

# Silencing lentiviral vector cargo gene to aid cell therapy manufacturing

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## Introduction

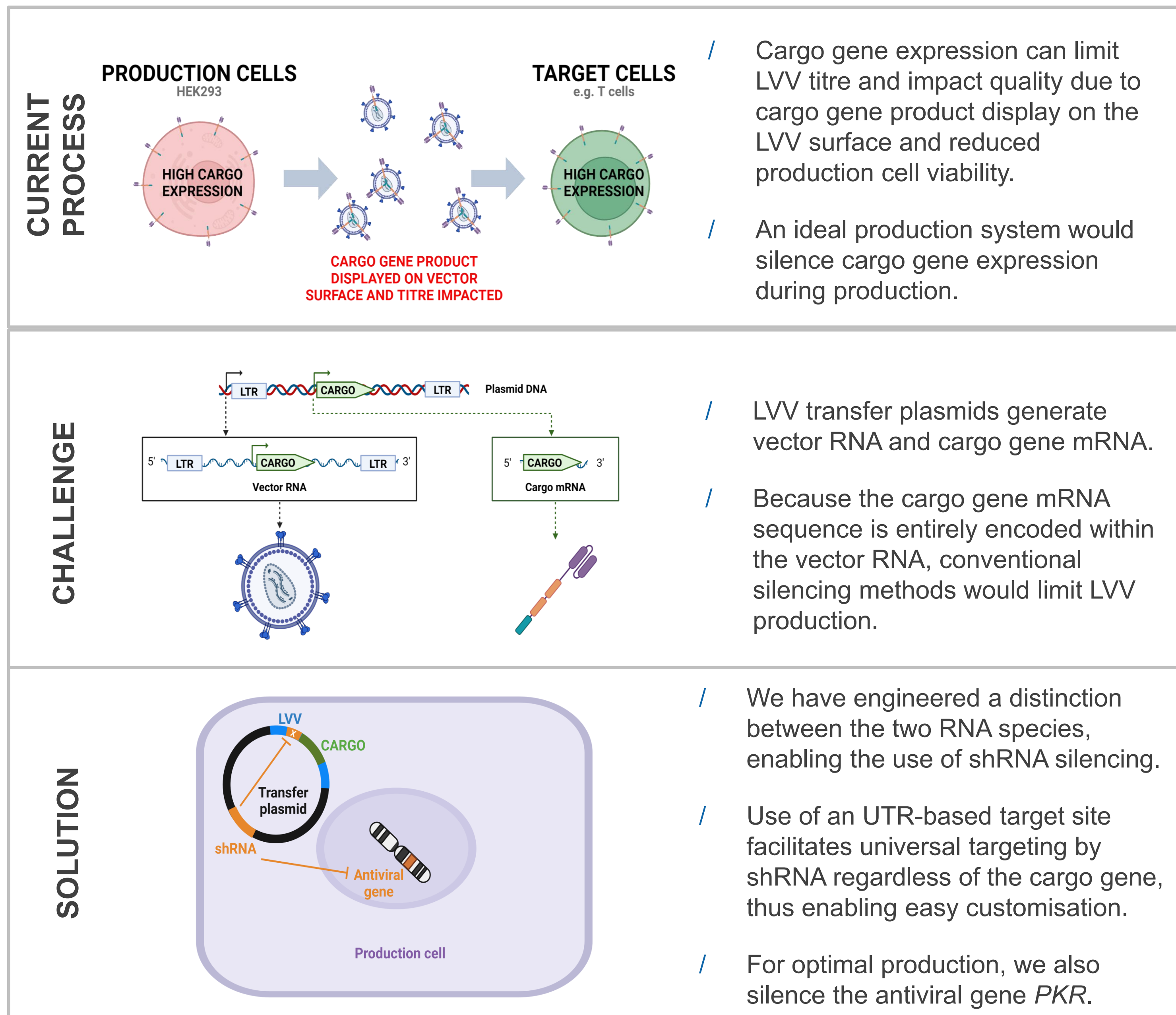


Figure 1. Challenge associated with silencing LVV cargo gene, and schematic of cargo gene-silencing system.

## Cargo gene knockdown with various promoters

- XOFLX™ packaging cells were transfected with either conventional or silenced plasmids with the cargo gene (EGFP) expression driven by various promoters.
- Substantial cargo gene silencing was observed throughout the production window (Figure 2).
- Thus, the system functions with various cargo gene promoters, allowing flexibility in its application.

