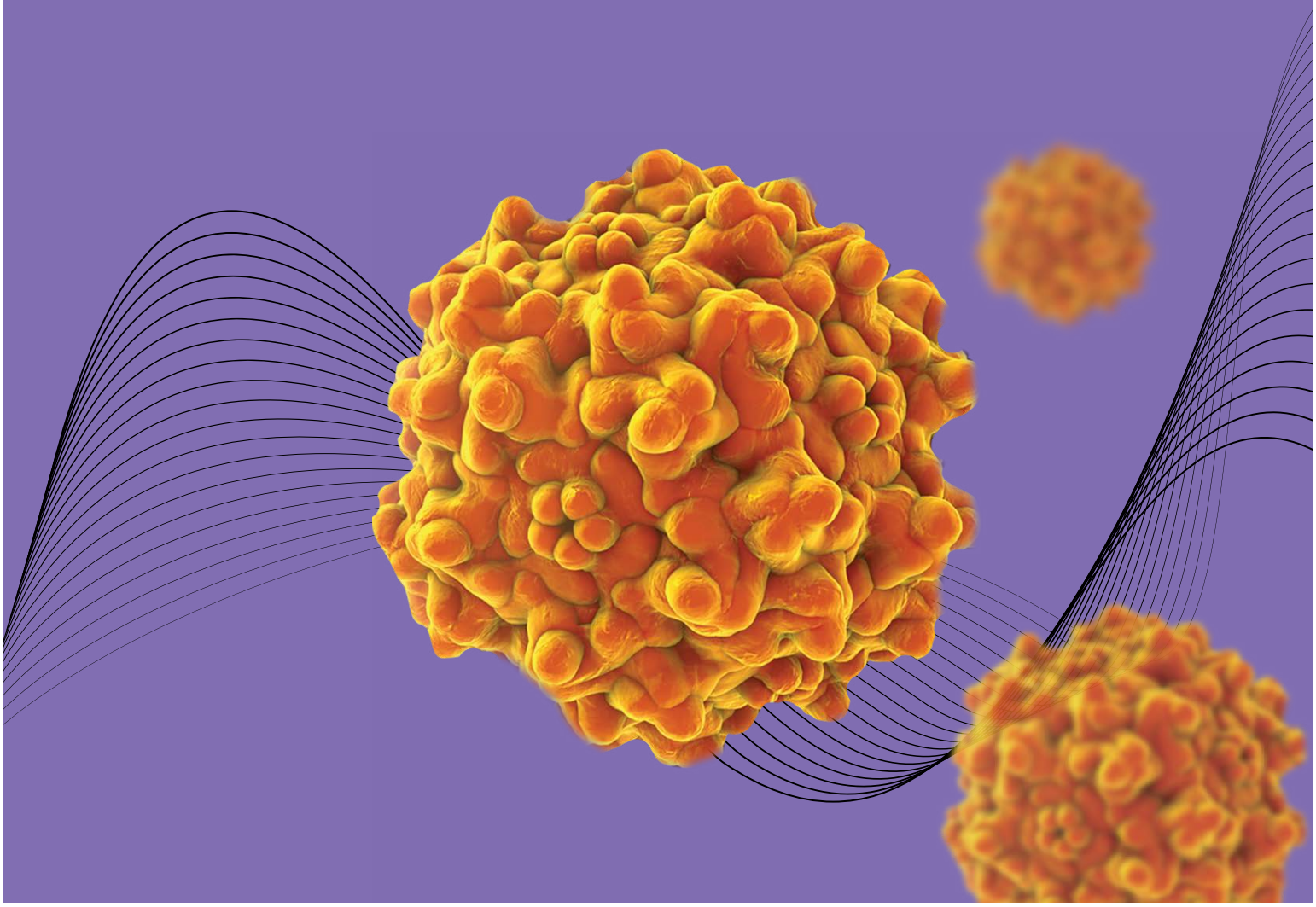
Sample Analysis & Multiomics Solutions

TECH NOTE

An Efficient and Reliable Approach to AAV Packaging



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Abstract

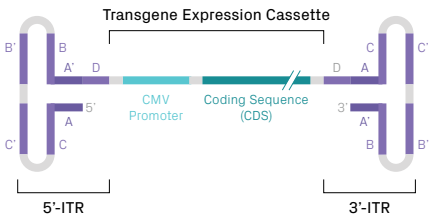
Adeno-associated virus (AAV) is a promising gene therapy vector due to its low pathogenicity and immunogenicity[1]. It can deliver a genetic payload of up to 4.7 kb, and multiple serotypes are available to target specific cell types or tissues in humans. The recombinant AAV (rAAV) genome comprises a transgene construct flanked by a pair of 145 bp inverted terminal repeat (ITR) sequences. Previous reports have shown that the ITRs are necessary to produce viral particles[2,3], but their structure is problematic for conventional cloning workflows. ITR sequences in plasmids are unstable, often acquiring deletions during propagation in bacteria[4,5]. Because of the high risk of mutation, sequence verification of ITR sequences is a critical part of rAAV quality control. This too is challenging, however, as standard Sanger sequencing fails to read through most ITR sequences. Here, we study the effect of partial