Optimizing Helper Plasmids by Removing Unnecessary "Junk" DNA Significantly Increases AAV Productivity

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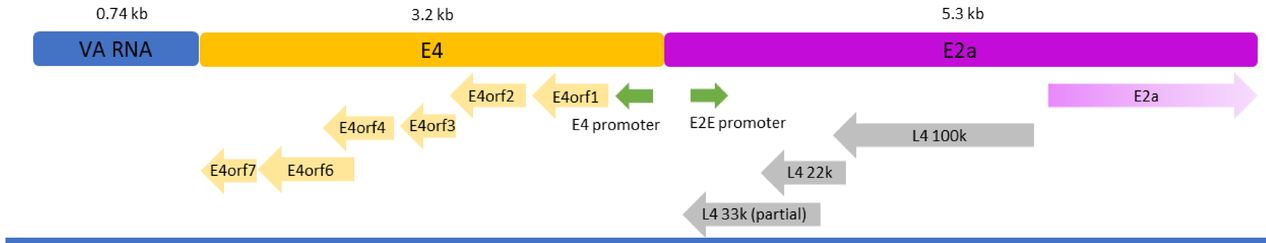
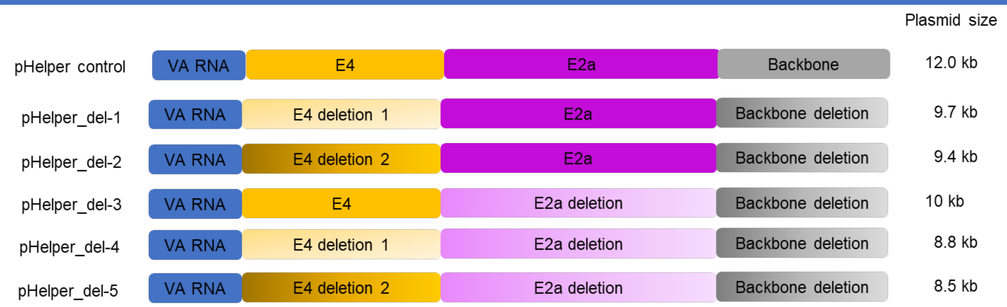
INTRODUCTION

Production of adeno-associated virus (AAV) vectors using live adenovirus infection has largely been replaced with a plasmid containing the necessary adenovirus genes to facilitate AAV replication for safety and logistical considerations. Helper plasmid (pHelper) contains three adenovirus genes: E2a, E4 and VA RNA. Although there are a number of different pHelper plasmids available, they all minimally contain these three genes, along with various additional sequence structure. These genes can contain larger regions than the promoter and coding sequence due to the nature of the adenovirus genome structure, that could be referred to as "junk" DNA as it pertains to AAV replication. Since the adenoviral genome is tightly packed and highly regulated, transcriptional units are overlapping or intertwined. For example, adenoviral E2a and L4 gene overlap on opposite strands, which resulted L4 regions being included in Helper plasmids. The E4 transcriptional unit contains 6 open reading frames (ORFs), encoding 6 individual proteins.



In our experience, pHelper is challenging to produce at large scale with high density fermentation, resulting in low yields and increased manufacturing costs. Therefore, we sought to reduce the size of our pHelper by removing regions of the adenoviral genes that were not essential to their function, in an effort to improve the manufacturability of the plasmid. We generated five pHelper deletion constructs aimed to reduce the size of E4 and E2a transcriptional units as well as reducing the plasmid backbone and tested for comparability with the control pHelper. Surprisingly, all five of these smaller Helper plasmids increased vector genome (VG) productivity to various extents across multiple genomes. Capsid production also increases proportionally to VGs using the smaller pHelpers. We observed statistically significant increases in VG production through the removal of "junk" regions of our current Helper plasmid. Our working hypothesis is that the removal of non-coding regions of the adenoviral genes is altering the expression of E2a and E4 proteins, which enables higher capsid expression and in turn more VGs are packaged into the available capsids.



