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Original Article

An optimizing lentiviral titer determination assay based on Raji cells

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Abstract: Objectives: Accurate lentiviral titer determination is crucial for optimizing transduction efficiency in gene therapy. Traditional titration methods based on the human embryonic kidney 293T (HEK293T) cell line often encounter issues with fidelity and reproducibility. This study assessed the potential of suspension cell lines, such as Raji cells, as a more reliable platform for lentiviral titration. Methods: Transduction efficiencies were compared between HEK293T cells and various suspension cell lines, including Raji, across a range of viral doses and infection conditions, both with and without infection enhancers. Lentiviral titers were quantified using quantitative polymerase chain reaction (qPCR) with primers targeting human reference genes Albumin (ALB), Poly(RC) Binding Protein 2 (PCBP2), and lentiviral sequences including Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE) and Group-specific antigen (*gag*). Results: Raji cells demonstrated significantly higher transduction efficiency than HEK293T cells, particularly at lower viral inputs, and maintained robust infection rates at high cell densities. qPCR-based titration revealed that functional titers in Raji cells were substantially higher than those in HEK293T cells. Moreover, infection of primary T cells using Raji-derived titers showed greater sensitivity, achieving saturation at lower viral loads compared to HEK293T-derived titers. Conclusions: Raji cells offer a more reliable and efficient platform for lentiviral titration compared to the conventional HEK293T-based method. This suspension cell-based approach holds potential for enhancing the scalability and consistency of lentiviral vector production in gene therapy.

Keywords: Lentiviral transfection efficiency, optimal transduction conditions, Raji cells, titration

Introduction

Lentiviruses (LVs), a subclass of retroviruses, are indispensable in molecular biology and therapeutic research due to their distinctive capability to integrate genetic material into the host genome [1, 2]. This feature enables stable gene expression and allows for the modification of various cell types, including non-dividing cells, setting lentiviruses apart from other viral transfection methods [3, 4]. In particular, lentiviral vectors have demonstrated notable advantages in chimeric antigen receptor (CAR)-based therapies due to their high transduction efficiency, stable transgene expression, and relatively low immunogenicity. These properties make LVs ideal candidates for gene therapy applications targeting diseases such as cancer, refractory systemic lupus erythematosus, systemic sclerosis, and human immunodeficiency virus (HIV) [5, 6]. The quantity and quality of LV products are critical factors influencing the efficiency of exogenous gene transfer. Accurate titration is essential for ensuring optimal transduction of target cells without compromising their viability or functionality [7, 8]. Thus,

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precise determination of LV titer is a fundamental step in achieving effective target gene delivery mediated by lentiviral vectors.

Historically, lentiviral titration has relied on the use of human embryonic kidney 293T (HEK-293T) cells, due to their high transfection efficiency and rapid growth rate. The typical process involves transfecting HEK293T cells with a lentiviral vector encoding a target gene and then quantifying the gene expression as a surrogate for viral titer [9]. However, this method presents several challenges, including variability in transfection efficiency due to passage differences in the cell line and the potential overestimation of functional viral particles [10, 11]. Furthermore, while HEK293T cells are suitable for small-scale lentiviral production, scaling up to meet clinical demands is problematic. The relatively slow adhesion rate of HEK293T cells necessitates at least 6 hours of cell adhesion and growth before viral titer detection can occur [12, 13]. Collectively, the adherent and serum-dependent nature of HEK293T cells limits its production scalability and introduces time constraints.

Recent studies have highlighted the potential of suspension cell lines as a viable alternative for lentiviral titration, offering several advantages over traditional HEK293T cell-based methods. Suspension cultures enable scalable, high-throughput titration processes, addressing the increasing demand for efficient and reliable lentiviral production systems [13, 14]. Additionally, the use of suspension cells can streamline the titration workflow, reduce variability, and enhance the overall accuracy of lentiviral quantification [15, 16]. Suspension cell lines present a promising alternative by facilitating easier scale-up and potentially higher throughput analyses, while also minimizing dependence on adherent cell culture conditions. This provides a more uniform and reproducible platform for viral vector quantification [17]. The ability to culture cells in suspension simplifies handling large volumes of cells, a key advantage for industrial-scale viral vector production, where efficiency and consistency are critical [18].

However, optimizing conditions for suspension cell-based titration remains challenging. Suspension cultures of HEK293T cells, for instance, use serum-free media, and due to differences in cell density, nutrient availability, or interactions with transfection reagents, transfection efficiency may be lower in suspension cultures [19, 20].

In this study, the transfection efficacy of suspension cell lines was compared with the traditional HEK293T cell line. Flow cytometry demonstrated that Raji cells exhibited significantly higher lentiviral transfection efficacy. Further RT-qPCR analysis of lentiviral titers across different cell lines revealed that titers determined using Raji cells provided greater precision, particularly when transfecting primary T cells. Collectively, the transfection efficacy of Raji cells surpasses that of HEK293T cells, and this strategy for lentiviral titration could be implemented in laboratories with minimal equipment and reduced costs.

