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Supplemental Information

Production of Lentiviral Vectors Using Suspension Cells Grown in Serum-free Media

Matthew Bauler, Jessica K. Roberts, Chang-Chih Wu, Baochang Fan, Francesca Ferrara, Bon Ham Yip, Shiyong Diao, Young-In Kim, Jennifer Moore, Sheng Zhou, Matthew M. Wielgosz, Byoung Ryu, and Robert E. Throm

Figure S1

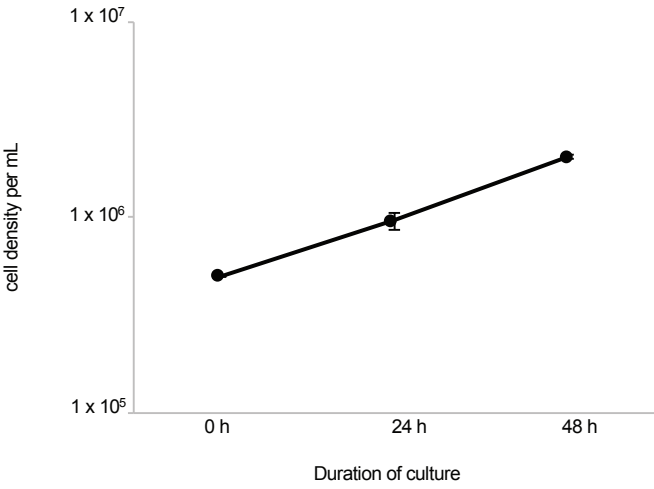

Figure S1. Growth rate of SJ293TS cells in shaker flasks. SJ293TS cells were seeded at 5 × 10^5 cells per mL in 20 mL media and counted every day for two days. n=3. Data are expressed as the mean ± the standard deviation.

Figure S2

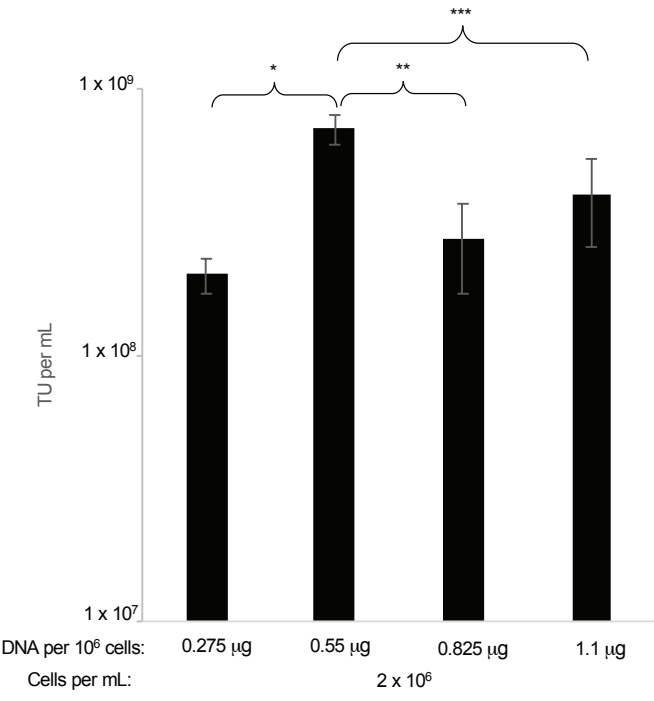

Figure S2. Optimizing the total amount of transfected DNA during lentiviral vector production using SJ293TS cells. The total amount of transfected DNA was titrated in while SJ293TS cell density remained constant at 2 × 10^6 cells per mL. * p-value = 0.0034, ** p-value = 0.0028, *** p-value = 0.0227. n=3. Data are expressed as the mean ± the standard deviation.

Figure S3

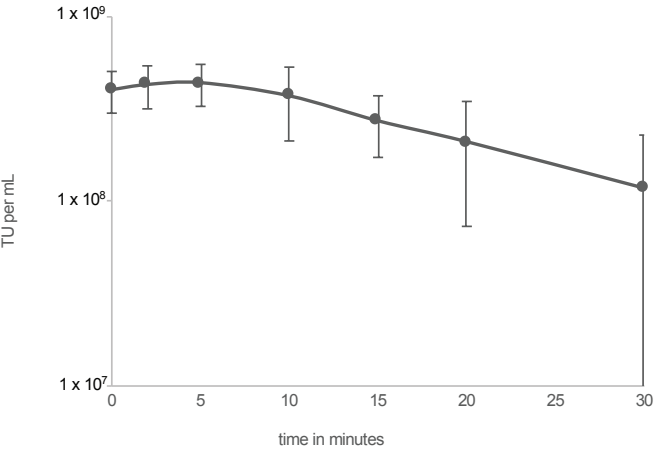

Figure S3. Titer obtained from SJ293TS cells after polyplex formation time course. Polyplex formation between plasmid DNA and the transfection reagent, PEI, proceeded for the time indicated before addition to SJ293TS cells. No significant differences were detected for any of the time points tested. n=3. Data are expressed as the mean ± the standard deviation.

