Case study | Lentivirus

Improving manufacturing conditions: use of DOEs for optimal LV production

Context

OXB partnered with a client that had a T-cell directed CAR product and needed lentiviral vector (LV) supply for this product. The objective was to evaluate the LentiVector™ platform for their specific transgene to select process conditions that would maximise the LV yield.

The client was keen to maximise the number of potential patient doses and as part of the optimisation process wanted further to assess performance of the LV on T-cells. As T-cell protocols and requirements vary from product to product, OXB suggested examining a range of conditions using a Design of Experiments (DoE) approach to achieve optimal parameters for LV production. Performance of each condition was assessed at OXB. In addition, the vector was shipped to the client for examination in their own T-cell assays.

Our solution

A custom DoE was designed by OXB, based on a central composite design, analysing 4 factors to determine the best production conditions. Each of these 4 factors - amount of Genome, GagPol, and the VSV-G envelope plasmids, and the concentration of transfection reagent - was varied over 4 or 5 levels. The levels were chosen partly from our client's observation and experience, and partly from OXB historical data. Additionally, OXB assessed the impact of our LV titre enhancing technology: U1 snRNA. In total, the DoE consisted of 23 conditions each in duplicate. To ensure that the small-scale LV production was as similar as possible to the scale-down model bioreactor process, the cells used for LV production were obtained from the bioreactor on the day of transfection. LV from each condition was quantified for transgene expression by flow cytometry and for LV integration following transduction onto both HEK293T indicator cells and primary T-cells.

Spotlight on the U1 snRNA technology

U1 snRNA plasmid is a modified U1 snRNA engineered to target the lentiviral vector RNA (vRNA) packaging sequence. Co-transfection of this additional plasmid can increase vRNA abundance in the cell and enhance functional lentiviral vector titre.

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DoE results

The LV titre results from the HEK293T integration assay demonstrated that several conditions surpassed the standard platform conditions for this LV. The best performer was Condition 21, though there were 5 other conditions with high performance (Figure 1).

When LV was used to transduce primary T-cells, the LV from several conditions outperformed LV generated using the platform conditions. The overall pattern between the two assays was similar, with high performers in the HEK293T cells also performing well in the T-cell assays, although the changes were much more pronounced in the T-cells. The best condition in T-cells was Condition 4, while in the HEK293T integration assay it was Condition 21 (Figure 2).

Analysis of DoE conditions: HEK293T Integration Assay Titre
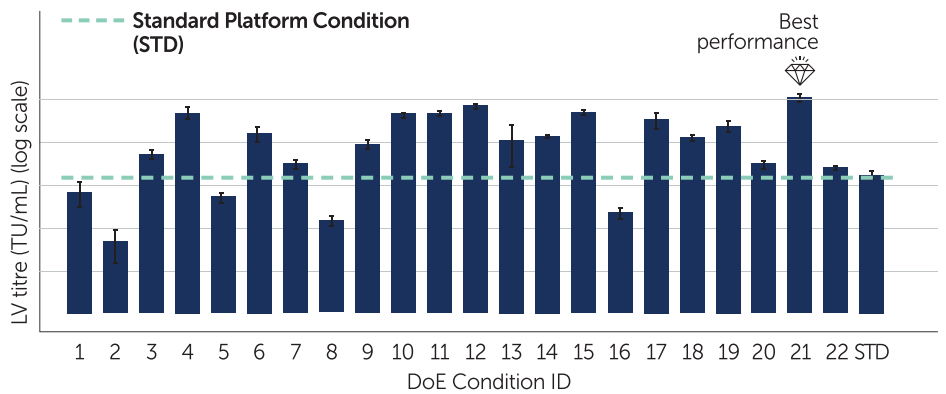DoE condition — different ratios of plasmids & transfection reagent

Figure 1

Analysis of DoE conditions: % CAR Expression in T-cells
DoE condition — different ratios of plasmids & transfection reagent
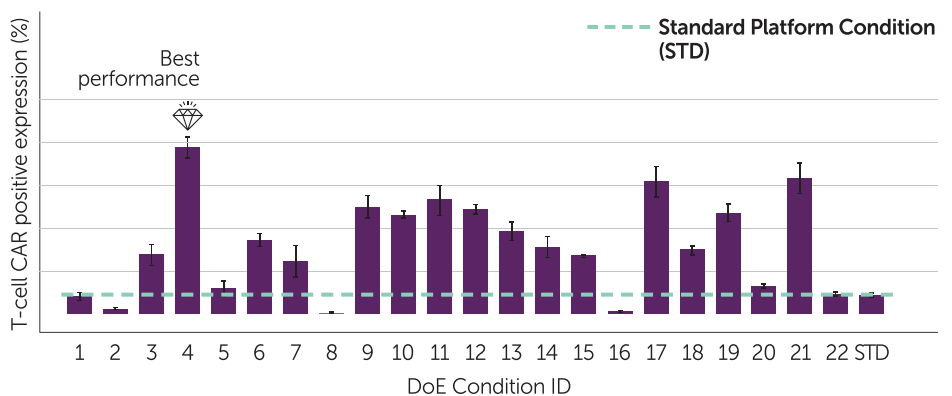

