Poster #447

Smartphone-based titration of lentiviral vectors

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Abstract

Lentiviral vectors are flexible delivery tools that possess attractive features for gene delivery, including permanent integration into the host genome, the ability to infect both dividing and nondividing cells, a broad tropism for transducing a wide range of cell types, as well as easy manipulation and production. Extensive use in basic research and translational studies, including gene editing and chimeric antigen receptor (CAR) expression, have increased the need for accurately determining the infectious titer of a lentiviral vector preparation. Successful transduction of a target cell relies on knowing the infectious titer of the lentivirus preparation, as it permits calculation of the multiplicity of infection (MOI) and influences the final proviral copy number within the transduced cells. Ironically, many researchers choose not to quantify vector titer due to the labor-intensive and time-consuming assays that take from four hours to two weeks to complete. In this work, we present an iOS- and Android-compatible smartphone application that analyzes a p24-specific lateral flow assay and delivers infectious unit values (IFU/ml) in approximately 10 minutes. The simplicity of the assay facilitates easy monitoring and optimization of lentiviral production processes to ensure consistency and confidence in downstream applications. The two-step assay consists of adding a small amount of lentiviral supernatant to the lateral flow device followed by imaging and analysis of the results using a smartphone. Densitometric analysis of the observed bands is performed by the intuitive Lenti-X™ GoStix™ software that compares the results to an automatically downloaded, lot-specific standard curve. The result is a GoStix Value (GV) that, like an ELISA assay, can be used to normalize virus stocks prior to transduction. In addition, a reference virus with a known infectious titer can be applied to generate an IFU/ml titer for additional unknown samples. Analyzing several vectors made with different packaging parameters, including a lentiviral sgRNA library, we obtained accurate titer values with R^2 values of greater than 0.99 and with coefficients of variation of less than 15% when compared to FACS-based GFP titers. In summary, this highly convenient titration technology can quantify lentiviral vector preparations in approximately 10 minutes, reduce expenses related to labor and materials, and accelerate lentiviral production processes and transduction experiments.

1 Standard titration method timelines

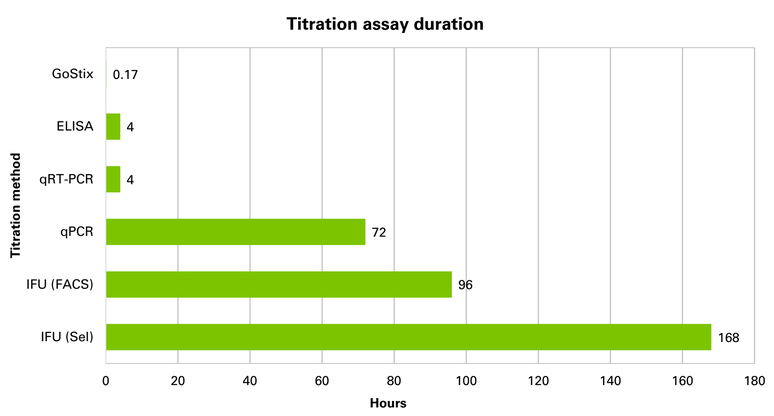

Figure 1. Most commonly used methods for lentiviral vector titration and their associated timelines (in hours). Lenti-X GoStix: a lateral-flow-based method for the detection of lentiviral p24 in supernatants. **ELISA:** detection of p24 capsid protein by sandwich assay. **qRT-PCR:** quantitation of viral RNA genomes by qRT-PCR. **qPCR:** detection of integrated DNA proviruses by qPCR. **IFU (FACS):** determination of the percentage of infected cells via FACS analysis of RFP/GFP positive cells. **IFU (SeI):** infectious units determined by quantifying the number of drug resistant colonies in transduced populations.

