gibco

Large Scale Serum Free Suspension Lentiviral Production System for Gene Therapy Application

Yongchang Ji, Xin Yu and Xavier de Mollerat du Jeu

Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad CA, 92008

ABSTRACT

Lentiviral vectors have become the center of attention for its use as gene transfer vectors in gene therapy. Here, we have developed a new lentiviral production system for the clinical grade production of Lentiviral vectors on a large-scale serum-free suspension platform. This technology employs a newly developed propriety set of GMP reagents comprising of culture media, suspension cells, transfection reagent and boosting enhancers. With new culture media and media supplement, high density cell growth is optimized for maximum LVVs production. Customized lentivirus transfection reagents ensure highly efficient plasmids delivery. LVVs production is further elevated by the boosting enhancers. Taken together, our innovative system is able to deliver >2-3.0E+08 (TU/mL) functional titer with un-concentrated LVVs.

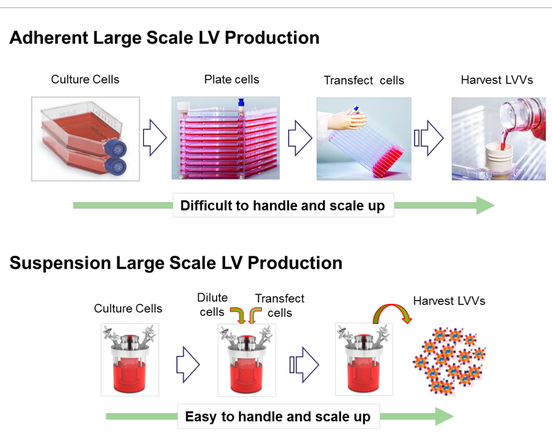
INTRODUCTION

Current new generation of therapies CAR-T requires the lentiviral vectors as efficient gene transfer tool to express engineered Chimeric Antigen Receptors on the surface of the T-cells to recognize and kill the cancer cells. One problem of developing CAR-T cell therapies is the high cost associated with lentiviral production. Therefore preclinical and clinical researchers have demanded their lentiviral production on a much larger scale, high-titer and in serum free medium. Current lentiviral production system use mainly adherent cells in fetal bovine serum to support cell growth, the system is suitable for research purpose at small scale, but not at large scale which requires large incubators. In this report, we describe the methods and DOE experiments that we used to identify this new suite of reagent and their application in the immune cell therapy field. The method can be easily scaled up to generate larger volumes of vector stocks in bioreactors.

MATERIALS AND METHODS

- Cells were transfected pLenti-GFP plus ViraPower™ Lentiviral Packaging Mix. Culture supplement medium was added at the time of transfection. Post-tfx 16hrs, LV enhancer was added to cells. Post-tfx 48hrs, LVVs were harvested and titer assay was performed by using unconcentrated cell supernatant on Ht1080 cells. After 4 days incubation, %GFP of infected Ht1080 cells were measured by Attune NxT Flow Cytometer.
- ViraPower™ Lentiviral Packaging Mix
- pLenti6.3/V5-GW/EmGFP Control Vector
- Opti-MEM™ I medium

Adherent vs. Suspension LV Production

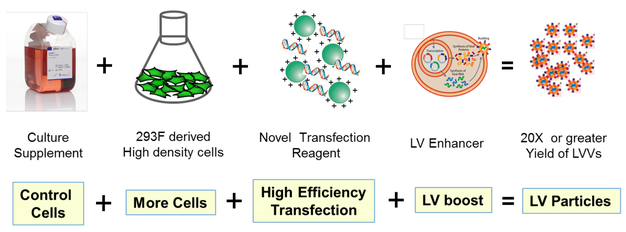


The advantages of suspension LV production:
- Simplicity
- Homogeneity of culture
- Easy to scale up
- Feasible for sealed culture system

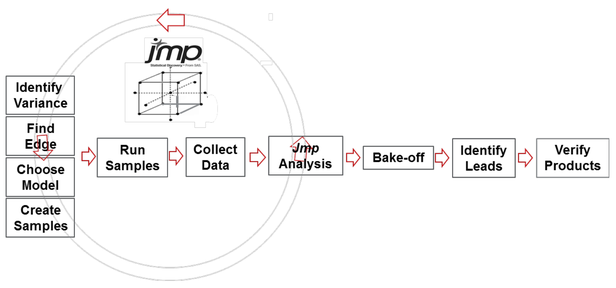
DEVELOPMENT STRATEGY

- Culture Supplement - Optimize cell growth
- New Transfection Reagent - Efficiently deliver DNA into high density cells
- LV Enhancer - Boost LV production

