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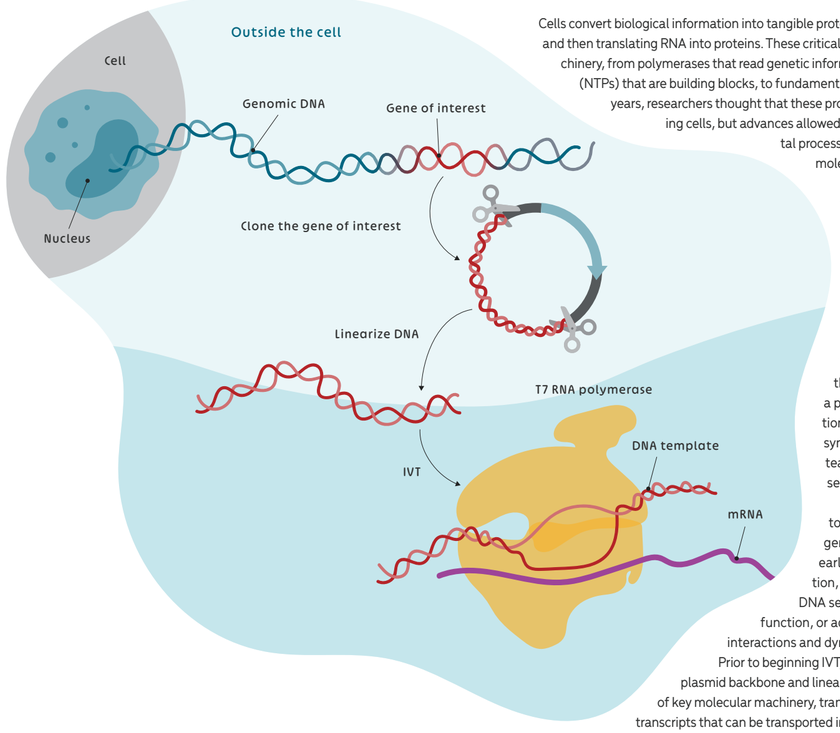
explained

How Do Scientists Make mRNA for mRNA Vaccines and Therapeutics?

Utilizing the technology behind mRNA vaccines begins with understanding and harnessing one of the most fundamental processes in cell biology: transcription.

If DNA is the blueprint of a cell, then its transcription into messenger RNA (mRNA), the intermediate single-stranded molecules containing the instructional information for specific genes, is the first step in making that blueprint a reality. The idea of leveraging mRNA to introduce new or modified gene copies not naturally expressed within cells dates back more than 50 years (1), culminating in the research behind the recently approved mRNA vaccines against SARS-CoV-2. Today, researchers are invigorated by the possibility of transcribing mRNA outside of cells, in a process known as *in vitro* transcription (IVT), and using these exogenously transcribed products for the development of vaccines and therapeutics.

What is IVT?



Cells convert biological information into tangible proteins by first transcribing DNA into RNA and then translating RNA into proteins. These critical processes rely on suites of molecular machinery, from polymerases that read genetic information, to ribonucleoside triphosphates (NTPs) that are building blocks, to fundamental chemical elements like magnesium. For years, researchers thought that these processes could only be performed within living cells, but advances allowed researchers to perform these fundamental processes outside living organisms using selected molecular machinery.

In particular, IVT is the process of converting DNA into mRNA without a living cell. This frees researchers from having to maintain fickle cells in a viable state or account for unpredictable biological processes that might alter protein outcomes. Researchers can standardize and control the entire transcription process. Once researchers have identified and isolated a gene of interest, they then clone the DNA sequence into a plasmid backbone for further manipulation and amplification (2). With the dawn of synthetic biology technology, many research teams now synthetically generate DNA sequences for their genes of interest.

The versatility of IVT lies in the ability to make numerous mRNA copies of any gene. Because IVT recapitulates one of the earliest steps in the path to protein production, researchers can introduce mutations in DNA sequences to enhance downstream protein function, or add molecular tags to investigate protein interactions and dynamics.

