Intensified lentiviral vector perfusion bioprocessing with a spiral inertial microfluidic cell retention device

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Supplementary Information

Supplementary Figures and Descriptions

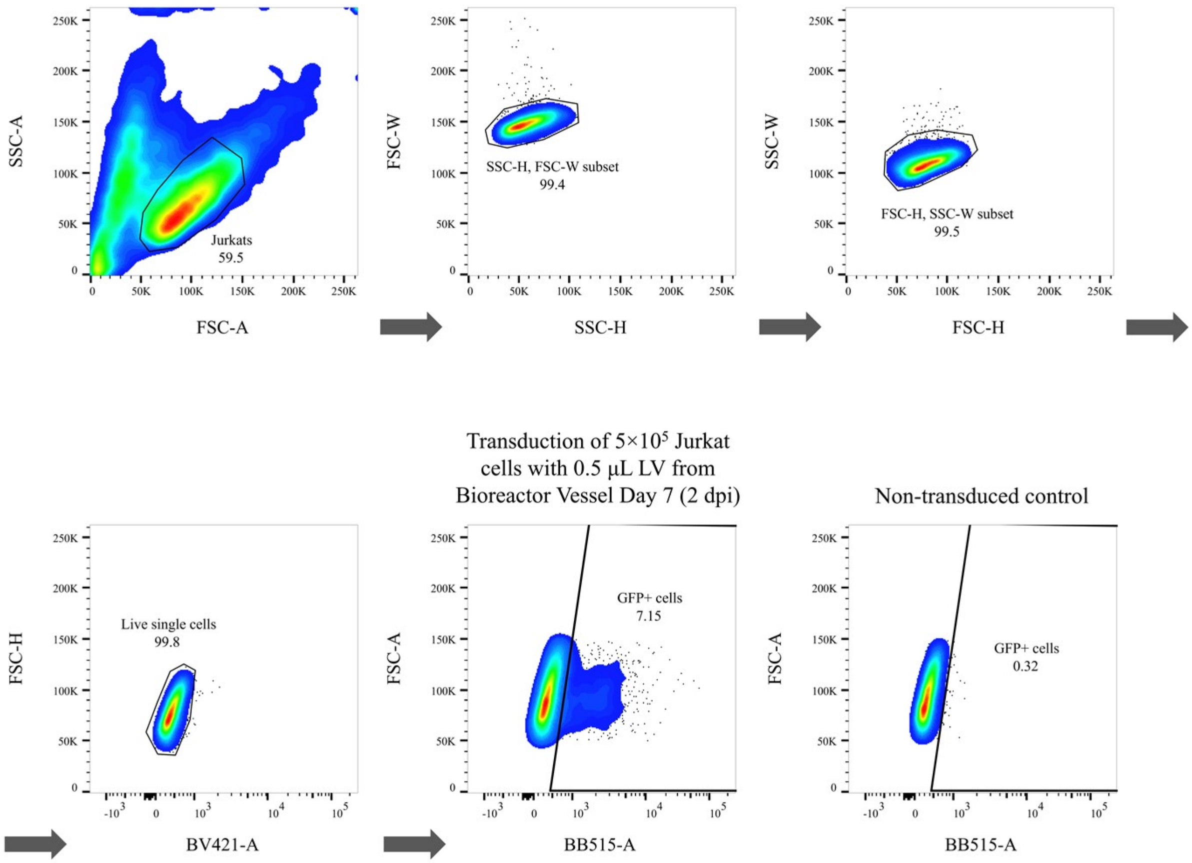

Figure S1: Jurkat T cell transduction assay gating strategy.

Multiplicity of infection (MOI) is defined as the number of infectious LV particles divided by the number of Jurkat cells. If the MOI is well-approximated, the number of cells introduced into each well at the time of transduction can be used to determine the number of infectious units of LV. Functional titer is calculated from flow cytometry plots, which have a $\%GFP^+$ | Live Jurkat cells (Percentage of GFP positivity, given a live cell) of ideally 5 to 25%, since by the Poisson Distribution, this offers greater likelihoods that one cell is not infected multiple times.

$$P(n) = \frac{\lambda^n e^{-\lambda}}{n!}$$

λ is the average number of LVs per cell or the MOI, $P(n)$ is the probability that a cell is infected by n LVs. The probability that a cell is infected by zero LVs is $P(n = 0) = e^{-MOI}$, the probability that a cell is infected by one LV is $P(n = 1) = MOI \cdot e^{-MOI}$, and the probability that a cell is infected by more than one LV is $P(n > 1) = 1 - e^{-MOI}(1 + MOI)$.

