Enhanced lentiviral vector characterization using digital PCR: Genome titer, VCN and RCL detection

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Enhance your LV analytics with fast, reliable and resource-efficient solutions

The advancement of cell and gene therapies is essential for treating a variety of diseases. Lentiviral vectors (LVVs) have become a key technology for delivering therapeutic genes into cells. Ensuring the safety, efficacy and consistency of LVs requires thorough characterization. One important aspect is accurately quantifying viral vector genome titers and vector copy number (VCN), which indicates the number of transgene copies in the target cell genome. The effectiveness of viral transduction depends on factors such as viral dose and target cell type, making precise VCN measurement crucial for optimizing viral doses in therapy applications.

After extracting genomic DNA from transduced cells, the number of lentiviral gene copies is compared to a reference gene. Traditional methods often use qPCR, which requires external standard curves.

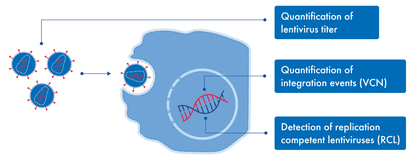
Digital PCR is a powerful tool for absolute quantification of LVVs, addressing the limitations of qPCR with its precision, tolerance for inhibitors, accuracy, specificity and reproducibility. We propose a rapid dPCR method to determine VCNs, providing insights into transgene stability and expression. Consistent VCNs are vital for maintaining uniform product quality in biopharmaceutical production.

Another significant safety concern with lentiviral systems is preventing the creation of replication-competent lentivirus (RCL) through recombination. Regulatory bodies, such as the FDA and EMA, require comprehensive RCL testing in retroviral vector products.

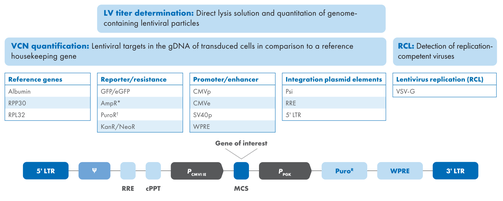
We demonstrate a streamlined dPCR workflow for quantifying lentiviral genome titers, VCN, and RCL detection, enhancing characterization with greater precision, accuracy and robustness.

Characterization of LVs is key to safe and effective therapies

Lentiviruses are widely used as viral vectors in research and cell and gene therapies. Their generation and purification require stringent quality control for safe and effective treatments. Analytical methods are essential for characterizing and monitoring critical quality attributes, helping to ensure product efficacy and safety. Digital PCR (dPCR) provides accurate quantification of these attributes.



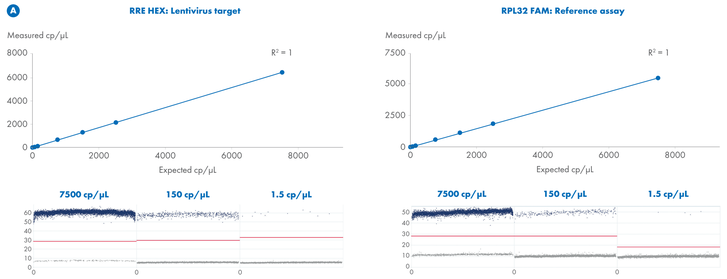
Comprehensive characterization of lentiviruses (LVs). The schematic illustrates the LV production workflow. dPCR provides precise and absolute quantification of critical quality attributes (CQAs), including viral vector genome quantification (physical titer), vector copy number quantification (VCN) and detection of replication-competent lentivirus (RCL).

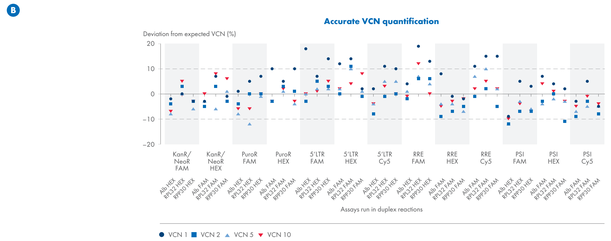


Overview of assays available for LV physical titer quantification, VCN determination and RCL detection.
*AmpR only residual plasmid testing; † PuroR very high GC content – not recommended for RNA applications.

Accurate VCN and LV titer determination using the QIAcuity® dPCR System

Determination of VCN and LV physical titer is a crucial quality attribute. dPCR facilitates accurate and precise quantification of both.

