Scalability of CTS LV-MAX Lentiviral Production System in bioreactors

Introduction

The Gibco™ CTS™ LV-MAX™ Lentiviral Production System is the first optimized high-titer lentiviral vector (LV) platform for the production of GMP lentivirus in suspension cells. The system enables scalable production of greater than 1 x 10^8 TU/mL LV (unconcentrated) using high-density HEK293F-derived suspension cells adapted to a chemically defined medium, a proprietary transfection reagent, a production supplement, and an enhancer. All components of the CTS LV-MAX system are serum-free, xeno-free, manufactured in conformity with current GMP guidelines for medical devices (21 CFR Part 820), and follow USP <1043> and European Pharmacopoeia (Ph. Eur.) 5.2.12 recommendations. All Gibco™ CTS™ products will have a Drug Master File (DMF) in the United States, Canada, and Japan or a Regulatory Support File in other regions available per request.

Here we demonstrate the scalability of the CTS LV-MAX system in a 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor.

Materials

- Gibco™ CTS™ Viral Production Cells (VPCs) (Cat. No. A3152801)
- Gibco™ CTS™ LV-MAX™ Production Medium (Cat. No. A4124002)
- Gibco™ CTS™ LV-MAX™ Transfection Kit (Cat. No. A4132602)
- Gibco™ CTS™ Opti-MEM™ I Medium (Cat. No. A4124802)
- Thermo Scientific™ HyPerforma™ Glass Bioreactor (Cat. No. F100-2680-002)
- Thermo Scientific™ HyPerforma™ G3Lab™ Controller (Cat. No. F100-2695-002)
- Gibco™ LV-MAX™ Lentiviral Packaging Mix (Cat. No. A43237)
- Invitrogen™ Vivid Colors™ pLenti6.3/V5-GW/EmGFP Expression Control Vector (Cat. No. V37006)
- HT1080 cells (ATCC Cat. No. CCL-121)
- Gibco™ DMEM, high glucose, GlutaMAX™ Supplement, pyruvate (Cat. No. 10569010)
- Gibco™ Fetal Bovine Serum, qualified, United States (Cat. No. 26140087)
- Polybrene (Sigma-Aldrich Cat. No. TR-1003-G)
- Gibco™ TrypLE™ Select Enzyme 1X, No Phenol Red (Cat. No. 12563011)
- Invitrogen™ Attune™ NxT Flow Cytometer (Cat. No. A24858)

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Methods

1. Bioreactor control and process parameters

The following set points are used when producing lentivirus in 3 L HyPerforma Glass Bioreactors.

Parameter	Setting
Temperature	37 ± 0.5 ˚C
Vessel	3 L HyPerforma Glass Bioreactor
Controller	HyPerforma G3Lab Controller
Working volume	2 L
Sparge	L–shaped drilled-hole macrosparge
Impellers	1 x Rushton (bottom), 1 x 3 pitched blade (top)
Impeller diameter	55 mm
Impeller power number	1.4
Agitation	140 rpm, P/V 4.5 W/m^3, tip speed 0.4 m/sec
Headspace gassing	Air—0.05 lpm
Dissolved oxygen (DO)	40% controlled by O_2 through the sparge
pH for growth	≤7.25 controlled by CO_2 through the sparge
pH for production	6.80 ± 0.05 controlled by CO_2 through the sparge

2. Bioreactor inoculum preparation and inoculation

Prepare cells (day –6)

CTS VPCs were grown and maintained in shake flasks as directed by the CTS LV-MAX Lentiviral Production System user guide (Pub. No. MAN0018450). Three days prior to inoculation of the bioreactor, a seed culture was prepared by inoculating CTS VPCs at a density of 0.5 x 10^6 cells/mL at a culture volume of 400 mL. This seed culture yielded the 1 x 10^9 cells that were needed to seed one stirred-tank bioreactor.

Prepare the bioreactor (day –4 to day –3)

On day –4, the 3 L HyPerforma Glass Bioreactor was assembled. The pH and DO probes were calibrated and attached to the bioreactor, which was then sterilized in an autoclave. Following sterilization, the bioreactor was allowed to cool to room temperature before 700 mL of CTS LV-MAX Production Medium were added. Stirring speed, temperature, and headspace gassing were set to 145 rpm, 37°C, and 50 mL/min of air, respectively. DO was set to 40%, controlled by on-demand O_2 through the macrosparge. The pH was set to 7.25, with an upper dead band of 0.05, and was controlled by on-demand CO_2 through the macrosparge. The bioreactor was allowed to equilibrate to 37°C before cells were seeded.

Inoculate bioreactor (day –3)

The bioreactor was inoculated by adding CTS VPCs from the seed culture to a final density of 1 x 10^6 cells/mL in a total volume of 1,000 mL. The pH was checked offline on a blood gas analyzer, and a one-point calibration was performed.

