Manufacturing of a Broad Range of AAV Capsids Using our Suspension Platform Process

E SCHWEIGERT, R DERLER, M OHME, S HENZE, K BREUNIG, M HAUBNER, F DUNKER, M HOERER, A SCHULZE, F SONNTAG

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Abstract

A broad range of naturally occurring and synthetically engineered adeno-associated virus (AAV) capsid sequences have been reported until today and are applied in AAV gene therapy programs to target specific tissues and organs with high efficiency. There is an increasing demand to manufacture high quality AAV vectors based on various AAV capsids in large quantities for pre-clinical and clinical trials as well as approved therapies. Yield and quality parameters can differ from one AAV capsid to another, impacting the manufacturing processes and requiring modularity of a manufacturing platform to be able to address product dependencies.
Based on our proprietary HEK293 cell line and split two plasmid system, we have developed a robust suspension platform process, that is optimized towards yield at best possible quality with full scalability.

We present here the comparison of a range of AAV capsids (amongst others AAV1, AAV2, AAV3B, AAV8 and 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.
Manufacturability of the AAV capsids was demonstrated in the Ambr® 15 platform which has been shown to be predictive for the larger production scales of our manufacturing offering (currently up to 200 L).

Analytical methods

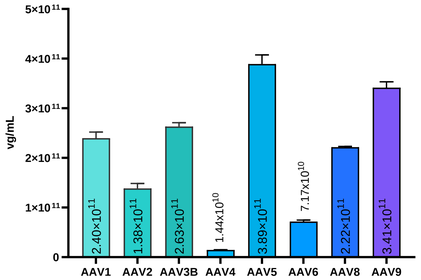
Vector genome (vg) yields were determined by transgene specific digital droplet polymerase chain reaction (ddPCR).

AAV capsid (cap) yields were quantified by automated immunoassay.

Mispacked plasmid derived DNA or host cell DNA (HCD) were quantified by ddPCR with primers binding to regions in the cap, kanamycin resistance (kanR) or 18S ribosomal RNA sequence in one-step affinity purified samples.

Total packaged DNA in one-step affinity purified samples was analyzed by sequence agnostic nanopore-based next generation sequencing (NGS).

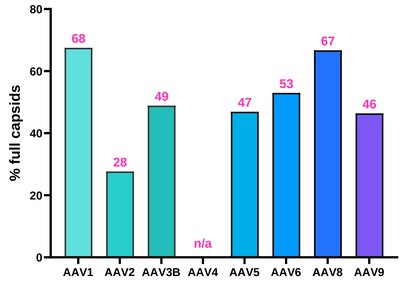
Our production platform results in comparable high yields for most capsids



Depicted are vector genome yields of the total harvest:

- Less than 3-fold yield differences for all capsids analyzed except AAV4 and 6
- Work to boost AAV6 productivities are ongoing

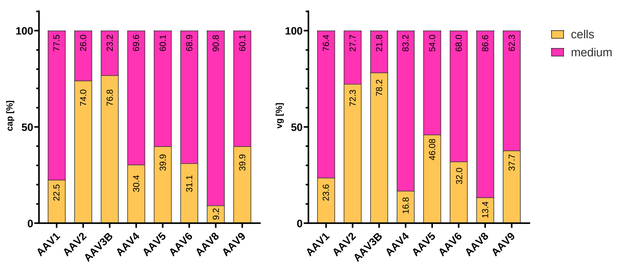
Percentage full particles in comparable range for most capsids



Percentage full particles were calculated based on vg and cap yields of total harvest:

- High % full particles up to 70% for all serotypes without additional enrichment steps
- Percentage for AAV4 inconclusive due to low overall yields (not depicted)

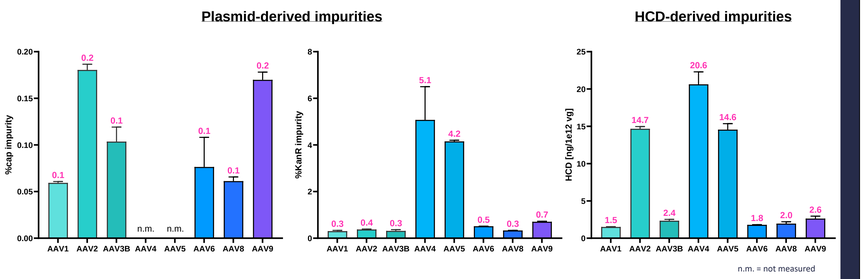
Capsid type-dependent release of AAV particles into culture medium



AAV particle distribution was evaluated on the level of vg and cap yields in “cell” and “medium” fraction following cell lysis and nuclease treatment:

- AAV2 and AAV3B particles are primarily located in the cell fraction
- AAV1 and AAV8 particles are primarily located in the culture medium

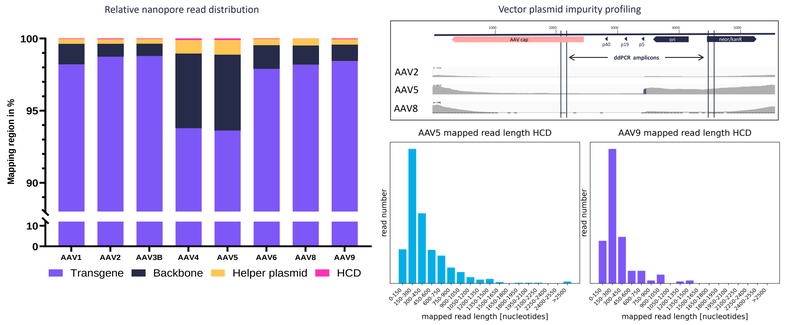
Very low DNA mispacking for most capsid types



Impurity levels are indicated relative to the vg yields:

- Capsid type-specific differences in plasmid and host cell DNA derived impurities
- Very low levels of DNA mispacking for all except AAV2 (only HCD), AAV4 and AAV5 reducing the risk of immunogenicity (Brimble *et al.*, Hum Gene Ther. 2024)
- Residual cap gene assays for AAV4 and AAV5 are in development

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



- Vast majority of reads are vector cassette derived
- Confirmation of observed impurity levels
- Comparable HCD read lengths and coverage independent of capsid type and absolute levels of mispackaged DNA
- Mainly short and thus HCD reads encoding incomplete genes

Summary

Overall, very similar high vector genome yields within a 3-fold range are obtained for the majority of AAV capsids. Serotype-dependent release of AAV particles from cells into cell culture media during production as described in literature was seen, opening the possibility to develop “perfusion/repeated harvest-based processes” to further increase yields. Despite the same vector genome cassette of approx. 3kb (typical length of therapeutic vectors) used for all productions, differences in the levels of plasmid- and host cell DNA-derived mispackaging were determined in a serotype-dependent manner.

These data generated in the AMBR® 15 bioreactor system are highly valuable as they inform on specific development opportunities and underpin the importance of a modular platform enabling quick reaction to special process-related challenges. Our results demonstrate the strength of our suspension platform rAAV production process with respect to high yield & quality vector manufacturing across a broad range of AAV capsids.
Please refer to Poster #1498 for details concerning our versatile suspension-based platform for AAV manufacturing.

Aim higher

Ascend Advanced Therapies GmbH | Fraunhoferstraße 9b | 82152 Planegg | Germany

www.ascend-adv.com
Markus.Hoerer@ascend-adv.com

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