Materials and methods

Cell isolation and culture

Human cell lines Raji, Jurkat, NALM-6, K562, and HEK293T were cultured under specific conditions to ensure optimal growth and viability. Suspension cell lines Raji, Jurkat, and K562 were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (BioInd, Israel) supplemented with 10% fetal bovine serum (FBS; BioInd, Israel). HEK293T cells, an adherent cell line, were cultured in Dulbecco's Modified Eagle Medium (DMEM; BioInd, Israel) supplemented with 10% FBS. For suspension culture of 293T cells, the cells were adapted to grow in suspension by gradual acclimatization to FreeStyle™ 293 Expression Medium (Thermo Fisher Scientific, USA), supplemented with 10% FBS, and incubated under standard conditions [21]. After obtaining informed consent, 15 mL of peripheral venous blood was collected from three healthy donors [22]. T cells were isolated using Ficoll-Paque solution (Biolegend, USA) through density gradient centrifugation. The isolated T cells were activated with anti-CD3/CD28 beads (Miltenyi Biotec, Germany) for 24 hours and cultured in RPMI 1640 medium supplemented with 10% FBS and 200 U/mL interleukin-2 (IL-2; SinoBiological, China) to promote T cell growth and survival. All cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Lentiviral infection

Lentiviral particles were produced by co-transfecting psPAX2, pMD2.G, and pCDH-EF1-MCS-T2A-CopGFP into HEK293T cells. For suspension cells (Raji, Jurkat, NALM-6, K562, and suspension-adapted 293T cells), cells were adjusted to an appropriate density, followed by lentiviral infection through the direct addition of viral supernatant to the culture medium. Adherent 293T cells were seeded in 12-well plates and allowed to adhere before adding lentiviral particles in varying volumes. After 24 hours, the medium was replaced with fresh one, and the cells were incubated for an additional 4 days. Additionally, the effects of various infection-enhancing solutions on infection efficiency were compared [23, 24]. The enhancers tested were Dextran (10 μg/mL; Sangon Biotech, China), Polybrene (5 μg/mL; Solarbio, China), Protamine Sulfate (5 μg/mL; Sangon Biotech, China), and HitransG P (40 μL/mL; GeneChem, China). Each enhancer was added to the culture medium at the time of infection, and the impact on transduction efficiency was evaluated.

For primary T cells, activation was performed before infection. Lentiviral particles (0.1 μL, 0.5 μL, 1 μL, 5 μL, and 10 μL) were added along with 40 μL/mL of HitransG P. Infection efficiency was assessed 24 hours post-infection by replacing the medium with fresh one and incubating the cells for an additional 4 days.

Flow cytometry analysis

Cells were harvested and washed twice with PBS containing 2% FBS. The cells were then resuspended in 100 μL PBS with 2% FBS and analyzed for GFP expression using a flow cytometer. At least 10,000 events were collected per sample, and infection efficiency was determined by calculating the percentage of GFP-positive cells.

DNA extraction

For genomic DNA extraction, cells were harvested and washed twice with PBS. DNA was isolated using the TIANamp Genomic DNA Kit (TIANGEN, China) following the manufacturer's protocol. Briefly, the cell pellets were resuspended in lysis buffer and subjected to proteinase K digestion. After digestion, the lysate was mixed with absolute ethanol and transferred to a spin column. The column was centrifuged, and the flow-through was discarded. The column was washed with the provided wash buffers, followed by DNA elution with the elution buffer. The eluted DNA was quantified and stored at -20°C for further analysis.

Real-time qPCR

Quantitative PCR (qPCR) reactions were set up using AceQ qPCR SYBR Green Master Mix (Vazyme, China) with primers targeting lentiviral genome integration sites. Each 20 μL reaction contained 10 μL Master Mix, 0.4 μL of each primer (10 μM), 2 μL template DNA, and 7.2 μL nuclease-free water. Ct values were used to quantify viral integration.

Calculation of lentivirus titer

Human reference genes ALB and PCBP2, along with lentiviral reference genes WPRE and gag, were used in this study [25, 26]. The oligonucleotide sequences are listed in Table S1. Lentiviral titers were calculated using the following formula [10]:

$$\text{Titer}\left(\frac{\text{TU}}{\text{ml}}\right) = \frac{2^{(\text{CT Value of PCPB2})-(\text{CT Value of WPRE})} \times 2 \times \text{Primary cell count per well}}{\text{Volume of used lentivirus (ml)}}$$

Statistical analysis

All experiments were conducted in triplicate (n = 3) to ensure reproducibility. Statistical analysis was performed using GraphPad Prism 9.0 software (San Diego, USA). An unpaired two-tailed Student's t-test was used for comparisons between two groups, and one-way ANOVA was applied for multi-group comparisons. Data are presented as mean ± SEM, with a P value < 0.05 considered statistically significant. *P < 0.05; **P < 0.01; ***P < 0.001.

Results

Primer selection and evaluation for lentiviral infection studies

Lentiviruses were integrated into the genomic DNA of target cells, enabling detection of their presence and relative expression by comparison with human reference genes. Primers were employed to assess the expression of target DNA in HEK293T cells infected with lentivirus. Amplification efficiency was compared across primers to identify those most suitable for con-