2 Rapid testing of lentiviral supernatants with Lenti-X GoStix Plus

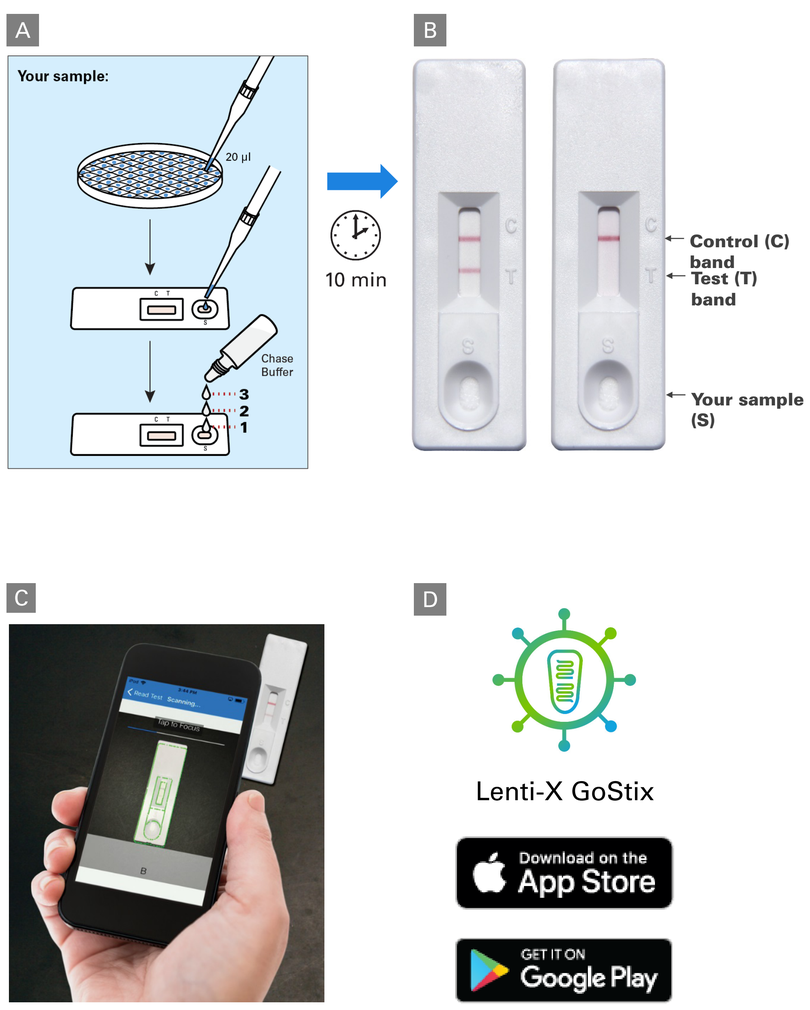

Figure 2. Rapid titration of lentiviral supernatants with Lenti-X GoStix Plus. Panel A. This lateral flow assay detects lentiviral p24 in lentiviral supernatants simply with the addition of 20 µl of lentiviral supernatant, followed by the addition of Chase Buffer, and then incubating at room temperature for 10 minutes. **Panel B.** During the 10-minute incubation time, test and control bands that indicate the presence of lentiviral p24 will develop. **Panel C.** The results on the cassette can then be analyzed using our free smartphone app, which quantifies lentivirus titer by comparing the intensities of the test and control bands. **Panel D.** The Lenti-X GoStix smartphone app can be downloaded from the Apple App Store or Google Play.

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3 Densitometric analysis of Lenti-X GoStix Plus

