SCALABLE LENTIVIRAL VECTOR PRODUCTION
USING STABLE PRODUCER CELL LINES IN PERFUSION MODE

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Lentiviral vectors (LVs) are becoming an important tool in gene and cell therapy and are being utilized in several clinical studies for rare and more frequent genetic and acquired diseases, as well as in cancer therapies. However, two major challenges need to be overcome in order to generate enough material to treat patients: First, current production platforms result in low titers (stable producer cell lines from adherent cell lines) or are not amenable to large scale production (LV produced by transfection). Next, LVs are known to have a low temperature stability. To address these two challenges, the National Research Council Canada has developed packaging cell lines and stable producer cell lines for the production of LVs which can grow in suspension in serum-free media and produce LV in the 10^6 TU/ml range without optimization. Furthermore, productions are performed in perfusion mode in order to operate at high cell densities and address the low LV stability.

To facilitate titration, a producer cell line for LV expressing GFP regulated by the strong constitutive CMV promoter was generated (HEK293SF-LVP-CMVGFPq-92). Transcription of Rev and the envelope protein (VSVG) is under the control of the tetracycline and cumate switches, which means that addition of doxycycline and cumate is required to induce the production of LV. Results obtained demonstrate that the system is scalable (Fig. 1A), and up to 15 fold increase in total yield was obtained in perfusion mode when compared to batch mode (Fig. 1B), using perfusion rates of 0.65 to 1 vessel volume exchange per day after induction.

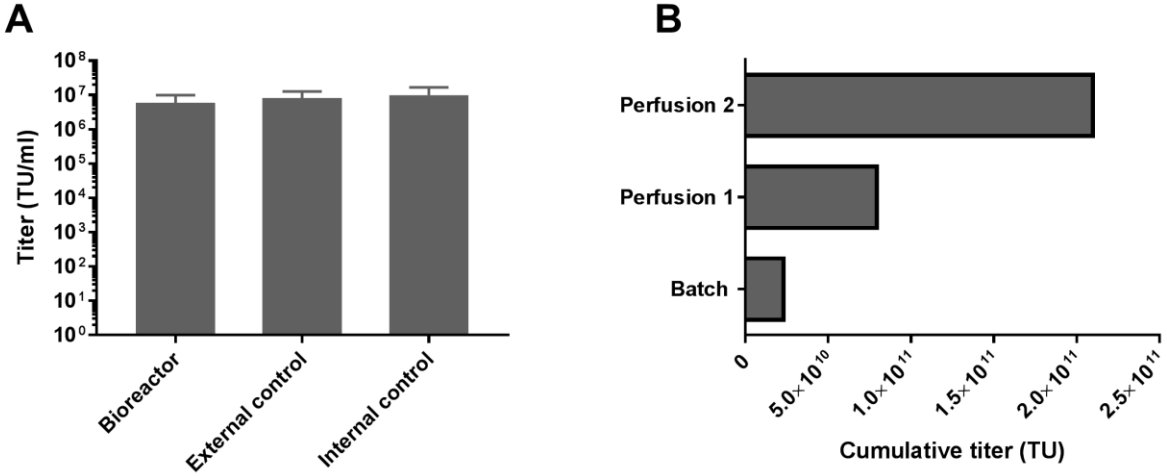

Figure 1 – A) Average titers obtained at 3 days post-induction in 3L bioreactors operated in batch mode (n=4) compared to 50ml shake flasks. The internal control was extracted from the bioreactor at the time of induction and the external control was performed in shake flask throughout the experiment. B) Comparison of the total amount of LV produced in 3L bioreactor in batch mode (n=4) using about 1.3E06 cells/ml at induction, and during two runs operated in perfusion mode using an acoustic filter (with 10E06 and 5E06 cells/ml at induction respectively). Titers were obtained using a gene transfer assay.

Additional improvements are expected by optimizing the perfusion rate and cell density. In conclusion, through a combination of induction at high cell density and operation in perfusion mode, cumulative functional LV titers were increased by >15-fold, reaching up to 1E11/L of bioreactor culture. The approach described brings us closer to a process that could be used in the manufacturing of LVs for gene and cell therapy applications.