

# Determination of genome titer and integrity of adeno-associated virus (AAV) reference standards using the QIAcuity® Digital PCR System



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## Standardization and reproducibility in gene therapy analytics using digital PCR (dPCR)

Recombinant adeno-associated virus (rAAV) vectors are widely used as vehicles for gene therapy in both clinical and research applications. To help ensure product safety, reference standard materials (RSMs) are key. The use of viral vector RSMs is particularly important to normalize laboratory internal controls, for the calibration of medical products and procedures, and as best practices for manufacturing and testing of advanced therapy medicinal products (ATMPs). Additionally, AAV RSMs are often used for assay validation and optimization.

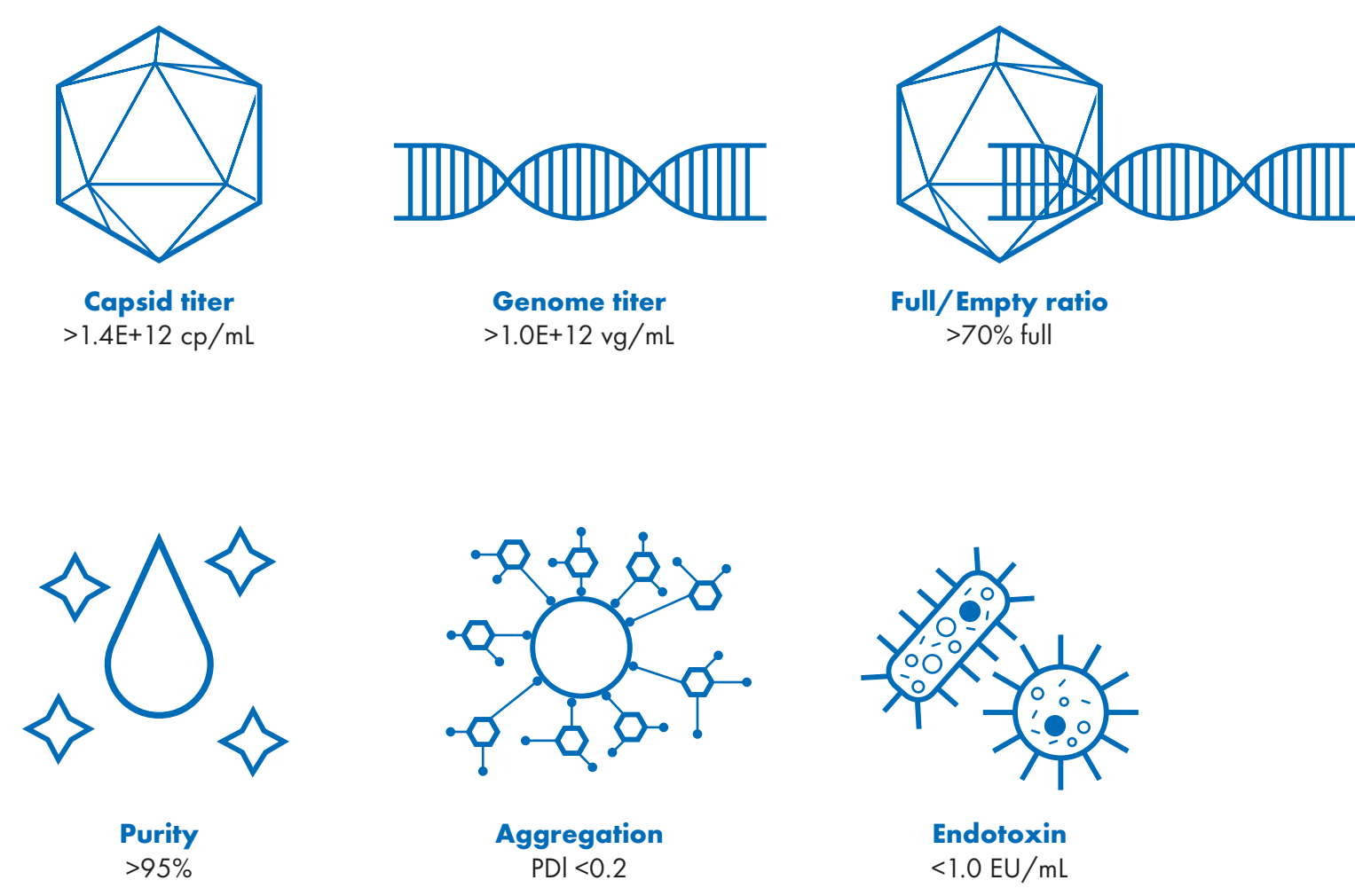
In general, the use of well-characterized AAV RSMs and orthogonal methods is indispensable to meet current authority guidelines. Since the production of these is not achievable for many companies, it is important to have commercially available AAV RSMs for the most frequently used AAV serotypes. Underlying these standards is a

