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Virus Counter® Plus Platform Enables Fast and Precise Quantification of Lentiviral Vectors

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Abstract

ABL is developing a production and purification process for gene expression using lentiviral vectors. Precise monitoring of critical process parameters at each stage is critical to optimize both the yield and quality of the vector, ensuring the process is consistently high-performing. Traditional virus quantification methods for physical titer such as qPCR (quantifying genomic content) and p24 ELISA (detecting viral capsid protein) can be laborious and fall short in providing direct particle counts due to their indirect detection mechanisms. Transduction assays assess viral activity but take weeks to produce results and are often highly variable, reducing confidence in data.

The Virus Counter® Plus platform can quickly and precisely determine the total particle count for a given virus sample. This study demonstrates how the Virus Counter® Plus system can be used to follow the production and purification process steps in near real-time and deliver quick and precise results throughout the process. The obtained results correlate strongly with ABL's current physical titer techniques with less labor and faster time to result. This study also shows that the Virus Counter® system can accurately estimate functional titer measurements after establishing a predictive model. Overall, the Virus Counter® Plus platform limits the need for highly variable, time-consuming assays and accelerates development timelines.

Learn more about the
Virus Counter® Plus

Introduction

Lentiviral vectors are important delivery vehicles for emerging cell and gene therapies. However, producing the virus in the volume and quality required for clinical applications can be challenging. The lack of analytics that deliver rapid and reliable insights into the bioprocess can be a significant bottleneck when optimizing production and purification steps. Results are often only available days or weeks after samples are taken, which makes fine-tuning the process difficult, and slows the development of potentially lifesaving therapies.

Current quantification methods often measure the building blocks of the virus to derive a titer measurement. qPCR quantifies the viral genome to assess the virus titer, while p24 ELISA methods quantify viral envelope proteins to estimate the lentivirus concentration. Neither of these methods targets the glycoprotein (most typically VSV-G) used for pseudotyping lentiviruses. This glycoprotein is crucial for successful transduction, which is monitored through assays that measure the functional titer of a virus. As these methods depend on cell cultures and replicating viruses, their results typically take multiple weeks.

The Virus Counter® platform is purpose-built for the direct, rapid, and simple quantification of viruses. The platform consists of a system, reagents, and software that work together to provide a user-friendly experience, enabling quicker, more robust titer data acquisition with less hands-on time. The Virotag® VSVG assay, which uses highly specific fluorescently labeled anti-VSV-G antibodies, is used to label VSV-G pseudotyped lentivirus for quantification on the system, which directs virus particles in suspension through an optical detection system for direct quantification with high precision. The time-to-result ranges from two hours to overnight, depending entirely on the number of samples analyzed.

Here, we investigate if the Virus Counter® system can be utilized in ABLs' process to eliminate analytical bottlenecks by replacing indirect quantification methods and building predictive models to reduce the requirement for functional assays. This study focuses on understanding how the Virus Counter® Plus platform can be used during ABL's process development activities by delivering robust and rapid analytics, allowing confident decision-making and optimization of the viral vector production process.