3. Bioreactor transfection and harvest

Dilute and pH shift (day 0)

The culture reached an approximate cell density of 5–7 x 10^6 cells/mL three days after inoculation. Viability remained above 95%, which is necessary to proceed with the transfection process. Next, pre-warmed CTS LV-MAX Production Medium was added to dilute the culture to a final viable cell density of 4 x 10^6 cells/mL. The pH was shifted down to 6.8, with an upper dead band of 0.05, controlled by CO_2 only. The bioreactor was allowed to equilibrate to the optimal temperature and pH before transfection.

Transfect (day 0)

To make the transfection complex, CTS Opti-MEM I Medium was added to a 250 mL Thermo Scientific™ Nalgene™ PETG bottle at 5% v/v of cell culture to be transfected. The CTS LV-MAX Transfection Reagent bottle was gently inverted 4–5 times to mix and then added to a final concentration of 6 mL/L of culture to be transfected to the 250 mL Nalgene bottle. The bottle was then swirled briefly to mix.

To a second 250 mL Nalgene PETG bottle, CTS Opti-MEM I Medium was added at 5% v/v of cell culture to be transfected. Plasmid DNA was then added to a final concentration of 2.5 mg/L of the culture to be transfected (1 mg/L packaging vector and 1.5 mg/L transfer vector for a combined total of 2.5 mg/L). The bottle was then swirled briefly to mix.

The two Nalgene bottles were combined by decanting the second bottle containing the diluted plasmid DNA into the first bottle that contained the diluted CTS LV-MAX Transfection Reagent. The mixture was then swirled briefly and incubated at room temperature for 10 minutes. Following the incubation, a transfer cap was attached to the bottle and then sterile tube–welded to the bioreactor. The mixture was then transferred to the bioreactor through a peristaltic pump.

Addition of enhancer and supplement (day 0)

Four hours posttransfection, Gibco™ CTS™ LV-MAX™ Enhancer and Gibco™ CTS™ LV-MAX™ Supplement were combined in a 250 mL PETG Nalgene bottle with a transfer cap and added to the bioreactor. CTS LV-MAX Enhancer was added at 4% v/v of transfected cell culture and CTS LV-MAX Supplement was added to a final volume of 5% v/v of transfected cell culture.

Harvest (day 2)

The bioreactor was sampled daily. Cells were counted on an automated cell counter. The pH, O_2, and CO_2 levels were checked on a gas analyzer. Metabolites produced in the bioreactor were checked on a bioanalyzer.

Produced LV was harvested 48 hours posttransfection. One mL of crude culture was centrifuged for 15 minutes at 3,000 x *g*. The supernatant (denoted as "LV sample") was filtered through a 0.45 µm syringe filter and then stored at –80°C for further analysis.

4. Determination of infectious titer

HT1080 cells were seeded in a 96-well culture plate at a density of 1 x 10^4 cells/well in 100 µL DMEM (high glucose, GlutaMAX Supplement, pyruvate) and 10% FBS. The cells were placed in a 37°C incubator with 8% CO_2. The following day, the LV samples (from step 3) were allowed to thaw on ice for 2 hours. During this time, dilution medium was prepared by combining 25 mL of DMEM (high glucose, GlutaMAX Supplement, pyruvate) supplemented with 10% heat-inactivated FBS containing 20 µL of 10 mg/mL of polybrene for a final concentration of 8 µg/mL. The dilution medium was vortexed to mix. A 1:10 serial dilution of each LV sample was performed by adding 40 µL of LV sample to 360 µL of dilution medium. Serial dilutions were performed in a 96-well plate from 10^{-1} to 10^{-5}. After the 96-well plate containing HT1080 cells was retrieved from the incubator, the medium was aspirated and replaced with 100 µL of diluted LV sample. The transduced cells were then centrifuged at 900 x *g* for 30 minutes at room temperature followed by incubation for 72 hours at 37°C.

After 72 hours, the plate was removed from the incubator, medium was aspirated, and 150 µL of TrypLE enzyme was added to each well. The plate was incubated for 5–10 minutes at 37°C before the cells were resuspended by gently pipetting up and down. LV titer (TU/mL) was determined by processing the cells through a flow cytometer.

Results

Previous pH control optimization experiments in the CTS LV-MAX system using an Ambr™ 15 bioreactor revealed that controlling pH to 6.8 posttransfection is the optimal set point for the plasmid construct used in these experiments (note that additional pH optimization may be required when using a different plasmid construct). In the 3 L stirred-tank bioreactor, the pH shift only required the use of CO_2 and no base was required. In the control shake flasks, the pH dropped to 6.6–6.7 posttransfection (Figure 1A). Glucose utilization was similar at both scales, with the CTS LV-MAX system consuming slightly less glucose posttransfection in the 3 L stirred-tank bioreactor than in the 125 mL shake flask (Figure 1B). Lactate levels were significantly lower posttransfection in the 3 L stirred-tank bioreactor than in the 125 mL shake flask (Figure 1C).

