Quantification and qualification of adeno-associated virus (AAV) using dedicated CGT assays and the QIAcuity® Digital PCR System

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Accurate and precise vector genome titration is key for gene therapy applications

Adeno-associated virus (AAV) is a widely used viral vector in gene therapy applications. However, the process of generation and purification of the viral vectors requires precise quality control to enable safe and reliable dosing during clinical studies or patient care.

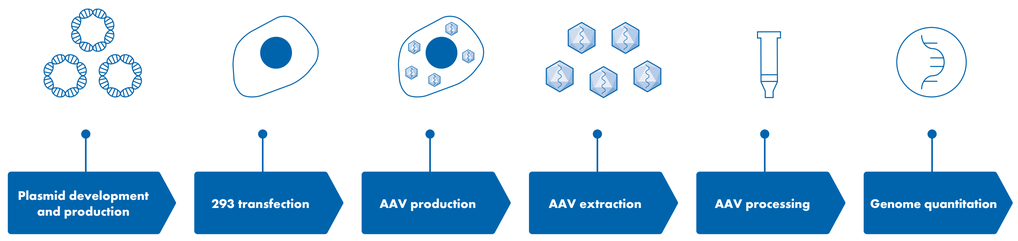
Viral vector monitoring is an increasingly important tool for Biopharma in cell and gene therapy (CGT). The ability to accurately quantify vector titers as well as to determine contaminations is key for safe and effective AAV-based gene therapies.

qPCR is a widely used method for AAV quantitation due to its sensitivity and ease of use. Nevertheless, well-characterized DNA standards and assays are needed for accurate quantification. The ability to accurately quantify vector titers as well as to determine contaminations is key for safe and effective AAV-based gene therapies. The QIAcuity Digital PCR System, with its dedicated CGT assays, enables vector genome titration with outstanding accuracy, reproducibility and speed with an easy workflow comparable to qPCR.

The QIAcuity One.

The AAV genome is a key component for vector quantification

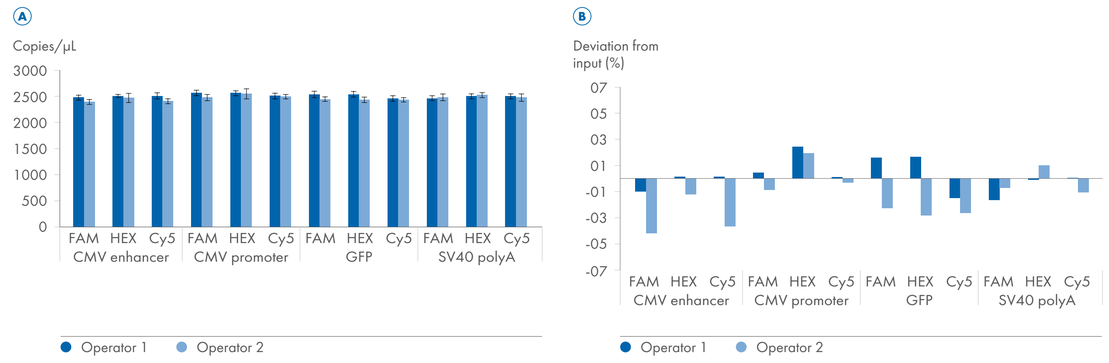
HEK293 cells are typical producers of AAVs of various serotypes. After production of AAVs, the particles must be extracted and processed. Upstream and downstream processes are strictly monitored. Vector genome titration is performed at various steps throughout the process. Digital PCR enables robust titer determination of AAV samples of different purities and concentrations.



Schematic illustration of AAV production, processing and quantification.

