The ultimate flex: pseudotype specific LV titer with Leprechaun

Introduction

VSVG is the most common envelope protein used for pseudotyping lentivirus (LV) due to the broad cell tropism and high transduction efficiency it provides. While ideal for *ex vivo* modification of selected cells, these properties make VSVG pseudotyped LV poorly suited for *in vivo* therapies due to the high chance of off-target delivery. Modifying the LV pseudotype by replacing VSVG with an alternative envelope protein with a more specific cell tropism can increase LV delivery efficiency and reduce off-target effects[1]. As a result there is growing interest in manufacturing non-VSVG pseudotyped LV, engineered to target disease specific cell types.

Amongst the challenges for anyone modifying the LV pseudotype is the inability to easily check that the correct envelope protein is consistently expressed throughout production and across batches. Conventional methods for assessing LV quality, e.g. capsid ELISAs or qRT-PCR, don't look at the viral envelope, while alternative approaches such as western blotting are slow and only semi-quantitative. Consequently, confirmation of the viral pseudotype is often indirectly confirmed by functional cell-based assays which measure the efficiency of transduction of the target cells by the virus.

Leprechaun makes it easy to obtain a pseudotype-specific LV titer at any stage of your production process (Figure 1).

Leprechaun's Lentivirus Flex RNA Kit utilizes Abcam's Lightning-Link™ technology, allowing users to customize the antibody used for LV capture from the comfort of their own labs. All you need to do is start with an antibody which recognizes the envelope protein of your choice, incubate it with the linker protein provided in the kit (Figure 2A), add the quencher reagent and then incubate the linker-antibody conjugate on a Flex Luni. The linker protein is specifically bound by Flex antibodies spotted on the Luni surface (Figure 2B). Once the Luni has been functionalized with the desired antibodies the sample is added, and immuno-staining performed to check for the presence of the viral capsid and RNA (Figure 2C).

Figure 1: Leprechaun is the only platform that simultaneously checks your LV has the correct envelope protein, contains a capsid and RNA, and is the right size.

The number of viral particles with and without capsid or RNA is combined with single particle sizing data to generate a LV titer for each structural population – LV w/ capsid & RNA, LV w/ capsid w/o RNA, LV w/o capsid w/ RNA, LV w/o capsid w/o RNA and aggregates. As an alternative to the

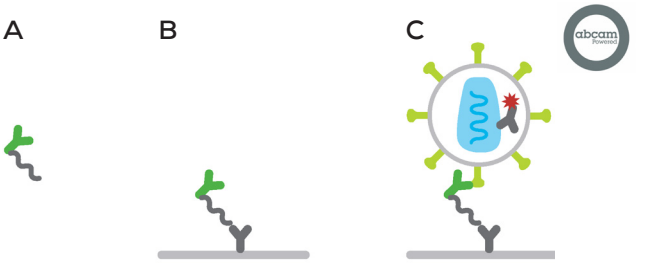

Figure 2: Step by step functionalization of the Lentivirus Flex Luni. (**A**) An antibody specific for the desired envelope protein (green) is incubated with the Flex linker (gray) to form an antibody-linker conjugate. (**B**) The antibody-linker conjugate is then incubated on the Flex Luni and the linker specifically binds to the corresponding Flex capture antibody, which is pre-spotted on the Luni surface. (**C**) LV is captured by the custom antibody via the viral envelope protein.

