Utilization of Tenaya's AAV Productivity Boosting Small Molecule (SMB) for Intelligent Design of HEK293 Expression Platforms

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Objectives

Tenaya has developed a proprietary Small Molecule Booster (SMB) that significantly improves AAV productivity in HEK293 transient transfection without affecting product quality and transduction efficiency both *in vivo* and *in vitro*. RNA-Seq was used to further analyze the pathways affected by SMB in order determine the viability of using this data for intelligently designed experiments that target genes and pathways that may be utilized for cell line engineering or second-generation SMB

Materials and Methods

- HEK293 Suspension Cells were used along with commercially available transfection agent using standard triple transfection
- Titer was determined by ddPCR and commercially available ELISA kits
- RNAseq was prepare using commercially available kits on Illumina Nextseq 550

SMB Improves Scale-Up Productivity

To improve the scalability of HEK293 process, Tenaya screened potential small molecule boosters (SMB) in shake flasks. Tenaya has developed a proprietary SMB to improve yield. SMB addition process and titer improvements improve productivity at 3L, 50L, and 200L bioreactor (BRX) based on power per unit volume (PV). Five different capsids were tested and boosting effect was seen across the majority of serotypes in shake flask (SF) and for all BRX.

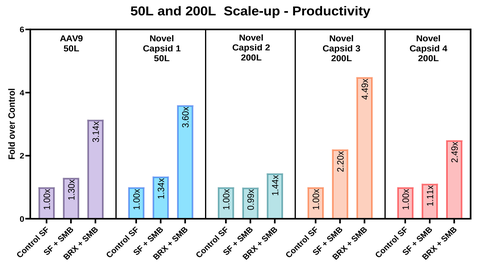


Figure 1: Scale-up to 50L and 200L

Successful SMB scaling was achieved at 50L and 200L bioreactor scale. Vg titer boosting is seen in all shake flask scale productions except Novel Capsid 2. However, boosting is shown in AAV9 and all other novel capsids at BRX scale.

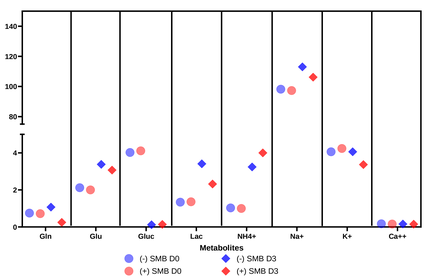


Figure 2. 3L Bioreactor Results with and without SMB

Metabolites were comparable with and without SMB in the shake flask satellites.

No Impact on Purification Performance

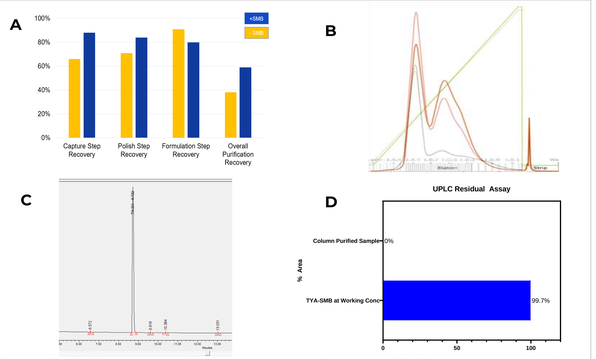


Figure 3. (A) Purification Recovery comparison and (B) Chromatograph of polishing step with and without SMB (C) UPLC Chromatogram of SMB stock (D) UPLC analysis of SMB at working concentration compared to 2 column purified product produced with SMB

No Impact on Product Quality

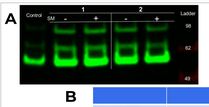


Figure 4. Western blot (A), images of cardiomyocyte transduced with AAV:GFP, and capillary electrophoresis electropherograms (B) to verify the small molecule effects on AAV quality.

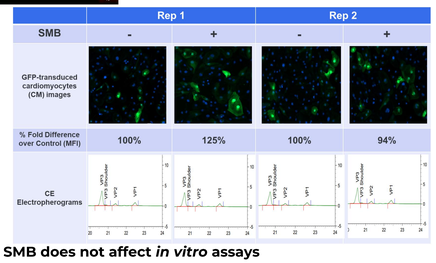


Figure 4 SMB does not affect *in vitro* assays

Based on cardiomyocyte transduction with AAV9:GFP, there is no significant impact of SMB addition on AAV infectivity. Additionally, from the CE electropherograms, VP1/VP2/VP3 ratios are found to be similar with SMB added samples vs. no SMB.

