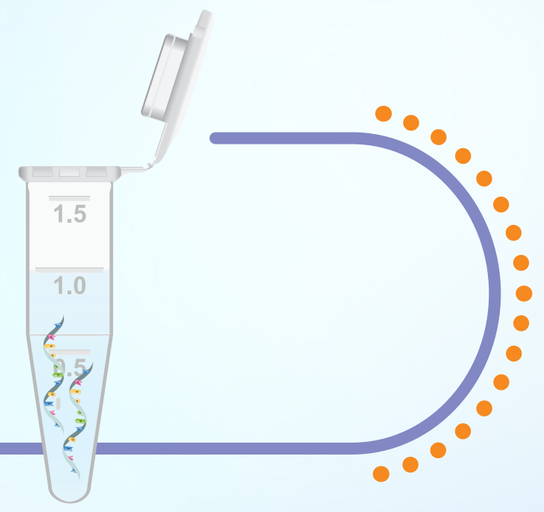
Create the Perfect mRNA for Translation in Cells

Key Considerations

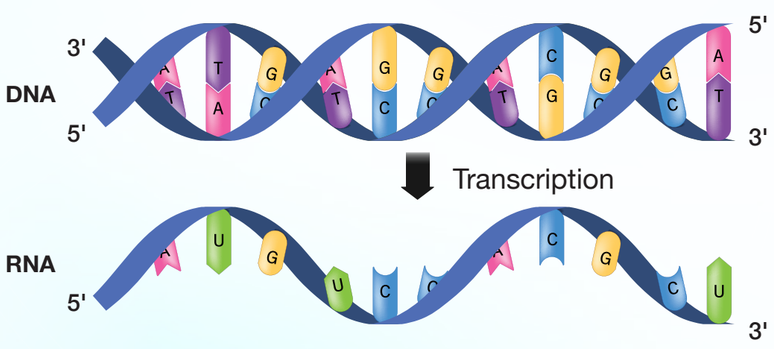
Messenger RNA (mRNA)-based therapeutics are transforming molecular medicine, offering rapid, scalable, and cell-free production of biologically active proteins. *In vitro* transcription (IVT) can often generate double-stranded RNA (dsRNA) byproducts, which may trigger immune responses and reduce protein expression, compromising the safety and efficacy of mRNA-based therapeutics. This infographic outlines the various design steps and key considerations of mRNA synthesis including:

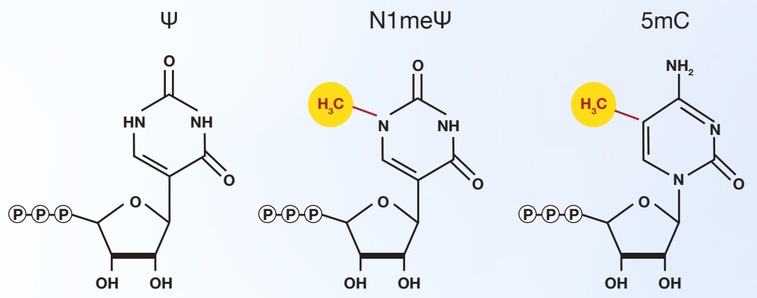
- **Optimization of mRNA expression** (nucleotide selection, 5´ capping, 3´ tailing)
- **Minimization of immunogenicity** (modified nucleotide selection, 5´ capping, dsRNA removal)
- **mRNA stability and longevity** in cells (5´ capping, 3´-poly(A) tail length)



Transcription

- **Step 1:** Determine the mRNA sequence including the gene of interest for optimal translation of the desired protein.
- **Step 2:** Transcribe this sequence with incorporation of modified nucleotides to reduce immunogenicity and increase mRNA stability.[1-6]
  Example modified nucleotides: • Pseudouridine (Ψ) • N1-methyl-pseudouridine (N1meΨ) • 5-methyl-cytidine (5mC)

