Start With Stable: Stepping Onto TetraVecta™ Packaging Cells To Make A Clearer Pathway To LentiStable™ Producer Cell Lines

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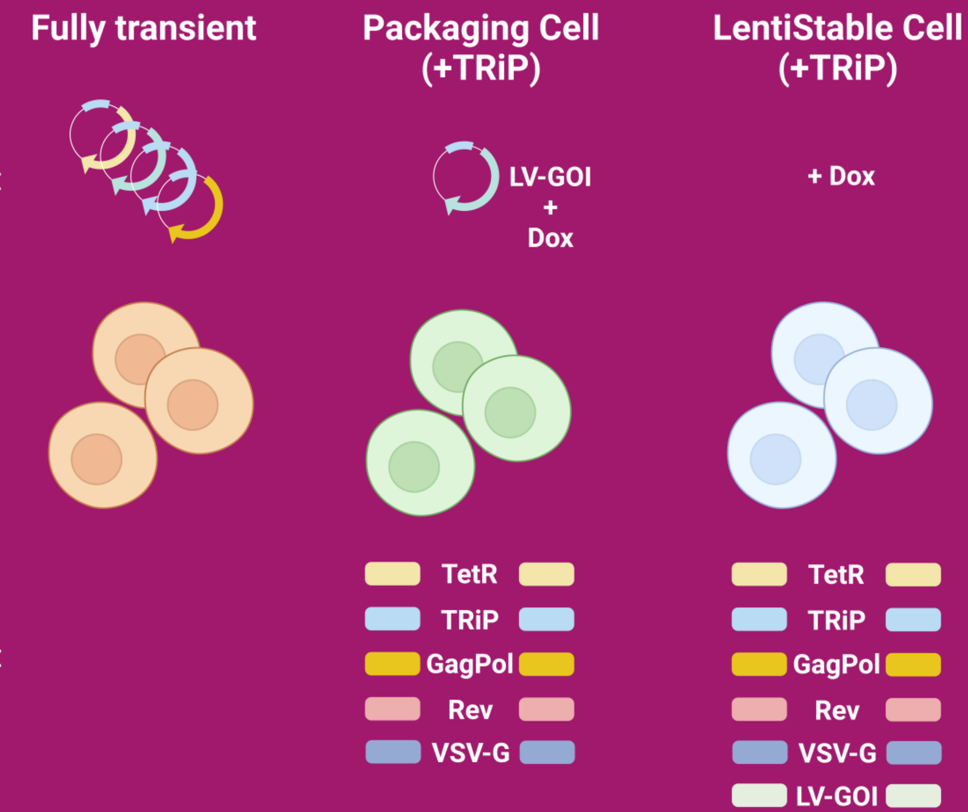
Why not start here?



Plasmids:

Stable components:

GMP pDNA sourcing/ costs

Access to large scale manufacturing

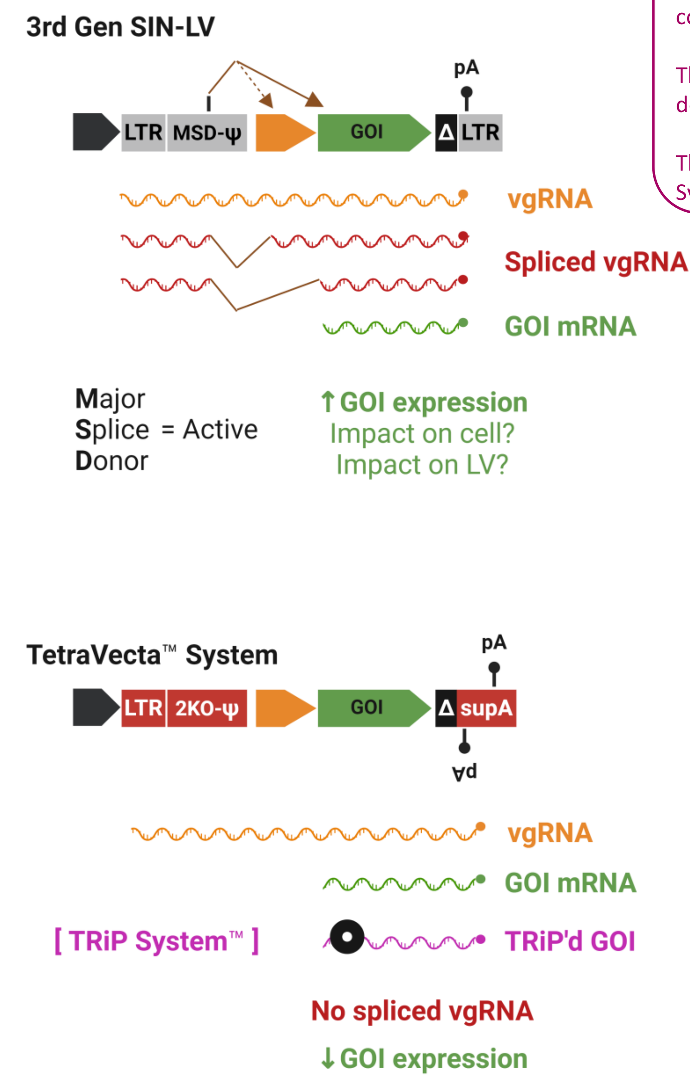
Bridging effort to transition to stable

LV batch-to-batch consistency

TetraVecta packaging cell use the TRiP System™ to repress the transgene to avoid GOI affecting output titres, and remove GOI protein from final LV product.

Some images created with BioRender.com

① 3rd Gen vs TetraVecta™ System

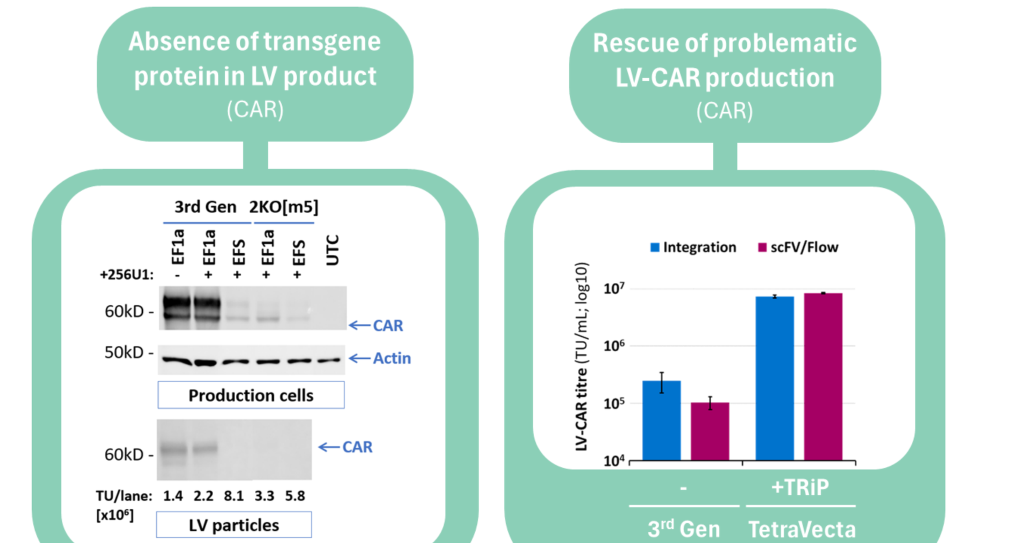


Aberrant splicing from the major splice donor (MSD) in 3rd Gen LVs leads to spliced vector genomic (vg)RNA that expresses the gene of interest (GOI) during production. This can happen for transgene cassettes containing constitutive or tissue-specific promoters.

These spliced vgRNAs are poorly repressed by the TRiP System™. Long-term GOI expression restricts the development of LV producer cell lines.

