Enhancing the Upstream Performance of Adeno-Associated Virus (AAV) Vector Manufacturing via Multivariate Data Analysis

A. Apezteguia[1], M. Iglesias[1], I. Arangoa[1], J. Keune[1].
[1]Viralgen S.L, San Sebastian, Gipuzkoa, 20009, Spain.

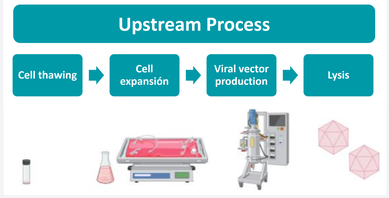
Abstract

Viralgen, a prominent AAV Contract Development and Manufacturing Organization (CDMO), has developed a comprehensive proprietary dataset from over 1000 batches produced using the Pro10 production platform. This study aims to enhance the AAV upstream process by analyzing which parameters significantly affect titer. The complexity of biological processes and the multitude of variables complicate process optimization, as traditional univariate techniques fall short in capturing multi-variable relationships.

To tackle these challenges, dimensionality reduction methods were employed, resulting in a multivariate data model based on 163 historical batches. Key findings reveal that higher VCD correlates with lower pH and glucose levels before transfection and impacts negatively to productivity.

Experiments confirmed the significant influence of viable cell density (VCD) and identified its optimal levels in relation to titer, To integrate this multivariate analysis into daily operations, an automated application was developed to identify factors affecting titer in historical batches. This tool will assist Manufacturing Sciences & Technology and R&D scientists in continuously reviewing performance and identifying process improvements.

Introduction

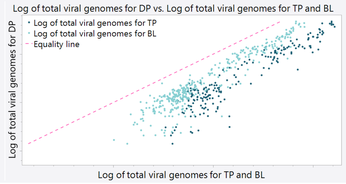


The field of cell and gene therapy (CGT) holds significant potential for treating various diseases, including genetic disorders and cancer. The diverse range of products, coupled with the small quantities needed for treatment and the high costs involved, underscores the importance of platform data for understanding process variability.

The following aspects are inherent to AAV industry:

- High number of sources of variability: e.g., manual manufacturing steps, raw materials, AAV product variety, analytical methods…
- Process complexity: critical process parameters are interrelated. E.g., viable cell density, metabolite concentration, pH…

The productivity of the batch at the end of the upstream process significantly impacts the chances for high drug product (DP) productivity. The TP is the first point at which the titer is measured and is closest to the transfection process. After clarification, a sample known as the BL is taken and the titer is measured again



Methods

Multivariate data analysis was conducted using Python[1)] and SIMCA[2)] software to identify upstream process improvements amidst high dimensionality and variability.

Two datasets were built: one containing data from 112 batches with titer measured after harvest and prior to lysis, and another with data from 94 batches with titer measured after clarification.

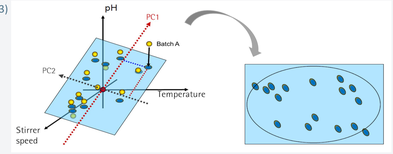
Dataset Composition:

- Data from batches manufactured using the fed-batch approach at scales of 50L, 250L, 500L, and 2000L.
- Includes process parameters measured off-line and in-line, from cell thawing to viral vector production. Off-line measurements in the vessels and transfection bioreactor were conducted daily, sometimes twice a day.
- Independent variable productivity defined as titer obtained via Integrated TaqMan Reverse Transcription Droplet Digital Polymerase Chain Reaction (ITRddPCR) method.

The comprehensive dataset serves as the foundation for our analytical techniques:

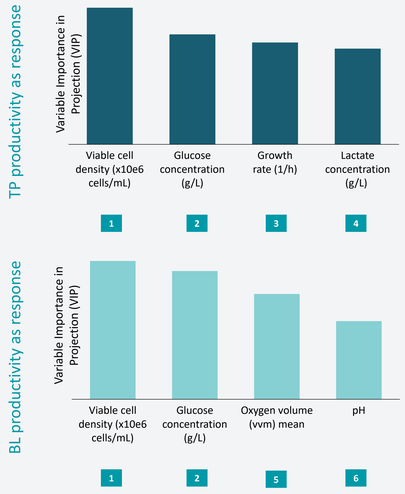
- Exploratory Data Analysis (EDA)
- Principal Component Analysis (PCA)
- Partial Least Squares (PLS) Modeling

3)

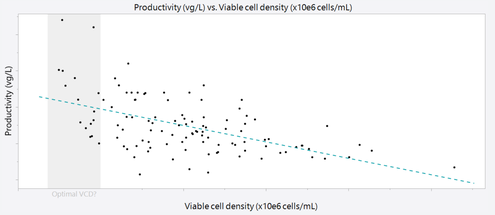


Results

Most Important Process Parameters in Relation with Productivity



1 Viable cell density (x10e6 cells/mL): significant negative correlation with ITRddPCR productivity.



2 Glucose concentration (g/L): significant correlation with ITRddPCR productivity..

3 Growth rate (1/h): significant correlation with ITRddPCR productivity.

4 Lactate concentration (g/L): significant correlation with ITRddPCR productivity..

5 Oxygen volume (vvm) mean: significant correlation with ITRddPCR productivity.

6 pH: significant correlation with ITRddPCR productivity.

Model Deployment for Daily Analysis: Why was Batch A Better Than B?

Several departments in Viralgen can understand which have been the process parameters that according to the model have impacted the productivity of a batch in comparison to others via Power BI[4)] dashboards. In addition, they can look at the behavior of the parameters of interest during the last stage of the cell expansion as well as the viral vector production phase.

3)

Select two of the most recent batches, e.g., batch A with worse productivity vs. batch B

Evaluate why one batch performed better (+) / worse (-), e.g. VCD more favorable for batch B

Review the corresponding data across time, e.g., VCD lower for batch B

Discussion

Key Findings

- VCD in the transfection bioreactor is significantly and negatively correlated with productivity.
- Cell counter measurement inaccuracies were observed at high cell densities, leading to efforts to improve measurement accuracy through new equipment and methods.
- Experiments were designed to identify the optimal seeding density and study the impact on the rest of the process parameters such as DNA concentration of the transfection cocktail[5)].
- Experiments were designed to test glucose injection and media supplementation strategies.
- The growth rate, lactate concentration, and oxygen volume (vvm) were identified as important parameters for predicting the productivity of a batch. Understanding the interplay between these factors can help refine production strategies and improve overall yield.

Next steps

Future enhancement of the model will include the addition of transfection process information as well as full/empty ratio estimation.

In addition, we plan to extend the model to explore mechanistic modeling, incorporating information on metabolic pathways and the virus production process. This will provide a more comprehensive understanding of the interactions and dynamics involved in cell expansion, transfection and virus production.

Conclusion

The analysis reveals several significant correlations between key parameters and ITRddPCR productivity. These findings underscore the importance of monitoring and optimizing these parameters in future production processes to improve overall yield.

References

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2) Umetrics. (n.d.). SIMCA. https://www.umetrics.com/simca
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