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Lentiviral Vector Reference Material – Upstream Development and Scale-Up

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Abstract

Main Objective: Develop an upstream process to produce a Lentiviral Vector Reference Material (LVV-RM)

- ~ 3000 vials of LV-GFP (0.5 mL/vial, 0.5E8 – 1.0E8 transducing units per mL (TU/mL)).
- The LVV-RM will be used by the scientific community.
- The LVV-RM is produced and purified at the National Research Council Canada (NRC) and shipped to the American Type Culture Collection (ATCC) for final filtration, vialing and distribution. The US Food and Drug Administration (FDA) offers overall support.
- Several organizations are involved in its characterization.
- The project is funded by donations (manpower and materials) by:

Introduction

Background and Novelty:

The American Society of Cell and Gene Therapy states in their Q4 - 2022 report[1] that 49% of a pipeline of 2,053 genetically modified cell therapies under development are CAR-Ts. While lentiviral vectors are the main tools for engineering CAR-T, they are produced using a costly 4-plasmid transfection process and there is currently no lentiviral vector reference material (LVV-RM) available. The NRC tackled both matters by developing a stable/inducible packaging cell line[2] and a pilot-scale process used to produce a LVV-RM encoding a GFP-reporter cassette.

Experimental approach:

- Phase 1: Develop the process at shake flask and 3 L single-use (SU) bioreactor (Fig. 1A) scale; compare batch and fed-batch production.
- Phase 2: Perform four pilot runs in 10 L SU bioreactors (Fig. 1B) to optimise the upstream and downstream process.
- Phase 3: Run a 100 L production in a 200 L SU bioreactor (Cytiva) (Fig. 1C) to fulfill the requirements of the project.

Methods

Upstream conditions used at different production scales:

- Shake flasks: 125 mL or 2 L; 37°C, 5% CO_2, 120 rpm
- Bioreactors (Fig. 1): 37°C, pH 7.1, 40% dissolved oxygen, agitation: P/V of 10 – 19 W/m³ (3 – 10 L), 125 – 176 W/m³ (100 L production)
- HyCell TransFx-H (Cytiva), with 4 mM L-glutamine, 0.1% Kolliphor

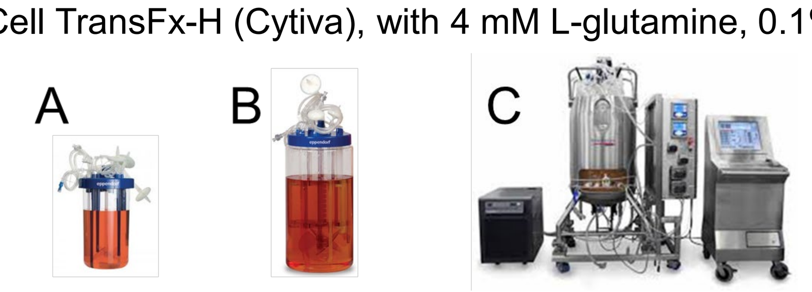

Figure 1. Single-use bioreactors used for scale-up

Single-use (SU) bioreactors from Eppendorf (A, BioBLU 3c, 3 L development; B, BioBLU 10c, 10 L pilot runs); and Cytiva (C, XDR-200, 100 L production) were used.

The stable inducible cell line was engineered using two molecular switches (Fig. 2A). The titers are measured using a gene transfer assay (GTA) (Fig. 2B).