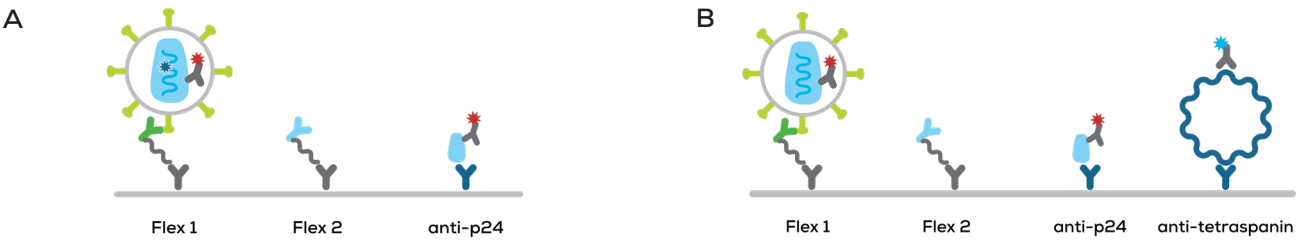

Figure 3: Available Lentivirus Flex Luni designs. (**A**) All Flex Lunis feature six Flex 1 spots for LV capture, three Flex 2 spots which can be functionalized with isotype controls and three anti-p24 spots for quantification of soluble p24. (**B**) Lentivirus Flex EV Contaminant Lunis also have an additional set of three anti-tetraspanin spots for analysis of non-viral vesicle contaminants.

Flex RNA Kit (Figure 3A), an EV Contaminant Kit is available which includes additional Luni spots for capture and quantification of non-viral vesicle contaminants alongside viral titer (Figure 3B). Each kit contains a pair of linker reagents, each of which recognizes a different set of Flex spots on the Luni surface. This design allows for conjugation of one antibody to capture the viral envelope, and a second antibody to function as an isotype control for the first.

This app note describes how Leprechaun's Lentivirus Flex RNA Kit and Lentivirus Flex EV Contaminant kit deliver pseudotype specific quantification LV and non-viral contaminants, providing a one stop shop for confirming the quality of your LV, whatever the pseudotype.

Methods

Leprechaun Analysis

LV samples were acquired from Vectorbuilder and Leprechaun analysis performed using the Lentivirus Flex RNA Kit or Lentivirus Flex EV contaminate Kit (Unchained Labs). Flex Lunis were functionalized, as per kit instructions, with SARS-CoV-2 spike protein RBD antibody (R&D Systems). Crude LV was diluted 1:5 and purified LV 1:30 in manufacturer supplied Incubation Solution with added 1% FBS. For RNA detection kit supplied SYTO14 was diluted to 10 µM in Incubation Solution and added to the diluted LV sample at a dye to sample ratio of 1:10. The dye-sample mixture was incubated for 16 hours at 37 °C. Post dye incubation, 50 µL SYTO14 labeled sample was incubated for 1 hour at RT on Lentivirus Flex RNA Lunis. For contaminant analysis diluted sample was incubated directly on the Luni for 1 hour at RT. After fixation and permeabilization by Solution C and D, all Lunis were incubated for 1 hour at RT with kit supplied anti-p24-CF647 (1:250). Lunis were washed, dried and run on Leprechaun using the Lentivirus RNA application or Lentivirus EV Contaminant application.

qRT-PCR Analysis

Analysis was performed by SydLabs using the Lenti-X qRT-PCR Titration Kit (Takara Bio), according to the kit protocol. Three dilutions of each sample were run in duplicate and results averaged to obtain genomic titer.

Results

Highly Specific

The specificity of the Flex system was confirmed by incubating Lunis with and without a flex-linked custom antibody in the presence of LV sample. In the case where the custom antibody was excluded the linker was still added to the Flex spots, but with no antibody attached. Signal was only detected on the fully functionalized Luni (linker and antibody both present), verifying that the linker alone is not sufficient for particle capture (Figure 4 – left pair of bars). A fully functionalized Luni was also analyzed without sample present and no signal recorded (right bars), confirming that particle counts are only detected when sample is bound to the Flex conjugate. EV contaminants were tracked across the three experimental designs, with readings taken from the same Flex Luni as LV titer. As the tetraspanin antibody is spotted directly onto the Luni rather than added via Flex, this acted as a positive control to confirm sample had been added where appropriate and there were no technical issues with the reading.