The MSD is inactivated in 2KO-LVs, and they do not produce spliced vgRNA. 2KO-LVs are part of the TetraVecta™ System technology, and are optimal LV genomes to host the TRiP System™

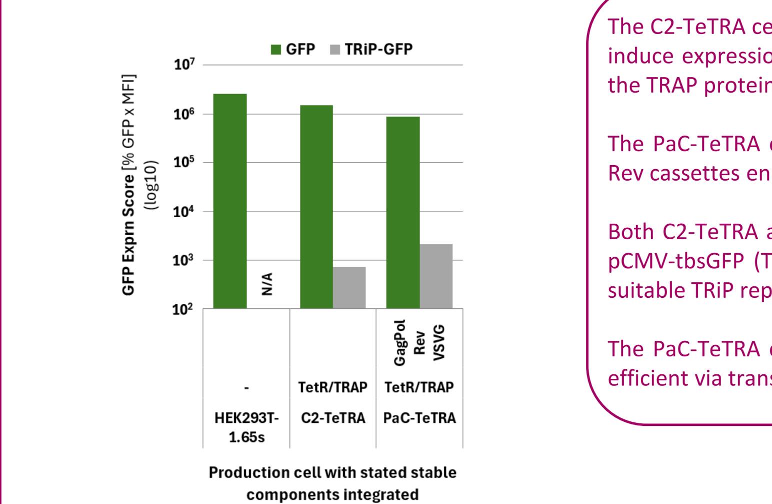
② Improved yields and quality using TetraVecta



TRiP & TetraVecta combine to rescue the output titres of LVs encoding problematic transgenes, for example certain Chimeric Antigen Receptor (CAR) proteins.

Transgene repression using TRiP substantially reduces the amount of transgene protein within LV product. This may be advantageous in reducing optimisation downstream processing and/or minimising immune response to the GOI.

③ TetraVecta Packaging Cells



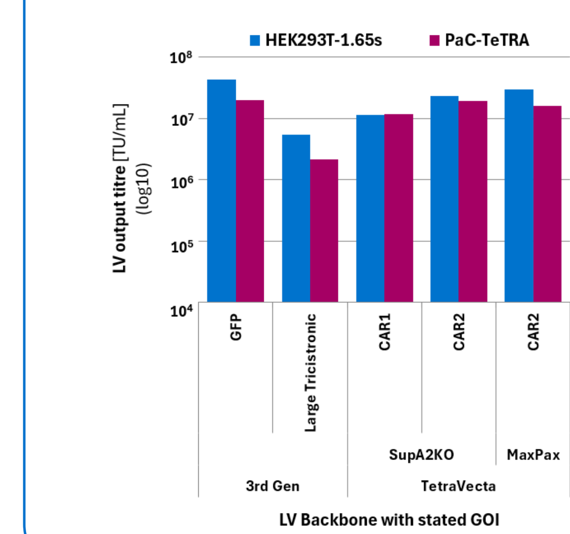
The C2-TeTRA cell line was generated first; this is stably expresses the tetR repressor protein to induce expression of CMV-tetO promoter-driven cassettes. This cell line also stably expresses the TRAP protein, which is the translation repressor protein of the TRiP System™.

The PaC-TeTRA cell line has stably integrated CMV-tetO promoter-driven GagPol, VSV-G and Rev cassettes enabling induction of their expression upon addition of Dox.

Both C2-TeTRA and PaC-TeTRA cell lines were assessed for TRiP repression by transfection of pCMV-tbsGFP (TRiP) or pCMV-GFP reporter plasmids. The PaC-TeTRA cell line demonstrated suitable TRiP repression capability.

The PaC-TeTRA cell line was also shown to be suitably stable, and inducible LV product was efficient via transfection of the pGenome and p256U1 enhancer plasmids with Dox addition.

