A Novel Scalable Production Platform for Lentiviral Vectors Based on Human Suspension Cell Lines

Martina Graßl, Kerstin Hein, Simon Fradin, Helmut Kewes and Nicole Faust

CEVEC Pharmaceuticals GmbH, Gottfried-Hagen-Straße 60-62, 51105 Cologne, Germany, www.cevec.com

Introduction

Background and Novelty

A rapid increase in the number of gene therapy trials and products has led to an equally increased need for industrial production of viral gene therapy vectors such as lentiviral, adeno-associated and adenoviral vectors. Current production systems are limited with respect to scalability and robustness. With our CAP and CAP-T cell lines, we have developed a novel system for high cell density suspension culture, efficient reproducible transfection and high efficiency production of viral vectors. By upstream process optimization we have obtained a robust high density fed-batch culture system, which can be scaled to any current bioreactor format.

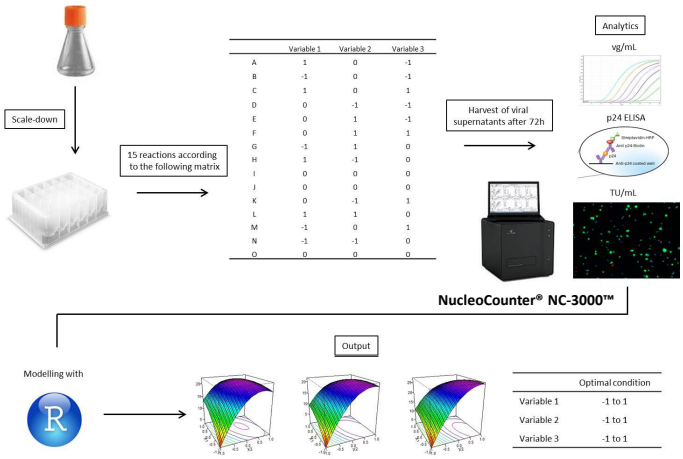
Experimental Approach

We applied a design of experiment approach to increase the lentiviral titers achieved with the CAP-GT platform. In a Box Behnken approach, parameters as cell density, DNA and PEI amount were optimized in regard to yielding the highest transducing units. In a further step, the addition of supplements, as sodium butyrate, was titrated and analyzed in regard to influence on viral production. The determination of cell count and viability as well as GFP transfection efficiency was performed using the NucleoCounter® NC-3000™, Chemometec A/S.

Results and Discussion

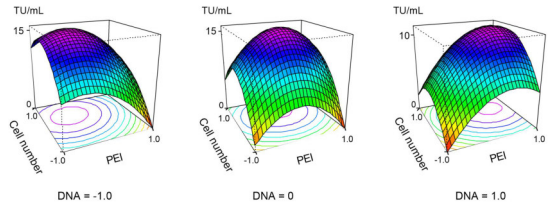
We indeed observed a significant increase of viral titers upon addition of supplements, as e.g. sodium butyrate. Using all the optimized conditions, we could increase the lentiviral titers about 10 fold compared to our previous standard protocol and also compared to adherent HEK293T cells. In addition, lentiviral production in shake flasks was shown to be scalable yielding comparable titers over a range of volumes.

Optimization Workflow for Lentiviral Production



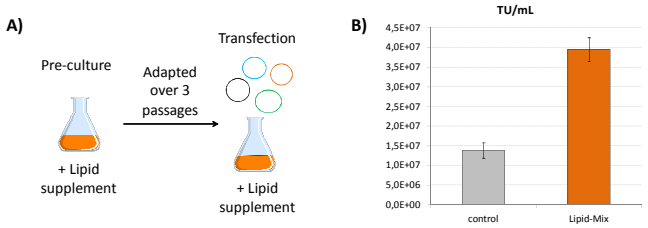
Optimization workflow for lentiviral production. Transient transfection (NucleoCounter® NC-3000™) of lentiviral packaging constructs was downscaled from shake flask to 24 deep well plate. According to the Box-Behnken design, defined combinations of three variables (-1 = low level; 0 = medium level; 1 = high level) were screened in a total of 15 reactions. After 72 h, viral supernatants were harvested and screened for viral titer (p24 levels, vector genomes/mL, Transducing units (TU)/mL). The resulting TU/mL were fitted to a secondary polynomial equation by non-linear regression analysis using R project for statistical computing (Rstudio). The output predicts the optimal concentration of the variables in order to achieve the highest lentiviral production.

