TriLink BioTechnologies

The Modified Nucleic Acid Experts

mRNA & Long RNA

trilinkbiotech.com/mrna

mRNA & Long RNA

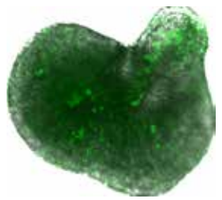
From The Modified Nucleic Acid Experts

For over 18 years TriLink has been an industry leader in manufacturing high quality RNA. We are pleased to offer the best mRNA and long RNA. These new product lines feature custom synthesis of mRNA and long RNA (up to multiple kilobases) with a wide array of modification services at scales ranging from µgrams to grams. We also offer stocked mRNA products including reporter gene, genome editing, gene replacement and reprogramming mRNA. All of our mRNA and long RNA products offer:

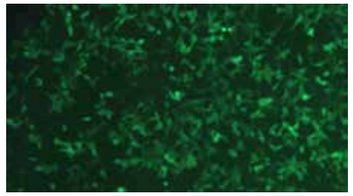
- High Quality at Competitive Prices
- Custom Tailored Support to meet Specific Application or Program Needs
- Wide Variety of Modification, Treatment and Purification Options
- Fully Traceable Documentation
- Path to Pharmaceutical GMP Manufacturing

Proven Expression

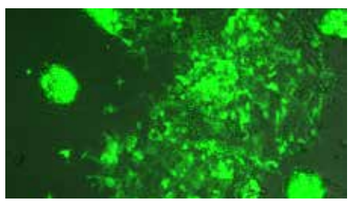
Very high levels of transfection with TriLink's modified mRNA has been observed in HEK-293, CHO and BJ Fibroblast cells. Even in difficult-to-transfect cells such as primary dendritic, CEM and primary CD34+ hematopoietic stem cells, expression has been demonstrated.



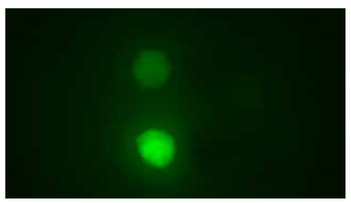
EGFP mRNA transfected *ex vivo* in mouse embryonic heart.
Image courtesy of George Porter, University of Rochester



EGFP mRNA transfected in HEK-293 cells.
Image courtesy of Jiehua Zhou, City of Hope



EGFP mRNA transfected in differentiating mouse embryonic stem cells.
Image courtesy of Alexandre Colas, Sanford Burnham Inst.



K562 cells microinjected with eGFP mRNA.
Image courtesy of Thomas Cradick, Georgia Tech

gallery.trilinkbiotech.com

Affordable Custom Synthesis

TriLink's RNA synthesis by transcription service is custom tailored to meet specific application or program needs. A wide variety of modifications, purifications and treatment options are available. Synthesis scales yielding µgrams to grams are offered. Our expert technical support team is available to guide you through the process from template source to final product.

Applications

- Genome Editing
- Gene Therapy
- Aptamer Research
- Stem Cell Reprogramming
- RNA Structural Studies and RNase Protection Assays
- Custom qPCR Standards

Request a Quote

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Types of RNA Transcripts

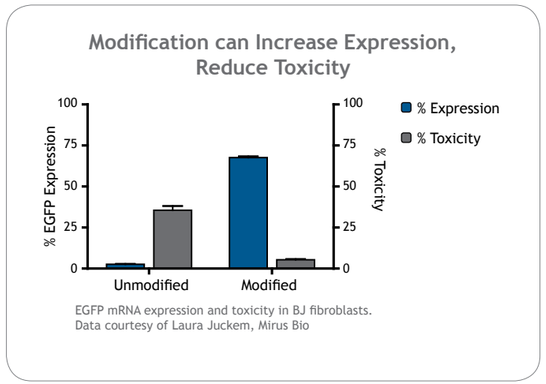
- Non-coding RNA: Long RNA, aptamers and other synthetic RNA for biochemical studies.
- mRNA (Coding RNA): Polyadenylated and capped mRNA offers the distinct advantage of integration-free gene expression.

