

# Next-generation transfection reagent for large scale therapeutic lentiviral vector production

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## Abstract

Lentiviral vectors are the carrier of choice for allogenic or autologous cell therapies (such as CAR-T) because of its capacity to permanently integrate viral genome into host cell DNA. To produce those vectors, cell therapy producers generally use a transient transfection system that is scaled-up during process development phases. FectoVIR®-LV is the next generation of transfection reagent, free of animal component, designed to improve LV productivity in HEK-293T cell systems. FectoVIR®-LV is made for large scale manufacturing with reduced complexation volume and increased complex stability. On top of the performance in titers and the scalability, FectoVIR®-LV is also compatible with standard expression booster such as sodium butyrate. These key benefits make FectoVIR®-LV transfection reagent a perfect match for lentiviral vector manufacturing.

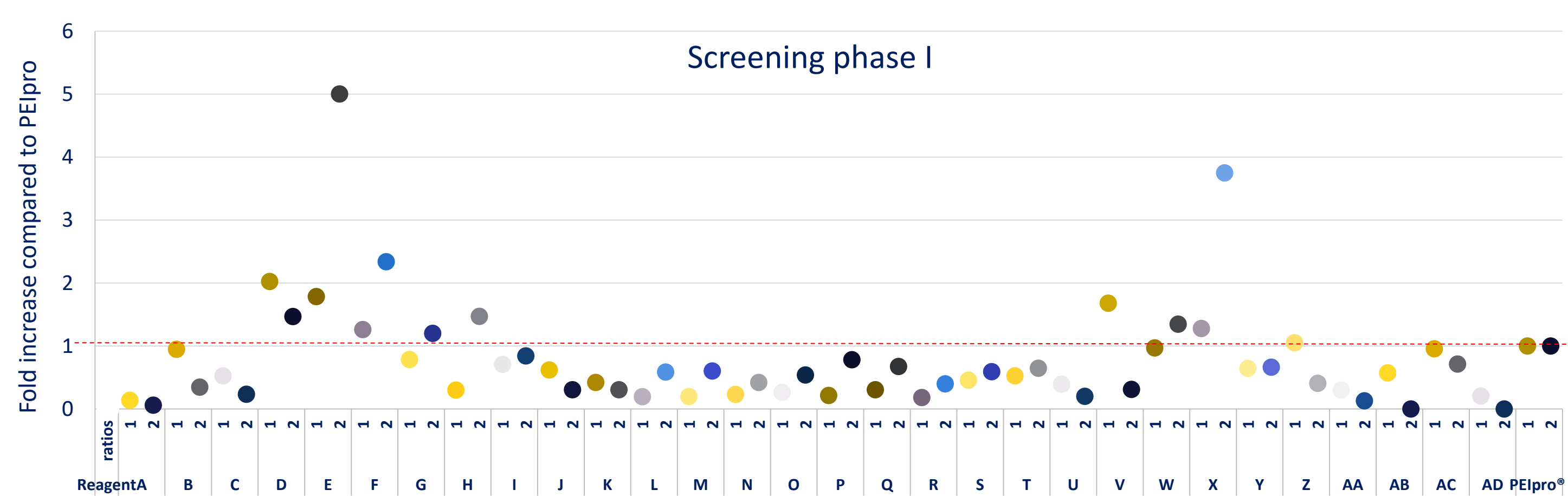
## FectoVIR®-LV development: A whole new screening

The gene and cell therapy field has exploded in recent years. Nearly 1,100 clinical trials investigating gene and gene-modified cell therapies were underway in mid-2022. Of the 1100 trials, the delivery vector was public information for 45%, with lentivirus predominating (48%), followed by AAV (26%). Despite these clinical advances, the cost to develop, manufacture, and deliver LV-based gene and cell therapies remains a significant issue.

The choice of the transfection reagent has a direct impact on manufacturing costs and at Polyplus® we have been continuously working to develop new transfection reagents that increase number of doses produce per batch while decreasing manufacturing costs.

