Process Development to improve batch yield of XOFLX® Lentiviral Producer Cell Lines

Jade Thomason, Robert Asatryan, Sherin Parokkaran Johny[1], Meenakshi Raghunath[1], Maria I Patrício, Qian Liu[1]
[1]Minaris Advanced Therapies, London, UK.

Minaris Advanced Therapies

Poster

1) Introduction

We have developed XOFLX® lentiviral packaging and producer cell lines to reduce or eliminate plasmid transfection during lentiviral vector (LVV) manufacturing, thus saving plasmid manufacturing cost, simplifying supply chain, and improving process robustness of LVV manufacturing. Previous LVV productions for several therapeutic cargo genes showed 1-9-folds higher titres from XOFLX® cell lines compared to the industry standard four-plasmid transfection method.

As the producer cell lines produce LVVs without the need for transient transfection, we explored to enhance batch productivity through various process strategies:

- High-density cell culture to support intensified vector production, and
- Implementation of multiple harvests over an extended production window to maximise cumulative yield.

2) XOFLX® Lenti platform engineering

- We developed lentiviral packaging and producer cell lines by integrating the lentiviral production plasmids into the suspension HEK293 cell line (**Figure 1**). The cytotoxic elements, VSV-G and GagPol, are under tight control of doxycycline-regulated promoters.

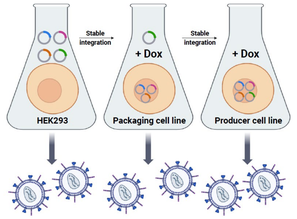

Figure 1. Schematics of XOFLX® technology

- We produced LVV encoding eight therapeutically relevant transgenes of various sizes and functions to reflect clinical needs, using all three production platforms (in 24 deep-well plate scale; **Figure 2**).

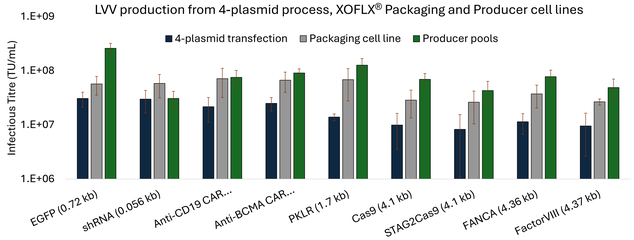

Figure 2. LVV production by four-plasmid transfection, packaging cell line, and various producer cell line pools encoding various transgenes. Productions were performed in 24 deep-well plates, and LVVs were harvested 72hr post transfection/ induction. LVVs were titrated in HEK293T cell line with ddPCR as readout. N = 12 biological replicates. Error bars indicate standard deviation.

3) XOFLX Producer process performance – platform process

- Clonal producer cell lines for EGFP and anti-CD19 CAR showed comparable upstream titres between shake flask and 1L STR productions (**Figure 3**), indicating good scalability of the platform.

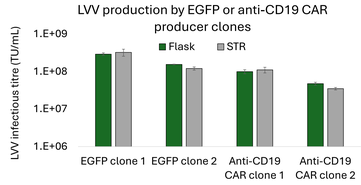

Figure 3. Scale-up LVV production from XOFLX® producer cell lines. Four clonal producer cell lines were induced to produce LV vectors in shake flasks and 1L stirred tank reactors. LVVs were harvested 72hr post induction, and titrated in HT1080 cell line with ddPCR readout.

- LV-EGFP vectors were produced from clonal producer cell line in 3L stirred tank reactor (STR). The harvested vectors were purified and concentrated following Minaris' platform processes without specific optimisation (**Figure 4**).

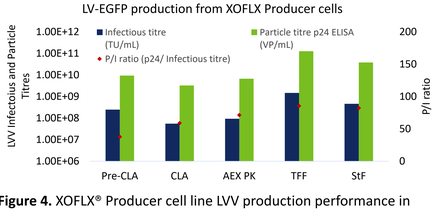

Figure 4. XOFLX® Producer cell line LVV production performance in platform processes. LV-EGFP producer cell line was seeded in 3L STR and LVVs harvested 48hr post Dox induction. Vectors were clarified (CLA), and purified and concentrated by AEX and TFF. Infectious titres were measured by flow cytometry from transduced HT1080 cell line, and particle titres determined by p24 ELISA.