Prior to beginning IVT, researchers enzymatically digest the plasmid backbone and linearize the DNA template. With the addition of key molecular machinery, transcription commences, yielding mRNA transcripts that can be transported into target cells for translation into functional proteins by *in vivo* cellular machinery.

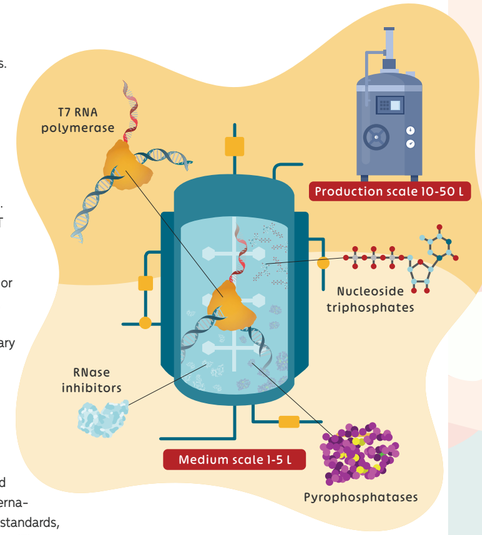
What materials are required for IVT?

To perform IVT researchers need several ingredients, including an RNA polymerase, NTPs, and supplementary enzymes. T7 RNA polymerase, originally derived from the T7 bacteriophage, is commonly used to produce high yields of *in vitro* transcribed RNA. During research and discovery, researchers place the DNA sequence for the T7 promoter upstream of their gene of interest, and the T7 RNA polymerase transcribes their linearized gene (2).

Other reagents also play critical roles in IVT success. As T7 RNA polymerase catalyzes phosphodiester bonds between nucleotides to form the newly synthesized RNA strand, it releases pyrophosphates (PPis) that can inhibit T7 RNA polymerase activity. Adding pyrophosphatase, which digests and removes PPis, increases transcription rate and is an essential component of an IVT reaction (3). Additionally, including an RNase inhibitor prevents premature RNA degradation. Utilizing a buffer solution containing dithiothreitol reduces oxidation, extending enzyme activity and increasing overall IVT efficiency (4). Finally, the ratio of magnesium (a cofactor in nucleic acid polymerization) and NTPs need to be optimized to obtain high yields of RNA (5).

Ancillary reagents such as these directly affect the purity and quality of mRNA therapeutics. Animal-origin byproducts or other impurities within ancillary IVT reagents can seep into the final mRNA preparations; if they are used in a therapeutic, they can cause undesirable effects like severe immune reactions and even death. From a company perspective, this could lead to high financial costs and loss of reputation and customer confidence. While researchers may use suboptimal ancillary products during the research and discovery phase, delaying the implementation of good manufacturing practice (GMP)-quality products until medium- to large-scale production could lead to manufacturing delays and increased costs.

GMP-grade products are generally verified to meet certain regulatory specifications but may not be validated for functionality. As such, researchers may find differences between product lots. Furthermore, some manufacturers of GMP-grade products may not be equipped to supply quality metrics or paper trails documenting products' animal-free origins for regulatory submission (6). In contrast, Thermo Scientific™ TheraPure™ GMP* IVT products come with comprehensive documentation to provide assurance on their analytical quality, animal-origin free, product specificity, and stability data. Furthermore, these TheraPure GMP products are manufactured and validated according to the relevant International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q2 standards, helping to ensure that they meet stringent quality standards for maximum manufacturing efficiency and therapeutic safety (7).