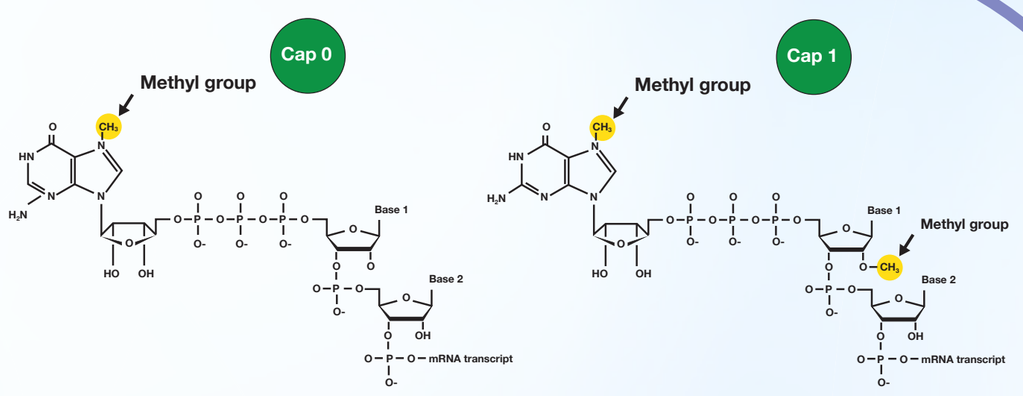


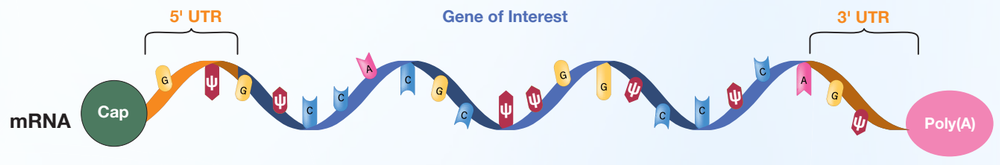


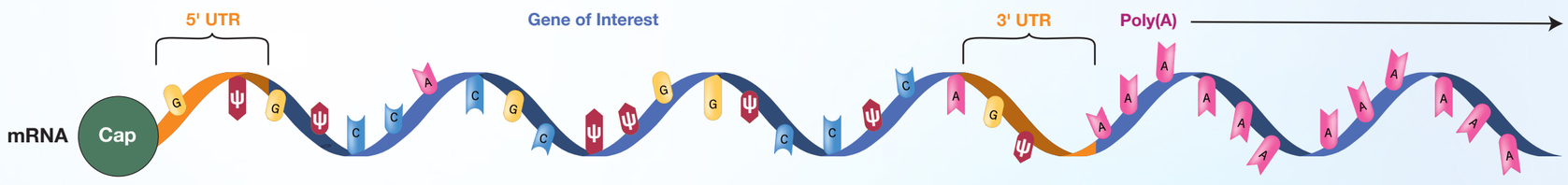
5′ Capping

5´ Capping protects the mRNA from 5´-3´ exonuclease degradation, improving mRNA stability and longevity. The 5´ cap also recruits translation initiation factors to the mRNA, which is essential for mRNA translation. The Cap 1 modification further increases mRNA translation efficiency by marking the mRNA as "self RNA", which is instrumental in reducing the innate immune response.[7,8] RNA can be capped by one of two methods, post-transcriptionally or co-transcriptionally. Both methods can produce either a Cap 0 or Cap 1 cap structure onto the RNA.

- **Post-transcriptional capping** uses enzymes for cap construction onto the primary transcript RNA. It is compatible with RNAs generated by A-start or G-start T7 promoters and offers ~100% capping efficiency.
- **Co-transcriptional capping** uses a cap analog molecule providing 80-95% capping efficiency depending on the cap analog used.







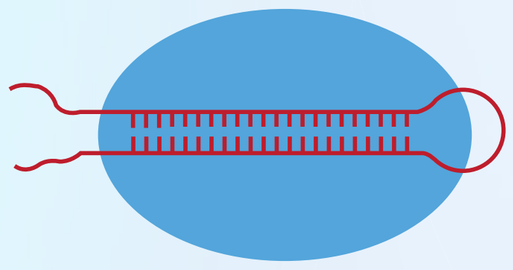
3′ Poly(A) Tailing

Polyadenylation at the 3´ end of mRNA increases the stability and longevity of mRNA in cells and enhances its ability to be translated. The mRNA 3´-poly(A) tail protects transcripts from 3´-5´ exonucleases that shorten 3´ tails in cells over time. Shortened tails lead to instability of the 5´ cap and decapping. The longer the 3´-poly(A) tail, the more stable the mRNA transcript will be.[9-11] Poly(A) tails can be added post-transcriptionally or co-transcriptionally.

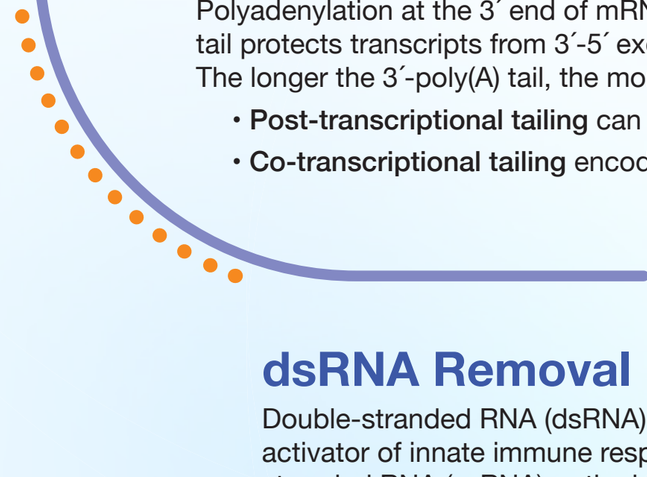
- **Post-transcriptional tailing** can generate tail lengths >300 bases using Poly(A) polymerase.
- **Co-transcriptional tailing** encodes the tail in the template, limiting tail length to ~60 bases.

