Gain and loss-of-function screens identify targets that ramp up AAV production

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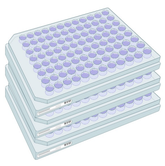
Abstract

Recombinant AAV (rAAV) is a promising gene therapy vector, but its current manufacturing methods result in still comparably poor yield and quality. In this study we present three different approaches to improve rAAV production: modulation of signaling pathways using small molecules: A library of over 3000 bioactive small molecules was screened using the ATLAS platform to identify compounds that increase the capacity of cells to produce AAV9. A novel compound (SM-016) was identified that increased rAAV9 production up to 3-fold, in a robust and dose-dependent manner. Engineering of HEK293 cells: A proprietary clonal suspension-adapted HEK293 cell line (AC001.230) was developed that shows improved productivity for 7 out of 10 tested serotypes compared to HEK293F cells and it´s parental polyclonal cells. Through a targeted CRISPR/Cas9 screen, three classes of targets were identified that significantly increased AAV9 production compared to the wild-type clonal cell line. Two independent suspension-adapted knockout cell lines (AC003 and AC010) were developed that show greater than 2-fold improvement in AAV9 production capacity. The findings from these approaches which next will be validated in a controlled small-scale bioreactor regarding yield and to also study any impact on quality have the potential to significantly reduce the cost of rAAV gene therapy, making it more widely accessible to patients.

Screens driving key verticals of innovation



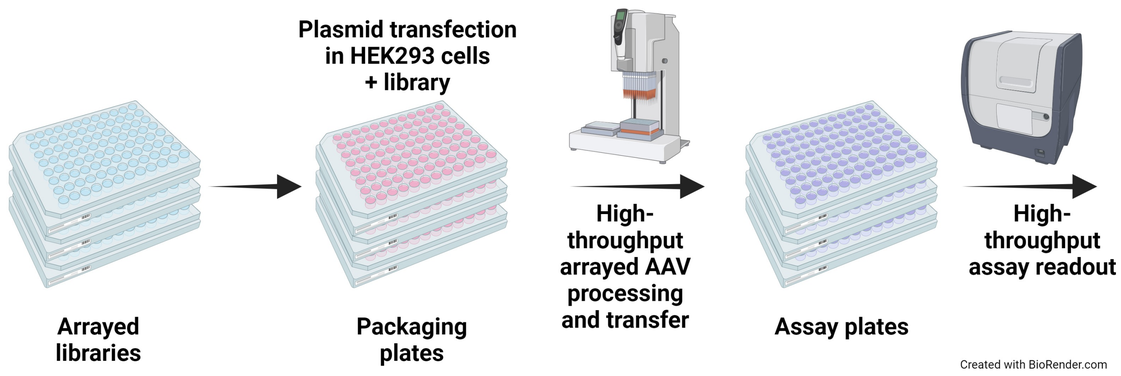
HTS

Plasmids
- Further plasmid evolution and genetic hit integration to optimize AAV yield, quality and potency

Cell lines
- Unbiased/targeted screens to identify genetic modifications to manufacturing cell lines
- Super-producer cell line generation and process intensification

Process
- Small molecule screening e.g. for enhanced transfection and/or cell survival
- Downstream purification innovations

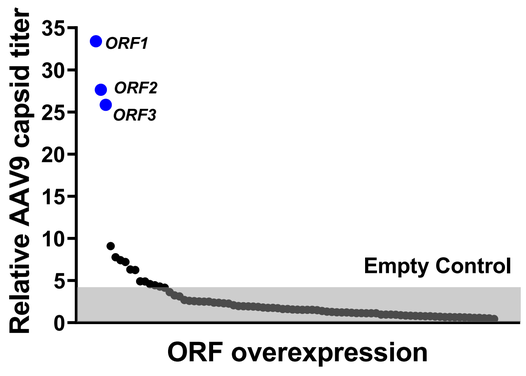
Arrayed targeted library for AAV screening (ATLAS)



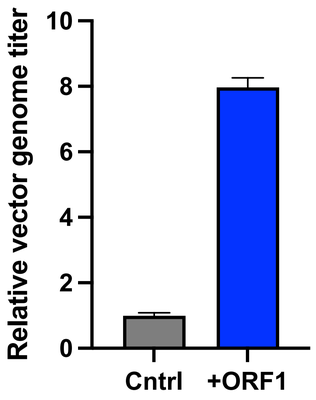
- Libraries consisting of ORFs, CRISPR/Cas9 and small molecules are screened using a proprietary cell-based reporter gene assay to identify factors that increase AAV yield (measured by capsid qPCR and ELISA) and functional titer assays.