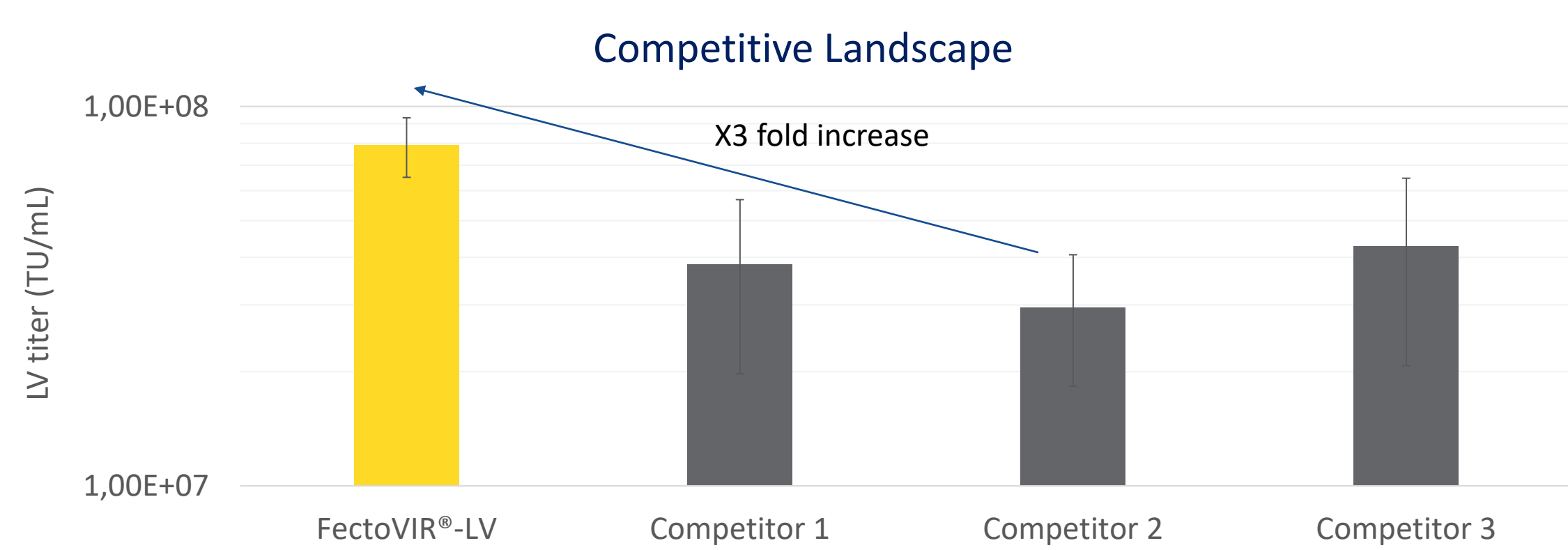
FectoVIR®-LV is the next generation of transfection reagent fruit of a discovery process and validation for suspension LV production.

### Screening phase I: More than 100 molecules from our internal library were tested



**Screening for a new transfection reagent dedicated to lentiviral production:** Lentiviral vectors were produced using molecules from our internal library and a 4-plasmid system in 125mL flask with HEK-293T cells cultivated in suspension in 30 mL. The transfection step was performed using 1µg of DNA per million cells with two ratios of DNA:transfection reagent. LV particles were harvested 72h post-transfection and functional titers were measured using an infectivity test on HT-1080 cells (ATCC-CCL121). Results are presented as fold increase in Transduction Unit (TU) titers compared to PEIpro®. Red line corresponds to the baseline.

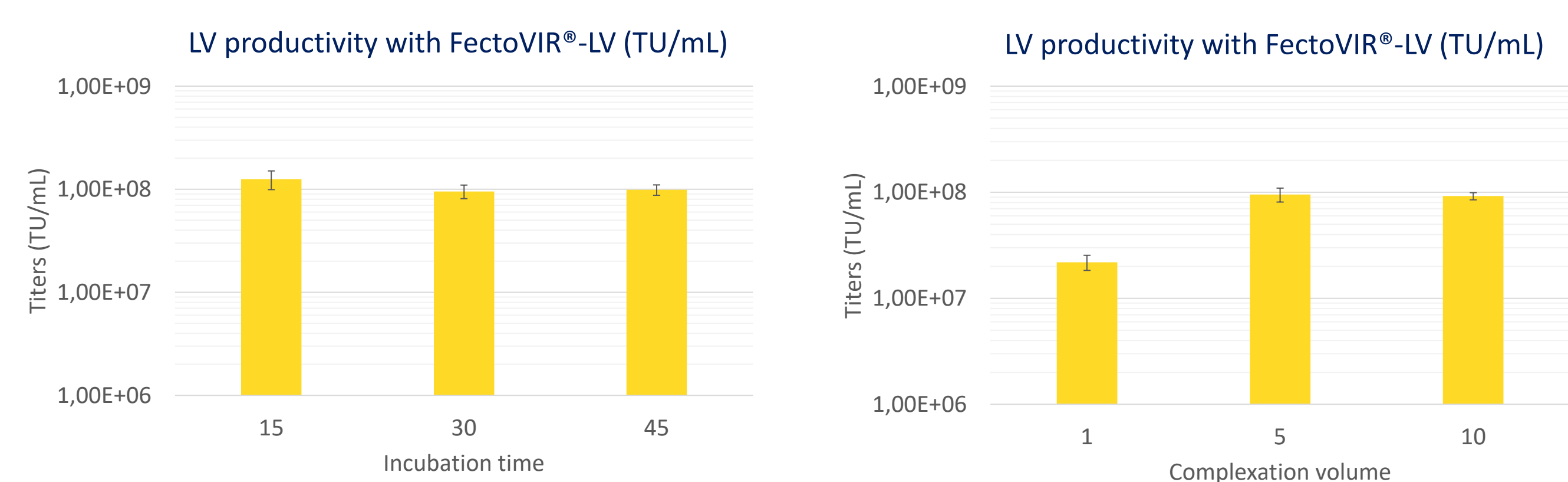
### Screening phase II: Optimization of chemical structure to meet key specifications



