Small Molecule Screen Identifies Targets that Increase AAV9 Production in Suspension HEK293 Cells

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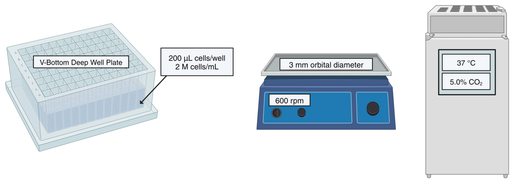
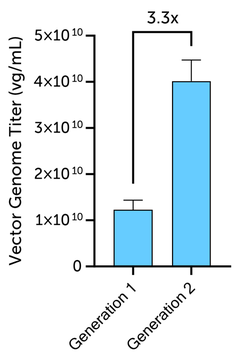
Abstract

The use of recombinant AAV as a vector for gene delivery is widespread, with over 900 pre-clinical and clinical programs underway. However, inefficient manufacturing methods result in high costs, limiting the availability of gene therapies. In this study we describe a high-throughput small molecule screening strategy to identify compounds that increase the capacity of cells to produce AAV9. Previously we had reported identification of a novel small molecule (SM-016) from a screen of 3,000 bioactive molecules that we validated in shake flasks and Ambr15 bioreactors. For our current study we wanted to further expand on the diversity of biological targets screened. In this study, we first developed a miniaturized suspension adapted high throughput screening strategy: ATLAS (Arrayed Targeted Library for AAV Screening). We performed optimization studies to show translatable, reproducible, and comparable AAV9 yields from 96 well to 125mL shake flask format. Next, we performed a screen using a curated compound library of over 700 small molecules. Targets identified include epigenetic modulators, DNA damage response, GPCR and transmembrane transporters, cell cycle modulators, anti-infection, and metabolic targets. Evaluation of top hits are currently underway.

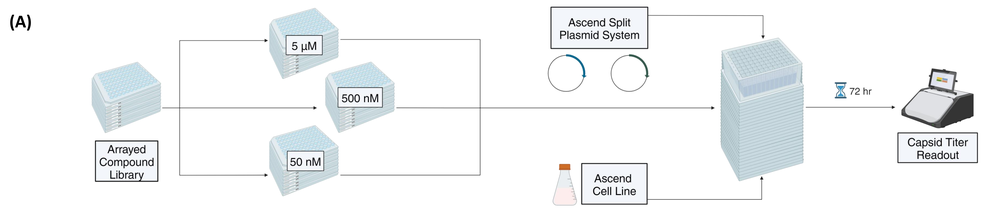
Development of a 96 deep well suspension platform for high throughput screening

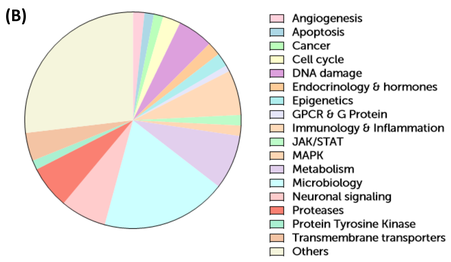




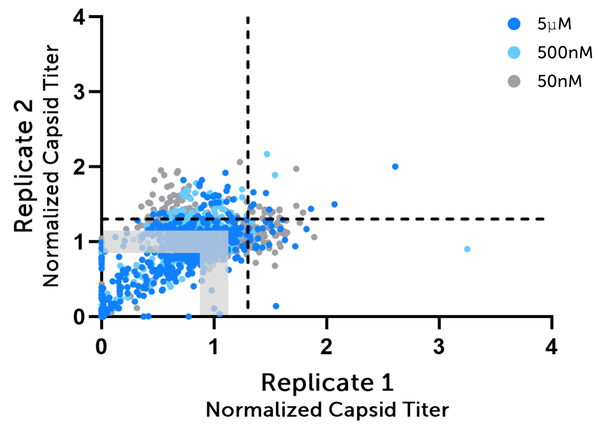
Optimized conditions in 96 well suspension format show 3.3-fold improvement in vector genome titer.
Optimized conditions were selected based on transfection efficiency and titer improvement from the generation 1 platform. Titers in the generation 2 platform are only about 10-fold lower than those obtained at the Ambr15 scale. The 96 well suspension format uses a V-bottom deep well plate containing 200 µL of cells per well at 2 M cells/mL. This plate is incubated for 72 hours on a 3 mm shaker at 600 rpm.