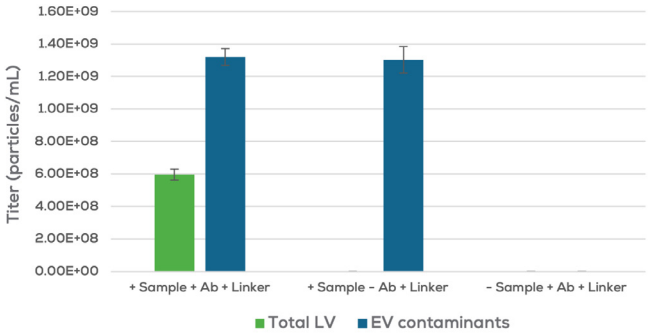

Figure 4: Validation of signal specificity from Lentivirus Flex Lunis. All components (linker, custom antibody and sample) must be present for LV titer to be recorded. n = 3 for each condition and output, error bars are SD.

Linear and Precise

LV engineered to express the SARS-CoV-2 spike protein was used to test the linearity and precision of Leprechaun analysis of non-VSVG pseudotyped LV. An antibody specific to the spike protein was immobilized on the Flex RNA Luni via the Flex conjugation system. Total LV titer and LV w/ capsid & RNA titer are linear down to $1x10^7$ particles/mL in both crude and purified samples (Figure 5), matching the performance of the VSVG Luni and application. Assay precision can be altered by the custom antibody clone used for capture, but for the spike protein antibody tested % CV was typically around 12%. Equivalent results were obtained for the Flex EV Contaminant Luni.

Pseudotype Specific LV Analytics

As soon as your Flex Lunis are functionalized and ready to go you can start digging into LV titer, RNA content, aggregation and contamination, using less than 25 µL of sample. Analysis of crude and PEG purified samples of SARS-CoV-2 spike protein pseudotyped LV reveals that purification successfully concentrates LV particles containing both capsid and RNA (Figure 6A). The titer of RNA-containing LV increased 100-fold in the purified sample, in line with the change in genomic titer as measured by qRT-PCR. LV containing both a capsid and RNA was enriched from 14% to 35%.

Contaminant analysis, however, shows that there are still a high titer of non-viral EV contaminants in the final product (Figure 6B). Standard approaches to LV purification tend to co-purify EVs due to their similar size and density. As a consequence, there is growing focus on the need to measure and characterize the EV content of LV related drug products in order to meet regulatory requirements[2]. Interestingly, the proportion of particles which are non-viral EVs rises from 58% in crude, to 72% in the purified sample, indicating that PEG purification is completely ineffective at removing these contaminants.

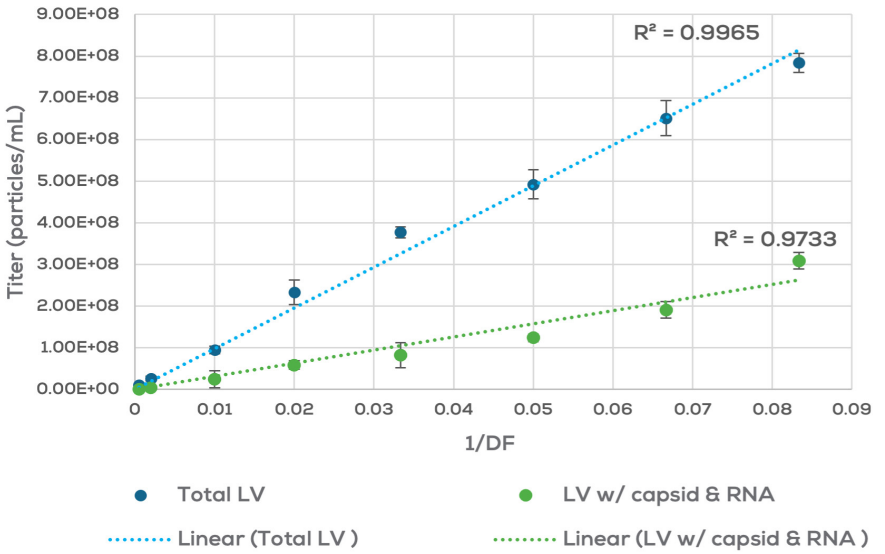

Figure 5: Linearity of LV titer for SARS-CoV-2 spike protein pseudotyped LV as measured by Leprechaun. Error bars are SD.