Methods

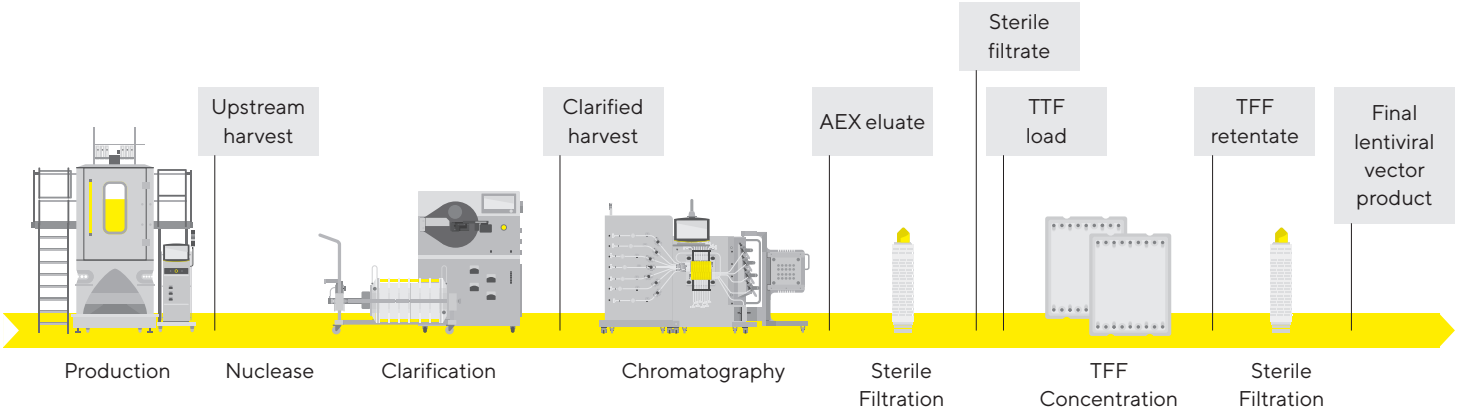
The Process Development group at ABL performed several development runs for lentivirus production and purification. The process is represented in Figure 1. Cells were grown in suspension in a 20 L bioreactor and transfected with lentiviral packaging, envelope, and GFP expression plasmids. After a two-day incubation, the harvested virus was treated with nuclease to clear unassociated nucleic acid and then clarified with depth filters to remove large particles and cellular debris. Following clarification, the virus was further purified by anion exchange (AEX) chromatography and filtered through a 0.22 µm filter. After an overnight hold, the filtrate was concentrated, and buffer exchanged into formulation buffer via tangential flow filtration (TFF) followed by sterile filtration.

Virus samples were analyzed at each step for physical titer using qPCR and p24 ELISA and for activity by transducing units (TU). The samples were also assessed on the Virus Counter® Plus with the Virotag® VSVG assay. Four independent production runs were tracked using these analytics throughout this study (Study 1, Study 2, Study 3, Study 4). Since the first sterile filtrate was held overnight, sampling of the TFF load was performed the following morning. Samples pulled for further testing were stored frozen.

The qPCR assay for physical titer in virus particles/mL (vp/mL) was performed using the Lenti-X™ qRT-PCR Titration Kit (TakaraBio). Each sample underwent qPCR in technical triplicates, and the results were converted from viral genomes/mL to vp/mL. In parallel, the p24 ELISA assay for physical titer was performed using the ABL p24 ELISA kit. The resulting p24 concentration value was multiplied by 10,000,000 (1×10^7) (conversion factor from Didier Trono) to determine the vp/mL. The transduction unit (TU) assay for functional titer in TU/mL was performed by transducing HT1080 cells with virus at three dilutions and quantifying the GFP-positive signal using a FACS instrument. While the qPCR and p24 ELISA assays can usually be run and analyzed within 1 – 2 days, the TU assay is much more time and labor-intensive and takes 2 – 4 weeks from the start of cell culture to data analysis. In contrast, results from the Virus Counter® Plus can be obtained in just a few hours.

The process for the titer evaluation with the Virus Counter® Plus is shown in Figure 2. Diluted viral samples were mixed with Virotag® VSVG, a primary antibody stain composed of a monoclonal antibody conjugated with fluorophores in a stabilizing buffer solution. Diluted negative controls were also prepared and stained in the same manner as the virus samples. For proper performance of the peak-finding algorithm, blanks composed of antibody stain in sample diluent were also prepared. The stained samples, negative controls, and blanks were loaded into a 96-well plate and incubated in the dark for a minimum of 30 minutes at room temperature. Following incubation, the plates were loaded on the Virus Counter® Plus system for analysis. During analysis, the plates are held at 4 °C. The blanks were analyzed prior to samples and negative controls.

Figure 1: *Lentivirus Production Process at ABL*



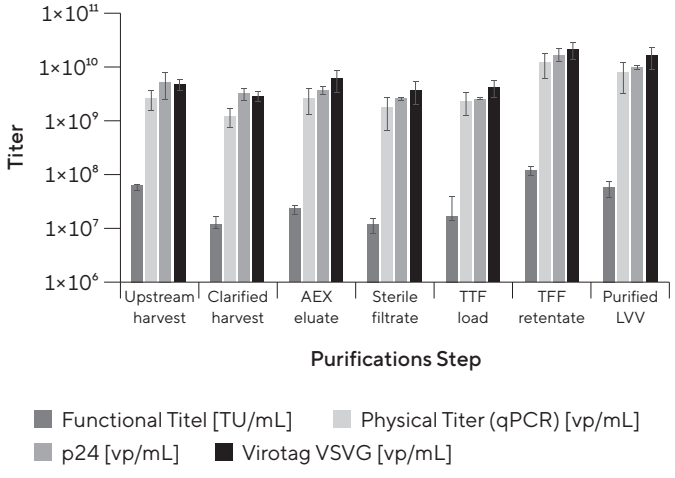
Note. Samples were taken throughout the production process and are labeled in grey.

