Performance Comparison of the Vericheck ddPCR™ Empty-Full Capsid Kit and Analytical Ultracentrifugation (AUC)

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Abstract

Adeno-associated viruses (AAV) are potent tools in gene therapy. AAV particles consist of a protein capsid containing an intended genome, the combination of which is referred to as a full capsid. The manufacturing process does not always produce full capsids and can instead lead to partial or empty capsids that lack a genome or contain unintended contaminant DNA. Analytical ultracentrifugation (AUC) is commonly used to analyze capsid content purity, yet it cannot differentiate between intended genomes and contaminant DNA. This application note compares results from AAV empty-full capsid testing using both Droplet Digital™ PCR (ddPCR) and AUC. The Vericheck ddPCR Empty-Full Capsid Kit (Bio-Rad Laboratories, Inc., catalog #17010082 and #17010072) specifically quantifies the therapeutic genome contents of AAV, providing a more precise analysis.

Introduction

The Vericheck ddPCR Empty-Full Capsid Kit can be used to quantitate AAV vectors that produce full, partial, and empty capsids. While AUC is an innovative technique for separating AAVs containing varied payloads, it cannot distinguish between capsids containing the intended payload and contaminant DNA. This is an important distinction as contaminants may lead to adverse health effects. In this study, capsids from two AAV serotypes were analyzed using the AUC method and the Vericheck ddPCR Empty-Full Capsid Kit. The intended contents were quantified by an AAV serotype 2 inverted terminal repeat (ITR-2) assay, while contaminants were quantified by additional specific assays.

Material and Methods

Two AAV samples, one serotype 5 (AAV5) and one serotype 9 (AAV9), were obtained from a commercial vendor. Both samples were created using the triple plasmid transfection method. The source vector maps are shown in Figure 1. Three tests were performed with each AAV serotype using the Vericheck ddPCR Empty-Full Capsid Kit corresponding to its serotype and the ITR-2 assay, as described in the kit user guide. The plate was analyzed using the QX600™ AutoDG™ ddPCR System (Bio-Rad, #17008371). The data were exported from the QX Manager Software, Premium Edition, version 2.2 (Bio-Rad, #12018108) and were analyzed using the Vericheck ddPCR Empty-Full Capsid Analysis Worksheet, which generates outputs for both genome and capsid titers. Each sample was diluted to approximately 10^{11} genome copies (GC)/ml and tested using the Vericheck ddPCR Empty-Full Capsid Kit to determine the percentage of capsids filled with ITR-2. Each sample was tested according to the manufacturer's instructions. For non-ITR-2 targets, the default ITR-2 assay was replaced with the corresponding contaminant assay (Table 1). Additionally, the *KanR* assay was tested in combination with the ITR-2 assay to determine if capsids contained both targets. Each assay–sample combination was tested with three antibody binding reaction replicates and three ddPCR wells per antibody binding reaction.

Table 1. Residual plasmid DNA assays.

Assay Target	Product Name	Description	Bio-Rad Catalog #
KanR	ddPCR Expert Design Assay: *KanR* (HEX)	Residual GOI plasmid	dEXD51863961
Rep	ddPCR RCAAV Assay	Residual Rep/Cap plasmid	16011505
E2A	ddPCR Copy Number Assay: dCNS919674709	Residual pHelper	dCNS919674709

GOI, gene of interest; RCAAV, replication competent adeno-associated virus.