High accuracy across assays and operators

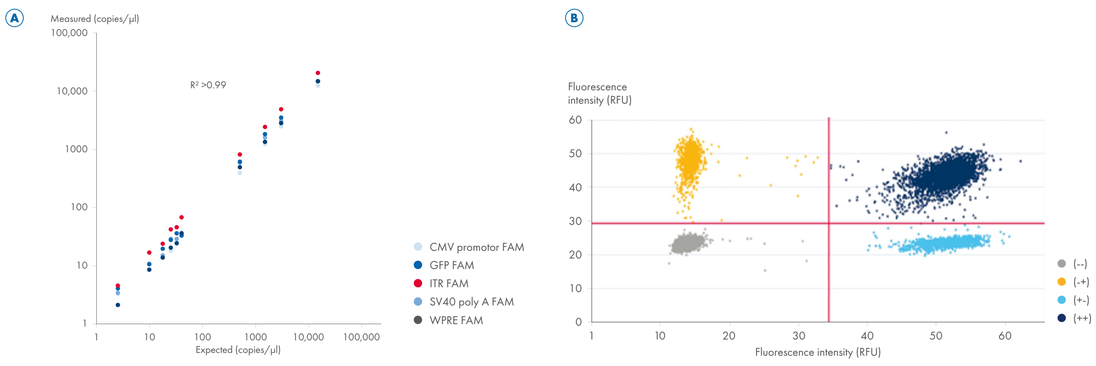
CGT dPCR assays show accurate quantification independent of fluorophores and operators. Therefore, higher multiplexing is possible without affecting titration. Titration with more than one assay gives a complete picture on the 'real' vector titer.



Highly accurate dPCR. A The CGT dPCR assays were run with AAV DNA. 2500 cop/µL was used as input on an 8.5k nanoplate. Mean number of copies of at least 13 replicates per operator are shown. Mean CV among all assays and operators ~2%. **B** Mean values of deviation from expected input copies of at least 13 replicates per operator are shown.

High inter-assay precision over a broad dynamic range

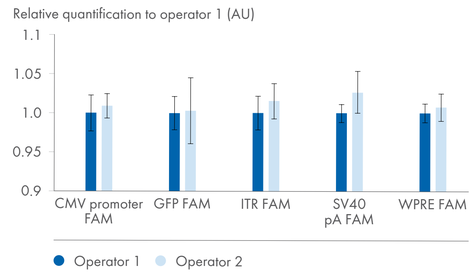
High linearity from 2.5 copies/µL to 15,000 copies/µL on 8.5k nanoplates enables titer determination of AAV samples originating from different in-process steps. Samples from early in the purification process are expected to have lower titers, whereas close to the release, product titers are expected to be highest.



High precision with dPCR. A The CGT dPCR FAM assays were run on a serial dilution of DNA extracted from AAV2 samples. The DNA input ranged from 2.5 copies/µL to 15,000 copies/µL. PCR was performed on an 8.5k nanoplate. Coefficients of determination >0.99. **B** 2D scatterplot of ITR FAM™ and CMV promoter HEX™ assays.

Robust PCR system with minimal variation between operators

High intra-assay precision is crucial for robust viral vector genome titration. High repeatability between operators is one of the key elements of titer determination analytics to ensure safe and effective AAV-based gene therapies.



Minimal inter-operator variation with dPCR. The CGT dPCR assays were run on AAV2 samples. 1000 copies/µL was used as input on an 8.5k nanoplate. Measured copy numbers of operator 1 were set as reference and the normalized quantifications of six replicates plotted accordingly. Operator 1 represents an experienced dPCR user whereas operator 2 represents a first-time dPCR user.

Intra-assay precision, %

CGT dPCR assay	Intra-assay precision (between operators), %
CMV promoter FAM	0.85
GFP FAM	0.29
ITR FAM	1.47
SV40pA FAM	2.56
WPRE FAM	0.74

Conclusion

- Titers of viral vectors such as AAVs can be accurately and precisely determined using digital PCR and QIAGEN's dedicated CGT dPCR assays without the need for references or standard curves
- The CGT dPCR assays in combination with the QIAcuity System provide a robust quantification with high repeatability between operators
- Viral genome titer can be determined in singleplex and multiplex reactions to gain a more complete picture on the genome state
- Broad dynamic ranges allow titration of samples with high and low titers

Sample to Insight