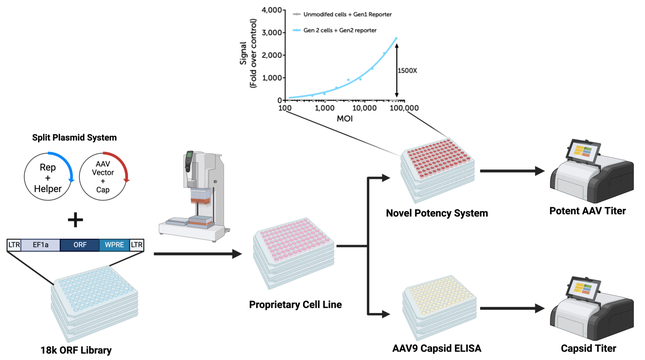
A Genome-Wide High-Throughput Gain-of-Function Screen Identifies Novel Targets for Improved AAV9 Production

Chad Wang[1], Muskan Sheth[1], Francesca Ispaso[1], Isha Ukani[1], Markus Hoerer[2], Mohammad A. Mandegar[1], Christopher A. Reid[1]

Abstract

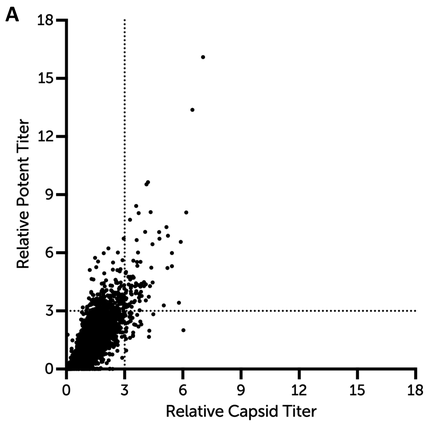
Recombinant AAV, a widely used vector for in vivo gene delivery, is being utilized in ongoing pre-clinical and clinical programs. Current manufacturing inefficiencies lead to high costs, poor yields and compromised quality in gene therapy products. The high costs restrict broader application, while quality issues affect the vector's potency, immunogenicity and overall safety, necessitating higher dosages and increasing the risk of severe adverse events following dosing. Our study utilized the ATLAS (Arrayed Targeted Library for AAV Screening) miniaturized screening platform to systematically over-express approximately 18,000 ORFs, aiming to enhance AAV9 production. Our screen identified transcription factors, epigenetic regulators, DNA replication factors, RNA regulators, protein ubiquitination, and metabolic targets as primary enhancers of AAV9 production. Confirmatory studies indicated a dose-dependent increase in AAV9 production, up to 3-fold from baseline. Moreover, we confirmed that these factors do not negatively impact the potency of AAV. Next, we confirmed these findings in other AAV serotypes including AAV2, 5, and 8. Further work is in progress to identify lead hits which next will be validated in our current manufacturing platform at Ambr15 scale towards yield, potency and key quality parameters focusing on product-related impurities.

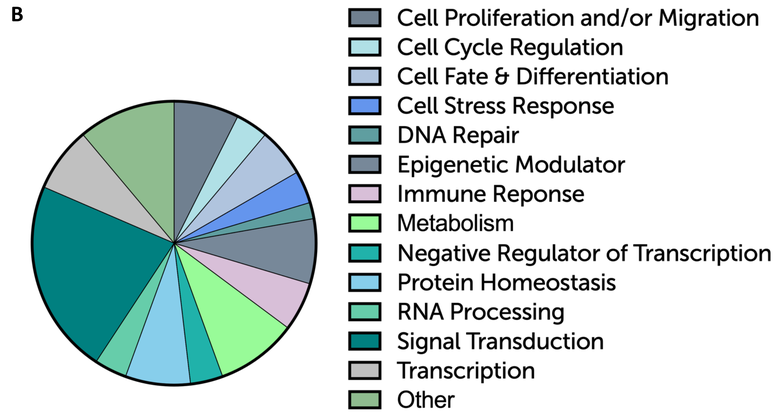
Employing the ATLAS screening platform to systematically over-express a large ORF library



The ATLAS platform was employed to screen a genome-wide library containing ~18,000 human ORFs, aiming to determine the effect of overexpression on AAV9 production. Vector production was performed utilizing Ascend's split plasmid system and proprietary suspension HEK293 cell line. AAV9 yields were measured using capsid ELISA and a novel and highly sensitive AAV9 transduction assay.