dsRNA Removal

Double-stranded RNA (dsRNA) is an undesirable byproduct of *in vitro* transcription (IVT) and a potent activator of innate immune responses when introduced into cells. It arises from the self-folding of single-stranded RNA (ssRNA) or the hybridization of complementary ssRNA strands during *in vitro* transcription. To defend against viral infections, cells have evolved pattern recognition sensors (PRSs) that detect non-self RNA molecules. Certain subclasses of these sensors specifically recognize dsRNA, interpreting its presence as a sign of viral invasion. As a result, dsRNA can trigger robust immune activation. Therefore, efficient removal of dsRNA is crucial to minimizing immunogenicity in RNA-based applications.[8,12-14]

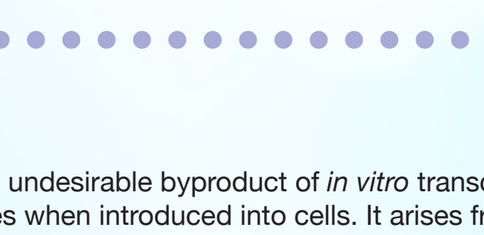


mRNA Downstream Applications



mRNA therapeutics are a class of medical treatments that use mRNA to instruct cells in the body to produce specific proteins that can prevent, treat, or cure disease. mRNA vaccines were successfully used during the COVID-19 pandemic. However, mRNA therapeutics can be used in a number of different disease areas as a wide range of proteins can be translated from specific mRNA transcripts.[15] Challenges posed by mRNA such as stability, degradation, immunogenicity, protein translation efficiency, and purity are being overcome, making this class of therapies promising. mRNA therapeutics are being explored to:

- Stimulate the immune system (e.g., vaccines),
- Replace missing or defective proteins (e.g., rare and genetic diseases),
- Function as therapeutic agents (e.g., cancer immunotherapy, CAR-T's),
- Edit genes to restore function (e.g., CRISPR-Cas9, Base Editing, Prime Editing)



CELLSCRIPT™
RNA for Translation in Cells

Explore the CELLSCRIPT™ Solutions for High-Efficiency mRNA Translation

The Future of mRNA Biomedicines

Academic researchers and industry alike are increasingly working on mRNA-based biomedicines. As of 2023, more than 190 companies and institutes are engaged in the development of more than 310 mRNA vaccines and therapeutics. Vaccines represent the majority of these drugs (65%), and more than 720 mRNA and interfering/modulating RNA candidate therapeutics are in the pipeline.[15]

The potential uses of mRNA as biomedicines are enormous and it is expected to become one of the major contributors of drug development in upcoming years in conjunction with the use of artificial intelligence (AI) technology. AI can aid in optimizing the design, selection, composition, and validation of DNA sequences, mRNA translation efficiency, purity, cellular stability, non-immunogenicity, non-toxicity, thermostability, cellular uptake efficiency, organ-specific targeting ability, control activation of immune system, and pharmacokinetics and pharmacodynamics (PK/PD) among other factors.[15] Thus, the combination of AI and high-throughput methodologies promises to revolutionize the mRNA therapeutic space and will continue to significantly impact drug development in the near future.