If a cell is infected by multiple infectious units, it becomes more difficult to calculate the viral titer. Since the readout from flow cytometry and the gating strategy are based on GFP expression, cells can be stratified into GFP^+ (the case where one or more LVs infected a Jurkat cell: $P(n \geq 1)$) and GFP^- (the case where no LVs infected a Jurkat cell: $P(n = 0)$). Uninfected Jurkat cells are therefore described in Poisson terms as $P(n = 0) = e^{-MOI} = 1 - \%GFP^+$, which may then be rewritten as $MOI = -\ln(1 - \%GFP^+)$. Ideally, for an accurate titer measurement, it is important to maximize the number of cells that are infected by zero LVs or one LV, which means that $P(n > 1) = 1 - e^{-MOI}(1 + MOI)$ should be reasonably low, while ensuring that $\%GFP^+$ is high enough to draw a conclusion based on a sufficient number of cells.

This scenario occurs when $P(n \geq 1)$ (the $\%GFP^+$) is between 5 and 25% (**Figure S2**). The $P(n \geq 1) = \%GFP^+$ model can be used for functional titer determination using these equations:

$$P(n \geq 1) = 1 - e^{-MOI} \rightarrow MOI = -\ln(1 - \%GFP^+)$$

$$LV\ Functional\ Titer = \frac{0.5 \times 10^6\ cells \cdot MOI}{(LV\ volume\ added)} = \frac{0.5 \times 10^6 \cdot (-\ln(1 - \%GFP^+))}{(LV\ volume\ added)}$$

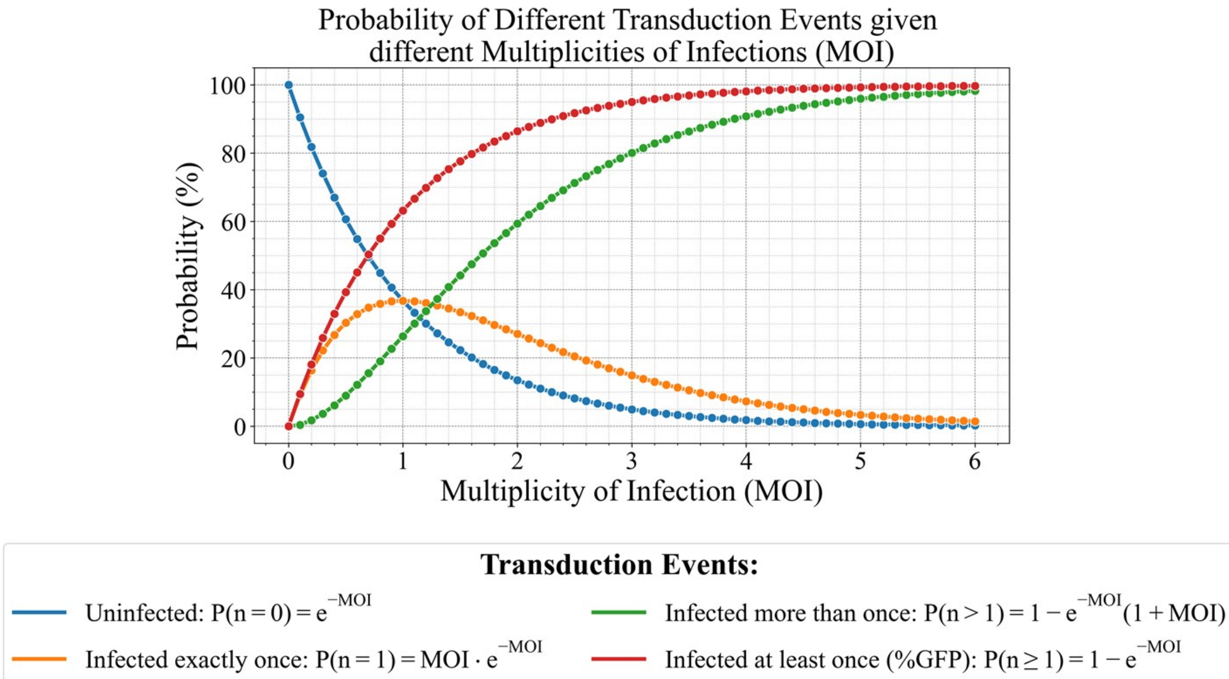

Figure S2: Poisson-distributed theoretical curves for probability of various transduction events occurring as a function of multiplicity of infection (MOI): $P(n) = \frac{\lambda^n e^{-\lambda}}{n!}$ for no LV ($n = 0$), single transduction ($n = 1$), more than one transduction ($n > 1$), and at least one transduction ($n \geq 1$).