comprehensive characterization process including determination of vector genome and capsid titer, filling grade, purity and aggregation. One of the critical quality attributes of AAV reference materials is the viral vector genome titer which is usually determined using qPCR.

Here, we demonstrate a streamlined digital PCR-based workflow for quantification of genome titers of commercially available, high-quality rAAV reference standards that enables complete characterization with increased precision, accuracy and robustness. Additionally, the integrity of the viral vector genome can be assessed, ensuring quality and safety of the final product.

## Quality attributes of PROGEN's AAV standard material

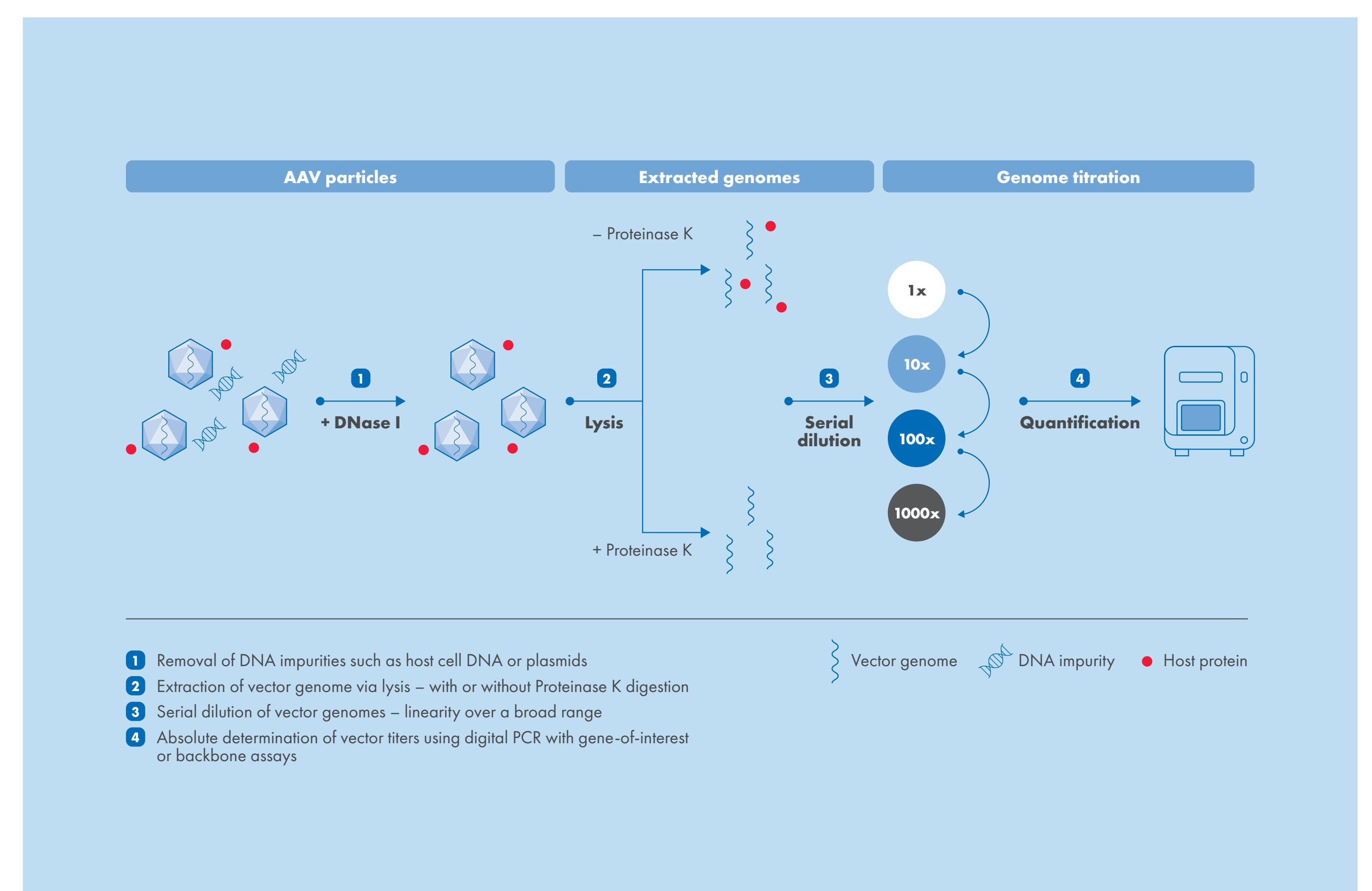
PROGEN has established a comprehensive characterization process for eGFP-filled AAV standards for the serotypes 1–3, 5–6, 8–9, rh10, and rh74. The quality control ensures well-characterized high-quality AAV standard material, which can be easily implemented as reference material in a variety of assays to prove the validity of the corresponding assay.