and full ITR deletions on AAV packaging and show how proprietary methods developed by GENEWIZ Genomics Services from Azenta Life Sciences can successfully detect and repair these mutations.

Results

TRANSFER PLASMID CONSTRUCTION

We designed five rAAV genomes that varied in ITR integrity and insert length (Figure 1). The constructs were synthesized and cloned into a plasmid vector. The resulting transfer plasmids were amplified using the GENEWIZ AAV plasmid preparation method, which employs a proprietary bacterial strain that enhances ITR integrity[6]. The sequences were then verified by GENEWIZ AAV-ITR Sanger sequencing, a proprietary protocol that can read through the challenging ITR regions[7].

A



B

Construct Name	5'-ITR	3'-ITR	CDS	Cassette Length
ΔB-S	ΔB	WT	CopGFP	2.4 kb
ΔITR-S	None			
WT-L	WT	WT	CopGFP-Luciferase Fusion	4.1 kb
ΔB-L	ΔB			
ΔITR-L	None			

C

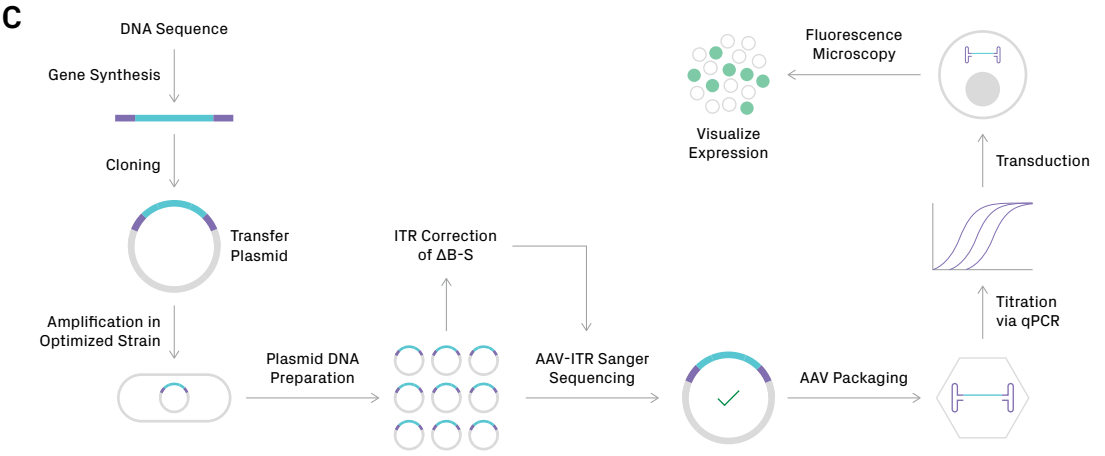


Figure 1. Transfer plasmid construction and testing. (A) Diagram of recombinant AAV genome. (B) Description of constructs. S = small cassette; L = large cassette; ΔB = B arm deletion (see Figure 2A for details). (C) Workflow of the study.

