The Impact of Individual Helper Genes on AAV Productivity

The path to developing engineered helper plasmids with optimal adenovirus gene expression

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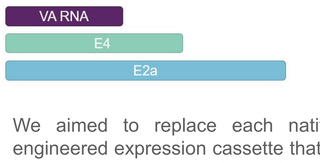
Introduction

The typical helper plasmid used for adeno-associated virus (AAV) manufacturing contains Adenovirus genes essential for AAV replication and gene expression: E2a, E4 and VA RNA. The E2a gene produces DNA-binding protein (DBP), however it has recently become clear that a fourth gene, L4 22K/33K, encoded on the opposite strand of the E2a gene, plays a critical role in AAV production. In the pursuit to optimize the helper sequences, each helper gene was split into four individual plasmids called native helper building blocks.

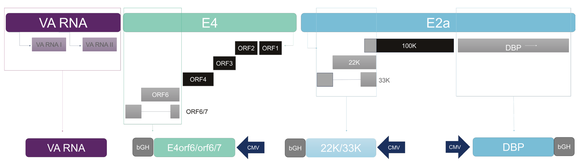
pHelper

Native Helper Building Blocks



We aimed to replace each native building block with an engineered expression cassette that provides more optimal gene expression levels than the native Adenovirus gene regulation. This resulted in engineered building blocks utilizing the cytomegalovirus (CMV) promoter and bovine growth hormone (bGH) polyadenylation signal to express E4orf6/orf6/7, L4 22K/33K and DBP individually. The VA RNA gene remains in the native configuration.

Engineered Helper Building Blocks

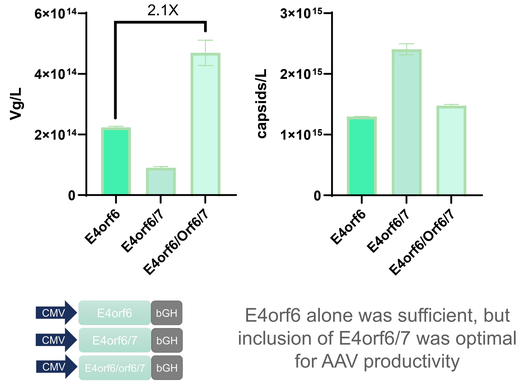


Methods

All experiments were completed using 125 mL shake flasks with a 50 mL working volume. VPC 2.0 cells (Thermo Fisher) were cultured at 2E6 cells/ mL in Expi293 media (Thermo Fisher) and transfected with 0.75 µg of DNA/ 1E6 cells at equal molar plasmid ratios using PEI. The OXB dual plasmid design (GOI+RepCap) bearing a CMV-GFP/Luciferase genome and AAV9 capsid was used for all experiments. Vector genome (VG) and capsid titers were analyzed by ddPCR and AAV9 ELISA (Progen) respectively.

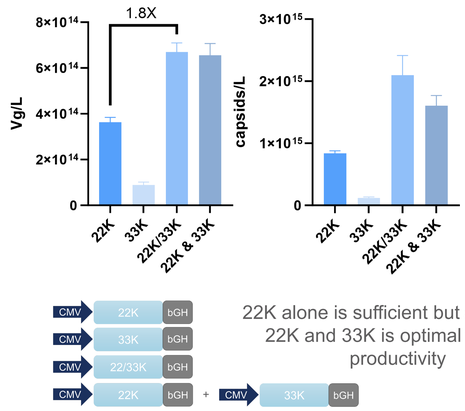
Results

A Engineered E4orf6 & E4orf6/7 Expression



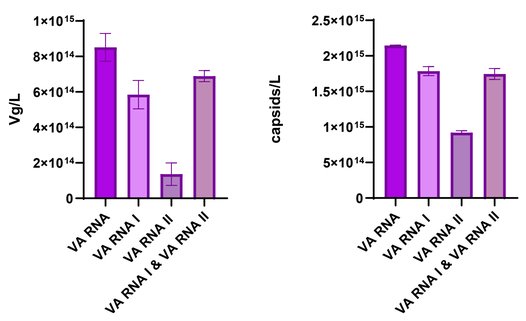
E4orf6 alone was sufficient, but inclusion of E4orf6/7 was optimal for AAV productivity

