mRNA & Long RNA:

Products & Custom Services

Leading the Way in mRNA™

TriLink BIOTECHNOLOGIES
part of Maravai LifeSciences

mRNA & Long RNA

For over 20 years, TriLink BioTechnologies has been facilitating customer application success as the industry leading manufacturer of nucleic acids, NTPs, and capping analogs. Our products are used in molecular biology research, diagnostics, therapeutics, and OEM applications. A combination of expertise in synthetic organic chemistry and pioneering knowledge in mRNA enables us to continually respond to customer needs by providing innovative tools and new solutions to the market.

Our dedication to innovation and continuous improvement results in a high quality product. These products include custom mRNA and long RNA synthesis at scales from µgrams to grams, with the most comprehensive array of modifications available. We offer stocked RNAs expressing reporter genes, antigens for vaccine studies, nucleases for genome engineering, and a model protein (EPO) for protein replacement studies.

Our high quality mRNA and long RNA products offer:

- ISO 9001:2015 certification
- Custom-tailored support to meet specific application or program needs
- A wide variety of modification, treatment, and purification options
- Fully-traceable documentation and audit trails
- A scalable path to pharmaceutical API cGMP manufacturing

Custom Transcription Services

To meet your specific application or program needs, TriLink has developed a portfolio of proprietary mRNA and long RNA production methods. Our expert technical support team is available to guide you through the process from template design to final product, including recommendations on base composition, determining appropriate scale, capping strategy, post transcriptional processing, and purification options. Common applications include:

- Gene editing
- Gene replacement
- Vaccines and immunotherapy

Advantages of mRNA

Efficient Delivery

Delivery to the appropriate compartment in the cell has been a major barrier in gene therapy research. mRNA targets the cytoplasm, and only needs to cross the plasma membrane. In contrast, plasmid DNA and most types of viral vectors must reach the nucleus, thus crossing an additional membrane barrier. In several cell lines, collaborators have observed higher transfection efficiencies with TriLink mRNA compared to DNA plasmids.

Controlled, Rapid Expression Levels

Plasmid DNA or viral vector expression can vary dramatically by cell type. The transient nature of mRNA also allows exquisite temporal control of expression. Furthermore, because they function post-transcriptionally, mRNAs eliminate the risk associated with ectopic promoters that are frequently silenced over time, and integrating viral vectors (retroviruses or lentiviruses), where the number of vector integrations and the location of integration can greatly influence expression in individual cells. Perhaps most importantly, mRNA is expressed more rapidly than DNA or virus-based approaches, as there is no wait for transcription, splicing, polyadenylation, or nuclear export.

Revolutionary Co-Transcriptional Capping Technology

CleanCap®, a groundbreaking technology developed at TriLink, is rapidly becoming the industry standard for mRNA co-transcriptional capping, as it offers clear advantages over legacy capping methods.

These advantages include:

- High levels of capping and increased yields
- A Cap 1 structure that is highly active *in vivo* and important for evading innate immune stimulation
- Alternative capped forms such as $^{m6}A_m$ Cap

It is essential to achieve a natural Cap 1 structure when manufacturing mRNAs for therapeutic applications, since this results in robust expression and reduced innate immune stimulation. As shown in the table (right), CleanCap represents a major improvement over previous generations of legacy cap analogs such as Anti-reverse Cap Analog (ARCA).

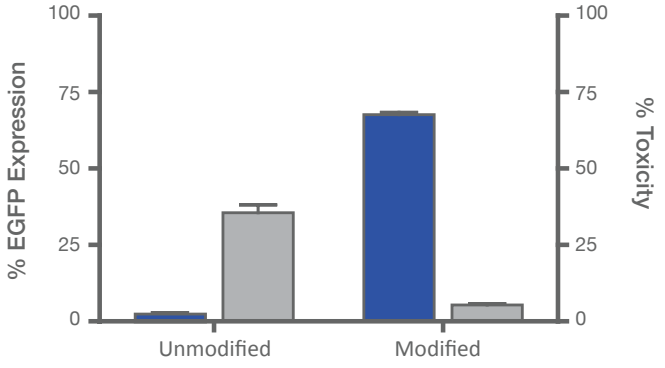
CleanCap is available as cap analogs to synthesize mRNAs, or via TriLink custom mRNA synthesis services. Catalog mRNAs manufactured with CleanCap technology are also available. For more information visit: **trilinkbiotech.com/cleancap**

	LEGACY CAP ANALOG	CleanCap®
Natural Cap	No	Yes
Immunogenic	Yes	Reduced Immunogenicity
Capping Efficiency	~70%	~95%
Yield/mL Transcription	1.5 mg/mL	4 mg/mL
Cost	3 X	1 X
Available Therapeutic Licenses	No	Yes

Modifications

TriLink offers an extensive catalog of modified nucleoside triphosphates (NTPs) that impart desirable characteristics to *in vitro* transcribed RNA, such as increased translation or altered interaction with innate immune receptors. For example, incorporation of pseudo-UTP has been shown to reduce innate immune stimulation in culture and *in vivo* while enhancing translation. RNA can be functionalized using biotin groups or aminoallyl NTPs for later conjugation to other molecules, such as dyes. TriLink custom mRNA can be manufactured with your choice of our modified NTPs. Custom NTP modifications are also available.

Modification Can Increase Expression, Reduce Toxicity



EGFP mRNA expression & toxicity in BJ fibroblasts.

Data courtesy of Laura Juckem, Mirus Bio.

Visit www.trilinkbiotech.com/mrna for details about our custom synthesis options.