ITR CORRECTION

Since ITR sequences are prone to mutate spontaneously when propagated in bacteria, GENEWIZ developed a method to correct the mutations efficiently. An aliquot of the ΔB-S plasmid prep underwent ITR correction: a wild-type 5'-ITR sequence was synthesized and introduced into the ΔB-S plasmid, followed by sequence verification (Figure 2).

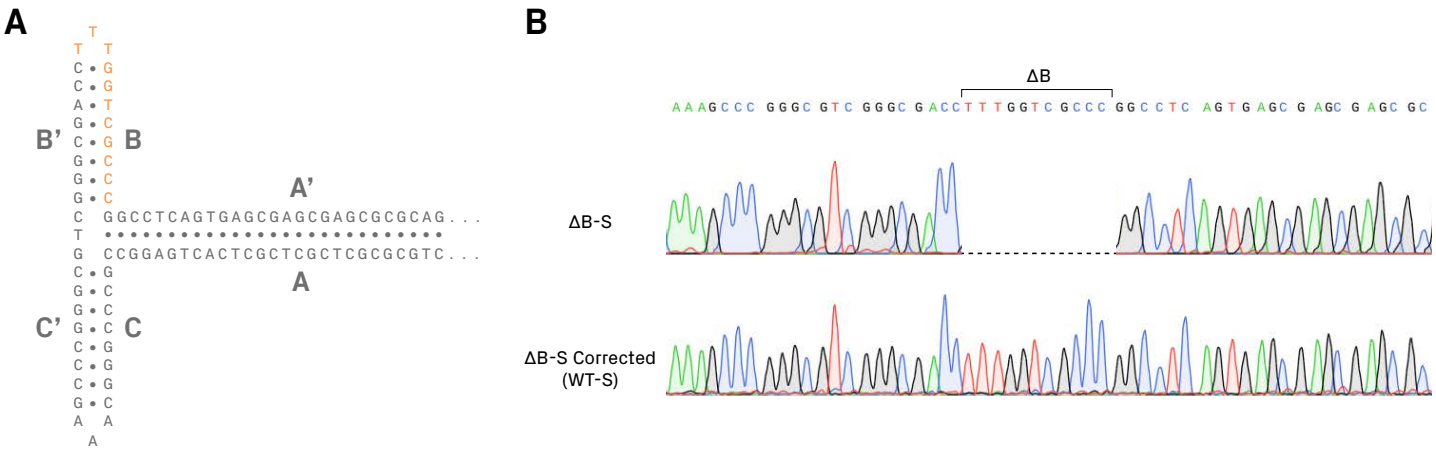


Figure 2. ITR correction. (A) Wild-type sequence of the 5'-ITR. The region highlighted in orange is absent in ΔB-S. (B) Chromatograms from AAV-ITR Sanger sequencing. The GENEWIZ ITR correction method restored the wild-type sequence in ΔB-S plasmid DNA.

VIRAL PACKAGING

HEK293T cells were co-transfected with the transfer, Rep/Cap (serotype AAV-DJ), and helper plasmids for viral packaging. AAV particles were purified, and viral titers were measured by quantitative PCR (qPCR). Recombinant AAV genomes with partial or full deletion of the 5'-ITR had significantly reduced titers (Figure 3). The drop in viral production was more pronounced in constructs with the larger ITR deletion and larger transgene cassette.