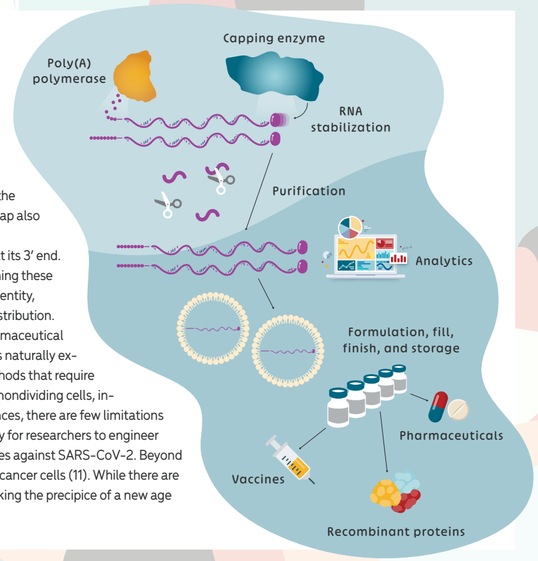


How do scientists make mRNA for mRNA vaccines and therapeutics?

Producing high-quality mRNA therapeutics extends beyond simply transcribing mRNA. The newly generated mRNA strand requires several molecular preparatory steps before it can serve as the basis for protein production in a potential therapeutic. Following IVT, capping enzymes add and methylate guanosine triphosphate, known as a cap, to the 5′ end of the mRNA. This cap stabilizes the mRNA and signals to the body that the mRNA is a "self" molecule, preventing its recognition and degradation by the innate immune system. The 5′ cap also ensures that the body's translation machinery recognizes the mRNA ready for protein translation (8).

The newly synthesized mRNA also receives a tail of adenosine monophosphates, known as a poly(A) tail, at its 3′ end. This tail helps ribosomes recognize the mRNA within the body's cells for protein production (9). After performing these essential molecular steps, researchers purify and analyze their final mRNA product to confirm its sequence identity, integrity, purity, potency, and safety. The mRNA product is then ready for final formulation, packaging, and distribution.

More researchers are turning to IVT mRNA technology to develop new gene therapies, vaccines, and pharmaceutical recombinant proteins. mRNAs do not require genomic integration to produce new proteins, because mRNAs naturally exist within the cytoplasm until their translation or degradation by cellular machinery. In contrast to other methods that require cells to be in an actively dividing state, mRNA-mediated protein expression occurs within both dividing and nondividing cells, increasing potential therapeutic efficiency. Lastly, unlike viral vectors that can only contain small gene sequences, there are few limitations to the size or number of mRNA sequences that researchers can deliver to cells (10). This opens the possibility for researchers to engineer gene sequences that yield more efficient recombinant proteins. Today there are two approved mRNA vaccines against SARS-CoV-2. Beyond vaccines, researchers have also used IVT mRNA technology to trigger the immune system to target specific cancer cells (11). While there are no currently approved mRNA therapeutics, there are several candidates in preclinical and clinical trials, marking the precipice of a new age of therapeutic development.



Ensuring quality

To navigate the inevitable demand for new vaccines and therapeutics, researchers need reliable access to quality ancillary reagents throughout the development of mRNA therapeutics. During the SARS-CoV-2 crisis, supply chain demand limited access to GMP-quality materials, prompting the need for a diversified global supply chain for purified, quality reagents [12]. TheraPure GMP products offer a reliable source for exceptional quality, speed, and scale to support the development of mRNA therapeutics. In fact, these products have been used in clinical and commercial mRNA therapeutics and vaccines that are in development or on the market (1). Using the same line of quality ancillary products used in present mRNA technology provides researchers with the assurance that their mRNA products are made with purity, quality, and functional reagents with the necessary documents to support a streamlined path to therapeutic discovery and application.

For raw materials from Thermo Fisher for mRNA production, please visit **thermofisher.com/therapure**

* "TheraPure GMP" refers to the quality level of the raw, ancillary, or starting materials to be used for further manufacturing. TheraPure GMP products are manufactured in facilities with ISO 9001–certified quality management systems that operate in accordance with relevant good manufacturing practice (GMP) principles, as outlined in ICH Q7 or equivalent guidance documents or standards.

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