The use of ShakeReactors™ for optimisation, characterisation, and predictive modelling in lentiviral vector bioproduction

An integrated study on scalable and efficient lentiviral vector production

Whitepaper

July 2026

Executive summary

As bioprocessing workflows demand faster development, greater scalability, and reduced cost, innovative platforms are required to bridge the gap between early-stage experimentation and manufacturing-scale performance. This study demonstrates that atSpiro ShakeReactors™ provide a powerful, cost-effective solution for scalable process development, specifically for lentiviral vector production.

Our evaluation shows that ShakeReactors™ deliver robust, precise, and highly reproducible performance, matching historical lentiviral production outcomes while significantly improving throughput compared with traditional shake flasks. Critically, they capture key bioreactor-like characteristics—most notably pH control and metabolic behaviour—without the complexity, expense, or footprint of stirred-tank systems.

By combining mechanistic modelling with advanced statistical optimisation, we show how ShakeReactors™ can be used not only to optimise critical process parameters (such as pH, cell density, media type, and transgene selection) but also to predict performance across multiple scales, from ShakeReactors™ to ambr®250 and from benchtop up to 5 L bioreactors. This hybrid modelling approach enables confident in silico exploration of previously untested conditions, accelerating development timelines and reducing experimental risk.

Most importantly, the ShakeReactors™ demonstrated clear scalability and translatability to larger-scale stirred-tank systems. Optimisation performed on ShakeReactor™ reliably predicted metabolic profiles and process behaviour at the 5-L bioreactor scale, validating ShakeReactors™ as a true scale-down model—not merely a screening tool.

In summary, atSpiro ShakeReactors™ uniquely combine the simplicity and low cost of shake flasks with the predictive power of bioreactors, enabling faster decision-making, fewer scale-up surprises, and more efficient development of viral vector processes. This positions ShakeReactors™ as a compelling platform for organisations seeking to accelerate bioprocess innovation while controlling cost and risk.

Background & rationale

OXB is one of the pioneers of cell and gene therapy, founded in 1995 as a spin out from the University of Oxford, UK. Today, OXB operates as a quality and innovation-led contract development and manufacturing organisation (CDMO), providing extensive expertise in the development and manufacture of lentiviral vectors (LVVs) and adeno-associated virus (AAV) products.

atSpiro is a biotechnology startup focused on addressing a well-recognised challenge in bioprocess development: the disconnect between shake-flask experimentation and stirred-tank bioreactor performance. Its flagship platform, the ShakeReactor™, is a smart laboratory device that incorporates patented sensor technology, transforming standard shake flasks into miniature bioreactor-like systems. The platform enables real-time online monitoring of pH and dissolved oxygen (DO), as well as automated feeding, all within a standard shaking incubator.

Bioprocess development for cell and gene therapies is inherently complex, involving multiple interacting biological, chemical, and engineering variables. Large-scale bioproduction is particularly resource-intensive, driven by high reagent costs and the operational complexity of conventional bioreactor systems. As a result, early-stage feasibility studies and process optimisation activities are typically conducted at shake flask scale, using working volumes ranging from approximately 10–250 mL.

Shake flasks are widely adopted at this stage because they are simple to operate, require minimal specialist training, support parallel experimentation across many conditions, and minimise the consumption of costly reagents. However, conventional Shake flasks lack active control or monitoring of critical process parameters such as pH and DO. This limitation leads to systematic differences relative to bioreactor operation, particularly in cell growth, metabolism, viral vector yield, and impurity profiles, including residual host-cell DNA and proteins.

The ShakeReactor™ platform

The ShakeReactor™ was developed to address these limitations by introducing key bioreactor-like capabilities at shake flask scale. By enabling real-time measurement and control of pH and monitoring of DO, ShakeReactors™ bridge the operational gap between SFs and stirred-tank bioreactors (Figure 1).

Shake Flask | Stirred-tank bioreactor | ShakeReactor

Parameter	Shake Flask	Stirred-tank bioreactor	ShakeReactor
DO Control	✗	✓	✗
DO Monitoring	✗	✓	✓
pH Control	✗	✓	✓
pH Monitoring	✗	✓	✓
Real Time Analysis	✗	✗	✓
Agitation	Shaking Incubator	Propeller	Shaking Incubator

Figure 1: Summary of bioprocessing. Shake flask (left) vs bioreactor (centre) vs ShakeReactor™ (right). Differences between the shake flask, the bioreactor, and the ShakeReactor™.

The ShakeReactor™ comprises a standard shake flask fitted with integrated sensors, wireless motorised controllers, and three sterile liquid reservoirs, all housed within a reusable sleeve. The reservoirs can be configured to deliver acid, base, and/or nutrient feeds, and the system can be freely programmed to add defined volumes from any combination of reservoirs. A typical configuration includes an acid and a base for automated pH control, along with a third reservoir for nutrient supplementation.

Importantly, ShakeReactors™ can be operated within the same incubator and under identical environmental conditions as conventional shake flasks. This enables direct, side-by-side comparisons of controlled versus uncontrolled pH operation at identical scales, an experimental setup that is not feasible using conventional bioreactor systems. Multiple ShakeReactor™ units can be accommodated within a standard shaking incubator, all of which communicate wirelessly with a central data hub positioned externally on the incubator (Figure 2). This architecture allows real-time data acquisition and monitoring while maintaining a high-throughput, small-footprint workflow.

