ENFINIA™ DNA, High-Complexity Synthesis

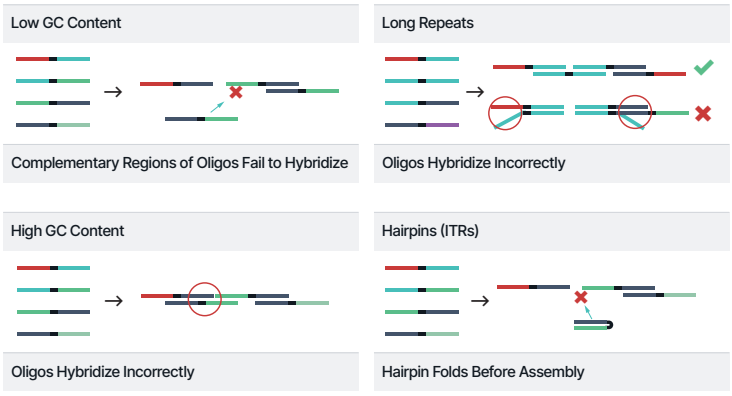
Patented Cell-Free Synthesis Platform Delivers Challenging Sequences that Many Gene Synthesis Suppliers Reject

High-complexity DNA sequences are notoriously difficult to synthesize and sequence. Conventional manufacturing workflows often fail during polymerase chain assembly (PCA) of complex sequences that contain extreme GC content; structural motifs like hairpins and short tandem repeats (STRs); homopolymers; or regions of long repeats. Elegen's cell-free process enables the synthesis of long, linear dsDNA despite such complexities making it possible to explore a more diverse sequence space.

LEGACY SYNTHESIS TECHNOLOGIES FAIL TO PRODUCE COMPLEX SEQUENCES

Why are high-complexity sequences so hard to build? The conventional synthesis process begins with chemically or enzymatically produced short oligos. Complementary single-stranded oligos are then annealed together during PCA to form double-stranded blocks, which are assembled into the final sequence by molecular cloning. When working with high-complexity DNA sequences, oligos can hybridize incorrectly, preventing downstream assembly or producing errors that persist throughout the synthesis process.

Assembly Errors Caused During PCA of Complex Sequences



INNOVATION OF CONVENTIONAL PCA DELIVERS HIGH-COMPLEXITY ENFINIA DNA

To minimize hybridization errors during the synthesis of high-complexity genes, Elegen developed a novel assembly method that limits the exposure of ssDNA during assembly to control the hybridization of complementary regions in complex sequences. Internal testing shows that incorporation of the novel assembly method into the cell-free production of ENFINIA DNA enables rapid and reliable access to the most challenging sequences including:

- GC-rich elements (promoters, enhancers, terminators)
- Long repeats
- Structural elements (ITRs, STRs)
- Homopolymers

Internal testing validated Elegen's ability to synthesize and assemble fragments that were more complex than permitted by their initial sequence acceptance criteria, resulting in expanded limitations for high-complexity DNA. For DNA sequences that fall outside of the new criteria, please contact info@elegenbio.com.

Expanded Limitations for ENFINIA DNA, High-Complexity Synthesis

To learn more about Elegen's DNA synthesis options, visit: https://elegenbio.com/high-complexity/

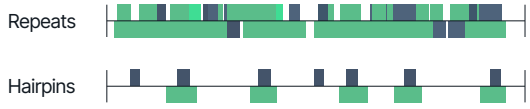
VALIDATING HIGH-COMPLEXITY DNA SYNTHESIS

To validate Elegen's novel DNA assembly method, 98 fragments (<1 kb length) representing a wide range of complexity were synthesized and separately ordered from 3 market-leading gene synthesis providers.

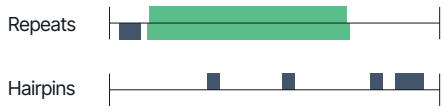
Using Elegen's new assembly method, 92% of the fragments were successfully synthesized. By comparison, the highest rate of acceptance from any other supplier was only 58%, demonstrating Elegen's ability to synthesize sequences rejected by other suppliers.

Challenging Sequences Successfully Synthesized with ENFINIA DNA, High-Complexity Synthesis

Hr5 Enhancer - Many mid-sized repeats, some short hairpins, low local GC%

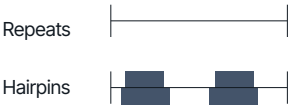


SV40 Promoter - Long repeats and high GC variation



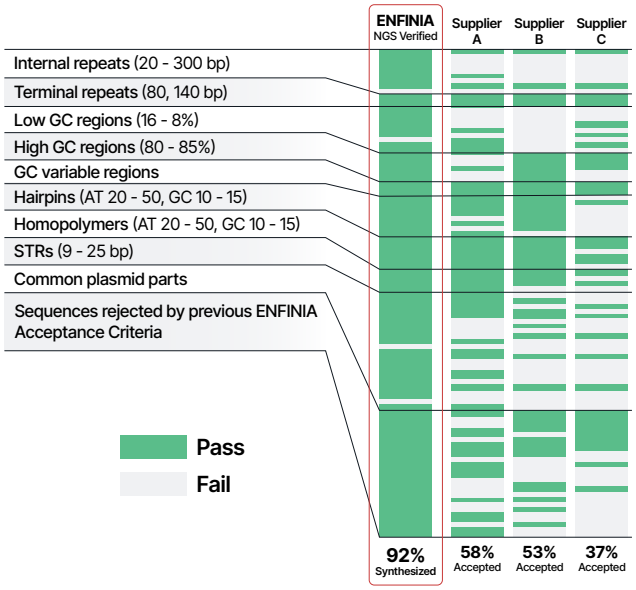
Tre3gs Promoter - Many mid-sized repeats, self-complementary sequence

AAV - ITR - High GC hairpin



Antibody expression sequences that contain a high percentage of local GC and long repeats can be challenging for conventional gene synthesis approaches. Results from the testing showed that a representative antibody expression sequence was successfully synthesized with 100% accuracy, high purity, and ample yield.

Successful Synthesis of 90/98 Sequences Commonly Rejected By Other Suppliers



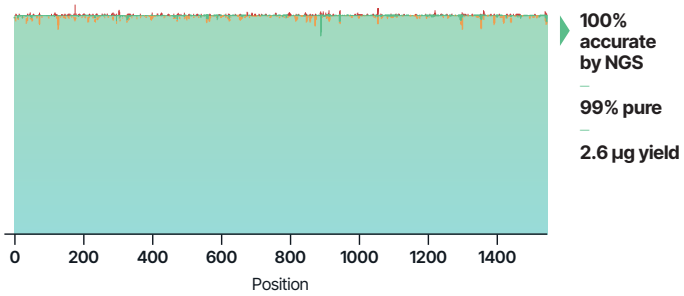
DEMONSTRATING HIGH PURITY AND YIELD FOR CHALLENGING CONSTRUCTS

A collection of 120 complex sequences of lengths of up to 5.5 kb were repeatedly synthesized to evaluate reproducibility.

During this testing, many uniquely challenging functional sequences containing a combination of high-complexity regions were synthesized including Hr5 enhancers, Tre3gs promoters, SV40 promoters, and AAV vectors.

NGS sequencing confirmed high levels of accuracy, purity, and yield.

Antibody Expression Sequence with High-GC and Repeats Successfully Synthesized



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