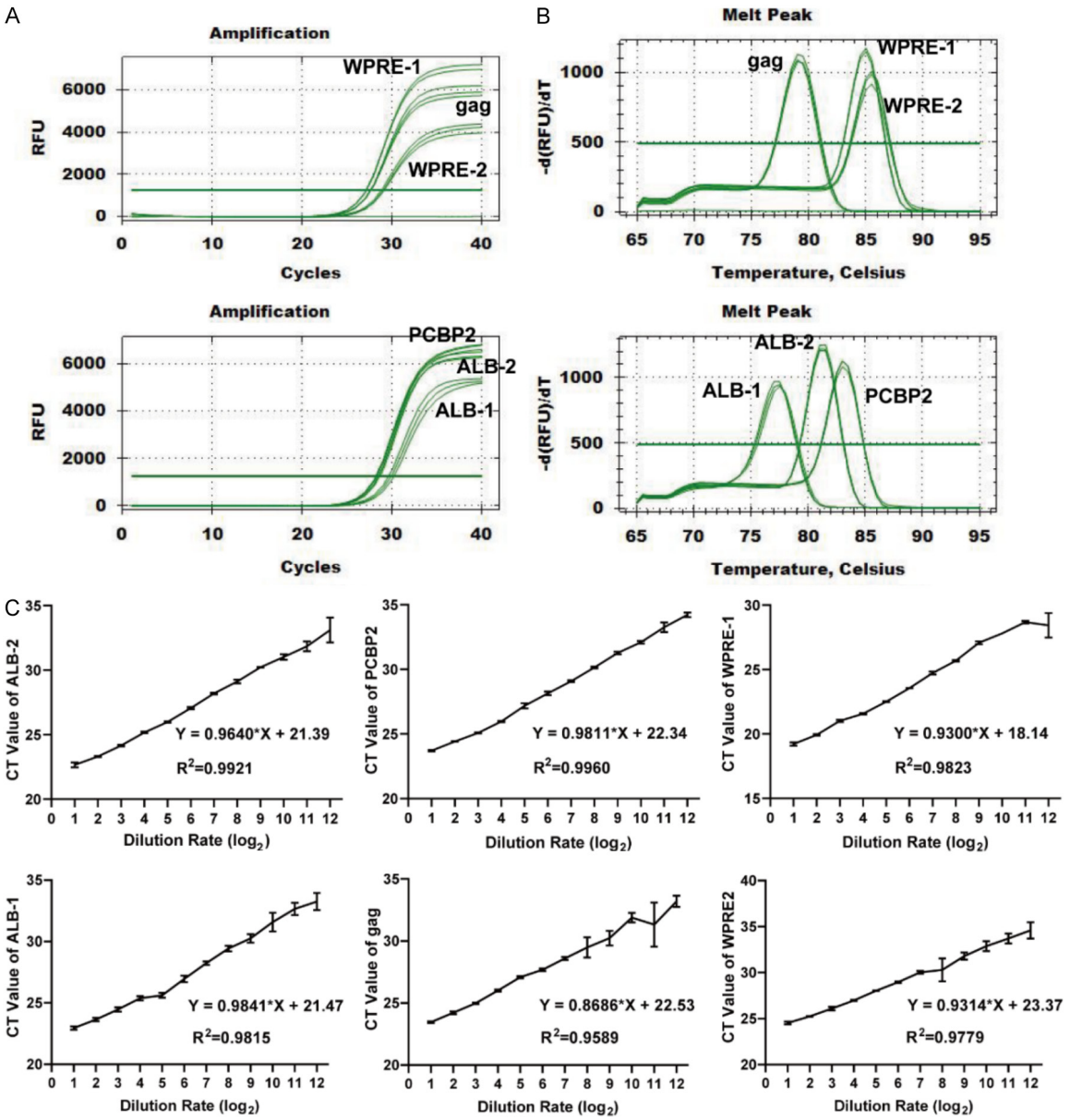

Figure 1. Primer selection and evaluation for lentiviral infection studies. A: Amplification curves for human reference genes ALB and PCBP2, and lentiviral reference genes WPRE and gag. The curves demonstrate the amplification efficiency of each primer set in HEK293T cells infected with lentivirus. B: Melting curves for the same genes, showing the specificity of the primers by identifying distinct melting peaks for each target. C: Standard curves for the optimal primers (ALB-2, PCBP2, WPRE-1, ALB1, gag and WPRE-2) based on dilution series. The equations and R^2 values indicate the linearity and efficiency of the qPCR assay for quantifying the initial quantity of target DNA in diluted samples. Data are presented as mean ± SEM of three independent experiments (N = 3).

structing standard curves. Analysis of amplification and melting curves, indicated ALB-2, WPRE-1, gag, ALB-1, WPRE-2 and PCBP2 as the potential primers (**Figure 1A** and **1B**). Subsequently, we plotted the standard curves of the dilution series and calculated the correlation coefficients. The strongest linearity and efficiency were observed with ALB-2, PCBP2, and WPRE-1 based on their R^2 values, supporting their use as the optimal primer sets (**Figure 1C**). To further evaluate viral gene background levels in various cell lines, WPRE-1 and PCBP2 primers were used to detect internal reference and viral genes in NALM-6, Jurkat, Raji, and K562 cells uninfected with lentivirus. The results revealed that higher CT values for the len-

Table 1. Baseline CT values for PCBP2 and WPRE in different cell lines

Infected Tool Cells	Average CT Value of PCBP2	Average CT Value of WPRE	Baseline CT values (PCBP2 - WPRE)
HEK293T	22.39	33.35	-10.96
Raji	20.09	34.57	-14.48
Jurkat	21.79	30.78	-8.99
K562	22.15	29.45	-7.3
NALM-6	22.74	33.37	-10.63

tiviral gene correlated with lower background levels in comparison to HEK293T cells (**Table 1**). Therefore, Raji, NALM-6, and HEK293T cells, which exhibited lower background levels, were selected for subsequent lentiviral infection experiments. This approach ensured the selection of the most appropriate primers and cell lines for accurate and reliable lentiviral infection studies.