LV Production System

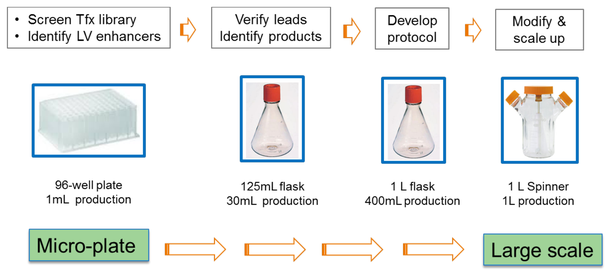


DoE Design



- Screen DoE designed transfection reagent library
- Identify new LV production enhancers

Experimental Format



Different experimental formats were applied, based on product development stages.

RESULTS

1. Culture Supplement Boosts LV Production

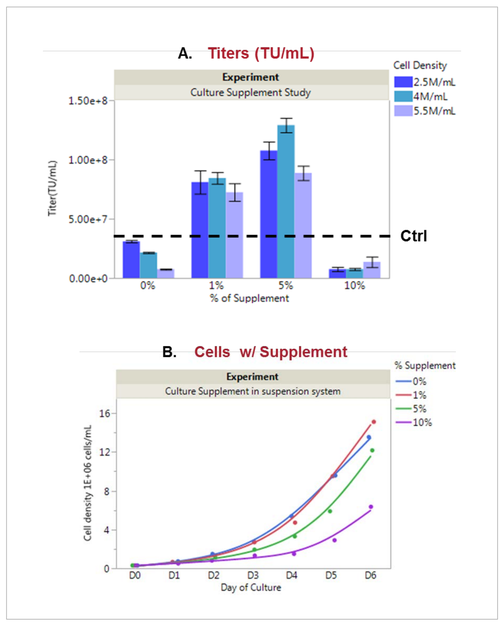


Fig 1. The amount of culture supplement in LV production. (A) At the time of virus production, culture medium was presented with 1%, 5% and 10% of culture supplement with various cell densities. Post-tfx 48hrs, LVVs were collected and titers were measured, respectively. Dot line is the control without the supplement. (B) Suspension cell viabilities were monitored in the presence of various amount of supplements.

2. Screening of New Transfection Reagents

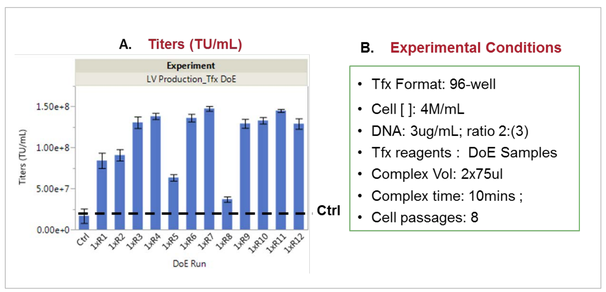


Fig 2. *jmp* DoE designed new transfection reagents were screened in this experiment. (A) Total 12 candidates were designed by *jmp*. 8 of 12 new tfx reagents were chosen for next DoE run. (B) The transfection conditions for the experiment. The dot line was control samples where linear 25K PEI was used as a transfection reagent.

3. Identification of Super LV Enhancers

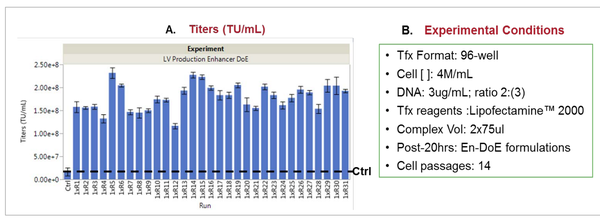
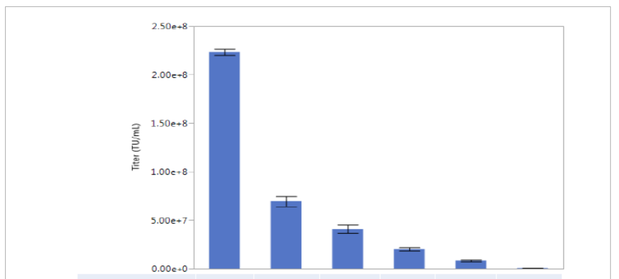


Fig 3. The Bake-off experiment for identifying new LV enhancer leads was designed by *jmp* DoE. (A) 32 candidates including a control without LV enhancer were tested. The samples with top 5 performance were chosen for the next step to identify leads. (B) Listed transfection conditions for this experiment.

