De novo DNA synthesis using polymerasenucleotide conjugates

Oligonucleotides are almost exclusively synthesized using the nucleoside phosphoramidite method, even though it is limited to the direct synthesis of ~200 mers and produces hazardous waste. Here, we describe an oligonucleotide synthesis strategy that uses the template-independent polymerase terminal deoxynucleotidyl transferase (TdT). Each TdT molecule is conjugated to a single deoxyribonucleoside triphosphate (dNTP) molecule that it can incorporate into a primer. After incorporation of the tethered dNTP, the 3’ end of the primer remains covalently bound to TdT and is inaccessible to other TdT-dNTP molecules. Cleaving the linkage between TdT and the incorporated nucleotide releases the primer and allows subsequent extension. We demonstrate that TdT-dNTP conjugates can quantitatively extend a primer by a single nucleotide in 10-20 s, and that the scheme can be iterated to write a defined sequence. This approach may form the basis of an enzymatic oligonucleotide synthesizer.

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