Showing some LoVe for Lentivirus with Maurice CE-SDS

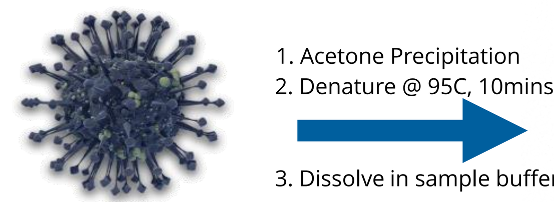
Priyanka Sarkar and Chris Heger

ProteinSimple, a Bio-Techne Brand, 3001 Orchard Parkway, San Jose, CA 95134, USA

INTRODUCTION

Lentiviral vectors (LVV) have become a prominent and popular gene delivery system for both *in vivo* and *ex vivo* therapies. It is crucial to identify, characterize, and quantify these LVV particles to ensure quality, safety, efficacy, and to meet all regulatory requirements. In this work, we leveraged the Maurice CE-SDS platform to develop a robust method for LVV analysis. We show the method can be used for LV identity and viral titer. Comparing different LV vendors, unique but similar profiles were obtained with the CE-SDS method. Assessing and characterizing the capsid core protein p24 offers an estimation of LVV quantitation as well as transduction efficiency. Using recombinant p24, we show accurate viral titer estimation. Together, the LV method provides a rapid and accurate analysis of lentiviral products.

METHODS



1. Acetone Precipitation
2. Denature @ 95C, 10mins
3. Dissolve in sample buffer

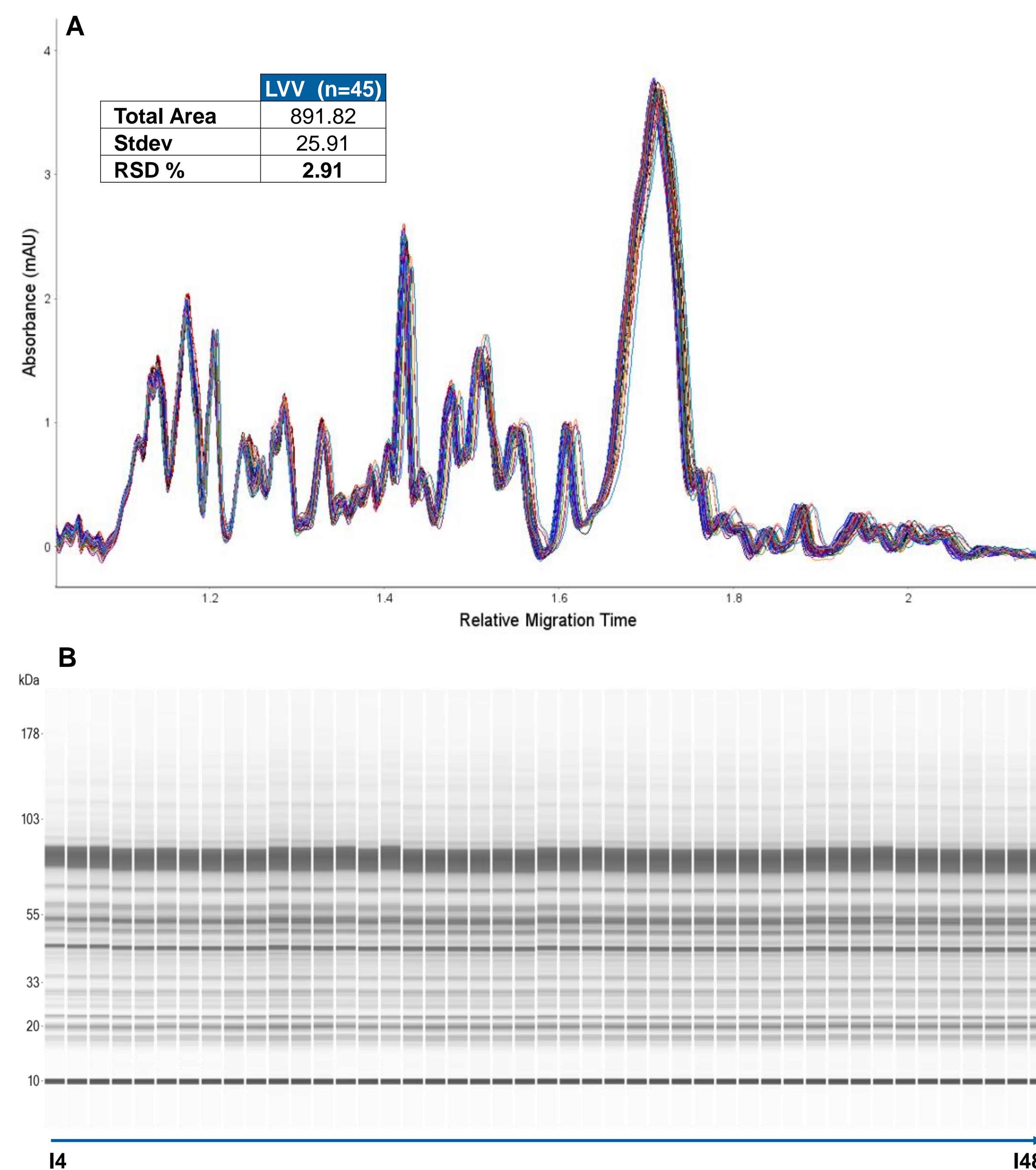
Sample preparation - LVV particles (1.1E+10 TU/mL) were heat inactivated at 95°C for 2 minutes. After inactivation, the sample was kept on ice for immediate use or at -80°C for later use. For protein extraction, cold acetone was added to the sample (10 times the sample volume) and briefly vortexed to precipitate the LV proteins. The sample was then spun in a centrifuge for 10 minutes at 15000xg to pellet the proteins. The supernatant was carefully removed, and the precipitate was allowed to dry for 5minutes. After 5 minutes, dissolved in 2% SDS containing 200mM bicine (pH 5.5).