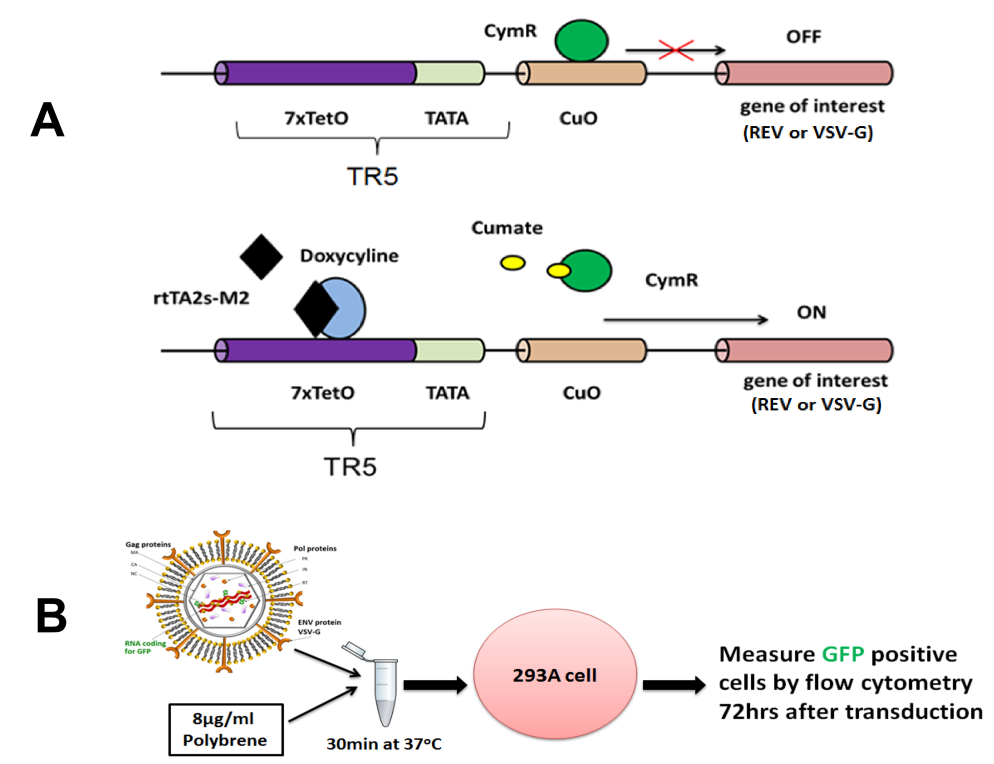

Figure 2. Schematic illustrations of inducible switches and gene transfer assay

A) Operation of the molecular switches used in the stable producer cell line:

The transcription of Rev and the envelope protein (VSV-G) is controlled by tetracycline and cumate switches. The cumate prevents binding of the cumate repressor (CymR) to the cumate operator (CuO) while doxycycline promotes binding of the reverse tetracycline transactivator (rtTA2s-M2) to the tetracycline promoter (TR5). The addition of cumate and doxycycline induces the production of LV.

B) Schematic of the gene transfer assay GTA assay:

Titers are measured using a flow cytometry–based assay. LV samples are serially diluted in DMEM supplemented with 8 µg/mL polybrene. Transduction is performed by adding the diluted LV to HEK293A adherent cells and incubating for 72 h at 37°C. GFP expressing cells are quantified by flow cytometry.

Results

Fed-batch improves the LV-GFP titer

To increase the titer, different fed-batch processes were tested in comparison to a batch process, from shake flask to 100 L bioreactor production-scale. The selected bioreactor process conditions are shown in Fig. 3:

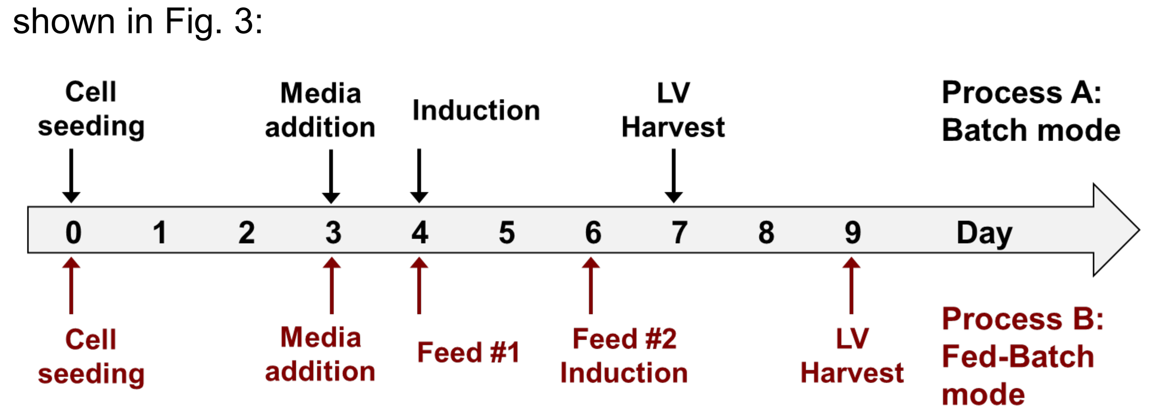

Figure 3. Main steps and timeline for the batch (top) and fed-batch process (bottom) tested with the inducible stable cell line

Cells are seeded in half the final volume. Media is added to bring up the volume 3 days after seeding. The fed-batch process includes two feedings of HEK-FS (Xell, Sartorius) and runs over a period of 9 days compared to 7 days for the batch process.

The viable cell count (VCC) and titer (infectious virus particles) increased by 1.6-fold (Fig. 2A) and 2.8-fold (Fig. 2B), respectively, in fed-batch *vs.* batch mode when compared in shake flask in (1) 26 mL and (2) 600 mL culture volume.

