HIGH CELL DENSITY PERFUSION PROCESS FOR rAAV9 PRODUCTION BASED ON TRANSIENT TRANSFECTION OF HEK293 CELLS

- Ye Zhang, GeneNova, Innovation Milieu for AAV gene therapy; AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden – email yezhang@kth.se

- Emil Sundäng Peters, GeneNova, Innovation Milieu for AAV gene therapy; AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden

- Véronique Chotteau, GeneNova, Innovation Milieu for AAV gene therapy; AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden

Key Words: rAAV, HEK293 cells, high cell density perfusion, transient transfection

Recombinant adeno-associated virus (rAAV) vectors are widely used for many current gene therapy applications. The fast growing worldwide demand for gene delivery presents a big challenge in rAAV viral vector manufacturing capacity. The cell culture platform remains a determining factor among all the efforts to lower the manufacturing cost and/or to increase the production yield. The aim of our study was to implement a novel approach to produce rAAV with enhanced productivity in high density perfusion cultures of suspension cells HEK293.
In our previous work with rAAV serotype 1, we have successfully established a mini bioreactor system (50 mL centrifuge tubes with vent caps) for transfection parameter screening. In the present study, we performed similar studies for rAAV serotype 9 and applied the optimized conditions in perfusion cultures in stirred tank bioreactors at 200 mL scale. Co-transfections of three plasmids pAAV-RC9 (plasmid providing the viral replication and capsid genes for AAV9), pHelper (plasmid carrying the adenovirus gene products required for the production of infective AAV), and pGFP (plasmid containing AAV inverted terminal repeats with the gene of interest replaced by gene coding for Green Fluorescent Protein) into suspension HEK293 cells were done at viable cell density > 70E+6 cells/mL. Transfection efficiency was daily monitored by quantification of the GFP expression level using a flow cytometer, while the production titers of viral particles were measured with ELISA and qPCR. Cell transduction assays were used to monitor infectious titers. After transient transfection the cultures were pursued in perfusion mode and harvested 10 days post transfection (dpT) at 1.25E+8 cells/mL. Figure 1 illustrates such a process, which enabled 8.4 times increase in volumetric productivity comparing to the reference (Ref) carried out in mini bioreactor where the cell density was 15E+6 cells/mL at harvest 3 dpT.

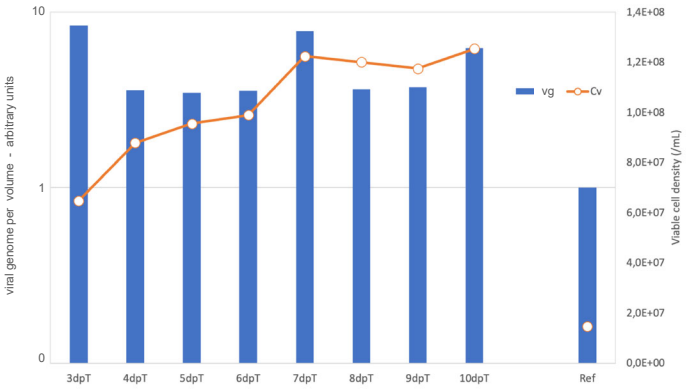

Figure 1: High density perfusion cultures of suspension cells HEK293 for the production of rAAV9, representing the viral genome productivity and the cell density in 200 mL bioreactor scale at days 3 to 10 post transfection, in comparison to the reference culture harvested at 3 dpT (Ref) performed in mini bioreactor system at 15E+6 cells/mL cell density