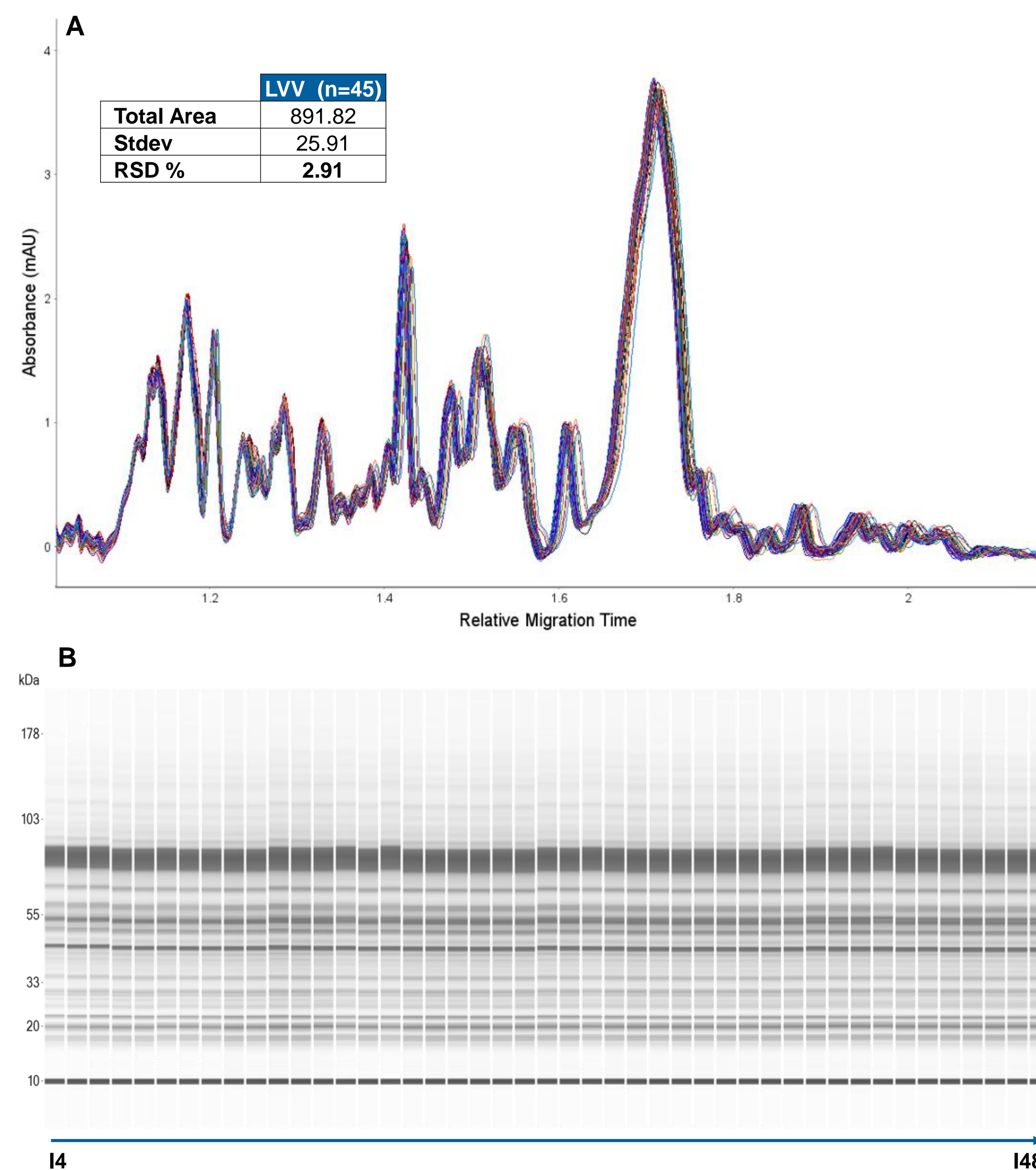
CE-SDS PLUS method - Maurice CE-SDS PLUS Application Kit (PS-MAK03-S). For the CE-SDS analysis (Figure 1-5), the samples were prepared using a reduced protocol. All samples were denatured with β-mercaptoethanol at 95°C for 10 minutes and cooled on ice for 5 minutes. Then samples were vortexed briefly and spun down with a microcentrifuge. On Maurice, samples were injected for 20 seconds at 4600 V and separated for 40 minutes at 5750 V.

