OVERCOMING pDNA SUPPLY CHALLENGES FOR mRNA MANUFACTURING

Demand for plasmid DNA (pDNA) is skyrocketing as mRNA and gene therapy candidates continue to progress from preclinical to early- and late-stage clinical studies. Sourcing challenges, quality issues, scaling difficulties, lack of standardization, and the need to meet specific and varying requirements for different applications are some of the hurdles that manufacturers must overcome if they are to meet that growing demand.

Plasmid DNA Plays a Critical Role in mRNA manufacturing

Production of messenger RNA (mRNA) begins with plasmid DNA (pDNA), which is a critical starting material for the process. Viral vector manufacturing also leverages pDNA as a critical starting material. With the dramatic increase in interest in both mRNA and viral vectors for gene and gene-modified cell therapies, the demand for pDNA has skyrocketed — particularly pDNA intended for use as a starting material rather than as a direct therapeutic.

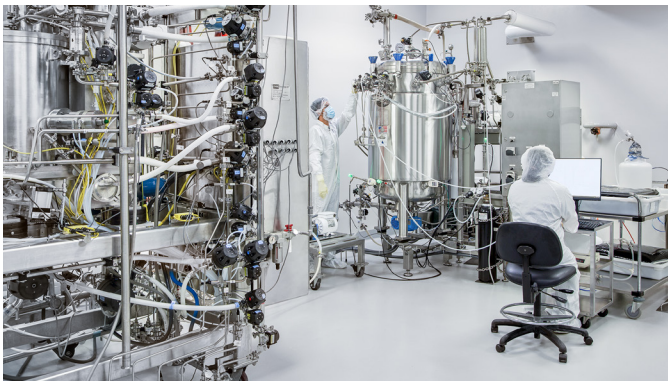
Complexities of Sourcing pDNA

Capacity for the production of pDNA, despite significant expansions by existing players and the entry of many new suppliers, remains constrained. Indeed, it is often necessary to order GMP-quality, custom-engineered pDNA at least one year in advance. Raw material supplies also remain limited, particularly single-use equipment components. Fortunately, additional capacity is expected to alleviate this situation in 2024, but those manufacturers that have formed partnerships with suppliers tend to have more supply chain security.

Need for High-Quality pDNA

Regulatory expectations for critical raw materials, including for ancillary materials that do not end up in the final product, have been increasing in recent years. Suppliers have responded by establishing three different product grades: research, an intermediate "high-quality" version that approaches but does not fully achieve GMP standards, and GMP.

For clinical production, most mRNA therapy and vaccine developers — and gene therapy developers as well — are requiring the intermediate grade, which is also variously referred to as "GMP-lite," "GMP-like," and similar names by different organizations. This material is generally produced under the same conditions as GMP material but with less involvement by quality assurance (QA) and therefore less extensive documentation. QA oversight can add significant cost to the product, so the high-quality grade offers the advantages of manufacture under GMP conditions but without the higher cost.



Wacker Biotech's pDNA production site in San Diego is equipped with 43-L single-use bioreactors and 650-L stainless-steel fermentation vessels, including continuous cell lysis.

One challenge facing both manufacturers and customers for the high-quality grade is the lack of a standard definition and hence consistent standards. Each supplier has its own specifications, with high-quality defined differently by each supplier. In some cases, it is likely that customers must specify the attributes of the high-quality pDNA they seek to purchase rather than it truly being a universal quality grade like GMP-grade pDNA. Going forward, there is an expectation that the industry will continue to move first to high-quality pDNA but will ultimately use GMP-grade material for most development work.

Scaling Challenges

Most pDNA is produced via bacterial fermentation using *Escherichia coli* strains in fed-batch mode. Cell lysis frees the pDNA, which is then purified via tangential-flow filtration and typically two chromatography steps, followed by sterile filtration and fill/finish operations.

Traditional pDNA fermentation processes are slow, afford yields much lower than those obtained for recombinant proteins and antibodies, and suffer from frequent batch failures. Yields and quality are also affected by the size of the plasmid and the nature of the genetic payloads. Purification is, meanwhile, typically time-consuming and complicated by the size and high negative charge of pDNA, which lead to low flow rates and difficulties achieving sufficient concentrations — problems that are magnified at larger scale.