Figure 2: *Titer Evaluation Process With the Virus Counter® Plus System*

Results and Discussion

Four growth and purification processes were evaluated for this study. Figure 3 shows the complied data for both the upstream harvest and downstream process (from clarified harvest to final purified virus) for all four studies. The p24 ELISA data was only available for Study 2 and 3. The Virus Counter® Plus data aligns most closely with the physical titer methods (qPCR and p24 ELISA). The Virotag® VSVG-derived particle count: TU/mL ratio was calculated to be ~215, well within the industry-accepted range of 100 – 1,000 and close to the ABL's qPCR: TU/mL ratio of ~190 (1 ng/mL of p24 $\approx 10^4 - 10^5$ TU/mL). These results suggest that the physical titer results obtained from the Virus Counter® Plus platform can be a suitable replacement for other methods measuring the physical virus titer. The Virus Counter® platform has the added strength of speed and minimal sample preparation, as the sample is simply diluted into diluent provided with the staining kit, mixed with stain, and incubated before reading on the instrument. Results can be obtained within 24 hours with less than an hour of hands-on time.

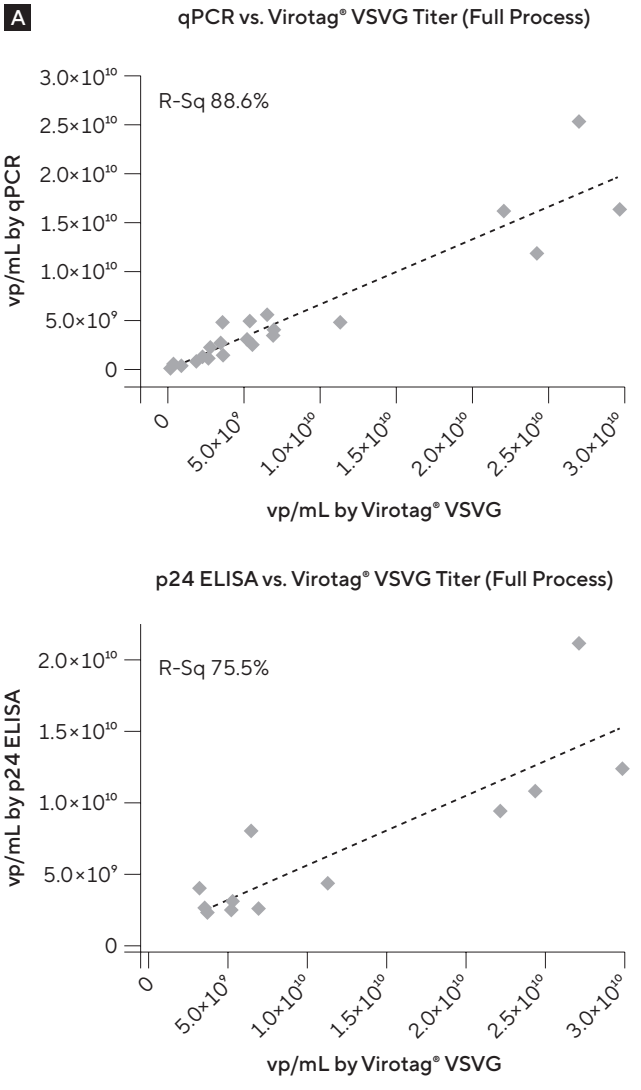
Figure 3: *Physical Titer (qPCR and p24 [vp/mL]) and Activity (Functional Titer [TU/mL]) Data for Each Process Step Compared to Virus Counter® Plus (Virotag® VSVG [vp/mL]) Measurements*

