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**Poster Presentation**  
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**Molecular Design and Characterization of Packaging Plasmid Sequences for Improved Production of Novel Clade F AAVHSCs**

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AAVHSCs are a group of Clade F AAV capsids isolated from human hematopoietic stem cells (HSCs). A scalable, transfection-mediated production process for novel Clade F AAVHSC vectors has been developed, demonstrated up to 2,000L production scale, and is being utilized for gene therapy and gene editing programs. The AAV plasmid production system involves sequences specific to the adenovirus helper genes, AAV replicase and capsid genes, and AAV genome plasmid containing the promoter and therapeutic transgene flanked by inverted terminal repeats (ITRs). To further optimize these components, molecular characterization and engineering of the packaging plasmids has been evaluated to improve design, target higher vector productivity, improve genome packaging, and better product quality. Here we demonstrate enhancements made to the Rep sequence (Rep 1.0→ Rep 2.0) that resulted in increased crude lysate productivity as well as product quality. AAVHSC15 and AAV5 packaging profiles comparing the same genome resulted in 24% less double packaged vectors using AAVHSC15. Furthermore, we demonstrated efficient packaging by AAVHSC15 with genomes between 2.3-4.8 kb by analytical ultracentrifugation. Lastly, we evaluated three alternative plasmid backbone designs to remove kanamycin selection from our process. Two of these alternatives yielded similar VG titers and percentage of full capsids to the traditional kanamycin plasmids, while alternative #2 resulted in an average crude lysate VG titer increase of 2.7X (n=3). Overall, here we show AAVHSC vector productivity and product quality was improved by optimizing the genetic sequence of the recombinant genome as well as the packaging components to allow for high quality production of a wide range of transgene sizes.