Determination of lentiviral titers and integrated lentiviral vector copy numbers in transduced cells using digital PCR

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Background and aims

Developing safe and effective cell and gene therapies is essential for treating various diseases. Lentiviral vectors (LVVs) are a robust technology for delivering therapeutic genes into cells. Accurate LVV characterization is crucial for ensuring safety, efficacy, and consistency. Besides quantifying viral vector genome titers, measuring vector copy number (VCN) is critical. VCN indicates the number of transgene copies in target cells' genomes and precise VCN measurement is vital for determining the optimal viral dose in cell and gene therapy. Another key safety concern with lentiviral systems for gene therapy is preventing the unintended creation of a replication-competent lentivirus (RCL) through recombination. Regulatory agencies, such as the FDA and EMA, mandate comprehensive testing for RCL in retroviral vector products.

Results

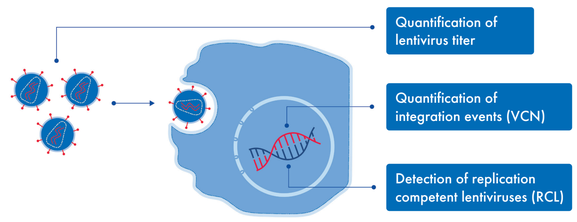
Genomic DNA from transduced cells is compared to a reference gene to determine lentiviral gene copies. Current methods often use qPCR, generating external standard curves. Digital PCR (dPCR) allows absolute quantification with high precision and tolerance for inhibitors, without standards. We propose a rapid dPCR method to determine VCN, ranging from 0 to >20 copies per genome, providing insights into transgene stability and expression. Consistent VCNs are crucial for uniform product quality in biopharmaceutical production. Furthermore, lentiviral quantitation is a key quality attribute in viral vector manufacturing for cell therapy. dPCR is highly suited for determining viral titers as it enables absolute quantification without a standard curve and with high precision, accuracy, specificity and reproducibility. Additionally, we have developed a rapid dPCR method for RCL detection that can identify VSV-G at concentrations as low as 0.35 copies/µL, even in the presence of high background genomic DNA.

Conclusions

We demonstrate a dPCR-based streamlined workflow for quantifying lentiviral genome titers, VCN and RCL detection, enhancing characterization 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.



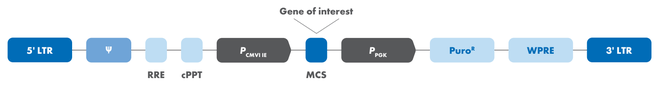
Comprehensive characterization of lentiviruses (LVs). The schematic illustrates the LV production workflow. dPCR provides precise and absolute quantification of critical quality attributes (CQAs), including viral vector genome quantification (physical titer), vector copy number quantification (VCN) and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

LV titer determination: Direct lysis solution and quantitation of genome-containing lentiviral particles

VCN quantification: Lentiviral targets in the gDNA of transduced cells in comparison to a reference housekeeping gene

RCL: Detection of replication-competent viruses

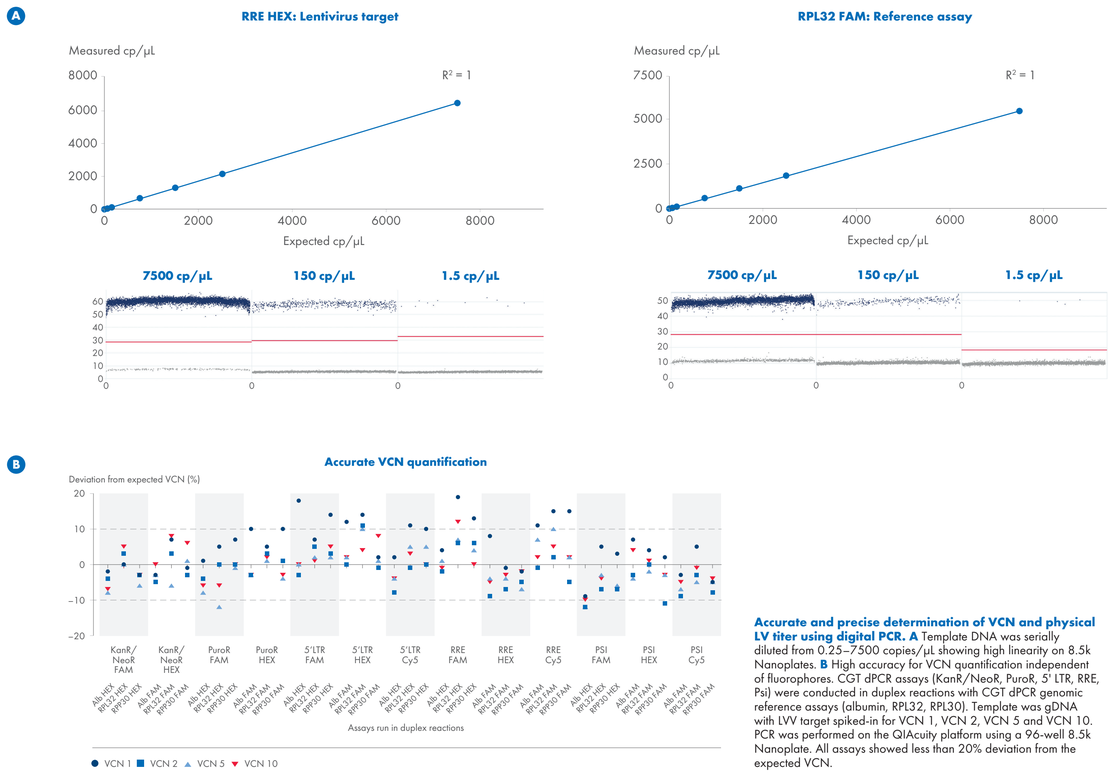
Reference genes	Reporter/resistance	Promoter/enhancer	Integration plasmid elements	Lentivirus replication (RCL)
Albumin	GFP/eGFP	CMVp	Psi	VSV-G
RPP30	AmpR*	CMVe	RRE	
RPL32	PuroR†	SV40p	5' LTR	
	KanR/NeoR	WPRE		