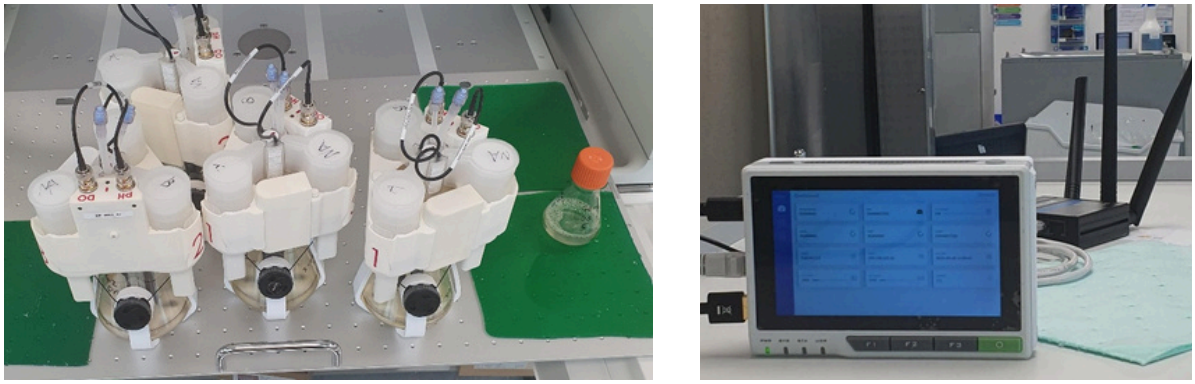


Figure 2: ShakeReactors™ in incubator (left), datahub for monitoring and control (right).

Study objectives

Given the functional similarities between ShakeReactors™ and stirred-tank bioreactors, this study evaluated the suitability of the ShakeReactor™ platform for lentiviral vector production. Prior studies have demonstrated that pH is a critical determinant of lentiviral vector productivity, influencing both transduction unit titres per millilitre (TU/mL) and cellular metabolic behaviour (Williams-Fegredo et al., 2024). We therefore hypothesised that ShakeReactors™ could be used as an effective scale-down model to support both mechanistic and statistical process development. Specifically, the objectives of this work were to:

1. Assess the reproducibility and performance of lentiviral vector production in ShakeReactors™ relative to historical OXB datasets.
2. Develop mathematical and statistical models to optimise TU/mL by manipulating pH and other key variables at the ShakeReactor™ scale.
3. Use these models to predict performance under both known and novel operating conditions at larger bioreactor scales.

The results of this work provide insight into the transferability of ShakeReactor™-based cultivations to larger-scale bioreactors, supporting their application as a predictive and cost-effective tool for bioprocess development. The experimental methods, modelling approaches, and outcomes are described in detail in the following sections.

Practical methods

ShakeReactor™ experiments were split into 8 runs, each one with varying pH, cell density, media ID or transgene ID. This study aimed to answer 4 primary questions.

1. Could the ShakeReactor™ generate lentiviral vector in a reproducible and robust fashion?
2. Could the ShakeReactor™ control cellular growth in a similar fashion to conventional bioreactors?
3. Could the ShakeReactor™ be used to optimise pH profiles and key variables (e.g., seeding density), with results that are scalable to larger conventional bioreactors?
4. Could the ShakeReactor™ be used to build a mechanistic model and predict bioreactor experiments?

The general experiment schema is given in Table 1. Each part was designed to answer one or more of the questions above, with Exp 1 and 2 assessing the feasibility of the ShakeReactor™ for making lentiviral vectors and growing cells in an acceptable manner. Experiments 3 onwards were used to develop both statistical and mechanistic models and to evaluate them at ShakeReactor™ and bioreactor scales.

Table 1: Experiment summary table

Experiment #	Variables explored	Scale	Purpose
1	Media type	Flask	Feasibility
2	Media type	Flask	Feasibility
3	Seeding density / pH profile / Media type	Flask	Development
4	Seeding density / pH profile / Media type	Flask	Development
5	Seeding density / pH profile / Media type	Flask	Development
6	Seeding density / pH profile / Media type	Flask	Development
7	Seeding density / pH profile / Media type	Flask	Predictive modelling
8	Unknown media	Flask	Predictive modelling
9	Unknown transgene	ambr®250	Predictive modelling
10	Unknown transgene	Bioreactor	Predictive modelling

Regardless of the purpose, each procedure was conducted in the same manner as illustrated in Figure 3.

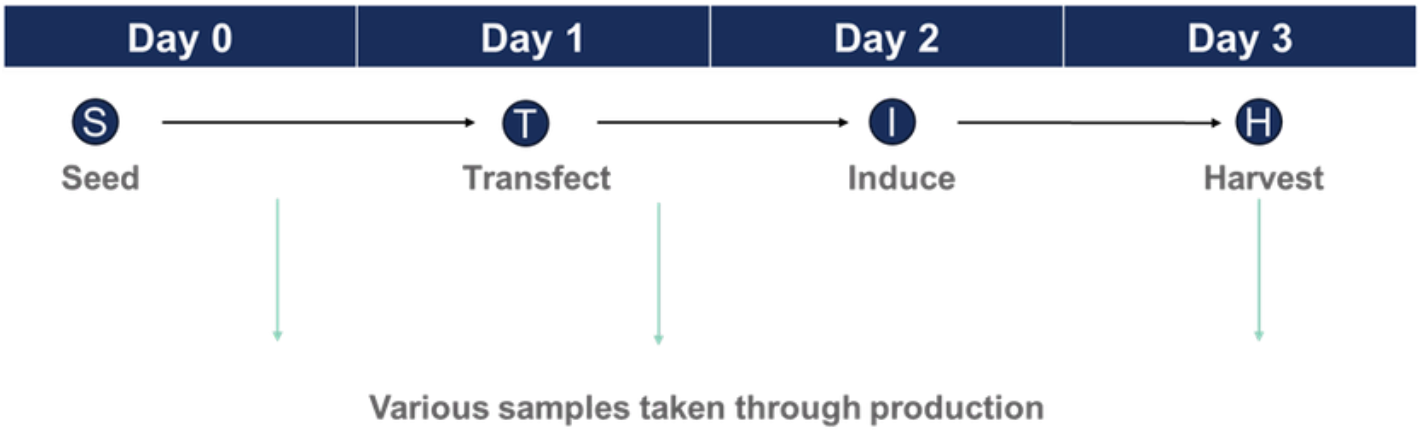


Figure 3: Experiment Workflow. Visual walkthrough of each experiment from seeding at day 0 to harvest at day 3.