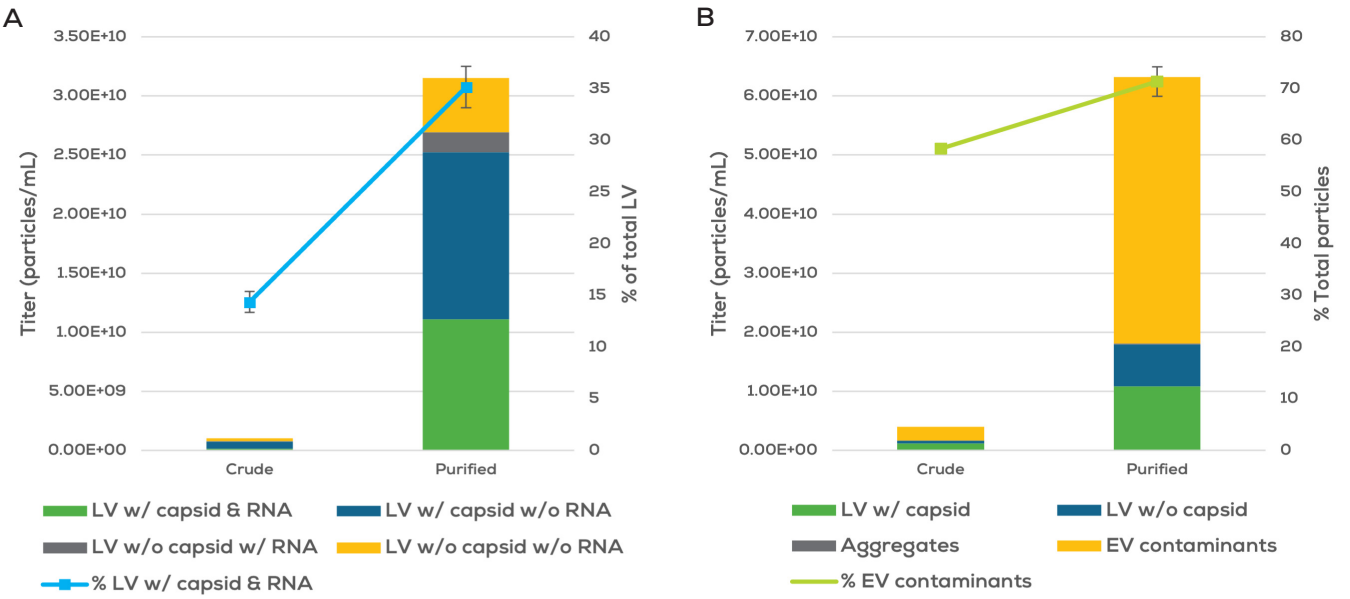

Figure 6: Leprechaun analysis of crude and purified SARS-CoV-2 spike protein pseudotyped LV. (**A**) Data from the Lentivirus Flex RNA Kit provides information on the structural composition and RNA content of LV particles. (**B**) The Lentivirus Flex EV Contaminant Kit delivers LV titer alongside contaminant analysis. n = 3 for each condition and output, error bars are SD.

Conclusion

Modification of LV pseudotype is a useful tool for increasing the specificity and efficiency of target cell transduction. The majority of LV analytical techniques focus on either the protein capsid or RNA payload and provide no information on the viral envelope protein. Leprechaun confirms LV pseudotype while simultaneously checking for the viral capsid and RNA, meaning you can be confident you're measuring the right stuff.

Flex Lunis are easily customized with any antibody and come in two flavors. If you want to know the titer of structurally complete RNA-containing LV then the Flex RNA Kit can provide it. Alternatively, to monitor LV purity and contaminant removal the Flex EV Contaminant Kit steps in.

Take your process and analytical development to the next level with the help of Leprechaun, the only platform to measure pseudotype specific LV titer, contamination and aggregation in both crude and pure samples. Whatever the envelope protein, no matter how complex the sample matrix, monitor your LV quality from harvest to final product.

References

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2 Critical assessment of purification and analytical technologies for enveloped viral vector and vaccine processing and their current limitations in resolving co-expressed extracellular vesicles. Vaccines, 2021. 9(8): 823

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