

Rapid Manufacturing of Long, Complex DNA Constructs Using Sequence-Agnostic Enzymatic Synthesis



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NEXT GENERATION DNA SYNTHESIS FROM ANSA BIOTECHNOLOGIES

Reliable DNA Regardless of Length and Complexity

Ansa Biotechnologies creates high-quality custom DNA constructs using enzymatic synthesis, enabling scientists to work with sequences previously deemed too large or complex. In an *in silico* study (Figure 1), randomly sampled intervals of the human genome were selected ranging in size from 500 bp to 5000 bp. For Vendor X, the percentage of sequences classified as “standard” dropped from 85% to 20% as the construct size increased. Ansa’s classification consistently remained “standard”. Regardless of construct size, over 97% of all submitted sequences to Ansa’s classified as “standard”.

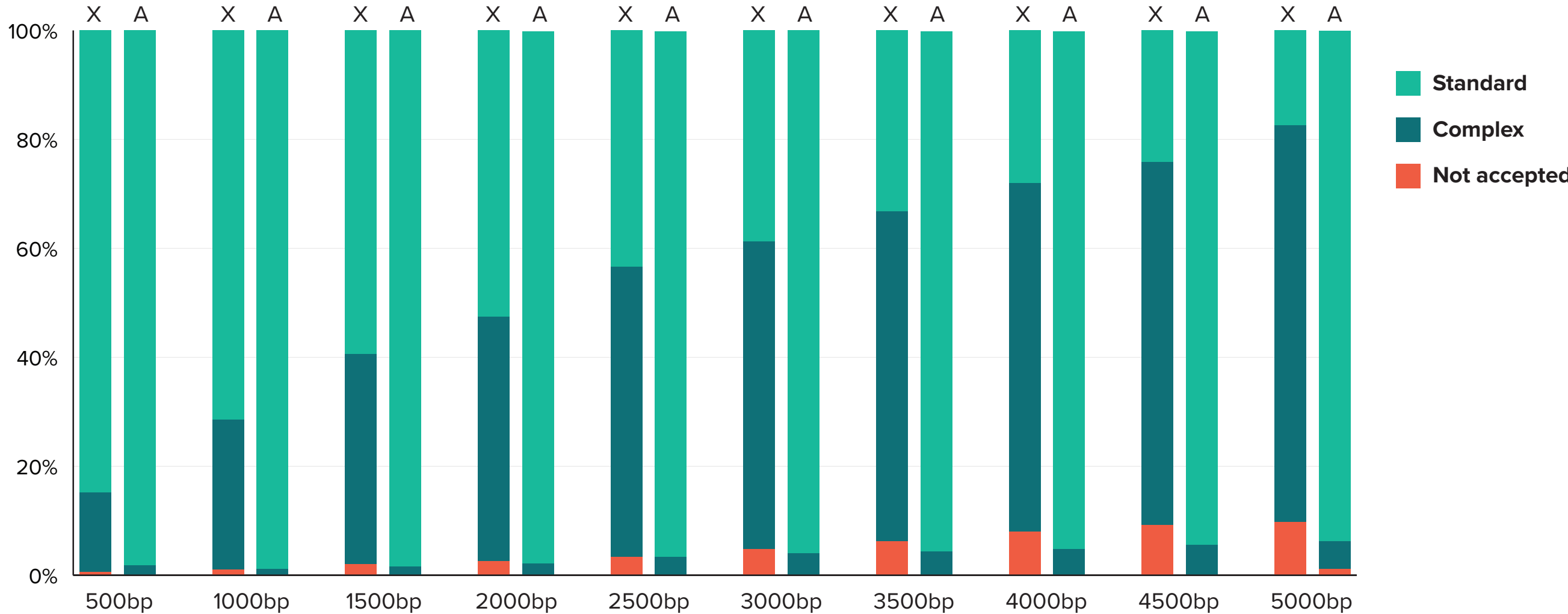


Figure 1. *In silico* comparison of randomly sampled sequences from the human genome classified by complexity by construct size.

Significantly Faster Delivery

Long turnaround times from legacy DNA vendors are a major bottleneck for scientists working in the drug discovery pipeline. Ansa’s next generation synthesis facilitates reliably rapid delivery (Figure 2). In a case study evaluating an order composed of 24 constructs ranging in size from 1.1 kb - 3.6 kb where 9 of the sequences contained complex elements (high/ low GC, repeats, and hairpin structures), Ansa delivered 100% of the order weeks before the next-fastest provider.

