

Overcoming Critical DNA Synthesis Challenges in Viral Vector-Mediated Gene Therapy Development

Gene therapies hold transformative potential for treating genetic diseases, cancers, and infections. Delays in research and development into these therapies can be caused by challenges in synthesizing viral vector DNA constructs - specifically inverted terminal repeats (ITRs) and regulatory sequences.

Ansa Biotechnologies uses its proprietary enzymatic DNA synthesis method to overcome these challenges, enabling faster and more reliable development of viral gene therapies.

Gene therapy holds great promise as a potential long-term treatment or cure for many diseases. Gene therapies work by delivering genetic material to treat disease, typically using viral vectors such as adeno-associated viruses (AAVs) as carriers. These therapies have primarily targeted monogenic diseases such as beta-thalassemia, sickle cell disease, and to a lesser degree, certain common diseases, cancers, and infectious diseases.^{1,2}

To identify the best candidates for development, scientists first start by creating several DNA constructs to experimentally determine which version produces the best therapy. Key non-coding DNA components, notably inverted terminal repeats (ITRs), promoters, and untranslated regions (UTRs) can be especially challenging to synthesize due to the nature of their sequences. Unlike coding sequence, which can be synonymously modified to improve synthesizability, non-coding sequence cannot be altered without compromising its function.

Highlights:

- **Industry Leading Success Rates**
Excellent track record of synthesizing complete sets of complex ITR and regulatory sequences, overcoming limitations of traditional chemical DNA synthesis
- **No Compromises**
Synthesizes repetitive, high-GC, or palindromic sequences without "optimizations" for synthesizability that risk degrading functionality
- **Proven Impact**
Demonstrates successful synthesis of previously rejected ITRs and regulatory elements, enabling critical research

However, such sequences are key to improving efficiency, efficacy and manufacturability of the gene therapies— in other words, whether they work, and critically, whether it is possible to affordably produce them in large enough quantities to treat the patient population. The DNA synthesis technology utilized could be the deciding factor as to whether that piece of DNA sequence can be created and become part of a potential development candidate. Ansa's novel enzymatic DNA synthesis method offers a unique approach for the creation and manufacturing of challenging sequences not possible using older chemical DNA synthesis methods.

ITR Synthesis: Enabling Optimization of AAV Production

Viruses, such as AAVs, are often used as carriers to deliver gene therapies. In addition to being efficient delivery vehicles, AAVs, more than other studied viral vectors, can be made in relatively large quantities for research and development studies, and later commercial manufacturing. AAV sequences can be encoded on plasmids that, when transfected into packaging cells together with helper plasmids, can produce AAV particles packaged with the recombinant DNA payload. One of the critical components of an AAV plasmid are the ITRs, which flank the viral genome and serve as its origin of replication, dictate how many copies are made, control the packaging of the genome into the virus, and how long the virus persists in cells. As the name suggests, ITRs are partially symmetrical DNA sequences containing three matching palindromic regions that form a T-shaped secondary structure. In recombinant AAVs, the two ITRs flank the transgene payload (Figure 1). This strong secondary structure causes challenges for DNA synthesis of ITR-containing sequences as well as their amplification by PCR and cloning using *E. coli*.

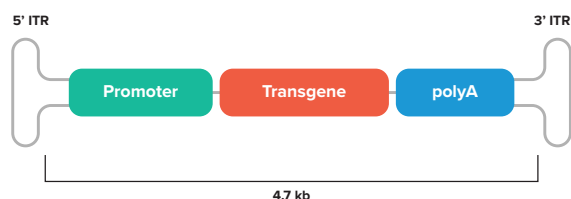


Figure 1: ITR secondary structure. ITRs are DNA sequences containing three matching palindromic regions that form a T-shaped secondary structure. ITRs flank the AAV genome, and in the case of AAV-based gene therapies, the DNA payload.

Successfully identifying the most effective ITRs can be game-changing for gene therapy development. Subtle changes in the ITR have been shown to improve packaging AAV yields, and thus optimizing ITRs represents an exciting opportunity for increasing the efficiency and lowering the cost of AAV manufacturing. In addition to directing replication and packaging, some ITRs function as mammalian promoters, and different serotypes of ITR sequences have been shown to control transgene expression levels³, suggesting that engineering ITR sequences could be a way to improve the safety and efficacy of AAV-based gene therapies.

Traditional chemical DNA synthesis can only produce high-quality oligos of 50–150 bp due to the chemicals involved damaging the oligos as they are synthesized. Thus, longer sequences must be stitched together from shorter oligos. However, strong secondary structures such as those found in ITRs can cause mis-assembly of the sequence, causing the build process to fail.

