Impact of the Genome Length of Adeno-Associated Viral Vectors on Yield and Quality Parameters

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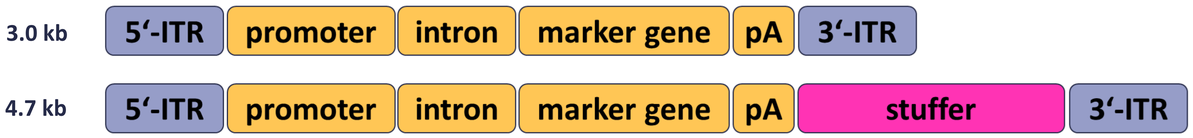
Abstract 1439

Background

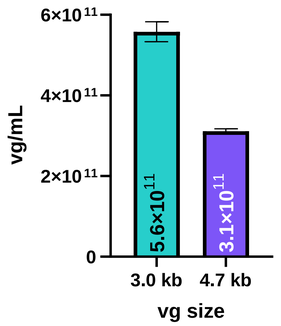
Wild-type adeno-associated virus (AAV) contains a single-stranded DNA genome with a length of approximately 4.7 kb. Yet even packaging of close-to wildtype sized transgene cassettes can lead to lower yields compared to smaller transgene cassettes. Production of rAAV with vector genomes in the range of the wild-type genome length is therefore challenging concerning vector yields and quality parameters.
We have developed a robust modular suspension platform process based on our proprietary HEK293 cell line and split two plasmid system, that is optimized towards yield at best possible quality with full scalability. The platform has already provided successful manufacturing of several distinct capsids. We continue to expand our data to underline the platform's universal application across both natural and engineered capsids.
We present here the impact of a wild-type genome length cassette (4.7 kb) in comparison to a medium-size vector genome (approx. 3.0 kb) in context of AAV9 produced using our suspension platform process. We analyzed yield and quality parameters that are known to be strongly impacted by the design and choice of the biological starting materials and the upstream process. A panel of analytical methods was applied to enable a comprehensive and detailed comparison of many different attributes.

Comparison of two rAAV vector genome sizes using our suspension platform process



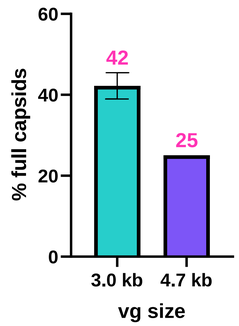
Large vector sizes for AAV are inevitable, such as FVIII vectors for hemophilia. For a large number of applications, 3 kb vectors are sufficient. Therefore, vector genomes of approx. 3.0 and 4.7 kb were used in this study. Sequences and cassette structure were the same with exception of a stuffer sequence included at the 3`-end of the vector genome.
rAAV9 production was performed using our proprietary HEK293 suspension cell line and split two plasmid system. A high throughput, automated bioreactor system (Ambr® 15) was applied to enable parallel analysis of multiple setups.

High, albeit somewhat reduced, yield and %full vector particles for 4.7 versus 3 kb vectors



Vector genome (vg) yields of total harvests were determined by transgene-specific ddPCR.

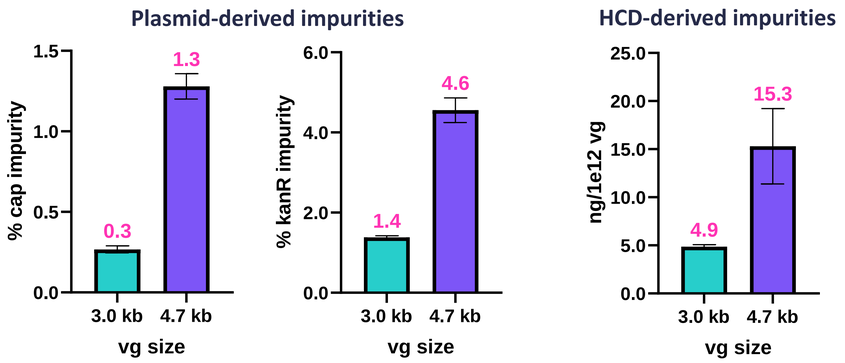
About 55% of yields of a 3kb vector are obtained for a full-length vector reflecting the relative sizes of both vectors.



Percentage of full capsids was determined based on vg yields (ddPCR) and capsid (cap, immunoassay) yields.

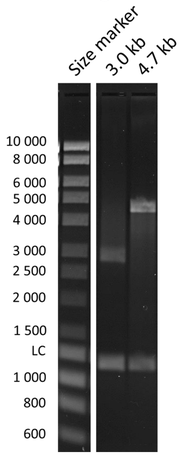
Decrease of % full particles in line with vg yield reduction and explained by comparable level of capsid expression at reduced vg packaging most likely due to slowed kinetics of larger vector genome replication.

Residual DNA packaging is low but elevated for full-length vectors



Mispacked plasmid-derived DNA or host cell DNA (HCD) was detected by ddPCR specific for regions in the cap, kanamycin resistance (kanR) or 18S ribosomal RNA gene in one-step affinity purified samples. 3- to 4-fold increase in plasmid- and HCD-derived impurities for full-length vector genome were observed.

Alkaline gel electrophoresis indicates packaging of intact vector genomes independent of vector length



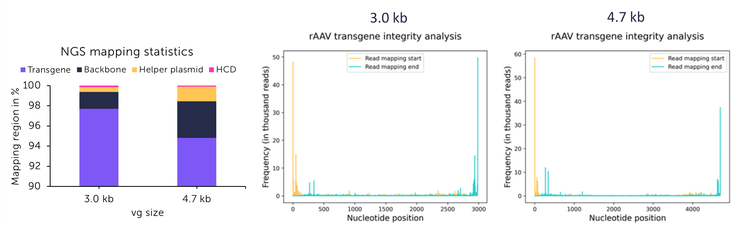
LC = loading control

Transgene cassette integrity was assessed by alkaline gel electrophoresis.

Bands at the expected sizes for both vector genomes were obtained.

Some smear is detectable especially for the large vector indicating some level of vector fragmentation. However, bands indicating full-length vector packaging are predominant.

Orthogonal, sequence-agnostic NGS confirmed very low levels of DNA mispackaging



Most NGS reads (> 94 %) were mapped against the transgene cassette for both constructs, indicating very high quality of the AAV vectors. Reads mapping against the vector plasmid backbone and helper plasmid were increased for the 4.7 kb construct .

NGS based transgene integrity analysis revealed high transgene integrity for both constructs. A slightly reduced integrity of the 4.7 kb construct is marked by the reduced mapping end frequency at the full-length vector payload size.

Summary

For the 4.7 kb construct, high vg and full/empty ratios were achieved using our proprietary AAV manufacturing platform and the AMBR® 15 bioreactor system. However, compared to a 3 kb vector a slight reduction of these parameters, reflecting the different vector sizes, was observed. Increased levels of mispackaged plasmid- and HCD-derived sequences were detected for the 4.7 vs the 3 kb vector genome using the same process parameters for both setups, with absolute levels still in a range observed for other platforms.
Integrity of both vector genomes was demonstrated by alkaline gel electrophoresis. Our results demonstrate the strength of our proprietary split 2-plasmid system and suspension platform process for challenging vector genome lengths at the rAAV packaging limit. We continuously invest in further CMC innovation to further improve yield and quality for all relevant genome lengths across capsids.
Please refer to Poster #1498 for details concerning our versatile suspension-based platform for AAV manufacturing.

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