ENGINEERING ENZYMES TO PRODUCE HIGH PURITY SYNTHETIC DNA

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Key Words: Terminal deoxynucleotidyltransferase, Enzymatic DNA synthesis

Phosphoramidite chemistry has been the gold standard in polynucleotide synthesis since the 1970s. While this mature technology is widely used in commercial oligonucleotide and gene synthesis, even heavily optimized processes have impractically low yield and purity on gene-length polynucleotides. Alternative strategies for template-free extension of polynucleotides can be found in nature, including using terminal deoxynucleotidyltransferases (TdT) to add nucleotides to the 3'-terminus of single-stranded DNA. Wild-type TdT enzymes are highly active on native nucleotides and under physiological conditions but have insufficient activity under conditions relevant to commercial polynucleotide synthesis. Evolving TdT to function under ideal process conditions may provide opportunities to exceed the limits of chemical synthesis methods. Codexis is leveraging its CodeEvolver® platform to generate engineered TdT variants with improved stability and activity on 3'-blocked nucleotides for the enzymatic synthesis of high purity synthetic DNA.