Ansa's proprietary enzymatic DNA synthesis method can produce oligonucleotides that are 5x longer (750+ bases) than those created using chemical DNA synthesis with essentially no limitations on the sequence, including strong secondary structures. These oligonucleotides can be more easily amplified and assembled into larger constructs, avoiding the challenges of assembling short oligos and enabling efficient synthesis of complex DNA, including ITRs.

Top Biopharma Case Study: Successful Synthesis of Complex ITRs

Highlights:

- 100% of 352 ITRs requested were delivered, compared to 0% by vendors using traditional chemical DNA synthesis
- ITRs were accurately synthesized using Ansa's proprietary enzymatic DNA synthesis technology and successfully propagated in *E. coli*.

Ansa was approached by a top biopharma company that wanted to synthesize a panel of 352 ITR variants but had all their sequences rejected for synthesis by other vendors. We were able to synthesize and clone 100% of their ITRs.

Using our enzymatic synthesis methodology made the creation of the initial DNA pieces straightforward. However, *E. coli* tends to mutate ITR sequences during propagation. Our initial sequencing results after assembly using our standard workflow showed that *E. coli* were often truncating the ITR. Extensive experience enabled our scientists to employ molecular biology approaches to ensure stable propagation of the complete ITR sequences (Figure 2). We were able to deliver 352 fully complete and clonally correct ITR sequences to the customer.

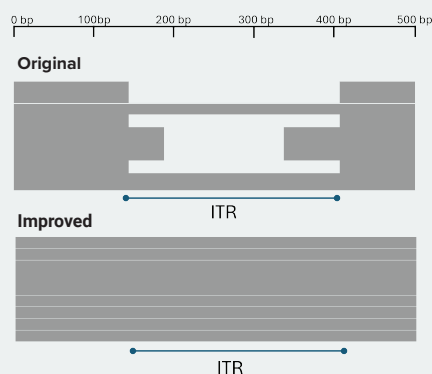


Figure 2: Graphical representation of long-read sequencing of the clone containing a complex ITR. The Original plot shows the ITR being truncated by the *E. Coli* whereas the ITR is retained in the Improved conditions.

Regulatory Element Synthesis: Optimizing Expression without Compromise

Regulatory elements, including promoters and UTRs, play a pivotal role in modulating the expression levels of transgenes, including those in AAV-based gene therapies. However, these sequences often contain repetitive elements needed to encourage binding of transcription factors and other regulatory proteins that drive expression, as well as large variation in GC content.

As with ITR sequences, this complexity poses issues for synthesis by assembly of short, chemically synthesized oligos.

Novel Bio Case Study: High Quality UTR Sequences for AAV-based Therapies

Highlight:

- Delivered 100% of 44 complex 5' UTR sequences using Ansa enzymatic DNA synthesis, while other vendors rejected up to 86%

Novel Bio is a biotechnology company that utilizes a proprietary microbial system to make high quality plasmid DNA for customers, many of whom are developing AAV-based therapies. The company was contracted to build 51 AAV gene-of-interest plasmids varying the 5' UTR of the transgene expression cassette. Novel attempted to order the 51 sequences from a leading DNA synthesis vendor ("Vendor 1"), but the vendor only accepted 8 of the sequences and ultimately delivered 7.

Subsequently, Novel submitted the 44 remaining sequences to Ansa, who accepted and delivered 100% within a week. (Ansa would have accepted and successfully manufactured the 7 sequences previously obtained by Novel). This enabled Novel to build all 51 AAV plasmids for their customer.

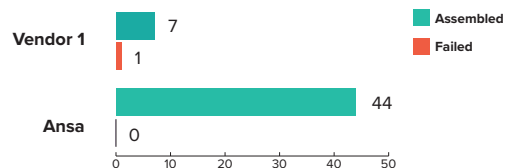


Figure 3: Acceptance and synthesis success rates for the 51' UTR sequences. Ansa accepted and delivered all requested all 44 5' UTR sequences by Novel Bio.

Conclusions: Get the DNA You Need, Fast and Reliably

The ability to synthesize complex DNA without any restrictions or modifications is critical for testing the full repertoire of sequences you can dream up, selecting the best candidates to take into development, and ultimately to achieve the best therapeutic outcomes.

Ansa's proprietary enzymatic DNA synthesis method resolves critical hurdles in viral vector-based gene therapy development, including synthesizing structurally complex ITRs and regulatory sequences. As a result, our workflow enables you to create the DNA you need, as specified, rather than the DNA that can be made.

By delivering complex DNA—with no sequence alterations—we empower researchers to focus on therapeutic efficacy rather than overcoming synthesis limitations.

For more information about Ansa and how we can accelerate your gene therapy research and development efforts, connect with us at ansabio.com.

References:

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Ansa Biotechnologies
1198 65th Street, #250
Emeryville, CA 94608