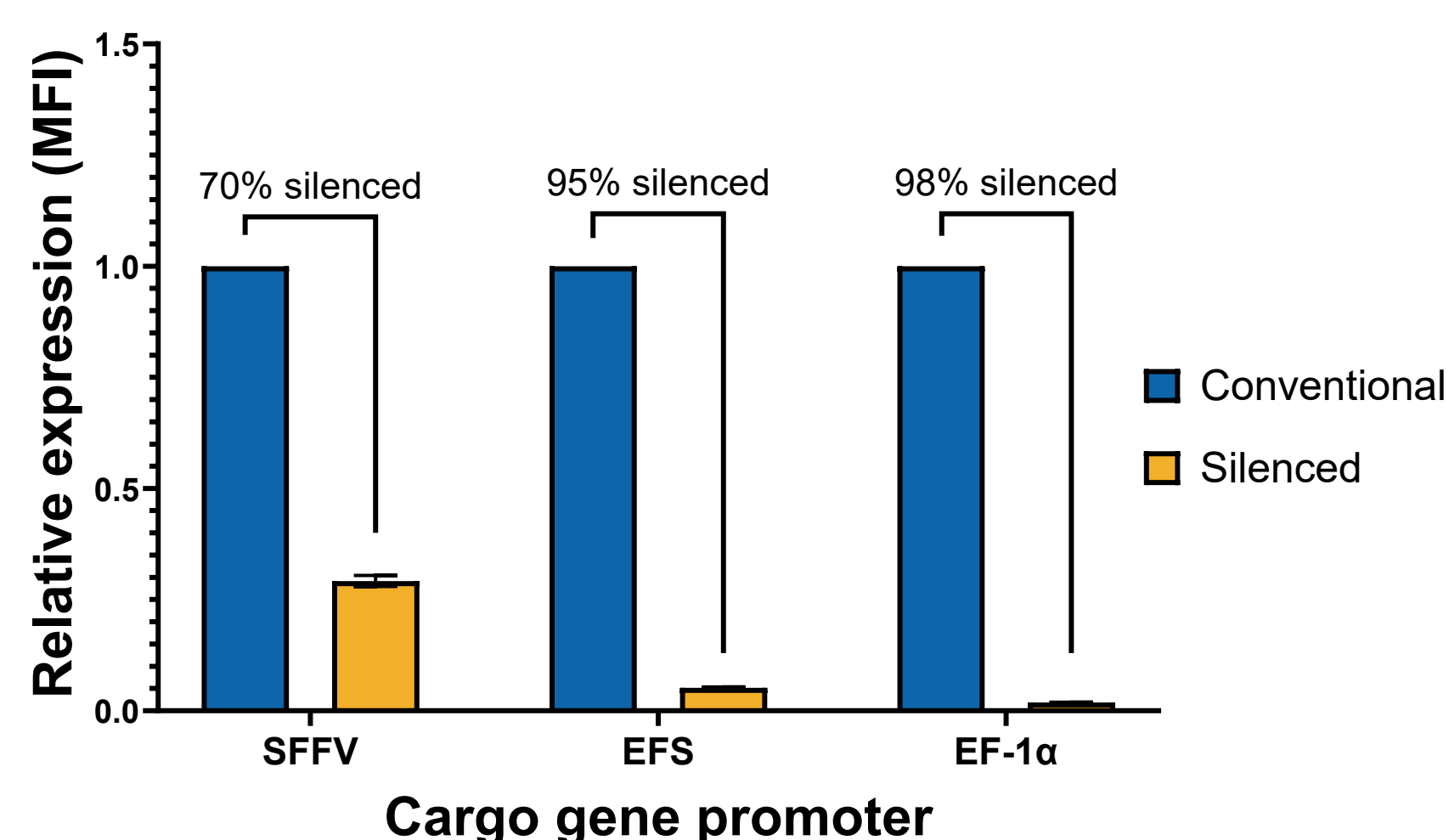


Figure 2. Cargo gene expression from various transfer plasmids, either conventional or with the cargo gene silencing technology. MFI by flow cytometry. NTC = non-transfected control. Error bars indicate standard deviation (n = 3).

## Silencing cargo gene increases titre up to four-fold

- XOFLX™ packaging cells were transfected with either conventional or silenced plasmids that encode either anti-CD19 CAR or the pro-apoptotic gene *BAX*.
- Production cell viability improved when the cargo gene was silenced (Figure 3).
- Titre increased up to four-fold due to use of the silencing system.
- Reduction of unwanted byproducts due to production cell death is highly desirable for effective downstream processing.