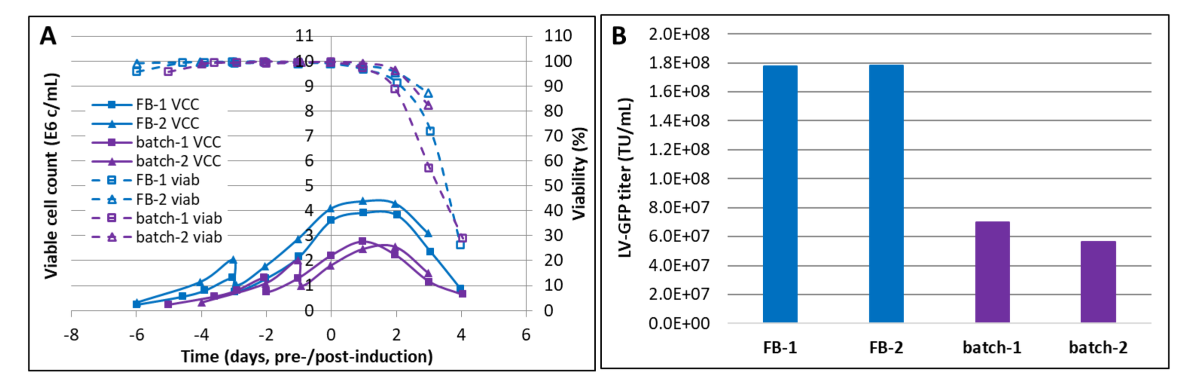

Figure 4. Batch vs. fed-batch process in shake flask

The viable cell count (VCC; A, solid lines), viability (A, dashed lines) and LV-GFP titer (B) at 3 days post-induction (dpi) are higher when operating in a fed-batch (blue) compared to batch mode (purple).

In 3 L bioreactor, the cell maximum (VCC) was 2.3-fold higher (Fig. 5A), while the titer was 3.3-fold higher (Fig. 5B) for the fed-batch compared to the batch process.

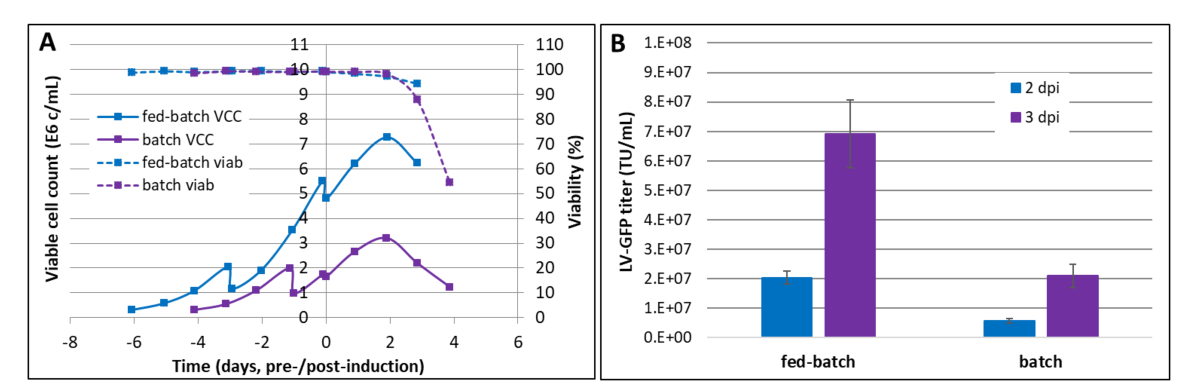

Figure 5. Batch vs. fed-batch process at 3 L scale in SU bioreactor

The viable cell count (VCC; A, solid lines), viability (A, dashed lines) and LV-GFP titer (B) are higher when operating in a fed-batch compared to batch mode. Previous data in shake flasks demonstrate a peak in titer at 3 dpi.

The fed-batch process is scalable to 10 L scale

Four consecutive pilot runs (P1 to P4) were performed in 10 L bioreactors using the fed-batch process described in Fig. 3. Viable cell counts, viability, and titers are shown in Fig. 6. Similar profiles were obtained for all runs.