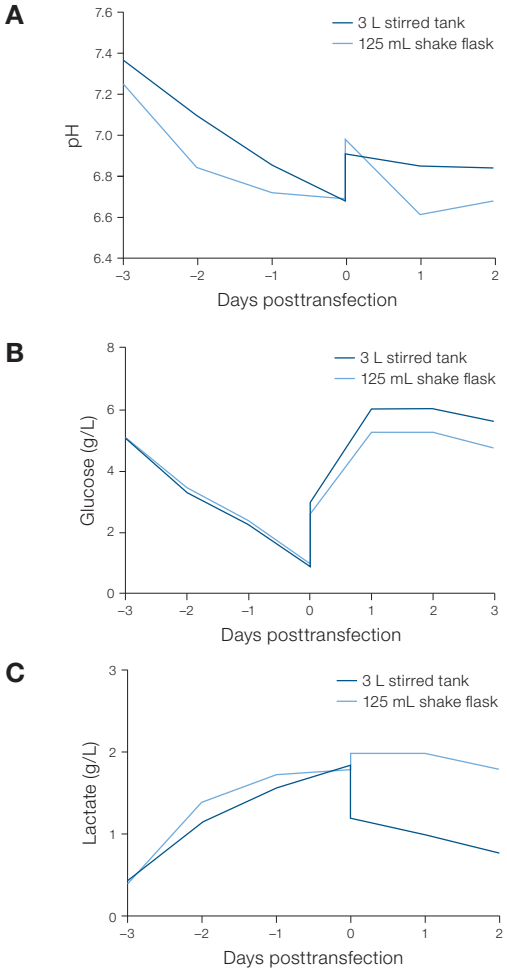

Figure 1. Analysis of metabolites and optimization of pH. (A) pH values, **(B)** glucose levels, and **(C)** lactate levels during growth and lentiviral production were measured in 3 L stirred-tank bioreactor (dark blue) and 125 mL shake flask (light blue).

During growth phase (day –3 to day 0), cells in both 125 mL shake flask and 3 L stirred-tank bioreactor grew to similar cell densities, reaching a peak cell density of 6.3 x 10^6 cells/mL and 7.0 x 10^6 cells/mL, respectively, while maintaining viabilities above 97%. VCDs remained similar at 5–6 x 10^6 cells/mL posttransfection (day 0 to day 2) and viabilities dropped to 80% at the time of harvest (Figure 2A). After harvest, LV titers obtained at both scales were comparable at 2.22 x 10^8 TU/mL in the stirred-tank bioreactor and 1.90 x 10^8 TU/mL in the 125 mL shake flask (Figure 2B).

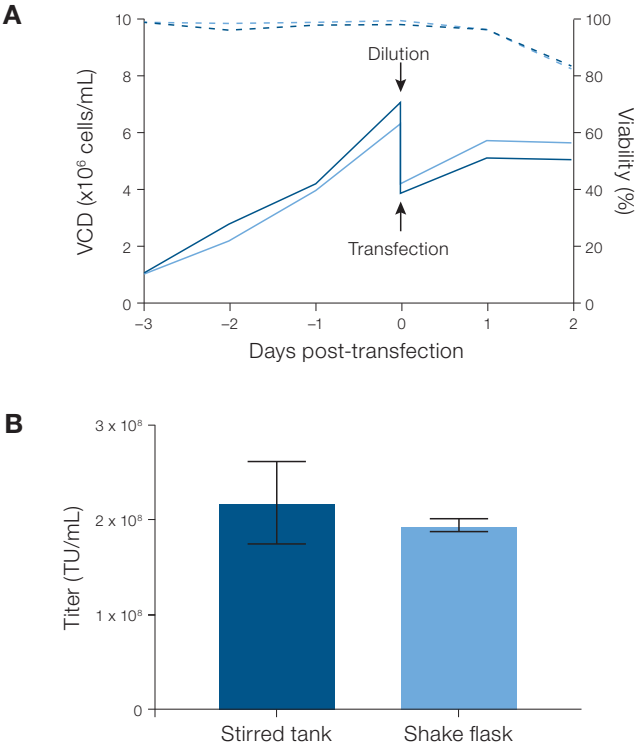

Figure 2. Scale-up of the CTS LV-MAX Lentiviral Production System in 3 L HyPerforma Glass Bioreactor (dark blue) and 125 mL shake flask (light blue). (A) Viable cell density (VCD) (solid lines) and percent viability (dotted lines). **(B)** Infectious titers obtained at both scales were comparable.