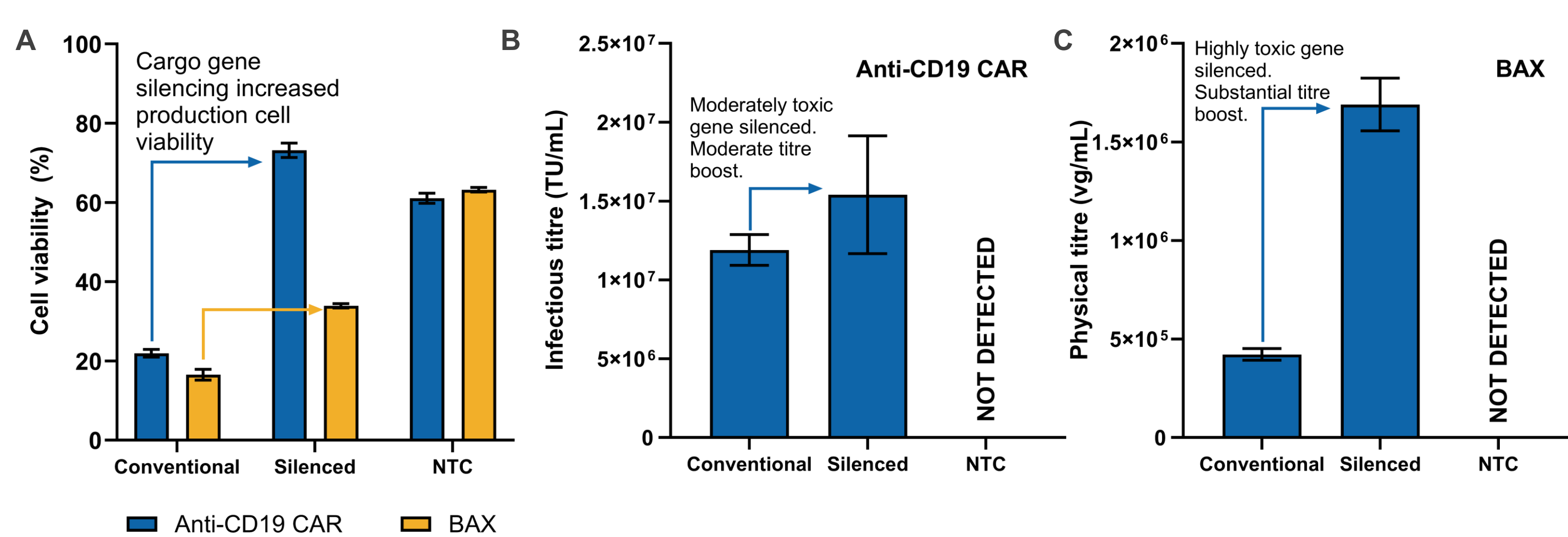


Figure 3. Anti-CD19 CAR- and *BAX*-encoding LVV production. (A) Viability of production cells at the point of LVV harvest. (B) Infectious titre of anti-CD19 CAR-encoding LVV supernatants. (C) Physical (RT-qPCR) titre of *BAX*-encoding LVV supernatants. NTC = non-transfected control. Error bars indicate standard deviation (n = 3).

## Cargo gene knockdown reduces downstream process burden

- XOFLX™ packaging cells were transfected with either conventional or silenced plasmids that encode either anti-CD19 CAR or anti-BCMA CAR.
- Residual host cell DNA (HCD) in lentiviral vector preparations was quantified by qPCR targeting Alu elements (Figure 4).
- Cargo gene knockdown during production decreased residual HCD in LVV preparations by up to 86%.
- Minimising unwanted byproducts due to production cell lysis is critical for improving the efficiency and robustness of downstream processing.