Evaluation of lentiviral infection efficiency in different cell lines

A comparative analysis of infection efficiency was conducted using HEK293T, Raji, and NALM-6 cells under different viral gradients following lentiviral infection. The results indicated that Raji cells consistently exhibited higher infection efficiencies than NALM-6 and HEK293T cells, particularly at lower lentiviral volumes (**Figure 2A**). Given that HEK293T cells can be maintained in both suspension and adherent cultures, this study examined whether these different states influenced infection efficiency. Although some studies suggest that cell culture state (suspension vs. adherent) may affect the infection positivity rate in HEK293T cells [14, 20], our analysis found no significant difference between suspension and adherent HEK293T cells in terms of infection positivity (**Figure 2B**). This suggests that the culture state does not substantially impact the infection efficiency of HEK293T cells. These results highlight the potential advantages of Raji cells in facilitating more efficient lentiviral integration and expression, making them a preferable choice for lentiviral transduction in gene delivery applications.

Optimal conditions for lentiviral infection efficiency in different cells

To further optimize infection conditions for lentiviral experiments and ensure accurate titer detection, the efficacy of various infection reagents across different cell lines was evaluated. Our results revealed that HEK293T cells exhibited the highest infection positivity rate when treated with Dextran, whereas Raji cells showed the best infection results with HitransG P treatment (Figure S1). Under these optimal infection conditions, even with the same viral load, HEK293T cells had a lower positivity rate than Raji cells (**Figure 3A**), reinforcing the notion that Raji cells possess inherent properties that enable superior infection efficiency, irrespective of optimized conditions for HEK293T cells.

The performance of Raji cells was assessed at a higher cell density of 6×10^6 cells/mL, which is six times the density typically used for HEK293T cells. Despite the increased density, Raji cells maintained high infection positivity rates (**Figure 3B**), indicating their ability to adapt to higher cell concentrations without compromising infection efficiency. Additionally, the infection efficiency of Raji cells at varying lentivirus volumes was compared (**Figure 3C**), and significantly better infection rates were observed at lower lentivirus volumes than at higher ones. These results suggest that Raji cells are more suitable for lentiviral infection experiments, offering higher intrinsic infection efficiencies and greater adaptability to diverse experimental conditions, including increased cell densities and varying viral loads.

Determination of lentiviral titer using qpcr in different cell lines

To determine optimal conditions for lentiviral titer detection, Raji and HEK293T cells were infected with varying lentivirus volumes. Five days post-infection, DNA was extracted from the cells, and qPCR was conducted to quantify the expression of internal reference genes and viral genes. Lentiviral titers were calculated using qPCR, with the CT values derived from