RESULTS

LVV CE-SDS Method Reproducibility

LVVs are composed of a range of structural and non-structural proteins. Because of the complex nature of its peak profile, it is crucial to test the reproducibility of the method.

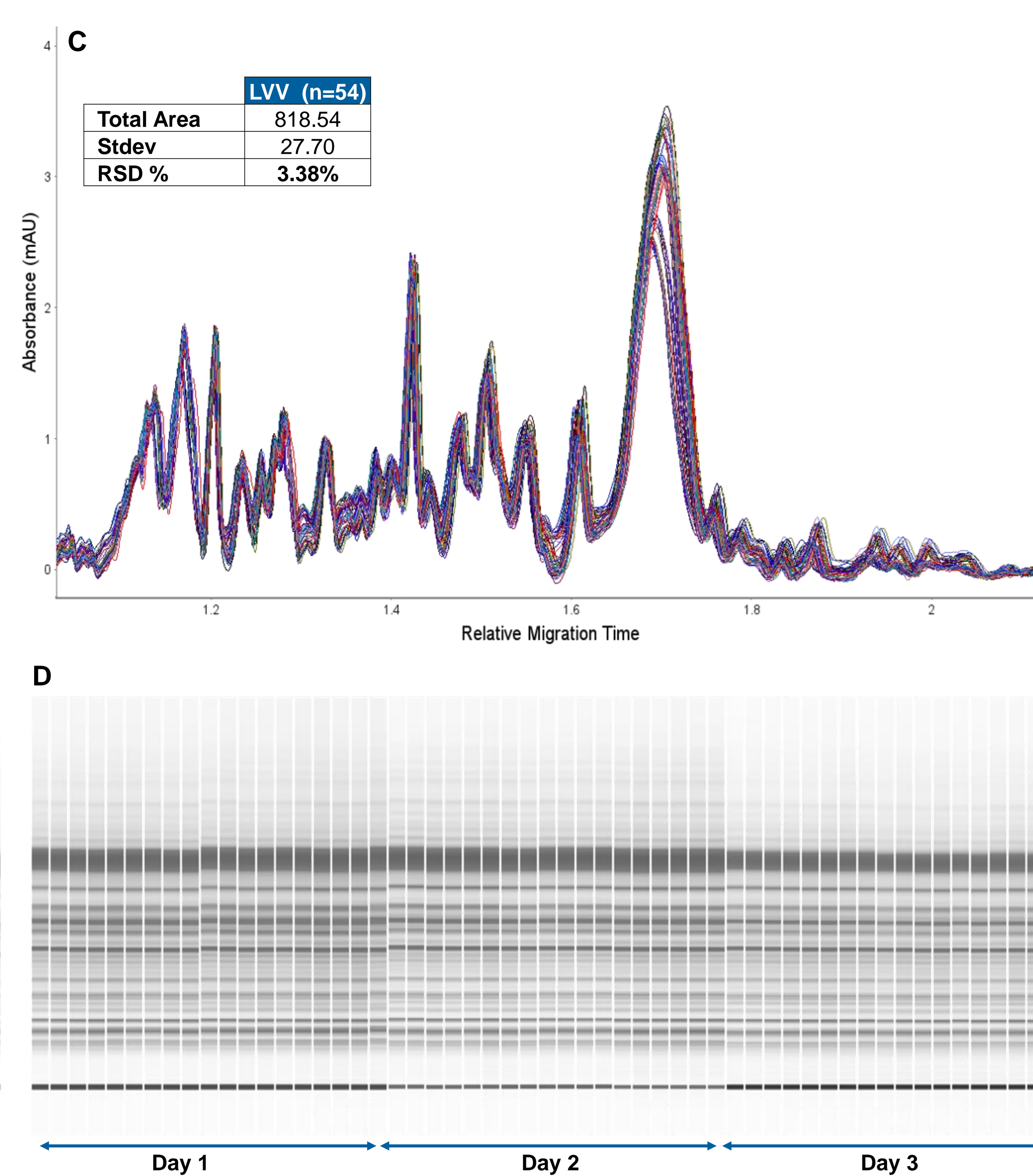




	LVV (n=45)
Total Area	891.82
Stdev	25.91
RSD %	2.91