Genome Editing mRNA

mRNA has emerged as a powerful tool in genome editing, as it has proven to transiently express required proteins with no risk of insertional mutagenesis. Editing with mRNA eliminates many of the risks associated with traditional methods that use plasmid and viral vectors, such as continuous expression of the nuclease, or inadvertent activation of a previously silent genomic location due to integration of a plasmid or virus.

CRISPR Mediated Genome Editing

The most popular method for genome editing, clustered regularly interspaced short palindromic repeats (CRISPR), was borrowed from a bacterial immune system. The CRISPR system was adapted to create RNA directed genome engineering tools. With CRISPR, the RNA guide sequence targets the Cas9 protein to a site of interest to perform the DNA cleavage. Previous generations of genome editing tools required selection of a new protein sequence each time a new locus was targeted. In contrast, CRISPR/Cas9 can be retargeted by simply changing the sequence of a small portion of the RNA guide sequence.

TALEN and Zinc Finger Nuclease mRNA

Zinc-finger nucleases (ZFNs) were the first widely applicable site-specific genome editing tools. In the last few years, transcription activator-like effector nucleases (TALENs) have emerged as another powerful tool. The effectiveness of both ZFNs and TALENs can be enhanced when delivered to cells as mRNA, as this reduces the off-target effects of the nucleases. For this reason, there is a move to transient expression using mRNA-based vectors. The TriLink team is experienced in both custom ZFN and custom TALEN mRNA synthesis. Additionally, we offer mRNA expression vectors designed to easily accept a TALEN cloned using the Golden Gate method.

For more information about our genome editing mRNA products, visit www.trilinkbiotech.com/genomeediting.

Reporter mRNA

Reporter mRNAs can be used as controls to study mRNA transfection and translation using a variety of assays, including fluorescence microscopy, quantitative fluorometry, and bioluminescent imaging, as well as fluorescence-activated cell sorting (FACS). TriLink offers reporter gene mRNA with a variety of the most popular standard and custom base modification patterns to:

- Reduce innate immune response
- Enhance expression
- Directly visualize mRNA delivery

To learn more about TriLink standard and proprietary capping technologies, and for a complete list of our reporter gene mRNA products, please visit us at trilinkbiotech.com/reportergenes.

Gene Replacement mRNA

In many cases, genetic disorders are recessive and gene replacement has the potential to replace the defective protein. Historically, DNA based non-viral and viral vector approaches have been used for gene replacement. However, insertional mutagenesis is a concern. mRNA transfection has gained popularity as a gene replacement tool because it poses no risk of insertional mutagenesis, and unlike plasmid and viral vector based approaches, it need only cross one membrane. This may reduce the delivery hurdles that must be overcome before gene replacement can become a reality in the clinic.

To view gene replacement mRNA, go to www.trilinkbiotech.com/genereplacement.

mRNA Antigens for Vaccines & Immunotherapy

mRNA offers several benefits over traditional plasmid- and viral-based approaches to vaccines and immunotherapy. Transient expression from mRNA reduces the risk of toxicity without compromising effectiveness. Additionally, mRNA immunotherapies trigger a more diverse and robust immune response than traditional subunit vaccines. The antigen sequence is easily customizable to target a specific epitope. Finally, unlike DNA, mRNA vaccines function in the cytoplasm, resulting in enhanced transfection efficiency, which is particularly useful for difficult to transfect cell types. Taken together, these benefits minimize the risk of toxicity while still providing a potent antigen to stimulate the immune system.

TriLink offers custom antigen mRNA synthesis as well as mRNA expressing the classical antigen, OVA.

Features of TriLink custom mRNA transcription services include:

- Gene synthesis and cloning into our custom plasmid for optimal stability and translation in mammalian systems
- Extensive customization and flexibility, making mRNA a viable option for personalized treatment
- Fast turnaround times enabling rapid deployment during pandemics
- Milligram to gram scales
- Research to therapeutic cGMP grade products

Self-Amplifying RNA Replicons

Recently, researchers have utilized self-amplifying RNAs for vaccination or protein over-expression. Self-amplifying RNAs are commonly derived from cytoplasmic RNA viruses such as alphaviruses. Viral structural proteins that form the virion are deleted, such that the virus can still replicate in the cytoplasm, but it cannot generate infectious virus. The structural proteins are replaced with an expression cassette for your desired protein/proteins. Because cytoplasmic RNA viruses replicate through a double stranded intermediate, they are strong inducers of the innate immune system, making them ideal vaccines. T7 RNA polymerase prefers to start with a guanosine residue. However, alphaviruses start with 5'AU... TriLink solved this issue by developing a novel proprietary method called CleanCap for co-transcriptional capping of alphavirus RNA with an 5'AU... start.

Diagnostic & Therapeutic cGMP Manufacturing

We have combined our expertise in nucleic acids with a new state-of-the-art cGMP production facility to offer therapeutic cGMP manufacturing for early phase clinical applications. With 12 cGMP manufacturing suites and two support labs, TriLink can quickly deliver therapeutic material that is aligned with your project timelines.

We offer a wide range of manufacturing scales, enabling you to specify the exact amount of material for pre-clinical IND toxicology studies or early phase clinical trials. TriLink delivers consistent quality API on schedule and on budget. In addition to drug substances, we will provide all the required supporting documentation to streamline the IND submission process and carry you through to clinical trials, making the process efficient and effective.

TriLink cGMP features include:

- Compliance with ICH Q7, section 19
- ISO Class 7 and Class 8 clean rooms
- Single-pass, HEPA filtered air system
- Routine monitoring of temperature, pressure, humidity, and particle count

For more details on our cGMP services, please visit us at trilinkbiotech.com/GMP.

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