Characterization of AAV Genomic Titer on the Applied Biosystems™ QuantStudio™ Absolute Q™ dPCR System

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Abstract

Purpose: Investigate the use of the QuantStudio Absolute Q digital PCR (dPCR) system for determining accurate recombinant adeno-associated virus (rAAV) genome titer.

Methods: Digital PCR (dPCR) using TaqMan chemistry and commercially available AAV particles treated with heat-lysis.

Results: Consistent quantitation of genomic copies observed over several experiments without the need for a standard curve and optimized using buffer containing surfactant and HEK293 cell lysate.

Introduction

mAbs are widely used for gene and cell therapies due to its low immunogenicity, vast tropism and both efficient and persistent gene transfer to treat human diseases. However, optimal concentrations of viral vectors are necessary for clinically effective dosages. Accurate, precise, and consistent viral titer measurement is required throughout routine quality control processes for rAAV vector preparations.

Quantitation of genomic copies (GC/mL) is important for characterizing rAAV. While quantitative PCR (qPCR) is considered the gold standard method for determining the genomic titer, this approach requires the use of standard curves and is thus more susceptible to variation from run to run. dPCR provides significant advantages for analytical assays necessary for viral vector production and characterization without the need for a standard curve. dPCR is also less sensitive to contaminants that affect amplification, including those present in solutions used during the development of AAV-mediated gene therapies.

In this work, we utilize the QuantStudio Absolute Q dPCR system to investigate the platform’s use for determining accurate AAV genome titer. Using commercially available purified AAV particles, we found consistent quantitation of genomic copies across several AAV serotypes when multiplexing assays that targeted different regions of the AAV genome. Results were obtained without the need for a standard curve, nor reference standard material. We also observed that while qPCR reactions are highly affected by the presence of HEK293 cell lysate, dPCR reactions are more tolerant to the presence of cell lysate and even less affected when cell lysate is diluted in TE buffer.

Workflow

Figure 1. Process map for the treatment of AAV samples.

Samples are diluted in buffer and heated to 95°C for 5 minutes, allowing open viral capsids to release DNA. qPCR reaction loading onto the QuantStudio Absolute Q dPCR platform is then performed for copy/uL results.

Results

AAV serotype titer

Multiplex reactions of custom dPCR assays with ITR2 viral titer assay were tested against several types of AAV serotypes to determine compatibility on the Absolute Q dPCR system. Serotypes were purchased as part of a kit containing different serotypes with the same genome (AMST04 catalog # C709002). Notably, SV40, CMV, and GFP were not detected in AAV8 samples.

Figure 2. Dilution buffer and AAV particle treatment

AAV particles treated with heat-lysis were diluted in TE buffer, Low TE 0.05% Tween-80 0.01% Pluronic-F68, or 1:50 diluted in TE buffer. Slight changes in copy/uL results were observed in samples containing lysate using dPCR. Slight changes in copy/uL results were observed in samples containing lysate using dPCR.

Figure 3. Multiplex dPCR reaction testing AAV2 particles

The average of three independent experiments on AAV2 particles showed CV of 1.8%.

Figure 4. Genomic titer of different AAV serotypes across three independent runs using TaqMan PCR

Table 1: Reproducibility of AAV serotype titer

<table>
<thead>
<tr>
<th>Sample</th>
<th>SV40</th>
<th>CMV</th>
<th>ITR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat Lysate</td>
<td>1.5 e+11</td>
<td>2 e+11</td>
<td>5 e+10</td>
</tr>
<tr>
<td>Lysate 1:10</td>
<td>6.5 e+11</td>
<td>1.1 e+11</td>
<td>4 e+10</td>
</tr>
<tr>
<td>Lysate 1:50</td>
<td>3.2 e+11</td>
<td>6.6 e+10</td>
<td>1 e+10</td>
</tr>
</tbody>
</table>

Figure 5. Cell lysate affects qPCR reaction

qPCR titer from Vigene (catalog #: RS-AAV2-FL) was reported on the certificate of analysis (1.82E11 GC/mL). The presence of surfactant may assist in viral particle lysis and prevent viral particles from clumping together and attaching to plastic walls.

Conclusions

In summary, we showcase several advantageous features of the QuantStudio Absolute Q™ digital PCR system for biopharma and gene therapy research:

1) simple and fast workflow
2) consistent quantitation of AAV genomic titer without the need for standard curves
3) higher resistance to PCR inhibition due to host cell lysis

References


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