Digital PCR

Fully-integrated digital PCR system for robust and consistent viral titer quantification

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Abstract

Adeno-associated virus (AAV) is a popular vector for use in gene and cell therapy research due to its high transduction efficiency, lack of pathogenicity and demonstrated efficacy and safety profile in several clinical trials and preclinical research. Recombinant AAV are produced in bulk quantities, and accurate quantification of the viral titer is essential in biopharma research for both production and tracking across the manufacturing process. Digital PCR (dPCR) has emerged as a leading technology for accurate and precise absolute quantification of nucleic acid targets. Researchers are using dPCR for robust viral titer quantification since it does not require a standard curve. Unlike other dPCR methods that rely on stochastic processes to generate micro-reactions by droplet formation, microfluidic array plate (MAP) technology facilitates automated sample distribution consistently into over 20,000 microchambers and utilizes >99% of the bulk reaction to minimize sample loss. Here we demonstrate how the increased robustness and consistency possible with MAP technology improves AAV viral titer quantification by performing comparison experiments using the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR system, as well as a comparison performed using a droplet digital PCR instrument. We found MAP-based digital PCR performed highly efficient digitization of the sample into 99% (±0.03% STDEV) of available microchambers compared to generation of 90% (±14.01% STDEV) of anticipated droplets on a droplet-based dPCR system. Paired with MAP technology, the QuantStudio Absolute Q Digital PCR system is a fully integrated, single instrument digital PCR system which has a simple qPCR-like workflow and can generate data in under 90 minutes. Reduced hands-on-requirements, consistent reagent digitization and fast time to answer serve the need for rapid, highly accurate and reproducible viral titer quantification required in the recombinant AAV production workflow.

Introduction

- Recombinant adeno-associated virus (rAAV) is produced in bulk at various low-titer concentrations and requires both concentration and quantification to meet the optimized concentration required for dose-escalation studies in cell and gene therapy research.
- Absolute quantification and high sensitivity with consistency and simple workflow are crucial for AAV production in cell and gene therapy research.
- In this study, we compared droplet-based digital PCR and the Microfluidic array plate (MAP) technology- based QuantStudio Absolute Q dPCR System to assess the reagent digitization consistency and quantification of AAV viral titer across four orders of magnitude of concentrations.

Materials and methods

Sample Preparation

A DNA fragment that contained the AAV two inverted terminal repeats (ITR-2) region was used and serially diluted 10-fold to create a total of 5 dilutions.

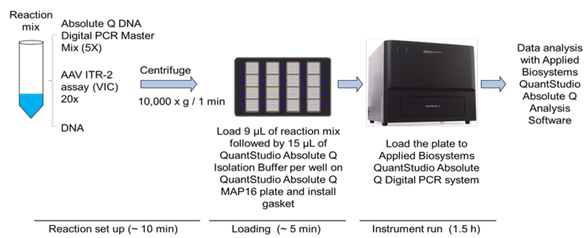
Test Methods

dPCR reactions were prepared as mentioned in Table 1 and were run on MAP technology-based Applied Biosystems QuantStudio Absolute Q Digital PCR system. Reactions (9 µL) were transferred to Applied Biosystems™ QuantStudio™ Absolute Q™ MAP 16 plates and then overlaid with 15 µL of Applied Biosystems™ QuantStudio™ Absolute Q™ Isolation Buffer per well. Following the addition of strip gaskets, the plate was transferred onto the system. A simple experiment workflow for MAP-based dPCR is illustrated in Figure 1.

Table 1. Reaction mix preparation for MAP-based dPCR.

Reagent	Volume for 1 reaction (µL)
Absolute Q DNA Digital PCR Master Mix (5X)	1.8
Absolute Q AAV ITR-2 assay (20X)	0.45
DNA sample	1.8
Water	4.95
Total	9

Figure 1. Rapid and easy workflow of the experiment on the MAP-based QuantStudio Absolute Q Digital PCR System.