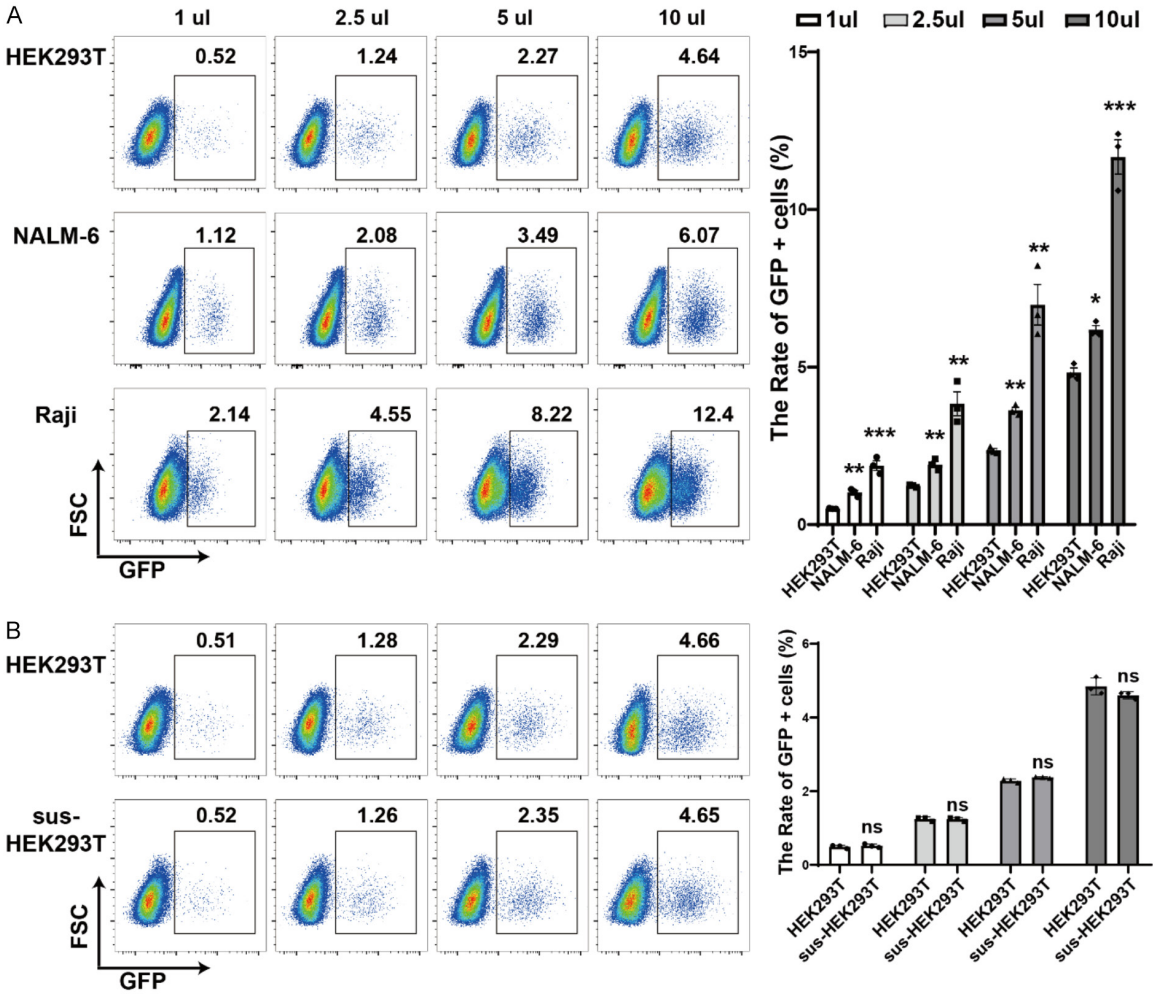

Figure 2. Evaluation of lentiviral infection efficiency in different cell lines. A: Flow cytometry analysis of GFP-positive cells in HEK293T, Raji, and NALM-6 cells following infection with varying volumes of lentivirus (1 µl, 2.5 µl, 5 µl, and 10 µl). The percentages indicate the proportion of GFP-positive cells in each condition (left). The bar graph (right) shows the rate of GFP-positive cells as a function of lentivirus volume. B: Comparison of GFP-positive rates in adherent HEK293T cells and suspension HEK293T cells infected with varying volumes of lentivirus (1 µl, 2.5 µl, 5 µl, and 10 µl). The flow cytometry plots (left) and corresponding bar graph (right) demonstrate similar infection positivity rates between the two culture conditions. Data are presented as mean ± SEM of three independent experiments (N = 3). Statistical significance was calculated by Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ns, non-significant.

primers and the virus volumes applied. The average titers for lentiviruses in HEK293T and Raji cells were 1.01 × 10^7 and 2.91 × 10^7, respectively (**Table 2**). Notably, titers measured in Raji and NALM-6 cells were significantly higher than those in HEK293T cells, highlighting their superior suitability for lentiviral transduction.

T cell infection efficiency using lentivirus titers from different cell lines

After determining lentiviral titers from HEK293T and Raji cells, T cells were infected with lentivirus derived from each cell line. Although the same viral stock was used, titers were assessed based on the different cell lines. Flow cytometry was employed five days post-infection to measure the positive rate of GFP expression in T cells. The results demonstrated a dose-dependent increase in GFP-positive T cells with rising lentivirus volumes from both cell lines. At lower volumes (0.1 µl to 1 µl), HEK293T-derived lentivirus achieved higher GFP-positive rates compared to Raji-derived lentivirus. However, at higher volumes (5 µl and 10 µl), the GFP-positive T cell rates were similar for both cell

