TetraVecta™ Packaging Cell Lines for LV-CAR Production

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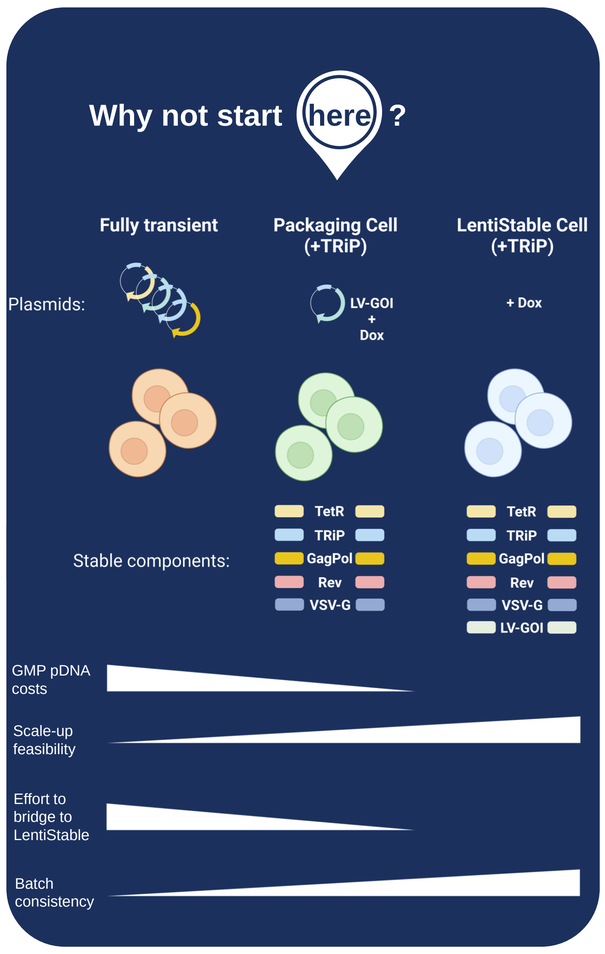
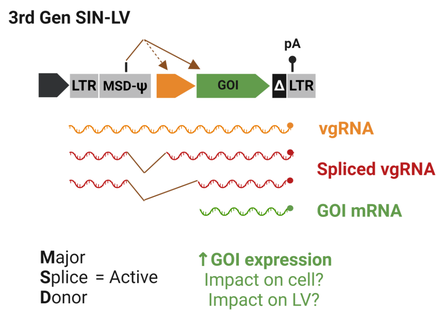
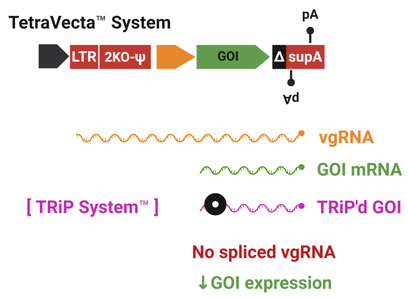


TetraVecta™ packaging cells have stably integrated inducible packaging components and use the TRiP System™ to repress the transgene. This prevents the gene of interest (GOI) negatively impacting lentiviral vector (LV) titres and removes GOI protein from the final LV product. Transfect only the pLV-GOI and induce with doxycycline (Dox) to stimulate LV production.

1 3rd Gen vs TetraVecta™ System



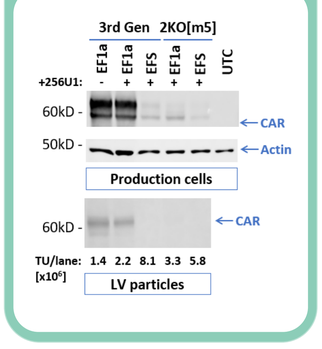


Long-term expression of the GOI restricts the development of LV producer cell lines which can be overcome by repression of GOI protein translation using the TRiP System™.

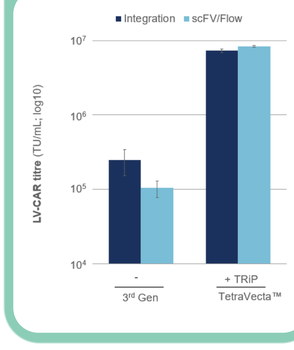
Aberrant splicing from the major splice donor (MSD) in 3rd Gen LVs generates spliced vector genomic (vg)RNA which is poorly repressed by the TRiP System™. LV genomes using TetraVecta™ System technology include MSD inactivation, preventing production of spliced vgRNA, and are optimal for the TRiP System™. Addition of SupA-LTRs imparts transcriptional insulation to integrated LVs and can enhance GOI expression in the target cell.

2 Improved yields and quality using TetraVecta™ System

Absence of transgene protein in LV product (CAR)



Rescue of problematic LV-CAR production (CAR)



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 the LV product. This may be advantageous in reducing downstream processing optimisation and/or minimising immune response to the GOI.

