Countable Labs.

Advances in genomics-based analytical characterization for cell and gene therapy

Introduction

Analytical Demands of CGT

Cell and gene therapies (CGT) are emerging as transformative therapeutic strategies for a wide range of diseases. Since 2021, there has been a sharp increase in the number of gene therapy clinical trials initiated globally (Park *et al*., 2025). Despite this momentum, the successful development of safe and effective CGT products depends heavily on robust analytical characterization throughout development and manufacturing.

Both viral and non-viral systems are used to deliver therapeutic genes into target cells. Among currently approved therapies, viral vectors remain the most widely utilized, although non-viral approaches—particularly lipid nanoparticles (LNPs)—are rapidly gaining traction (Shchukina *et al* 2025).

Adeno-associated virus (AAV) has emerged as one of the most extensively studied and clinically adopted delivery platforms, especially for in vivo applications. Other viral vectors, including retroviruses, lentiviruses, adenoviruses, and herpesviruses, are also actively being developed and used across a range of therapeutic modalities (Moldavskii *et al*., 2025).

However, viral vector production poses several analytical challenges. During production, it is essential

Table 1. Characteristics of genomics-based platforms for analytical characterization.

Attribute	qPCR	dPCR	Countable PCR	Long-read sequencing
Quantification	Relative - inferred via Ct values	Absolute estimated via Poisson statistics	True - direct single molecule counting	Limited
Reproducibility	Low (50-100% CV)	Moderate (~10% CV)	High (~ 1% CV)	Variable (depth dependent)
Sensitivity	Limited	High - requires sample splitting	High	Limited (depth dependent)
Inhibitor tolerance	Low	High	High	Sample-prep dependent
Multiplexing simplicity	Complex	Complex	Easy	Easy

to accurately quantify the amount of viral particles while also assessing attributes such as genome integrity and purity. Following transduction, understanding the biodistribution and persistence is also critical for evaluating both safety and therapeutic efficacy.

Reliable and efficient analytical assays are therefore essential for process development, scale-up, and batch release decisions. Several genomics-based technologies are used for these purposes, including quantitative PCR (qPCR), digital PCR (dPCR), single-molecule counting PCR, and next-generation sequencing (NGS). In this whitepaper, we review the strengths and limitations of these technologies across key CGT analytical workflows and discuss opportunities to streamline quality control assays for more efficient production decisions.

Genomics-Based Characterization of Viral Vectors

Viral Quantification

During viral vector production, quantifying the viral genome titer is a critical component of product characterization. Physical particle counting methods such as electron microscopy and mass photometry can estimate the number of viral capsids present. However, these approaches are expensive and do not discriminate between empty and full capsids and therefore prone to overestimation.

Genomics-based methods provide a more sensitive and informative approach to viral quantification (**Table 1**). These methods typically require capsid lysis and, in some cases, viral DNA purification prior to analysis. Despite these additional preparation steps, they enable molecular characterization of viral genomes with high analytical sensitivity.

PCR-based assays are widely used to quantify viral genome titers and vector copy number. In many workflows, crude lysates can be used directly as input material, minimizing biases introduced during DNA extraction. For example, while qPCR assays can be sensitive to inhibitors present in crude lysates, partition-based methods such as dPCR and single-molecule counting approaches such as Countable PCR are less susceptible to inhibition effects, enabling more accurate quantification with reduced technical bias.

PCR quantification methods such as qPCR require standard curves to calculate viral titer, or in the case of dPCR, Poisson statistics to account for loading of multiple targets per droplet. In addition, the presence of droplets with intermediate fluorescence (also known as “rain”) can make it challenging to confidently assign a threshold between positive and negative droplets, impacting dPCR accuracy and clean reporting. Countable PCR, however, eliminates the need for standard curves and Poisson correction due to the vast number of compartments available for single molecule amplification (1,000x greater than dPCR), and combined with high-resolution 3D imaging, offers direct counting of amplified targets.

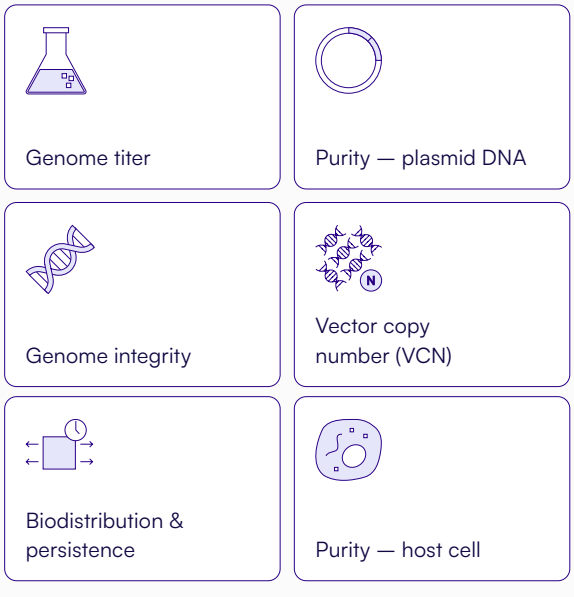

Figure 1. Applications of Countable PCR for CGT process development.