Statistical methods

General

Feasibility and robustness were assessed via t-tests and summary statistics. Principal Component Analysis (PCA) was used for data exploration. Data-driven optimisation was carried out via Hierarchical Mixed Model (HMM); assay ID was used as a blocking variable in order to control for assay-to-assay variation; media, transgene and pH regime were assessed as categorical variables, and seeding density was assessed as a continuous variable.

Mechanical model framework

Mechanistic modelling was set up by extending the Monod equation to account for pH and TU/mL. The final model had one compartment, over 15 parameters, and was based on an Ordinary Differential Equation (ODE). To optimise the parameters, the Broyden–Fletcher–Goldfarb–Shanno algorithm was used, with upper and lower bounds set based on expert guidance.

Figure 4 illustrates the overall structure of the model ODEs. Firstly, glucose causes cells to divide, and the cells convert glucose to lactate, which, being acidic, lowers the pH of the media. Changes in pH subsequently affect the cells and, by proxy, glucose and lactate. On the right-hand side of the diagram, we have the pH control, which externally alters pH and, in turn, cellular metabolism. This is not a factor in shake flasks because they lack a mechanism to control pH.

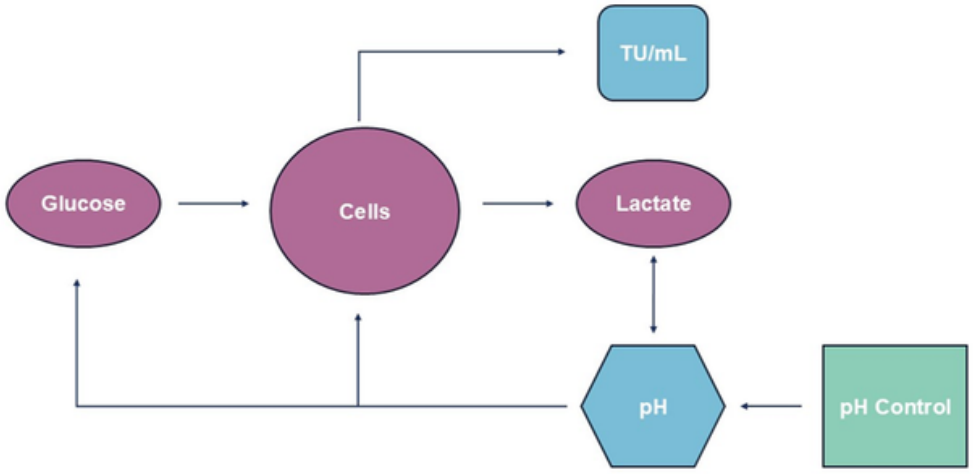


Figure 4: Mechanistic Model Graphical Representation. Visual representation of the mechanistic model illustrating the system's main effects.

Feasibility & robustness of the ShakeReactor™ as a viral vector platform

Feasibility

The initial two studies evaluated whether ShakeReactor™ cultivations could reliably produce lentiviral vectors under routine pH-controlled conditions across two distinct media formulations, referred to here as Media A and Media B. Conventional shake flask processes were conducted in parallel as controls.

The ShakeReactor™ platform supported robust cell growth in both Media A and Media B, maintaining expected metabolic behaviour across a range of pH control strategies. These strategies included continuous regulation at a mid-range pH set point, as well as a dynamic pH-shift approach in which the culture was loosely maintained at a mid-to-high pH prior to a tightly controlled transition to a lower pH. Continuous pH control at the mid-range set point resulted in metabolic profiles closely aligned with standard shake-flask processes, whereas dynamic pH reduction induced a distinct metabolic shift, characterised by reduced cell growth and slower lactate accumulation. Representative results are shown in Figure 5.

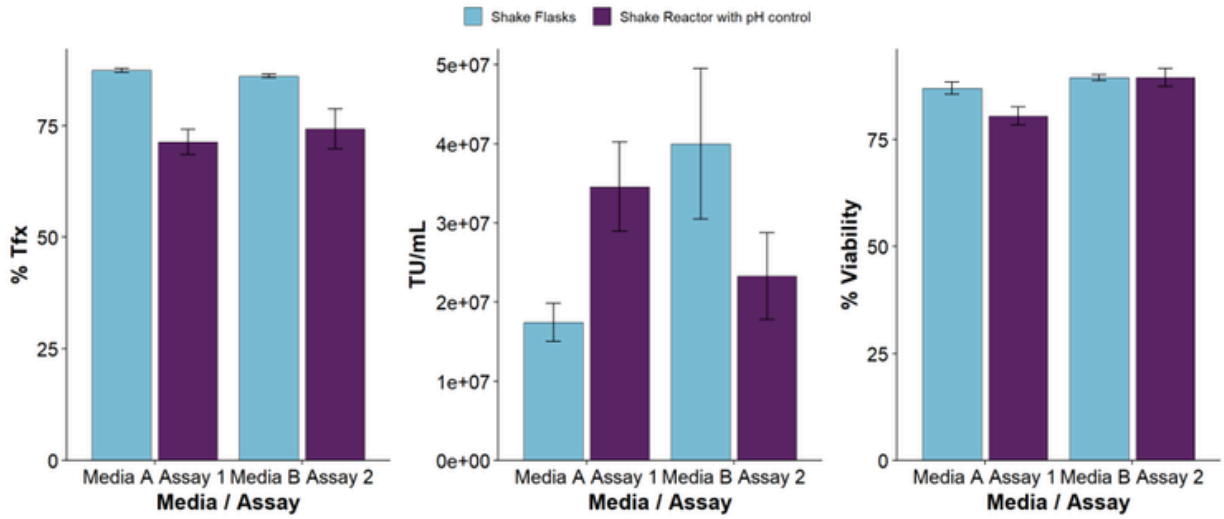


Figure 5. Comparison of ShakeReactors™ (Purple) and shake flasks (Blue) performance on % transfection efficiency (left), TU/mL (centre) and % cell viability at harvest (right). Facets are separated into Assay 1 (Media A) and Assay 2 (Media B).

