Analysis of Partial Capsids Using the Vericheck ddPCR™ Empty-Full Capsid Kit

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Abstract

Accurate quantification of empty and full adeno-associated virus (AAV) capsids is critical for optimizing viral vectors in gene therapy. This study aimed to extend the Vericheck ddPCR Empty-Full Capsid Kit by broadening its application to measure the percentage of AAV capsids filled with a range of genomic targets, thereby offering increased workflow flexibility and a more comprehensive assessment of AAV genome integrity. Using Droplet Digital™ PCR (ddPCR), we compared the percentage of filled capsids for various AAV targets. The kit offers a robust and reliable method for assessing capsid integrity, helping to ensure the consistency and effectiveness of AAV-based therapies.

Introduction

AAVs are widely used as viral vectors in gene therapy, consisting of a protein capsid and a recombinant DNA genome. During synthesis, some AAV particles become mispackaged, lacking some or all expected genes. The Vericheck ddPCR Empty-Full Capsid Kit includes an assay targeting viral packaging sequences from AAV serotype 2 inverted terminal repeat (ITR-2), a universal target for quantifying AAV genomes. Particles containing ITR-2 are reported as full capsids, whereas those without are reported as empty. However, as demonstrated here, alternate reference targets may be used to generate a percentage of capsids with each target. If the percentage of filled capsids is unequal across targets, this indicates the presence of partially filled capsids, offering a more detailed assessment of genome integrity during AAV development.

Materials and Methods

Summary

The following steps can be used to extend the Vericheck ddPCR Empty-Full Capsid Kits (Bio-Rad Laboratories, Inc., catalog #17010072, #17010082) to further targets:

1. Acquire ddPCR assay(s) against AAV targets in HEX.
2. Replace the included ITR-2 assay with the desired assay(s). No changes to the assay protocol or empty-full calculations are required. However, manual thresholding of ddPCR wells may be necessary.
3. Compare the percentage of full capsids between targets to assess genome integrity.

Assay Selection

The Vericheck ddPCR Empty-Full Capsid Kits contain a capsid detection assay in FAM and an ITR-2 assay in HEX. The ITR-2 assay can be replaced with an alternative HEX assay to test other genes within an AAV genome. Users can request custom assays using various predesigned vector backbones through the Bio-Rad Cell and Gene Therapy Assay Design Engine. Alternatively, users may design assays following the Droplet Digital PCR Applications Guide (bulletin 6407). Primers should be designed to have a melting temperature (T_m) of approximately 55°C, with the T_m of the HEX probe being 3–10°C higher than that of the primers. Each primer should be used at a 900 nM final concentration and probes at 250 nM.

Here, we tested AAV samples containing ITR-2, the cytomegalovirus (CMV) promoter, and enhanced GFP (eGFP) for the presence of partial genomes. HEX-labeled assays against ITR-2 (Bio-Rad, dEXD23004642), the CMV promoter (Bio-Rad, dEXD96423937), and eGFP (Bio-Rad, dCGTS863952655) were obtained from the Bio-Rad Cell and Gene Therapy Assay Design Engine.

Sample Preparation

Three AAV serotype 9 (AAV9) samples containing the genome ITR-2-CMV-eGFP-SV40-ITR-2 were used: Sample 1 (Charles River Laboratories, #CV10009), Sample 3 (Porton Advanced, #AAV9-DS230831-01), and Sample 4 (Charles River, #RS-AAV9-FL). Sample 2 (Charles River, #RS-AAV9-ET) consisted of empty capsids and was diluted to approximately the same capsid concentration as Sample 1 with dilution buffer by mixing equal volumes of the empty and full samples, as shown in Table 1.

Table 1. Samples tested with the Vericheck ddPCR Empty-Full Capsid Kit.

Sample	Description
1	Vendor A full capsids
2	50% Vendor A capsids + 50% empty capsids
3	Vendor B full capsids
4	Vendor C full capsids

ddPCR Assay Analysis

Each sample was tested with the Vericheck ddPCR Empty-Full Capsid Kit, following the Vericheck ddPCR Empty-Full Capsid Kit User Guide, using a QX200™ Droplet Digital PCR System. For the CMV promoter and eGFP analyses, the default ITR-2 assay was replaced and tested separately. Each assay and sample combination was tested using triplicates of the antibody binding reaction, with three ddPCR wells per antibody binding reaction. ddPCR wells were analyzed using QX Manager Software, Premium Edition, Version 2.2 (Bio-Rad, #12018108). The kit user guide describes using the positive control automatic thresholding option to analyze the data. However, the substituted genome assays may not be present in the positive control supplied in the kit, requiring manual thresholding instead. The threshold was manually placed one quarter of the total distance between the center of the double-negative cluster and the center of the capsid detection assay cluster and one quarter between the double-negative cluster and the gene of interest assay, as shown in Figure 1.