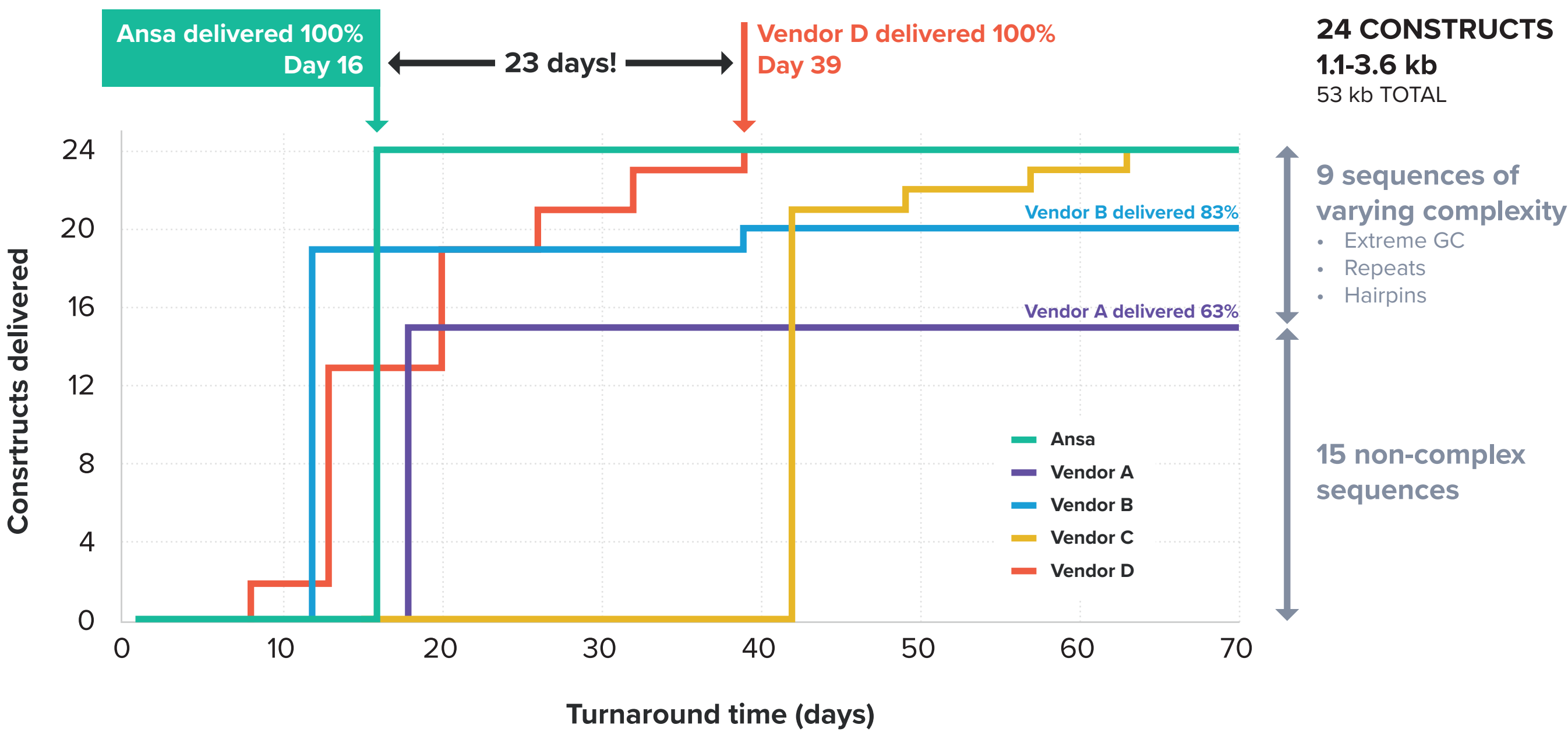


Figure 2. Ansa delivered a complex 24-construct order 23 days ahead of the fastest competitor.