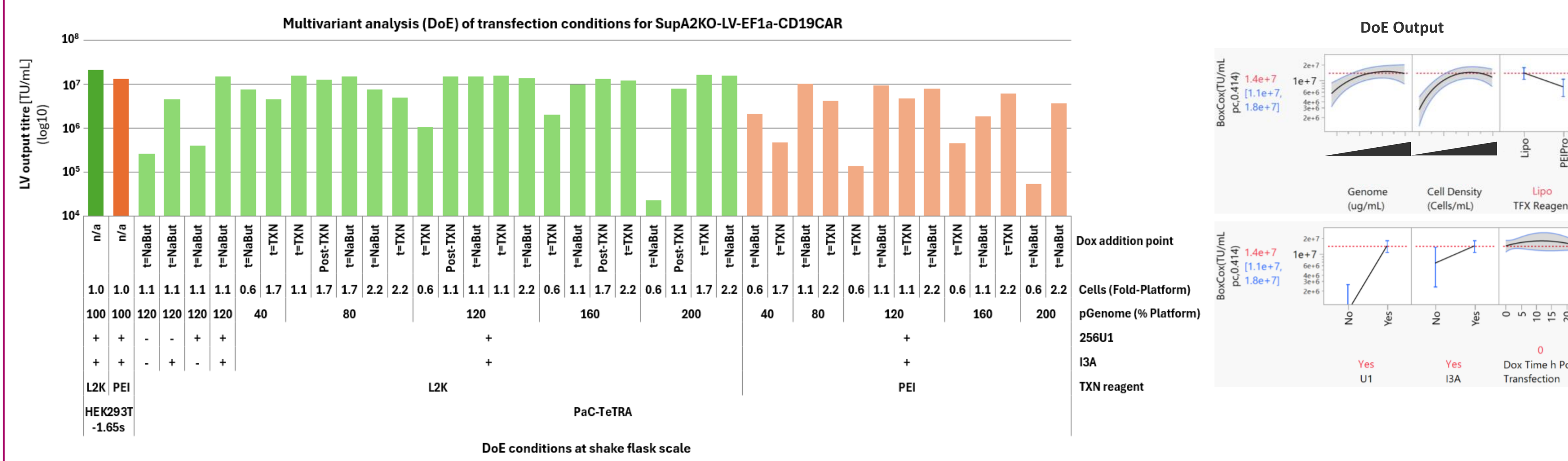
④ Validating PaC-TeTRAs with different LV-GOIs



The PaC-TeTRA cell line was compared to the HEK293T-1.65s base cell line for its ability to produce 3rd Gen LVs or TetraVecta™ System LVs encoding different GOIs.

PaC-TeTRA cells performed equivalently to the base cell line, demonstrating its potential utility in producing a broad spectrum of LV-GOIs without substantial optimisation.

⑤ Upstream parameter optimisation of LV production from PaC-TeTRA



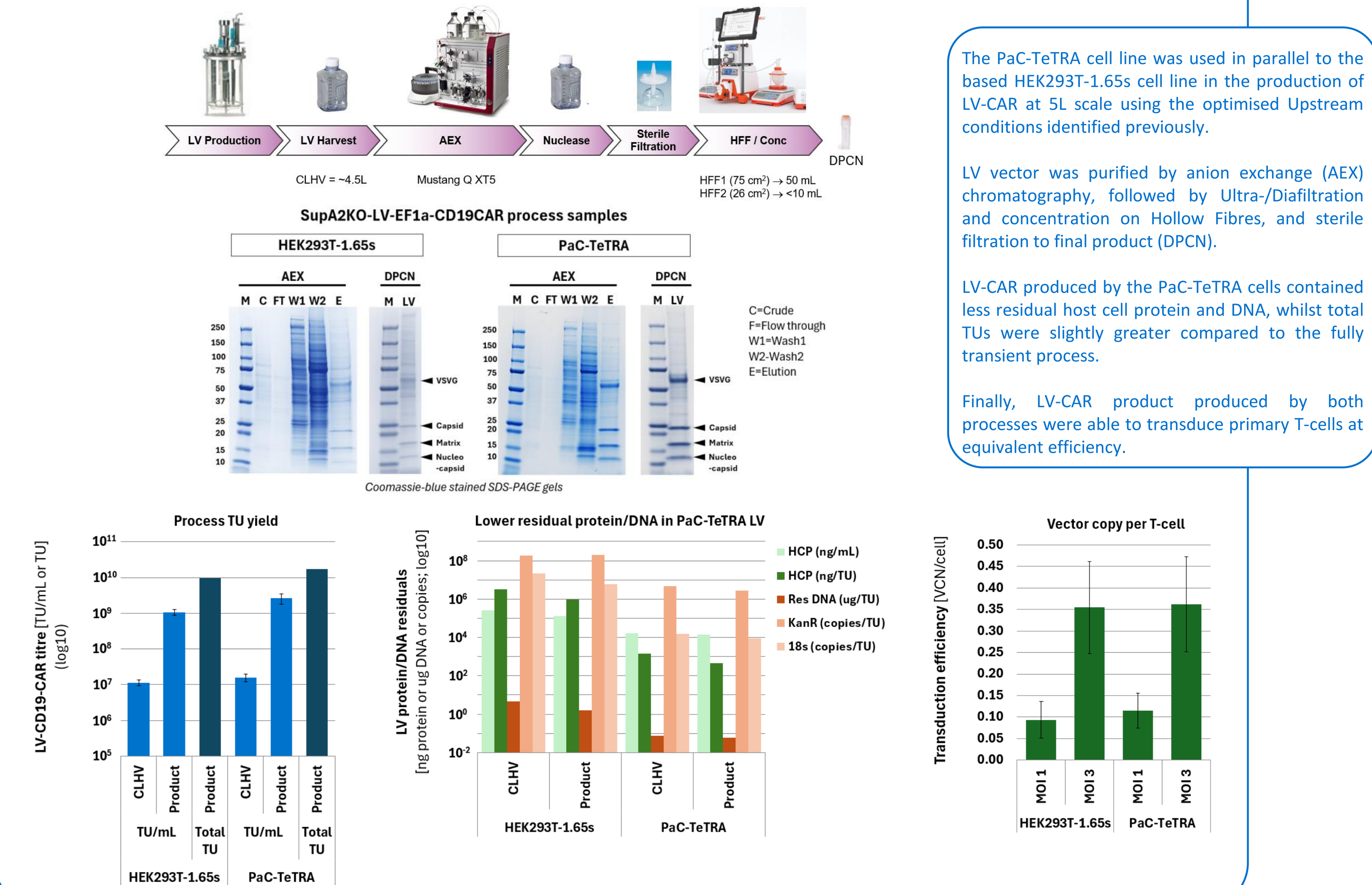
Upstream process parameters for LV production from PaC-TeTRA cells was initiated in 125mL shake flask scale using a TetraVecta genome encoding a CAR GOI: pSupA2KO-LV-EF1a-CD19CAR.

