Qualification of 4-plex assay for AAV vector genome integrity evaluation and its use to support process development and optimization

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The production of viral vectors for gene therapy is focused on pure, safe, and efficacious products. Absence of impurities and presence of full vector genomes play a crucial role. We have addressed the problem of AAV vector genome integrity by developing an advanced dPCR multiplex approach. The newly developed 4-plex assay was qualified on DNA level and tested on several different AAV vectors to show its applicability. The assay is an upgrade from simplex vector genome titering assay by providing accurate quantitative result on genome integrity. The use of such assay can better guide process development with a goal of having as much full vector genomes as possible.

Introduction

Current methods for viral vector titer quantification by simplex assays are targeting a small region of the whole genome and are thus unable to provide information on vector integrity. The titer is usually an overestimated value of actual number of full-length genomes. As there are no simple and easy-to-use tools available to help to increase the amount of capsids with full length genomes in the process development, the final product usually contains a mixed population of capsids with different genome fragments (Figure 1).

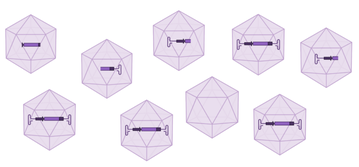

Figure 1: Shematic expample of different encapsidated viral vector genome fregments

Methods

To address these issues we have developed a 4-plex assay mix (Figure 2) named NibaPlex™, intended for quantification of viral vector integrity and for evaluating the presence of different fragment populations.

For each of the targets more assays were designed and tested in different combinations to select the best performing combination. Multiplex combinations were evaluated against simplex assays as well.

Figure 2: Positions of NibaPlex™ assays

4-plex assay qualification

Plasmid with AAV vector genome sequence containing target regions of interest was used for qualification. We prepared 11 different samples with different percentage of non-fragmented vector genome (restricted from plasmid).

Excellent linearity was observed for the developed 4-plex assay with R2 of 0.9997 over the whole range (Figure 3).

Limit of quantification was determined to be between 10 and 20%.

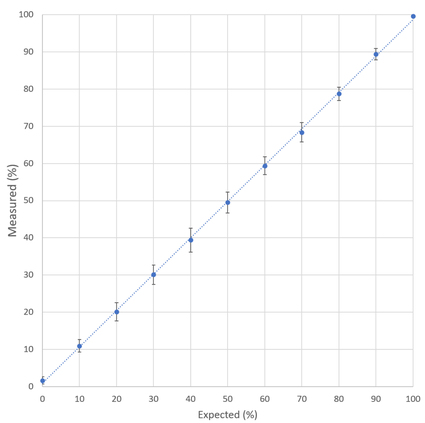

Figure 3: Linearity of the NibaPlex™ assay over the whole tested range.

Acknowledgement

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Key benefits of multiplex approach

Process optimization that leads to higher yield of full-length viral genomes in the capsids

Better characterized product

Lower production costs per dose

Improved process management & control

Improved safety attributes for the patient

Conclusions

- The titer of full-length vector genomes can be greatly overestimated by simplex assay, thus the use of multiplex assay for vector genome integrity quantification is needed.
- NibaPlex™ 4-plex assay gives better insight in the percentage of the full-length genomes and other fragment populations.
- The developed assay can be used to control different stages of process development, resulting in final drug product with lowest number of impurities in terms of genome fragments, thus providing more potent and safe drug to the patients.

Testing 4-plex assay on AAV samples

Eight different AAV samples were tested with developed 4-plex assay (NibaPlex™). Percentage of fragments for each AAV sample as quantified by NibaPlex ™ assay is presented on Figure 4.

Specificity of the developed NibaPlex™ assay was demonstrated on samples without the target regions (one example without two target regions is presented as Sample 1 on Figure 4).

We have shown the presence of different fragment populations in tested samples we have quantified their exact number and calculated their percentage of the total number of detected fragments. Surprisingly, the full-length vector genomes were present at relatively low percentages (ranging from 15% to 43%).

NibaPlex™ assay was also used to evaluate vector genome integrity during upstream process optimization (Figure 5).

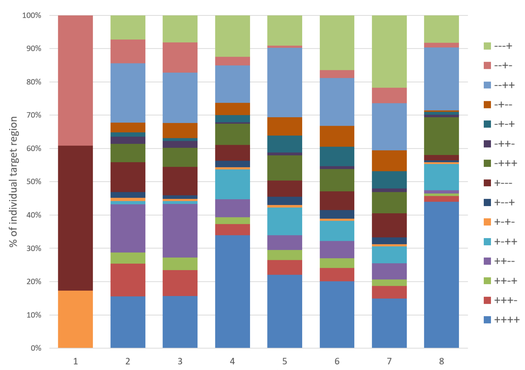

Figure 4: Results showing different fragment populations in tested AAV samples. Sample 1 served as specificity example as it harboured only two targets.

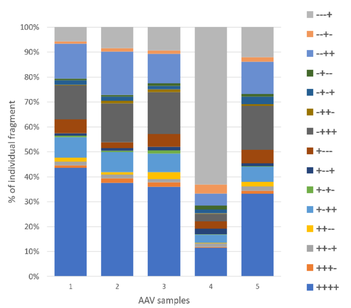

Figure 5: The influence of different up-stream conditions on vector genome integrity results.

We have shown that the titer obtained by individual assays overestimates the value of full-length genomes (Table 1).

Table 1: Example of vector genome titer obtained by using information from individual targets vs. the 4-plex result for the full-length genome

Target region	Average titer (vg/mL)
1	4.26E+12
2	4.40E+12
3	4.41E+12
4	3.98E+12
Full-length (1-2-3-4)	1.22E+12