Day to Day Reproducibility

Day-to-day reproducibility is a good measure to test the robustness of the method. The experiment was conducted for 3 days with 3 different sample preparations to understand intra- and inter-assay reproducibility day to day.



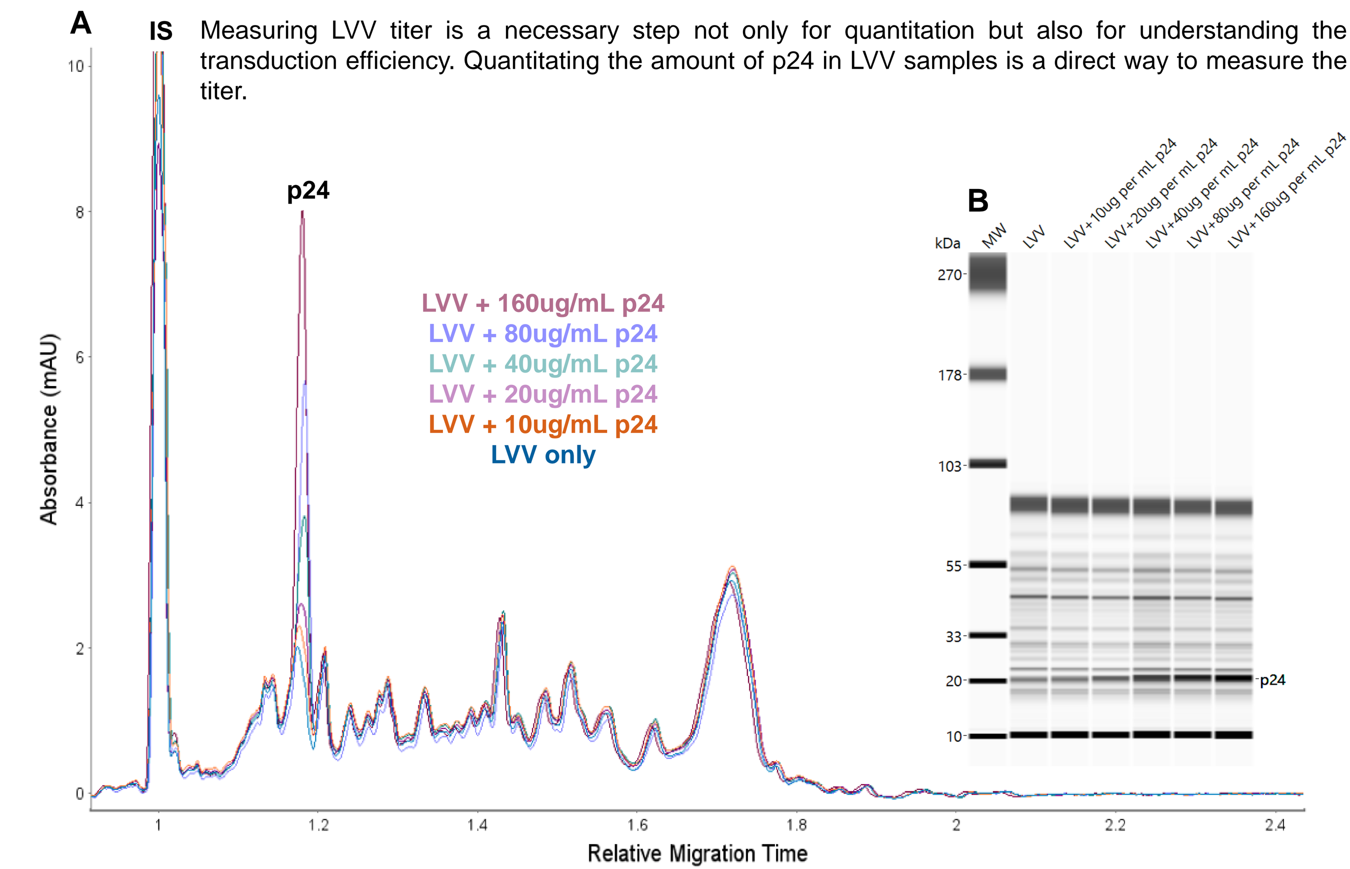
	LVV (n=54)
Total Area	818.54
Stdev	27.70
RSD %	3.38%

