Development and scalability of a robust suspension-based production process for AAV9

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A Schulze[1], R Derler[1], B Finkbeiner[1], A Youssef[1], M Langhauser[1], K Heller[1], F Sonntag[1], M Hoerer[1]

1: Ascend GCTx GmbH, Munich, Germany

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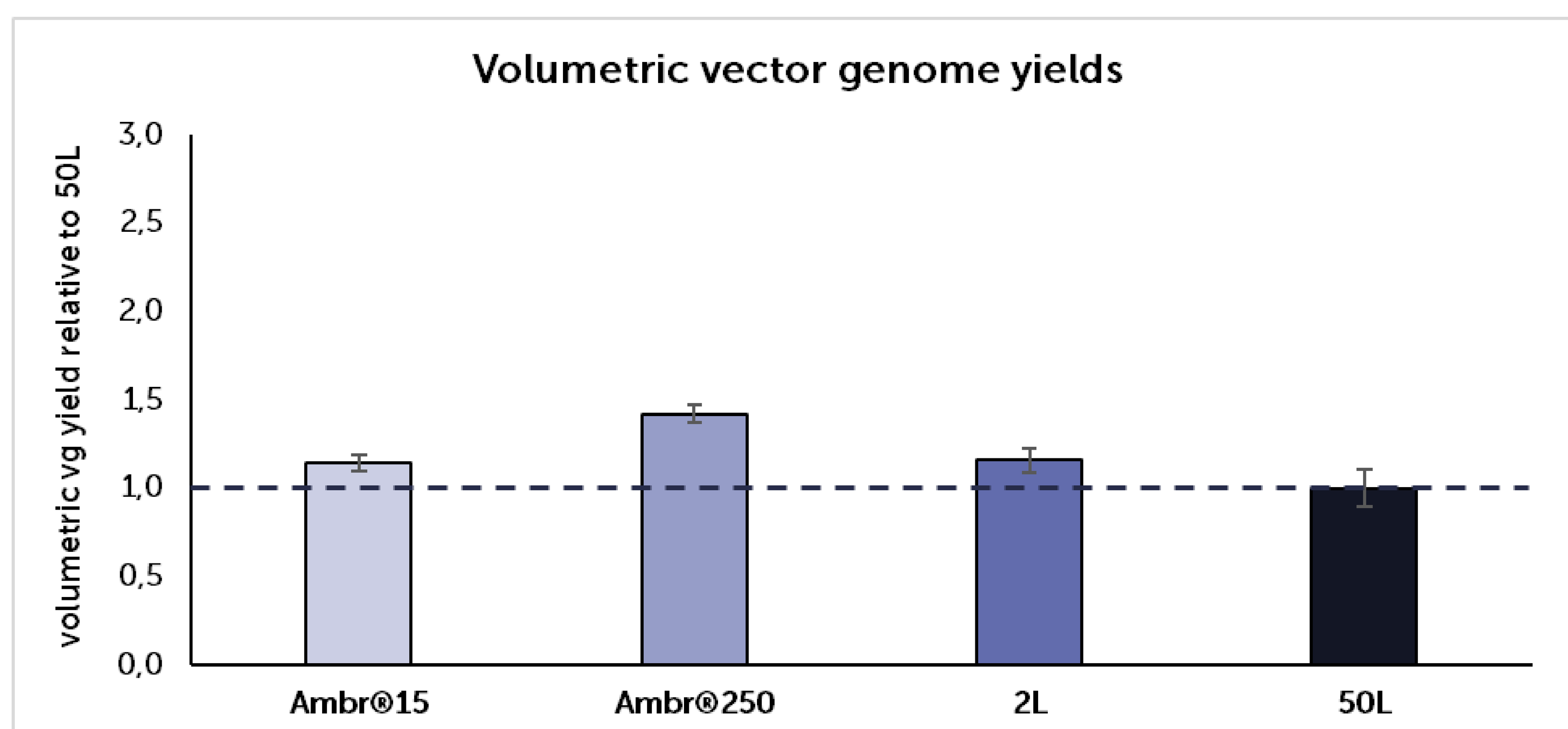
Abstract

