Primrose Bio
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primrose bio

Advancing the manufacture of protein and nucleic acid-based biologic vaccines and therapeutics

Primrose Bio's advanced technology for manufacturing nucleic acids and proteins makes it the partner of choice for pharma companies developing new vaccines, therapeutics and bio-based products.

Primrose Bio, recently created by the merger of Primordial Genetics and the Pelican Expression Technology group (formerly Pfenex), brings together complementary microbial and enzymatic manufacturing technologies for nucleic acids and proteins used in genomic medicines, vaccines and next-generation biologic therapeutics. Primrose's unique and broad capabilities in nucleic acid engineering, molecular evolution and protein expression allow the company to create powerful manufacturing platforms that address the production of increasingly complex therapeutic and vaccine molecules. Primrose Bio was launched in September 2023 with $15 million in startup funding from Ligand Pharmaceuticals.

The potential public-health impact of new genetic medicines has been dramatically demonstrated in the past few years by the development of messenger RNA (mRNA)-based vaccines against COVID-19, an achievement recently recognized by the 2023 Nobel Prize in Physiology or Medicine. As a now-proven vaccine technology, there is enormous interest in applying the same mRNA-based approach to create vaccines against other viral pathogens, such as influenza virus, human immunodeficiency virus, herpes simplex virus, rabies virus, and respiratory syncytial virus, as well as novel therapeutics and immunotherapeutics targeting a diverse range of other diseases and conditions including rare diseases and cancer.

Creating these mRNA-based medicines of the future requires robust and inexpensive ways to manufacture mRNA. Enter Primrose Bio with its offering of commercially available, proprietary, best-in-class RNA polymerases for mRNA synthesis by in vitro transcription (IVT) (Fig. 1). Primrose Bio's Prima RNApols offer significantly higher mRNA yields and integrity, and more efficient capping, compared with the standard T7 RNA polymerase (T7 RNApol), as well as lower levels of double-stranded RNA (dsRNA) and other undesirable by-products of IVT reactions. Improvements in these key IVT performance indicators translate into reduced manufacturing costs, higher quality RNA, greater drug efficacy and potentially lower dosage.

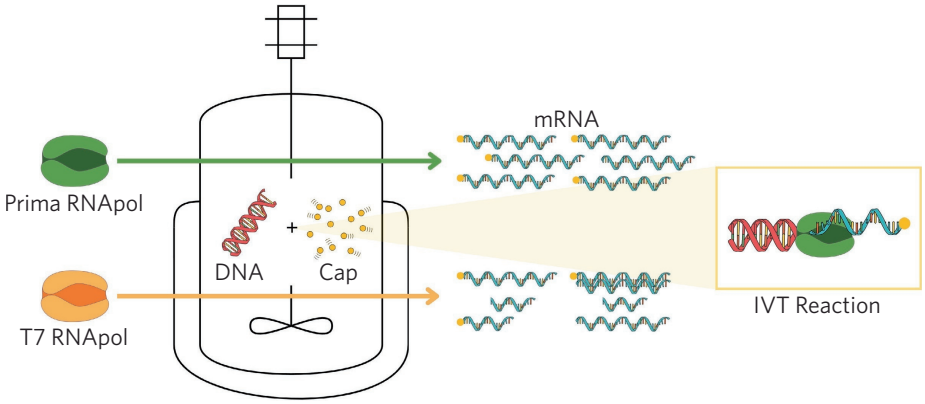

Fig. 1 | Messenger RNA (mRNA) synthesis by in vitro transcription (IVT). mRNA is produced by IVT of a DNA template (encoding the vaccine antigen or therapeutic protein of interest) by an RNA polymerase. Compared with industry standard T7 RNA polymerase, the use of Primrose Bio's Prima RNApols delivers a higher yield of target mRNA (full length and capped) with lower levels of undesirable side products such as truncated transcripts and double-stranded RNA (dsRNA).

Existing collaborations

Primrose Bio has research and development (R&D) collaborations with established pharmaceutical companies in the mRNA field and has licensed a Prima RNApol to Arcturus Therapeutics. Furthermore, five Prima RNApols were commercially launched in early 2023 to stimulate evaluations and collaborations with pharma companies and contract R&D organizations. In the quest to fuel the future of mRNA manufacturing, Primrose Bio has dozens of improved single-subunit Prima RNApols in development and is broadly working on other efficiency improvements of the IVT process.

