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Is Synthetic Plasmid DNA One of the Solutions for Meeting Growing Demand for Next-Gen Therapies?

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Next-generation vaccines and therapies in development, including those based on DNA, RNA, and viral vectors are all overwhelmingly manufactured using plasmid DNA (pDNA). The traditional method for the large-scale production of pDNA involves fermentation in *Escherichia coli* strains, which is a laborious, time-consuming, and expensive process, making plasmid production a bottleneck and potential inhibitor of growing success for next-generation therapies. Enzymatic routes to synthetic pDNA may address the problem.

Growing Plasmid Demand

DNA serves as a fundamental material for basic biological research, synthetic biology, and large-scale biological manufacturing and has potential uses in many other applications, from data storage to nanotechnology and beyond.[1,2] Before the COVID-19 pandemic, capacity was tight for both customized DNA sequence and plasmid DNA (pDNA) production for these various applications. The pandemic created considerably greater demand for DNA used for both research and manufacturing purposes, demand that continues to grow given the expanding interest in DNA- and RNA-based therapies and vaccines, cell-free protein synthesis, DNA origami, and other applications.

One estimate sees global demand for DNA rising from 3 kilograms per year to anywhere from 10 to 100 kilograms annually, a three- to 30-fold increase.[1]

Fermentation Limitations

DNA used for research and biopharmaceutical manufacturing purposes generally is produced using two very different methods. Solid-state synthesis leveraging phosphonamidite chemistry is used for the former to generate custom DNA sequences (see sidebar). Fermentation in *E. coli*, meanwhile, is the common process for the manufacture of pDNA.

Bacterial fermentation generates pDNA containing a bacterial backbone, antibiotic-resistance genes, and endotoxins, all of which can present issues for therapeutic applications.[3] Despite the development of platform processes by many contract manufacturers, production of pDNA in large bioreactors involves a considerable plant footprint and often affords low yields, and batch failures are not infrequent owing to the complexity and nature of upstream and downstream operations. With limited large-scale, GMP production capacity, lead times for GMP pDNA can also be many months or more.

Benefits of Synthetic DNA

Synthetic pDNA produced using enzymes in a cell-free manner can address the primary issues associated with fermentation. High yields, simpler purification, no concern about bacterial or antibiotic resistance genes, and a smaller footprint are among the most appealing advantages.[3]

One of the challenges hindering the embrace of enzymatic pDNA synthesis in the past was the high error rate associated with conventional polymerases compared with that of bacterial amplification systems.[3] Advances in enzymatic design and engineering have largely addressed this problem, reducing the risk of mutations to a level comparable to that observed using fermentation.

For pDNA destined to serve as a template for mRNA manufacturing, a further advantage of synthetic DNA is that most is directly generated in a linear format.[3] Plasmid DNA obtained via fermentation is supercoiled and must be enzymatically linearized before use in the *in vitro* transcription (IVT) reaction for mRNA production. In addition, it is easier to include longer poly(A) tails in synthetic pDNA, which enable the production of more stable mRNA constructs. The lack of unnecessary genetic material can lead to higher transcription yields.

Cell-free synthesis of pDNA has advantages beyond the biopharmaceutical industry. Cell-free pDNA synthesis could be beneficial for a variety of industrial, diagnostic, food, and agricultural applications.[4] For these uses, the avoidance of complex or unstable sequences combined with the shorter timeline, higher yields and quality, and lower cost for production at large scale are key benefits.

Different types of synthetic pDNA intended for use in the production of vaccines and therapeutics are discussed below.

Doggybone DNA from Touchlight

Doggybone DNA (dbDNA™) from Touchlight is linear, closed pDNA that is produced via rolling circle amplification from a plasmid template and contains no bacterial sequences.[1,5] In the process, an amplification enzyme and a processing enzyme are used to quickly produce high-quality product within a relatively small footprint.

The company is targeting many different therapeutic applications and can do so because its synthetic dbDNA can incorporate genes of interest up to 20 kilobases in size and with much higher complexity than can be produced using conventional bacterial fermentation, according to the company.

In May 2023, Touchlight announced the completion of an expansion of its production facility, which tripled capacity for dbDNA to 8 kilograms per year.[6] In November 2023, the company announced that its proprietary dbDNA as a critical starting material for manufacturing of VMB-100, a potency-enhanced mRNA encoding human insulin-like growth factor-1 (IGF-1) to support Versameb's first-in-human clinical study to treat chronic stress urinary incontinence (SUI).[7] Also in 2023, the U.S. Food and Drug Administration (FDA) cleared the first investigational new drug (IND) application for a therapeutic produced using dbDNA[8] and accepted Touchlight's drug master file (DMF) for GMP-grade dbDBA[9].