Novel small molecules that increase AAV9 production identified from anti-viral compound library

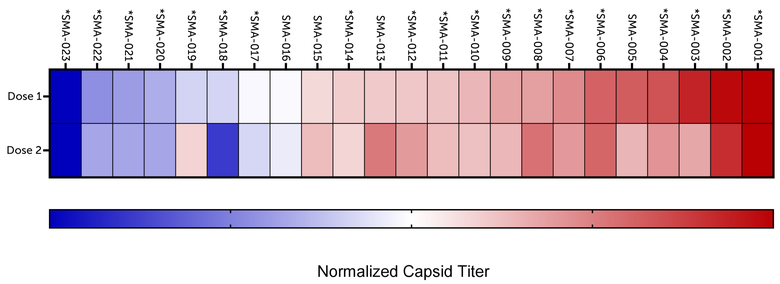




Primary screen of small molecule library identifies compounds resulting in increased AAV production.
(**A**, **B**) Arrayed small molecule library targeting distinct biological pathways transfected with Ascend's proprietary cell line and split plasmid system in the 96-well suspension platform. (**C**) Compounds of interest identified in primary screen via capsid titer improvement as an indicator of vector genome titer increase over DMSO only condition, shown as grey bar. Top 3.6% of compounds selected for confirmation studies.

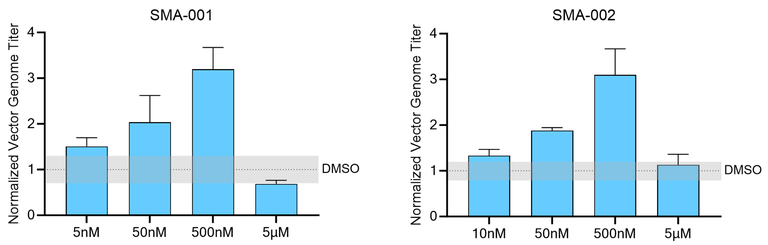


Confirmation studies demonstrated that inhibition of given biological pathway increases AAV9 titer



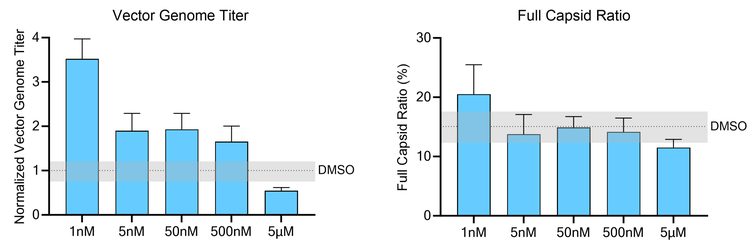
Confirmation of top hits from the primary screen hits identifies compounds targeting the same drug target.
Two doses selected for each compound based on initial screen. Several high performing molecules possess the same drug target, notated by an asterisk (*). SMA-001 and SMA-002 were identified for additional investigation.

Independent inhibition of two protein targets within the same biological pathway leads to a comparable, dose-dependent increase in AAV9 yield



Dose response of two molecules targeting the same pathway identifies optimal dose and validates inhibition of biological pathway.
Dosing of SMA-001 (Target 1i) or SMA-002 (Target2i) during AAV9 production led to a dependent increase in AAV9 yield up to 500nM.

SMA-024, a more potent analog of SMA-002, enhances AAV9 yield and the percentage of full particles at a 1 nM dose



The discovery of SMA-024, an analog to SMA-002, was facilitated by the screening of more potent molecules.
1nM dose of SMA-024 resulted in a 3.5-fold improvement in vector genome yield with an increased proportion of filled capsid.

Poster downloads

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