To further optimize the CTS LV-MAX system in 3 L stirred-tank bioreactors, the system was tested across a range of power inputs per volume (P/Vs). To this end, 3 bioreactors were set up using the following parameters: (1) 140 rpm (P/V = 4.5 W/m^3, tip speed = 0.4 m/sec), (2) 185 rpm (P/V = 10 W/m^3, tip speed = 0.5 m/sec), and (3) 210 rpm (P/V = 15 W/m^3, tip speed = 0.6 m/sec). The different stirred-tank bioreactors were compared to a 125 mL shake flask control (Figure 3). VCD and cell viability comparison among the three P/Vs and the shake flask control showed comparable growth kinetics across scales and P/V (Figure 3A). There was no significant difference in infectious LV titers across the range of P/Vs tested and all conditions were comparable to the 125 mL shake flask control (Figure 3B).

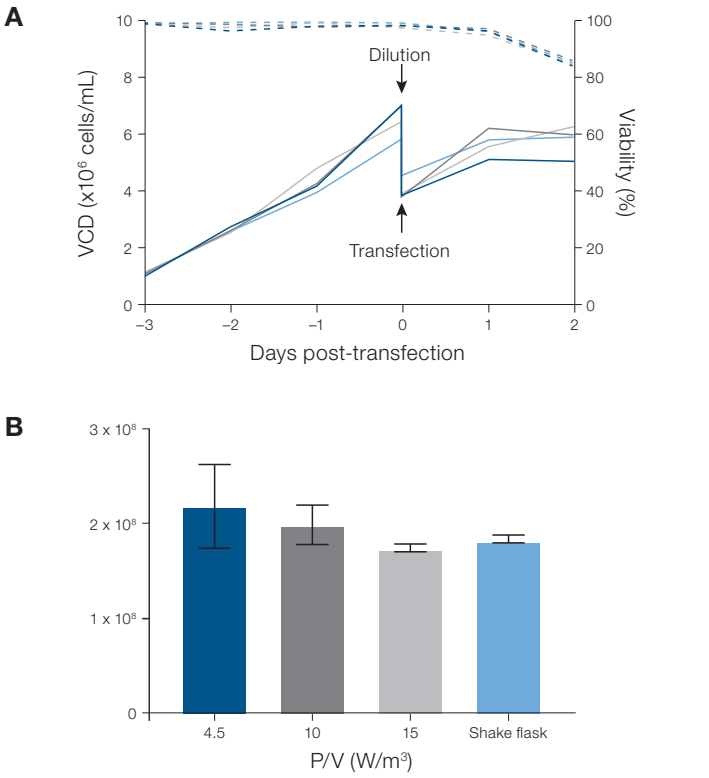

Figure 3. Optimization of P/V for the CTS LV-MAX Lentiviral Production System in the stirred-tank bioreactor. Different P/Vs were compared for the stirred-tank bioreactor—140 rpm (P/V = 4.5 W/m^3, dark blue), 185 rpm (P/V = 10 W/m^3, dark gray), and 210 rpm (P/V = 15 W/m^3, light gray); a 125 mL shake flask was used as a control (light blue). **(A)** VCD (solid lines) and percent viability (dotted lines) was comparable across scales and P/Vs. **(B)** There was no significant difference in infectious titer across the range of P/Vs tested and all are comparable to the 125 mL shake flask control.

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Conclusion

We demonstrate herein that the CTS LV-MAX Lentiviral Production System is readily scalable into a 3 L HyPerforma Glass Bioreactor with LV titers comparable to shake flask controls without the need for additional supplements or reagents outside of those provided in the CTS LV-MAX Lentiviral Production System. Optimal system performance was observed across a range of P/Vs, emphasizing the robustness of the CTS LV-MAX system for LV production. These results demonstrate the scalability of the CTS LV-MAX system and its adaptability to large-scale viral vector production processes.

Ordering information

Product	Cat. No.
CTS Viral Production Cells	A3152801
CTS LV-MAX Production Medium	A4124002
CTS LV-MAX Transfection Kit	A4132602
CTS Opti-MEM I Medium	A4124802
HyPerforma Glass Bioreactor (3 L)	F100-2680-002
HyPerforma G3Lab Controller	F100-2695-002
LV-MAX Lentiviral Packaging Mix	A43237
Vivid Colors pLenti6.3/V5-GW/EmGFP Expression Control Vector	V37006
HT1080 Cells	ATCC CCL-121
DMEM, High Glucose, GlutaMAX Supplement, Pyruvate	10569010
FBS, Qualified, United States	26140087
TrypLE Select Enzyme 1X, No Phenol Red	12563011
Attune NxT Flow Cytometer	A24858

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