New Circular DNA from OriCiro Genomics

OriCiro Genomics has developed a very different technology for production of synthetic pDNA. The company assembles DNA fragments into a circular plasmid that can then be rapidly amplified.[10] It offers the OriCiro Assembly Kit comprising different DNA fragments and the tools to combine up to 50 of them into a circular pDNA molecule in one step after just a few hours of incubation. The isothermal enzymatic reaction mimics the process used by *E. coli* but under cell-free conditions. The result: reduced cost and time for pDNA production and the ability to clone plasmids with difficult sequences not accessible via bacterial fermentation, such as those that are cytotoxic to cells and large, "genome-scale" DNA.

Furthermore, the process is scalable and provides a high concentration of pDNA.
The potential of OriCiro's technology was validated when mRNA developer Moderna announced that it was acquiring the company in January 2023.[11] Moderna's Chief Executive Officer Stéphane Bancel indicated the company made the move to gain access to "best-in-class tools for cell-free synthesis and amplification of plasmid DNA, a key building block in mRNA manufacturing."

ENFINIA DNA from Elegen Bio

Elegen Bio offers ENFINIA DNA, long linear DNA produced using "synthetic biology workflows with minimal or no cloning."[12] The process yields linear DNA as long as 7,000 bp in just 6–8 business days with error rates as low as 1:70,000 per bp. The company claims rapid access to its long DNA with low error rates can greatly accelerate mRNA therapy and vaccine development by shaving up to 15 weeks off development timelines.

Four Application-Specific DNA Construct Types from 4basebio

The synthetic DNA offered by 4basebio does not involve plasmid amplification, so there is no inclusion of bacterial sequences.[13] If used for viral vector manufacture, there is also no risk of reverse packaging. The cell-free process also avoids contamination from endotoxins and host-cell proteins. For mRNA applications, the process allows the amplification of long, complex sequences (up to 12 kb), such as inverted terminal repeats (ITRs) and long poly-A tails. The company offers four customizable, application-specific DNA construct types for mRNA, adeno-associated virus (AAV), and lentivirus production, as well as gene editing and other applications, and can produce microgram to multigram batches using its scalable enzymatic process.

The LinearDNA™ platform from LineaRx

The LinearDNA™ platform from LineaRx, an Applied DNA Sciences, Inc. company, leverages polymerase chain reaction (PCR) technology for the production of synthetic pDNA.[14] The process provides a

known/fixed yield in a matter of days at the microgram scale to weeks at the gram scale (compared with months for conventional pDNA), with DNA construct optimization achieved via primer modification and 100% of the generated DNA comprising the target therapeutic sequence. Sequences as long as 20 kb are possible while avoiding the inclusion of unwanted pDNA sequences. The company has also shown that its synthetic DNA provokes strong humoral and cellular immune responses at lower total DNA quantities than pDNA produced via fermentation.

Also noteworthy is LineaRx's Linea IVT Platform, which combines its enzymatically produced Linea™ DNA IVT templates and proprietary Linea™ RNA polymerase ("RNAP"), which the company obtained when it acquired Spindle Biotech.[15] Applied DNA announced at the end of 2023 that it is collaborating with the contract development and manufacturing organization (CDMO) Kudo Biotechnology to integrate the Linea™ IVT Platform into Kudo's mRNA manufacturing workflow.

In November 2023, meanwhile, the company reported the extension of its enzymatic synthesis technology to the production of self-amplifying mRNA (saRNA).[16] Earlier that year (March), Applied DNA announced the manufacture and shipment of a multi-gram quantity of linearDNA™ in under six weeks to a global manufacturer of *in vitro* diagnostics.[17]

Enzymatic Synthesis of Research Plasmids

The synthesis of customized DNA sequences for research and discovery purposes has traditionally been achieved via phosphonamidite synthesis. This technology enables the rapid production of oligos — or short, specifically coded sequences — but requires the use of hazardous chemicals and is highly energy- and labor-iintensive.[1] As with the manufacture of pDNA alternatives, enzymatic approaches are of growing interest in this area as well.

Enzymatic approaches allow for the generation of much larger sequences (up to 300 nucleotides) in a short period of time without the use of harmful reagents, and several startups are seeking to commercialize technologies that accelerate customized DNA synthesis.[18] Techniques include rolling circular amplification, which requires a plasmid template; Gibson assembly and polymerase cycling assembly (PCA), which are gene assembly approaches; and template-independent enzymatic oligonucleotide synthesis (TiEOS).[2]

Companies such as Molecular Assemblies, DNA Script, and Ansa Biotechnology have recently attracted notable investments.[18] Molecular Assemblies and DNA Script, along with Nuclera, are developing terminal deoxynucleotidyl transferase (TdT)-based TiEOS solutions. Ansa Biotechnologies elected to use modified TdT to address reliability and efficiency issues. Molecular Assemblies, meanwhile, is partnering with enzyme company Codexis to pursue engineering solutions as well.

