Lentivirus and AAV Production in Suspension and Adherent Cells with MaxCyte Flow Electroporation

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Abstract

Viral Vectors have been a core component of gene therapy since the 1980s. Since that time vector production technologies have not changed a great deal, leaving industry and academia alike to face the same challenges. MaxCyte's flow electroporation technology is a universal transient transfection platform that addresses many of the issues of viral vector production. The MaxCyte system can (co)transfect from 5E5 cells up to 2E11 cells with plasmid DNA and/or mRNA to generate a wide range of viral vectors, including vectors derived from lentivirus, adenovirus, adeno-associated virus, and alphavirus. Compared to reagent-based methods of viral vector production, which are typically labor-intensive, multi-step processes with low efficiency, MaxCyte's flow electroporation system requires minimal optimization and offers seamless scalability. In this poster, data will be presented showing that the MaxCyte system can transfect both adherent and suspension cells to produce AAV and Lentivirus rapidly and efficiently.

MaxCyte STX®

5E5 Cells in Seconds
Up to 2E10 Cells in <30 Min.

MaxCyte VLX®

Up to 2E11 Cells in <30 Min

The MaxCyte STX® and MaxCyte VLX® Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection with very high cell viability post transfection. Transfected cells support gram-scale production of antibodies and proteins for efficient biotherapeutic development.

Large-Scale Lentivector Production in Adherent HEK Cells

Four Plasmid Co-transfection with the MaxCyte STX

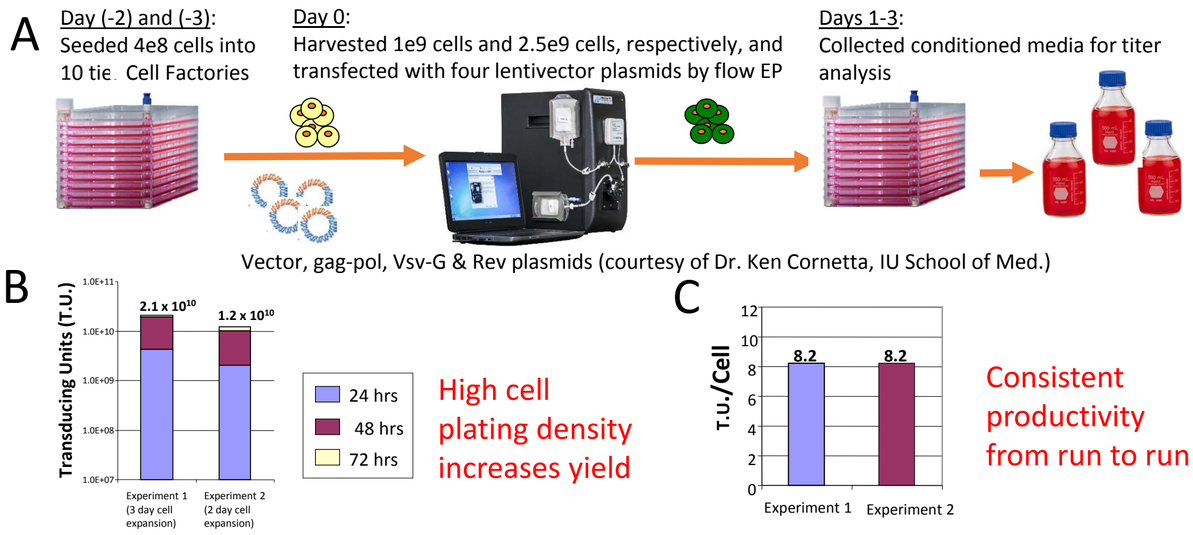

Figure 1. Production of Lentivirus in Adherent HEK Cells using Flow Electroporation. A: HEK 293FT cells were seeded in 10 tier Cell Factories 2-3 days prior to electroporation (EP). Cells were co-transfected with 4 plasmids (HIV-based lentivector system) using flow EP. Media were collected daily (complete replenishment each day), and transducing units were measured. B and C: Results for 2 independent transfections are shown.