References

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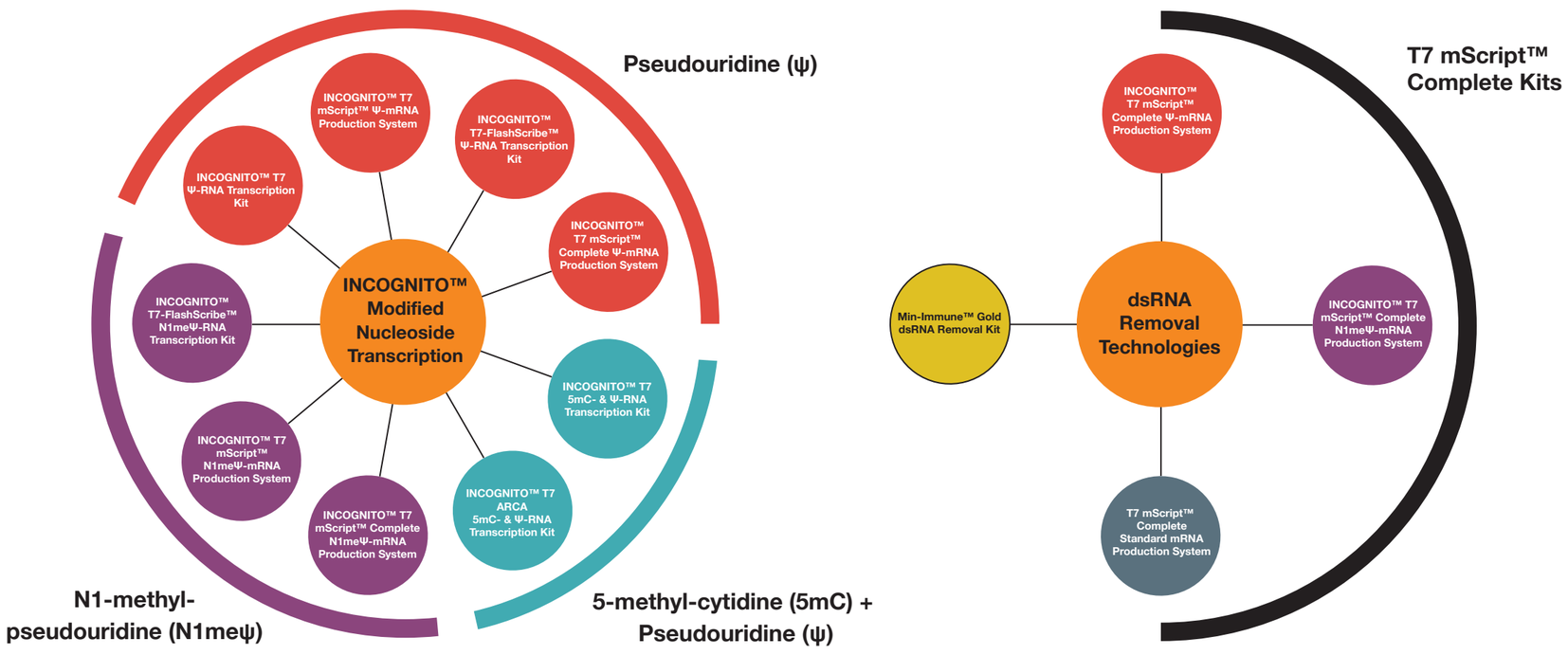
Optimize Your mRNA for Successful Translation Using High-Efficiency Kits



INCOGNITO™ mRNA & IVT RNA Synthesis Kits

These kits are available with either N1-methyl-pseudouridine (N1meΨ), pseudouridine (Ψ), and/or 5-methyl-cytidine + pseudouridine (5mC/Ψ) nucleotides for incorporation during RNA synthesis.

Catalog No.	Product Name
IMMY240625	INCOGNITO™ T7 mScript™ N1meΨ-mRNA Production System
IMY240525	INCOGNITO™ T7 mScript™ Ψ-mRNA Production System
IFMY240625	INCOGNITO™ T7-FlashScribe™ N1meΨ-RNA Transcription Kit
IFY240525	INCOGNITO™ T7-FlashScribe™ Ψ-RNA Transcription Kit
C-ICTY110510	INCOGNITO™ T7 Ψ-RNA Transcription Kit
C-ICTMY110510	INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
C-ICTAMY110510	INCOGNITO™ T7 ARCA 5mC- & Ψ-RNA Transcription Kit

T7 mScript™ Complete mRNA Production Systems

These kits include modules for (i) *in vitro* transcription of linear double-stranded DNA templates, (ii) post-transcriptional enzymatic capping of the RNA to Cap 1, (iii) post-transcriptional enzymatic 3′ poly(A) tailing, (iv) enzymatic removal of dsRNA and (v) mRNA purification.

Catalog No.	Product Name
IMCMY250225	INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System
IMCY250125	INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System
MSCC250225	T7 mScript™ Complete Standard mRNA Production System

Min-Immune™ Gold dsRNA Removal Kit

This kit is the gold standard in double-stranded RNA removal. Available as a standalone kit but also included as a module component in mScript™ Complete mRNA Systems.

Catalog No.	Product Name
MGDR250125	Min-Immune™ Gold dsRNA Removal Kit

Select Publications using INCOGNITO™ kits:

- Sambri, I. *et al.*, (2023) Nat Commun. 14(1):2775.
- Campostrini, G. *et al.*, (2023) Cardiovasc Res. 119(1):167–182.
- Zhang, Y. et al., (2023) Microbiol Spectr. 12(1): e02866-23.
- Li, D. *et al.*, (2023) Front Microbiol. 14:1145114.

The above CELLSCRIPT™ kits are for research use only (RUO).