Data Analysis

The concentrations reported by the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR Analysis Software for MAP-technology based dPCR and droplet-based dPCR associated software for droplet-based dPCR were then used in Equation 1 to calculate the concentration (cp / µL) of the AAV target in each dilution.

Materials and methods

Equation 1: Equation to calculate stock concentration. The total reaction volume was 9 µL and sample input was 1.8 µL for MAP-based dPCR, and for samples run on droplet-based dPCR system, the total reaction volume was 20 µL, and sample input was 4 µL.

$$\text{Stock concentration (cp / µL)} = \frac{\text{Concentration by the software } \left(\frac{\text{cp}}{\text{µL}}\right) \; x \; \text{Reaction volume µL}}{\text{Sample input (µL)}}$$

Results

Figure 2. 1D dot plots displaying dPCR-based quantification of AAV from serially diluted samples. Using the QuantStudio Absolute Q Digital PCR System, absolute quantification of AAV copies is possible with a dynamic range of 4 orders of magnitude for 10-fold serially diluted samples by counting the total number of microchambers positive for the fluorescent label.

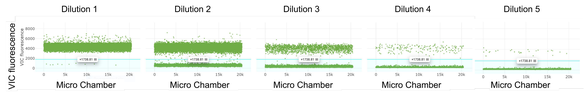
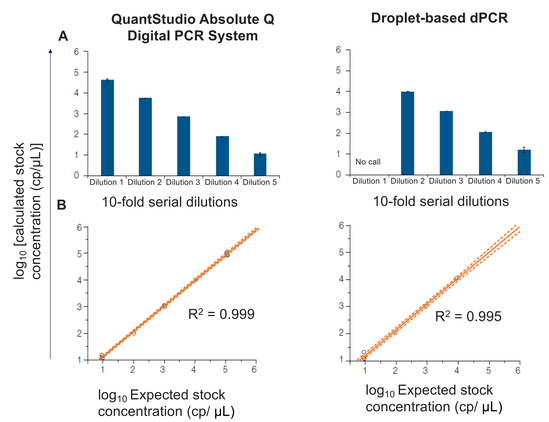
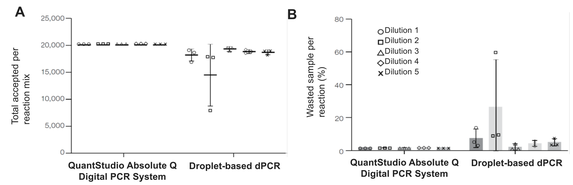


Figure 3. Dynamic range of AAV quantification on the QuantStudio Absolute Q Digital PCR System and droplet-based dPCR. (A) Calculated AAV concentration using the QuantStudio Absolute Q Digital PCR System and droplet-based dPCR using equation 1. Quantification is in cp/µL across five dilutions. **(B)** Correlation between the values of expected stock concentration and dPCR absolute quantification values. For the calculation of linear regression in droplet dPCR, only data within the detected dynamic range were considered. The regression line (solid line) with its associated 95% CI (dashed lines) is shown.



Results

Figure 4. Comparison of digitization consistency and percentage of sample wasted between the MAP-based QuantStudio Absolute Q Digital PCR System and a droplet-based dPCR platform. (A) Distribution of total number of accepted microchambers or droplets per dPCR reaction for five AAV dilutions run in parallel on the MAP-based QuantStudio Absolute Q Digital PCR System and a droplet-based dPCR platform. **(B)** Percentage of wasted sample per reaction in the QuantStudio Absolute Q Digital PCR System and a droplet-based dPCR platform.



Conclusions

- The easy workflow and rapid turnaround time on the QuantStudio Absolute Q digital PCR System are well suited to fulfilling the needs to rapidly and accurately quantify rAAV for cell and gene therapy research.
- Microfluidic array plate (MAP) technology facilitates robust reagent digitization with utilization of >99 ± 0.03% of available microchambers, compared to droplet-based technology generating only ~90 ± 14.01% of expected droplets, leading to a reduced dynamic range
- Sensitive and accurate quantification of AAV on the QuantStudio Absolute Q Digital PCR System could be valuable in viral vector production for biopharma and biomedical research.

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