Figure 1. LVV method reproducibility. (A) A single 50 µL LVV sample (3.67E+09 TU/mL) was analyzed with 45 replicate injections on Maurice. The total area under the curve for each injection was obtained, and the average total area, standard deviation, and relative standard deviation were calculated. The method is highly reproducible with an RSD = 2.91% for 45 injections. **(B)** Lane view of 45 replicate CE-SDS injections of LVV from a single well. The peak profile in the lane view also shows good reproducibility and allows easy evaluation of multiple samples simultaneously. **(C)** For inter assay reproducibility testing, 6 replicate injections of 3 different LVV (3.67E+09 TU/mL) sample preparations were run on Maurice for three days. When all 54 injections from 3 different days were compared, the peak patterns shows good reproducibility presenting an RSD of 3.38%. **(D)** Lane view of inter assay reproducibility.

RESULTS

Estimate LVV Titer with CE-SDS

Measuring LVV titer is a necessary step not only for quantitation but also for understanding the transduction efficiency. Quantitating the amount of p24 in LVV samples is a direct way to measure the titer.



C — p24 titration

y = 0.885x + 106.69
R^2 = 0.9848

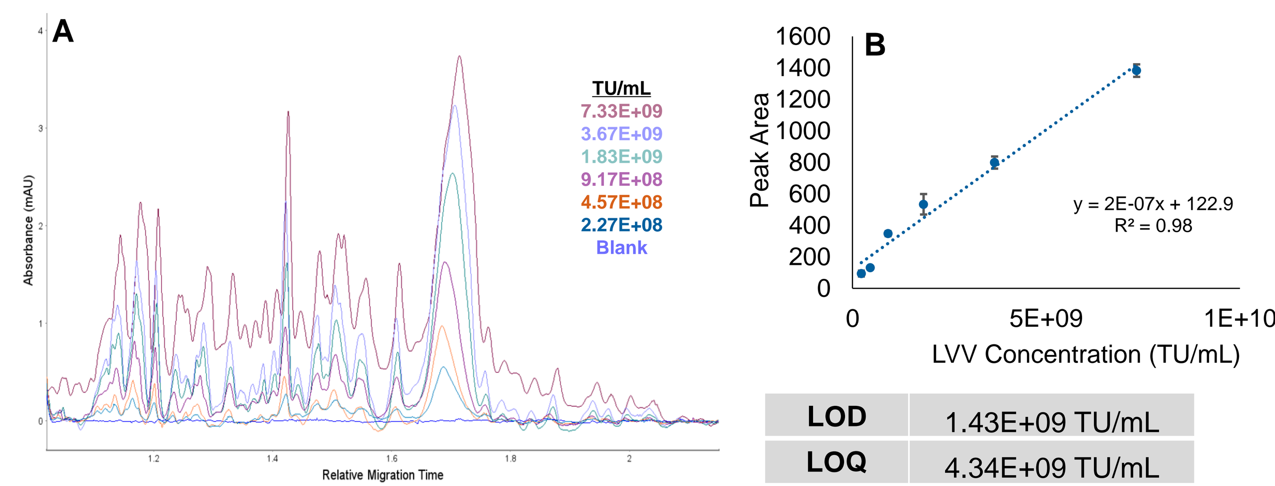
Titer determination:
LVV p24 area = 99.2
~8.5 mg/mL p24 in LVV at 10^{10} TU/mL

