N1-Me-pUTP has DMF filed at FDA

Nucleotides

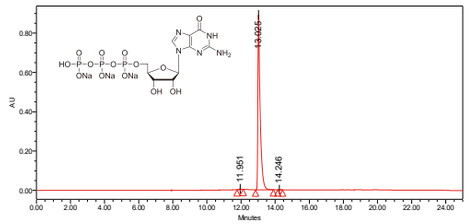
Synthgene offers GMP grade NTPs in sodium salt or Tris solution, at 100mM or 200mM.

High Purity Nucleotides≥99%

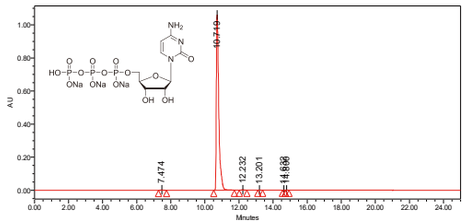
ATP | Purity : 99.89%, HPLC



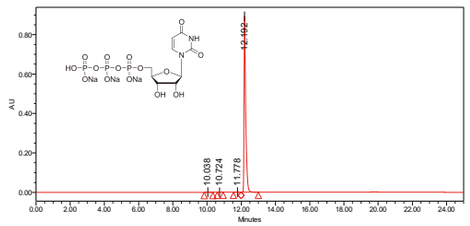
GTP | Purity : 99.62%, HPLC



CTP | Purity : 99.53%, HPLC



UTP | Purity : 99.60%, HPLC



✓	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
GMP-ATP001-1、GMP-ATP001-10、GMP-ATP001-100、GMP-ATP001-500	ATP 100mM Sodium Solution, GMP grade
GMP-GTP001-1、GMP-GTP001-10、GMP-GTP001-100、GMP-GTP001-500	GTP 100mM Sodium Solution, GMP grade
GMP-CTP001-1、GMP-CTP001-10、GMP-CTP001-100、GMP-CTP001-500	CTP 100mM Sodium Solution, GMP grade
GMP-UTP001-1、GMP-UTP001-10、GMP-UTP001-100、GMP-UTP001-500	UTP 100mM Sodium Solution, GMP grade
GMP-ATP002T-1、GMP-ATP002T-10、GMP-ATP002T-100、GMP-ATP002T-500	ATP 200mM Tris Solution, GMP grade
GMP-GTP002T-1、GMP-GTP002T-10、GMP-GTP002T-100、GMP-GTP002T-500	GTP 200mM Tris Solution, GMP grade
GMP-CTP002T-1、GMP-CTP002T-10、GMP-CTP002T-100、GMP-CTP002T-500	CTP 200mM Tris Solution, GMP grade
GMP-UTP002T-1、GMP-UTP002T-10、GMP-UTP002T-100、GMP-UTP002T-500	UTP 200mM Tris Solution, GMP grade