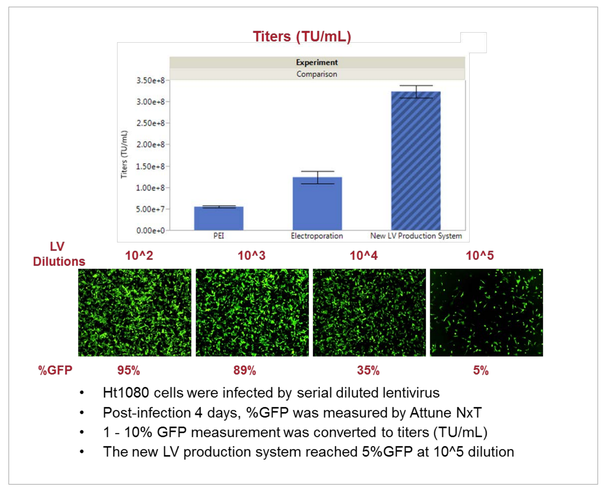
4. Superior LV Production in the New System



Cell	LV 293	LV 293	LV 293	LV 293	293F	293F
Transfection reagents	LVR2	PEI	LVR2	PEI	LVR2	PEI
Supplement	+	+	-	-	-	-
Enhancer	+	+	-	-	-	-
Sodium butyrate	-	-	+	+	+	+

Fig 4. LV production improvement compared to different production components. LV was produced in a 125mL-30mL format.

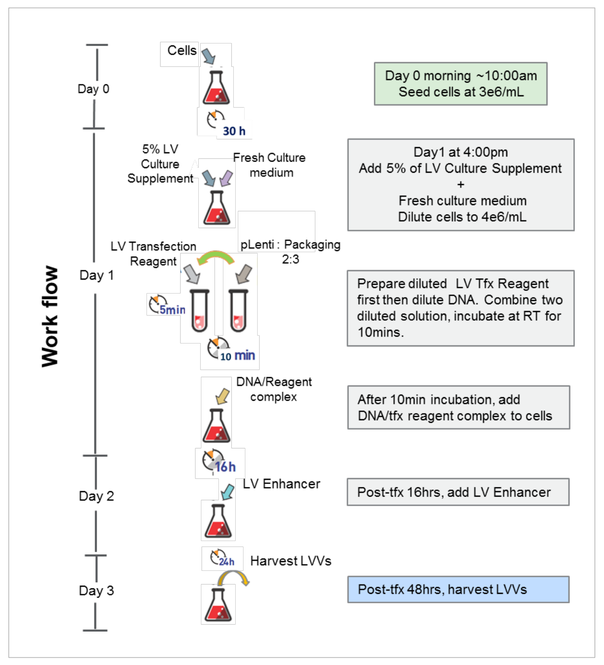
5. LV Production System Comparison



- Ht1080 cells were infected by serial diluted lentivirus
- Post-infection 4 days, %GFP was measured by Attune NxT
- 1 - 10% GFP measurement was converted to titers (TU/mL)
- The new LV production system reached 5%GFP at 10^5 dilution

Fig 5. Compare our new developed suspension LV production system with PEI mediated and electroporation technique LV production. Our LV production system with combination of all new findings is superior than other two system.

Suspension LV Production Protocol



CONCLUSION

- We developed a new serum free suspension LV production system. It's easy to use, scalable and highly efficient. Combination of all the new findings, the system can produce 20X more LV than current methods.
- We identified a new generation of transfection reagent, which can efficiently deliver lentiviral expression and package vectors into serum-free suspension-growing cells at a density of 4E+06 cells/mL.
- We developed a special culture supplement, which keeps cells in the middle log phase, yielding a maximum amount of lentiviral particles.

LICENSE CONTACT INFORMATION

R&D
de Mollerat du Jeu, Xavier
Work: +1 760 476 6642
E-mail: xavier.demolleratdujeu@thermofisher.com

Licensing and Commercial Supply
McCarty, Aaron
Work: +1 760 268 7428
E-mail: Aaron.McCarty@lifetech.com

ThermoFisher SCIENTIFIC