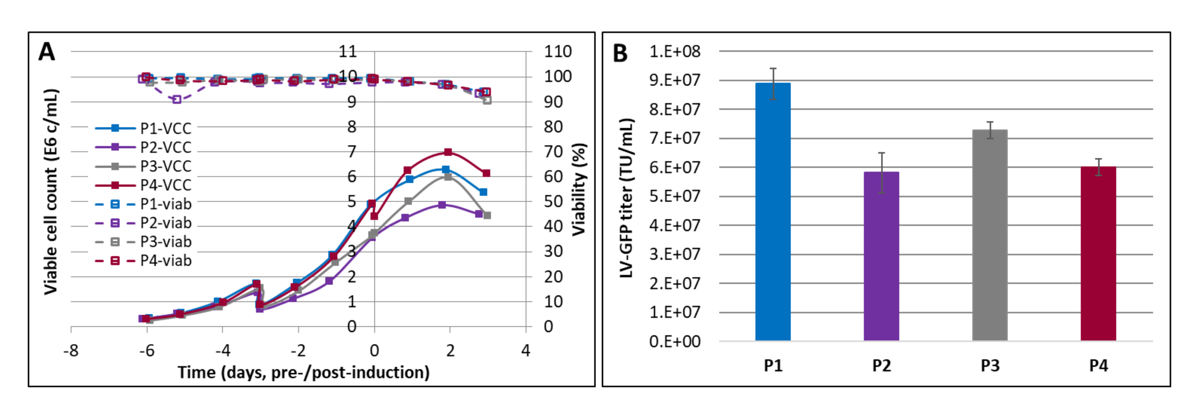

Figure 6. Scaling-up the fed-batch process to 10 L scale in SU bioreactor

A) The bioreactors were seeded at slightly different cell densities:

- 3.3E05 cells/mL (P1) • 3.0E05 cells/mL (P2, P4) • 2.51E05 cells/mL (P3)

Lower cell growth for P2 can be linked to slower cell growth of the seed train. The viability at time of harvest was between 91% and 94%.

B) Infectious titers of ≥ 6E07 TU/mL were obtained at time of harvest. The lowest titer (P2, 5.8E07 TU/mL) was most likely due to lower cell growth.

Scaling up to 100 L: LVV-RM production

The 10 L fed-batch process was transferred to an XDR-200 bioreactor (Cytiva) with a production volume of 100 L. Cell growth (Fig. 7A) was slower than at 10 L scale. Despite similar VCCs at induction, the LV-GFP titer was almost 5-fold lower at production scale (Fig. 7B).

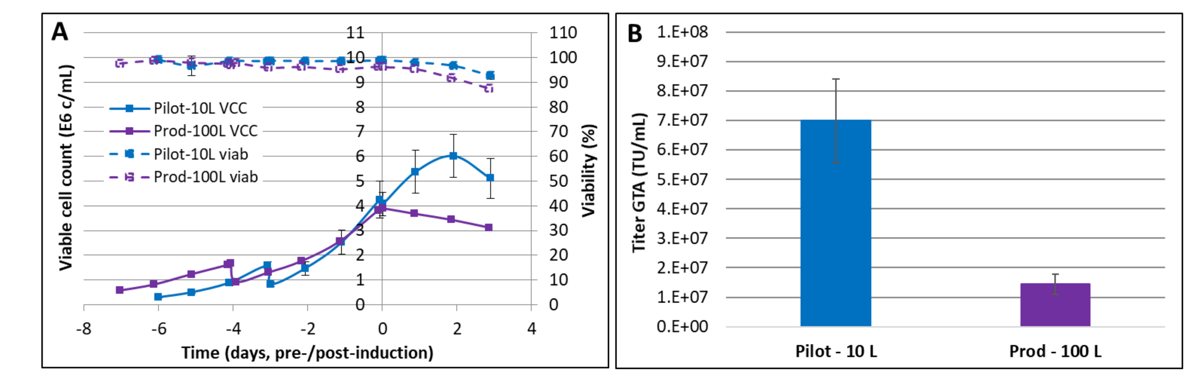

Figure 7. LV-GFP production at 100 L compared 10 L scale

A) Cell growth (solid lines) was slower at 100 L production scale (Prod) compared to 10 L pilot scale (Pilot) despite the higher seeding density; viability (dotted lines) was comparable during the growth phase, but slightly lower (87%) at harvest in the XDR.

B) The lower titer at 100 L scale was most likely due to lower specific productivity.

Conclusions

It is widely recognized in the field of cell and gene therapy that an international LV standard has become crucial for harmonization between institutions and regulatory agencies. In order to succeed in producing required quantities of LVV-RM, we have developed an upstream fed-batch process using a NRC proprietary stable/inducible cell line that produces a LVV encoding a GFP-reporter cassette.

The timely addition of feed to our original batch process supported a higher VCC and a titer increase of about 3-fold. Similar titers were obtained at 10 L pilot scale in four independent runs, demonstrating that the process is reproducible. Although the VCC and titers obtained at 100 L scale were lower, enough material was produced to meet the original goal. This material is expected to be available to the scientific community through ATCC in 2023.

References

1. Gene, Cell and RNA Therapy Landscape, Q4 2022 Quarterly Data Report
2. Brousseau et al., Mol. Ther. vol.16 no.3, March 2008

Acknowledgements

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* (https://isbiotech.org/ReferenceMaterials/pdfs/LVRMparticipants082420.pdf)