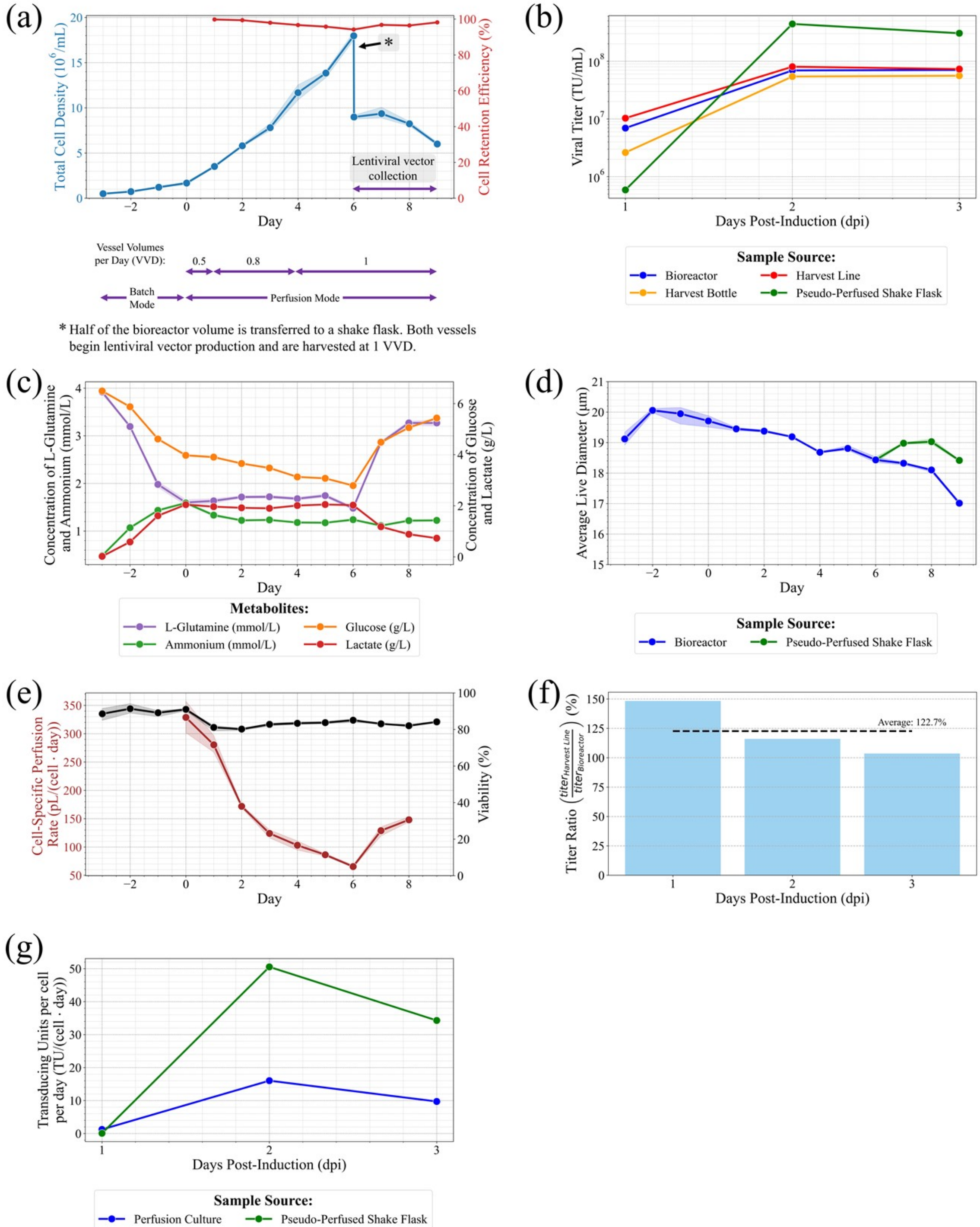

Figure S3: Perfusion Culture Run 2. **(a)** Time-series data of the total cell density of HEK293 cells in the bioreactor and cell retention efficiency of the spiral microfluidic device. Once the target cell density was achieved, half of the bioreactor volume was transferred to a shake flask, and both vessels were simultaneously induced, filled back to the working volume of 350 mL and then maintained at 1 VVD (as indicated by *). **(b)** Functional unconcentrated viral titer measurements at multiple sample sources during the lentiviral vector production phase. **(c)** Concentrations of L-glutamine, ammonium, glucose, and lactate in the bioreactor. **(d)** Average cell diameter in the bioreactor and the pseudo-perfused shake flask. **(e)** Cell-specific productivity and viability in the bioreactor. **(f)** Titer ratio, represented as the quotient of the functional titers measured in the harvest line and the bioreactor. **(g)** Cell-specific productivity (TU $cell^{-1}$ day^{-1}) of the perfusion bioreactor and pseudo-perfused shake flask. Perfusion bioreactor: Peak cell-specific productivity of 16.03 TU $cell^{-1}$ day^{-1} at 2 dpi, cell-specific yield of 27.0 TU $cell^{-1}$. Pseudo-perfused shake flask: Peak cell-specific productivity of 50.55 TU $cell^{-1}$ day^{-1} at 2 dpi, cell-specific yield of 84.92 TU $cell^{-1}$.