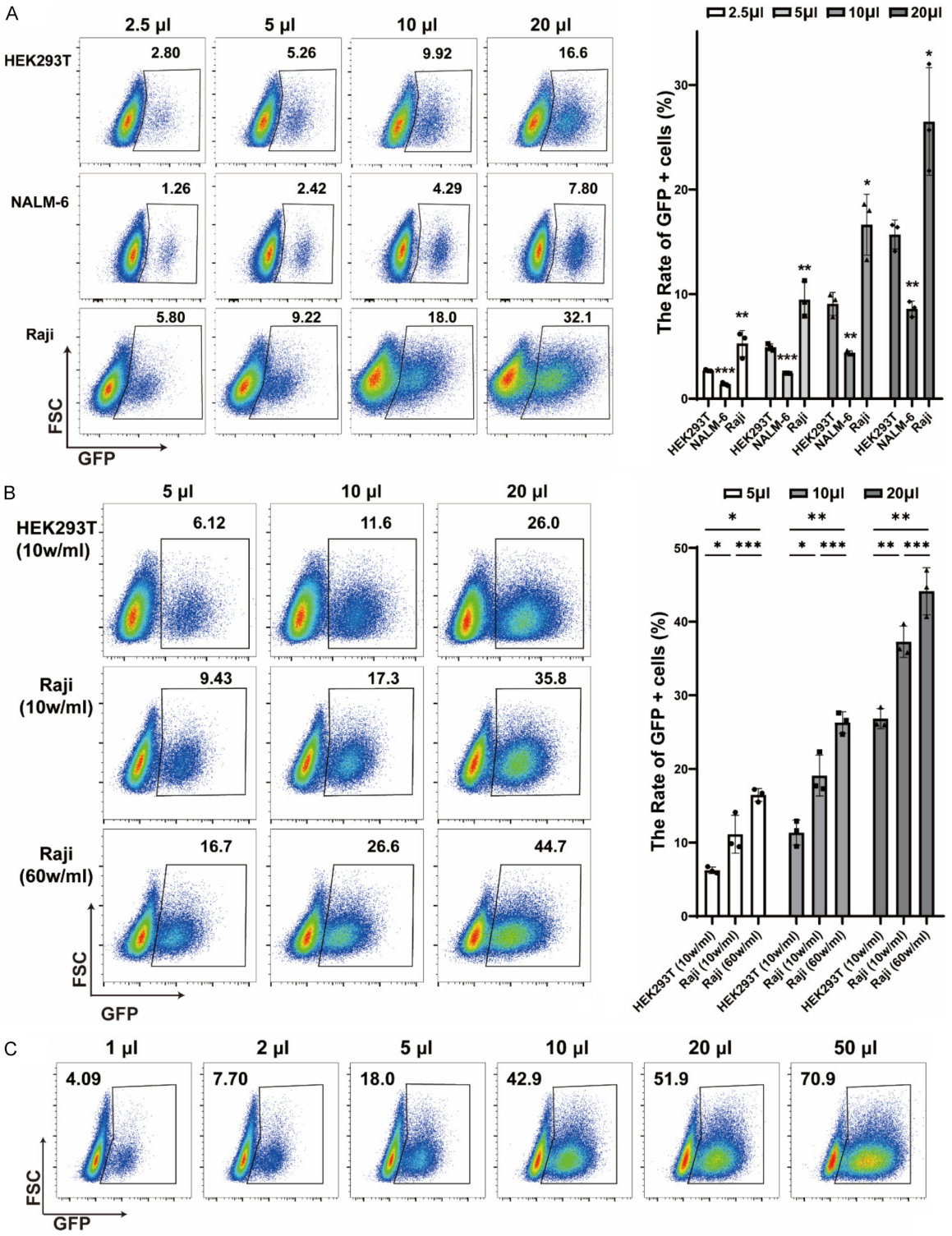

Figure 3. Optimal conditions for lentiviral infection efficiency in different cells. A: Flow cytometry analysis of GFP-positive cells in HEK293T, NALM-6, and Raji cells following infection with varying volumes of lentivirus (2.5 μl, 5 μl, 10 μl, and 20 μl) after transfection enhancer treatments. The bar graph shows the rate of GFP-positive cells for each cell line. B: Comparison of GFP-positive rates in HEK293T and Raji cells at two different densities, infected with varying volumes of lentivirus (5 μl, 10 μl, and 20 μl) after transfection enhancer treatments. C: GFP-positive rates in Raji cells infected with increasing volumes of lentivirus (1 μl, 2 μl, 5 μl, 10 μl, 20 μl, and 50 μl) treated with HitransG P. Data are presented as mean ± SEM of three independent experiments (N = 3). Statistical significance was calculated by Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ns, non-significant.

Table 2. Lentivirus titer calculation in 293T and Raji cells using PCR detection of PCBP2 and WPRE genes

Cell line (Counts)	PCBP2 (CT value)	WPRE-1 (CT value)	LVs Volume (ul)	Titer	Average Titer
293T (1×10^5)	22.33	24.21	5	1.09×10^7	1.01×10^7
	22.61	23.65	10	9.73×10^6	
	22.49	22.12	20	1.29×10^7	
	22.56	21.76	50	6.96×10^6	
Raji (3×10^5)	21.72	22.99	5	2.91×10^7	2.91×10^7
	22.17	22.86	10	3.72×10^7	
	22.27	22.18	20	3.19×10^7	
	22.04	21.44	50	1.82×10^7	

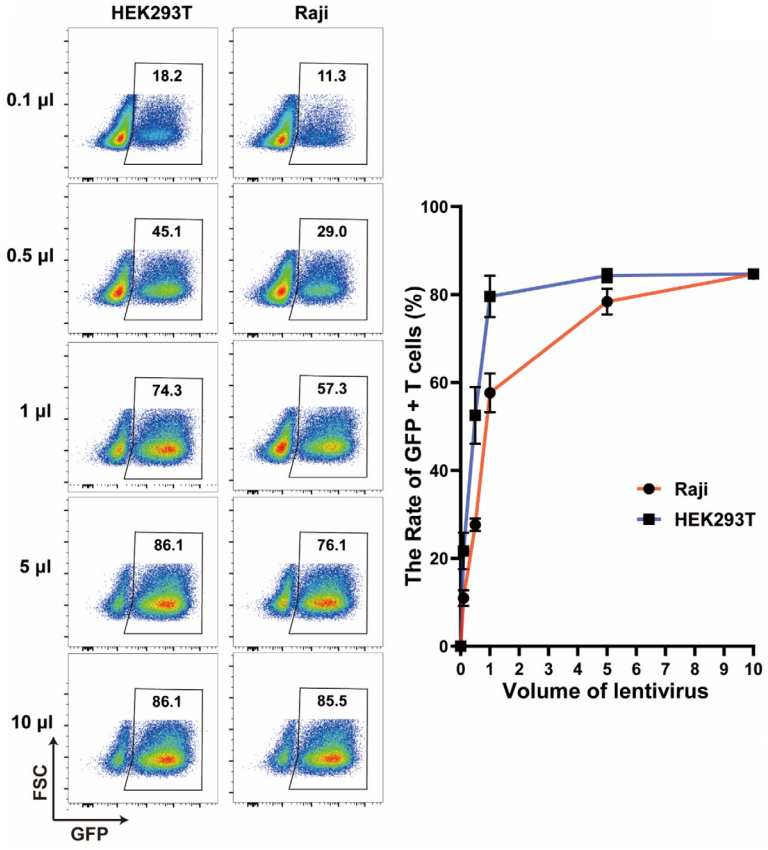

Figure 4. GFP-positive rate of T cells infected with different virus titers from HEK293T and Raji cells. Flow cytometry analysis of GFP-positive T cells five days post-infection with varying volumes (0.1 µl, 0.5 µl, 1 µl, 5 µl, and 10 µl) of lentivirus derived from HEK293T and Raji cells. The percentages indicate the proportion of GFP-positive T cells in each condition (left). The graphical representation shows the rate of GFP-positive T cells as a function of lentivirus volume, illustrating a dose-dependent increase in GFP positivity for both cell lines. HEK293T-derived lentivirus produced higher GFP-positive rates at lower volumes, while both cell lines showed similar rates at higher volumes (right). Data are presented as mean ± SEM of three independent experiments (N = 3).

lines, with HEK293T reaching saturation slightly earlier (**Figure 4**). The graphical representation illustrates that while HEK293T-derived lentivirus initially exhibited higher transduction efficiency, the titer measured in Raji cells was ultimately higher (**Figure 4**). This suggests that Raji cells offer superior sensitivity in titer measurements compared to HEK293T cells. Overall, these findings indicate that lentiviral titers measured in Raji cells provide higher sensitivity, making them particularly advantageous for applications requiring precise titer quantification.