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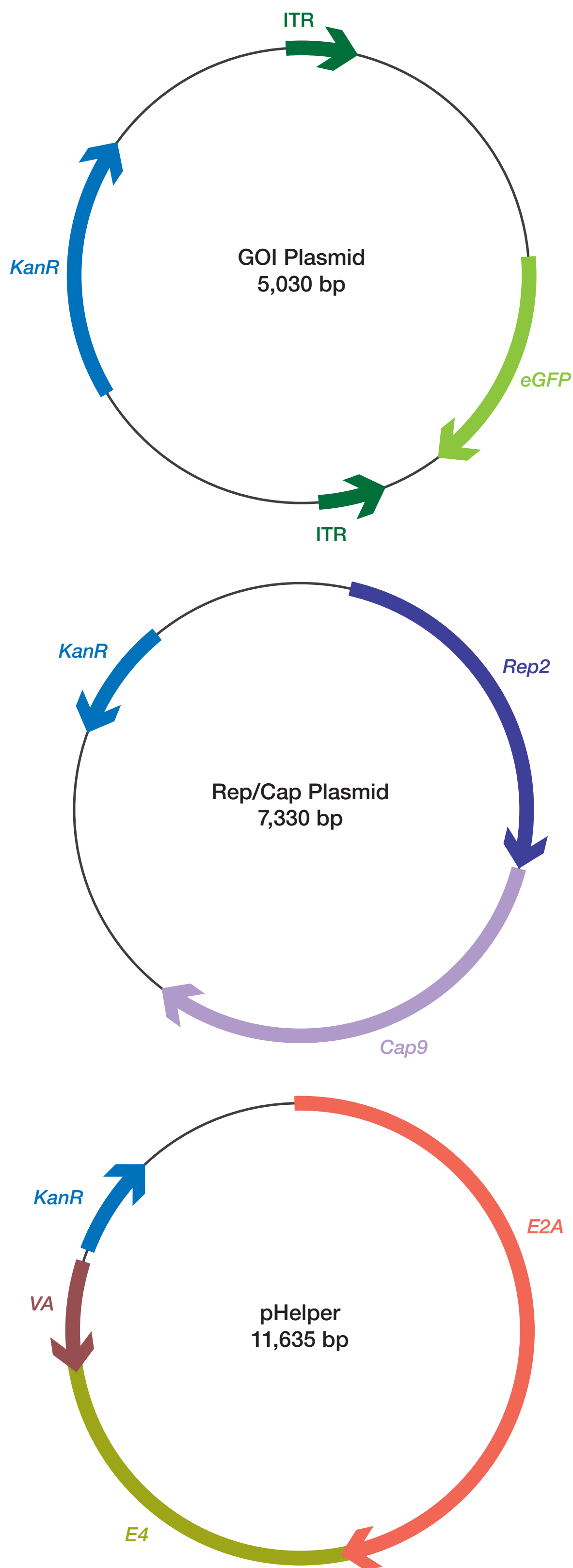

Fig 1. Vector genome maps for plasmids used in the AAV triple plasmid transfection assay. AAV, adeno-associated viruses; eGFP, enhanced green fluorescent protein; GOI, gene of interest; ITR, inverted terminal repeat.

Experimental Analysis

All the wells from the ddPCR assay were analyzed using QX Manager Software, Premium Edition. For each channel, the threshold was manually placed one quarter of the distance between the center of the negative cluster and the center of the positive cluster, as shown in Figure 2. To determine the percentage of full capsids in the ITR-2 assays and the residual plasmid targets, the output was exported and analyzed with the Vericheck ddPCR Empty-Full Capsid Analysis Worksheet, as described in the kit user guide.