**FectoVIR®-LV demonstrates a high productivity for lentiviral vector manufacturing compared to competitors in the field.** Lentiviral vectors were produced using FectoVIR®-LV / 3 other competitors and a 4-plasmid system in 125 mL flask with HEK-293T cell cultivated in suspension. The transfection step has been performed using the recommended conditions and LV were harvested 72h post-transfection. Functional titers were measured using an infectivity test.

## Made for large scale

FectoVIR®-LV has been designed to increase complex stability and decrease complex volume allowing sufficient time to transfer the mix into large bioreactors (>200 L). FectoVIR®-LV has proven its efficiency using the **recommended 30 min of complexation time and 5% complexation volume**, which can be further optimized by a DOE study.

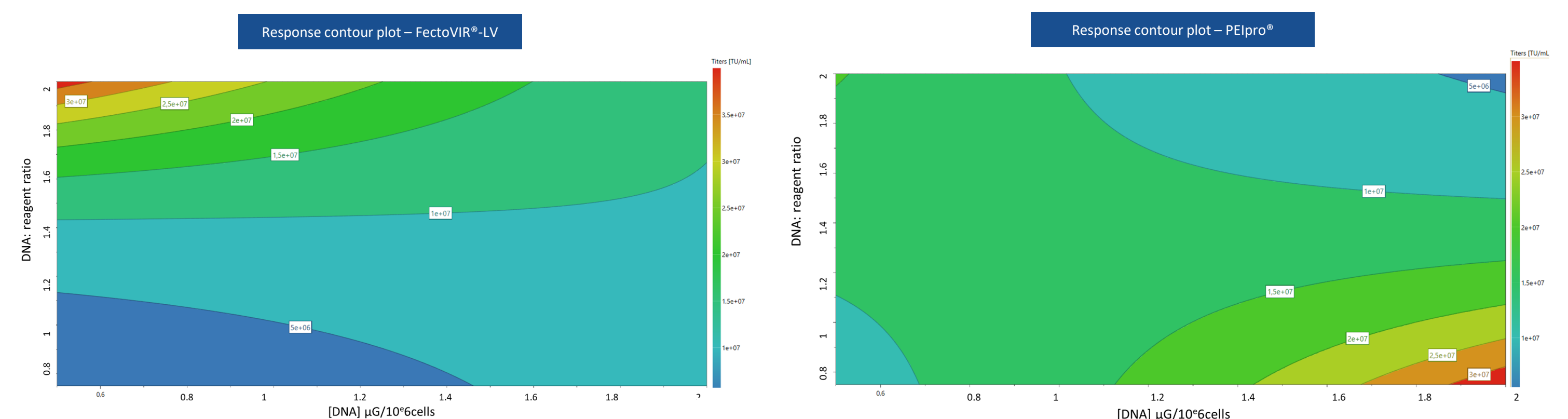


**FectoVIR®-LV is highly scalable with recommended conditions allowing to transfect large bioreactors.** Lentiviral vectors were produced using FectoVIR®-LV and a 4-plasmid system in 125 mL flask with HEK-293T cell cultivated in suspension. A/ The transfection mix was incubated during 15, 30 or 45 min, using the recommended conditions (ratio DNA:transfection reagent of 1:1, 1 µg of DNA per million cells, 5% complexation volume) and LV were harvested 72h post-transfection. Functional titers were measured using an infectivity test. B/ The transfection mix were complexed using 1, 5 or 10% of the total volume (30 mL), using the recommended conditions (ratio DNA:transfection reagent of 1:1, 1 µg of DNA per million cells, 30 min incubation time) and LV were harvested 72h post-transfection. Functional titers were measured using an infectivity test.