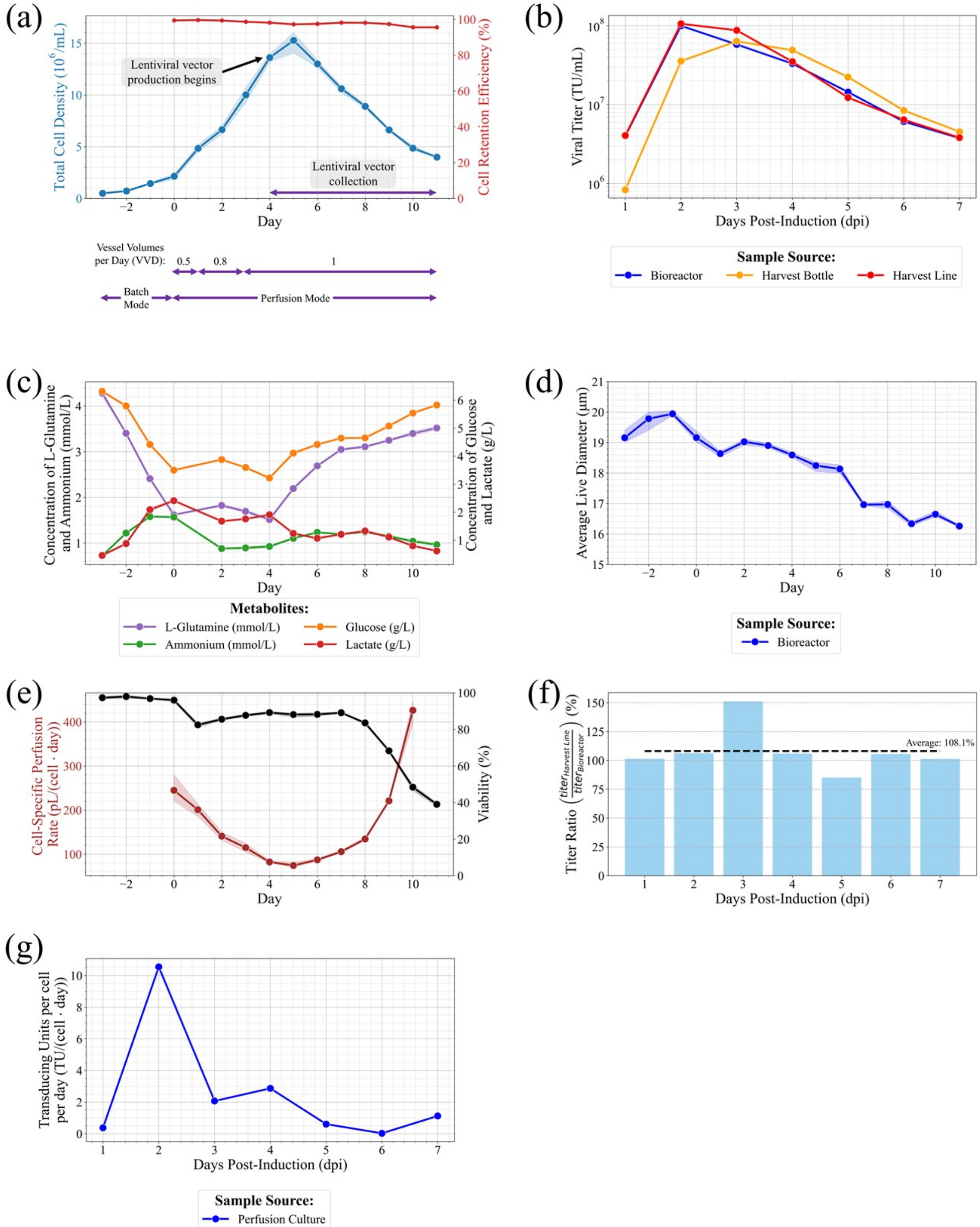

Figure S4: Perfusion Culture Run 3. **(a)** Time-series data of the total cell density of HEK293 cells in the bioreactor and cell retention efficiency of the spiral microfluidic device. **(b)** Functional unconcentrated viral titer measurements at multiple sample sources during the lentiviral vector production phase. **(c)** Concentrations of L-glutamine, ammonium, glucose, and lactate in the bioreactor. **(d)** Average cell diameter in the bioreactor. **(e)** Cell-specific productivity and viability in the bioreactor. **(f)** Titer ratio, represented as the quotient of the functional titers measured in the harvest line and the bioreactor. **(g)** Cell-specific productivity (TU $cell^{-1}$ day^{-1}) of the perfusion bioreactor: Peak cell-specific productivity of 10.55 TU $cell^{-1}$ day^{-1} at 2 dpi, cell-specific yield of 17.63 TU $cell^{-1}$.

