Improvement in Baseline Lentivirus Production using Design of Experiment (DoE) Tool and Scale-up in Bench-Top Bioreactors

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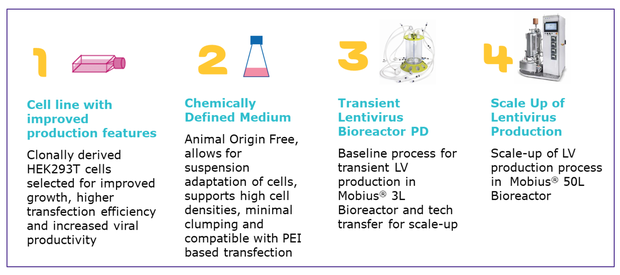
Introduction

The use of viral vectors to facilitate gene delivery into cells has offered a wide variety of advantages over the traditional non-viral methods. The two widely used viral vectors are recombinant adeno-associated virus (rAAV) and lentivirus (LV). Traditionally, adherent cell culture has utilized generic, unoptimized media supplemented with fetal bovine serum to produce virus. Planar cell culture has scalability limitations, it requires physical manipulations, and it may introduce potential contaminants into the manufacturing process. As a result, current LV manufacturing processes are labor intensive.

Baseline LV production was established using our VirusExpress® Lentiviral Production Platform. The system consists of a suspension-adapted HEK293T cell line, chemically defined (CD) medium compatible with PEI based transfection and a process with proven performance in large scale bioreactors. This platform offers reduced process development and scale-up time for speed to market. Here, we describe improvements in baseline lentivirus production using a Design of Experiment (DoE) to study multiple factors affecting transient transfection and lentivirus titers. Using shaker flasks, we explored the effects of transfection cell density, plasmid DNA and PEI ratio, complex volume, complex hold time and plasmid molar ratios. DoE data modeling was done using JMP® software and the optimal level for each factor was determined. Confirmation runs were then performed in shaker flasks and scale-up was done at bench-scale using Mobius® 3 L Single-Use Bioreactors.

Our Next Generation VirusExpress® Lentiviral Production Platform



The baseline lentiviral production process was scaled-up in Mobius® 50 L Bioreactor multiple times. The historical infectious titer ranged from 7.8E+06 TU/mL to 9.6E+06 TU/mL.

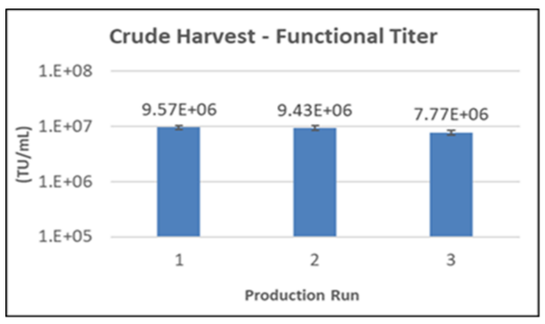
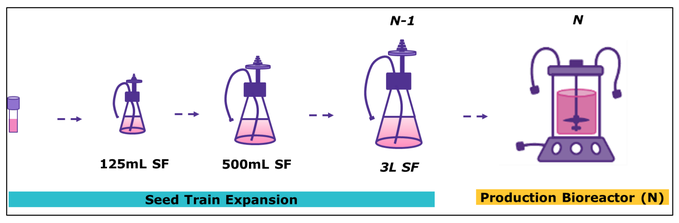