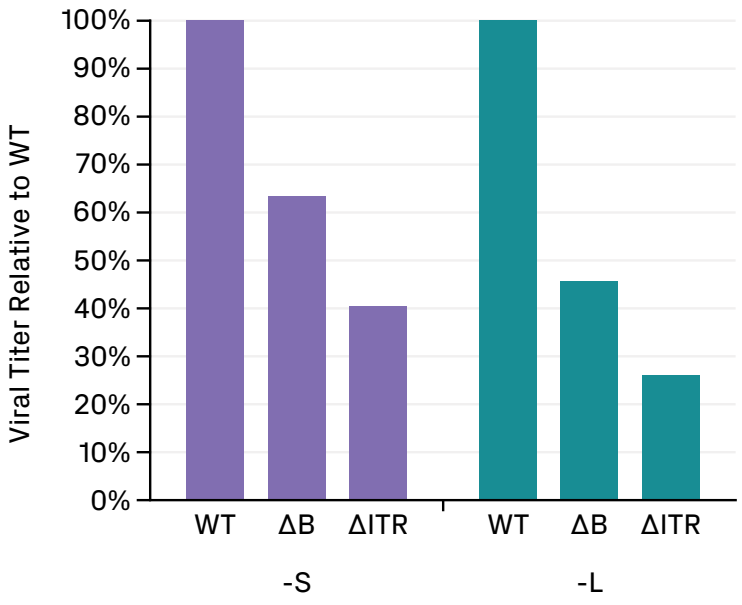


Figure 3: Viral titration, as measured by qPCR of the CMV promoter. Values normalized to the WT construct per cassette. WT-S = ΔB-S Corrected.

TRANSDUCTION

Equal volumes of the viral preps were used to transduce HEK293T cells, followed by fluorescence imaging. Consistent with the titration data, the constructs with compromised ITR sequences showed lower transduction efficiency (Figure 4).

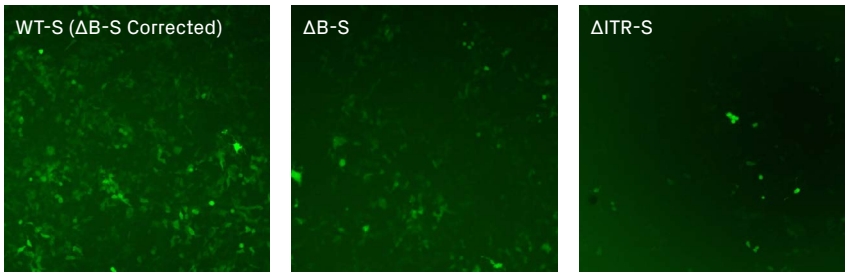


Figure 4: Transduction. HEK293T cells were incubated with equal volumes of the viral preps, and CopGFP expression was visualized by epifluorescence microscopy.

Conclusion

While AAV is a powerful vehicle for gene delivery, unstable ITRs threaten the fidelity of rAAV genomes. Loss of ITR integrity diminishes the packaging efficiency of rAAV particles and thus viral titer. With proprietary DNA sequencing and synthesis technologies from GENEWIZ, mutations in ITR regions can be reliably identified and effectively corrected. The upshot is improved rAAV quality and performance.

LEARN MORE ABOUT AAV PACKAGING →

References

[1] Li, C. & Samulski, R. J. Engineering adeno-associated virus vectors for gene therapy. Nature Reviews Genetics vol. 21 255–272 (2020).

[2] Wang, X. S., Ponnazhagan, S. & Srivastava, A. Rescue and replication of adeno-associated virus type 2 as well as vector DNA sequences from recombinant plasmids containing deletions in the viral inverted terminal repeats: selective encapsidation of viral genomes in progeny virions. Journal of Virology vol. 70 1668–1677 (1996).

[3] Savy, A. et al. Impact of Inverted Terminal Repeat Integrity on rAAV8 Production Using the Baculovirus/Sf9 Cells System. Human Gene Therapy Methods 28, 277–289 (2017).

[4] Gray, J. T. & Zolotukhin, S. Design and Construction of Functional AAV Vectors. Adeno-Associated Virus 25–46 (2011).

[5] Wilmott, P., Lisowski, L., Alexander, I. E. & Logan, G. J. A User's Guide to the Inverted Terminal Repeats of Adeno-Associated Virus. Human Gene Therapy Methods 30, 206–213 (2019).

[6] An Efficient and High-Fidelity Approach to AAV Plasmid Preparation. GENEWIZ Tech Note (2022). https://web.azenta.com/tech-note/aav-plasmid-prep.

[7] Reading Through the Inverted Terminal Repeats (ITRs) of Adeno-associated Virus (AAV). GENEWIZ Tech Note, (2020). https://web.azenta.com/aav-itr-tech-note.

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