Discussion

Lentiviral vectors are essential tools in molecular biology and therapeutic applications, particularly in gene therapy and the development of chimeric antigen receptor (CAR) T-cell therapies [27, 28]. These vectors enable stable gene insertion into target cells, providing a robust platform for genetic studies and interventions. The success of CAR T-cell therapy, a transformative cancer treatment, further emphasizes the critical role of precise lentiviral titration [29]. Accurate lentiviral titration is pivotal for successful gene therapy outcomes, as demonstrated by the recent FDA approvals of lentiviral vector-based treatments for β-thalassemia and cerebral adrenoleukodystrophy [30]. One study highlighted the impor-

tance of controlled titration conditions - specifically the careful selection of multiplicity of infection (MOI) - in predicting gene transfer events [31]. Inaccurate titer measurements, however, can lead to suboptimal transduction rates, compromising the efficacy and safety of therapies like CAR T-cell treatment. These inaccuracies may result in insufficient therapeutic gene expression or, conversely, cytotoxicity and off-target effects from excessively high vector doses [32, 33]. In the present study, the lentiviral transduction efficiency of Raji and NALM-6 cells was significantly higher than that of HEK-293T cells. By employing titration strategies tailored to Raji and NALM-6 cells, more precise viral titer measurements were achieved, leading to more effective transduction of primary T cells. The use of suspension cell lines represents an innovative approach to improving the precision and efficiency of lentiviral titration. Unlike the traditional adherent HEK293T cell line, suspension cells offer a more homogeneous environment for lentiviral production and titration, facilitating scalable processes and potentially higher titers [13, 34]. Studies have shown that lentiviral transduction is both effective and stable in leukemia-derived cell lines such as Raji and NALM-6, suggesting high gene delivery efficiency in these cells [35, 36]. In alignment with these findings, our experiments demonstrated that the lymphoma-derived Raji and NALM-6 cell lines exhibited significantly higher lentiviral transduction efficiency than HEK293T cells. This increased efficiency is likely due to their suspension growth, which enhances virus-cell interactions and provides a better approximation of *in vivo* conditions for hematological applications. Furthermore, studies suggest that suspension cultures create a dynamic environment favorable to lentiviral entry and integration, likely due to increased availability of surface receptors and more efficient viral uptake mechanisms in these cell types [37, 38]. In adherent cultures, cells at the bottom of the vessel may experience greater exposure to viral particles settling due to gravity, leading to uneven infection rates. In contrast, suspension cells are more uniformly exposed to lentiviruses, promoting a more consistent and efficient infection process. Suspension cultures provide continuous mixing of cells and viral particles, ensuring that viruses have an equal opportunity to interact with all target cells, potentially increasing viral entry efficiency [21, 39]. Furthermore, the adaptation from adherent to suspension growth induces phenotypic changes that may influence viral susceptibility, including differences in gene expression related to cell adhesion and membrane composition [40]. Traditional lentiviral titering methods include p24 antigen ELISA, flow cytometry measurement of transducing units, and qPCR for estimating vector copy number [32, 41]. The p24 assay quantifies retroviral Gag protein (e.g., HIV p24) in the supernatant to measure total viral particles (both functional and non-functional), but it has a limited linear range and high costs [42, 43]. Flow cytometry-based measurement of transducing units provides insights into viral production and infection efficiency; however, it may not correlate well with functional titers or accurately reflect the number of integrating viral particles [41, 44]. Additionally, flow cytometry is limited to vectors encoding fluorescent reporters and requires expensive equipment [25, 26]. In contrast, qPCR directly measures integrated proviral copies, potentially offering a more accurate functional titer. This method amplifies viral genetic sequences, such as the WPRE element, in host-cell DNA using specific primers and is cost-effective with minimal specialized equipment [10, 45]. In the present study, integrated lentiviral copy number per cell was calculated by comparing the abundance of the WPRE sequence to that of a single-copy human gene (ALB) in infected cells. This approach ensured that only viruses successfully integrated into host genomes were quantified, accurately reflecting infectivity without the need for fluorescent markers. Using inaccurate lentiviral titers can significantly affect target cell transduction efficiency and therapeutic outcomes. Excessively high titers can result in cytotoxicity and off-target effects, while insufficient titers may lead to inadequate gene transfer [46, 47]. In this study, titers derived from Raji and NALM-6 cells led to higher transduction efficiencies and better T-cell viability compared to HEK293T-derived titers. This finding emphasizes the importance of precise titration for effective target cell infection, ensuring optimal expression of therapeutic genes, which is crucial for the success of CAR T-cell therapies [48]. Our results align with the documented benefits of using suspension cells for lentiviral production and titration, underscoring their suitability for applications demanding high transduction efficiency

and cell viability [49, 50]. Despite these advantages, suspension cell lines present certain limitations in lentiviral titration. They often require specific growth conditions and may exhibit different viral production dynamics compared to adherent cell lines, which can present challenges [51, 52]. Additionally, the optimal choice of suspension cell line may depend on the target cell type and intended application, necessitating a tailored approach to vector production and titration [7, 53]. These factors suggest that suspension-based titration is most beneficial under specific conditions and for particular target cell types. Furthermore, each titration method has distinct sensitivity, specificity, and equipment requirements, meaning that the selection of the appropriate cell line and technique should be driven by the needs of the study or therapeutic application. In conclusion, using Raji cells - a suspension cell line - for lentiviral titration offers substantial advantages in terms of both efficiency and precision, particularly for hematological applications. Our findings contribute to the growing body of knowledge on optimizing lentiviral vector production and titration, potentially advancing the development of more effective and safer gene therapies and CAR T-cell therapies.

