Whole Genome High-Throughput Screen Identified microRNAs Enhancing rAAV Production

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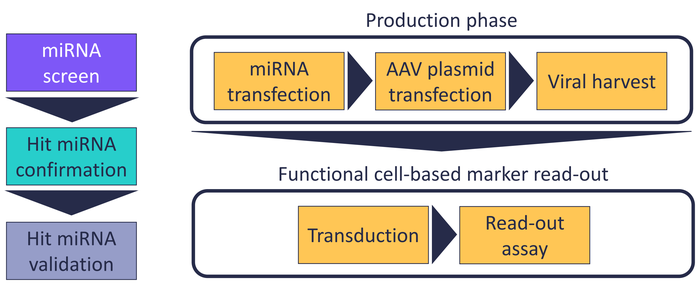
Abstract

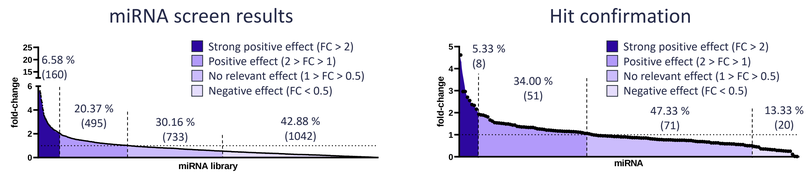
Recombinant adeno-associated viruses (rAAV) have become the leading vector for gene therapy in recent years. However, manufacturing bottlenecks pose a severe challenge to provide the required high quality rAAV amounts for increasing clinical and commercial demands. Scale-up of vector manufacturing and additional capacities are now commonly observed. However, further increase in cell-specific and volumetric rAAV productivity are still neglected strategies to drive rAAV yields and beyond that potentially rAAV quality. MicroRNAs are short non-coding RNAs playing a crucial role in the regulation of gene expression during virtually all cellular processes and were already successfully exploited to boost the manufacturing of classical biologics. Therefore, microRNAs are considered a useful tool to overcome critical bottlenecks in rAAV vector production that, if addressed, could drive rAAV production efficiency towards the required levels.
Based on the Ascend HEK293 cell line and split two plasmid system we have developed a robust modular suspension platform process for rAAV production, that is optimized towards yield at best possible and consistent quality from bench to 200 L scale.
For a systematic identification of host cell factors that significantly impact rAAV productivity of our HEK293 cells, we performed a high-throughput screen using a genome-wide library of human microRNA mimics.

miRNA screen & transient validation setup



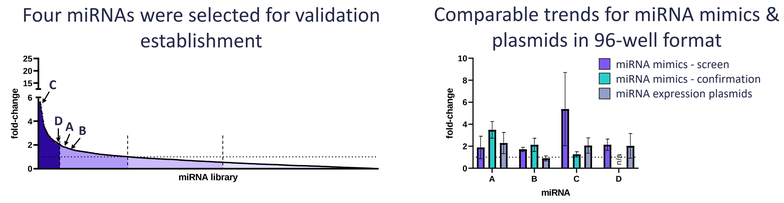
A high-throughput screen based on a genome-wide library of human miRNAs was performed to identify miRNAs that significantly impact rAAV productivity. Hits were confirmed in 96-well plate-based experiments. Top candidates were validated using a high-throughput bioreactor system (AMBR® 15) that is predictive for our large-scale manufacturing platform. A flow-chart of the experimental process used for the miRNA screen and the confirmation / validation studies is depicted.

miRNAs with positive effect on rAAV productivity were identified in the miRNA screen



The miRNA screen resulted in approx. 6.6 % miRNAs (160) with a strong positive effect on rAAV productivity (fold-change (FC) > 2).
Hit confirmation was performed with 150 of these miRNAs in 96-well plate format, resulting in approx. 5.3 % miRNAs (8) with confirmed strong positive effect on rAAV productivity (FC > 2) and approx. 34 % (51) with positive effect (2 > FC > 1).
Depicted are the fold-changes of functional marker read-out levels normalized to a non-targeting reference control.

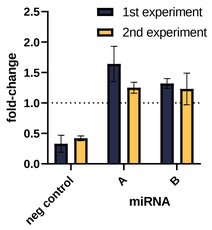
miRNA expression plasmids for hit validation in larger bioreactors result in comparable trends to miRNA mimics



For miRNA hit validation in larger bioreactors and possible use in the next generation manufacturing platform, four miRNA hits with increased productivity from the library were selected. miRNA mimics used for the initial screening and confirmation studies were compared with miRNA expression plasmids containing the respective sequences.
Comparable trends were obtained in 96-well plate-based experiments concerning functional marker read-out levels for miRNA mimics and expression plasmids, demonstrating applicability of the miRNA expression plasmids for further validation.

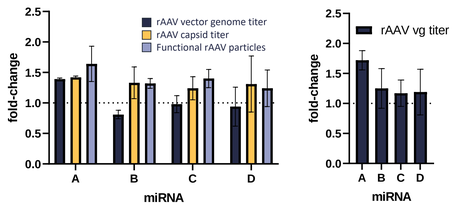
miRNA hits with positive effect on rAAV productivity were successfully validated using an automated bioreactor system

Productivity of our high yield platform is further increased by lead miRNA candidates



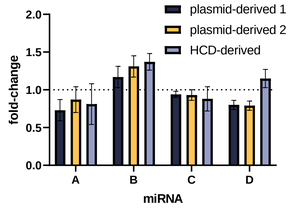
Two independent experiments with two miRNA hit candidates were performed. A siRNA inhibiting rAAV production was included as control. Depicted are the fold-changes normalized to a non-targeting reference control.

miRNA effects on rAAV productivity can be demonstrated using different read-out systems



Four miRNA hits were analysed for their effect on rAAV productivity. Vector genomes were quantified by promoter-specific qPCR. Capsid yields were determined by immunoassay. Generation of functional rAAV particles was determined by cell-based marker read-out assay. Depicted are the fold-changes normalized to a non-targeting reference control for non-purified (left) and purified (right) samples.

Only one miRNA hit candidate results in a moderate increase in DNA impurity levels



One-step affinity purified vectors from productions using the four selected miRNAs were analysed by qPCR for plasmid-derived impurities or ddPCR for host-cell DNA (HCD)-derived impurities. Two markers for plasmid derived impurities flanking the vector genome were used. Depicted are the fold-changes normalized to a non-targeting reference control.

Validation of miRNAs identified in the high-throughput screen and confirmed in 96-well format was performed using a high-throughput, automated bioreactor system (AMBR® 15). Positive effects of the miRNA hits on rAAV productivity were robustly demonstrated. The effects could be shown for the AAV yields as well as in cell-based function marker read-out assays. No detrimental effect on mispacking of plasmid- or host-cell DNA derived DNA was observed for the selected miRNA hit candidates.

Summary

160 microRNAs increased rAAV yields by more than 2-fold while over 1000 microRNAs decreased rAAV yields.
More significant positive effects on rAAV productivity are expected upon stable expression of the miRNAs as shown for CHO cell-based protein manufacturing.
miRNA hits will also be validated across several serotypes.
In summary, the top microRNAs identified from the genome-wide screen provide a very promising platform to further improve rAAV vector yields for Ascend's next generation AAV manufacturing platforms.

Aim higher

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