ascend gene & cell therapies
Poster downloads | ASGCT 2023
Password: AIMHIGHER

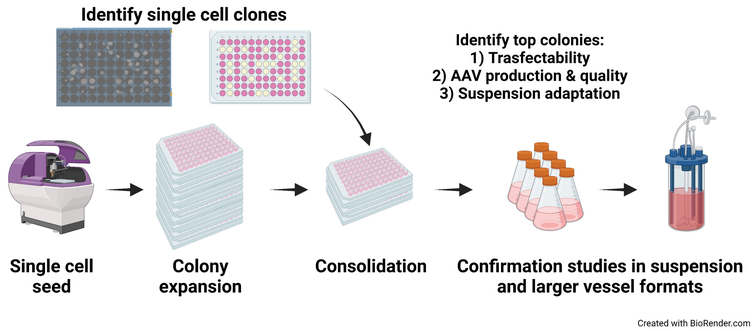
Targeted CRISPR/Cas9 Screen Identifies Superior HEK293 Cell Lines for AAV9 Production

Francis Grafton, Ryan Feitzinger, Kaylin Fisher, Lily Leveque-Eichhorn, Christopher A. Reid, Mohammad A. Mandegar

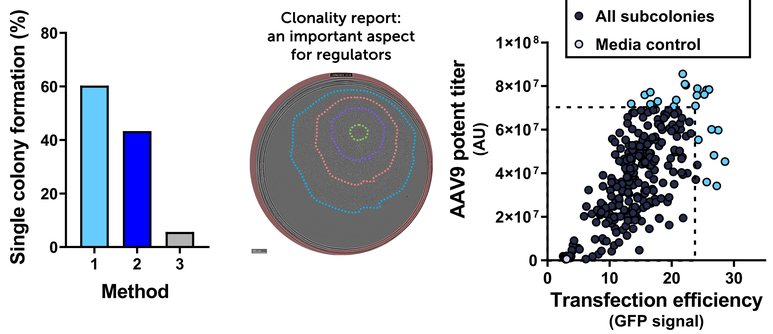
Abstract

There are two dominant AAV production platforms: transient transfection of mammalian cells and baculovirus infection of Sf9 insect cells. Academic and industry-led studies have shown Sf9 cells produce higher titers of rAAV, but it comes at a cost of vector quality and potency for some rAAV serotypes. Few studies have reported on systematically engineering cell systems to enhance both viral production capacity and potency. To address this, we used clonal isolation and high-throughput screening to develop a proprietary clonal suspension-adapted HEK293 cell line (AC001.230) that shows improved productivity for 7 out of 10 tested serotypes compared to 293F cells. Through a targeted CRISPR/Cas9 screen, we identified 3 classes of targets that significantly increased AAV9 production compared to our wild-type clonal cell line. From this work, we have developed two independent suspension-adapted knockout cell lines (AC003 and AC010) that show greater than 2-fold improvement in AAV9 production capacity. Our data suggests increase in AAV9 production can be achieved using synergistic effects of single cell cloning and genetic knockouts. We have confirmed these findings using capsid titer and vector genome quantification using a reporter construct. Studies are underway to evaluate these cell lines for production of vectors with different lengths using multiple AAV serotypes and in a large-scale bioreactor system. Learnings from this study are readily transferable to customer cell lines or for cloning and engineering of our GMP banked HEK293 suspension cells.

General workflow for isolation of an improved AAV9 producer suspension HEK293 cell line

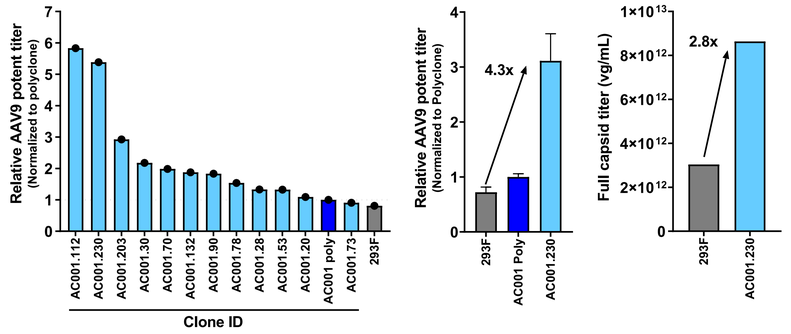


- Clonal cell line generation and suspension optimization workflow.
- Various methods were tested for optimal single cell colony formation and expansion