Across all conditions, the ShakeReactor™ achieved high transfection efficiency (>70%), final lentiviral titres exceeding 2 × 10^7 transduction units per mL (TU/mL), and strong cell viability at harvest (>75%). When compared with shake flask controls, ShakeReactor™ cultivations exhibited a modest reduction in transfection efficiency. In Media A, mean transfection efficiency decreased from 87.4% (CV: 0.47) in SFs to 71.4% (CV: 3.94) in SRs, while in Media B, the corresponding decrease was from 86.2% (CV: 0.49) to 74.3% (CV: 6.05).

Despite this reduction, ShakeReactor™ cultivations in Media A achieved approximately two-fold higher TU/mL relative to shake flask counterparts, whereas the opposite trend was observed in Media B. While the underlying cause of this difference remains unclear, it is likely attributable to a more favourable response of Media A to active pH control, consistent with historical pH optimisation efforts conducted using this formulation. Importantly, no meaningful differences in final cell viability were observed between the ShakeReactor™ and shake-flask processes.

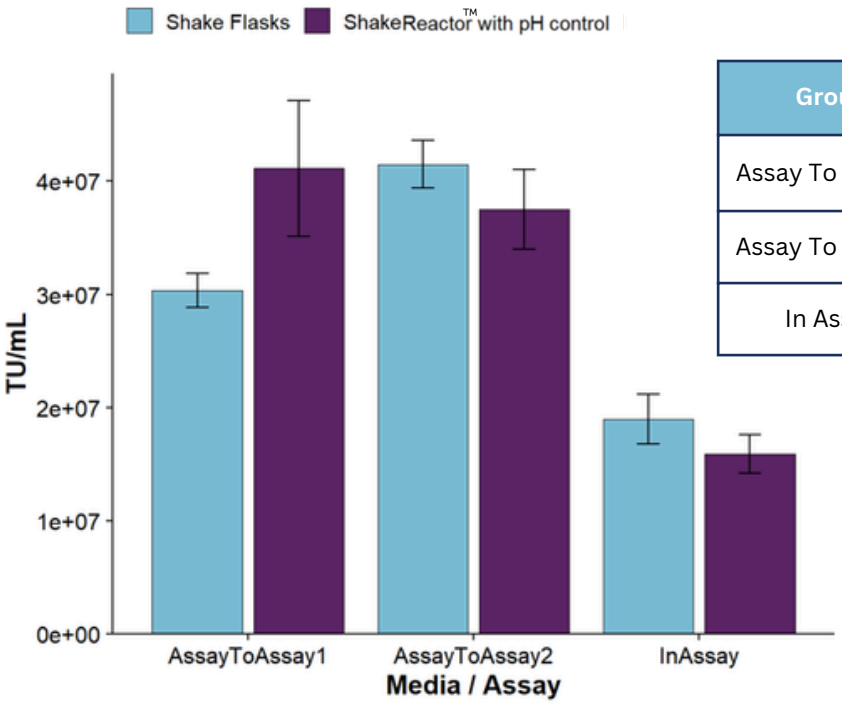
Overall, these findings demonstrate the feasibility of the ShakeReactor™ platform for lentiviral vector production under controlled pH conditions and highlight its ability to modulate metabolic behaviour in a manner distinct from conventional shake flasks.

Robustness

To assess the accuracy, precision, and robustness of the ShakeReactor™ platform, a defined subset of samples was selected for detailed comparison. Six samples, comprising three matched pairs, were analysed across multiple assays. These samples were deliberately distributed across different seeding densities and pH control profiles to ensure a representative and statistically meaningful evaluation of platform performance.

Table 2: Samples highlighted for accuracy, precision and robustness purposes; the first 5 columns give sample information. The last 2 indicate how samples were allocated into testing groups.

Assay ID	Vessel ID	pH	Media	Cells	Replication purpose	Sample ID
1	2	Control	Media A	1 x	In Assay	A
1	4	Control	Media A	1 x	In Assay	B
3	1	Drop	Media A	1 x	Assay To Assay 1	A
7	1	Drop	Media A	1 x	Assay To Assay 1	B
3	2	Drop	Media B	1.35x	Assay To Assay 2	A
4	2	Drop	Media B	1.35x	Assay To Assay 2	B



Group	Overall %CV	p-value
Assay To Assay 1	20	0.13
Assay To Assay 2	8	0.3
In Assay	14	0.26

Figure 6: TU/mL and Statistics of Robustness and Reproducibility

Across all comparisons, lentiviral titres were consistent between SR and shake flask cultivations. All sample groups exhibited less than a two-fold difference in transduction units per millilitre (TU/mL), with coefficients of variation (%CV) below 25%. In addition, all statistical comparisons yielded p-values greater than 0.05, indicating no statistically significant or practically meaningful differences in TU/mL between ShakeReactor™ and shake flask conditions.

Viral vector production in the ShakeReactor™

Taken together, these results demonstrate that the ShakeReactor™ is both a feasible and robust platform for lentiviral vector production under controlled pH conditions across multiple media types. The ShakeReactor™ enables reproducible performance comparable to conventional shake flasks while providing enhanced process insight and control, with substantially lower operational complexity than stirred-tank bioreactor systems.

Building on this validated foundation, the next section leverages the ShakeReactor™ platform to develop mechanistic models capable of predicting larger-scale stirred bioreactor behaviour and establishing quantitative links between ShakeReactor™ performance and bioreactor operation.

Differences in metabolism between shake flask & ShakeReactor™

Effect of pH control on cell growth and metabolism

Here, we summarise the core experimental datasets used to inform model development and highlight the most informative trends. Figure 7 presents cell growth and metabolic profiles from Assay 3, in which media identity, cell seeding density, and pH control strategies were systematically varied.

