Development of an Upstream Process for a Scalable Lentiviral Vector Manufacturing Platform Using Curator® Cell Lines

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LVV CURATOR® PLATFORM

Introduction

Lentiviral vectors (LVVs) have become important tools for CAR T cell therapies and other gene-modified cell therapy programs due to their large payload capacity and stable genomic integration, enabling long-term transgene expression. However, LVV fragility presents significant manufacturing challenges, including low production titers. This study systematically evaluated key upstream parameters including cell line performance, growth media, transfection conditions, and supplement addition to rapidly develop a robust, scalable LVV manufacturing platform.

Methods

Lentivirus production: Lentiviral vectors were prepared using four-plasmid transient transfection of Curator® suspension or adherent cells using the envelope protein VSV-G and eGFP as the gene of interest. Culture supernatants containing viral particles were collected at appropriate timepoints and clarified by centrifugation.

Vector genome, capsid, and infectious titer: Vector genome titer was determined using an Andelyn-developed RT-qPCR assay utilizing a standard curve of known quantities run on the same plate. Capsid titer was determined using a commercially available p24 ELISA kit with removal of free p24 prior to performing the assay. Infectious titer was determined using a standard transduction assay utilizing a standard curve with known MOI.

Statistical analysis: For experiments with less than three conditions, an unpaired two-tailed t test was performed. For experiments with greater than three conditions, a one-way analysis of variance (ANOVA) was performed followed by a Tukey test. Statistical significance levels are declared as $p \leq 0.01$ (**), $p \leq 0.001$ (***), $p \leq 0.0001$ (****).

Findings

An RT-qPCR assay was developed to consistently quantify LVV vector genome. Using this assay, the Andelyn-developed Curator® suspension and adherent cell lines were found to produce comparable LVV titers to commercially available cell lines, indicating their suitability for LVV production and were further characterized and optimized.

Two transfection reagents were screened with *Mirus Bio® VirusGEN® LV Transfection Kit* showing the most robust LVV production. Screening of transfection conditions led to the ideal timing of transfection and harvest in the LVV Curator® platform, as well as the identification of a suspension cell growth medium producing consistent, high-titer LVV.

Media supplements were tested to determine ways to increase LVV production and a transfection enhancer was tested to identify the best time of addition to increase LVV production. The stability of infectious LVV during freeze/thaw was tested and a final product buffer was developed for enhanced stability.

Using these established upstream parameters, a downstream purification process was developed for robust, scalable LVV manufacturing in the Curator® adherent platform.

Assay Development

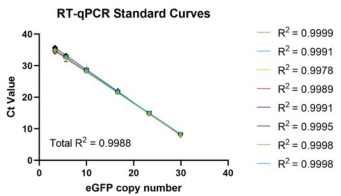

Figure 1. Representative standard curves from Andelyn-developed RT-qPCR assay for eGFP to determine LVV vector genome titer.

Cell Line Selection

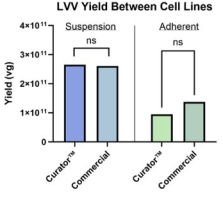

Figure 2. LVV vector genome yield at harvest between Andelyn Curator® cell lines and commercially available cell lines for both suspension and adherent platforms.

Transfection Reagent

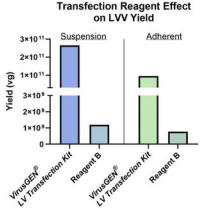

Figure 3. LVV vector genome yield using either *Mirus Bio® VirusGEN® LV Transfection Kit* or other commercially available transfection reagent in both suspension and adherent platforms.

Transfection Conditions

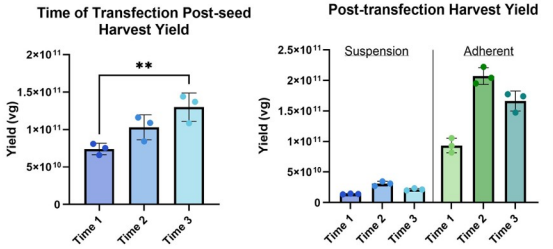

Figure 4. Transfection and harvest timing. Left: LVV vector genome yields at harvest after transfecting at 3 different timepoints post-seeding of flasks in suspension cells. **Right:** Vector genome yield from harvesting at 3 different timepoints post-transfection for both suspension and adherent platforms.

Growth Medium

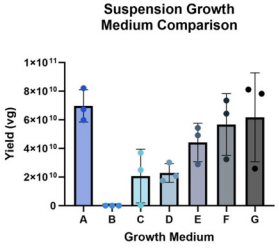

Figure 5. LVV Vector genome yields compared between seven commercially available growth media.

Media Supplements and Enhancer Additions

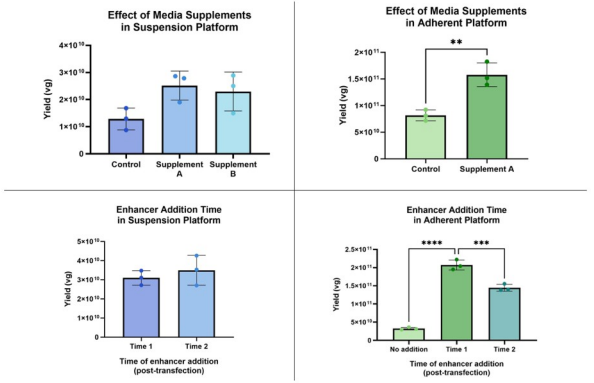

Figure 6. Enhancer and supplement addition. Top Left: LVV vector genome yields for different supplements added to suspension culture post-transfection. **Top Right:** Vector genome yields for addition of a media supplement to adherent culture post-transfection. **Bottom Left:** Vector genome yields for addition of transfection enhancer at different timepoints post-transfection in suspension culture. **Bottom Right:** Vector genome yields for addition of transfection enhancer at different timepoints post-transfection in adherent culture.

Freeze/Thaw Stability

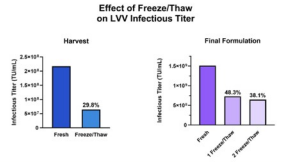

Figure 7. Effect of freeze/thaw cycles on functional titer. Left: Infectious titer determined by transduction assay using harvest supernatant collected fresh and after one freeze/thaw cycle at -80°C. **Right:** Infectious titer determined by transduction assay using fully purified LVV in storage buffer developed for increased LVV stabilization during freezing collected fresh, and after one or two freeze/thaw cycles at -80°C.

Downstream Purification Process

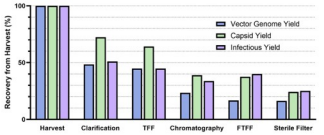

Figure 8. Percent recovery from harvest in an adherent production of LVV in two 10-CellSTACKs at each step of developed downstream purification strategy measured by RT-qPCR (vector genome titer), p24 ELISA (capsid titer), and transduction assay (infectious titer). Final percent recovery from harvest was 16.3% vector genome yield, 24.2% capsid yield, and 25.1% functional yield with a final infectious titer of 2.6×10^8 TU/mL.

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