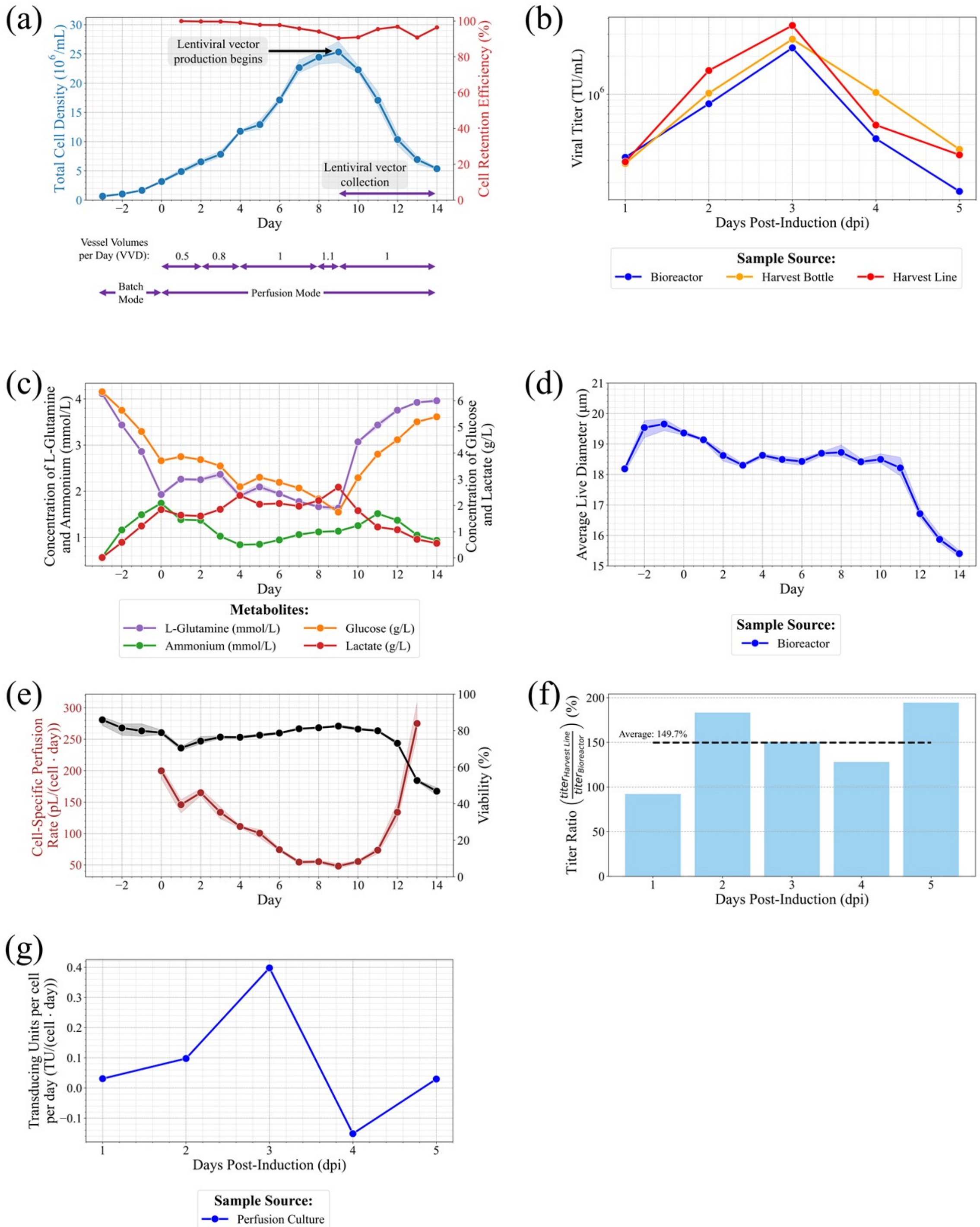

Figure S5: Perfusion Culture Run 4. **(a)** Time-series data of the total cell density of HEK293 cells in the bioreactor and cell retention efficiency of the spiral microfluidic device. **(b)** Functional unconcentrated viral titer measurements at multiple sample sources during the lentiviral vector production phase. **(c)** Concentrations of L-glutamine, ammonium, glucose, and lactate in the bioreactor. **(d)** Average cell diameter in the bioreactor. **(e)** Cell-specific productivity and viability in the bioreactor. **(f)** Titer ratio, represented as the quotient of the functional titers measured in the harvest line and the bioreactor. **(g)** Cell-specific productivity (TU cell^{-1} day^{-1}) of the perfusion