No Impact on *In Vivo* Transduction

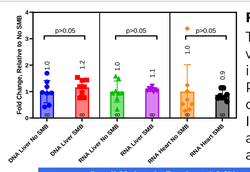
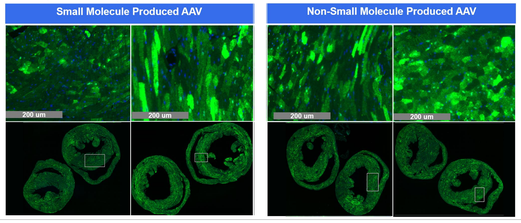


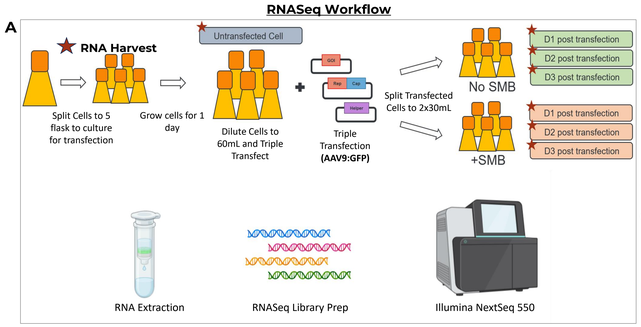
Figure 5. SMB does not affect in vivo tests

Two arms consist of AAV produced with and without SMB. Mice were euthanized 3 weeks post injection and harvested for heart and liver tissue. RNA and DNA were extracted and analyzed using qPCR to determine fold change in Gene of Interest (GOI) relative to a process without SMB addition. Tissue sections were imaged from each arm, no difference is observed



SMB Demonstrates Unique and Novel Mechanism of Action

Experimental design was done using two arms, No SMB and +SMB. Each arm included five replicates and were harvested for RNA before transfection and daily throughout transfection. Tenaya's SMB provides unique and novel mechanisms that affect many different pathways during the production of AAV9:GFP in HEK293.



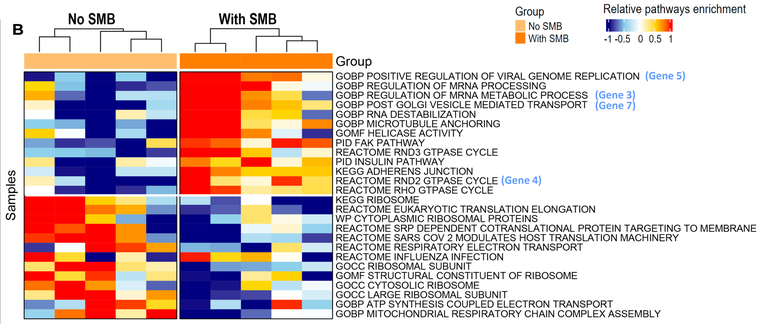


Figure 6. (A) RNASeq workflow (B) Matched heatmap of top 13 enriched and depleted pathways in SMB Production Culture relative to no SMB from RNAseq analysis

Utilization of SMB RNASeq to Intelligently Design Knock-Out and Overexpression Targets

Knock-out (KO) Genes were selected based on statistical testing in order to investigate their impact on viral titer compared to wildtype cells with SMB. KO cell lines resulting in reduced viral titers are potential candidates for further studies or cell line engineering. Gene 3 stood out with the most significant decrease in titer, aligning with RNASeq findings in volcano plot. Genes in the red have potential for further evaluation and may be good targets for Cell Line Engineering (CLE) or Cell Line Development (CLD).

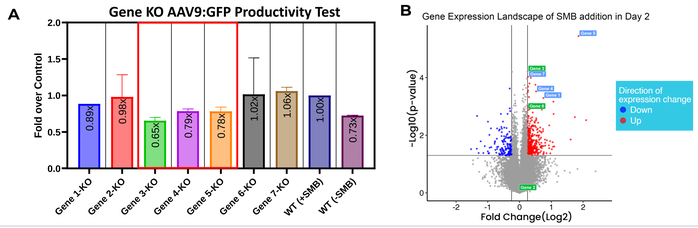
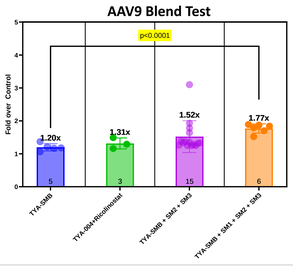
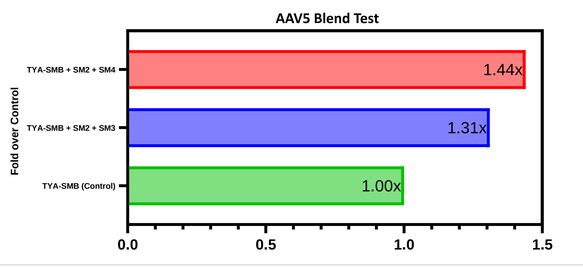


Figure 8. (A) KO adherent cell lines were benchmarked with small molecule booster added (B) Volcano plot for Day 2 production and indicated genes selected for KO (C)Gene 5 was titrated using an expression plasmid to determine the optimal expression level. Increasing expression improved titer further confirming RNAseq and KO data.

Utilization of SMB RNASeq to Intelligently Design Second Generation SMB



Through data obtained from our RNAseq, we were able to further iterate our SMB through blending it with other synergistic small molecules. This data allowed us to understand which pathways are or are not targeted and further increase productivity of our AAV productions by targeting unrelated pathways that benefit viral production.



Conclusions

- Tenaya's proprietary SMB consistently shows a substantial increase in vg productivity with no observable impact on product quality and purity and transduction efficiency (*in vivo* and *in vitro*)
- At BRX scale, boosting affects of SMB is shown independent of capsid serotype
- SMB can plug-and-play into existing HEK-based process
- Overexpression of genes prove to be complicated, titration for gene expression must be done to determine optimal expression levels
- Further work can be done to examine other genes from RNAseq
  - Examine under expression and create KO lines or RNAi lines and multigene interactions

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