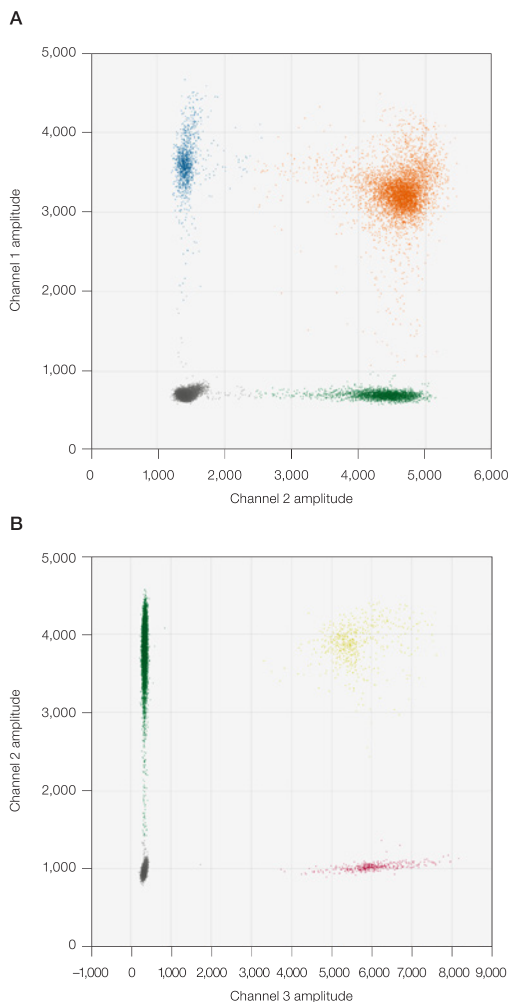

Fig. 2. Example of thresholding. A, capsid detection assay in FAM (●), ITR-2 assay in HEX (●), and double-positive clusters (●). **B**, *KanR* assay in FAM (●), ITR-2 assay in HEX (●), and double-positive clusters (●).

As the concentration of the *Rep* and *E2A* assays were considerably lower than the concentration output of the capsid detection assay, these targets and the capsid detection assay could not be analyzed in the same well. Instead, the percentage of capsids containing *Rep* or *E2A* was determined by dividing the measured concentration of *Rep* by the capsid titer determined in the ITR-2 assay. The proportion of capsids containing both the plasmid backbone target and ITR-2 was determined using the QX Manager Software linkage calculation.

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Results

The percentage of full capsids for all methods are shown in Table 2. While the percentage of capsids filled with ITR-2 was less than the total percentage of filled capsids detected by AUC, ddPCR analysis using the QX600 ddPCR System detected significant amounts of contaminant fragments in both samples.

Table 2. Percentage of full capsids calculated by AUC and ddPCR analysis.

		Percent of capsids filled with ITR-2 and contaminant fragment DNA*			
Sample	**AUC, percent full capsids**	**ITR-2**	***KanR***	***E2A***	***Rep***
AAV5	69.8%	54.8%	4.9%	0.1%	0.14%
AAV9	81.8%	71.8%	2.6%	0.1%	0.11%

AUC, analytical ultracentrifugation.
* Determined by ddPCR analysis using the QX600 System.

Further analysis identified the proportion of capsids containing both ITR-2 and a contaminating fragment, as shown in Figure 3. For both samples, the majority of capsids containing contaminant fragments did not also contain ITR-2.

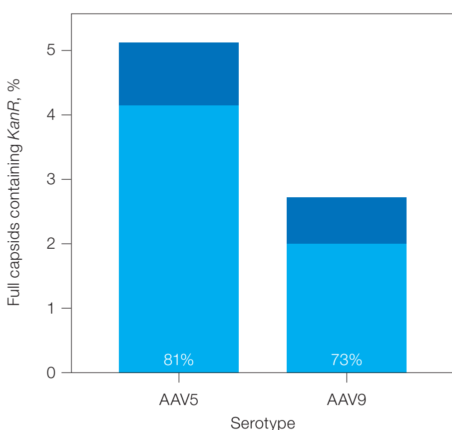

Fig. 3. Percentage of filled capsids in the plasmid backbone assays containing *KanR*, with and without ITR-2. The proportion of filled capsids containing a contaminant fragment but not containing ITR-2 is shown in light blue and represented by the percentage listed at the base of the bar.

Conclusions and Discussion

The percentage of full capsids containing ITR-2 was 10–15% less than the total percentage of full capsids detected by AUC. However, contaminant fragments from the plasmid backbone were found in both samples and in up to 5% of full capsids, partially accounting for the discrepancy. Linkage analysis of the plasmid backbone and ITR-2 assays showed that approximately 70–80% of capsids containing the tested plasmid backbone assays did not contain ITR-2. These capsids would not be considered full using the Vericheck ddPCR Empty-Full Capsid Kit but may still be considered full by content-blind methods such as AUC. It is possible that additional capsids are filled with contaminant fragments from other sources, such as residual host cell DNA, which would further overstate the number of full capsids detected by AUC (Lecomte 2015, Schnödt 2016, McColl-Carboni 2024).

Only capsids containing the intended vector genome have the desired therapeutic effect. For biophysical methods like AUC, all DNA is considered equivalent. In contrast, the Vericheck ddPCR Empty-Full Capsid Kit allows for measuring capsids filled only with the intended vector genome, which may have a stronger correlation with product quality.

References

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

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