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All participants provided written informed consent before data collection.

Disclosure of conflict of interest

None.

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Table S1. The primers used in qRT-PCR

Name	Sequence (5'-3')
human PCBP2-F	TTGTGTCTCCAGTCTGCTTG
human PCBP2-R	AGGTGGTGGTGGTGGTA
human-ALB-F	TTTGCAGATGTCAGTGAAAGAGA
human-ALB-R	TGGGGAGGCTATAGAAAATAAGG
human-ALB-2F	GCTGTCATCTCTTGTGGGCTGT
human-ALB-2R	ACTCATGGGAGCTGCTGGTTC
HIV-WPRE-F	GGCACTGACAATTCCGTGGT
HIV-WPRE-R	AGGGACGTAGCAGAAGGACG
HIV-WPRE-2F	CCGTTGTCAGGCAACGTG
HIV-WPRE-2R	AGCTGACAGGTGGTGGCAAT
HIV-gag-F	GGAGCTAGAACGATTCGCAGTTA
HIV-gag-R	GTTGTAGCTGTCCCAGTATTTGTC

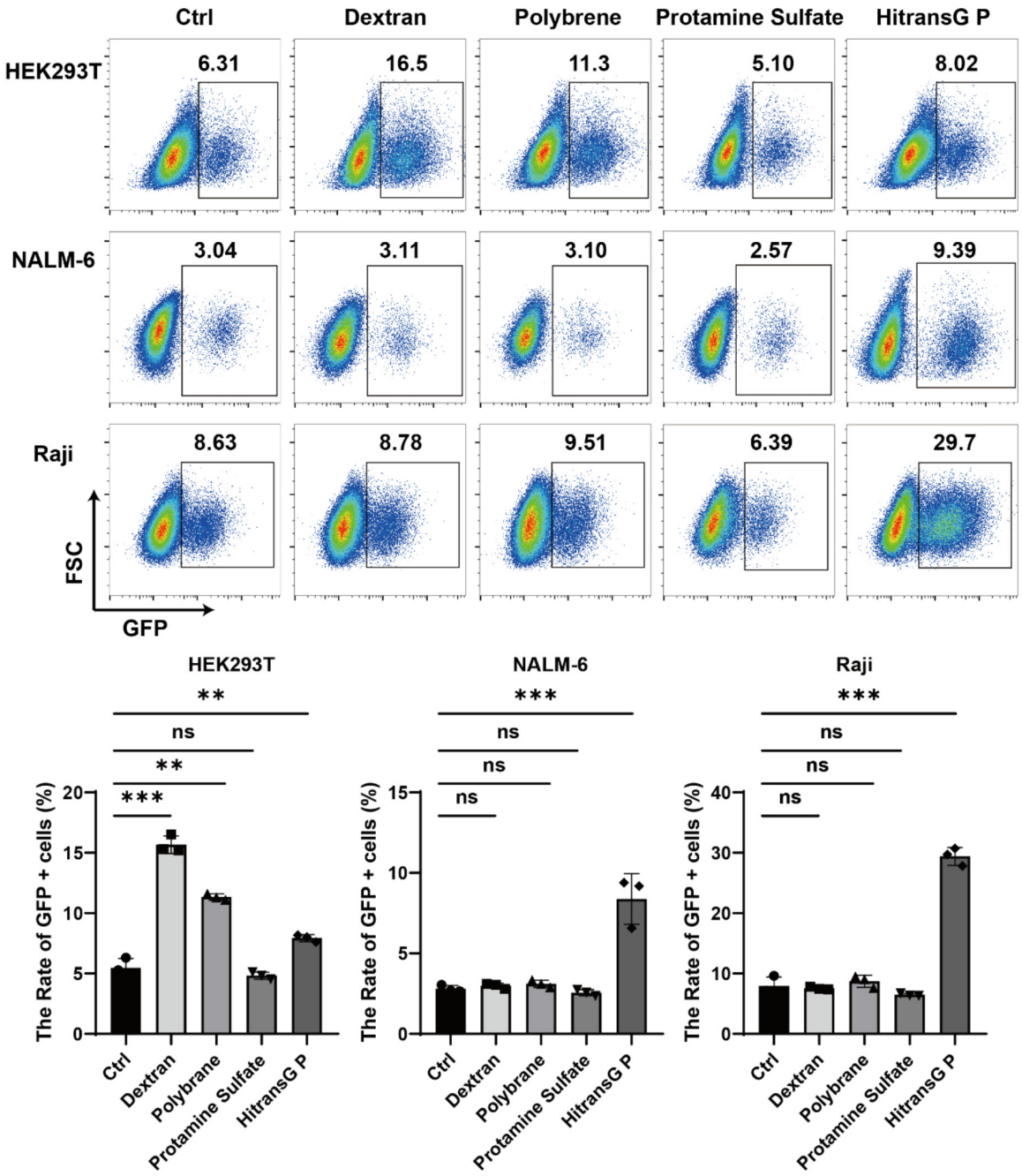

Figure S1. Optimization of infection conditions using various reagents across different cell lines. Flow cytometry analysis of GFP-positive cells in HEK293T, NALM-6, and Raji cells following treatment with different infection reagents (Ctrl, Dextran, Polybrene, Protamine Sulfate, HitransG P). The percentages indicate the proportion of GFP-positive cells in each condition. The bar graphs display the rate of GFP-positive cells for each cell line. Data are presented as mean ± SEM of three independent experiments (N = 3). Statistical significance was calculated by Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ns, non-significant.