

Overcoming Challenges in Adeno-Associated Virus (AAV) Inverted Terminal Repeat (ITR) Sequencing with a novel AccuGenomics Accukit

Adeno-Associated Virus (AAV) are integral components in gene therapy and molecular biology research. However, the inverted terminal repeats (ITR), a key requirement for AAV replication, are difficult to characterize due their repetitive nature and structural variability. Here we will first outline some of the challenges encountered during AAV ITR sequencing, and then introduce our Accukit as an innovative solution to improve ITR characterization via sequencing. This Accukit represents a novel QC tool for NGS to estimate if the ITR and internal sequence count is similar, which until now has been a key challenge for accurate AAV ITR sequencing for QC as well as research purposes.

AAV ITR: Biology & Sequencing Background

ITR (Inverted Terminal Repeat) sequences are vital components of AAV (Adeno-Associated Virus) gene therapy. They facilitate the packaging of therapeutic genes into AAV vectors and serve as origins of DNA replication. Additionally, ITRs play a role in the integration of AAV DNA into the host cell's genome, allowing for stable, long-term expression of the therapeutic gene. The ITRs also contain regulatory elements that control the transcriptional activity of the therapeutic gene, enabling precise control over gene expression levels. Characterization of ITR by sequencing is a challenge due to their extensive secondary structures and repetitive sequence. For example, NGS of ITR is impeded by these structures, leading to regions of low sequence quality or even the complete failure to obtain accurate or usable data.

In AAV ITR's, the terms "flip" and "flop" refer to two alternative orientations of the ITR sequence that play a crucial role in forming a double-stranded DNA "replication hairpin", directly impacting the efficiency and accuracy of the replication and packaging processes. The flip orientation is the predominant orientation found in AAV vectors, is crucial for the production of functional AAV vectors for gene therapy applications, and is characterized by a specific arrangement of nucleotides within the ITR. The flop orientation is less commonly observed and represents the complementary arrangement of nucleotides; it plays a role in regulating specific aspects of AAV biology and in the generation of mutant AAV vectors for research purposes. Improved methods that support more accurate characterization of AAV particles flip & flop could improve our knowledge of these biological processes.

Introducing the AccuGenomics ITR-26Index-CC DNA IS Accukit

AccuGenomics, in collaboration with a team of industry experts at a large biopharma, has developed the *ITR-26Index-CC DNA IS Accukit*. This is a cutting-edge solution specifically designed for AAV ITR sequencing to accurately measure the per base 3-prime flip ITR abundance, allowing comparison to internal sequences to ensure a substantial proportion of the ITR remains intact. The *AccuGenomics ITR-26Index-CC DNA IS Accukit* requires only a single pipette step reagent addition into your current NGS workflow. By using the internal standards provided in this Accukit, you can precisely monitor ITR yield, sample index tracking (26 unique indexes), and also obtain a measurement of complexity capture for each sample. This Accukit provides assurance that the AAV

vector has the ITRs sequenced at the same level as internal sequence. This approach may be extended to accurately measuring the flop levels, abundance determination of contaminating cell line oncogenes, levels of AAV manufacturing vectors, or quantification of known adventitious agents all in the same NGS sample. These benefits may open new avenues for the development of improved AAV vectors and enhance the understanding of AAV biology and its applications in gene therapy and molecular biology research.

Note: The pharma client specified a single Accukit that would combine the AAV ITR standards, the complexity control standards, and the index tracking standards. However, each of these specific functionalities can also be provided separately, based on the needs of the client.

The *AccuGenomics ITR-26Index-CC DNA IS Accukit* offers several key improvements that empower QC scientists and researchers to achieve more accurate and reliable AAV sequencing results. These include:

1. **Enhanced Monitoring of ITR Yield:**

The Accukit introduces an innovative approach to accurately monitor the yield of 3-prime flip ITRs. By leveraging the read depth ratio between the native template (NT) and internal standard (IS) based on ITR position, researchers can precisely track and assess ITR yield. This empowers scientists to make informed QC release decisions and optimize their experimental approaches with greater confidence by accurately quantitating and comparing the genomic abundance of the AAV ITR and internal regions, ensuring robust results.