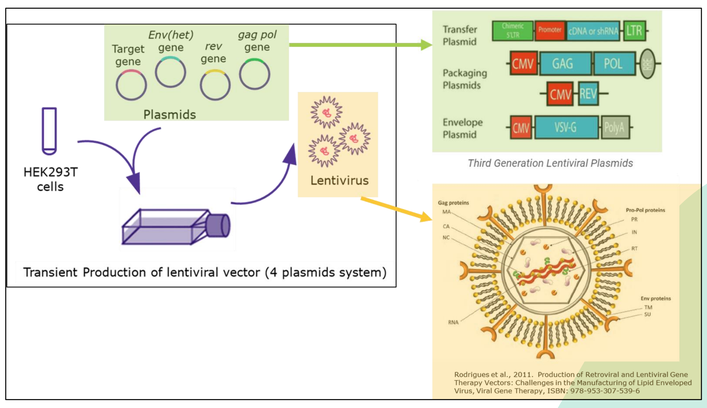
Figure 1: Average infectious titers achieved during multiple 50L bioreactor runs using the baseline process for Lentiviral production.

Methods

- A shaker flask lentivirus process was initially used as baseline for the DoE experiments. Shaker flasks were incubated at 37°C, 80% RH, 8% CO_2 and 135 rpm. **Seed train expansion** - carried out as shown in the diagram below.

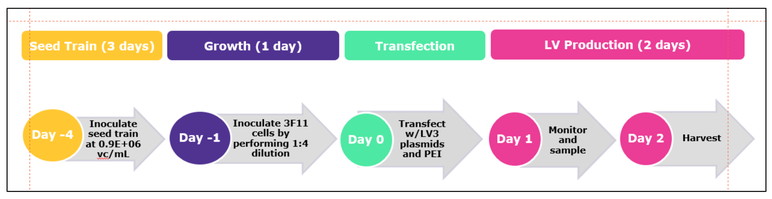


- **Third generation lentivirus plasmid system** was used for viral vector production with a lentivirus transfer vector that contained eGFP transgene. PEIpro® (Polyplus transfection, FRANCE) was used as the transfection reagent.



- For lentivirus production, VirusExpress® 293T Lentiviral Production Cells were inoculated followed by transfection at 24 hrs. Cell counts and lentivirus harvest samples were collected on Days 1 & 2 post-transfection.

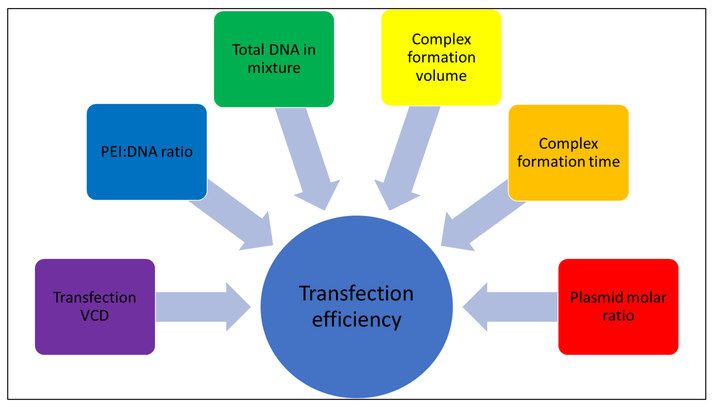
Lentivirus Production Process & Timeline



- For routine seed train maintenance, cells were passaged at 0.5E6 vc/mL and cultured for 3-days or at 0.25E+06 vc/mL for 4-day duration. For lentivirus production, cells were seeded at 0.5E+06 vc/mL, 0.7E+06 vc/mL or 0.9E+06 vc/mL for DoE #1. For DoE #2, cells were inoculated at seed density recommended by JMP® data modeling.

- Optimal process parameters for pH, agitation and dissolved oxygen and gassing configuration were previously determined for lentivirus production in Mobius® 3L Single-Use Bioreactor.

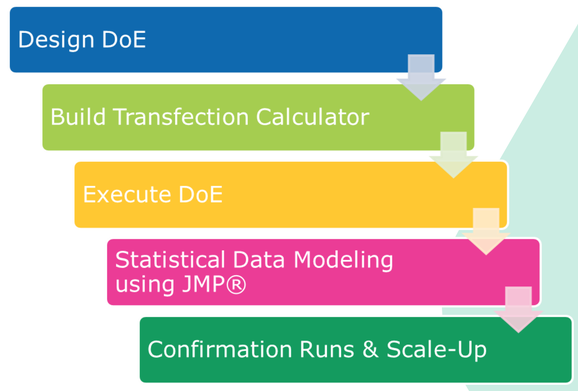
Factors that Impact Transfection Efficiency



- Using **Statistical analysis tool JMP®,** experimental design and data modeling software provided the insights regarding experimental approach for DOE and Scale-up.