Peak Area vs. p24 Added (µg/mL)

Figure 2. p24 spike enables titer determination. (A) A titration of p24 spiked LVVs show a gradual increase in peak intensity with an increase in the recombinant p24 protein concentration. **(B)** Lane view of the spiked LVV titration. **(C)** The data was then fit into a linear regression model. The straight-line equation leads to the concentration of p24 in LVV only and with an established relationship of 1ng p24 = 10^5 TU (*Tiscornia et al.*, Nature Protocols 2006) we estimated the titer concentration to be ~8.5 mg/mL p24 in LVV at 10^{10} TU/mL.

LVV Linearity Determination

To understand the concentration as well as the response range of this method we designed a linearity assay with a target to determine the LOD & LOQ also.



LOD	1.43E+09 TU/mL
LOQ	4.34E+09 TU/mL

Figure 3. Linearity, LOD & LOQ determination of LVVs. (A) The electropherograms show an increase in peak area with an increase in concentration. **(B)** The regression model fitting shows that the data points are fitted well presenting a R-squared value of 0.98. The LOD and LOQ is determined to be 1.43E+09 TU/mL and 4.34E+09 TU/mL.

LVV Identity

Comparison of LVVs from different vendors can be achieved with this method which can present distinct electropherograms exhibiting peak similarities and differences.

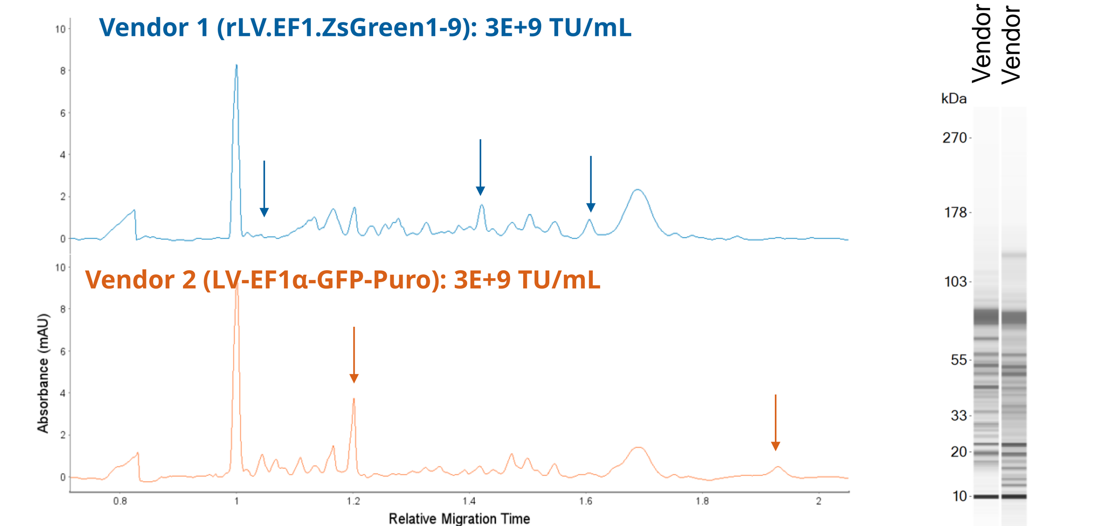
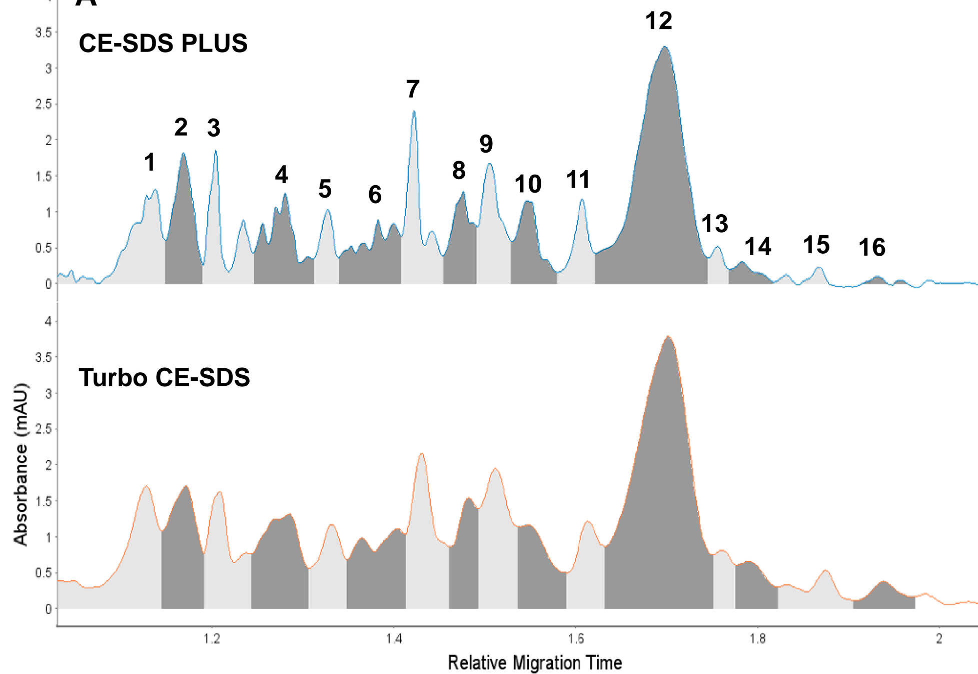