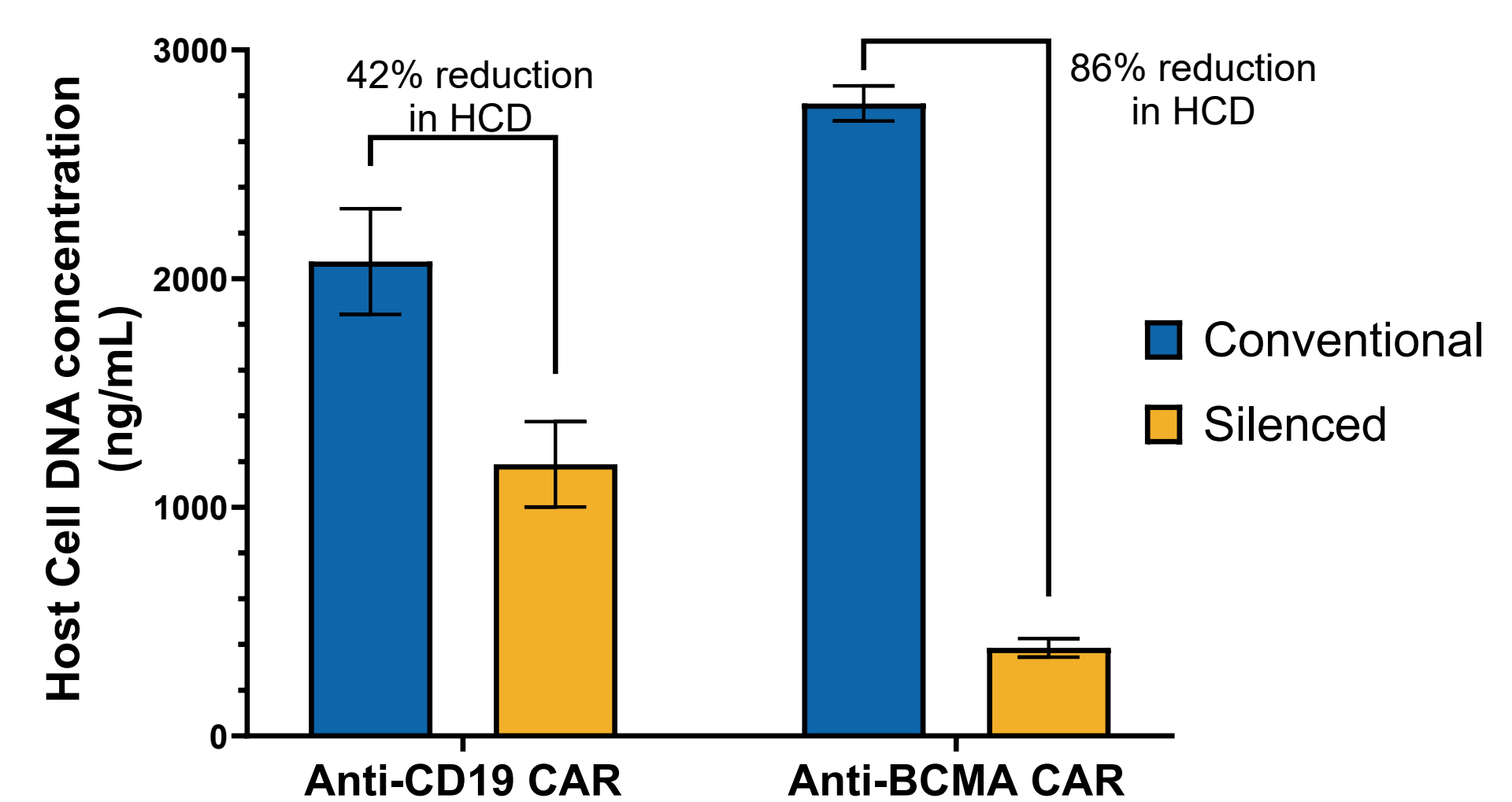


Figure 4. Residual HCD in LVV preparations using conventional and silenced transfer plasmids. qPCR targeting Alu elements. Error bars indicate standard deviation (n=3)

## Cargo gene knockdown facilitates producer cell line development

- LVV producer cells stably encode all LVV genetic components (Figure 5 A).
- Constitutive expression of certain cargo genes can limit producer cell growth.
- We stably integrated either conventional or silenced anti-CD19 CAR transfer plasmids into XOFLX™ Packaging cells (Figure 5 A).
- Silencing the cargo gene enabled generation of producer cells that otherwise were unrecoverable (Figure 5 B-D).