> **The Pfenex Expression Technology platform has been successfully used to produce thousands of novel proteins, enzymes, vaccine antigens, antibody derivatives and other complex proteins**

Beyond mRNA-based vaccines and therapeutics, many other therapeutic modalities, from small-interfering RNA and other oligonucleotides to peptide- and protein-based therapies, depend on high-quality, cost-effective manufacturing processes and reagents. Primrose Bio intends to apply its broad competencies in nucleic acid synthesis and protein expression to provide novel manufacturing solutions to these molecules as well. By offering affordable manufacturing to developers of new drug modalities, the company hopes to support the development of new vaccines and cures and eliminate the manufacturing bottlenecks that currently prevent many promising new molecules from being advanced from the laboratory into clinical development.

Currently, oligonucleotide synthesis is built around phosphoramidite synthesis, which has inherent cost and scale limitations and involves the use of toxic organic solvents. Primrose Bio's enzymatic oligonucleotide synthesis (EOS) technology is a water-based process that eliminates the toxic waste stream and the permitting challenges of chemical synthesis. The company's unique EOS approach allows sequential nucleotide addition without blocking groups—specific chemical modifications to nucleotides that are employed by other companies developing EOS processes—which complicate the synthesis process and increase its cost. Primrose uses unblocked, natural nucleotides which promise a simpler, more robust and lower-cost oligonucleotide-synthesis process.

The platforms

Primrose Bio's commercial offerings are fueled by two core platforms: Pfenex Expression Technology and Function Generator genetic technology. The Pfenex Expression Technology platform, originally created by The Dow Chemical Company and perfected by Pfenex scientists over more than 20 years, is based on proprietary, modified strains of *Pseudomonas fluorescens* engineered to increase protein titers and quality, shorten development timelines, and reduce costs for the production of proteins and peptides used in human and animal

health, as well as in nutritional, consumer or industrial products.

The Pfenex Expression Technology platform has been successfully used to produce thousands of novel proteins, enzymes, vaccine antigens, antibody derivatives and other complex proteins (Table 1). Scalable up to 100,000 l and boasting soluble protein titers exceeding 20 g/l in multiple cases, the platform excels in the production of complex therapeutic and vaccine proteins that are difficult to make in conventional protein-production hosts.

Five products approved by pharmaceutical regulatory agencies use Pfenex's *P. fluorescens* as a production host, including Jazz Pharmaceuticals' Rylaze (which went from cloning through approval in less than six years) and Alvogen's Teriparatide Injection, the first FDA-approved alternative to Eli Lilly's FORTEO. Teriparatide Injection was developed by the Pfenex group itself through clinical development and FDA approval. These and other legacy programs, ranging from discovery through chemistry, manufacturing, and controls (CMC) and clinical manufacturing, give Primrose a deep expertise in protein drug development from start to finish.

Pfenex Expression Technology is also at the heart of the manufacturing process for Primrose Bio's PeliCRM197 vaccine-carrier protein based on CRM197, a non-toxic mutant of diphtheria toxin that is coupled to polysaccharide vaccine antigens, making these molecules more immunogenic and the vaccines more efficacious in generating an immune response. Primrose Bio's PeliCRM197 is the highest purity CRM197 commercially available and the only source of this protein in marketed vaccines for which customers can purchase or license an established process to manufacture their own supply.

PeliCRM197 production has been successfully scaled to kilogram quantities of research grade and good manufacturing practice (GMP) grade CRM197. Pneumococcal vaccines, including Merck's VAXNEUVANCE, Serum Institute of India (SII)'s Pneumosil (Pneumococcal Conjugate Vaccine—adsorbed, 10-valent) and SII's MenFive meningococcal vaccine, each of which uses CRM197 produced by Pfenex's *P. fluorescens*, have been approved and marketed by the FDA, European Medicines Agency (EMEA) and World Health Organization (WHO) for their respective territories. PeliCRM197 customers are exploring a wide variety of other applications for this promising carrier protein in immunotherapies, Alzheimer's disease drugs and anti-addiction vaccines.

