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Scalable Manufacturing Platform for Highly Potent AAV Vectors Preparations

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Manufacturing high quality of recombinant Adeno-associated vectors for pre-clinical and clinical applications has become one of the major bottlenecks that limit clinical and commercialization expectations for this gene therapy biologic. Over the past few years, we have established a robust, high yield and highly scalable method in a mammalian cell suspension and serum-free platform. The process relies on a highly efficient herpes simplex virus (HSV) shuttle system to deliver AAV and helper functions to the producer cells by infection. In this presentation, we will describe a series of optimization steps that enabled the production of high yield and high quality AAV9 stocks, including unexpected findings showing that optimization of the salt concentration in the production media resulted in a significant increase in AAV particle production. Quantitative and qualitative data resulting from side-by side comparison of the HSV suspension system with the traditional $CaPO_4$ transfection method in adherent HEK293 cells as well as with a recently implemented PEI transfection protocol using suspension-adapted 293 cells will be presented. Across the three methods, the HSV-generated AAV9 stocks showed an average volumetric yield increase for most constructs tested side-by-side, and, more importantly, displayed a similar or improved potency when compared to transfection in adherent cells. Current limitations of all systems will be discussed with a special emphasis on process- and product-related impurities, including but not limited to, HSV proteins and rcHSV contaminants, HEK293 DNA and protein residual, ratio full-to-empty capsid. Downstream processes established to support removal of the majority of HSV-related contaminants will be discussed as well as analytical methods for their detection in final AAV stocks. Based on our data, the HSV and the transfection methods using suspension 293 provide robust platforms to enable semi-to large scale vector manufacturing for gene therapy applications.