4) Process innovation – high cell density supports intensified LVV production

- Higher cell seeding density (5E6 cells/mL) boosted LVV titre significantly at early harvest (48hr post induction), but the titre at 72hr harvest was comparable to standard seeding density (2E6 cells/mL) (**Figure 5A**).
- At the optimal harvest point (48hr), the viable cell density (VCD) of the high seeding density condition was 2.1-fold of the standard seeding density condition (**Figure 5B**), but the titre was 4.1-fold higher (**Figure 5A**), indicating a significant improvement on per-cell productivity with the high seeding density.
- Cell viability of the two seeding conditions remained comparable during the course of LVV production (**Figure 5B**), so the tested high cell density did not introduce much additional burden for downstream process.

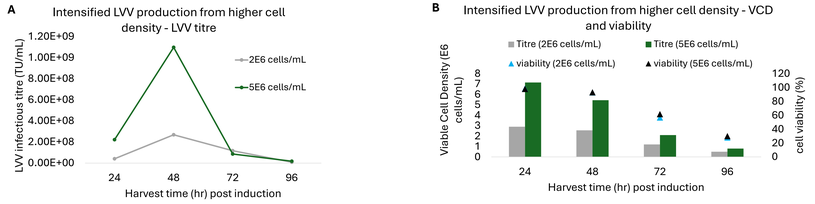

Figure 5. Intensified LVV production from XOFLX LV-EGFP Producer Cell Line. The LV-EGFP producer cell line was seeded at high (5E6 cells/mL) or standard (2E6 cells/mL) density, and Dox and NaBu were added to induce and enhance LVV production, respectively. LV vectors were harvested at 24hr, 48hr, 72hr and 96hr post induction, and titrated in HT1080 cell line followed by flow cytometry.

5) Process innovation – extended production window benefits LVV production without NaBu

- To test how long the Producer Cell Line can continue producing LVVs, we kept the cells in production mode until cell viability dropped below 70%.
- Cells with sodium butyrate (NaBu) as production enhancer did not maintain >70% cell viability beyond 72hr post induction (**Figure 5B**), but without NaBu, the high seeding density condition kept >70% cell viability for 7 days (168hr post induction), and the low cell density condition kept LVV production and high viability for 11 days (264hr post induction) (**Figure 6**).
- Without NaBu enhancement, the LVV production had a much slower start, so the total LVV yield of the batch was significantly lower than the production with NaBu. This 'slow and steady' extended harvest strategy may benefit the productions where NaBu cannot be used for various considerations.

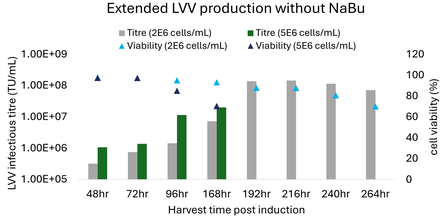

Figure 6. Extended LVV production from XOFLX® LV-EGFP Producer Cell Line. Cells were seeded at 2E6 cells/mL or 5E6 cells/mL, and induced by Dox to produce LVVs without NaBu enhancer. LVVs were harvested at indicated time points and titrated in HT1080 cell line followed by flow cytometry.

6) Conclusion

- XOFLX® Packaging and Producer Cell Lines reduce/ eliminate plasmid transfection dependency, reducing manufacturing cost and process variability.
- The Producer cell lines adapted well to Minaris' standard upstream and downstream processes without further optimization, demonstrating the robustness, scalability and flexibility of the cell line and process.
- Producer cell line allowed the development of an intensified LVV production process with high cell seeding density, increasing LVV yield from each production batch.
- Extended LVV harvest window with multiple harvests were also demonstrated feasible with XOFLX® Producer cell line, showing the potential for the development of a perfusion process.
- Producer cell lines may enable further process innovations as the process is not limited by transfection.
- XOFLX® Packaging and Producer Cell Lines are available for evaluation. Please contact https://minaris.com/contact for more information.

Corresponding author:
Qian.liu@minaris.com

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