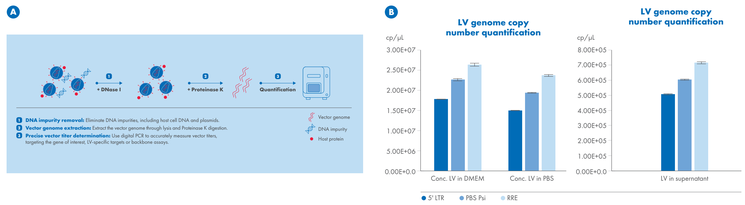




Accurate and precise determination of VCN and physical LV titer using digital PCR. A Template DNA was serially diluted from 0.25–7500 copies/µL showing high linearity on 8.5k Nanoplates. **B** High accuracy for VCN quantification independent of fluorophores. CGT dPCR assays (KanR/NeoR, PuroR, 5' LTR, RRE, Psi) were conducted in duplex reactions with CGT dPCR genomic reference assays (albumin, RPL32, RPL30). Template was gDNA with LVV target spiked-in for VCN 1, VCN 2, VCN 5 and VCN 10. PCR was performed on the QIAcuity platform using a 96-well 8.5k Nanoplate. All assays showed less than 20% deviation from the expected VCN.

LV genome titer determination following the CGT Lentivirus Lysis Kit protocol

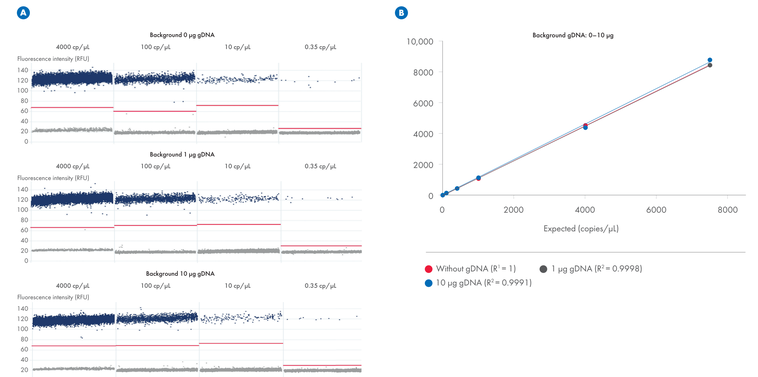
The determination of physical LV genome is essential for safe, stable and effective therapies. Different LV samples are efficiently processed in three standardized steps for genome quantification on a QIAcuity dPCR System.



LV sample processing and titer determination using QIAGEN CGT Lentivirus Lysis Kit and CGT dPCR Assays: A Schematic overview. In a first step, LV samples are treated with DNase I to remove DNA impurities. In step 2, DNase I-treated samples are lysed in the presence of Proteinase K. Finally, in step 3, the viral titers are determined using a QIAcuity dPCR instrument. **B** LV samples are processed using the CGT Lentivirus Lysis Kit and quantified on a QIAcuity dPCR System with 8.5k nanoplates and CGT dPCR Assays. The CGT dPCR Assays were run in triplex reactions in the FAM™, HEX™ and Cy5® channels. Assays for LTR, Psi, and RRE were used for the determination of the LV genome titer. LV samples from supernatant (DMEM) or concentrated LV samples resuspended in DMEM or PBS were processed.

RCL detection

Accurate detection of RCL is essential for the safety and efficacy of LV-based therapies. Digital PCR, using tools like QIAcuity RCL Quant Kit, provides unmatched sensitivity and accuracy compared to other methods, such as qPCR, making it an ideal choice for detecting RCLs.



Robust RCL detection using the QIAcuity RCL Quant Kit. A Different concentrations of positive control (4000 cp/µL, 100 cp/µL, 10 cp/µL, and 0.35 cp/µL) were spiked with increasing amounts of genomic background DNA (0 µg/rxn, 1 µg/rxn, and 10 µg/rxn). Increasing amounts of human background genomic DNA did not affect the detection of the VSV-G target. **B** The assay demonstrated high linearity and a broad dynamic range of 0.35–7,500 copies/µL, even in the presence of up to 10 µg of genomic DNA per reaction, without the need for prior dilution.

Conclusions

The data show a simplified dPCR process for measuring lentiviral genome titers, VCN and RCL detection. This improved workflow enhances characterization with greater precision, accuracy and reliability.

- Flexible assay options: Utilize wet-lab tested QIAcuity CGT dPCR Assays for measuring LV titer and VCN.
- Optimized LV titer assessment: Use assays designed for integration plasmid elements, regulatory elements or reporter/resistance genes.
- Integrated vector copy assessment: Combine these assays with genomic reference assays to easily determine the number of integrated vector copies.
- Robust RCL detection: The QIAcuity RCL Quant Kit, with positive and internal controls, enables sensitive and robust detection of VSV-G absence in cell material.
- Efficiency and reliability: Eliminate the need for standard curves, reducing hands-on time, turnaround time and errors, while increasing accuracy and consistency.
- Standardized and tested: All assays are standardized and wet-lab tested.
- High throughput and scalability: Designed with high throughput and scalability in mind.

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