Optimization of DNA-PEI-Cell Density



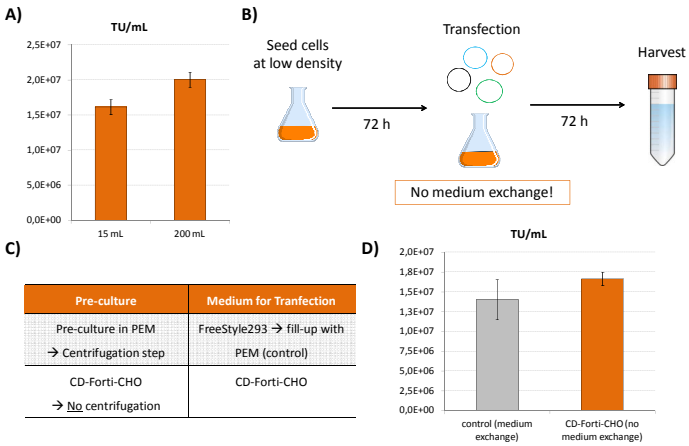
Response surface graphs based on Box-Behnken experimental TU/mL results (relative values). For each graph, DNA amount/cell is constant while the relation between cell density and amount of PEI under this condition is depicted in the graph in coded values.

Influence of Lipid Supplementation on Lentiviral Production



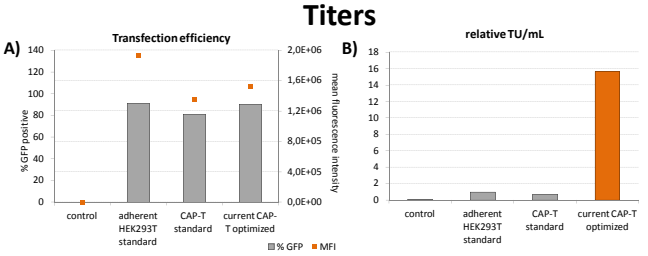
Lipid supplementation significantly increases production of infectious LV particles. A) Cells were adapted to lipid supplementation over 3 passages and then transfected in presence of lipid. B) Transducing units/mL were measured of viral supernatants of either untreated or lipid supplemented cultures.

Lentiviral Production with the CAP-GT Platform Is Scalable without Medium Exchange Step



Lentiviral transfection and production is scalable to different volumes without medium exchange step. A) Transfection (NucleoCounter® NC-3000™) of lentiviral constructs in 15 mL or 200 mL scale yields comparable TU/mL. **B)** Experimental set-up for scalable transfection without medium exchange step. **C)** Pre-culture and transfection medium for standard control transfection in FreeStyle293/PEM and scalable protocol in CD-Forti-CHO. **D)** Scalable transfection in CD-Forti-CHO results in comparable TU/mL to the control protocol.

Optimized Protocol Results in 10-fold Increased Titers



Optimized protocol results in 10-fold increased titers. A) Transfection efficiency (GFP-positive cells and mean fluorescence intensity; NucleoCounter® NC-3000™) of HEK293T standard protocol, CAP-T standard and CAP-T optimized protocol samples. **B)** Comparison of relative transducing units per mL between HEK293T, CAP-T standard and optimized protocol.

Conclusion

- Conditions for lentiviral production using the CAP-GT platform were optimized by a design of experiment approach in regard to cell density and DNA and PEI amount.
- Lipid supplementation significantly increased production of infectious LV particles.
- A scalable protocol without medium exchange step was developed using CD-Forti-CHO medium.
- The optimization process resulted in significantly improved titers, also compared to adherent HEK293T cells.
- Using the NucleoCounter® NC-3000™, fast and reliable quantification of cell numbers and GFP expression for a large amount of samples was possible.