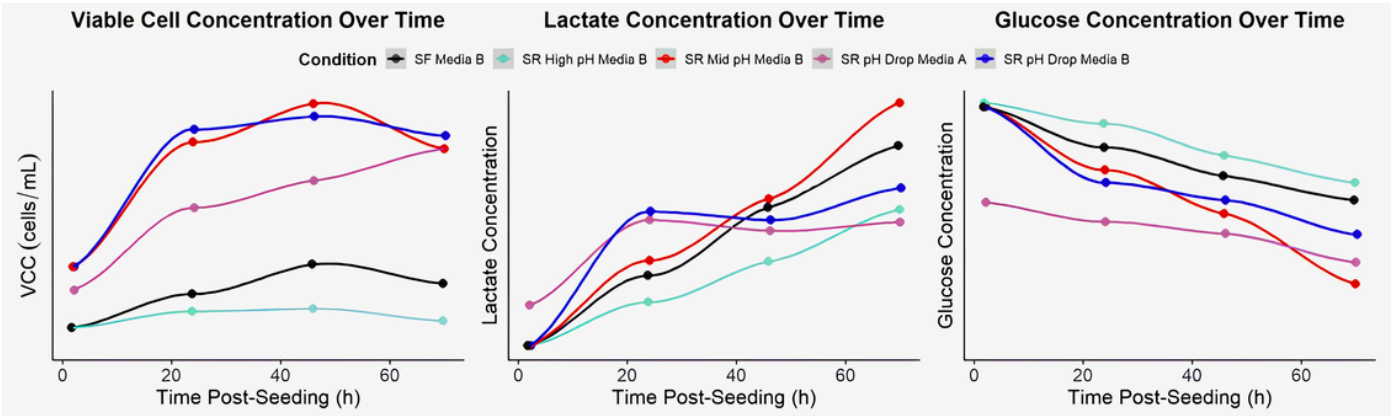


Figure 7: Viable Cell Counts (VCC) (left), Lactate (Mid) and Glucose (Right) Over Time – Assay 3

As shown, cultures initiated at low seeding density failed to reach the cell concentrations achieved by higher-density inoculation, remaining at least three-fold lower in viable cell concentration (VCC) throughout the process (Figure 8, black and aqua-green profiles). This lower biomass accumulation was reflected in reduced glucose consumption and lactate production. In addition, a clear metabolic shift was observed following implementation of a dynamic pH reduction at approximately 24 hours (blue and pink profiles), demonstrating that temporal pH manipulation can directly influence cellular metabolism. Furthermore, comparison of the black and green profiles highlights the inhibitory effects of elevated pH on both cell growth and metabolic activity.

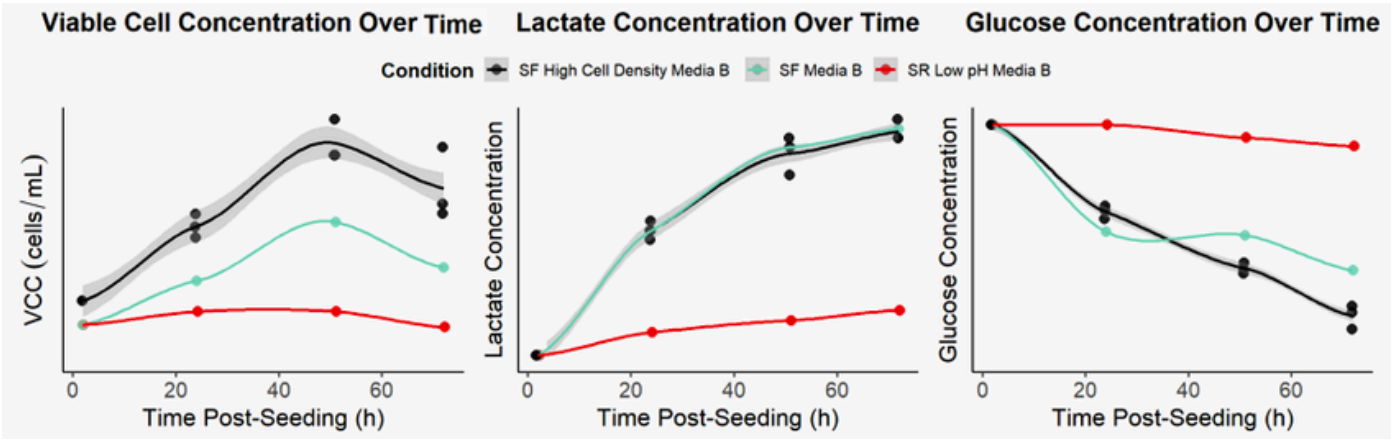


Figure 8: Viable Cell Counts (VCC) (left), Lactate (Mid) and Glucose (Right) Over Time – Assay 6

Building on these findings, the subsequent figure examines the impact of sustained high-pH conditions on growth and metabolism. As illustrated by the red profile, fixation at a low pH setpoint resulted in pronounced metabolic suppression, characterised by slower cell proliferation, reduced glucose uptake, and markedly lower lactate accumulation compared with non-pH-controlled cultures. These profiles closely resemble those observed under high-pH conditions. An additional trend evident in the data is that while elevated seeding density alone (black profile) substantially alters VCC, its effect on glucose and lactate profiles is minimal when compared with the standard seeding density control (aqua-green profile).

To further explore system-wide metabolic relationships, multiway correlation analysis was performed across all measured metabolites, with data filtered to end-of-production samples. Historic stirred-tank bioreactor datasets were included for contextual comparison.

