Introduction

- Recombinant adeno-associated virus (AAV) is produced in bulk at various low-titer concentrations and requires both concentration and quantification to meet the optimized concentration requirement 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 for the QuantiStudio Absolute Q Digital PCR System to assess the droplet quantification 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 qPCR technology-based Applied Biosystems QuantiStudio Absolute Q Digital PCR System. Reactions (9 μL) were transferred to Applied Biosystems® QuantiStudio™ Absolute Q™ 10-plex plates and then overlaid with 15 μL of Applied Biosystems® QuantiStudio™ Absolute Q™ Isolation Buffer per well. Following the addition of strip gaskets, the plate was transferred onto the system. A simple experiment workflow for dPCR-based qPCR is illustrated in Figure 1.

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume for 1 reaction (μL)</th>
</tr>
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<tbody>
<tr>
<td>Absolute Q DNA Digital PCR Master Mix (MIX)</td>
<td>1.5</td>
</tr>
<tr>
<td>Absolute Q AAV/ITR-2 assay (STA)</td>
<td>0.5</td>
</tr>
<tr>
<td>DNA sample</td>
<td>1.5</td>
</tr>
<tr>
<td>Water</td>
<td>4.05</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
</tr>
</tbody>
</table>

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

Results

Figure 2. ID dot plots displaying dPCR-based quantification of AAV from serially diluted samples. Using the QuantiStudio 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 QuantiStudio Absolute Q Digital PCR System and droplet-based dPCR. (A) Calculated AAV concentration using the QuantiStudio Absolute Q Digital PCR System and droplet-based dPCR using equation 1. Quantification is in pg/g across the dilutions. (B) Correlation between the values of quantification across dPCR and the droplet-based dPCR, using 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 line) is shown.

Conclusions

- The easy workflow and rapid turnaround time on the QuantiStudio Absolute Q digital PCR System are well suited to fulfilling the needs to rapidly and accurately quantify AAV for cell and gene therapy research.

- Microfluidic array plate (MAP) technology facilitates robust reject dilution with utilization of <15% ± 0.03% 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 QuantiStudio Absolute Q Digital PCR System could be valuable in viral vector production for biopharma and biomedical research.

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