Design of experiment (DoE) based high throughput process optimization for rAAV manufacturing development

Rupert Derler, Kathrin Breunig, Sandra Lange, Martina Ohme, Markus Hörer, Florian Sonntag, Andreas Schulze

Background

Recombinant adeno-associated virus (rAAV) is among the most widely used viral vectors for gene therapy. However, vector manufacturing remains a challenge as the choice of upstream production systems represents a trade-off between flexibility, scalability, quality and speed to clinic. Currently, adherent and suspension cell-based rAAV manufacturing processes are used by the industry. While different large-scale adherent production systems, such as the iCELLis® 500, are in use, suspension cell-based rAAV production platforms come with a number of additional benefits.. Suspension processes allow for better scalability (scale-up instead of scale-out) resulting in lower operational costs. Furthermore, suspension-based processes eliminate the need for animal-derived component supplementation. Resource- and labor-intensive approaches for process development and optimization, such as the use of shake flasks or bench-top bioreactors, limit the throughput required to efficiently improve rAAV production processes. Furthermore, these systems do not easily allow for changing of multiple parameters at a time (e.g., DoE - design of experiment) to determine the individual and combined effects of these changes on rAAV production. Therefore, high-throughput, automated microbioreactor systems represent ideal tools for effective bioprocess development and optimization. Here, we present the results of a DoE-based study evaluating the impact of process parameters, such as cell seeding density, total DNA per cell at transfection, and pH setpoint of the bioreactors, on rAAV yield and quality.

Aim

Optimization of process parameters to increase rAAV production yields and reduce plasmid and host cell DNA (HCD)-derived impurity mispackaging.

Methods

- Single-stranded rAAV was produced in the Ambr® 15 bioreactor system (Sartorius®) using our proprietary HEK293 suspension cells and 2-plasmid split system.
- A 3-parameter 5-level DoE optimization study design was applied that allowed for quadratic modelling.
- Purification of rAAV was performed by affinity chromatography using AVB Sepharose (Cytiva®).

DoE design space

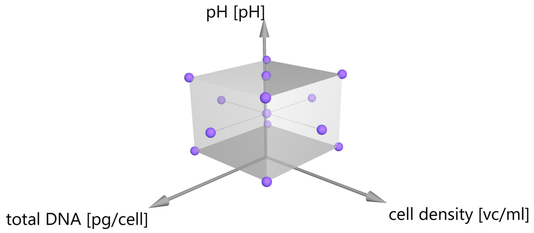

Figure 1. Design region of the central composite orthogonal (CCO) design applied to optimize rAAV production parameters.

Cell density at transfection, total DNA per viable cell at transfection and pH setpoint of bioreactors during production were evaluated as factors to determine the impact on rAAV yield and quality. A full-factorial central composite orthogonal (CCO) design was applied.

Analytics

- Vector genome (vg) yields were determined by qPCR with transgene cassette specific primers.
- Capsid (cap) yields were determined by ELISA.
- Mispackaged plasmid-derived DNA impurities were quantified using qPCR specific for the AAV capsid (cap) and kanamycin resistance gene (kanR). Impurity levels were expressed as percentage of vector genome yield.
- Host-cell DNA -derived impurity levels were determined using droplet digital PCR specific for 18S ribosomal RNA gene. HCD impurity levels were calculated as fraction of vector genome yield.

Modelling of vg and cap yields

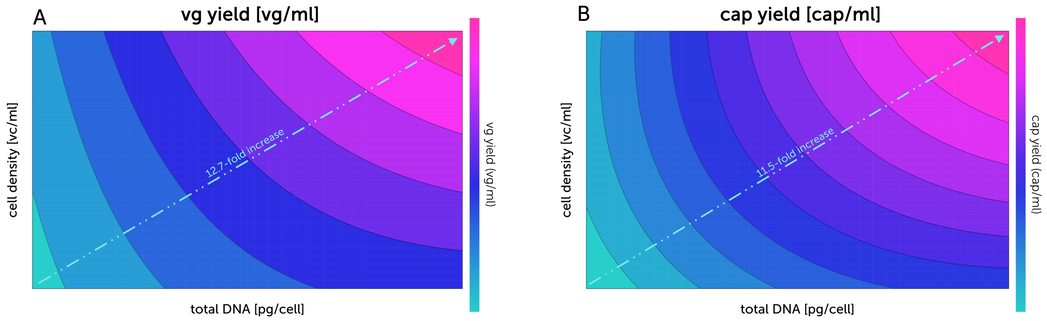

Figure 2. Response contour plots of (A) vg yield and (B) cap yield. The x-axes represent increasing total DNA [pg/cell] used for transfection. The y-axes represent increasing viable cell density at transfection [vc/mL]. All results are visualized as colors from lowest response (blue) to highest response (pink).

