BIOCHEMISTRY

An enzymatic oligonucleotide synthesizer

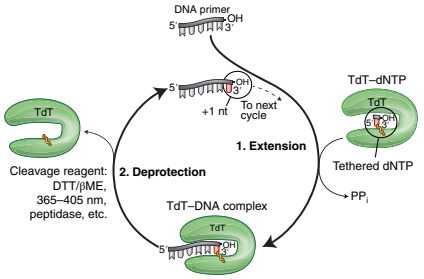
Reversible primer termination, enabled by polymerase–nucleotide conjugates, provides an enzymatic method for the de novo synthesis of oligonucleotides.

Over the past decade DNA-sequencing technologies have transformed the capacity for genome reading. In contrast, the ability to write genes still relies largely on the assembly of short oligonucleotide fragments into a defined, full-length sequence.

Short oligonucleotides are generally synthesized via the solid-phase phosphoramidite method pioneered by Marvin Caruthers and colleagues in 1981. Despite the advances made in solid-phase synthesis since then, the accumulated errors inherent in the stepwise addition of phosphoramidite nucleosides limit cost-effective synthesis to oligonucleotides about 200 nucleotides in length. To generate full-length genes, it is necessary to combine assembly protocols with oligonucleotide synthesis.

"To get full-length gene, you synthesize small pieces, put them together, and do sequencing that can take time," says Jay Keasling from UC Berkeley. He and his team developed an enzymatic approach that they hope will circumvent some of the limitations inherent in phosphoramidite chemistry. They take advantage of a unique, template-free DNA polymerase, terminal deoxynucleotidyl transferase (TdT), which catalyzes the stepwise addition of the nucleotide onto the 3′ OH of an oligonucleotide primer.

Specifically, polymerase TdT is conjugated with a single deoxyribonucleoside triphosphate (dNTP) via a cleavable linker. The TdT–dNTP conjugate serves as a reversible terminator for chain extension. Once the TdT–dNTP is incorporated at the 3′ end, the primer is inaccessible to another TdT–dNTP, thus generating a temporary terminated primer +1-mer. The linkage between TdT and dNTP is then cleaved by a cleavage reagent (e.g., UV light), which releases the primer +1-mer for a subsequent extension. Iteration of the reaction cycle extends the primer to a sequence defined by the order of TdT–dNTP addition. In each reaction cycle, polymerase TdT is treated as not only a catalyst but also a reagent. "This is pretty novel thinking," comments Jay Keasling; "rather than having a catalytic turnover, we have a stoichiometric turnover."



Reaction cycle for reversible termination of chain extension. Reproduced with permission from Palluk et al. (2018), Springer Nature.

Although the photocleavable conjugates leave a scar, an unnatural modification, on nucleobases, DNA containing scarred bases can still be used as a template for accurate amplification by PCR. As a proof of concept, a 10-mer was de novo–synthesized and confirmed by Sanger sequencing. The average yield of all steps can reach 97.7%, with errors arising from deletion (1.3%) and insertions (1.0%). Keasling also added, "You can imagine someday we will have a machine doing the reaction cycles, and the error certainly will further go down."

Ultimately, only a few DNA molecules, serving as an original copy, are required from de novo synthesis. If a larger amount is desired, then subsequent PCR reactions can be used to amplify the synthesized DNA molecules. Yet, practical gene synthesis by means of de novo DNA synthesis still has a long way to go. Keasling commented that automating the process, finding a solid support for the growing chain, and optimizing the linker chemistry are the critical, forwarding steps.

The use of de novo DNA synthesis to write any desired gene sequence, in a faster and cheaper fashion, will find many applications in genomics.

Lei Tang

Published online: 31 July 2018
https://doi.org/10.1038/s41592-018-0096-x

Research papers

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