Development of a scalable downstream process for lentiviral vector purification from Curator® cell lines

Candice Crilly, Tiffany Claeys, Michael Mcilhatton, John Ketz, Samir Acharya

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LVV CURATOR® PLATFORM

Introduction

Lentiviral vectors (LVVs) are a widely used modality in the manufacturing of gene and cell therapies due to their ability to deliver and stably integrate large transgenes into dividing and non-dividing cells. To meet the projected increase in demand for LVV, more manufacturing organizations will need to offer large-scale GMP LVV production. Here, we show the stage-gated workflow used to develop a scalable purification platform for LVV and demonstrate the scalability of the optimized downstream process using a feed stream produced with Andelyn's Adherent Curator® cell line.

Methods

Approach: A stage-gated approach was used to develop a scalable downstream process for purifying LVV. Candidate modules for each process step were selected based on their potential for scalability and then subjected to an experimental screen; the best-performing modules were chosen for further characterization. All processes were performed on VSVG-pseudotyped eGFP LVV produced in-house using the Curator® cell lines (both adherent and suspension).

Analytics: LVV yields were monitored using (i) ELISA to quantify LVV capsid-associated p24 and, from there, calculate an estimated physical titer (viral particles or vp/mL), (ii) RNA extraction followed by RT-qPCR to track genomic titer (vector genomes or vg/mL), and iii) a flow cytometry-based transduction assay to quantify functional titer (transducing units or TU/mL).

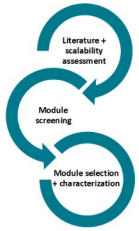


Figure 1. Approach toward developing a scalable LVV purification platform.

Clarification

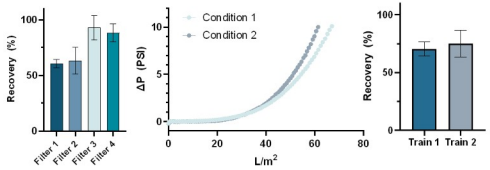


Figure 2. Clarification filter screening and characterization. Left: Percent LVV viral genome recovery from an adherent feed stream through four filters with differing chemistries and nominal pore sizes. **Center:** Pressure profiles of the selected filter for adherent feed streams under two conditions. **Right:** Percent LVV viral genome recovery from a suspension feed stream through two filter trains with differing chemistries and nominal pore sizes. Error bars represent %CV from duplicate RNA extractions and RT-qPCR measurements.

Tangential Flow Filtration

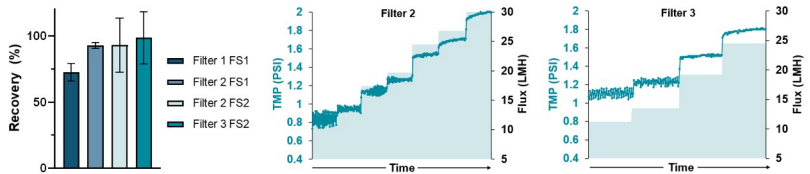


Figure 3. Tangential flow filtration module screening and characterization. Left: Vector genome recoveries after performing ultrafiltration-diafiltration over TFF modules that differed in membrane chemistry and/or molecular weight cut off. Two different feed streams of LVV produced using the Suspension Curator® cell line (FS1 = post-chromatography, FS2 = pre-chromatography) were processed for this test. Error bars represent %CV from duplicate RNA extractions and RT-qPCR measurements. **Center:** Transmembrane pressure (TMP) across Filter 2 as a function of permeate flux, using a feed stream of LVV produced with the Suspension Curator® cell line (FS3). **Right:** TMP across Filter 3 as a function of permeate flux, using FS3.

Anion Exchange Chromatography

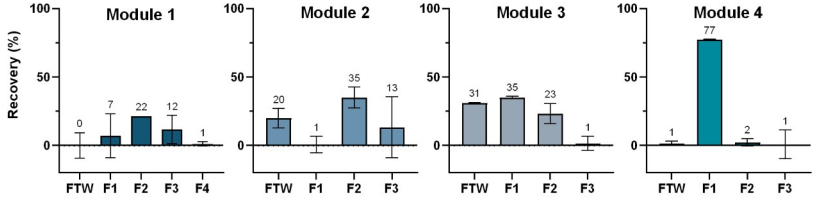
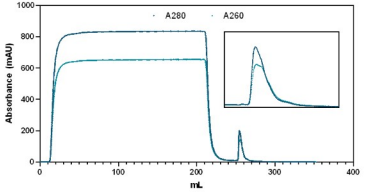


Figure 4. Anion exchange chromatography module screening.

Top: Percent LVV viral genome recovery from 4 different modules. For screening, all modules were loaded with a diluted, clarified feed stream of LVV produced with the Adherent Curator® cell line and washed - the flow-through and wash were collected together (FTW). Subsequently, a gradient elution was performed, and the eluate collected in fractions (F1-F4). Error bars represent %CV from duplicate RNA extractions and RT-qPCR measurements.

Right: Chromatogram of the load and elution profiles for Module 4, with the inset showing a zoom of the elution.



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Process Recoveries: 1 L Scale

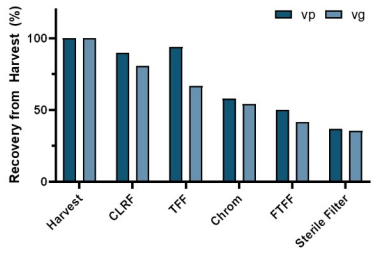

Figure 5. Total LVV recoveries from 1 L of adherent feed stream. LVV was harvested from a 10-chamber CellSTACK® and processed using the modules selected during screening; modules were scaled up to accommodate the larger feed stream. Samples were taken after each process step and analyzed via p24 ELISA and RT-qPCR to quantify total viral particles (vp) and viral genomes (vg), respectively.

Process Recoveries: 20 L Scale

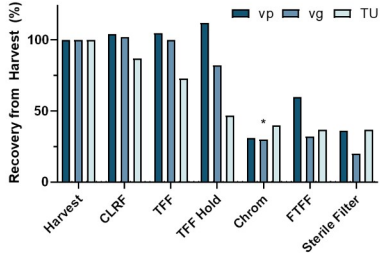

Figure 6. Total LVV recoveries from 20 L of adherent feed stream. LVV was harvested from five 36-layer HYPERStacks® connected in tandem and processed using the modules selected during screening; modules were scaled up to accommodate the larger feed stream. Samples were taken after each process step and analyzed via p24 ELISA, RT-qPCR, and a flow-cytometry based transduction assay to quantify total viral particles (vp), viral genomes (vg), and transducing units (TU), respectively.

**Root cause analysis identified conductivity measurement error to be the primary reason for higher-than-anticipated losses during chromatography.*

Conclusions & Future Directions

- Using a stage-gated approach, we successfully developed a scalable purification process for LVV produced with Andelyn's Adherent Curator® cell line.
- Large-scale runs informed risk mitigation and scaling strategies that will be implemented during technology transfer to maximize recovery of functional LVV.
- Development of an analogous platform for purification of LVV produced using Andelyn's Suspension Curator® cell line is ongoing.