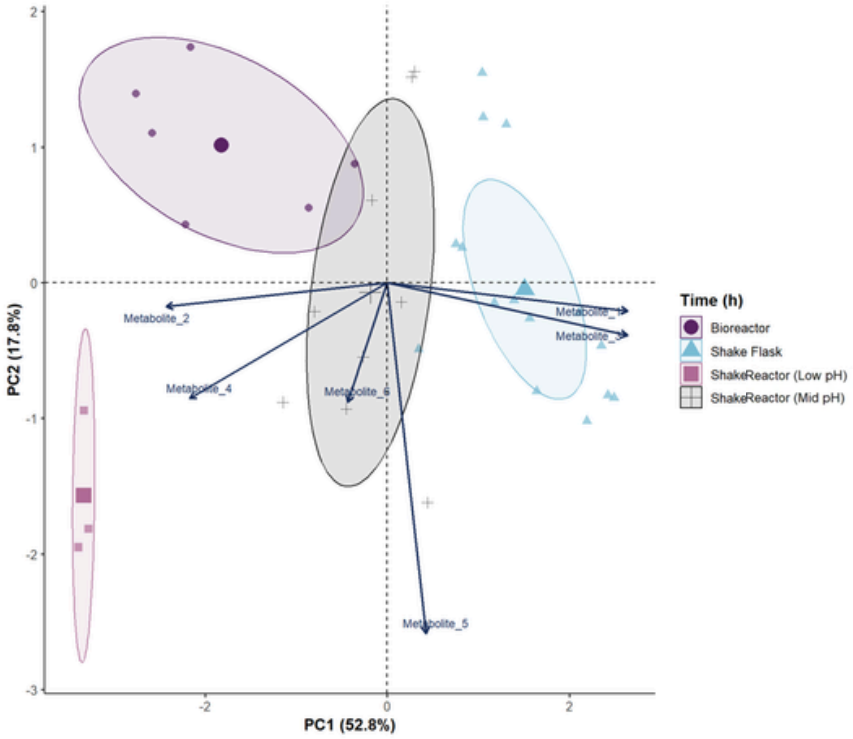


Figure 9: PCA Biplot of metabolites and cell properties, highlighted by shake flask or ShakeReactor™ or bioreactor, with 95% CI ellipses overlaid

As shown, conventional shake flask samples cluster distinctly on the far right of the scores plot (light-blue triangles), whereas ShakeReactor™ cultures operated at mid-range pH occupy a central position (black crosses). Both bioreactor samples (dark-purple circles) and low-pH ShakeReactor™ samples (light-purple squares) cluster on the left.

Principal component analysis indicates that the first principal component (PC1) accounts for over 50% of the variance among six key metabolites. The clear separation of shake flask samples from both ShakeReactor™ and bioreactor data indicates that pH control within the ShakeReactor™ enables a more bioreactor-like metabolic phenotype at small scale.

Collectively, Figures 7–9 demonstrate that pH control alone is sufficient to significantly alter cellular growth and metabolic behaviour, underscoring the critical importance of this parameter during early-stage bioprocess development and highlighting the value of controlled small-scale systems for predictive process modelling.

Predictive modelling of ambr®250 and 5L stirred bioreactor

Mechanistic modelling framework

Following the definition of the governing equation sets and parameter bounds, a mechanistic modelling framework was applied to all samples generated across the first six experimental studies. For each sample, model parameters were independently optimised within the predefined bounds, enabling systematic exploration of variability in kinetic behaviour and identification of subtle effects attributable to vessel type, seeding density, media formulation, and pH control strategy.

Marked differences in fitted parameter values were observed between Media A and Media B. To further interrogate these differences, principal component analysis (PCA) was performed on shake-flask samples only, with data stratified by media type to control for vessel-specific effects. The resulting PCA biplot illustrates how distinct parameter loadings—primarily associated with glucose consumption and lactate production kinetics—drive clear separation between the two media groups.

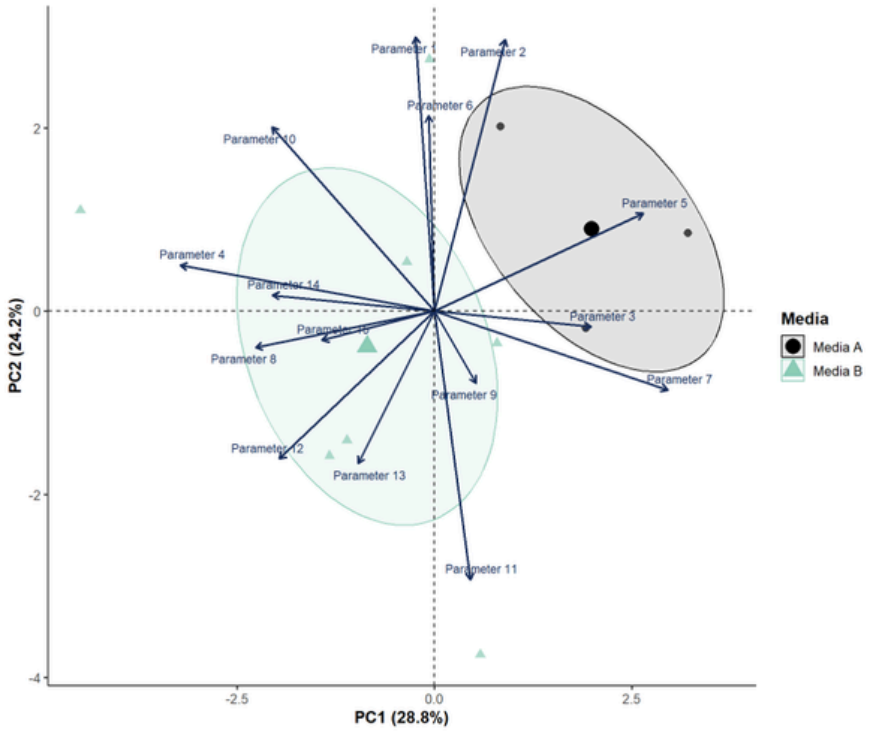


Figure 10: PCA Biplot of mechanistic modelling parameters highlighted by Media ID, with 95% CI ellipses overlaid.

As shown, the media formulations segregate strongly along the first two principal components, indicating a multivariate and mechanistically meaningful difference in metabolic behaviour. These differences are effectively captured by the mechanistic model, demonstrating its ability to exploit media-specific kinetic signatures for predictive purposes.

Following development, refinement, and extensive evaluation of model performance across the prospective design space, the mechanistic framework was used to predict process outcomes across multiple cultivation systems, including ShakeReactors™, ambr® 250-mL bioreactors, and 5-L stirred-tank bioreactors. This establishes a quantitative basis for translating small-scale experimental insights to larger-scale bioprocess operation.