## FectoVIR®-LV protocol optimization using DOE

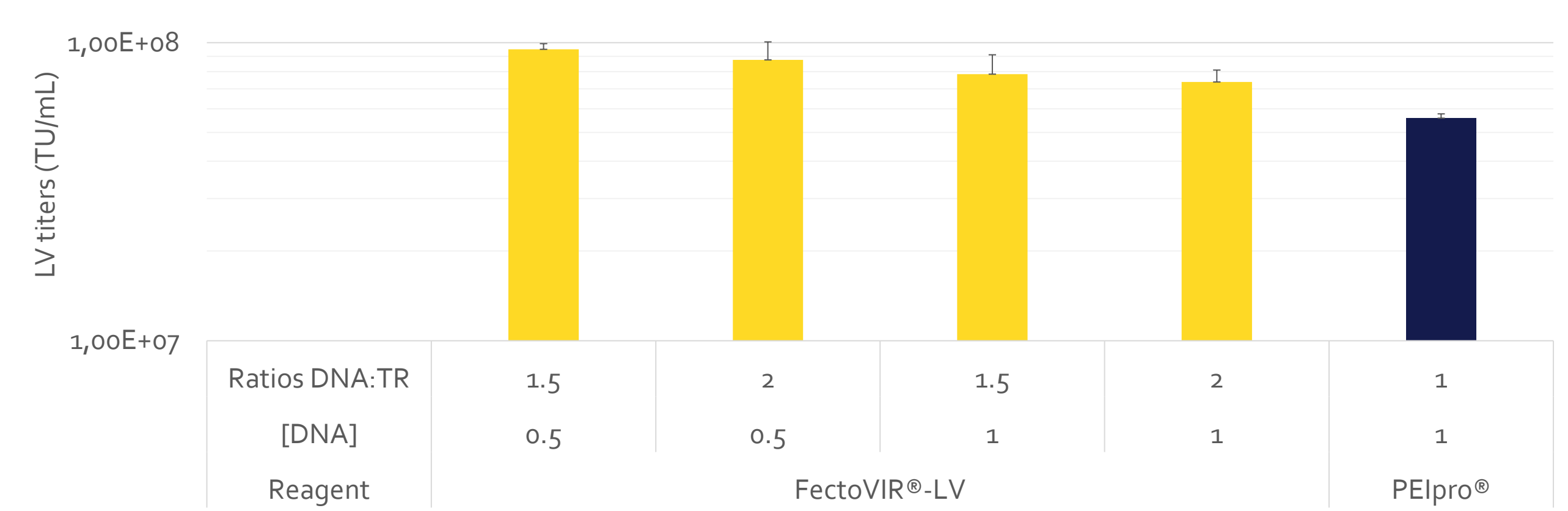
During transfection, different factors (such as DNA amount, DNA:reagent ratio, complexation time, complexation volume, etc. ) will impact complexation efficiency and therefore transfection efficiency and titers. To explore the impact of these parameters and gain time during optimization of the process, we recommend using Design of Experiment (DoE) \*.

### DoE optimization of FectoVIR®-LV based transfection using MODDE®: different behavior compared to PEIpro® and 2-fold increase in titer



**DoE results show different optimization range for FectoVIR®-LV and PEIpro®:** A DoE experiment was designed using MODDE® Software with a full factor design. The factors were DNA concentration (0.5 to 2 µg per million cells), DNA to transfection reagent ratio (1:0.75 to 1:2) and the measured output was the transduction unit in TU/mL. HEK-293T cells in suspension were transfected following the different conditions as recommended by MODDE®. Transfection complexes were prepared in a 10% or 5% complexation volume and added 15 min or 30 min after transfection with PEIpro® and FectoVIR®-AAV, respectively. LV particles were harvested 72h post-transfection and functional titers were measured using infectivity test on HT-1080 cells.