Recombinant adeno-associated virus (rAAV) is the most widely used viral vector for in vivo gene therapy today. A broad range of different capsids have been used in clinical trials with AAV9, 2, 8 and 5 being the most prominent serotypes applied in that order. AAV manufacturing processes typically comprise platform modules which work universally across serotypes, whereas other steps require specific development and optimization for a given serotype or capsid variant such as molecular plasmid design, plasmid ratios, affinity and full/empty chromatography. We have developed a robust suspension platform process based on our HEK293 cell line, that is optimized towards yield at best possible quality with full scalability. The platform is proven for several capsid serotypes. We are continuously expanding our data to show its universal applicability.

We present here the development and scalability of a robust suspension-based production process for AAV9. Using our split 2-plasmid system we applied a screening strategy on the Ambr®15 bioreactor system to determine the best parameters for AAV9 production. The most promising conditions were then tested and validated in scaled-up bioreactors representative of large-scale production formats. A broad range of analytical methods were applied demonstrating comparable yields to rAAV control batches at best possible quality.

The data presented here demonstrate that our production process developed for an AAV3-like capsid has now successfully been proven for AAV9 with limited adjustments needed. This illustrates that our established suspension production process is a platform for manufacturing several AAV serotypes.

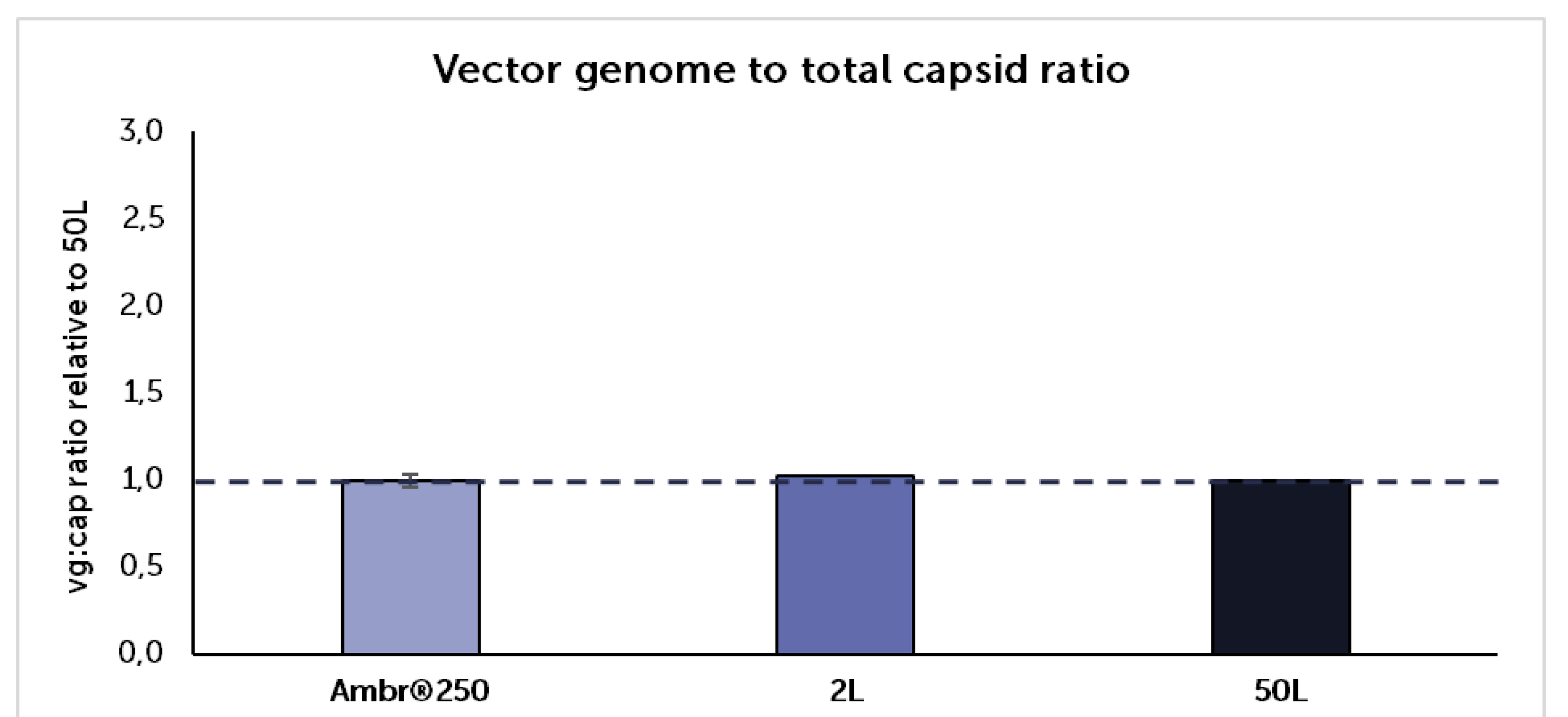
AAV9 yield is scalable across our robust suspension-based production process