Seamless Scale-up of Lentivector Production in Suspension Cells

Consistency of Static and Flow Electroporation using the MaxCyte STX and VLX

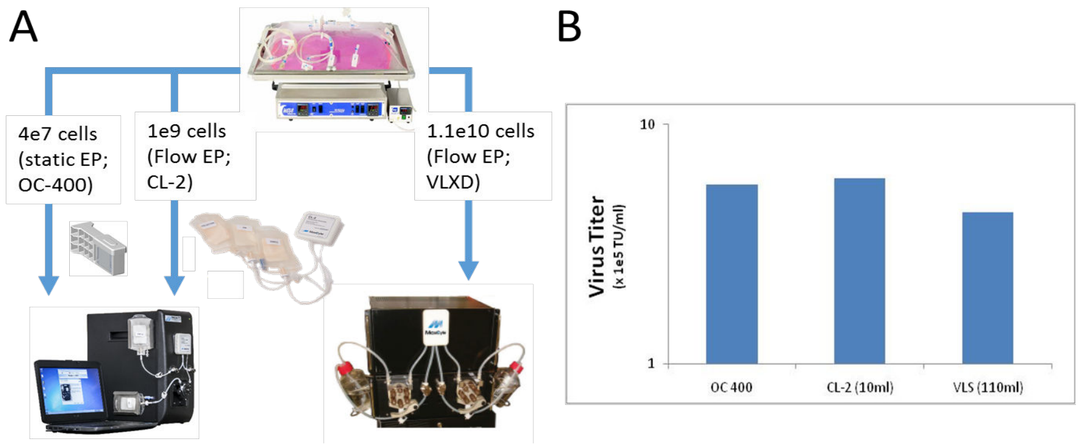

Figure 3. Production of Lentivirus in HEK Cells at Three Different Scales. A: Suspension HEK 293FT were transfected with lentivirus component plasmids via static EP and flow EP with the MaxCyte STX and via flow EP with the Maxcyte VLX. B: Lentiviral titers measured 48 hr post EP show consistent productivity regardless of the scale.

Lentivector Manufacturing in Suspension HEK Cells

Transfecting Suspension-adapted Cells Facilitates Scalability of Vector Production

B

Table 1. Titer Results of Large-Scale Lentivirus Productions in 10-L Cellbags[a]

Production no.	Volume (mL)	Total cells	48-hr titer (IU/mL)	Cumulative titer (IU)	Productivity (IU/cell)	p24 (ng/mL)	IU/ng of p24
1	2300	6.0×10^9	9.8×10^7	2.2×10^{11}	37	771±58	1.3×10^5
2	2300	4.8×10^9	8.8×10^7	2.0×10^{11}	42	614±76	1.4×10^5
3	2100	7.4×10^9	1.3×10^8	2.7×10^{11}	36	739±33	1.7×10^5

Source: Witting et al. (2012). Hum. Gene Ther. 23:243–249.

Figure 2. Scalable Lentivector Production in Suspension HEK cells. A: Suspension-adapted HEK 293FT cells were cultured in 10L Wave Cellbags, transfected via flow EP with lentivctor component plasmids and then returned to Cellbags. B: Result for 3 production runs in a GMP-compliant core facility show consistently high titers in each production run.

High Titer AAV Production in HEK Cells

Three Plasmid Co-transfection Yields High Efficiency and Robust Titers

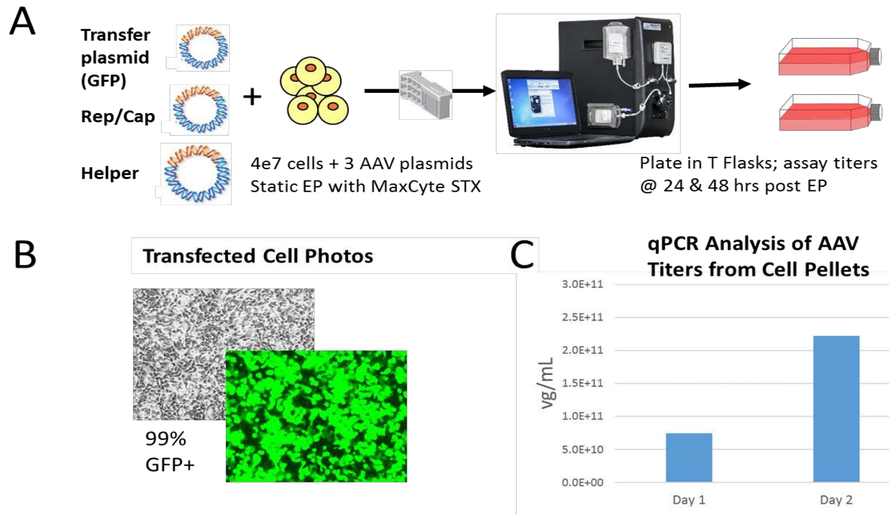

Figure 4. Production of AAV in HEK Cells A: Adherent HEK cells were transfected with three plasmids encoding AAV vector components (GFP transgene) via static EP using the MaxCyte STX. B: Nearly 100% of the transfected cells exhibited robust transgene expression 48 hr post EP. C: High AAV titers were measured in cell pellets via qPCR analysis.

Summary

- MaxCyte's scalable electroporation technology allows highly efficient transfection of adherent- and suspension-adapted cells.
- The ability to co-transfect multiple plasmids and large plasmids enables manufacturing of complex viral vectors with the MaxCyte system.
- Consistency of the MaxCyte transfection process results in reproducible production yields, regardless of the culture scale.
- Efficient transfection of suspension cells allows for simplified scalability of viral vector manufacturing using the MaxCyte process.
- High AAV titers generated via MaxCyte electroporation illustrate the diversity of manufacturing applications that can be facilitated with MaxCyte's flow EP process.