Modifications

TriLink offers an extensive catalog of modified nucleoside triphosphates (NTPs) that impart desirable characteristics to *in vitro* transcribed RNA such as increased nuclease stability, increased translation or altered interaction with innate immune receptors. For example, incorporation of 5-Me-CTP and 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.

Purification and Treatment Options

- Silica-gel Membrane Spin Column Purification
- HPLC Purification
- Lithium Chloride Precipitation
- Acidic Guanidinium Thiocynate Phenol Extraction
- DNase Treatment
- Phosphatase Treatment
- Poly-A Polymerase Tailing
- Enzymatic Capping, Cap Methylation



EGFP mRNA expression and toxicity in BJ fibroblasts.
Data courtesy of Laura Juckem, Mirus Bio

Pharmaceutical GMP Manufacturing

TriLink recently built a 2,000 square foot pharmaceutical GMP production suite. TriLink's quality system now includes research, diagnostic and therapeutic GMP production capabilities. The facility contains nine labs and, in addition to mRNA and long RNA, it will be equipped to manufacture and process:

- Oligonucleotides
- Aptamers
- Small Molecules
- Nucleoside Triphosphates

Request a Consult

trilinkbiotech.com/gmp/consult.asp

Reporter Gene mRNA

Reporter genes are commonplace tools in cell biology research. TriLink has developed optimized capped (Cap 0) and polyadenylated mRNA expressing reporters. These reporter mRNAs can be used as controls to study mRNA transfection and expression in mammalian cells using a variety of assays including fluorescence microscopy, quantitative fluorometry and bioluminescent imaging, as well as fluorescence-activated cell sorting (FACS). TriLink's standard reporter gene mRNA is modified with 5-methylcytidine (5meC) and pseudouridine (Ψ) to reduce immune stimulation. Unmodified FLuc and ß-gal mRNA are also offered for systems where immunomodulation is not a concern.

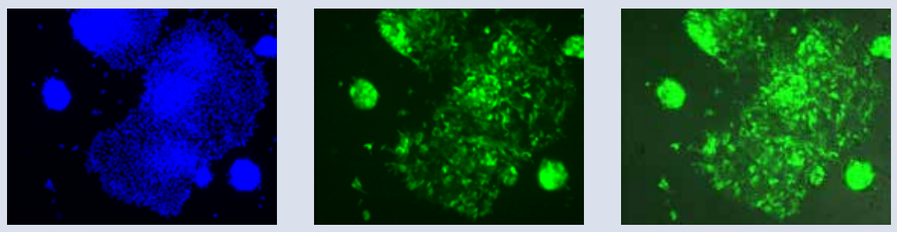
Products

EGFP mRNA (5meC, Ψ); L-6101
FLuc mRNA (5meC, Ψ); L-6107
ß-gal mRNA (5meC, Ψ); L-6109
mCherry mRNA (5meC, Ψ); L-6113
Eira CFP mRNA(5meC, Ψ); L-6114
Blaze YFP mRNA(5meC, Ψ); L-6115
Guassia Luc mRNA(5meC, Ψ); L-6123
Renilla Luc mRNA(5meC, Ψ); L-6124
EGFP mRNA(5meC); L-6126
FLuc mRNA(5meC); L-6127
Cyanine 5 FLuc mRNA (5meC, Ψ); L-6401
Cyanine 5 EGFP mRNA (5meC, Ψ); L-6402
EGFP mRNA ; L-6301
FLuc mRNA; L-6307
ß-gal mRNA;L-6309

trilinkbiotech.com/reportergenes

Order: trilinkbiotech.com/reportergenes

Robust Expression of EGFP mRNA



DAPI EGFP Overlay

Differentiating mouse embryonic stem cells transfected with EGFP using Lipofectamine® RNAiMax.
Image courtesy of Alexandre Colas and Mark Mercola, Sanford Burnham Research Institute

Customer Testimonials

"TriLink is my go-to source for mRNA expression. I placed a first-time order with TriLink after looking for a company to fulfill my mRNA needs to launch a recent company project. My initial experience with TriLink was very positive because: 1) the product I received was high quality and fit my requirements perfectly; 2) the customer service was exceptional, it addressed my particular project needs professionally and responsively and 3) the turnaround time for deliverables was rapid and exceeded expectations. It is this type of business service that has compelled me to scale-up my orders with TriLink for additional projects without hesitation."
- Tim Johnstone, Anvyl LLC