bioreactor: Peak cell-specific productivity of 0.4 TU cell^{-1} day^{-1} at 3 dpi, cell-specific yield of 0.4 TU cell^{-1}.

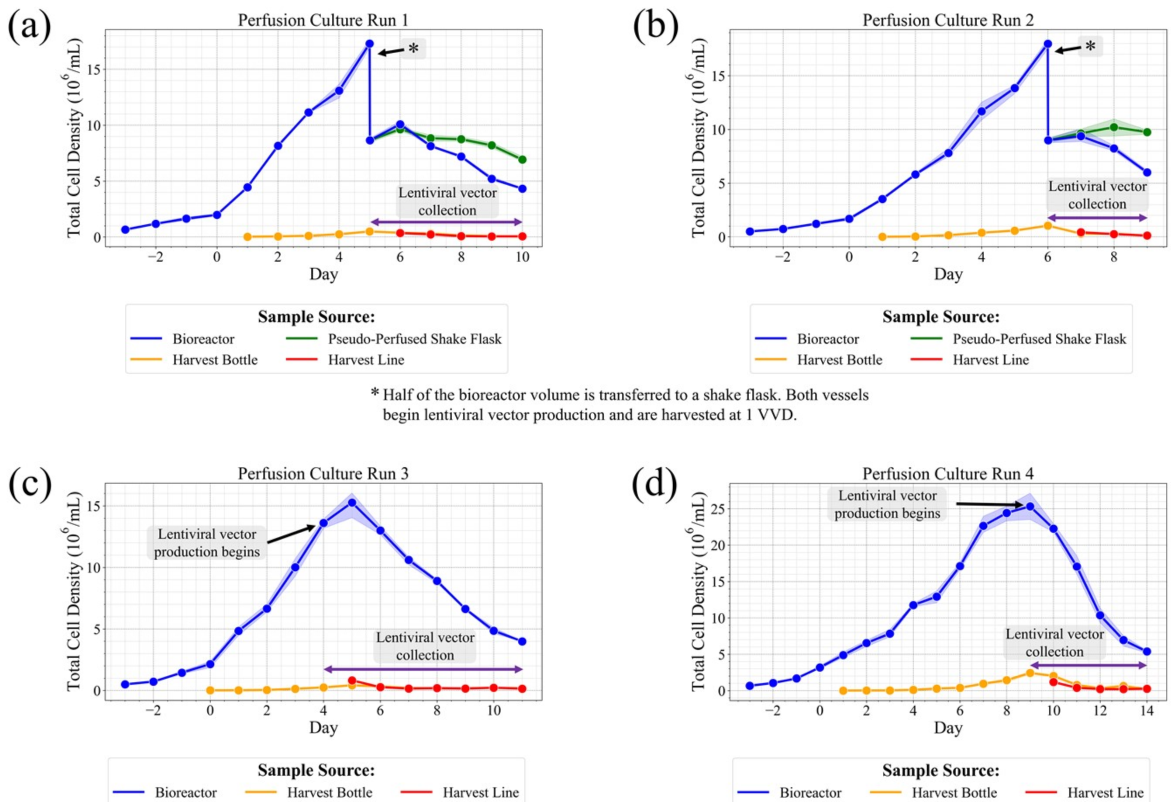

Figure S6: Time-series data of the total cell density of HEK293 cells in the bioreactor, harvest bottle, harvest line, and pseudo-perfused shake flask (if applicable). The plots are annotated to show the time points when lentiviral vector production was initiated and when samples were drawn and frozen at −80°C to be titered later. During Runs 1 and 2, once the target cell density was achieved, half of the bioreactor volume was transferred to a shake flask, and both vessels were simultaneously induced, filled back to the working volume of 350 mL and then maintained at 1 VVD (as indicated by *).

