

Accelerating the Development of Designer Polyketides

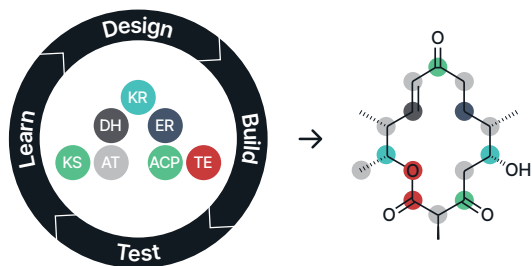
Elegen's cell-free ENFINIA DNA accelerates projects with high sequence complexity from the drawing board to the incubator, saving weeks to advance research goals faster.

Complex polyketides are a class of natural product synthesized by large, multi-domain enzyme complexes known as modular polyketide synthases (PKSs). The domains within a synthase are organized into functional modules that carry out extension and modification of the growing polyketide product. The polyketide molecule is passed from domain to domain; each domain either extends it by one polyketide unit or performs one chemical modification

Within diverse microbes, the DNA-encoding modules assemble into gene clusters that produce synthases that biosynthesize a diverse range of compounds used for medicinal, agricultural, and industrial purposes (e.g., the antibiotic erythromycin). In fact, polyketides represent approximately 20% of the top-selling drugs. Because polyketides are often used by microbes as chemical weapons against other microbes, they are a good source of bio-active molecules.



The Keatinge-Clay Lab at The University of Texas Austin



The design-build-test-learn (DBTL) iterative cycle is inherent in the bio discovery process. It can take weeks or even months to manufacture highly complex DNA sequences, including designs with high or low GC content, long hairpins, and repeat regions.

Until recently, the ability to engineer polyketide biosynthesis for the discovery of new compounds has been limited by a lack of technology and know-how. With the availability of thousands of bacterial genomes and an improved understanding of how PKS modules are organized, researchers like those in the Keatinge-Clay lab use synthetic DNA to engineer and heterologously express PKSs for the in vivo biosynthesis of designer polyketides. The Keatinge-Clay lab primarily works with PKSs from *Streptomyces* bacteria, which are encoded by long, repetitive, GC-rich sequences difficult to amplify from genomic DNA.

BY THE NUMBERS

14,713 bp
de novo plasmid synthesis

As little as
3 weeks
turnaround for delivery

CHALLENGES OF PKS GENE CLUSTER SYNTHESIS

The high GC-content and length of PKS gene clusters have historically proved challenging for conventional gene synthesis suppliers. Even with modern codon-optimization tools, sequences are often very complex and littered with near-repeats that lead to assembly errors. Codon optimization can also have detrimental effects on protein translation and product yield, making native-like sequences paramount for reconstitution.

To mitigate these risks, the Keatinge-Clay lab prefers to isolate the gene clusters directly from genomic DNA. When that is not feasible, the lab asks external gene synthesis providers to produce synthetic gene fragments to their longest length with as little alteration as possible. Even with this approach, the lab typically must order multiple 5kb gene fragments to build PKS gene clusters for insertion into their custom expression vectors. Due to the length and complexity of each fragment order, it can take three or more weeks from the time of order to receive the set of fragments from external suppliers, and another one to two weeks to build the final expression vector internally.

EVALUATING ENFINIA DNA WITH PLASMID SYNTHESIS

The Keatinge-Clay lab joined Elegen's Early Access Program in Q2 2023 and ordered de novo synthesis of a 14,713 bp plasmid containing a PKS gene cluster. After receiving the full-length ENFINIA DNA plasmid just three weeks after ordering, the Keatinge-Clay lab transferred the gene cluster into a custom plasmid for biosynthesis.

Having observed successful biosynthesis in their heterologous host of choice, the Keatinge-Clay lab plans to expand their pool of syntheses for testing. With Elegen's ability to synthesize regions of high complexity, the Keatinge-Clay lab is able to move projects with difficult, complex sequences, including repetitive GC-rich regions, from the drawing board to the incubators in a matter of weeks.

Elegen's rapid, full-length plasmid synthesis saved the lab one to two weeks of assembly time and labor when compared to traditional approaches. Furthermore, Elegen synthesized the entire sequence with minimal changes to avoid the risks of yield loss, as described earlier.

LEARN MORE

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Acknowledgments

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