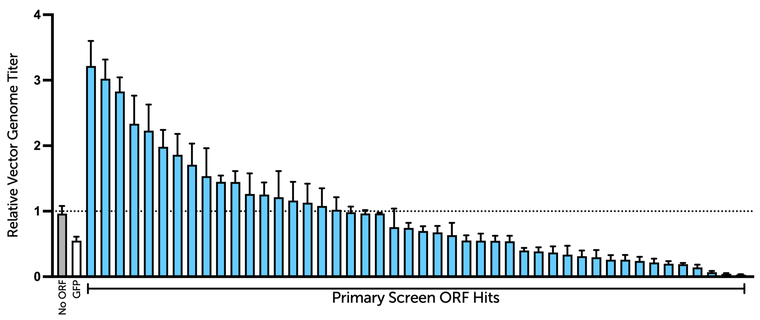
Primary screen of human ORFeome identifies numerous biological factors that improve AAV9 production





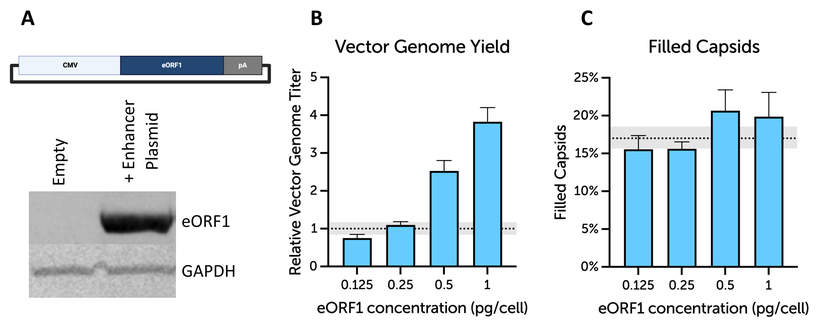
(**A**) Capsid titer and potency from the primary screen. ORFs were classified as a hit if both capsid titer and potency were greater than a 3σ increase from the mean of the plate.
(**B**) Hits from the primary screen were clustered within several biological pathways including DNA repair, transcription, signal transduction and epigenetic modulation

Confirmation studies demonstrate that multiple ORFs significantly enhance AAV9 production



A secondary screen was conducted to further examine the effect of ORFs initially identified in the primary screen. This study evaluated productivity by measuring encapsulated AAV9 vector genomes from crude lysate via qPCR. Raw titers were normalized to the control group (no ORF) that consisted of cells solely transfected with the split plasmid system.

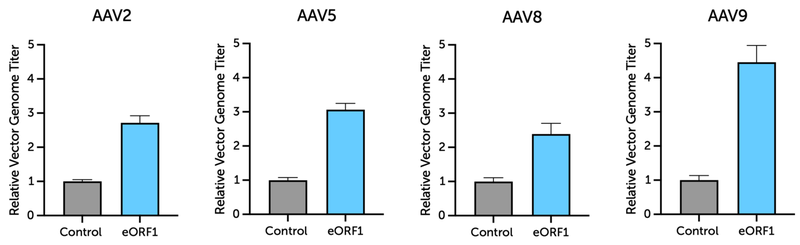
Expression of eORF1 results in a dose-dependent increase in AAV9 yield



(**A**) In the confirmation study, eORF1 emerged as one of the lead candidates and was subsequently cloned into a compact enhancer plasmid equipped with a CMV promoter, enabling swift and potent expression.

Over-expression of eORF1 resulted in a dose-dependent increase in (**B**) vector genome titer (normalized to no ORF control) and (**C**) the percentage of filled AAV capsids

Over-expression of eORF1 significantly increases AAV yield for multiple serotypes



AAV yield for AAV2, AAV5, AAV8 and AAV9 were assessed either with (blue) or without (grey) over-expression of eORF1. The assessment of vector genome yield from crude lysate was performed via qPCR. An enhancement in yield across all four serotypes was observed upon the addition of eORF1, with AAV5 and AAV9 resulting in the greatest increase in yield.

Poster downloads

1 - Ascend Advanced Therapies, Innovation Hub, 1010 Atlantic Ave. Alameda, CA, 94501
2 - Ascend Advanced Therapies GmbH | Fraunhoferstraße 9b | 82152 Planegg | Germany

www.ascend-adv.com
chad.wang@ascend-adv.com
reid@ascend-adv.com