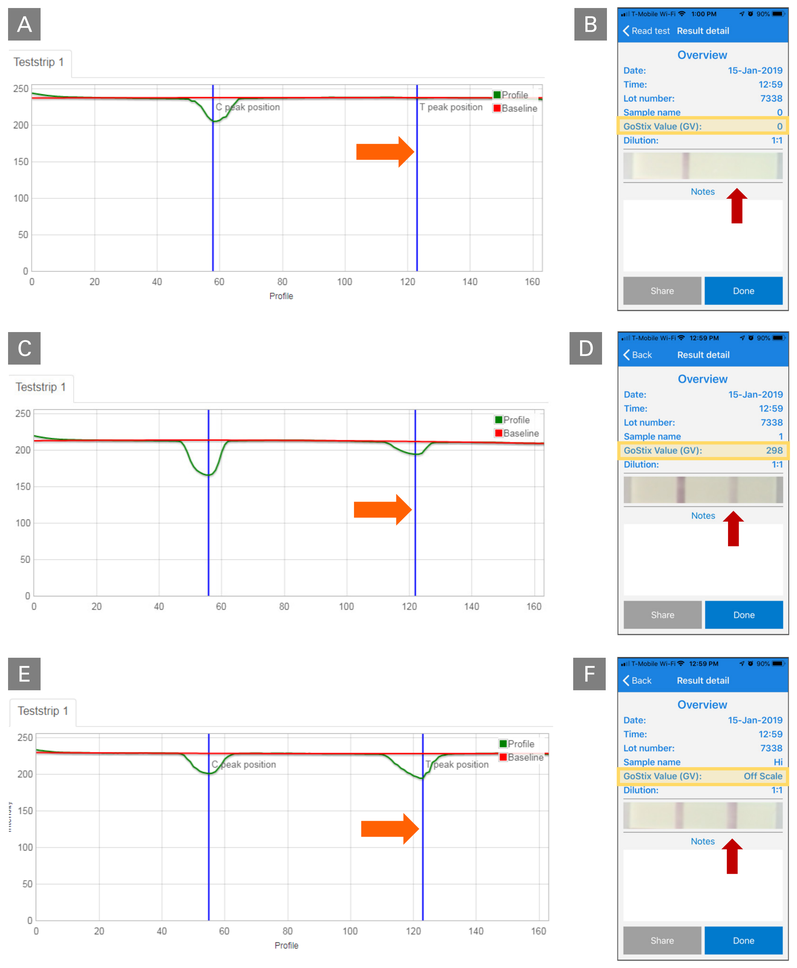

Figure 3. Densitometric analysis of Lenti-X GoStix Plus. Dilutions of recombinant p24 were prepared and applied to Lenti-X GoStix Plus, developed for 10 minutes, and then analyzed using the Lenti-X GoStix smartphone app (v1.2.7(34) for iOS). Test (T) and Control (C) line signals were acquired automatically by the software and GoStix Values (GV) determined by densitometric analysis. The intensity of each T band (orange arrows) and C band are represented in the plots on the left. The software then calculates the T/C ratio and compares it to a previously downloaded, lot-specific control curve. The output within the smartphone app (seen in the screenshots on the right) corresponding to the densitometric analysis includes a time/date stamp, the lot number used for the control curve, sample name, the GV (yellow boxes), and the dilution used for the sample. An image of the read showing the C band and T band (red arrows) is also included, along with an area to make note of any special conditions related to the sample. This data can be distributed by either email or SMS formats via the "Share" button. **Panels A–B.** Example of negative readout. **Panels C–D.** Example of on-scale readout. **Panels E–F.** Example of off-scale readout. The software indicates when the sample is off-scale (when the T/C ratio is nearing 1) and thus requires further dilution.

4 Establishment of the control curve

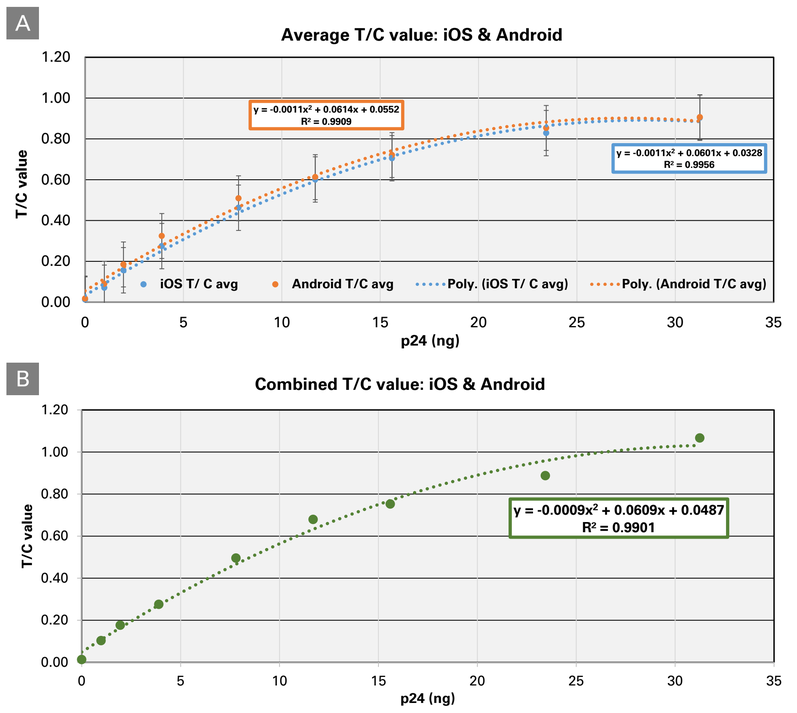

Figure 4. Generation of the internal standard curve. Panel A. A dilution series of recombinant p24 was prepared and added to Lenti-X GoStix Plus in triplicate, developed for 10 minutes, and then analyzed using the Lenti-X GoStix Plus smartphone app on both iOS (iPod6)- and Android (LG MP260)-based devices. T/C ratios for each amount of p24 were then plotted to generate the standard curve. **Panel B.** The combined results from the two devices were then used to create the final lot-specific control curve to be uploaded to the app upon startup on any smartphone with an active internet connection.