Multivariant analysis (Design of Experiment [DoE]) allowed the assessment of cell density, transfection reagent, use of proprietary LV enhancers 256U1 and I3A, mass of pGenome and timing of Dox induction.

The output of DoE provided insights for optimal parameter ranges for further study in AMBR-15 bioreactors. Further characterisation of viable cell densities, [pGenome] and pH control (not shown) in the bioreactors allowed refinement of controlled Upstream conditions.

A more focussed AMBR-15 bioreactor study identified Upstream conditions that enabled slightly higher output LV titres compared to the HEK293T-1.65s fully transient transfection process. These conditions formed the basis for scale-up into the 5L bioreactor and our model Downstream process.

⑥ PaC-TeTRA process scale-up for LV-CD19-CAR production and T-cell transduction



The PaC-TeTRA cell line was used in parallel to the based HEK293T-1.65s cell line in the production of LV-CAR at 5L scale using the optimised Upstream conditions identified previously.

LV vector was purified by anion exchange (AEX) chromatography, followed by Ultra-/Diafiltration and concentration on Hollow Fibres, and sterile filtration to final product (DPCN).

LV-CAR produced by the PaC-TeTRA cells contained less residual host cell protein and DNA, whilst total TUs were slightly greater compared to the fully transient process.

Finally, LV-CAR product produced by both processes were able to transduce primary T-cells at equivalent efficiency.

Summary

- The advantages of stable LV producer cell lines (such as LentiStable™) are the economies of scale-up to >1000L for larger indications, and improved batch-to-batch consistency.
- However, commercial pressures typically dictate the pursuit of a fully transient transfection approach for early clinical supply.
- After product approval, the switch of LV manufacturing from transient transfection to stable cell lines becomes extremely challenging to due significant regulatory hurdles.
- We advocate the initial onboarding of LV process development using inducible packaging cell lines with transfection of pLV-GOI.
- We show that equivalent LV output titres are achievable with packaging cells compared to base HEK293T cells, with minimal process optimisation.
- Our PaC-TeTRA packaging cell line additionally expresses the TRAP protein as part of the TRiP System™, which suppresses GOI during production.
- The TRiP System™ negates GOI effects, such as long-term toxicity of CARs in HEK293T-based LentiStable™ cells.
- Parallel development of LentiStable™ cell lines from PaC-TeTRA will narrow the gap between LV product profiles, enabling a late switch to stable LV production prior to commercialisation.

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