Quantitative evaluation of vector genome integrity by multiplex digital PCR assay can facilitate process development

David Dobnik[1,2,*], Nejc Jakoš[2], Nejc Košir[2], Rebecca Vollmeier Kovačić[2]

[1] National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia; [2] Niba Labs d.o.o., Ljubljana, Slovenia; *david.dobnik@nib.si, david.dobnik@niba-labs.com,

Scan the QR code below to download the poster as pdf file:

In the production of viral vectors for gene therapy, the emphasis is on pure, safe, and effective products. The absence of impurities and the presence of a complete vector genome play a crucial role. We have previously developed and qualified a 4-plex digital PCR assay to address the problem of AAV vector genome integrity. We have tested the assay on several different AAV vectors to demonstrate its applicability in process development. We have shown that different conditions in upstream processes can lead to up to 4x differences in the amount of full-length vector genome. The assay is an improvement over the simplex vector genome titer assay, as it provides accurate quantitative results on genome integrity. The use of such an assay can better guide process development with the goal of obtaining as many full-length 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 (Table 1). Whichever AAV production process is being used, the final product usually contains a mixed population of capsids with different genome fragments. The full, partial and empty capsid particles can be more or less efficiently identified by different techniques, however, they do not provide a quantitative value for the population of the encapsidated fragments (Figure 1).

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

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

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

Figure 2: Positions of NibaPlex™ assays. More targets will be added in the future to enable a diverse Mix & Match approach.

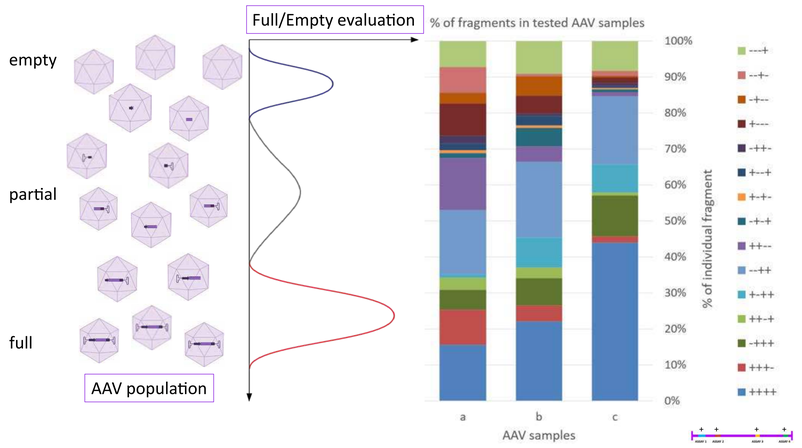

Figure 1: Current approaches for evaluation of full/empty ratio can determine a ratio between different populations, but not their actual content. NibaPlex™ assays enable absolute quantification of different fragment populations, providing an accurate quantitative results on vector genome integrity.

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 and control

Improved safety attributes for the patient

Testing of NibaPlex™ assay on AAV samples

Several different AAV samples were tested by NibaPlex™ assay. An example of results in terms of percentage of fragments for different AAV sample in the process of transduction optimization as quantified by NibaPlex™ assay is presented on Figure 3. We have shown the presence of different fragment populations in the tested samples, 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, but up to 4-fold difference between the sample in terms of full-length genomes indicates that our assays can guide process development towards higher yield of full-length vector genomes.

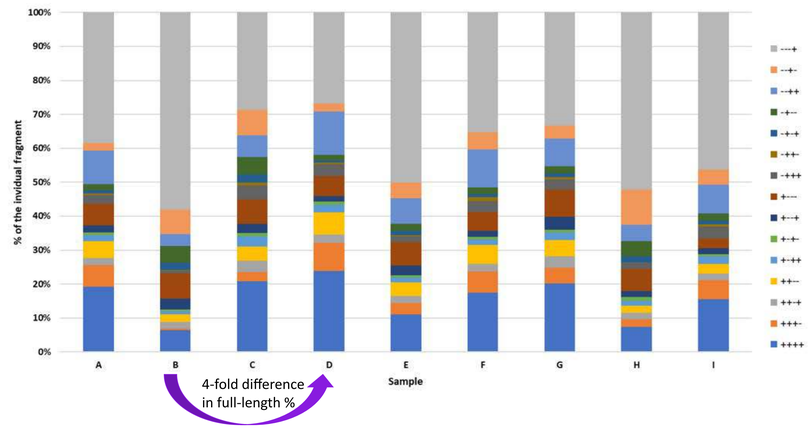

Figure 3: Results showing different fragment populations in AAV harvest samples from transduction optimization experiment. The maximum difference in terms of full-length genomes, was between sample B and D (4-fold difference). The grey area represents encapsidated polyA fragment – these capsids might be considered as empty or light and could possibly be removed during downstream purification.

Conclusions

1. NibaPlex™ 4-plex assays provide accurate absolute titer of full-length genomes and other fragments and offer a better insight in the populations of the full-length genomes and other fragments. The result is a much better characterized product.
2. The NibaPlex™ assays 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.