5 Consistent results regardless of device

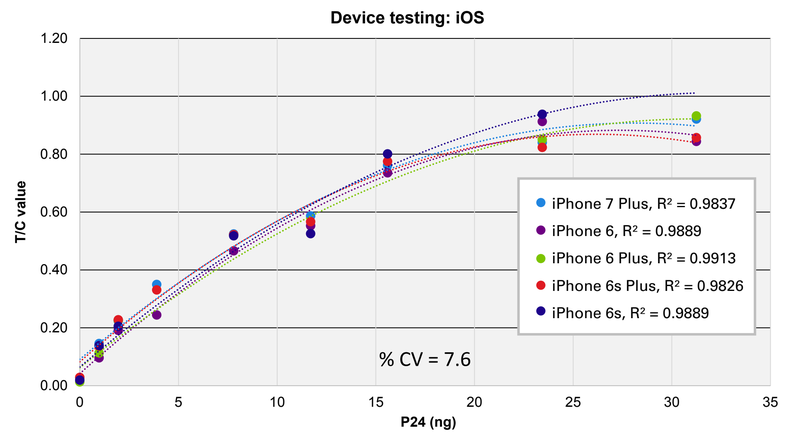

Figure 5. Variation is minimal between different devices. A dilution series of recombinant p24 was prepared and added to Lenti-X GoStix Plus in triplicate, developed for 10 minutes, and then analyzed using the Lenti-X GoStix smartphone app on multiple iOS and Android devices (not shown) with different operating systems in place. %CVs ranged from 3.3 to 16.4 with an average of 7.6 for all dilutions.

6 Low inter- and intra-assay variation with Lenti-X GoStix Plus

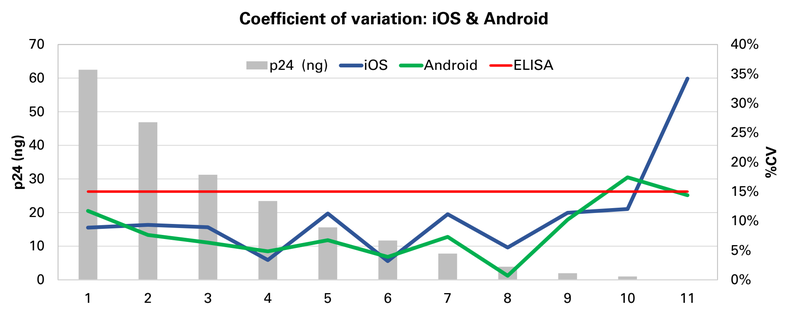

Figure 6. Low %CVs are observed using Lenti-X GoStix Plus. A twofold dilution series (1–11) of recombinant p24 was prepared and added to Lenti-X GoStix Plus in triplicate, developed for 10 minutes, and then analyzed using the Lenti-X GoStix smartphone app (v1.2.6) on both iOS and Android devices. The combined results were analyzed for variance at each p24 dilution. The %CVs observed in the dilution series are in the same range as those generally expected for ELISA kits (red line).

7 Lenti-X GoStix Plus-based titers are comparable to ELISA-based titers

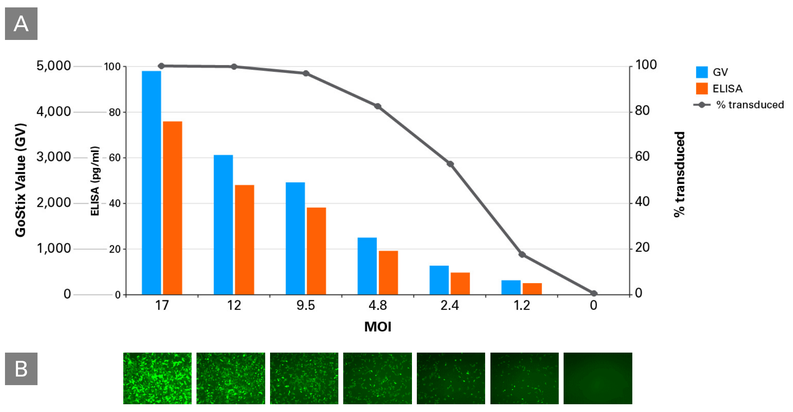

Figure 7. Lenti-X GoStix Plus-based titers can be used in a similar fashion to ELISA-based titers. Panel A. Lenti-X ZsGreen1 lentivirus was prepared using the Lenti-X packaging single shots lentiviral packaging system. Percentages of cells transduced (% transduced) were determined for HT-1080 cells followed by FACS analysis. In addition, the viral p24 value was assessed using the Lenti-X p24 Rapid Titer Kit and the GoStix Value (GV) was determined using the Lenti-X GoStix smartphone app (v1.2.6). Transductions were performed on HT1080 cells using a range of MOIs and the resulting transduction efficiencies were plotted against the associated ELISA and GV equivalents. **Panel B.** Fluorescent images of the cells at varying MOIs were taken at 48 hours post-transduction.