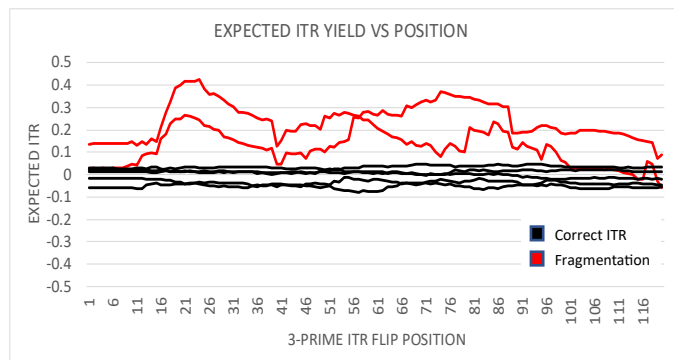


Figure 1. Measuring ITR:Internal Sequence Yield of AAV Particles. The IS ITR biochemically covaries with the sample (NT) ITR, with the resulting NT:IS read depth ratio reflecting the genomic NT:IS ratio at the time IS is added to the sample. Because the IS is a known molecule, both in abundance and sequence, examining the relative behaviors of IS and NT in sequencing reads can be used to calculate ITR abundance, or sequencing error biases. The data in this plot was generated by DNA from AAV particle samples mixed with 1 ul of AccuGenomics ITR-26Index-CC DNA IS Accukit prior to fractionation and non-targeted NGS short read sequencing, FASTQ aligned using BWA mem to a reference genome of AAV and IS sequence, the depth by position of an inner region and the 3-primer ITR flip region for both sample (NT) and IS was extracted using samtools depth, a NT or IS ratio between each ITR position and a median internal depth was calculated (NT-Ratio & IS-Ratio), and the NT-Ratio / IS-Ratio plotted by ITR position. Each line represents a sample with the fold ITR deviation from inner sequence indicated on y-axis (log10) with nominal ITR yields (black) and suspicious ITR yields (red).

2. **Sample Index Tracking (IX):**

Beyond ITR yield monitoring, this Accukit provides sample index tracking capabilities for up to 26 samples in parallel. Researchers can ensure the sample input corresponds to the bioinformatic output. Additional indexes can be created for higher volume workflows.

3. **Comprehensive Complexity Capture (CC) Measurement:**

The Accukit offers a novel complexity capture measurement, quantifying the extent to which each sample has been captured as sample template in the sequencing reads. This valuable information enables researchers to assess the efficiency and completeness of their sequencing approach, and by optimizing and monitoring sample preparation based on the

CC, researchers can ensure the reproducibility of each complex NGS test, especially during routine testing.

4. **Accelerate Discovery and Development:**

AccuGenomics' Accukit has the potential to revolutionize AAV ITR sequencing, expediting discovery and development in this critical field. By addressing the challenges associated with repetitive sequences, structural variability, secondary structure formation, read length limitations, and sequence misinterpretation, the Accukit empowers researchers to obtain accurate and reliable results to unlock their full potential of AAV research and development.

5. **A More Collaborative Industry Approach:**

The development of the Accukit exemplifies the power of collaboration in advancing scientific research. AccuGenomics' partnership with industry experts has resulted in a tailored solution that specifically addresses the challenges faced in AAV ITR sequencing. This collaborative effort demonstrates a commitment to pushing the boundaries of genomics research and delivering innovative solutions to the scientific community.

6. **Unlock Your QC Potential:**

The *AccuGenomics ITR-26Index-CC DNA IS Accukit* offers a comprehensive solution that improves assay sensitivity, accuracy, and efficiency in AAV ITR sequencing. By incorporating innovative features such as ITR yield monitoring, sample index tracking, and complexity capture measurements mixed into a single reagent, QC scientists are provided with the tools they need to overcome AAV ITR sequencing challenges to achieve groundbreaking results.

The *AccuGenomics ITR-26Index-CC DNA IS Accukit* represents a significant advancement in the field of AAV ITR sequencing. By enabling accurate abundance measurement of AAV ITR, the Accukit enables more reliable data and quantitative results to support work in QC and vector biology / genome structure research. With this Accukit you can accelerate discoveries, optimize experimental approaches, and make remarkable strides in the field of AAV research.

Experience the power of the *AccuGenomics Accukit ITR-26Index-CC DNA IS* and embark on a journey of new discovery in AAV ITR sequencing. AccuGenomics is committed to empowering researchers with cutting-edge solutions to advance genomics research, and this Accukit enables more accurate and standardized data to propel your groundbreaking advancements in gene therapy and AAV sequencing.

AccuGenomics – A Higher Standard of Accuracy

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