ORF screen identifies hits improving AAV9 production

96-well format



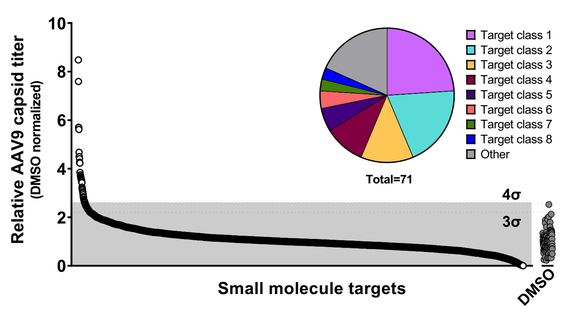
Shake flask



- Targeted ORF screen identified key factors that significantly increased AAV9 production
- An expression cassette for ORF1 was inserted into a non-split rep-cap (3-plasmid system) plasmid;
- Efforts are underway to evaluate the potency of vector produced in conjunction with single/multiple ORF over-expression

SM-16 improves AAV9 production

96-well format



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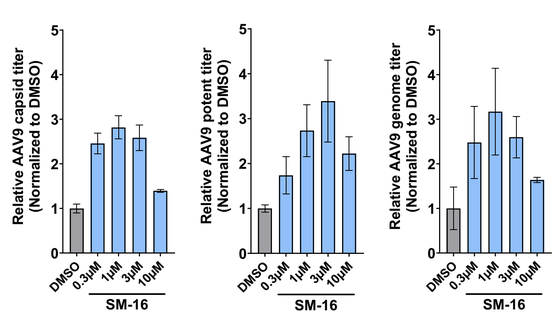
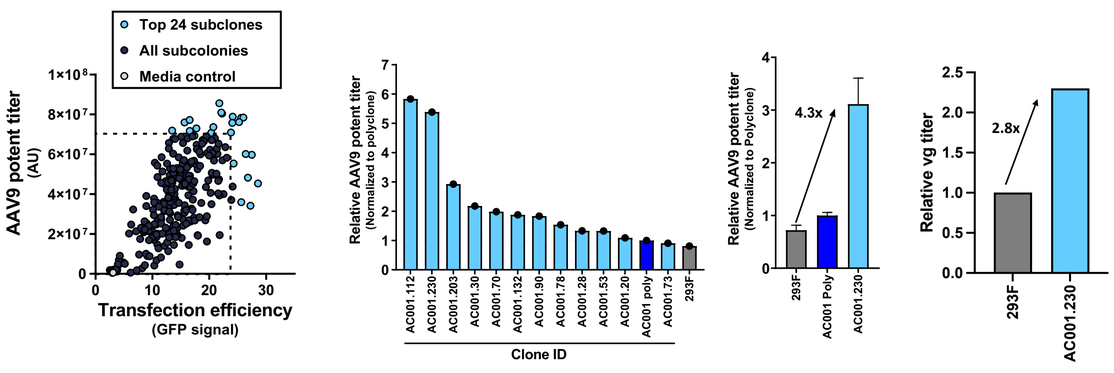


- A high-throughput small molecule screen of bioactive compounds was performed on HEK293 cells. AAV9 capsid titer was measured 3 days post transfection and normalized to DMSO.
- Top performing compounds consisted of transmembrane and DNA repair proteins, cell-cycle, and epigenetic modulators.
- SM-16 increases AAV9 production on the level of capsid (ELISA) and vector genome (qPCR) yield as well as in a cell-based reporter gene assay

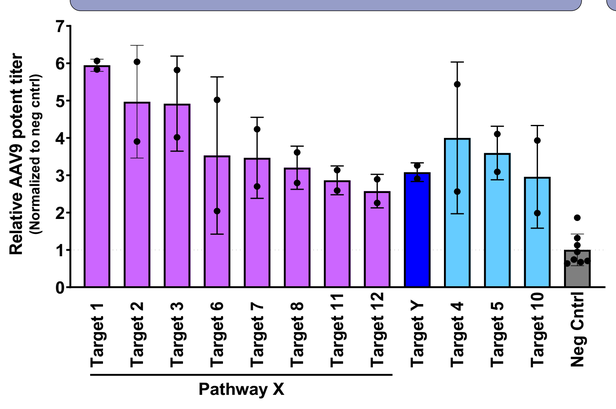
AC001.230 identified as top producer of AAV9 using multiple assays



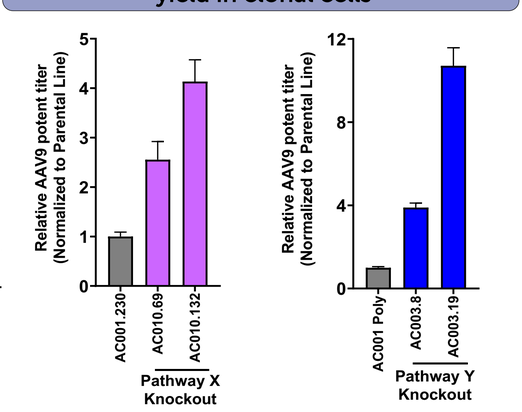
- Using the Solentim VIPS™ (Verified in-situ plate seeding) and whole well imaging, a clonality report is generated for all derived subclones
- Using single cell seeding and high-throughput screening we have identified a clonal suspension-adapted HEK293 cell line with a favorable growth and viability profile (data not shown) that shows higher productivity for AAV9 compared to it's parental polyclone (dark blue) and HEK293F (grey) cells

Knockout of pathways X and Y in separate clonal HEK293 cells improves AAV9 production

CRISPR screen in polyclonal population



Genetic modifications improve AAV9 yield in clonal cells



- A follow-up study confirmed that knockout of targets in pathways X and Y improves AAV9 productivity analysed on transducing titer level
- Clonal HEK293 cells with knockouts in pathways X and Y showed improved AAV9 production

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