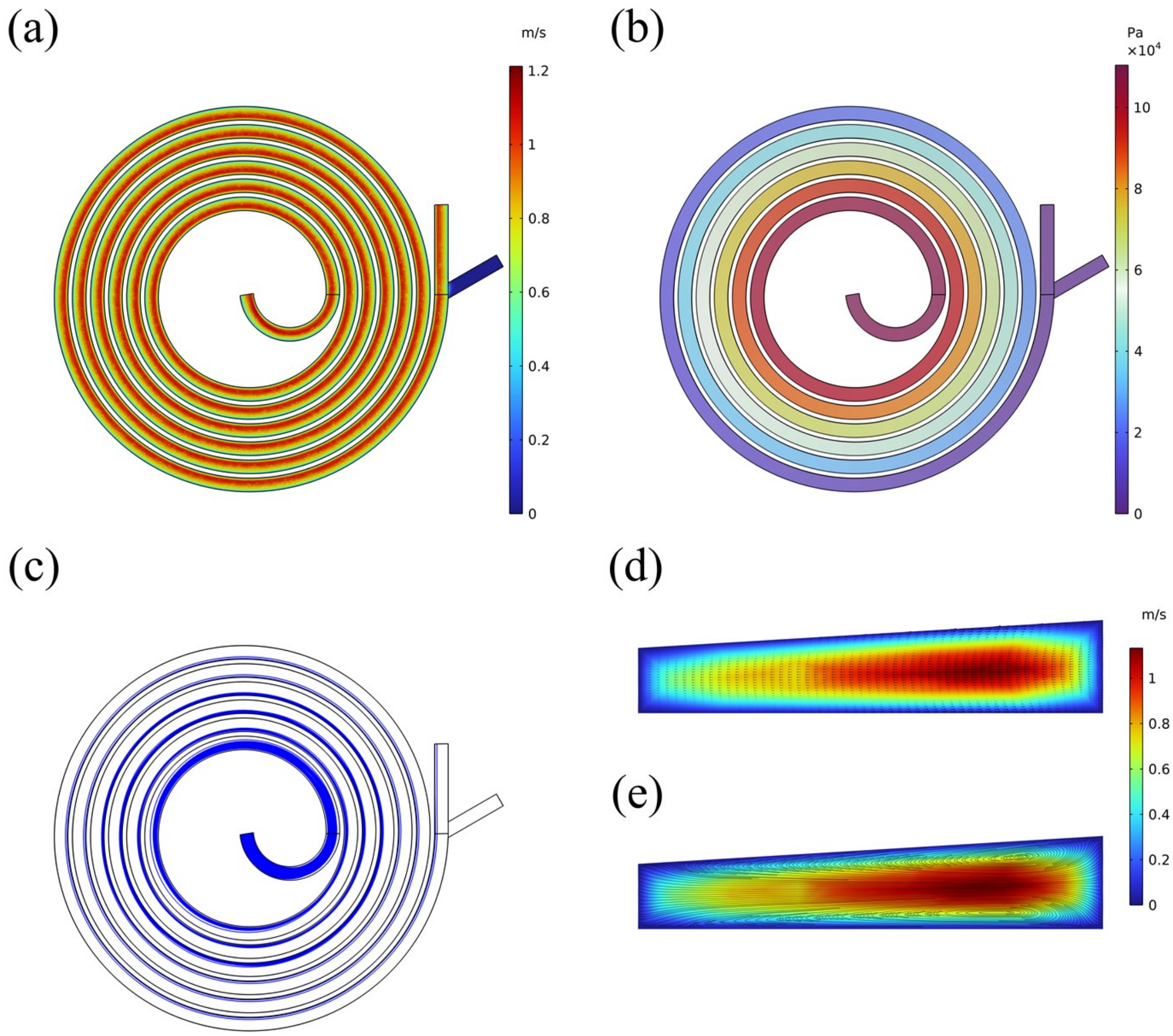

Figure S7: Fluid flow simulation results. The channel cross-section has a 1000 μm channel width, a 200 μm inner wall height, and a 140 μm outer wall height. **(a)** Fluid velocity profile at a slice located 70 μm from the glass face (1/2 the height of the outer wall height). **(b)** Pressure profile along the walls. **(c)** Particle tracing of 150 particles with a diameter of 20 μm undergoing inertial focusing. **(d)-(e)** Visualization of the channel cross-section with counter-rotating Dean vortices represented by **(d)** arrows and **(e)** streamlines.

Supplementary Calculations

All calculations presented in the following sections use variables from **Table S1**.

Table S1: Variables used in calculations within the Supplementary Information.