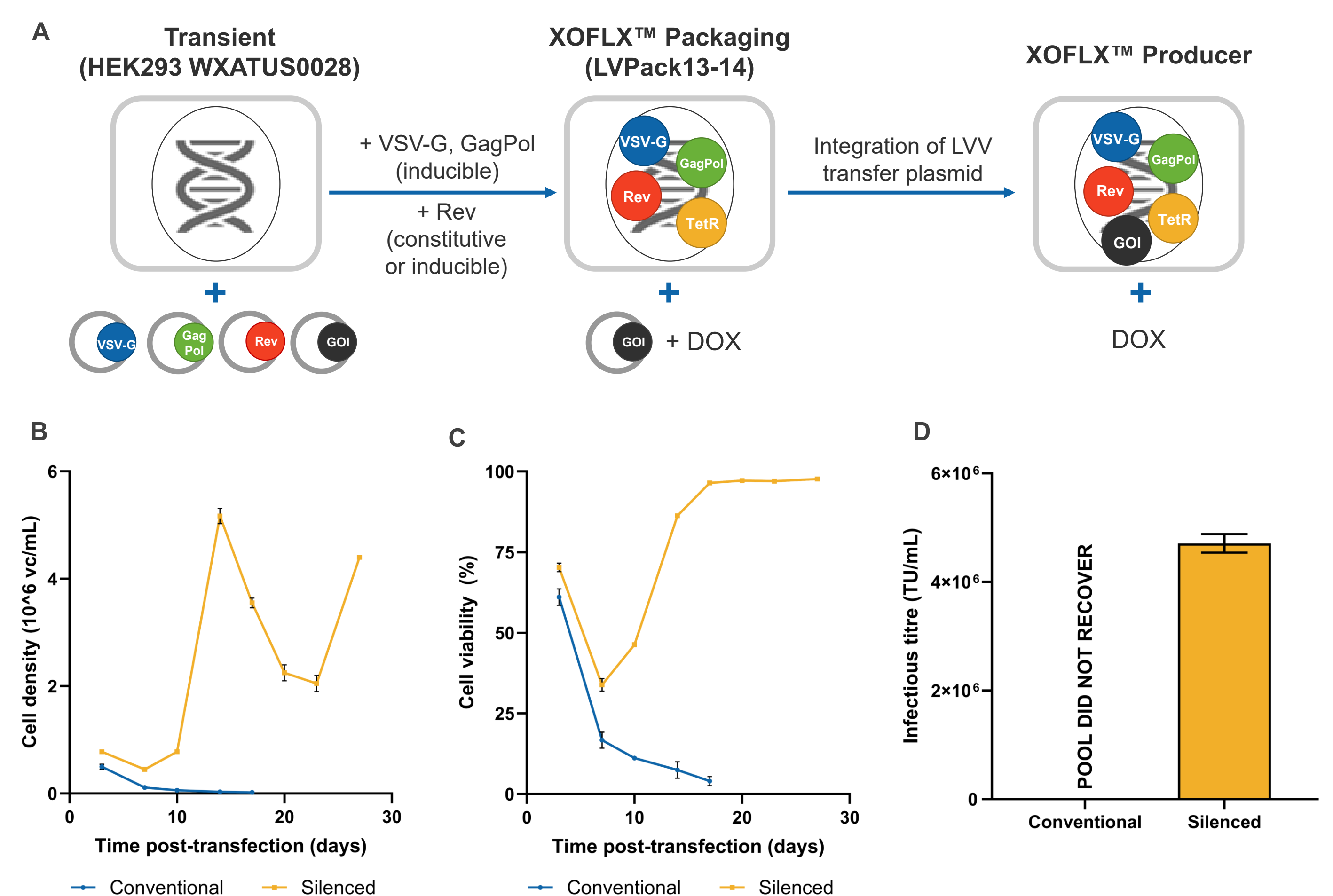


Figure 5. Generation of stable producer cell lines with silenced cargo gene. (A) Schematic of producer cell line development. (B) Growth and (C) viability of producer cells following stable integration of transfer plasmids. (D) Infectious titre of LVV produced by recovered producer cell line. (n = two integration replicates, three production replicates; error bars indicate standard deviation).

## Conclusions

- Our cargo gene silencing system reduced SFFV-, EFS-, and EF-1α-driven cargo gene expression during production up to 95%.
- LVV titre was boosted up to four-fold.
- This cost-effective LVV production approach can be used for a wide range of cargo genes, including those that are cytotoxic.
- Cargo gene knockdown during LVV production window reduced residual HCD by up to 86%.
- The silencing system enabled the recovery of otherwise unrecoverable producer cell lines, de-risking this simplified manufacturing modality.
- This silencing system mitigates the risks associated with development of novel LVV-based therapeutics, resulting in decreased costs and improved patient access to LVV-based therapies
- The cargo gene silencing system is available for evaluation. Request at [www.minaris.com](http://www.minaris.com).