Our HEK293 suspension cells were transfected with our split 2-plasmid system (capsid/transgene & rep/AdV) to produce rAAV9 vector in Ambr®15, Ambr®250, 2L Biostat B and 50L Biostat STR bioreactors. AAV vector genome yields were determined via qPCR and AAV capsid yields were determined via an AAV capsid-specific immunoassay.

Volumetric rAAV vector genome and capsid yields (data not shown) were consistent over all tested scales showing the robustness of our suspension-based production process for AAV9 quantity.

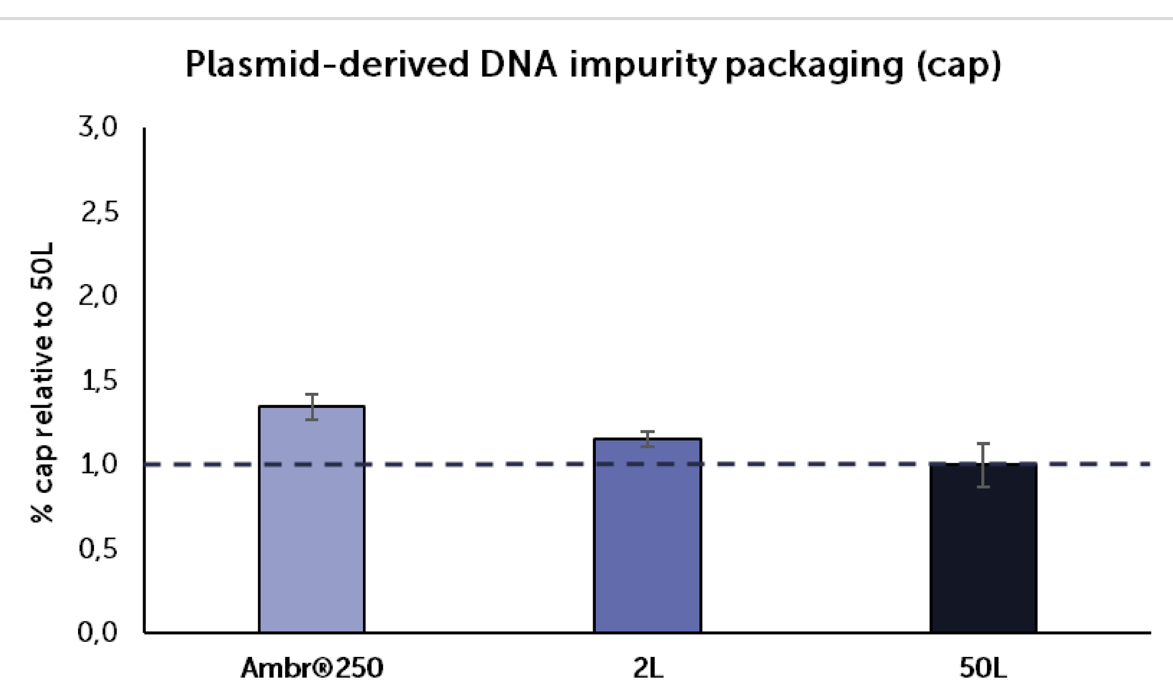
AAV9 vector genome to total capsid ratio is consistent across our robust suspension-based production process

