

# Designing Compact and Efficient Adeno-Associated Virus Vectors For Higher Transgene Expression

Ian O'Connor and Renping Zhou  
Rutgers, The State University of New Jersey

Adeno-associated viruses (AAV) is an established vector for gene therapy. However, there are still many struggles associated with AAV and one is improving the gene cargo capacity inside of the capsid protein. With only 4.4 kb to fit between the inverted terminal repeats (ITRs), larger transgene expression cassettes struggle to show strong efficiency. Therefore, we designed experiments to identify whether some of the common regulatory elements can be deleted. In our experiments, we focused on the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) and produced three different vectors: one with WPRE, another with shortened WPRE (CW3SL), and lastly without WPRE. All of these vectors express eGFP driven by a CMV promoter as well as containing a chimeric intron upstream of the transgene. Additionally, a positive control using the original vector containing the CMV promoter, eGFP reporter gene, and a beta-globin poly(A) signal was used. Our results show that the WPRE element can be left out of the vector and still achieve reasonable expression and CW3SL showing inhibitory effects in the infected HEK293 cells. In addition, the original vector was observed to be noticeably stronger than the WPRE. The findings of this study show that WPRE expression may differ between different AAV constructs and in different cell lines but can be left out for larger transgenes. The need to create more efficient packaging systems still remains but not dire as there are now many unique and creative methodologies to produce efficient packaging systems. Supported by NIH R25ES020721 and the ASPET SURF Program.