Vg and cap yields increased with higher cell density and total DNA per cell used for transfection. pH did not have an effect on vg and cap yields

Modelling of plasmid- and HCD-derived mispackaging

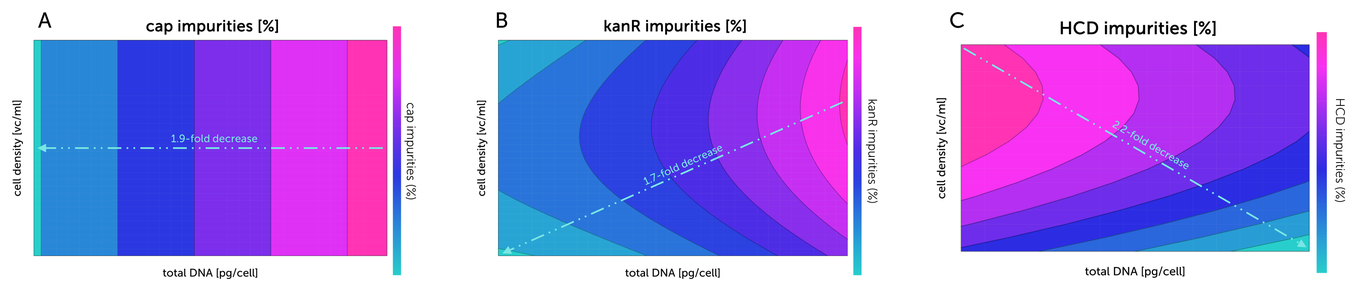

Figure 3. Response contour plots of (A) AAV cap gene plasmid derived impurity levels (B) kanR gene plasmid derived impurity levels and (C) HCD impurity levels. The y-axes represent increasing cell density at transfection [vc/mL]. All results are visualized as colors from lowest response (blue) to highest response (pink).

Cap gene impurity mispackaging increased only with increasing amount of DNA per cell used for transfection. KanR impurity mispackaging increased mainly with increasing amount of DNA per cell used for transfection and a minor effect of cell density was observed. Higher cell densities and lower total DNA for transfection increased HCD mispackaging. pH did not have an effect on cap, kanR and HCD impurities.

Summary

We present the results of a DoE-based study evaluating the impact of process parameters cell density at transfection, total DNA per cell at transfection, and pH setpoint of the bioreactors during production on rAAV yield and quality. We applied a full-factorial central composite orthogonal (CCO) design allowing for quadratic modelling of vector yields as well as plasmid- and host cell-derived DNA impurity packaging. Variation of cell density and total DNA per cell at transfection had a clear impact on both, rAAV yields (12.7-fold increase for vg yields and 11.5-fold increase for capsid yields) and plasmid (1.9-fold decrease for cap DNA impurities and 1.7-decrease for kanR DNA impurities) and HCD-derived impurity packaging (2.2-fold decrease).

These results allowed for the definition of key parameters and their set-points to enable further scale-up activities for our rAAV production processes to larger-scale suspension manufacturing systems (Ambr® 250, 2L and 50L). Furthermore, the study provided insight into opportunities to optimize rAAV quantity and quality by adjusting these parameters. Transferability for different serotypes and vector genome sizes has already been successfully demonstrated with comparable yields (data not shown). Overall, application of the DoE methodology to improve rAAV quantity and quality parameters confirmed usage of the Ambr® 15 system as a useful tool for our manufacturing process development, optimization and starting material development.

Aim higher

Ascend GCTx, Fraunhoferstr. 9b, 82152 Planegg, Germany

www.ascend-gctx.com
Kathrin.Breunig@ascend-gctx.com
Andreas.Schulze@ascend-gctx.com

Poster downloads
Password: AIMHIGHER