KEY FINDINGS

- Complexity is no longer a barrier.** Ansa classified over 97% of randomly sampled human genomic sequences (500–5,000 bp) as standard, while acceptance by a legacy vendor fell from 85% to 20% as length increased.
- Faster delivery, even for difficult orders.** Ansa delivered a 24-construct order containing 9 complex sequences in 16 days, 23 days ahead of the next-fastest provider.
- Design for expression, not manufacturability.** All 21 Trias-optimized antibody sequences were scored standard by Ansa, while Vendor X rejected 11 and flagged the remaining 10 as complex.
- Better designs yield better titers.** Trias-optimized sequences synthesized by Ansa improved transient CHO expression for 19 of 21 antibodies (90%), with an average 1.9-fold increase in titer.

CODON OPTIMIZATION FOR EXPRESSION, NOT EASE OF SYNTHESIS

Sequence-Agnostic Synthesis Preserves Optimal Codon Choice

When considering downstream gene expression, the best codon selection is not necessarily the easiest DNA sequence to build via traditional chemical synthesis. For legacy providers, low complexity may take priority over downstream applications. With Ansa’s sequence-agnostic enzymatic synthesis, compromising downstream optimization is not necessary.

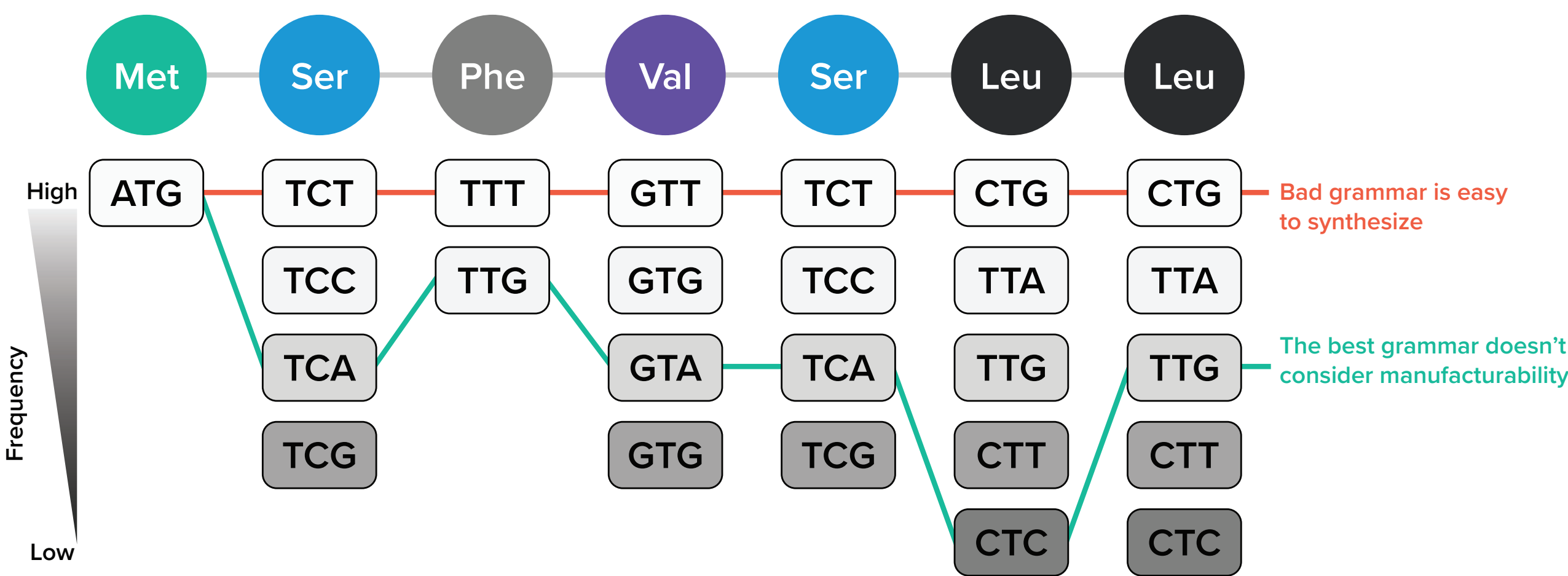


Figure 3. Optimal codon choice can conflict with ease of synthesis.

Sequence Manufacturability Screening

To examine how codon optimization affects DNA synthesis, 21 monoclonal antibody sequences were codon optimized using Trias¹, an open-source large language model trained on the coding sequences of vertebrate genomes. The DNA sequences generated by Trias were screened for manufacturability by Ansa and Vendor X using each company’s standard ordering platform. Ansa scored all 21 sequences as “standard” complexity, meaning customers could submit them directly for synthesis with high confidence in on-time delivery. By contrast, Vendor X scored 11 sequences as “not manufacturable” because of sequence complexity and suggested that the user modify the sequences using its own codon optimization algorithm. Vendor X scored the remaining 10 sequences as “complex,” a classification that can mean additional optimization, extra time, special handling, and higher pricing.