**Comprehensive characterization of PROGEN's AAV reference standards.** Characterization procedure includes determination of capsid and viral genome titer using PROGEN EUSA and qPCR, respectively. The filling grade is measured by charge detection mass spectrometry and purity by silver staining. Aggregation is analyzed by dynamic light scattering and endotoxin levels by spectrophotometric measurement [AL kinetic chromogenic assay]. cp/mL: Capsid particles per milliliter; EU/mL: Endotoxin units per milliliter; PDI: Polydispersity index; vg/mL: Vector genomes per milliliter.

## AAV standards genome titer and integrity determination following the CGT Viral Vector workflow

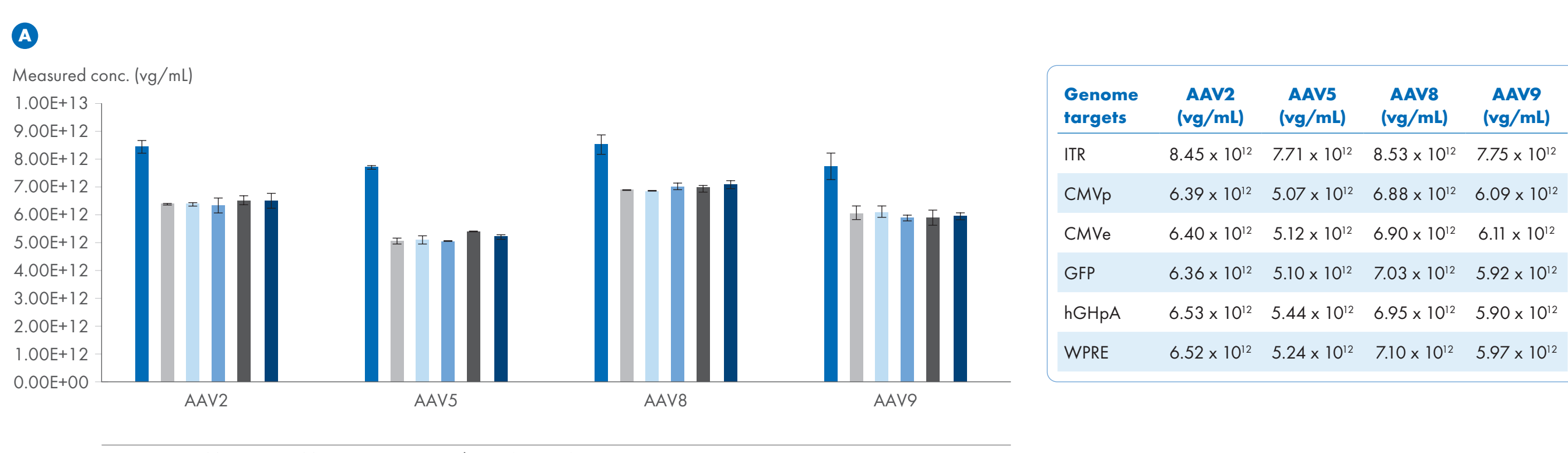
AAV reference standards of different serotypes and suppliers are efficiently processed in four standardized steps for genome titration and genome integrity determination on a QIAcuity dPCR System.