For more information, please visit Synthgene's website: www.synthgene-bio.com

*ATP has DMF filed at FDA

* CTP has DMF filed at FDA

* GTP has DMF filed at FDA

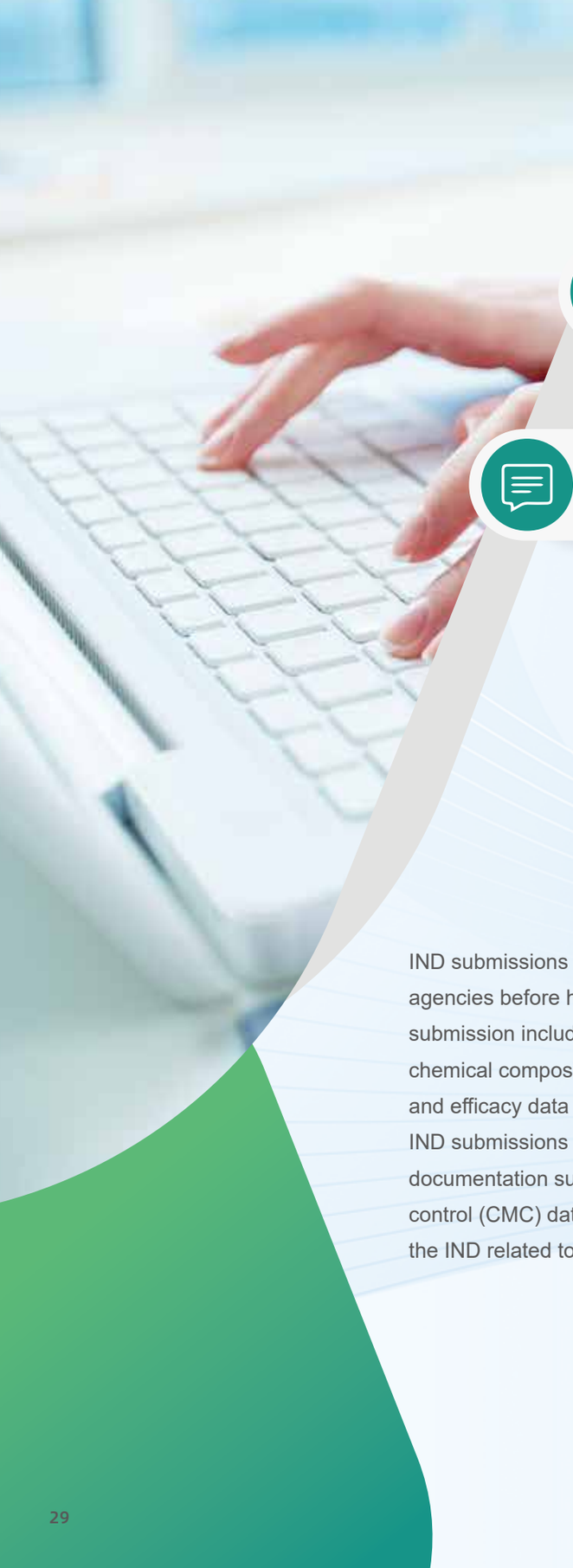
Enzymes

Synthgene offers enzymes required for mRNA synthesis, from linearization enzymes, Dnase I, to T7 RNA Polyermase and vaccina capping enzyme.

Enzymes Product List

Product Name	Unit Size
BspQI	10U/μL
BsaI	20U/μL
XbaI	20U/μL
XhoI	20U/μL
T7 RNA polymerase	250U/μL
10×Transcription Buffer	400mM Tris-HCl, 450 mM Mg $(CH_3COO)_2$, 20 mM spermidine, 100 mM DTT
10×Transcription Buffer	400mM Tris-HCl, 350 mM Mg $(CH_3COO)_2$, 20 mM spermidine, 100 mM DTT
Inorganic Pyrophosphatase	1U/μL
Murine RNase Inhibitor	40U/μL
DNase I	2U/μL
T7 RNA polymerase mix	—
Proteinase K	—
M-MuLV Reverse Transcriptase (RNase H-)	200U/μL
Bst Plus DNA Polymerase	40 U/μL
Uracil DNA Glycosylase(UDG)	1U/μL
RNA Isolation beads	—
DNA Isolation beads	—

For more information, please visit Synthgene's website: www.synthgene-bio.com



Synthgene Services
Preparation of Regulatory Documents

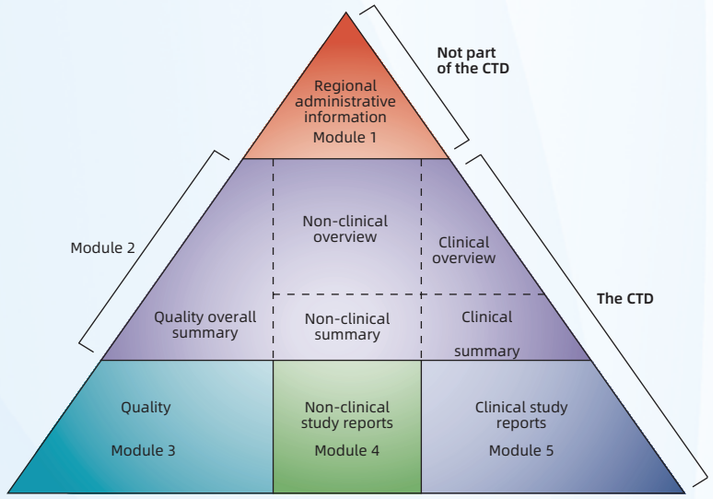
Synthgene Services
Consultation on Process Compliance

Chapter Nine
IND Support

IND submissions must be filed and approved by regulatory agencies before human clinical trials can begin. The submission includes detailed information on the drug's chemical composition, proposed clinical trial design, 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. Synthgene can author certain sections of the IND related to CMC and mRNA raw materials used.

Preparation of Regulatory Documents SYNTHGENE SERVICES

Synthgene 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.



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Consultation on Process Compliance

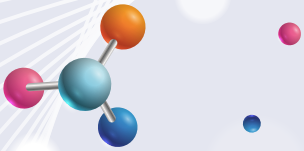
Regulations and Guidelines for mRNA Vaccines and Drugs

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 quality, safety and efficacy of mRNA vaccines.

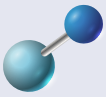
In April 2023, the US Pharmacopoeia (USP) published the second edition of "Analytical Procedures for mRNA Vaccine Quality". The draft guideline proposed testing panels for plasmid DNA, mRNA drug substance and mRNA drug product with both release assays and characterization assays.

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.



Raw Materials and Services to
Enable mRNA and Oligo Therapeutics



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