- Scale-up in Mobius® 3L Single-use Bioreactor was done using linear scale-up of transfection parameters (transfection mix to culture volume).

Experimental Approach from DoE to Scale-Up



- A **high-throughput cell-based transduction assay** was used to determine lentivirus functional titer. % GFP(+) cells was determined at 72 hours post-transduction using Cytation 5 imager. Samples were assayed post-freeze thaw for harvest titer determination.

- All the graphs showing infectious titer data (Fig 1-6) are the averages of 2 dilutions plated in triplicates.

Results

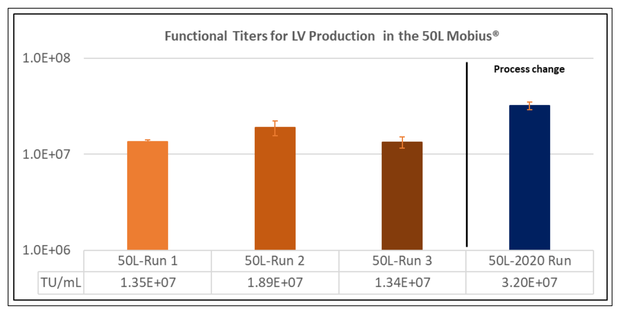


Figure 2: Average infectious titer of 1.5E+07 TU/mL was obtained with CD Prototype 1 (orange/brown bars) while a 2-fold improvement in production was achieved with improved formulation, EX-CELL® CD HEK293 Viral Vector Medium (catalog # 14385C, blue bar) in 50 L bioreactor.

Results: Lentivirus Infectious Titer @ 48 hr PT

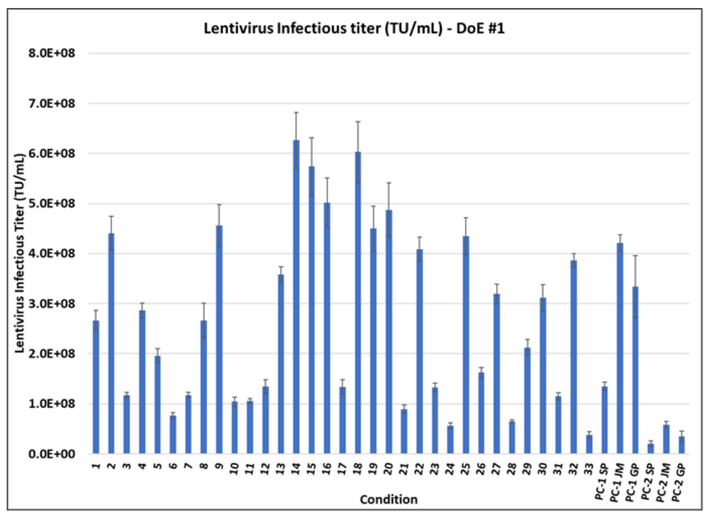


Figure 3: The 5 factors (transfection VCD, PEI:DNA ratio, total DNA/mL, complex formation volume and complex formation time) tested in DoE #1 had significant effect on lentivirus titer production. The highest titer achieved was 6.3E+08 TU/mL (condition 14) while the lowest titer was 3.8E+07 TU/mL (condition 33).