### Reduce LV manufacturing costs using DoE: FectoVIR®-LV allows gain in productivity while reducing by 2-fold DNA consumption

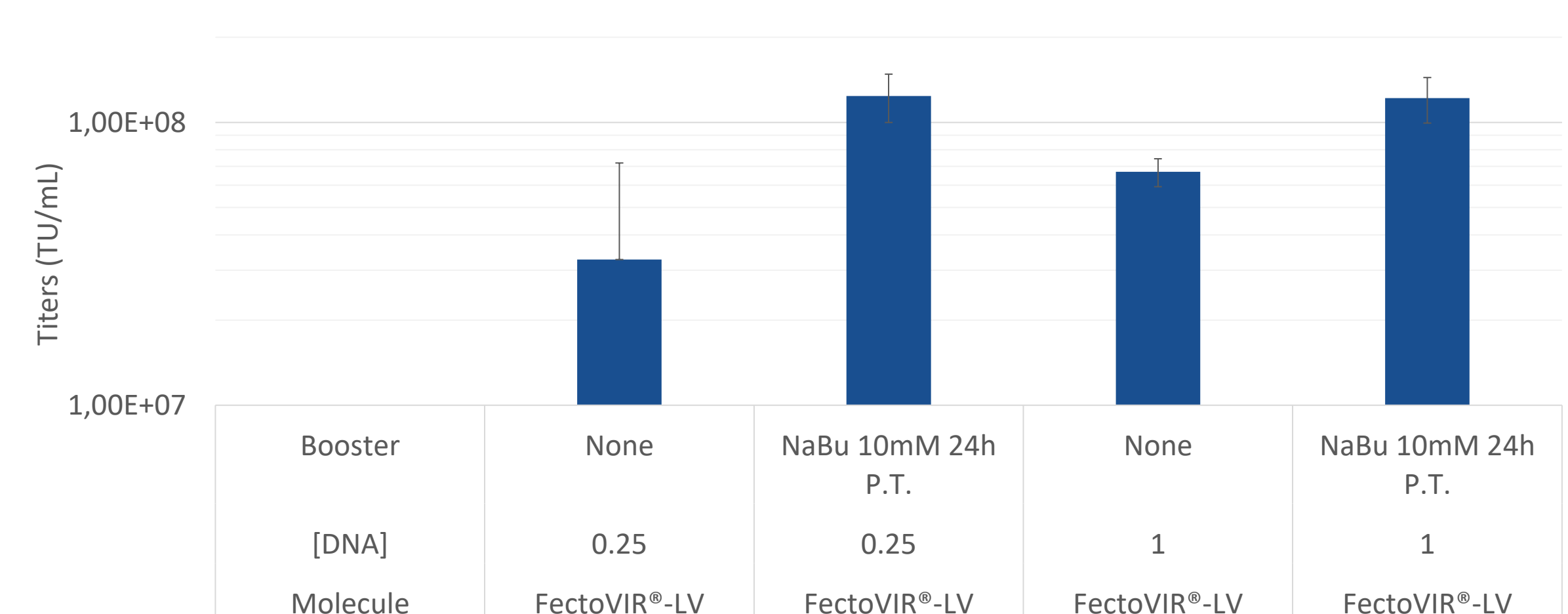


**FectoVIR®-LV transfection protocol uses less DNA quantity compared to PEIpro®, while improving titers for better process economics.** Lentiviral vectors were produced using FectoVIR®-LV or PEIpro® and a 4-plasmid system in 125 mL flask with HEK-293T cell cultivated in suspension. The transfection mix was incubated during 30 min, using different ratio DNA:transfection reagent (from 1.5 to 2), DNA quantity (from 0.5 to 1 µg/10E6 cells) and FectoVIR®-LV or PEIpro®, using their recommended transfection protocol. LV particles were harvested 72h post-transfection. Functional titers were measured using an infectivity test.

\*To support our customers, Polyplus offers a comprehensive DoE service to optimize key parameters of transfection step to reach higher titers.

## Compatible with booster

Sodium butyrate, an agent known to affect the expression of a number of viral and cellular genes, is commonly added to the culture medium to increase lentiviral vector production. FectoVIR®-LV has been shown to be compatible with the use of sodium butyrate, resulting in higher viral titers, especially under low DNA conditions.



**The use of sodium butyrate (booster of lentiviral production) is compatible with FectoVIR®-LV.** Lentiviral vectors were produced using FectoVIR®-LV and a 4-plasmid system in 125 mL flask with HEK-293T cell cultivated in suspension. The transfection mix was incubated during 30 min, using a DNA quantity from 0.25 to 1 µg/10E6 cells. NaBu 10 mM was added 24 post-transfection. LV particles were harvested 72h post-transfection. Functional titers were measured using an infectivity test.

Productivity: Reach highest Lentiviral vectors titers in suspension systems

Cost-effectiveness: Reduce your cost per batch with high titers and low DNA consumption

## Conclusion Advantages of FectoVIR®-LV

Scalability: Produce at large scale with low complexation volume and long complex stability

Time saving: Use DOE service to help you optimize your process in a record time

GMP development & residual test development ongoing