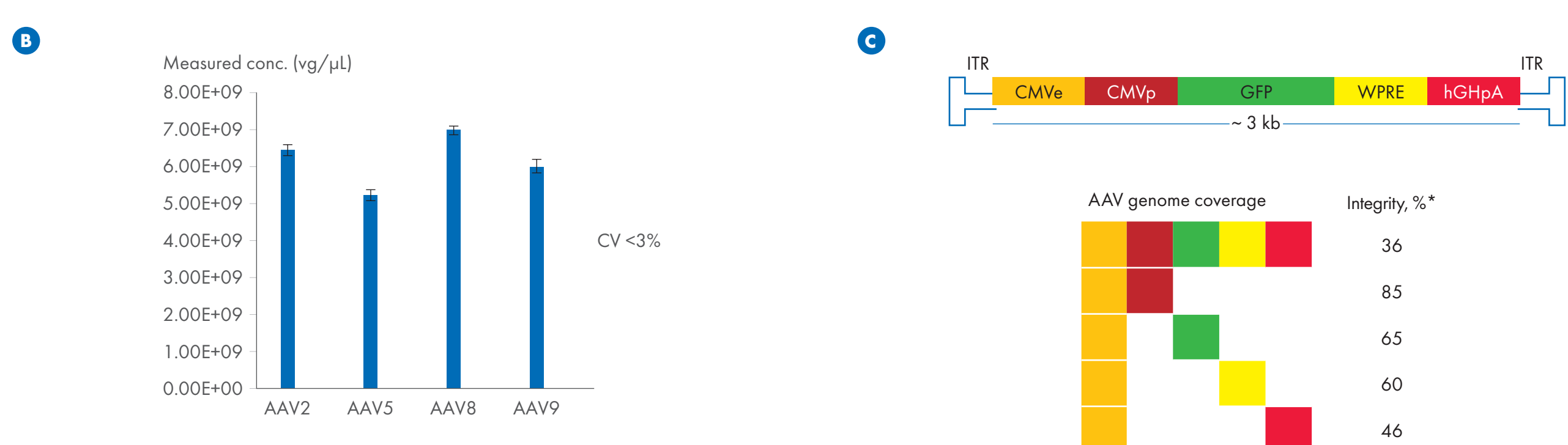
**AAV standards processing, titer and integrity determination using the QIAGEN CGT Viral Vector Lysis Kit and CGT dPCR Assays:** In a first step, AAV samples are treated with DNase I to remove DNA impurities. In step 2, DNase I-treated samples are lysed in the presence or absence of Proteinase K. In step 3, the lysates are serially diluted. Finally, in step 4, the viral titers and integrity status are determined using a QIAcuity dPCR instrument.

## Accurate, precise determination of genome titers and integrity of PROGEN's AAV standards using the QIAcuity dPCR System

AAV reference standards are crucial tools for CGT analytics. Besides the determination of the viral vector genome titer, the assessment of genome integrity is playing an increasingly important role. Digital PCR with high multiplex capabilities enables the analysis of both critical attributes.



**The non-ITR genome targets of PROGEN's AAV standards show comparable quantification.** AAV2, AAV5, AAV8 and AAV9 standards were processed using the CGT Viral Vector Lysis Kit and quantified on a QIAcuity dPCR System using 8.5k nanoplates and CGT dPCR Assays. The CGT dPCR Assays were run in 2-plex reactions in the FAM<sup>®</sup>, HEX<sup>®</sup> and Cy5<sup>®</sup> channels (ITR, CMVe, CMVp and GFP, WPRE, hGHpA). The samples were serially diluted and 2 dilutions with 3 technical replicates each were used for the determination of the genome titer.