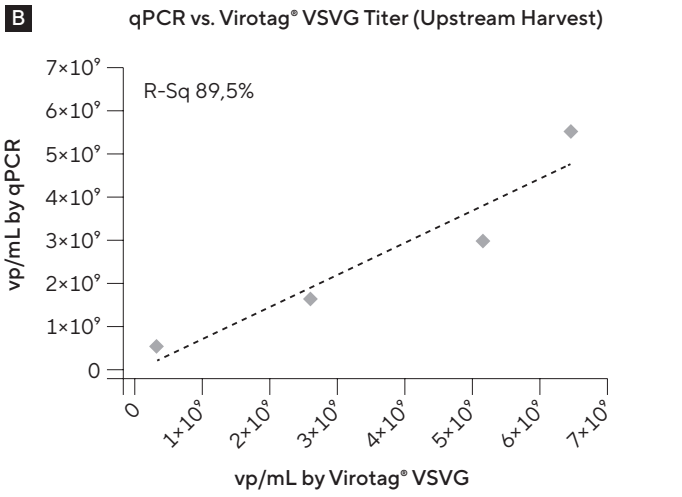


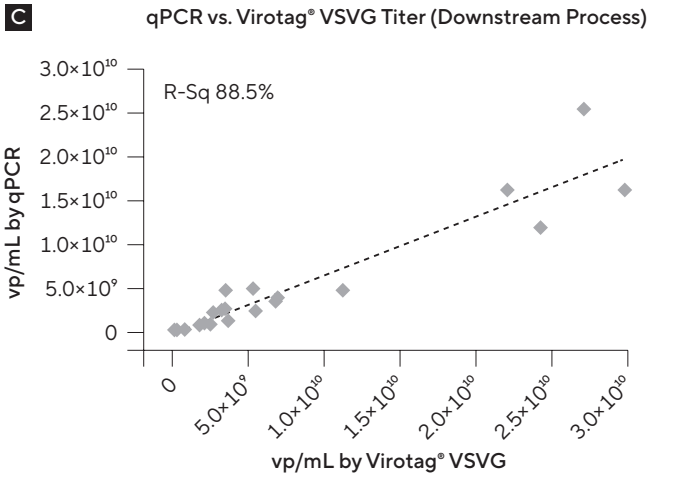
Note. Columns represent the mean across four growths. Error bars represent the standard error of the mean.

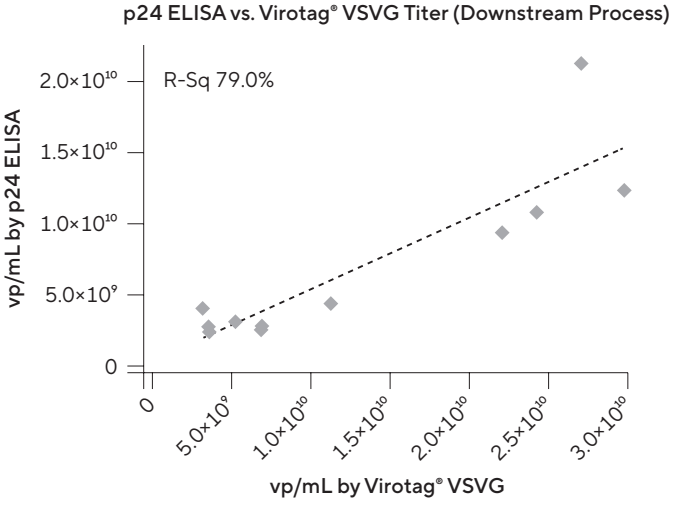
We then correlated the results from different methods with those obtained using the Virus Counter® Plus. Figure 4 illustrates the strong correlation between physical titer (from genome copies and p24 ELISA) and Virus Counter® Plus data for the overall process (Figure 4A). The correlation for both methods maintains strength when the upstream harvest is separated from every downstream step (Figures 4B and 4C). Since the ELISA measurements were only performed for two of the four studies, there was insufficient data to find an upstream harvest correlation. These datasets indicate that the Virus Counter® Plus assay targeting the VSVG pseudotype is quantifying the same particles as the other methods.