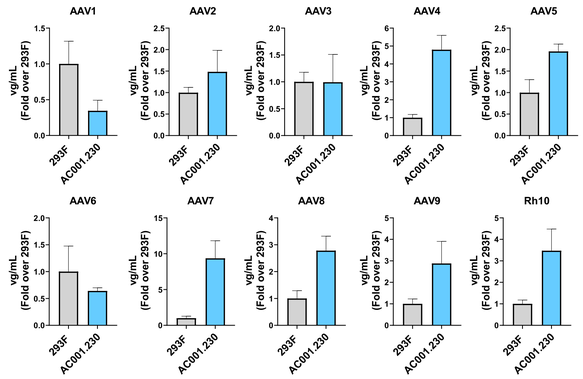
- Using the Solentim VIPS™ (Verified in-situ plate seeding) and whole well imaging, a clonality report is generated for all derived subclones
- Top performing colonies were selected based on transfection efficiency and AAV9 production using a cell-based reporter gene assay

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



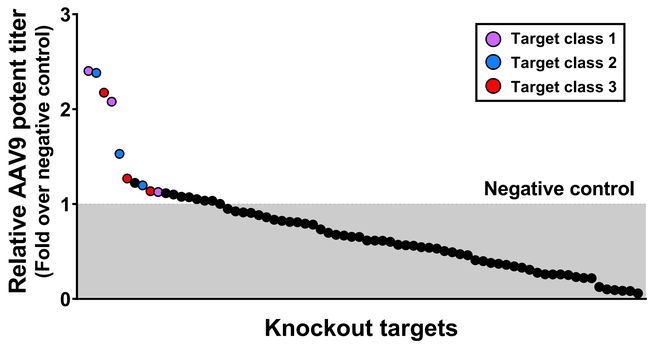
- Using single cell seeding and high-throughput screening we have identified a clonal suspension-adapted HEK293 cell line with a favorable growth and viability profile that shows higher productivity for AAV9 compared to it's parental polyclone (dark blue) and 293F (grey) cells

AC001.230 outperforms 293F cells in production of AAV2, AAV4, AAV5, AAV7, AAV8, AAV9, and Rh10



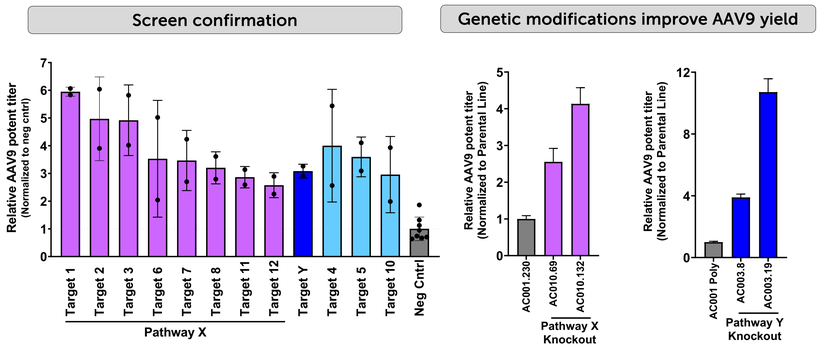
- AAV production capacity of AC001.230 was tested using additional AAV serotypes and compared to 293F. Production capacity was highest for AAV7 followed by AAV4, AAV9, Rh10, AAV8, AAV5 and AAV2.

CRISPR/Cas9 knockout screen identifies pathways improving AAV9 production



- Targeted CRISPR/Cas9 screen identified 3 target classes that significantly increased AAV production

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



- A follow-up study confirmed knockout of targets in pathways X and Y improve AAV9 productivity analysed on potency level
- Clonal HEK293 cells with knockouts in pathways X and Y showed improved AAV9 Production

ascend gene & cell therapies

Ascend GCTx, Innovation Hub, 1010 Atlantic Ave. Alameda, CA, 94501

www.ascend-gctx.com
Grafton@ascend-gctx.com
Mandegar@ascend-gctx.com