Figure 4. Unique LVV peak profile can serve as an identity assay when comparing materials from different vendors. (A) Similar yet singular peak patterns obtained from two different vendors are quite evident in the electropherograms. **(B)** Lane view of the electropherograms.

LVV on Turbo CE-SDS



The new Maurice CE-SDS Turbo cartridge takes your LVV analysis to another level with enhanced speed. The CE-SDS Turbo cartridge can reduce the separation time ~5X without compromising the peak pattern and percent peak area when compared to the PLUS data.

B

	Percent Peak Area															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CE-SDS PLUS	10.8	8.7	7.3	7.7	3.1	6.6	7.3	4.9	6.1	4.8	3.4	26.1	1.2	1.1	0.7	0.3
Turbo CE-SDS	11.3	8	6.6	7.6	4.1	6.6	7	4.2	6.8	4.5	3.6	22.8	1.6	2	2	1.2

Figure 5. Comparison between CE-SDS PLUS and Turbo CE-SDS. (A) LVV (4E+09 TU/mL) was analyzed on both a Turbo CE-SDS and PLUS cartridge using the same Maurice instrument. For Turbo CE-SDS, the LVV sample was dissolved in 0.5% SDS containing 50mM bicine and was injected for 8s, 3500V and separated for 8mins, 4200V. Data is obtained in only 8 minutes with Turbo CE-SDS, whereas it takes 40 minutes to get a full peak profile for LVV on CE-PLUS. **(B)** Percent peak area comparison between CE-SDS PLUS and Turbo CE-SDS shows overall comparable data is obtained with either separation mode.

CONCLUSION

Here we used Maurice CE-SDS PLUS as an analytical tool to investigate LVV peak profile. We have demonstrated that we can use this method to identify related changes in LVVs from different vendors and thus can serve as an identity assay. We have also shown that it is possible to estimate the LVV titer with spiked p24 titration. Lastly, using the brand-new Turbo CE-SDS cartridge, we obtained comparable CE-SDS data for LVV nearly 5 times faster. Together, these data show that Maurice CE-SDS is a powerful analytical platform to characterize lentiviral vectors.

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Contact: Priyanka Sarkar
Priyanka.Sarkar@bio-techne.com

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