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

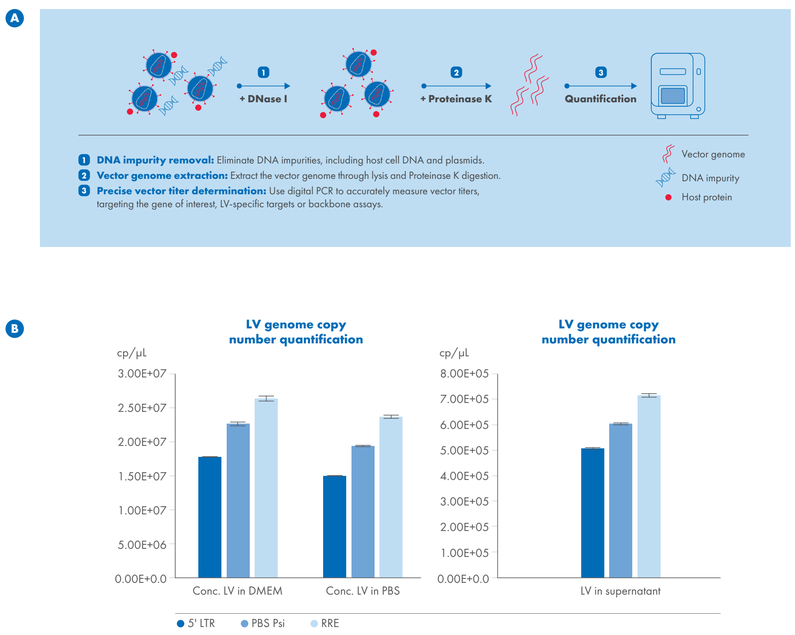
Determination of VCN and LV physical titer is a crucial quality attribute. dPCR facilitates accurate and precise quantification of both.



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

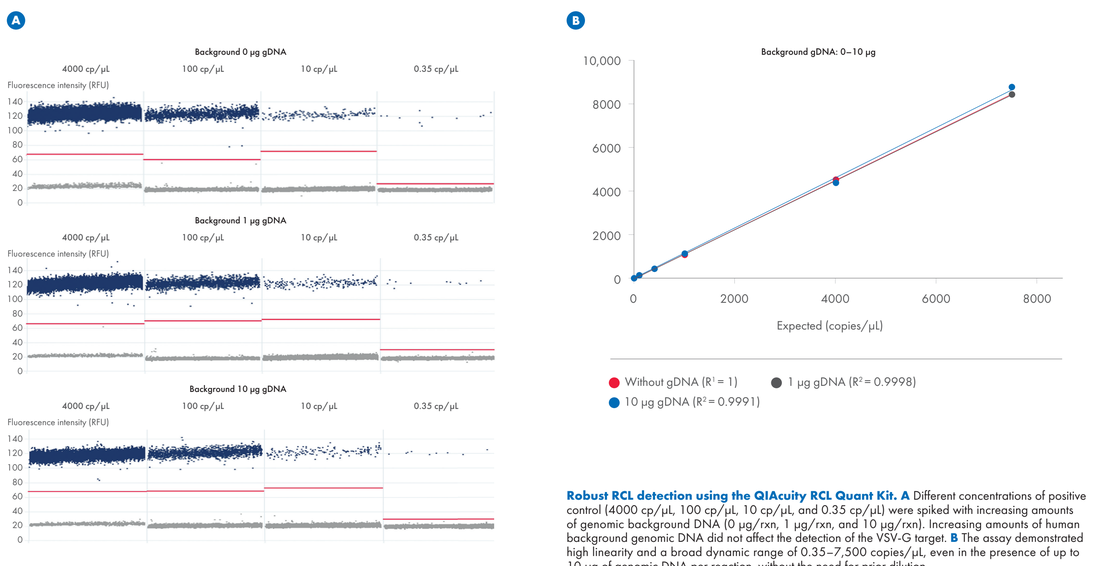
The determination of physical LV genome is essential for safe, stable and effective therapies. Different LV samples are efficiently processed in three standardized steps for genome quantification on a QIAcuity dPCR System.



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: 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.

Learn more about QIAcuity Cell and Gene Therapy dPCR Assays

Sample to Insight