Figure 2

The LV titre results from the HEK293T Integration assay, demonstrated that several conditions surpassed the standard platform for this vector
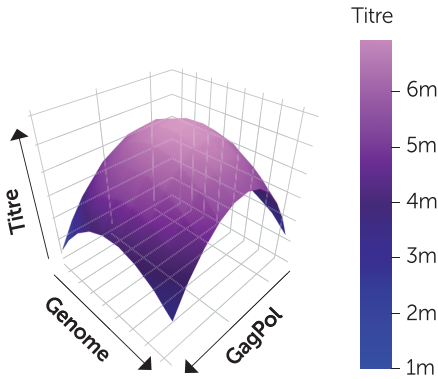
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3D visualisation of vector titre for 2 variables

Figure 3

Prediction profiles for all 4 variables using HEK293T data

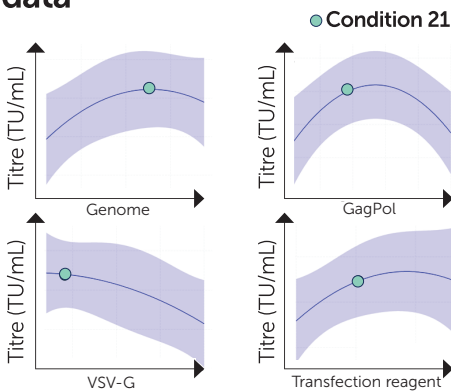

○ **Condition 21**

Figure 4
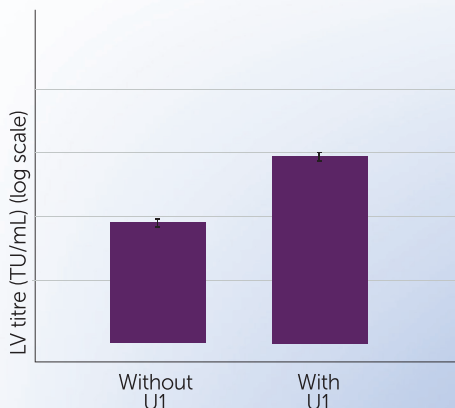
U1 technology increases titre 11-fold

Figure 5

DoE results
continued

DoE modelling utilised both sets of data to identify the best production conditions for this product.

Figure 3 shows the 3D model data for two components; this produces a clear parabolic curve with the best titres at the centre of the model and the lowest titres at the edges of the model.

Figure 4 shows the desirability curves for each of the 4 components. Furthermore, we have mapped Condition 21, which produced the best HEK293T Integration assay titre, onto these conditions with the blue circle. It sits very close to the optimal point for all 4 factors, which explains its high performance.

In this study, inclusion of OXB's titre enhancing technology, U1 snRNA, increased LV titre by 11-fold over the standard OXB platform condition without U1 snRNA.

Conclusion

Both the OXB analytical data and data from the client's own T-cell assay (data not shown) using OXB-generated LV exhibited similar patterns of best conditions.

OXB was able to recommend several possible conditions for scale up. The discussion with the client focused on the benefits of each recommended condition for scale up while considering the optima for both the HEK293T integration and T-cell assays, the feasibility of the selected conditions at large scale and the potential batch costs with different plasmid quantities.

The client selected the condition which best fitted their requirements, and this scaled up well using OXB's Stirred Tank Bioreactor (STR) perfusion process, where around 2E10 TU of LV was produced following downstream purification and concentration. This was sufficient for the client to perform the studies they needed, and this project has now advanced to our GMP manufacturing facilities.

Let's deliver life-changing therapies together

We are a global quality and innovation-led CDMO in cell and gene therapy with 30 years of experience, committed to helping our clients deliver therapies that transform patients lives. We offer end-to-end capabilities, from plasmid design and optimisation to clinical and commercial GMP manufacturing, accompanied by robust control systems, analytical methods, and deep regulatory knowledge.

We know the challenges and we're ready.

To discuss your project, please contact our team at partnering@oxb.com

www.oxb.com

Oxford Biomedica PLC, Windrush Court, Transport Way, Oxford, OX4 6LT, United Kingdom