Simplifying Progress

# Expediting Lentiviral Vector Development with Automated Bioreactors and Design of Experiments

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## Introduction

Development of new lentiviral vector (LV) bioprocesses is often slow due to the difficulty in establishing optimal values for critical process parameters and the large time required to run analytical assays. Adding to the development time is the extended work required with external manufacturing partners. The ability to make accurate, data-driven product estimations can greatly speed up development timelines.

Leveraging tools and expertise from Sartorius, Seattle Children's Therapeutics (SCTx) developed a new in-house LV upstream production process for the SCTx VectorWorks program. The application of design of experiments (DOE) in combination with automated multi-way bioreactors with Ambr® 15 supported rapid and efficient experimentation to identify ideal operating conditions that were able to be directly scaled to a 50 L Biostat STR® bioreactor.

## Experimental Approach

An Ambr® 15 24-vessel bioreactor system was used for all small-scale cell growth and LV production experimentation. Cell growth, viability, and analyte measurement was performed via BioProfile® FLEX2. LVs were produced in HEK293 cells, transiently transfected, and infectious titers were calculated by LV transduction on H9 cells measured using flow cytometry. Scale-up results were validated in a 50 L Biostat STR® bioreactor. Scale-up calculations were performed on Sartorius BioPAT® Process Insights software.

Experimental design was done using DOE methodology in MODDE® software to ensure statistically valid experimental planning. DOE was used to systematically explore a variety of controlled parameters in the Ambr® 15 bioreactors and other growth and transfection conditions, including DNA load, PEI:DNA ratio, pH, DO, stir speed, media concentration, and more.

**Figure 1:** *The Creation of a New LV Upstream Production Process Utilized a Scalable Bioreactor Platform With Ambr® 15, Biostat STR® 50 L, and Umetrics® Suite Data Analytics Software*

## Results

### Effect of process conditions on lentiviral vector production

Early experiments were focused on identifying bioreactor conditions that yielded high lentiviral vector production using the HEK293 cell line. Using the Ambr® 15, up to 24 experiments were simultaneously performed in a structured manner using factorial or multilevel DOEs from MODDE® software. The factors that were interrogated over a number of experiments included pH, DO, and stirring speed. To interpret the results from designed experiments, it is important to fit a model that objectively measures against stated goals. The design space plot shows the favorability of the full design space toward a goal of improving infectious titer. The fit model clearly shows an optimal operating region for future experiments. The observed-versus-predicted plot shows strong predictive power for the fit model.

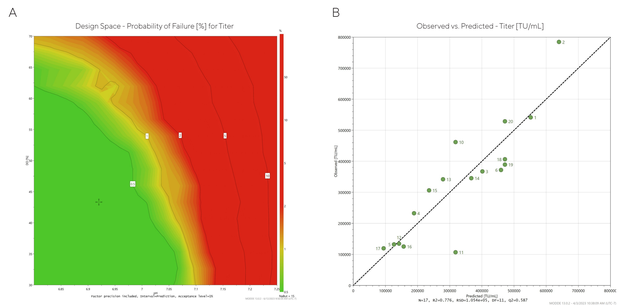


**Figure 2:** *A) A Design Space Plot From MODDE® Software Showing the Optimal Experimental Conditions for pH, DO, and NaBut to Yield Target Titer Levels.*
*(B) Observed-Versus-Predicted Plot That Displays the Observed Experimental Values Compared to the Fitted Values for the Fit Model. $R^2 = 0.776$*

### Optimizing lentiviral vector production

After establishing the bioreactor and media conditions that are optimal for lentiviral vector production over a series of designed experiments, further experimentation was performed to identify the optimal conditions for lentiviral production, as measured by final titer. The results are shown here. The first plot shows a design space plot for the probability of failure for achieving target titer when varying total DNA content and PEI:DNA ratios. The second shows the observed-versus-predicted plot that compares the observed experimental values to the fitted values for the fit model. Data for models such as these were able to be performed in a single programmed experiment with the automated bioreactor platform. Each designed experiment can optimize multiple parameters at once, accelerating development time.

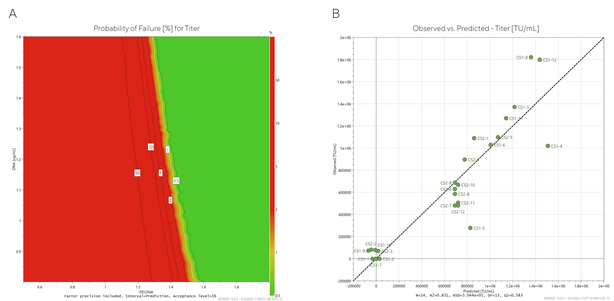


**Figure 3:** *(A) A Design Space Plot From MODDE® Software Showing the Probably of Failure for Achieving a Target Titer When Varying Total DNA Content and PEI:DNA Ratios.*
*(B) Observed-Versus-Predicted Plot That Displays the Observed Experimental Values Compared to the Fitted Values for the Fit Model. $R^2 = 0.831$*

### Confirmation of large-scale production success

After all parameters were optimized, the lentiviral platform was ready for scaled-up production at the 50 L, a 3,000x increase that was possible through BioPAT® Process Insights scaling calculations. With the inputs of the parameters that were optimized on the Ambr® 15, Process Insights yielded the associated control parameters for 50 L production in the Biostat STR® bioreactor. The chart below shows the progression from the earliest achieved infectious titer to optimized production in Ambr® 15 scale. Results from manufacturing scale show further improved infectious titer.

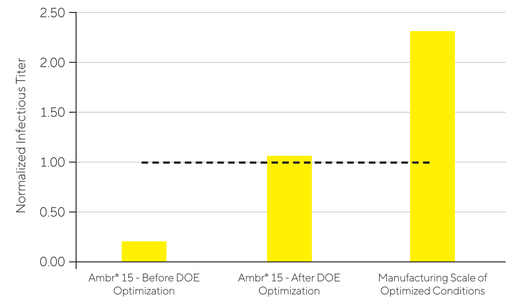


**Figure 4:**
*Infectious Titers Achieved From the Earliest Ambr® 15 Experiment, the Optimized Ambr® 15 Experiment, and 50 L Batch Utilizing Parameters Identified in Ambr® 15 With Samples Collected 48 h Post-transfection*

## Conclusion

DOE methodology and automated multi-way bioreactors sped up the development timeline of a new LV platform by testing and optimizing multiple factors in each experiment. After development at the 15 mL scale, a 50 L scale-up experiment was performed to confirm results. First phase titers at 50 L scale were comparable to those produced at 15 mL, and the final optimized process improved upon the initial results. The use of data-based software and automated multi-way bioreactors enabled rapid development of a new LV manufacturing process by reducing the number of experiments and time required to complete development and enabling direct scale-up from 15 mL to 50 L. This project design allows for rapid deployment of the process for initial GMP facility operations to happen in tandem with rapid process optimization experiments.

## Acknowledgements

Seattle Children's Therapeutics VectorWorks and Sartorius teams.
The authors acknowledge that David Ede and Finn Watt contributed equally to the work.

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