Figure 4: *Physical Titer Correlations for A) Full Process, B) Upstream Harvest, and C) Downstream Process*







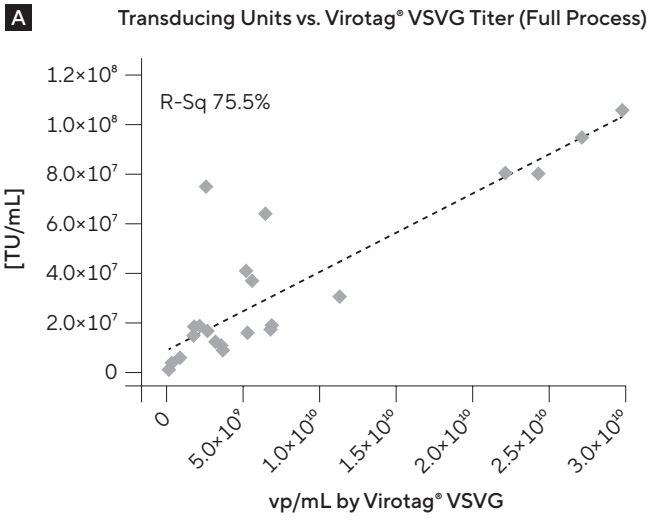


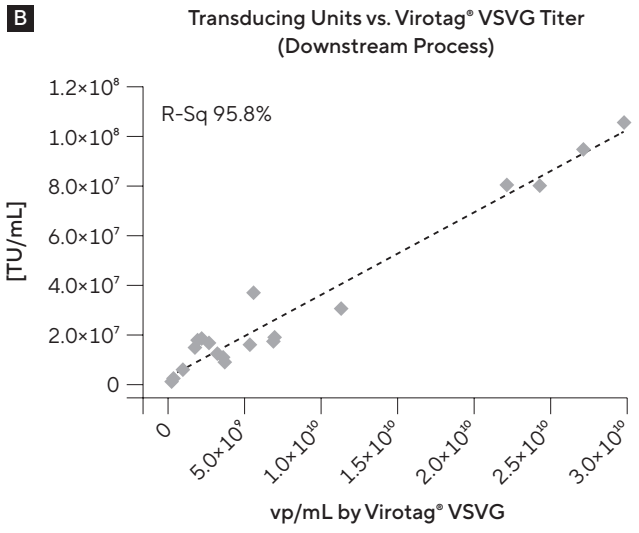
Note. Due to insufficient data, no p24 ELISA correlation was performed for the upstream harvest.

Next, we evaluated how the Virotag® VSVG data correlates with the functional titers (Figure 5). The overall correlation between the Virotag® VSVG and the transduction assay results is weaker than the correlations with the physical titers (Figure 5A). The correlation strengthens when upstream data is excluded, and only the downstream process data are plotted (Figure 5B). This is because of a single, relatively high point in the upstream harvest data, observable when the upstream harvest data is isolated (Figure 5C). It is also important to note that TU/mL results are inherently more prone to statistical noise than any physical titering method, and more data points would likely improve the results. The mean ratio across the process for the Virotag® VSVG results in vp/mL to the functional titer in TU/mL is 214.7 with a standard deviation of 111, an expected result for lentivirus preparations.

Because the Virotag® assay targets VSV-G — the glycoprotein involved in infecting cells for transduction — the correlation raises the possibility of using Virus Counter® data to generate a predictive model for transducing unit data. Such a development would save significant time and costs, limiting the number of transducing unit assays required during process development.

Figure 5: *Functional Titer Correlations for A) Full Process, B) Downstream Process, and C) Upstream Harvest*





