SCALABLE DOWNSTREAM PURIFICATION OF RECOMBINANT ADENO-ASSOCIATED VIRAL VECTORS

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Scalable manufacturing technologies are essential for ensuring modern medicines can be produced to meet the needs of clinical trials, process development, and commercial manufacture. Recent advances in *in vivo* gene therapies have resulted in multiple regulatory approvals of rAAV vectors for gene transfer in humans. These vectors can be produced using transient transfection of mammalian cells, baculovirus infection of insect cells or produced via engineered stable producer cells. These production methods are performed in single-use bioreactors and utilize other scalable technologies as used in commercial monoclonal antibody manufacture.

In this work, we evaluated the use of existing single-use filtration and separation technologies for downstream purification of an rAAV5 viral vector. rAAV5 vector was produced by transient transfection of HEK293 cells in the Pall iCELLis® Nano bioreactor. Bioreactor harvest lysis material was clarified using direct flow filtration with both depth and sterilizing grade filters. The product was concentrated 10x using 100kD Omega™ flat-sheet tangential flow-filtration (TFF) before primary purification using affinity chromatography. The rAAV5 vector was then polished using Mustang® Q membrane chromatography to enrich for full capsids. A second TFF step was performed to concentrate and buffer exchange with flat sheet TFF with the same 100KD Omega membrane. Final sterile filtration was performed using Supor® EKV validated sterilizing grade filters.

All downstream unit operations resulted in acceptable performance. Feasibility of a complete downstream process was established with a theoretical whole process yield of ~25%. This process results in a very low contaminant profile as host cell protein (HCP) and host cell DNA were reduced to near and below the assays' limits of quantitation during purification. Of particular interest, Mustang Q polishing resulted in retention of only ~10% of total capsids, while recovering ~50% of full capsids enriching the ratio of full capsids to empty capsids by 4.5 fold.