Genome Integrity

Beyond measuring viral genome titer, it is also critical to determine whether packaged genomes contain the full set of functional elements required for therapeutic activity. During vector production, viral genomes may undergo recombination, truncation, or partial packaging events (Green and Lee, 2021). These processes can generate incomplete genomes or rearranged sequences that reduce therapeutic potency and introduce variability across batches.

Monitoring genome integrity therefore represents an important quality attribute during process development and manufacturing. Early detection of incomplete or rearranged genomes enables rapid identification of low-quality production batches and prevents unnecessary downstream processing.

Sequencing-based approaches such as long-read sequencing and capillary sequencing provide comprehensive characterization of viral genome structure by detecting single nucleotide polymorphisms (SNPs), insertion-deletion events (INDELs), and larger structural variants across the entire viral genome. These approaches provide an unbiased view of the diversity of genome variants present. However, sequencing workflows introduce biases during DNA extraction and library preparation that limit their quantitative accuracy. As a result, sequencing is typically used for variant discovery and structural characterization rather than precise measurement of genome completeness.

In addition, sequencing-based characterization is typically performed separately from quantitative assays. This requires splitting a sample across multiple workflows and correlating results generated by different methods, each with its own sources of variability. This can introduce inconsistency in data interpretation and make it more challenging to establish clear relationships between genome integrity and quantitative metrics such as titer.

PCR-based assays offer an alternative approach for routine monitoring of genome integrity with higher throughput and lower cost. One strategy with dPCR involves multiplex amplification of two genomic regions—for example the 5' and 3' ends of the gene-of-interest. Genome completeness is inferred by detecting droplets that contain both targets (**Figure 2**).

However, droplet-based partitioning introduces statistical ambiguity. Two separate DNA fragments containing the 5' and 3' targets may randomly occupy the same droplet even if they originate from different molecules. This multi-

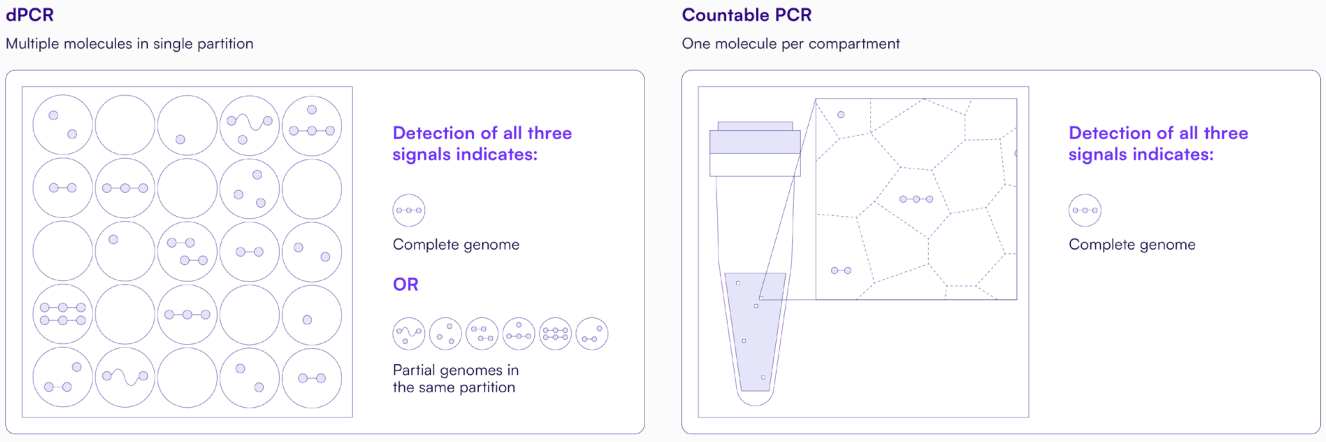

Figure 2. Comparison of genome integrity measurement approaches using dPCR and Countable PCR.

occupancy effect requires mathematical modeling to estimate the true fraction of complete genomes (Duong *et al.*, 2025).

Single-molecule counting approaches such as Countable PCR enable direct linkage analysis by isolating individual DNA molecules prior to amplification. Multiple genomic regions can be interrogated simultaneously, and the presence of multiple signals indicates the targets originate from the same molecule. Because individual molecules are resolved prior to amplification, genome integrity can be measured directly without complex statistical correction.

Impurity Testing

Ensuring the purity of viral vector preparations is a critical requirement during manufacturing and scale-up. Common impurities include residual plasmid backbone DNA from vector production systems as well as carry over from host cell genomic DNA.

Next-generation sequencing (NGS) provides a discovery tool for identifying potential contaminants present in viral preparations. Because sequencing does not rely on predefined targets, it can reveal unexpected DNA species that may arise during vector production.