In addition, pDNA is shear-sensitive and can undergo topological changes, leading to higher levels of non-supercoiled isoforms, the risk of which rises as process scale increases. Furthermore, many impurities present after the lysis step have properties similar to those of the desired plasmid and can be quite difficult to remove without significant product losses.

Downstream processing of pDNA in Wacker Biotech's San Diego facility is available to suit various customer needs along the manufacturing path.

A final complication is the wide variation in genetic payloads and other requirements of plasmids for different applications. Transient transfection to produce different viral vectors requires different plasmids with varying components and payloads, depending on the target virus. Those properties are different still from those of the pDNA required for the manufacture of mRNA vaccines and therapeutics, each of which will have a unique sequence. Furthermore, research has shown that careful engineering of plasmids to eliminate non-necessary genetic material can be highly beneficial.

Wacker Biotech: An Experienced, Stable, and Integrated pDNA and mRNA Outsourcing Partner

Through its pDNA Center of Excellence in San Diego, California, Wacker Biotech has decades of experience in pDNA development and production. Our highly experienced team has been manufacturing GMP-compliant pDNA since 2003 and has released over 100 GMP batches.

Wacker Biotech's San Diego location provides pDNA production and has been manufacturing GMP-compliant pDNA since 2003.

Wacker Biotech's versatile plug-and-play PLASMITEC® platform leverages innovative, cutting-edge pDNA technologies and high-performing strains to produce excellent yields of supercoiled pDNA a consistent and reproducible manner. Even complex plasmids are produced efficiently, and the same process is leveraged to produce material of different grades in small to large volumes for seamless scaling.The proprietary platform process includes continuous lysis and pDNA quality control and enables efficient production of research, high-quality, and GMP grades of pDNA, including clinical and commercial material, for applications ranging from preclinical safety and tox studies to viral vector, mRNA, and injectable DNA product manufacturing.

The combination of our expertise in pDNA and efficient production platform with extensive capacity enables Wacker Biotech customers to benefit from shortened lead times and reduced project turnaround times. Those clients with mRNA products can realize additional advantages by leveraging Wacker Biotech's integrated mRNA development and manufacturing services, which include *in vitro* transcription, lipid nanoparticle production, and fill/finish operations from research to commercial scale.

Value of Platform Processes

To overcome these challenges, many manufacturers have focused on developing platform manufacturing processes for the production of pDNA. The key to success of these platform approaches, particularly for contract manufacturers, is the incorporation of sufficient flexibility to accommodate a wide range of pDNA molecules. Developing a platform for which the impacts of *Escherichia coli* strain characteristics and plasmid size and complexity on product yield and quality are understood eliminates the need to optimize individual processes for different plasmids, providing both cost and time advantages.

Benefits of Integration with End-Use Applications

For developers of novel drug products that leverage pDNA as a critical starting material, there can be tremendous benefits to partnering with a contract development and manufacturing organization (CDMO) that can support not only pDNA development and production but also development and manufacture of the ultimate therapeutic or vaccine product.

Combining capabilities in pDNA engineering, design, and production with mRNA manufacturing, for instance, ensures development of the optimal pDNA sequence for the specific mRNA therapeutic or vaccine product. In addition to simplification of planning and oversight of each process step, all materials are produced under the same quality, management, and regulatory systems. Furthermore, close collaboration between R&D, analytical, and production teams ensures alignment of all activities with reduced timelines, cost, and risk associated with transfer from pDNA to mRNA production.

About the Author

Mack earned his B.S. in Biology from Baylor University (Waco, TX) and his Ph.D. in Biochemistry from Texas A&M University (College Station, TX), where he focused on X-ray crystallography and structure-based drug design of proteins essential to tuberculosis and malaria. Following his tenure at several biotech companies, including Thermo Fisher, he joined Genopis as the Process Development Manager, where he added plasmid manufacturing to his knowledge base. WACKER then acquired Genopis and established WACKER Biotech US in San Diego, where Mack has been Associate Director of Process Development since 2021.

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