Figure S4

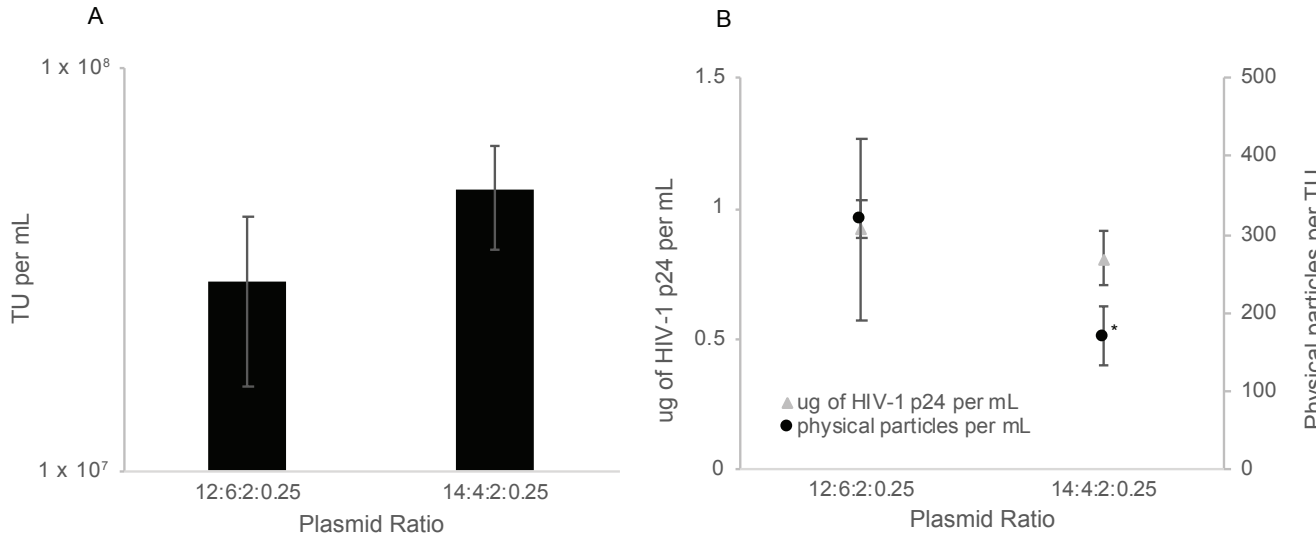

Figure S4. Optimization of plasmid ratio for production of lentiviral vectors by SJ293TS cells. SJ293TS cells were transiently transfected with plasmid ratio of either 12:6:2:0.25 or 14:4:2:0.25 consisting of transfer vector:HIV-1 *gagpol*:Env:HIV-1 *rev* to produce the pre-clinical lentiviral vector, SJL644. Harvested vector supernatants were A) titered and B) analyzed for HIV-1 p24 levels from which a physical particles per TU value was calculated by estimating 10^4 physical particles per picogram of HIV-1 p24. * p-value = 0.0071. n=3. Data are expressed as the mean ± the standard deviation.

Figure S5

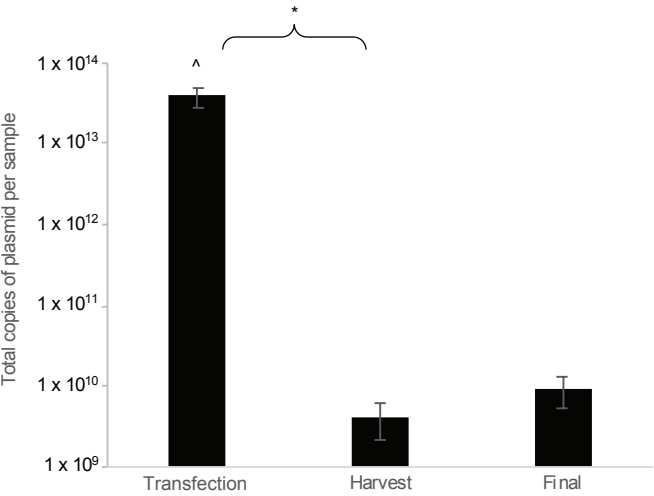

Figure S5. Determination of contaminating plasmid in vector preparation. Total plasmid copies in one-liter of 293TS-derived lentiviral vector preparations at different stages. ^ calculated from known values. * p-value ≤ 0.001.n=4. Data are expressed as the mean ± the standard deviation.

Table S1. HIV-1 p24 per mL and physical particles per transducing unit.

	Unprocessed		Processed[a]	
	μg p24 per mL	PP per TU[b]	μg p24 per mL	PP per TU[b]
Prep 1-SJL643	0.4	99	8.4	130
Prep 2-SJL643	0.3	90	9.9	99
Prep 3-SJL643	0.9	117	19.5	137
Prep 4-SJL643	1	145	30.3	168

[a] 50-fold concentration post–bulk filtration, ion-exchange, ultra-filtration, and sterile filtration

[b] physical particles per transducing unit