Sequence Classification	Vendor X	Ansa
Not Manufacturable	11	0
Complex	10	0
Standard	0	21

Table 1. Complexity Classification for sequences optimized by Trias.

Antibody Expression Testing in CHO

To compare expression outcomes from different codon optimization tools, Ansa worked with Axio BioPharma to benchmark the antibody sequences codon optimized by Trias or Vendor X’s program. Mammalian expression vectors were synthesized by Ansa and provided to Axio BioPharma for testing. Axio used its standard CHO cell expression system to test transient expression of the monoclonal antibodies and measured titers using protein A HPLC. The results showed that the Trias-generated sequences produced higher antibody titers for 19 of the 21 sequences and improved expression by an average of 1.9-fold. The figure below shows a representative sample of six sequences that were either codon optimized by Trias (green) or by Vendor X’s tool (red). These results show how Ansa can help customers preserve high-expression designs that might otherwise be constrained by a DNA manufacturability-first optimization.

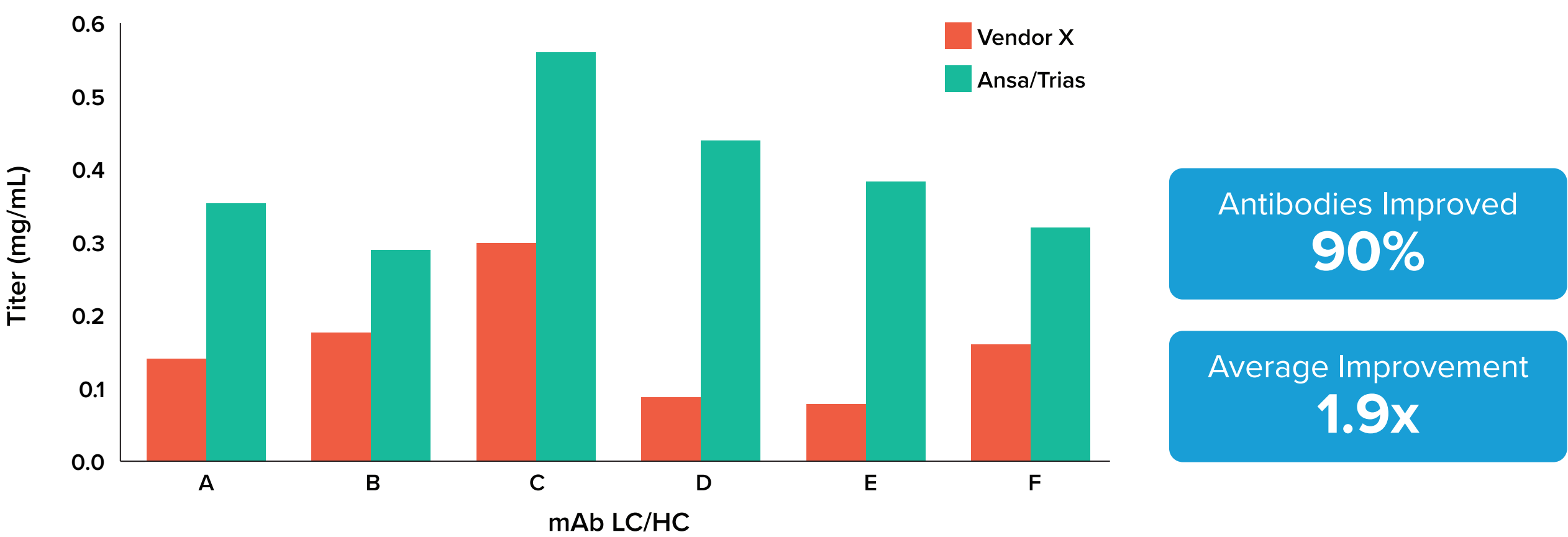


Figure 4. Monoclonal Antibody Expression in CHO Cells.

¹ Faizi M, Sakharova H, Lareau LF. A generative language model decodes contextual constraints on codon choice for mRNA design. bioRxiv [Preprint]. 2025 Jun 6:2025.05.13.653614. doi: 10.1101/2025.05.13.653614.

