Complex DNA; No Compromises

How can you ensure your gene therapy research won't be slowed down by synthesis problems?

By Daniel Lin-Arlow, Co-founder and chief scientific officer of Ansa Biotechnologies. A former synthetic biologist who grew frustrated by being unable to access the DNA he needed to conduct experiments, he is now committed to helping other scientists access all the DNA they need for their experiments.

Gene synthesis, the construction of DNA molecules longer than a few hundred base pairs, is essential for the discovery, development, and manufacture of cell and gene therapies, as well as for assay development, target validation, and model organism development. For gene therapies, researchers rely on gene synthesis for constructing transgene payloads, modulating their expression in cells, and engineering viral vector delivery systems. For cell therapies, synthetic genes are also used to build transgene expression constructs and more complex genetic circuits that can sense and process multiple signals to trigger a context-sensitive therapeutic response within the patient.

Designing these sequences on a computer is often considerably more straightforward than actually obtaining the needed DNA constructs. However, once you input your designed sequences into the order form of traditional gene synthesis vendors, you'll often immediately encounter limitations around guanine-cytosine (GC) content, homopolymers, repeats, and many other elements that fall under the broad category of "complex DNA." Across the board, synthesis vendors seem to have convinced scientists that they have no choice but to accept suboptimal practices such as redesigning sequences to meet manufacturing constraints, abandoning desirable sequences for being too complex, or resorting to tedious, labor-intensive, and failure-prone workarounds to build the constructs in house from small pieces.

This should never have become acceptable. When it comes to the development of critically needed cell and gene therapies, nobody should have to compromise their science just because synthesis vendors aren't up to the challenge. From rejected or failed orders to long turnaround times and delays, researchers should expect more from vendors who play such a pivotal role in the process of therapy discovery and development. After all, we cannot realize the full potential of cell and gene therapies without being able to explore a broader design space – and reliably get the actual DNA we want to test all of those interesting ideas.

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Complexity

As the most common reason sequences are rejected or eventually failed by legacy vendors, the ability to build complex DNA sequences is probably the most important factor to consider. Most gene synthesis vendors build DNA constructs by stitching together dozens of chemically synthesized oligos that are roughly 80-150 bases long, but the assembly process struggles with complex sequences. By contrast, enzymatically

synthesized oligos can be much longer – 600 bases or more – enabling vendors who use them to produce a much broader range of sequences than can be reliably manufactured starting from short, chemically synthesized oligos. If exploring a larger design space would be helpful for your cell and gene therapy work, look for a synthesis vendor that not only claims to build complex DNA, but also backs it up with specific parameters for how they define (and consistently deliver on) complexity.

Quality control

Synthesizing complex DNA isn't the only tough task for vendors – sequencing the resulting DNA can be challenging too. Short-read sequencers can be stymied by extreme GC content, repetitive DNA, and other hallmarks of complex sequences. Sanger sequencing and other conventional tools, such as gel electrophoresis, often aren't precise enough for high-confidence quality control. If your order involves complex DNA sequences, make sure the vendor has a robust workflow to validate the purity of the products, ideally with long-read sequencing that can get through difficult DNA elements. Even for clonal DNA, this is the only way to know for sure that your synthetic DNA is homogeneous and matches the sequence you ordered.

Turnaround time

Faster is almost always better when you're trying to get a new therapy into the clinic. Consider the financial impact of a delayed program because the DNA constructs you need take weeks or months longer than expected to arrive. Were your cells or animals ready to receive a product by a certain date, but then your DNA got delayed? You could find yourself in a situation that feels like missing a connecting flight and ruining your vacation.

With most existing gene synthesis vendors, the chance of delays and failures increases with the length and the complexity of the sequences requested. This again can be traced back to the limitations of chemical DNA synthesis; in building DNA from short oligos, one low-quality oligo could sink the build of the full-length sequence.

Some new gene synthesis approaches that rely on longer oligos can help reduce turnaround times, especially for long and complex DNA, but make sure you're looking at the time to receive some constructs, as well as the time it takes to receive your complete set. It's often not worth beginning an experiment until you have all the constructs, so getting half your order in the promised amount of time, and half your order a month later, can still be a costly issue. Check with vendors about their success metrics for the percentage of orders shipped complete within the committed delivery window.

In conclusion, cell and gene therapies represent a burgeoning field with incredible opportunity to address or even cure diseases and conditions that have never been targetable with traditional drug classes. For the best chance at success, however, the scientists creating them shouldn't be limited by arbitrary technical constraints in the DNA synthesis process. New types of synthesis are entering the market, ready to fill these gaps. It's time for scientists to have the freedom to focus on their research without being limited by their DNA synthesis vendor.