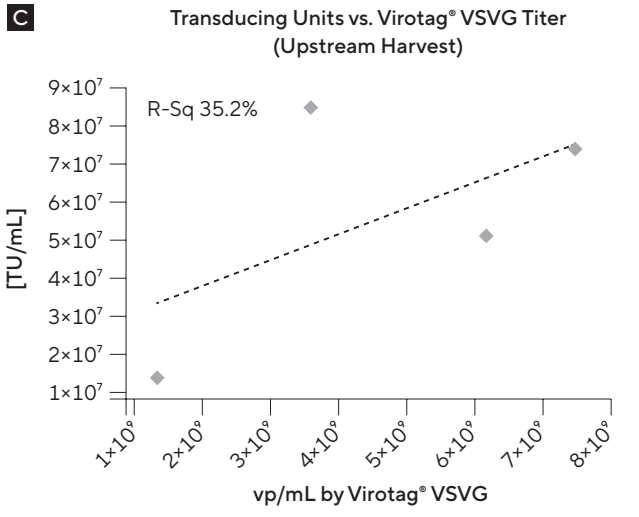
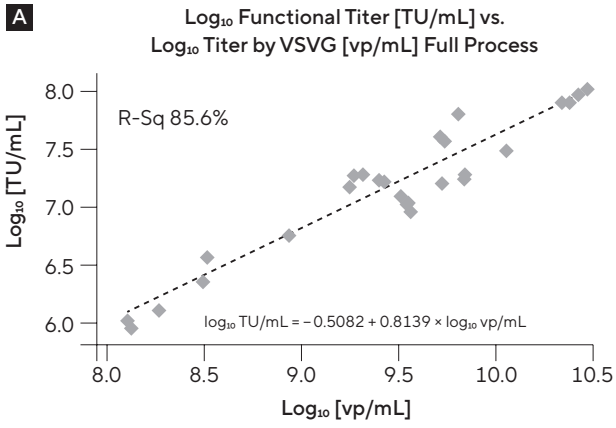
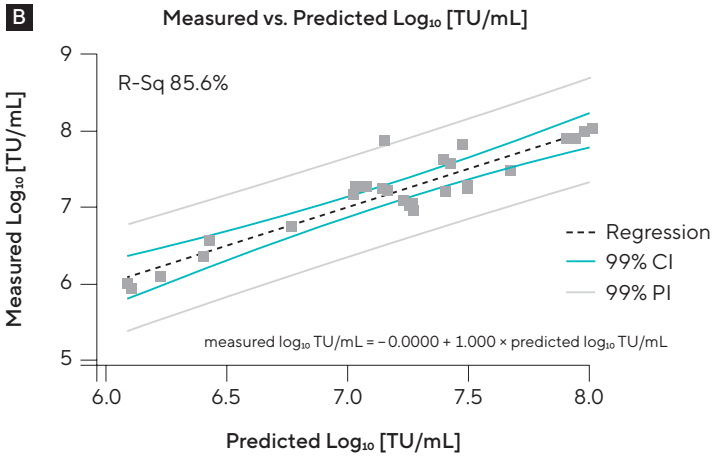


Figure 6: *Modeling of the Transducing Unit and Virotag® VSVG Titer*





Note. A) Correlation of the log-transformed data used to generate the model and B) the correlation between the measured TU/mL and the predicted TU/mL.

Alternative fitting techniques for the existing data sets were tested to develop the strongest possible predictive model. To maintain statistical power, the data was not separated into process stages. A $\log_{10}$ transformation of both the TU/mL and VSVG-derived vp/mL data yielded the most promising results for the overall data set, with the upstream harvest data included. Figure 6 shows the log-transformed dataset (Figure 6A) and the predicted results (Figure 6B). The correlation between the predicted and the actual TU/mL is about 86%, with a perfect slope and a y-intercept of 0, indicating that the model reliably reproduces the measured results. More data will be required to refine the model further and improve its reliability. Still, these results demonstrate how VSVG titer data can predict the functional titer, reducing the time to result by a factor of 14 or more. This application note can be used as a guideline for establishing predictive models for other vector platforms.

Conclusion

This study demonstrates a strong correlation of data from the Virus Counter® Plus platform (using the Virotag® VSVG assay) with other physical titer methods and transducing units through the downstream lentivurs production process. The data in this study was used to build a reliable predictive model for finding functional titers based on direct titer measurement.

The Virus Counter® system can accelerate time-to-result and deliver reliable results with less hands-on labor than existing methods, significantly reducing the analytical bottleneck and delivering relevant insights quicker and more robustly. The correlation of Virus Counter® data with transducing units offers a significant opportunity to further accelerate development timelines and enable fast and confident process optimization by lowering the demand for time-consuming functional assays.

"The Virus Counter® Plus offers distinct benefits compared to qPCR, particularly in its ability to provide rapid and direct quantification of virus particles without the need for nucleic acid extraction or amplification. This not only simplifies sample preparation but also reduces assay time significantly, making it a more efficient choice for high-throughput screening and real-time monitoring of viral titers in our applications."

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