"The staff was very helpful in my decision to have TriLink make modified mRNAs for our lab and great in helping to design them."
- George Porter, Jr. MD, PhD, Assistant Professor at the University of Rochester

"We are very happy with the quality of mRNA produced by TriLink."
- Jin Li, Research Scientist at Cellectis

Reprogramming mRNA

TriLink manufactures optimized capped (Cap 0) and polyadenylated mRNA used in stem cell reprogramming. Reprogramming mRNA mimics fully processed mRNA which is a substrate for translation by the ribosome. For some applications, mRNA is preferred over DNA or viral vectors because there is no risk of integration into the genome which can lead to insertional mutagenesis.

iPS Cell Generation

Eliminating the risk of insertional mutagenesis is especially important in the generation of induced pluripotent stem cells (iPS cells). Once iPS cells are made, they are typically expanded, differentiated and sometimes implanted in animals. During this process, an insertional event can lead to a cancerous phenotype. For safety reasons, the stem cell community has moved to non-integrating approaches, such as mRNA expressing factors.

Efficient Delivery

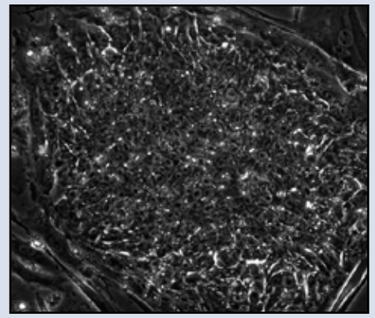
Delivery to the appropriate compartment in the cell has been a major barrier in gene therapy research. mRNA targets the cytoplasm, and hence only needs to cross the plasma membrane. In contrast, plasmid DNA and most types of viral vectors must reach the nucleus and therefore need to cross an additional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

Controlled, Rapid Expression Levels

With reprogramming mRNA, it is easy to control gene dosage. The transient nature of mRNA expression allows exquisite temporal control of expression. Expression levels achieved from different promoters used in plasmid DNA or viral vectors can vary dramatically from cell type to cell type. Ectopic promoters are also frequently transcriptionally silenced over time. With integrating viral vectors such as retroviruses or lentiviruses, the number of vector integrations and the location of integration can greatly influence expression in individual cells. All of these concerns are eliminated with mRNA because they function post-transcriptionally.

Lastly, mRNA is expressed more rapidly than DNA or virus based approaches since there is no waiting for transcription, splicing, polyadenylation and nuclear export.

TriLink Reprogramming mRNA Effectively Forms Stem Cell Colonies



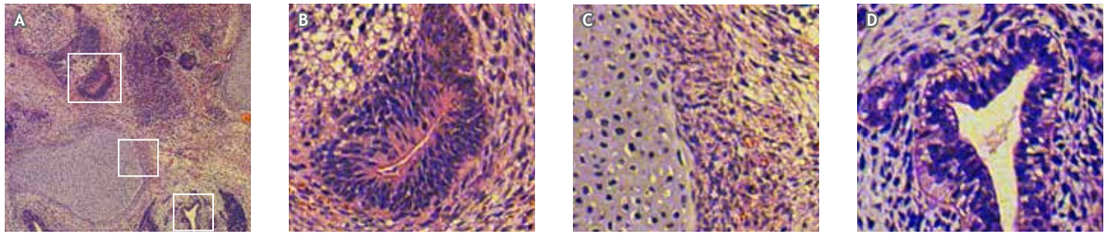
A stem cell colony formed from patient-specific fibroblasts transfected with TriLink mRNA displaying characteristic nucleoli and big nuclear cytoplasmic ratio.
Image courtesy of Karl-Dimiter Bissig, Baylor College of Medicine

Products

Oct4 mRNA (5meC, Ψ); L-6102
Klf4 mRNA (5meC, Ψ); L-6103
Sox2 mRNA (5meC, Ψ); L-6104
c-Myc mRNA (5meC, Ψ); L-6105
Lin28 mRNA (5meC, Ψ); L-6106