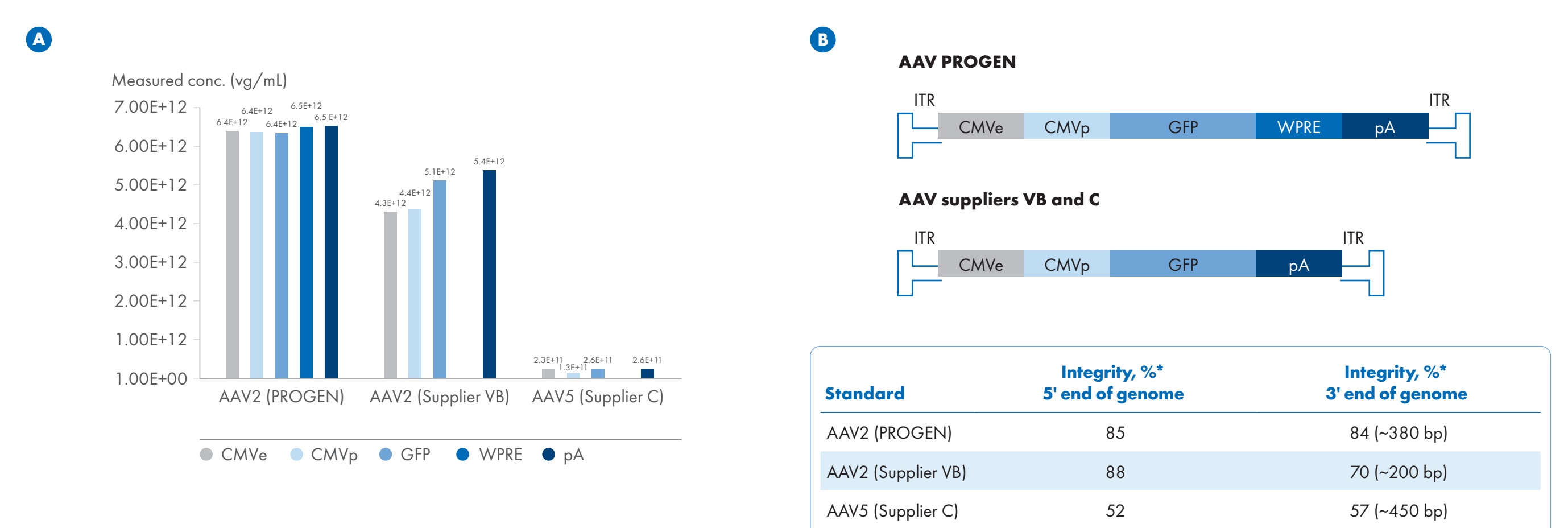


**Genome titer determination with high precision.** Reference standards (PROGEN) were processed using the CGT Viral Vector Lysis Kit and quantified using the CGT dPCR Assays in 2-plex reactions (ITR, CMVe, CMVp and GFP, WPRE, hGHpA) on a QIAcuity dPCR System. Coefficient of variation (CV) between the non-ITR targets is shown. Maximum deviation from mean between targets <5% for AAV5 and <2% for AAV2, AAV8 and AAV9. **C** Genome integrity was determined using the QIAcuity Software Suite version 2.5. Genome targets were run in a 5-plex reaction. The analysis of genome integrity was performed including all 5 channels or in groups of 2 spanning different parts of the genome.

\* AAV2 standard was processed using CGT Viral Vector Lysis Kit. Processing includes enzymatic removal of ITR secondary structures and takes place outside of the QIAcuity nanoplate partitions, which may lead to reduced integrity scores.

## Comparing genome titers and integrity of different AAV reference standards using dPCR

Quantification of multiple targets on the genome increases insights on sample titer and quality. AAV packaging is error prone and elimination of mispackaged or incomplete particles is difficult to achieve during manufacturing and downstream clean-up. Digital PCR allows in-depth analysis of AAV samples and standards.



**The viral vector genome titer does not necessarily reflect the genome integrity status.** AAV standards from different suppliers (PROGEN, VB, C) were processed using the CGT Viral Vector Lysis Kit and quantified using the CGT dPCR Assays in multiplex reactions on a QIAcuity dPCR System. **B** Genome integrity of the 5' and 3' end of the genomes was determined using the QIAcuity Software Suite version 2.5. Integrity was analyzed in a duplex setup (5' end towards GFP and GFP towards 3' end). Distance between targets is indicated in case of differences between standards.

\* AAV standards were processed using the CGT Viral Vector Lysis Kit. Processing includes enzymatic removal of ITR secondary structures and takes place outside of the QIAcuity nanoplate partitions which may lead to reduced integrity scores.

## Conclusions

- Viral vectors, such as AAVs, can be reliably processed using the CGT Viral Vector Lysis Kit. Genome titers and integrity can be accurately determined using dPCR and the CGT dPCR Assays.
- Thoroughly characterized AAV reference standards like PROGEN's standard material are crucial for accurate analytics of viral vectors.
- QIAcuity Software Suite version 2.5 enables access to insights into genome integrity.
- Viral vector genome titers do not necessarily reflect the genome integrity status.
- The more targets are analyzed in a multiplex reaction, the more comprehensive the integrity score.

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