Transcriptomic analysis identifies differential expression patterns in cellular stress response, signal transduction, and extracellular matrix proteins during AAV production

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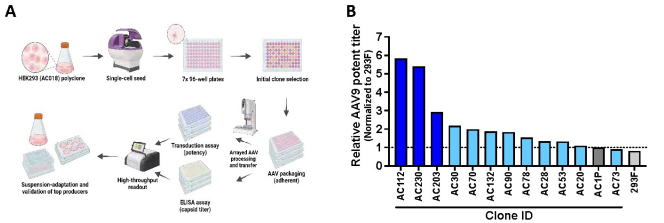
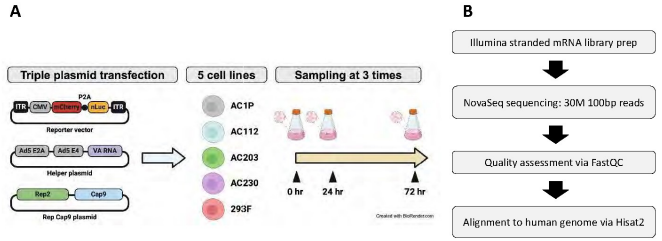
Recombinant adeno-associated virus (rAAV) is a widely used viral vector for gene therapy. Despite its clinical efficacy, the manufacturing of rAAV faces challenges in productivity and vector quality, leading to high costs and limited availability of gene therapies. To meet the growing clinical and commercial demand, mechanistic understanding of the cellular response to rAAV production is required to develop next-generation rAAV production processes. In this study, we performed transcriptomic analysis at multiple stages of rAAV production to better understand the pathways altered during this process. RNA-sequencing was performed on suspension-adapted HEK293 cells originating from two polyclonal populations during rAAV9 production. Polyclonal and clonally derived cells were included in our analysis to identify robust transcriptional signatures independent of clonal differences. Differential expression analysis across timepoints revealed that heat shock and inflammatory proteins, as well as Golgi organization, spindle assembly, and other cytoskeleton-associated components were among the most significant upregulated genes, while transcriptional repressors were most consistently downregulated during AAV9 production. A variety of extracellular matrix-associated proteins also exhibited significant changes in expression across AAV production. These data uncover novel and previously identified pathways that may affect rAAV productivity, potentially enabling Ascend a path to engineer improved processes and cell lines for higher yields and better quality rAAV production."

Clonal HEK293 cell line selection



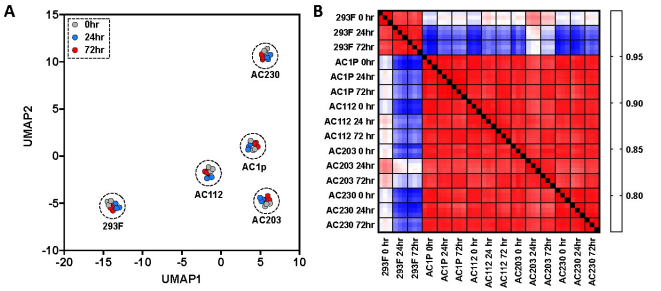
(**A**) Schematic of workflow for single-cell cloning and isolation of HEK293 cells with improved AAV production capacity. The Solentim VIPS™ single cell seeder was used to isolate clonal HEK293 cells using a two-step clonality verification process.
(**B**) 274 single-cell derived HEK293 clones were identified, and top 24 performing colonies were selected based on transfection efficiency and AAV9 production. After suspension adaptation, 12 clones were selected based on morphology, growth rate and viability and further screened. The top performing clonal cell lines (AC230, AC112, AC203) along with the parental polyclone (AC1P) and 293F were selected for this study.

RNA-Seq experimental design during AAV9 production



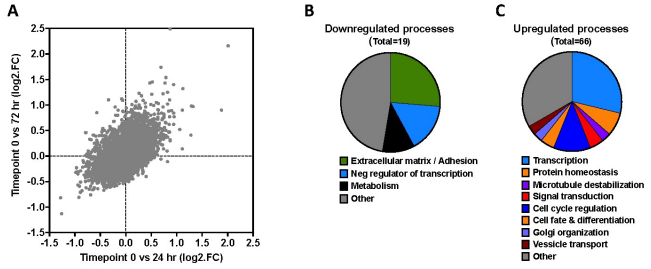
(**A**) Experimental design for RNA-Seq analysis during AAV9 production. 2 cell lines (293F, AC1P) and 3 clonal cell lines derived from AC1P (AC112, AC203, AC230) were used for the analysis. Samples were collected pre transfection and at 24 and 72 hrs post triple transfection. 3 shake flasks were used per condition.
(**B**) Bioinformatic and RNA-Seq processing workflow.

Cell clustering and principal component analysis



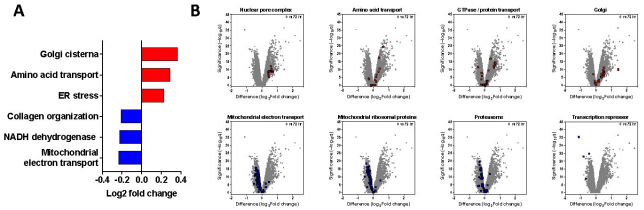
(**A**) Principal component analysis and (**B**) gene correlation matrix of the 5 HEK293 cell lines shows high reproducibility among replicates. Although RNA expression differences between cell lines are greater drivers of variability than differences between timepoints post-transfection, we focus our analysis on the subset of time-dependent differences which are shared across cell lines to identify common pathways for AAV production.

Activation of transcription, protein homeostasis and cell cycle regulation during AAV production



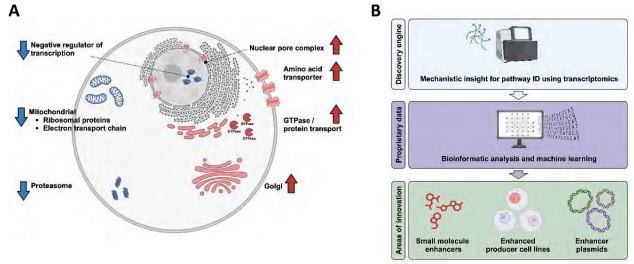
(**A**) Differentially expressed genes at 24 and 72 hr post transfection
(**B**) Downregulated processes include extracellular matrix organization and transcription repressors.
(**C**) Upregulated processes include transcriptional activation, protein homeostasis, microtubule destabilization, cell cycle regulation, Golgi organization and vesicle transport.

Downregulation of mitochondrial, proteasome and transcription repressors during AAV production



(**A**) Go term analysis post transfection shows amino acid transport, stress response pathways and Golgi cisterna are upregulated during AAV production. While mitochondrial electron transport chain and collagen fibril organization are downregulated during AAV production.
(**B**) Volcano plots of select processes at 72 hours show nuclear pore complex, amino acid transport, GTPase / protein transport and Golgi are upregulated during AAV production. While mitochondrial electron transport, mitochondrial ribosomal proteins, proteasome and negative regulators of transcription are downregulated during AAV production.

Mechanistic insights to engineer cell lines and processes for improved AAV production



(**A**) A model of altered biological processes during AAV production potentially point to pathways that could be modulated for improved AAV yield and quality.
(**B**) Our discovery engine incorporates transcriptomics to provide mechanistic insights and identify key pathways during AAV production. These studies provide a rich source of proprietary data that can be mined to develop the next generation of AAV manufacturing.

Poster downloads

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