Order: trilinkbiotech.com/reprogramming

iPS Cells Reprogrammed with TriLink mRNA Demonstrate Pluripotency



iPS cells reprogrammed with TriLink mRNA form teratomas containing all three germ layers. (A) Low magnification overview of subcutaneous teratoma, (B) neural tissue, ectoderm, (C) cartilage and muscle, mesoderm (D) intestinal epithelium, endoderm. (B), (C) and (D) are high magnifications of insets in (A).
Image courtesy of Dr. Karl-Dimiter Bissig, Baylor College of Medicine

Genome Editing mRNA

Plasmids and viral vectors have traditionally been used in genome editing to express the required proteins inside cells or in an organism. However, editing DNA carries risk. Double stranded DNA breaks catalyze insertion of DNA at the cut site. At some substantial frequency, the protein expression vectors can integrate and lead to continuous expression of the nuclease or a previously silent sequence.

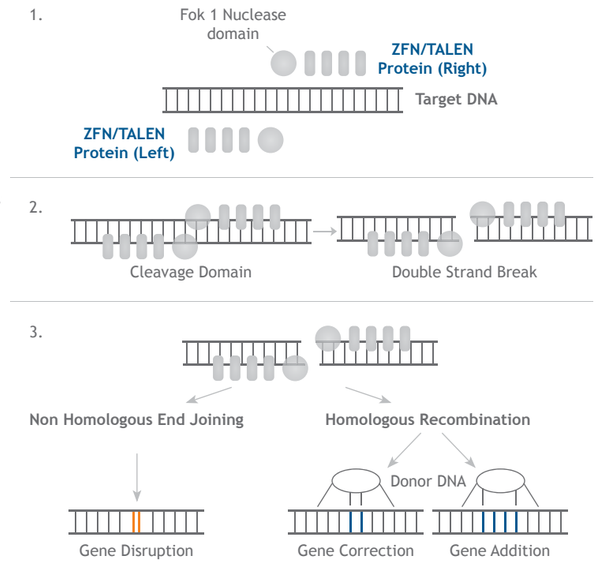
mRNA is being used in genome editing to transiently express the required proteins. With no risk of insertional mutagenesis, it is a powerful tool. Additionally synthetic mRNA can be produced in large quantities by *in vitro* transcription and modified to reduce innate immune stimulation.

trilinkbiotech.com/genomeediting

Zinc Finger Nuclease and Transcription Activator-like Effector Nuclease mRNA

Zinc-finger nucleases (ZFN) were the first widely applicable site specific genome editing tools. In the last few years, transcription activator-like effector nucleases (TALEN) have emerged as a more accessible than ZFN. Like ZFN, TALEN utilize a modular DNA binding motif that can be modified to introduce new DNA binding specificities. Unlike ZFN, TALEN are not as prone to sequence context effects which greatly complicate the *de novo* design, making them a more practical tool for the general scientific community. Additionally, several recent studies have shown that ZFN can have off-target effects at non-targeted chromosomal sites that are similar in sequence to the intended target site. For this reason, there is a move to transient ZFN 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.



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trilinkbiotech.com/mRNAquote

CRISPR Mediated Genome Editing

The newest 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. Due to recognition of the target DNA sequence being RNA mediated, rather than protein mediated, this new tool has generated considerable interest. With CRISPR, the RNA guide sequence targets the site of interest and the Cas9 protein is employed each time to perform the DNA cleavage.

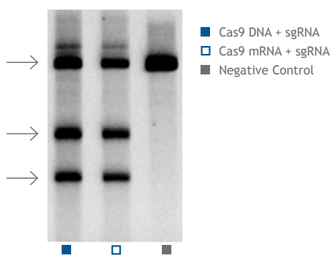
Products

Cas9 mRNA (5meC, Ψ); L-6125
Cas9 mRNA (Ψ); L-6129
Cas9 Nickase mRNA (5meC, Ψ); L-6116

Order: trilinkbiotech.com/genomeediting

CRISPR RNA guide sequences may be custom ordered through OligoBuilder®, go to oligobuilder.com.