Table 1 | Pfenex Expression Technology. Improvement in the expression titer, quality, or both for customer and internal program proteins expressed in traditional expression systems versus Pfenex Expression Technology.

Protein type	Alternative host	Pfenex results
Fab	Yeast: quality issues, low yield	10–20× yield and quality improvement
Growth factor	*E. coli*: insoluble expression; poor quality	20 g/l soluble active expression
Microbial outer membrane protein	Yeast: low yield, degradation, glycosylation	20× yield, quality, and activity improvement
Therapeutic enzyme	*E. coli*: undesirable isoforms, quality issues	10× yield improvement; no isoform issues
Human cytokine	*E. coli*: inclusion bodies; no soluble expression	Soluble active expression; elimination of refold
Multimeric antibody derivative	CHO: low expression (<10 mg/l)	20× yield improvement of soluble, active protein in 8 weeks
Vaccine antigen	Undisclosed; cost issues	10× yield improvement

Expanding the evolutionary genetic space with Function Generator

Function Generator, a unique and powerful molecular-diversification approach contributed to Primrose Bio by Primordial Genetics, is a genetic platform for creating novel and improved proteins and enzymes with desirable but hard-to-engineer characteristics. Conventional approaches to protein engineering operate within the confines of a limited genetic space defined by a known protein sequence and navigate this space for improvements. While effective, these methods are limited to proteins of known activity and function, which constrains the available solutions to a discovery or improvement challenge. Function Generator creates a vast and unexplored evolutionary genetic space to search for broader and better solutions.

> "Five products approved by pharmaceutical regulatory agencies use Pfenex's *P. fluorescens* as a production host

Function Generator was originally created to maximize an organism's genetic potential but is equally applicable to protein evolution. New proteins built by the technology can achieve quantum-leap improvements in microbial and enzyme performance. Function Generator uses thousands of protein-coding sequences from microbial genomes to generate tens of millions of novel genes. These sequences have never actually existed before in nature and have therefore remained hidden in the dark depths of evolutionary genetic space. This revolutionary method of enhancing biological performance can be applied to any microbe, as long as there is an annotated genome available.

The novel genes are then reintroduced into the original microbe, with one new gene combination represented per cell. Candidates with improved function are identified using ultra-high-throughput genetic screening assays which allow for the isolation of, for example, any desirable qualities or traits such as improved protein synthesis and solubility, resistance to or tolerance of toxic products or abiotic stresses, or enhanced productivity and metabolic efficiency. Function Generator has been used to generate microbes with greatly enhanced alcohol tolerance and resistance to heat and acidic pH.

The technology has also been used to create microbes that produce methionine, an amino acid that currently cannot be produced by fermentation, at commercially useful titers. The random, combinatorial nature of Function Generator allows the discovery of functionally novel proteins that would be impossible to predict or rationally engineer from first principles. Crucially, the genes and proteins represented in Function Generator libraries can be covered by composition of matter patents, ensuring stringent intellectual property protection.

> "Function Generator uses thousands of protein-coding sequences from microbial genomes to generate tens of millions of novel genes

Primrose is applying its molecular evolution platform to develop not just microbes used in industrial bio-production but also new enzymes and proteins. In combination with proprietary ultra-high-throughput screens, the company can isolate enzymes with enhanced activity, increased resistance to inhibitors or altered substrate specificity. Primrose can select improved RNA polymerases from huge numbers of starting variants: up to two billion enzyme variants can be screened in a single sample of a few milliliters in volume. The company has isolated Prima RNApols with activity many-fold higher than the parental proteins from which they were derived.

Through the broad capabilities of its platforms, representing many decades of scientific discovery and ingenuity, and its existing product offerings, Primrose Bio already stands as the go-to provider of mRNA manufacturing and protein expression solutions, which pharma companies can deploy through partnering and licensing arrangements. The company plans to continue innovating in these areas to provide its customers with steady efficiency gains. Primrose Bio will also leverage its new-found technology synergies into new areas of bio-discovery and bio-production that will allow powerful new active drug and vaccine molecules to be developed, manufactured and brought to market.

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