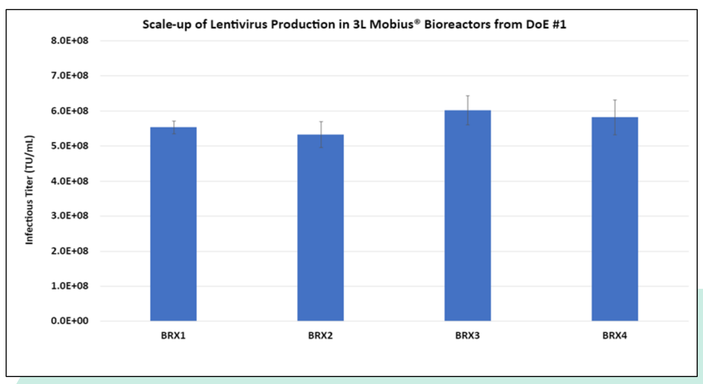


Figure 4: Scale-up in 3L bioreactors using the optimal levels of the 5 factors obtained through statistical data modeling from DoE #1 resulted in infectious titers ranging from 5-6E+08 TU/mL. Data model predicted harvest titer of ~7E+08 TU/mL.

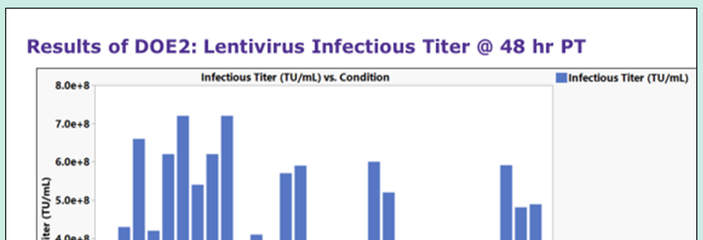


Figure 5: Plasmid molar ratio of each of the 4 plasmids used for lentivirus production was varied in DoE #2. Maximum harvest titer of 7.2E+08 TU/ml was obtained with conditions 6 & 9 while the lowest titer of 1E+08 TU/mL resulted with condition 10.

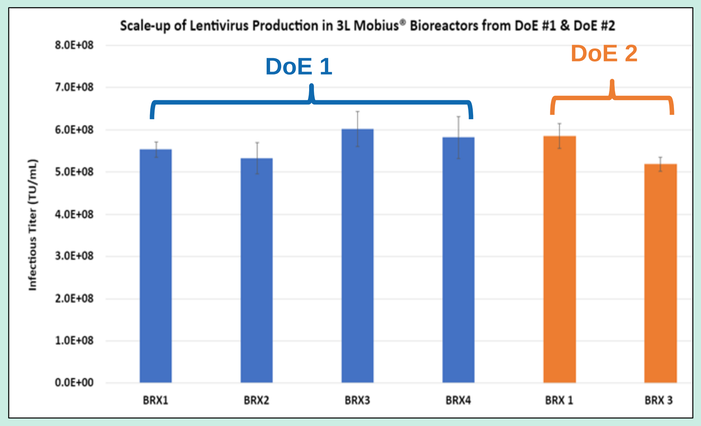


Figure 6: Optimal plasmid molar ratio of each plasmid from DoE #2 recommended through statistical modeling using JMP® was scaled up in 3L bioreactors. Infectious titers achieved in 3L bioreactors were comparable to DoE1 in the range of 5-6 E+08 TU/mL

Summary

- Many factors affect transient transfection efficiency. Using DoE tool and JMP® statistical analysis software, we studied these factors simultaneously rather than one-factor-at-a-time (OFAT). Using this approach, we were able to determine interactions between factors.
- Improved lentivirus production has been developed through DoE & 3L scale-up experiments resulting in harvest titers of 5-6E+08 TU/mL. Historical lentivirus production process resulted in harvest titers of 1.5E+07 TU/mL in 50L bioreactor.
- Using the 3L bench-scale bioreactors, we are conducting process development experiments to optimize gassing strategy and use of multiple sparger types as well as method for addition of transfection complex mix. These parameters need to be understood for large scale bioreactor lentivirus production.
- Future work will consist of scaling up the process to 50L pilot scale and above.

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