Camena Bioscience took a totally different approach, developing a proprietary mix of enzymes for the synthesis of DNA from trinucleotides.[18] Both Camena and DNA Script report coupling efficiencies greater than 99% for short sequences at small scale. Some companies are looking to offer synthesis services, while others are creating benchtop instruments for use in laboratories. DNA Script and Nuclera are two examples of the latter.

There are other interesting approached to DNA synthesis as well.[18] Evonetix is using thermally controlled synthesis to facilitate parallel DNA synthesis. Ribbon Biolabs is synthesizing >10 kb duplex DNA via the convergent assembly of double-stranded oligonucleotide pools. Twist Bioscience leverages a silicon microarray chip and ink-jet printing.

Potential for Significant Impact of Synthetic pDNA

Progress in the manufacture of large quantities of DNA for use in the production of therapeutics, vaccines, and diagnostics cannot be disregarded. Therapies produced using synthetic DNA have begun to enter the clinic. Leading pDNA manufacturer Aldevron (part of Danaher) is developing a process for large-scale production of cell-free DNA via enzymatic synthesis.[19] While the space remains nascent, the benefits of cell-free pDNA, from time and cost savings to smaller footprints and greater sustainability, could eventually drive the biopharma industry away from cell-based manufacturing — not just for plasmids, but proteins and other biomolecules as well.

References

1. **"**The DNA Industry Is Going Cell-Free**."** Symbiota Emerging Technologies Blog. 4 May 2021.
2. **Hoose, Alex et al.** "DNA synthesis technologies to close the gene writing gap." *Nature Reviews Chemistry.* 7: 144–161 (2023).
3. **Dhir, Ashish and Amy Walker.** "'Ready-to-use' enzymatically produced cell-free synthetic DNA in mRNA manufacturing." *Labiottech.* 10 Jul. 2023.
4. **Cumbers, John.** "A Pioneer Of Cell-Free Genome Technology Is Unlocking Biology's Potential." *Forbes*. 6 Jul. 2021.
5. "Give A Dog A Bone: Touchlight's Cell-free DNA Vector Synthesis Ignites Genetic Medicine Manufacturing." *Eng. News.* 20 Jun.2022.
6. *Touchlight completes expansion, creating one of the world's largest DNA manufacturing facilities in London, UK.* 24 May 2023.
7. *Touchlight's enzymatic doggybone DNA used in the manufacture of Versameb's VMB-100 for first-in-human clinical study.* 21 Nov. 21, 2023.
8. *Touchlight announces first FDA clearance of an IND utilising doggybone DNA*. Touchlight. 28 Feb. 2023.
9. *FDA accepts Drug Master File, signifying a new milestone for Touchlight's enzymatic doggybone DNA technology.* 24 Jan. 2023.
10. **Cumbers, John.** "A Pioneer Of Cell-Free Genome Technology Is Unlocking Biology's Potential." *Forbes*. 6 Jul. 2021.
11. *Moderna to Acquire OriCiro Genomics.* 4 Jan. 2023.
12. "ENFINIA Long Linear DNA." Elegen Bio. Accessed 17 Jan. 2024.
13. "A faster and safer alternative to plasmid DNA." 4basebio. Accessed 17 Jan. 2024.
14. "A simple cell-free enzymatic platform for large-scale DNA manufacturing." LineaRx. Accessed 17 Jan. 2024.
15. *Applied DNA Announces Joint Development Agreement to Integrate Linea™ IVT Platform into CDMO Kudo Biotechnology's mRNA Manufacturing Workflow.* Applied DNA. 14 Dec. 2023.
16. *Applied DNA Extends Application of Linea™ IVT Platform to saRNA (self-amplifying mRNA).* Applied DNA. 16 Nov. 2023.
17. *APDN Achieves Milestone for Large-Scale linearDNA™ Production, Delivers Largest Single Shipment by Volume to Date.* Applied DNA. 29 Mar. 2023.
18. **Eisenstein, M.** "Enzymatic DNA synthesis enters new phase." *Biotechnol.* 38: 1113–1115 (2020).
19. **Dutton, Gail.** "Synthetic DNA and RNA Can Open New Manufacturing Workflows." *Eng. News.* 5 Oct. 2023.