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Wildtype AAV2 Rep Protein Produces Higher Titer AAVHSC Vectors with Improved Packaging Profiles Compared to Clade F Associated Chimeric Rep

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A chimeric adeno-associated virus (AAV) Rep gene associated with AAV9 RepCap packaging plasmids has been observed in Genbank sequences in addition to plasmids from vector cores and DNA vendors. Compared to wild type AAV2 Rep, this chimeric Rep contains 14 nucleotide mutations resulting in seven silent mutations and four amino acid changes within the 3' end of the open reading frame. The chimeric Rep functions to produce AAV vectors of various serotypes, including AAVHSCs, Clade F vectors originally isolated from human hematopoietic stem cells. When compared, these of the wild type (WT) AAV2 Rep sequence results in higher vector yields and improved vector packaging profiles.

Vector production utilizing WT AAV2 Rep resulted in up to an 80% increase in vector genomes and up to a 40% increase in full capsids generated in crude lysate when compared to vector production using the chimeric Rep. A substantial increase in DNA containing vectors by analytical ultracentrifugation was observed as well as a reduction in partial genomes packaged. In addition, vector produced with WT AAV2 Rep resulted in an increase of vector infectivity and up to a 50% reduction in packaged host cell DNA compared to production with the chimeric Rep. Furthermore, vector quality attributes including aggregation, purity, residual host cell protein and residual packaged plasmids remained in process range. Similar trends were observed for both adherent and suspension HEK293 production platforms ranging across scales up to 500L and using multiple gene of interest constructs.