Engineering of 5’ UTR to Control the Expression and Incorporation Level of VP1 During rAAV Vector Production Using a Baculovirus System

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Introduction

• Recombinant Adeno-Associated Virus (rAAV) is a growing vector of choice for gene therapy and clinical trials are ongoing for multiple diseases. Until a baculovirus/Sf9 cells expression system was developed for AAV, a lack of efficient and cost-effective methods for production was a bottleneck. The baculovirus/Sf9 system is fully scalable, enabling the production of AAV from 25ml flask up to 10,000l, with excellent reproducibility and quality. Nevertheless, published production reports of AAV using the baculovirus/Sf9 system have described reduced level of VP incorporation into the AAV capsids for several tested serotypes. Additionally, when compared to HEK293 cell production, baculovirus/Sf9 vector production appears to result in lower cellular transduction. To overcome this problem, increase stability and homogeneity of baculovirus/Sf9 produced AAV vector, we altered the ACG stating codon to ATG for translational modulation of VP1 expression. This modification leads to lower cellular transduction. To overcome this, we introduced a new modular to modulate the VP1:VP3 ratio for multiple serotypes through 5’ UTR engineering.

• Improving the VP expression in baculovirus/Sf9 has been achieved using the 5’UTR for AAV2 constructs, but preliminary results demonstrate the feasibility to modulate the VP1:VP3 ratio for multiple serotypes through 5’ UTR engineering.

Summary

• Most starting sequences for VP1 expression in baculovirus systems are non-canonical codons such as ACG, UAG, GUG or CUG. Unfortunately, because most eukaryotic cells tend to block expression at a non-AUG starting codon, it is difficult to understand the mechanism for expression of VP1 and a universal system for different serotypes.

• We engineered the 5’ UTR to modulate the expression of VP1, VP2 and VP3.

• The modified 5’UTR allows for modulation of VP1/VP3 expression ratios within a range between 1 to 34-fold. Additionally, these new structures can also modulate other proteins in the same open reading frame.

• A precise tuning of VP1 expression can be achieved through modulation of hairpin size, hairpin GC content, hairpin position and loop size.

• Data below was obtained with AA2 constructs, but preliminary results demonstrate the feasibility to modulate the VP1:VP3 ratio for multiple serotypes through 5’ UTR engineering.

Table 1. Examples of 5’ UTR design

<table>
<thead>
<tr>
<th>Sample</th>
<th>5’UTR Design</th>
<th>VP1</th>
<th>VP2</th>
<th>VP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP6-3dpi</td>
<td>ACG start codon</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>HP6-4dpi</td>
<td>ACG start codon</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>HP6-5dpi</td>
<td>ATG start codon</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Examples of 5’UTR: We investigated the effect of loop size and position of the hairpin at different distances from the first ATG start codon for VP1.

1. In 3’ of ATG, the loops contain the ATG and we modified the size and composition of the hairpin.
2. In 10bp of ATG, the loops contain the ATG and we modified the size and composition of the hairpin.
3. In 3bp from ATG, the loops contain the ATG and we modified the size and composition of the hairpin.

Sample 14: the hairpin size is 15bp from the ATG.
Sample 15 and 16: hairpins are at 7bp from the ATG with a mismatch in the hairpin for the sample 16.

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Figure 4. Examples of 5’UTR tested

Figure 5. Purification using A260 column chromatography

To investigate the composition of the capids from our different constructs, we used microfluidic capillary electrophoresis for analysis. A high throughput system allows to run 6 samples at once. On Figure 6, we analyzed four capids for different concentrations to see the banding and separation of the library. In this experiment, we found a library of 24 different in vitro transcripts for VP1 but a level of deviation around 40-50% to detect VP1 and VP2 (data not shown).

Figure 6. High-throughput method for capid protein analysis

Figure 7. Electropherogram from microfluidic

Figure 8. Protein VP ratio: Labchip

Using the Labchip instrument, we compared the ratio of the capids produced with 2 different constructs using an AAV2 start codon (VLP1/P002 and VLP1/P002) as our reference construct (VLP1/P002).

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