8 sgRNA Library titration with Lenti-X GoStix Plus

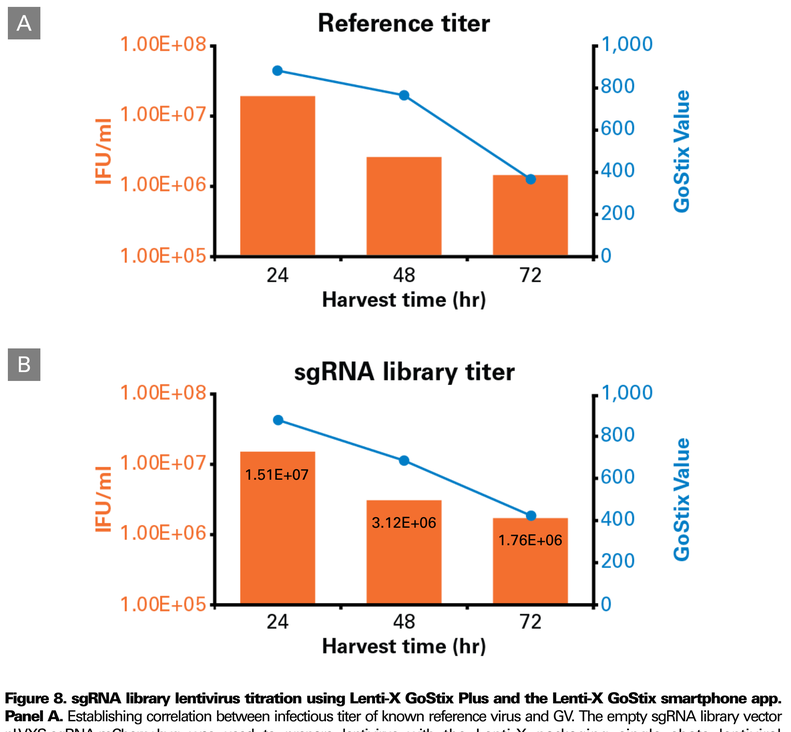

Figure 8. sgRNA library lentivirus titration using Lenti-X GoStix Plus and the Lenti-X GoStix smartphone app. Panel A. Establishing correlation between infectious titer of known reference virus and GV. The empty sgRNA library vector pLVXS-sgRNA-mCherry-hyg was used to prepare lentivirus with the Lenti-X packaging single shots lentiviral packaging system. Lentiviral supernatant was serially harvested at the times indicated. Percentages of cells transduced (% transduced) were determined for HT-1080 cells followed by FACS analysis for mCherry expression. In addition, the viral p24 value was assessed using the Lenti-X GoStix Plus lateral flow devices and associated smartphone app. From this data, the ratio of IFU to GV was calculated for each timepoint. Orange bars represent actual titers. **Panel B.** The Guide-it™ CRISPR Genome-Wide sgRNA Library System was used to produce a genome-wide sgRNA lentivirus library with supernatants harvested at the same times as the reference virus (empty vector). These supernatants were analyzed using the Lenti-X GoStix Plus lateral flow devices and smartphone app. The GV produced at each timepoint from this analysis was then multiplied by the ratio from each timepoint from the reference virus (Panel A) to determine the IFU/ml. Orange bars represent calculated titers. Actual IFU/ml is shown as text for each sample. Using a reference lentiviral supernatant with known IFU/ml, the titer of an unknown lentiviral stock produced in the same manner can be titrated in 10 minutes.

Conclusions

- Lenti-X GoStix Plus lateral flow tests are an easy method to verify the relative quantity of your lentiviral supernatants in just 10 minutes
- The Lenti-X GoStix smartphone app can read p24 values with high reproducibility and low, consistent %CVs between different devices and different GoStix cassettes
- Lenti-X GoStix Plus titration results are in agreement with standard titration methods (e.g., ELISA), and with the use of a reference virus, can determine the IFU/ml titer of an unknown preparation
- The free Lenti-X GoStix smartphone app is an easy, quantitative tool that yields consistent, effective analysis of lentiviral titers via Lenti-X GoStix Plus cassettes

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