A priori prediction of cultivations in ShakeReactor™, Ambr®250, and 5 L stirred bioreactors

Forward predictions were generated by systematically sampling parameter values from the fitted distributions to simulate hypothetical process conditions. For example, to represent a previously untested media formulation expected to exhibit characteristics intermediate between Media A and Media B, model parameters were interpolated between the fitted parameter distributions of the two media types. This approach constitutes a hypothesis-driven predictive framework, rather than a purely model-driven extrapolation, as all parameter selections are explicitly motivated by prior experimental knowledge and biological rationale.

The predicted experimental scenarios are summarised in Table 3. Three forward-prediction cases were evaluated: one at ShakeReactor™ scale using a previously uncharacterised media formulation, and two at large scale involving an unknown genome. In each case, model parameters were deliberately adjusted based on mechanistically justified assumptions regarding how these unknown factors were expected to influence process performance. This strategy enabled informed in silico exploration of novel conditions while maintaining transparency and interpretability of the underlying assumptions.

Table 3: Variables assessed in final experiments.

Scale	Media	Transgene
ShakeReactor™	Media C (unknown)	Genome A
ambr®250	Media A	Genome B (unknown)
5L Bioreactor	Media A	Genome B (unknown)

Predictions of new experiments in SR

As shown in Figure 11, the mechanistic model accurately predicts the performance of ShakeReactor™ for a previously untested media formulation. This predictive capability arises from the compositional similarity of the new media to Media A. Accordingly, bioproduction outcomes were forecast using parameters derived from Media A as a reference, systematically adjusting them based on informed assumptions about specific compositional differences. This approach enables reliable prediction of process behaviour under novel conditions while maintaining transparency in parameter selection and biological rationale.

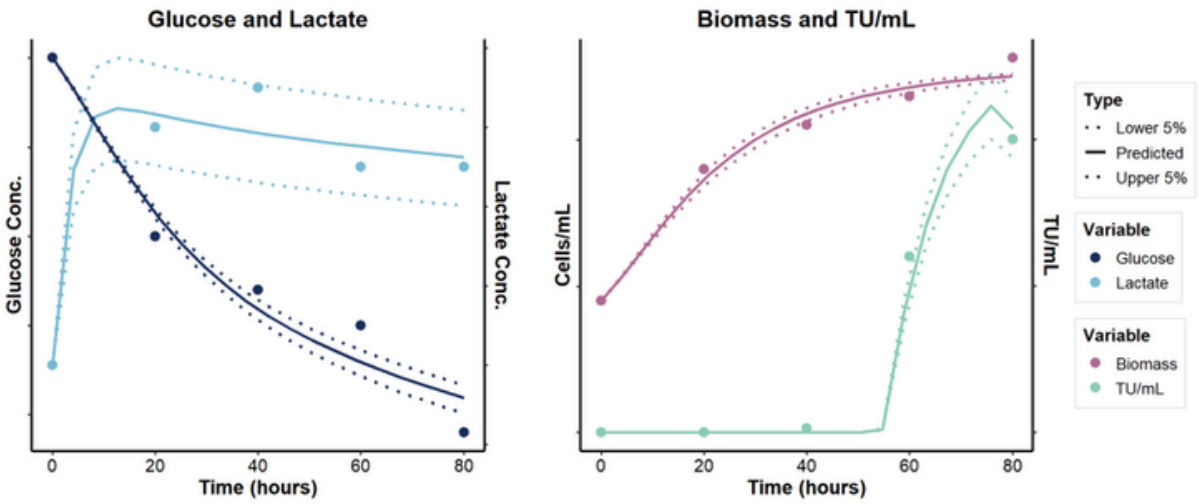


Figure 11: Predictions and experimental data of ShakeReactor™ scale LV production in media C.

Predictions of new experiments in ambr®250 bioreactors

We next extended the modelling framework to predict lentiviral vector production in the ambr®250 system. Model parameters were defined based on prior assumptions informed by ShakeReactor™ experimentation and the underlying mechanistic structure derived from SR cultivations. In addition to the change in scale, the transgene of interest was also varied, introducing an additional layer of novelty to the prediction challenge.

As shown in Figure 12, the model accurately reproduced ambr®250 performance across all predicted conditions. These results indicate that the ShakeReactor™ functions as a scalable and representative small-scale platform, and that both the governing equation set and parameterisations established at ShakeReactor™ scale can be successfully transferred to ambr®250 bioreactor systems. This further supports the suitability of ShakeReactor™-derived data for informing larger-scale bioprocess development and predictive modelling.

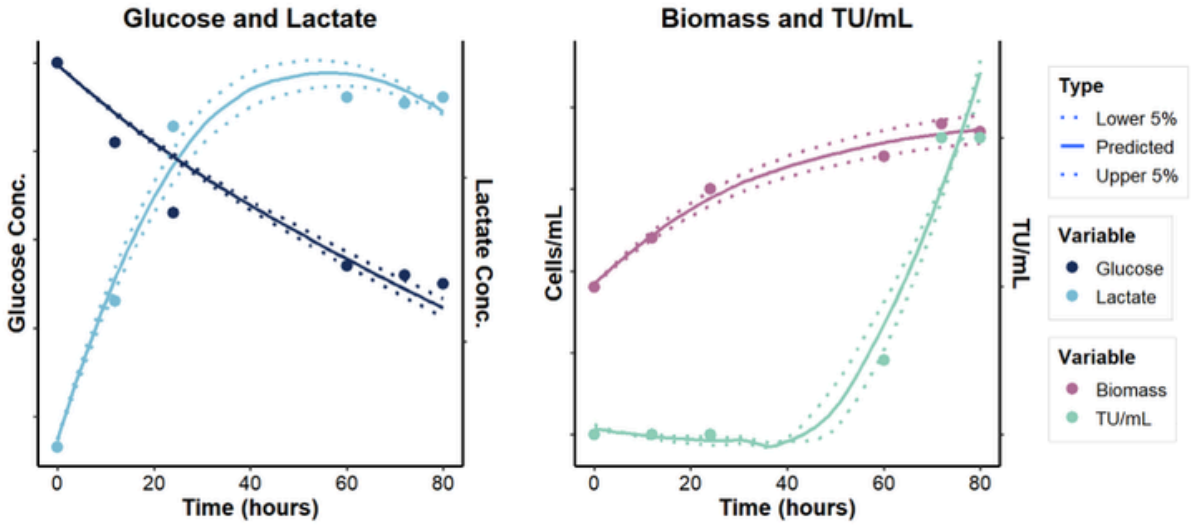


Figure 12: Predictions and experimental data of ambr®250 data using unknown genome