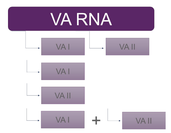
B Engineered L4 22K & 33K Expression



22K alone is sufficient but addition of 22K and 33K is optimal for AAV productivity

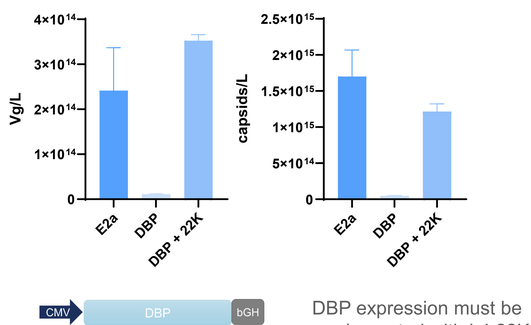
C Engineered VA RNA Expression





VA RNA I is the major contributor to VA RNA function and VA RNA II plays a more minor role

D Engineered DBP Expression



DBP expression must be supplemented with L4 22K

Removal of Each Engineered Building Block

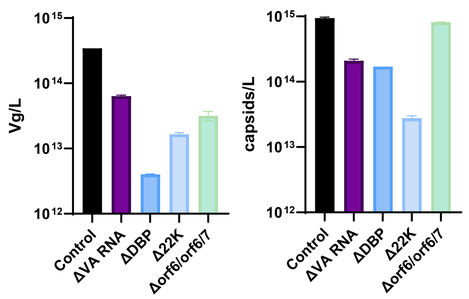
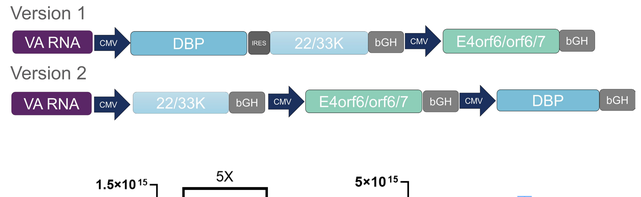


FIGURE 1.
AAV vector genome and capsid titers in crude lysate to determine the optimal expression cassette for each engineered building block: (A) L4 22K/33K, (B) E4orf6/orf6/7, (C) VA RNAI/II and (D) DNA binding protein (DBP).

Engineered Helper Designs



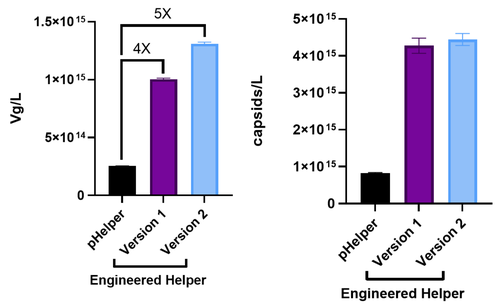


FIGURE 3.
AAV vector genome and capsid titers in crude lysate comparing the original pHelper to two versions of engineered helper plasmids. These engineered helper plasmids combine the engineered building blocks back into a single plasmid. Both engineered helper version 1 & 2 increase VG productivity by ~4X, demonstrating the currently used pHelper plasmids based on native Adenovirus sequences are not optimal for AAV production. Engineered helper version 1 utilizes an internal ribosome entry site (IRES) to express DBP and L4 22K/33K from a single CMV promoter. Engineered helper version 2 employs three distinct CMV expression cassettes to express L4 22K/33K, E4orf6/orf6/7 and DBP separately.

Conclusion

L4 22K, E4orf6 and VA RNA I expression are all sufficient for AAV production, however these alone are not optimal. Using engineered building blocks, it was determined that L4 22K/33K, E4orf6/orf6/7 and native VA RNA provide the most optimal conditions. These findings led to the design of engineered helper plasmids that yield higher AAV productivity, suggesting a more optimal helper gene expression profile was achieved compared to standard pHelper.

FIGURE 2.
AAV vector genome and capsid titers in crude lysate following removal of each engineered building block. VA RNA, DBP, L4 22K and E4orf6/orf6/7 each play a functional role in AAV productivity. The lack of DBP was the most impactful at decreasing genome productivity (86X) while VA RNA was the least impactful (5X). 22K was the most effective at decreasing capsid productivity (30X) while removing E4orf6/orf6/7 showed minimal impact on capsid productivity.