SCALING UP LENTIVIRAL VECTOR PRODUCTION FROM STABLE PRODUCER CELLS

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Lentiviral vectors (LVs) are commonly used for gene and cell therapies where long-term, sustained expression of therapeutic genes is needed. Legacy methods for LV production include the use of adherent cell lines, transiently transfected with viral packaging genes and the gene of interest (GOI), and cultured in media supplemented with animal sera. However, large scale production is severely limited in adherent cell culture and commercial manufacturing of LV is moving to scalable, serum-free suspension systems. In addition, stable-inducible producer cell lines may eventually replace transient systems for LV production, as this approach circumvents the costs of continually obtaining high quality or cGMP-grade plasmid DNA, the cost of the transfection reagents and the inherent variability of transfection efficiencies. Accordingly, we focused our efforts on workflows that are likely to define the LV manufacturing space in the future and to develop strategies for process development that could enable their uptake and application sooner. For our model, we used an HEK293 derived stable-inducible LV producer cell line (developed by the viral vector production team at the National Research Council Canada in Montreal and described in Manceur et al., 2017) that has been engineered to produce a third generation LV harboring the GFP transgene. A double-inducible system tightly controls the transcription of the envelope glycoprotein *VSV-G* and the viral *Rev* genes and allows for normal maintenance and expansion of cultures during seed train development, without loss of viability. This cell line is stable over many passages in culture and no antibiotics were used to maintain a selection pressure. Our goal was to bring the baseline production protocol closer to an industrial workflow that could be closed, scaled and make use of fully chemically defined media and supplements. Optimization of media formulations and feeding regimes at small scales (in shake flasks) led to the development of a multiple harvest, perfusion enabled process in a 1 L stirred tank reactor (STR) as well as a 5-25 L batch process in single use STRs. We demonstrated that to achieve high yields in multiple harvests, cell density needed to be intensified prior to induction and the medium regularly replaced with fresh medium during the production window. To avoid perfusion in the lead up to induction, while reaching a relatively high density of cells (5E6 cells/mL) in the exponential growth phase, we added GE HyClone Cell Boost 5 Supplement (3.5 g/L) to the basal media. After induction, medium was exchanged by continuous perfusion, using an acoustic filter for cell retention, at a rate of one reactor volume per day. High titer (≥1E7 TU/mL) harvests were observed at three, four and five days post induction, including the reactor contents on the fifth day, resulting in four reactor volumes of high titer product at the end of an 11-day process (including the pre-induction culturing time). For higher production scales (5 L and greater) in single use bioreactors, we failed to identify suitable single-use filtering technology that allowed LV to pass freely into the harvest, while retaining the cells in the culture vessel. Therefore, we developed a simple batch process for large scale production that consisted of inoculation, induction and a single harvest at the end of a 6-7-day process (including the pre-induction culturing time). To reach an acceptable volumetric titer (≥1E7 TU/mL) in a batch process, we supplemented the basal medium with GE HyClone LS250 lipid supplement, which resulted in a greater than 3-fold improvement in LV yield over basal media alone. A multiple harvest production mode is higher yielding than a single harvest mode for equivalent culture vessel volumes, however, the single harvest is technically simpler, uses five times less media and supplements, requires less specialized equipment, and has advantages for downstream processing. Therefore, each harvesting mode offers unique advantages and can be used to address specific production needs. In conclusion, we successfully demonstrated a process development path for an industrial workflow for LV manufacturing based on a stable-inducible producer line in fully chemically defined, serum-free media with high volumetric titers.

Reference:

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