

DNA Synthesis Needs A Paradigm Shift

To accelerate research, scientists need longer and more complex DNA sequences than traditional synthesis techniques can build.

When the very first gene was synthesized by H. Gobind Khorana in 1970, the response from the research community was a collective shrug. An editorial in *Nature New Biology* at the time stated, “Like NASA with its Apollo Programme, Khorana’s group has shown [that synthesizing a gene] can be done, and both feats may well never be repeated.”¹

Fast forward to the present, and scientists are engineering metabolic pathways in cells to turn them into factories for valuable chemicals, developing personalized cell-based therapies to treat cancer, and deploying an mRNA vaccine to overcome a global pandemic. All of this work has been powered by our ability to write DNA sequences. No human has set foot on the moon since the Apollo Program finished in 1972, but scientists use millions of synthesized genes every year.

All of these advances can be traced back to the laboratory creation of the first man-made DNA molecule, a thymidine dinucleotide. Over the following decades, a small community of chemists developed various iterations of reaction schemes to increase the lengths of DNA molecules that could be synthesized, ultimately establishing the nucleoside phosphoramidite method in the early 1980s. To this day, the phosphoramidite-based synthesis approach developed by Marvin Caruthers — also known as chemical DNA synthesis — remains the primary method for DNA manufacture.

Pushing the boundaries

While phosphoramidite chemistry has been a workhorse for many applications, it is insufficient for the life sciences industry of the future. To truly unlock the genomics revolution, employ synthetic biology for sustainable food and chemical production, and make groundbreaking advances in healthcare, the ability to write DNA requires significant improvement.

As scientists’ understanding of DNA has expanded, so too has the need for longer and more complex sequences to power modern experiments. From designing new drug candidates to synthesizing full genomes, the demand for diverse sequences is constantly increasing. However, researchers still face the vagaries of chemical DNA synthesis, which cannot produce long DNA constructs or complex DNA sequences, suffers from long turnaround times, and becomes cost-prohibitive when perfect accuracy is required.

Reliable access to long DNA constructs of any sequence could substantially accelerate the rate of research progress, particularly in the area of cell and gene therapies. Building complex biological systems requires rapid iterations, and accelerating this cycle of learning and improvement is essential for realizing the promise of engineering biology.

To be sure, there have been improvements in the field of chemical DNA synthesis — but they have offered only incremental change. Efforts to tweak processes, apply new techniques for oligo assembly, and isolate correct molecules have not led to the kind of breakthrough change required.

The future is enzymatic

What the research community needs isn't incremental change, it's a full paradigm shift. To realize the potential of the life sciences, DNA synthesis must outperform the current state of the art by orders of magnitude.

The answer lies in enzymatic DNA synthesis. Unlike chemical synthesis, an enzymatic approach harnesses the power of molecular biology to do what it does best: make highly accurate DNA sequences. Enzymes catalyze chemical reactions at unmatched efficiency by selectively binding molecules and arranging them with picometer precision to initiate the formation of new chemical bonds. This allows for perfect specificity, the ultimate requirement to enable the next generation of DNA synthesis.

In contrast, chemical synthesis requires 10,000-fold higher concentrations of reagents and operates by forcing molecules to randomly collide with each other. Activating and protecting groups are used to guide the process toward the formation of desired bonds, but unavoidable side-reactions occur that damage the growing strands and limit the quality of product that can be achieved. This sets a ceiling for chemical DNA synthesis that is much lower than what enzymatic approaches can accomplish.

But enzymatic DNA synthesis on its own is only part of the answer. Dozens of attempts at developing enzymatic DNA synthesis technologies haven't been able to clear the high bar set by phosphoramidite synthesis. It isn't just about using enzymes, but about how they are used that matters.

A new approach

Based on novel enzymatic DNA synthesis concepts developed in the Keasling Lab at the University of California, Berkeley, and Lawrence Berkeley National Laboratory, Ansa Biotechnologies aims to give scientists access to the long or complex DNA sequences that cannot be produced by traditional synthesis vendors.

The Ansa approach relies on an innovative conjugate technique that enables faster cycle times for rapid, accurate synthesis of kilobase-long DNA sequences.² The method employs the same terminal deoxynucleotidyl transferase (TdT) enzyme used in other enzymatic DNA synthesis workflows but overcomes challenges associated with speed, purity, and scalability to deliver higher-quality products to customers faster.

Ansa's technique also outperforms chemical DNA synthesis. In addition to producing the kinds of complex DNA sequences that stymie phosphoramidite-based approaches, the Ansa method has no toxic byproducts for a more sustainable solution.

Ansa's synthesis technology is currently available to scientists through an early access program. To learn more, please visit www.ansabio.com.

References

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2. Palluk S, Arlow DH, et al. 2018. De novo DNA synthesis using polymerase-nucleotide conjugates. *Nat Biotechnol*. 36(7):645–650