RESULTS

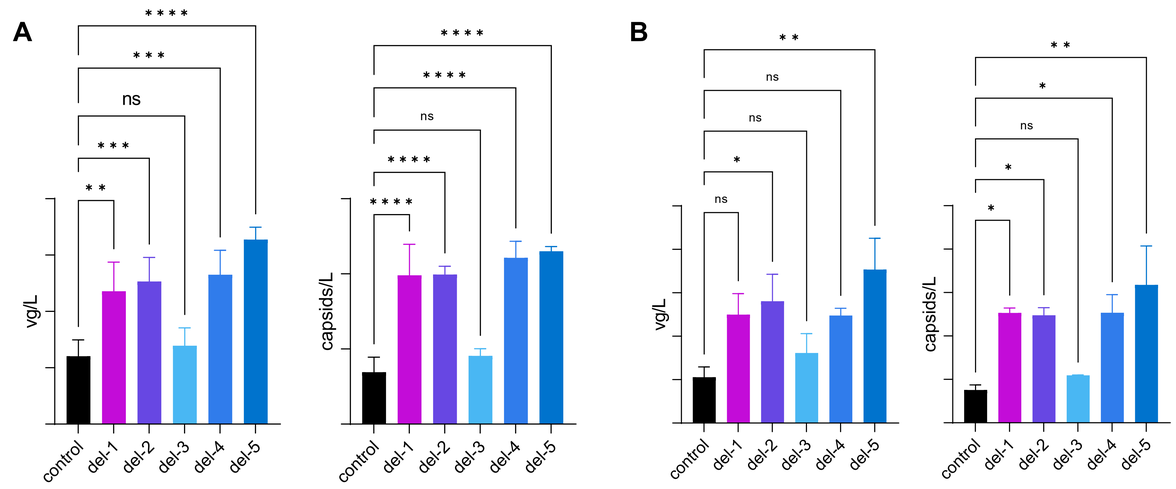


Figure 1. *Deletions of regions in pHelper resulted in an increase in vg and capsid production across multiple genomes of Clade F capsids.* Five new pHelper plasmids were tested for upstream productivity in shake flasks. Vector genome titer (vg) was measured in crude lysate by ddPCR. Capsid titer was measured by capsid ELISA. (A) AAV-HSC15 and genome 1. (B) AAV9 and genome 2.

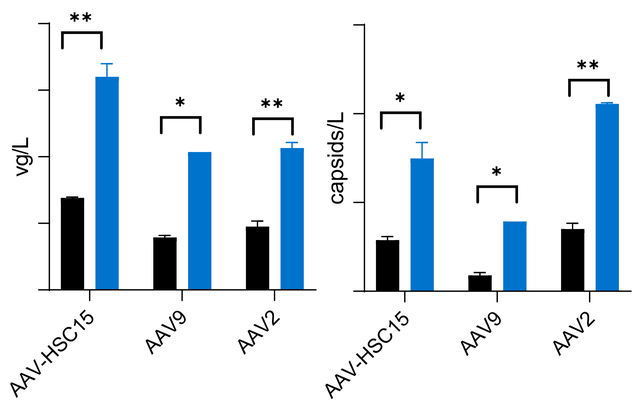


Figure 2. *Deletions of regions in pHelper resulted in an increase in vg and capsid production across multiple AAV serotypes.* The smallest pHelper plasmid containing both E2a and E4 deletion 2 was tested for upstream productivity in shake flasks packaging the same genome (genome 1) in AAV-HSC15, AAV9, and AAV2. Vector genome titer (vg) was measured in crude lysate by ddPCR and capsid titer was measured by capsid ELISA.

CONCLUSIONS

Removing unnecessary sequences in the adenoviral helper for the purpose of improving plasmid manufacturability plasmid lead to an unexpected increase in recombinant AAV productivity (vector genome and capsid) in multiple genomes and AAV serotypes.

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