Predictions of new experiments in 5 L bioreactors

We subsequently generated final forward predictions for a 5 L stirred-tank bioreactor operating with Media A under a dynamic pH-drop strategy for lentiviral vector production. Given the strong predictive performance observed at the ambr®250 scale and the established empirical evidence that ambr®250 systems scale consistently to 5 L bioreactors, parameter selection required only minimal adjustments to the ambr®250 model configuration.

As shown in Figure 13, the model reproduced overall process behaviour with good fidelity. Some deviations were observed, most notably in glucose consumption and lactate accumulation around the 20-hour time point. These discrepancies may reflect factors not explicitly captured within the current model structure, such as differences in oxygen transfer, agitation regime, or scale-dependent hydrodynamics. Nevertheless, the predictions remained sufficiently accurate to support their practical utility.

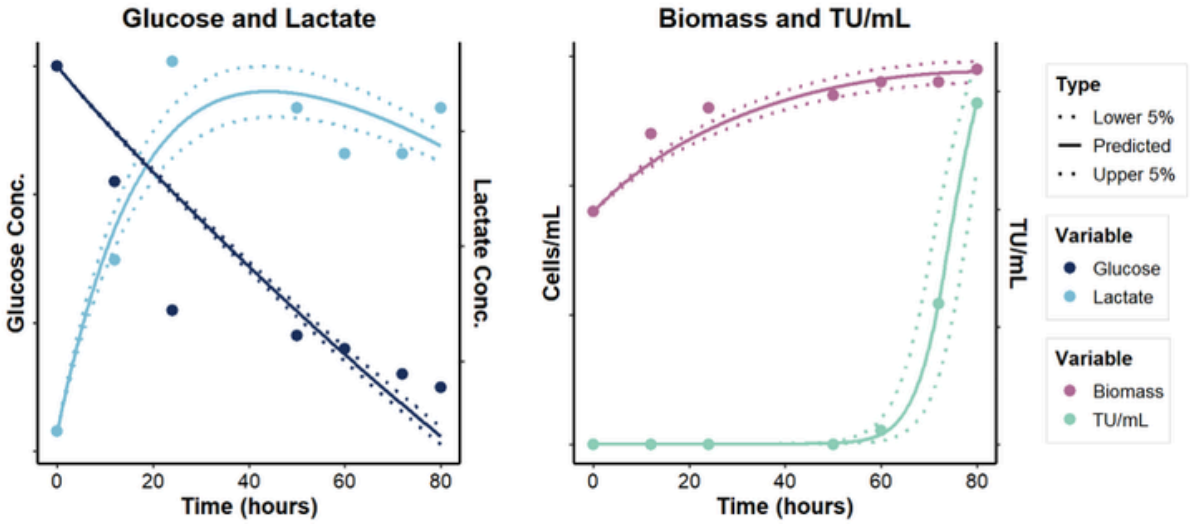


Figure 13: Predictions and experimental data of 5L data using an undisclosed genome.

Importantly, key performance indicators—including biomass accumulation and transduction unit titres (TU/mL)—were predicted with high accuracy. As these outputs are of primary relevance to viral vector manufacturing, the results demonstrate that a ShakeReactor™-derived mechanistic model can be effectively used to predict large-scale bioreactor performance, thereby validating its use as a scalable and informative platform for process development.

Conclusion

In this study, we evaluated the ShakeReactor™ platform for the production of lentiviral vectors in transiently transfected mammalian cell systems. Our results demonstrate that lentiviral vector titres achieved in the ShakeReactor™ are comparable to those obtained in conventional 5 L stirred-tank bioreactors. In addition to achieving equivalent productivity, the ShakeReactor™ delivered reproducible cell growth and metabolic profiles, reinforcing its reliability as a small-scale cultivation platform.

A key differentiating capability of the ShakeReactor™ is its ability to monitor and control pH, enabling tighter regulation of the cellular environment compared with traditional shake flasks. This capability enables the platform to serve as a cost-effective, information-rich optimisation tool, supporting the systematic evaluation of critical process parameters, such as pH control strategies and seeding density. Importantly, optimisation performed at ShakeReactor™ scale translated effectively to larger stirred-tank bioreactors, demonstrating strong scalability and predictive relevance.

Leveraging this scalability, we developed a mechanistic modelling framework that used ShakeReactor™ data to describe system behaviour and predict performance across multiple production scales. The model showed strong agreement with experimental data from larger-scale bioreactor runs, highlighting the ShakeReactor's™ value not only as an experimental system but also as a foundation for scalable, model-informed process understanding and decision-making.

Beyond its technical performance, the ShakeReactor™ offers significant practical advantages. The platform requires substantially less specialist training and operational expertise than conventional 5 L bioreactors and can be readily deployed within existing laboratory infrastructure. Unlike larger bioreactor systems, it does not require additional utilities, complex installation, or extensive calibration, further reinforcing its utility for early-stage development, feasibility studies, and rapid process optimisation.
Collectively, these findings position the ShakeReactor™ as a robust, scalable, and accessible platform for lentiviral vector process development, bridging the gap between shake-flask experimentation and conventional bioreactor manufacturing while reducing cost, complexity and development risk.

References

Thomas Williams-Fegredo, Lee Davies, Carol Knevelman, James Miskin, Kyriacos Mitrophanous, Qasim A. Rafiq (2024) Auto-transduction in Lentiviral Vector Bioprocessing: A Quantitative Assessment and a Novel Inhibition Strategy (Biotechnology and Bioengineering) https://doi.org/10.1002/bit.28834

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