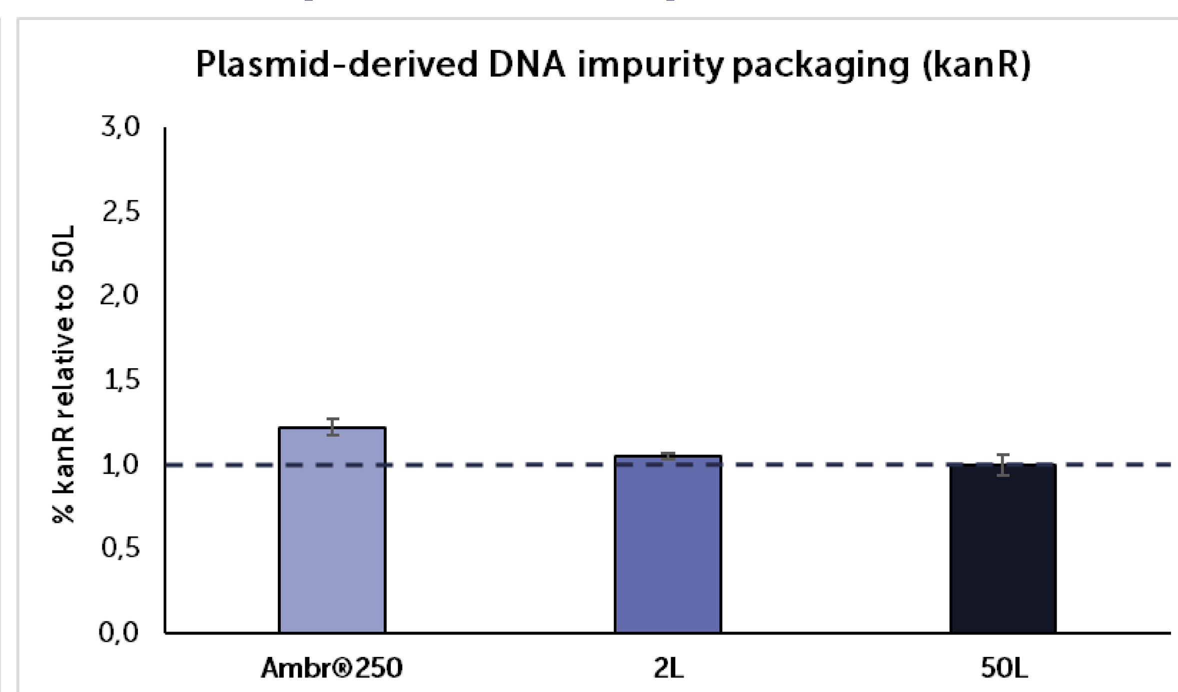


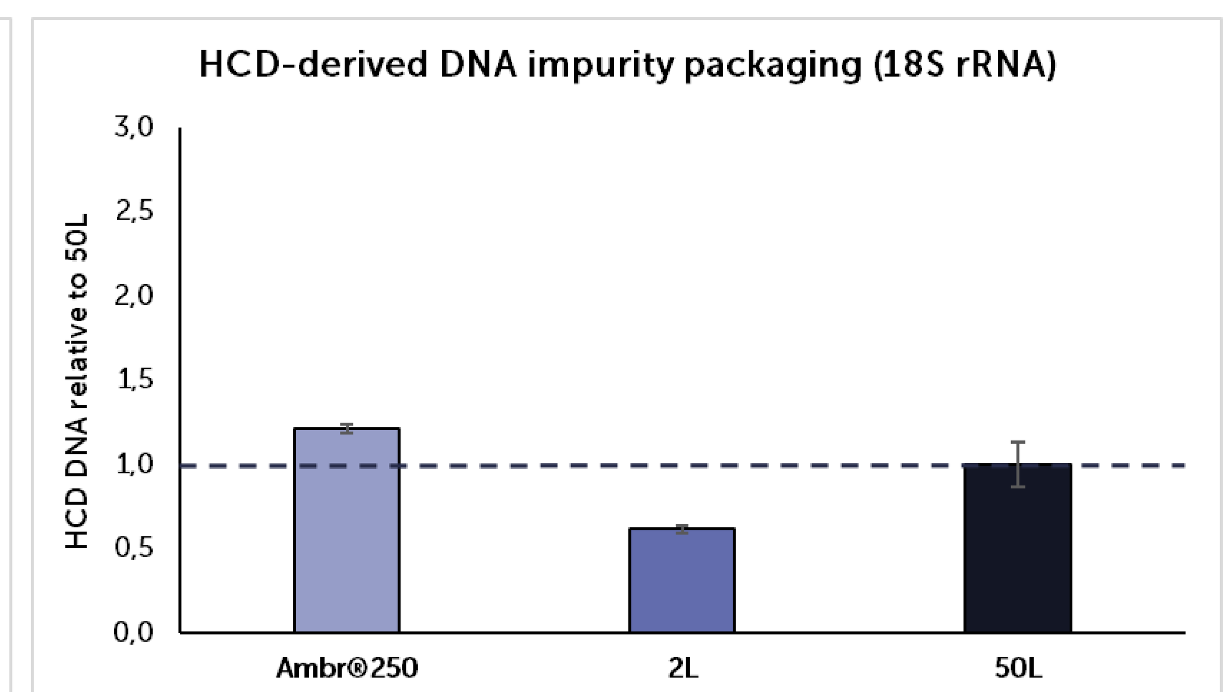
After demonstrating the scalability of our suspension-based production process for AAV9 regarding quantity, vector genome to total capsid ratios were analyzed for selected samples of larger production formats as one critical quality parameter.

The vector genome to total capsid ratios were consistent over all tested scales showing the robustness or our suspension-based production process for AAV9 quality.

AAV9 quality indicated via mispackaged DNA is consistent across all scales of our robust suspension-based production process







After demonstrating the consistent AAV quality of our suspension-based production process for AAV9 indicated via vector genome to total capsid ratios, selected samples of larger production formats were purified by affinity chromatography to allow for the analysis of other key quality parameters such as mispackaged DNA. Mispackaged plasmid DNA using AAV cap and kanR as marker sequences was determined by qPCR and mispackaged host cell derived DNA (HCD) was determined by ddPCR.

Mispackaged plasmid DNA indicated via AAV cap and kanR marker sequences was consistent over all tested scales. Levels of mispackaged host cell DNA indicated by 18S rRNA gene sequences as marker were close to the detection limit which explains some method-related variability of the data. Mis-packaged DNA data showed again the robustness of our suspension-based production process regarding AAV9 quality over scales. This study is at current extended to a 200L scale.

Summary

In this study we have demonstrated that our suspension-based production process that was initially developed for an AAV3-like capsid serotype also shows yield scalability at consistent quality over all scales for AAV9 vector manufacturing. This illustrates the broad applicability and robustness of our well-established suspension production platform for AAV manufacturing. For a demonstration of the robustness of our production platform across other AAV serotypes see poster P155.

Poster downloads

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Ascend | Fraunhoferstraße 9b | 82152 Planegg | Germany

www.ascend-gctx.com
bettina.finkbeiner@ascend-gctx.com