Variable	Name	Condition	Value	Units
ρ	Fluid density	Various	993	kg m^{-3}
μ	Dynamic viscosity	Various	0.000692	kg m^{-1} s^{-1}
Re_{spiral}	Reynolds Number	Spiral channel	245.1	
u	Average fluid velocity	Spiral channel	0.588	m s^{-1}
D_h	Hydraulic diameter	Spiral channel	0.000290375	m
$Re_{Bioreactor}$	Reynolds Number	Bioreactor	9338 to 13163	
$n_{impeller}$	Impeller's rotational speed	Bioreactor	8.3 to 11.7	s^{-1}
$d_{impeller}$	Impeller's diameter	Bioreactor	0.028	m
Re_{P-PSF}	Reynolds Number	Pseudo-perfused shake flask	77569	
n_{P-PSF}	Orbital shaker's rotational speed	Pseudo-perfused shake flask	2.25	s^{-1}
d_{P-PSF}	Shake flask's diameter	Pseudo-perfused shake flask	0.155	m
De_{spiral}	Dean Number	Spiral channel	24.7 to 37.1	
R_{spiral}	Spiral's radius	Spiral channel	0.00635 to 0.01435	m
$\dot{\gamma}_{spiral,eff}$	Effective shear rate	Spiral channel	8642	s^{-1}
$\dot{\gamma}$	Local shear rate	Spiral channel	Varies	s^{-1}
U	Local velocity magnitude	Spiral channel	Varies	m s^{-1}
$P_{Bioreactor}$	Power input	Bioreactor	0.00391 to 0.01094	W
N_p	Power number	Bioreactor	0.4	
V_{vessel}	Working volume	Bioreactor	0.00035	m^3
$\dot{\gamma}_{Bioreactor,eff}$	Effective shear rate	Bioreactor	127 to 213	s^{-1}

Reynolds Number and Dean Number Calculations

<u>Reynolds Number (Spiral Microfluidic Device)</u>:

$$Re_{spiral} = \frac{\rho\, u\, D_h}{\mu} = 245.1$$

Fluid densities and dynamic viscosities at 37°C are used. The fluid velocity reported is an average velocity using volumetric flow rate and the channel's cross-sectional area. Hydraulic diameter is calculated using the channel's cross-sectional area and the wetted perimeter of the channel.

Reynolds Number (Perfusion Bioreactor):

$$Re_{Bioreactor} = \frac{\rho \, n_{impeller} \, d_{impeller}^{\;2}}{\mu} = 9338 \; to \; 13163$$

Fluid densities and dynamic viscosities at 37°C are used. The impeller's rotational speed, $n_{impeller}$, is rewritten into a per-second form from its original form (500 to 700 rpm). The impeller has an outer diameter of 28 mm (Part Z813140511, Applikon Biotechnology B.V., The Netherlands).

Reynolds Number (Pseudo-Perfused Shake Flask):

$$Re_{P-PSF} = \frac{\rho \, n_{P-PSF} \, d_{P-PSF}^{\;2}}{\mu} = 77569$$

Fluid densities and dynamic viscosities at 37°C are used. The orbital shaker's rotational speed is rewritten from 135 rpm into a per-second form.

Dean Number (Spiral Microfluidic Device):

$$De_{spiral} = Re_{spiral}\sqrt{\frac{D_h}{2R_{spiral}}} = 24.7 \; to \; 37.1$$

Effective Shear Rate in the Spiral Device

The flow-weighted volume-averaged shear rate within the spiral device geometry normalizes the local shear rate by the local convective transport over the volume of fluid occupying the spiral device (V) using the formula below.

$$\dot{\gamma}_{spiral,eff} = \frac{\iiint_V \dot{\gamma} U \, dV}{\iiint_V U \, dV} = 8642 \; s^{-1}$$

This value was calculated using COMSOL Multiphysics Version 6.3.

Effective Shear Rate in the Bioreactor

The scaling relationships and constants used to define power input (P) and effective shear rate ($\dot{\gamma}_{eff}$) are adapted from References 22, 24, and 25 in the main article file.

The power input (by the impeller in the bioreactor) is defined as:

$$P_{Bioreactor} = N_p \, \rho \, n_{impeller}{}^3 \, d_{impeller}{}^5$$

The effective shear rate in the bioreactor ($\dot{\gamma}_{Bioreactor,eff}$) is defined as:

$$\dot{\gamma}_{Bioreactor,eff} = \sqrt{\frac{P_{Bioreactor}}{\mu \, V_{vessel}}} = 127 \; to \; 213 \; s^{-1}$$

in the range of impeller speeds from 500 rpm to 700 rpm.