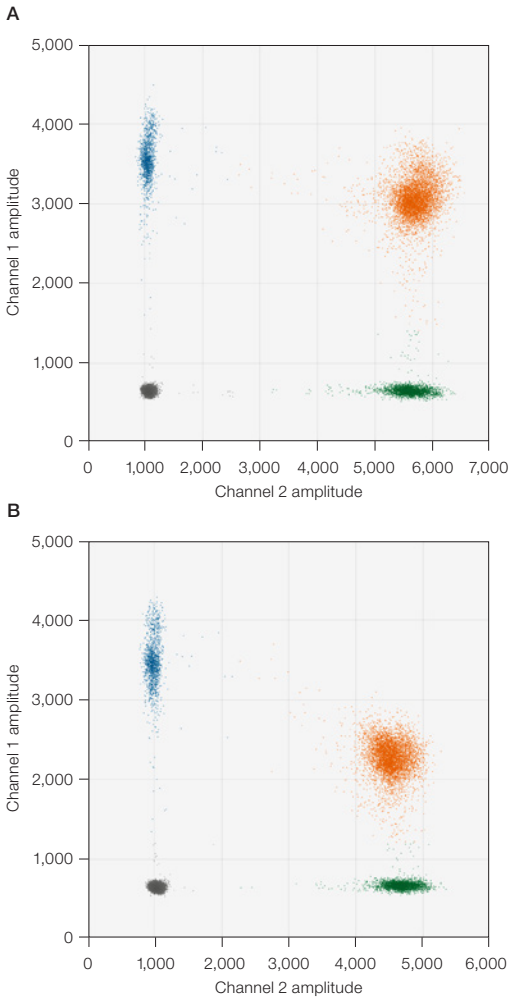

Fig. 1. Example of manual thresholding for genome assays. A, eGFP in HEX. **B**, CMV promoter in HEX. Capsid detection assay in FAM, channel 1 (●); genome assay in HEX, channel 2 (●); double-positive cluster (●).

Analyzing Percentage of Full Capsids

No modifications to the analysis described in the Vericheck ddPCR Empty-Full Capsid Kit User Guide were needed to determine the percentage total of each gene of interest. All samples were analyzed using the Vericheck ddPCR Empty-Full Capsid Analysis Worksheet. The percentage full for all genes of interest was compared to the percentage of capsids filled with ITR-2.

Results

The capsid titer for each sample using each genome reference is shown in Figure 2. Capsid titer was similar regardless of which assay was used as a genome reference.

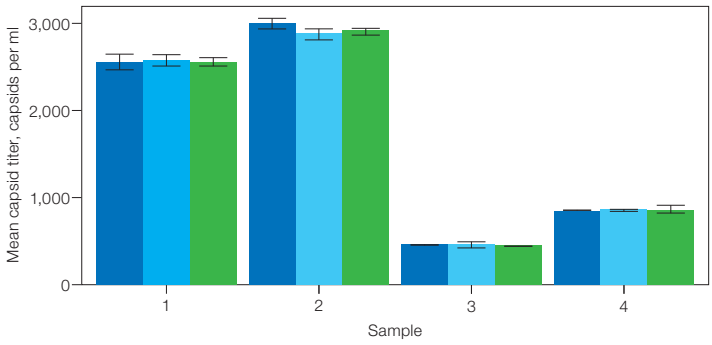

Fig. 2. Capsid titer by sample and genome reference. The concentration of AAV capsids using each genome reference is presented as a bar. Error bars mark one standard deviation. Targets: ITR-2 (■), eGFP (■), and CMV promoter (■).

The percentage of filled capsids is shown in Figure 3. Sample 4 had larger differences between each target, indicating the presence of partial capsids.

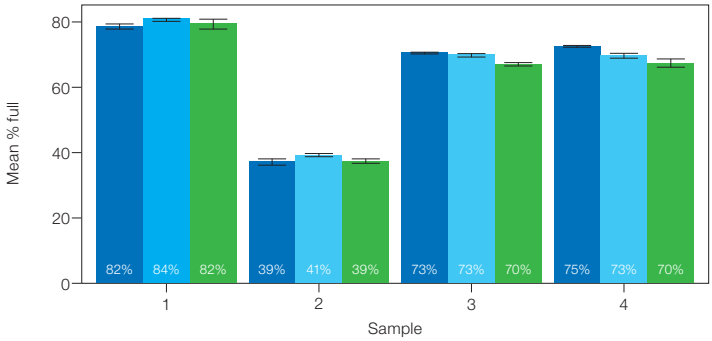

Fig. 3. Percentage of full capsids for all samples. The percentage of capsids filled with each target is presented as a bar. Error bars mark one standard deviation. Targets: ITR-2 (■), eGFP (■), and CMV promoter (■).

Conclusions

When comparing the results of the genome assays, all assays produced nearly identical capsid titers for each sample. This finding suggests that any present AAV genomic target is sufficient for calibrating capsid measurement, regardless of fill rate. When measuring samples from different vendors, differences in fill rate by gene were noted with Vendors B and C, perhaps indicating the presence of partial genomes.

In conclusion, this study successfully extended the Vericheck ddPCR Empty-Full Capsid Kit to measure the percentage of AAV capsids filled with various genomic targets beyond ITR-2. This expanded capability can be used with diverse AAV genomes to characterize partial capsids for a more comprehensive assessment of AAV integrity. By incorporating alternative reference targets, the kit provides greater flexibility and insight into packaging efficiency, extending its use in gene therapy applications.

Visit **bio-rad.com/ddPCR-Vericheck-EFcapsid** for more information.

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