Once contaminants have been identified, targeted PCR-based assays are typically developed for routine monitoring and quantification. These assays enable rapid and sensitive measurement of known contaminants across multiple manufacturing batches.

qPCR, dPCR, and Countable PCR can all be used for contaminant quantification. However, qPCR assays can be sensitive to inhibitors present in crude lysates, while dPCR workflows often require individual assays for each contaminant target, increasing assay complexity.

Single-molecule counting approaches enable flexible multiplexing using universal probe strategies. This allows multiple contaminants to be quantified simultaneously within a single reaction, reducing assay optimization time and simplifying routine impurity testing workflows.

Biodistribution and Toxicology Studies

For gene therapies, regulatory guidance requires characterization of viral vector biodistribution and persistence across multiple tissues and biological fluids (FDA, 2023). These studies often involve quantifying viral genomes across samples and tissues where vector presence may be extremely low.

PCR-based methods are widely used for biodistribution studies due to their high analytical sensitivity. dPCR can provide higher sensitivity than qPCR for vector quantification; however, its sensitivity is limited by practical limitations in sample input. In particular, the entirety of sample input is not able to be analyzed due to dead volume (up to 50% volume loss), reducing the total number of molecules assessed. In low-copy-number samples, this loss can increase the risk of false negatives, which is a key concern in biodistribution and toxicology assessments.

Single-molecule counting approaches such as Countable PCR provide an alternative strategy designed to maximize sensitivity and molecular recovery. By isolating and analyzing a substantially larger number of individual DNA molecules directly in the PCR reaction tube, these methods increase the probability of detecting rare targets and are not impacted by sample loss. This capability is particularly valuable for biodistribution studies, where reliable detection of low-abundance viral genomes is critical for accurately assessing tissue distribution, persistence, and safety.

Opportunities in Analytical Process Development

During viral vector manufacturing and process scale-up, rapid and reliable analytical feedback is essential for informed decision-making. This becomes especially important when establishing comparability between batches, where subtle differences in product quality can impact safety, efficacy, and regulatory acceptance. One emerging opportunity is the consolidation of multiple

analytical measurements into a single assay, enabling more comprehensive and consistent characterization across development and manufacturing stages.

For example, impurity detection and viral genome titer measurement can potentially be combined in a single, multiplexed assay. Historically, this has been challenging because impurities are often present at much lower concentrations than viral genomes. Platforms with limited dynamic range may struggle to detect both abundant and rare targets within the same reaction.

Single-molecule counting approaches with broad dynamic range make this type of multiplex assay feasible. Viral genome titer and impurities can be quantified simultaneously, simplifying assay workflows and reducing analysis time.

Another opportunity lies in the integrating of genome integrity analysis with genome titer measurement, particularly in the context of comparability. Viral genome titer alone does not distinguish between full-length and partial genomes, meaning batches with similar titers may differ significantly in structural composition. These differences can be difficult to quantify with conventional methods but may become increasingly important as regulatory expectations for comparability and product characterization evolve.

By leveraging DNA linkage detection at the single-molecule level, Countable PCR assays enable simultaneous measurement of genome integrity and titer within a single assay. Because individual molecules are spatially isolated, amplified, and imaged, fluorescence signals indicative of linkage can be directly counted. This allows for direct quantification of full-length versus partial genomes and viral titer without requiring separate assays.

Incorporating genome integrity into routine analytical workflows provides a more complete view of product quality and strengthens comparability assessments between batches. It enables detection of shifts in genome composition that may arise from process changes, scale-up, or manufacturing variability - differences that may not be apparent from titer measurements alone. As regulatory expectations for CGT products continue to evolve, analytical approaches that combine titer, impurities, and genome integrity into a unified, high-resolution measurement framework can enable more robust control strategies and reduce uncertainty.

Conclusion

Robust analytical characterization of cell and gene therapy products requires both precise quantitative measurement and comprehensive quality assessment. Sequencing technologies provide powerful, unbiased discovery of structural variants, integration events, and unexpected contaminants, while PCR-based methods enable accurate and sensitive quantification of defined targets.

Digital PCR offers higher sensitivity than other qPCR methods but operates within a limited, droplet-based framework that can constrain dynamic range and precision. Single-molecule counting approaches such as Countable PCR provide an alternative strategy that directly counts individual DNA molecules without reference standards or statistical modeling enabling highly sensitive, reproducible quantification.

Countable PCR also opens up the possibliity of simplified multiplexing for applications such as genome integrity analysis, impurity testing, and biodistribution studies. By combining NGS based discovery with robust multiplex quantitative assays, integrated analytical workflows can provide the rigor and reproducibility required for the development and manufacturing of next-generation cell and gene therapies.

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To learn more about single-molecule counting approaches and integrated analytical solutions for CGT characterization, visit countablelabs.com.