Cas9 Expressed from TriLink mRNA Efficiently Cleaves Genomic DNA



Cas9 mRNA and Cas9 DNA expression plasmid (Cas9 DNA) were cotransfected with guide RNA expression plasmid (sgRNA) into HEK-293 cells. Genome editing assayed using SURVEYOR® Mutation Detection Kit.
Image courtesy of Feng Zhang and Fei Ann Ran, Broad Institute

Recombinase mRNA

Site specific recombinases are used in the manipulation of genomes and for conditional activation or de-activation of gene expression in cells and organisms. Recombinases recognize short target DNA sequences of approximately 30-40 nucleotides and catalyze directional DNA exchange reactions. Because the recognition sites are not commonly found in the genomes of higher organisms, they can be used as tools to engineer genomes. However, continued expression of a recombinase in a cell or *in vivo* can result in toxicity and undesired off-target recombination, making transient expression from mRNA an ideal method.

Types of Recombination

Products

NLS-Cre mRNA (5meC, Ψ); L-6108

Order: trilinkbiotech.com/genomeediting

Looking for a different recombinase mRNA? Contact us!

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. Recently, 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.

TriLink offers the following gene replacement markers that can be used to assess efficacy of mRNA delivery. Don't see what you need? Just ask!

Products

Human Coagulation Factor IX mRNA (5meC, Ψ); L-6110
Human Alpha 1 Antitrypsin (hAAT) mRNA (5meC, Ψ); L-6111
Erythropoietin (EPO) mRNA (5meC, Ψ); L-6118

Order: trilinkbiotech.com/genereplacement

Stocked mRNA Quick Guide

Cat.#	Name	20 µg	100 µg	1 mg	5 mg
L-6101	EGFP mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6102	Oct4 mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6103	Klf4 mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6104	SOX2 mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6105	c-Myc mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6106	Lin28 mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6107	FLuc mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6108	NLS-Cre mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6109	ß-gal mRNA (5meC, Ψ)	$105	$265	$1,375	$4,850
L-6110	Factor IX mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6111	hAAT mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6113	mCherry mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6114	Eira CFP mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6115	Blaze YFP mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6116	Cas9 Nickase mRNA (5meC, Ψ)	$105	$265	$1,375	$4,850
L-6118	EPO mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250

Cat.#	Name	20 µg	100 µg	1 mg	5 mg
L-6123	Guassia Luc mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6124	Renilla Luc mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6125	Cas9 mRNA (5meC, Ψ)	$105	$265	$1,375	$4,850
L-6126	EGFP mRNA (5meC)	$95	$225	$1,200	$4,250
L-6127	FLuc mRNA (5meC)	$95	$225	$1,200	$4,250
L-6128	OVA mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250
L-6125	Cas9 mRNA (Ψ)	$105	$265	$1,375	$4,850
L-6301	EGFP mRNA	$85	$195	$1,050	$3,700
L-6307	FLuc mRNA	$85	$195	$1,050	$3,700
L-6309	ß-gal mRNA	$95	$225	$1,200	$4,250
L-6328	OVA mRNA	$85	$195	$1,050	$3,700
L-6401	Cyanine 5 FLuc mRNA (5meC, Ψ)	$195	$450	$2,400	$8,500
L-6402	Cyanine 5 EGFP mRNA (5meC, Ψ)	$195	$450	$2,400	$8,500
L-6403	5-AA-U FLuc mRNA (5meC, Ψ)	$95	$225	$1,200	$4,250

Inquire for larger quantities.

TriLink BioTechnologies

The Modified Nucleic Acid Experts

Since 1996 TriLink has offered cutting edge services to researchers in the fields of gene therapy, nucleoside chemotherapy, oligonucleotide therapy and diagnostics. Our scientists and technicians have decades of collective experience in synthesizing modified nucleosides, nucleotides, oligonucleotides and transcripts for research, diagnostics and therapeutic applications.

TriLink operates a GMP laboratory with a QSR environment and provides:

- Milligram to multi-gram synthesis
- Highly-modified oligonucleotides and nucleosides
- Oligonucleotide radiolabeling services
- Custom chemistry
- Custom mRNA and long RNA synthesis
- Contract research services
- Industry-leading technical support

30%

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Printed with Soy-Based Inks

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