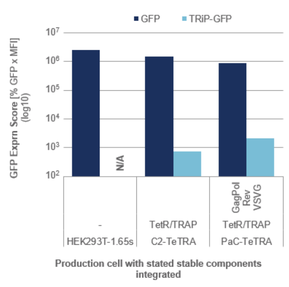
3 TetraVecta™ packaging cells

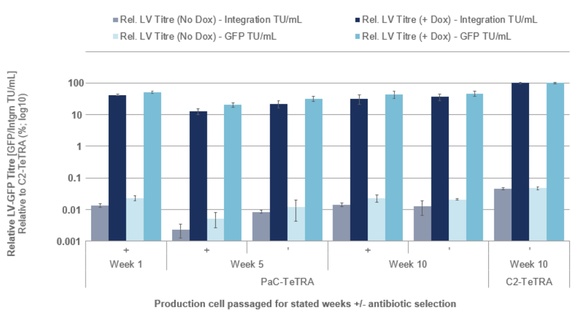
The C2-TeTRA cell line was generated first; this stably expresses the tet repressor (TetR) protein, to induce expression of CMV-tetO promoter-driven cassettes, and the TRAP protein, which is the translation repressor protein of the TRiP System™.

The PaC-TeTRA cell line was derived from C2-TeTRA and has stably integrated CMV-tetO promoter-driven GagPol, VSV-G and Rev cassettes enabling induction of their expression upon addition of Dox.

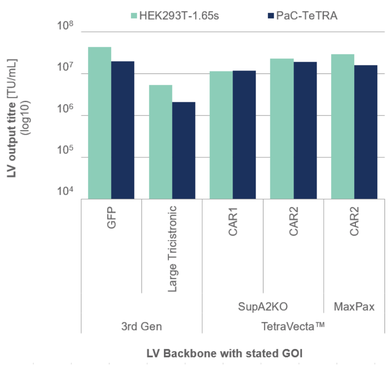
TRiP repression of translation was demonstrated in both cell lines by transfection of pCMV-tbsGFP (TRiP) or pCMV-GFP reporter plasmids.

The PaC-TeTRA cell line demonstrates stable and inducible LV productivity over 10 weeks following transfection of the pGenome and p256U1 enhancer plasmids with Dox addition.





4 Validating PaC-TeTRAs with different LV-GOIs



PaC-TeTRA cell line showed comparable LV productivity to the HEK293T-1.65s basal cell line for 3rd Gen LVs and TetraVecta™ System LVs encoding different GOIs. This highlights the potential utility of the PaC-TeTRA cell line in producing a broad spectrum of LV-GOIs without substantial optimisation.

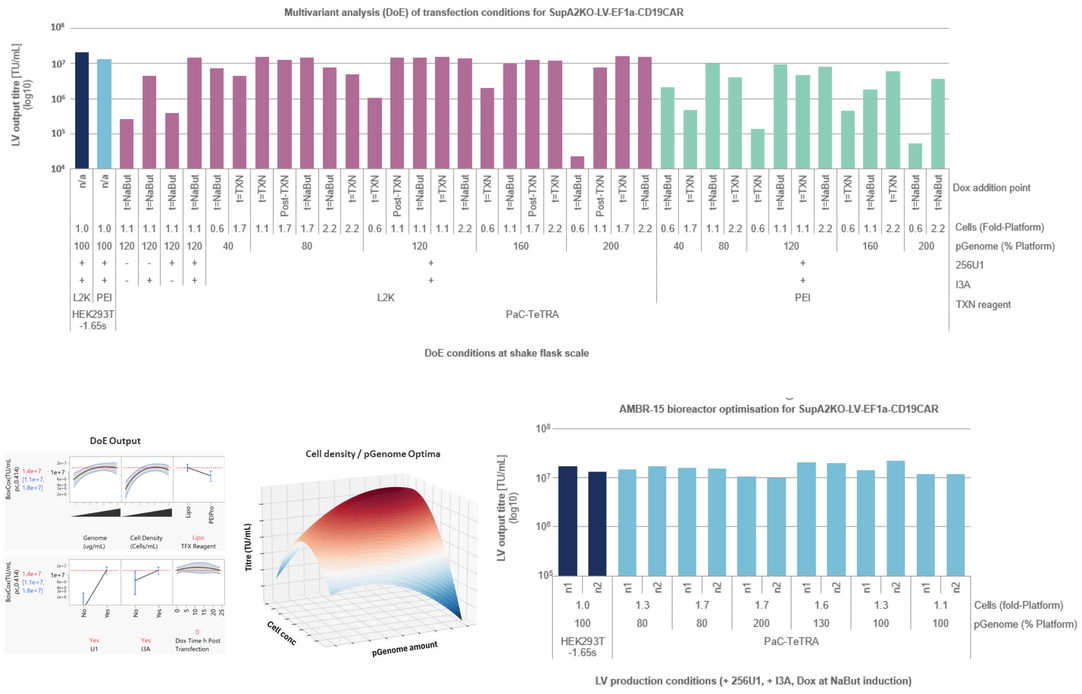
5 Upstream parameter optimisation of LV production from PaC-TeTRA

Optimisation of upstream process parameters for LV production from PaC-TeTRA cells was initiated at 125mL shake flask scale using a TetraVecta™ genome encoding a CAR GOI: pSupA2KO-LV-EF1a-CD19-CAR.

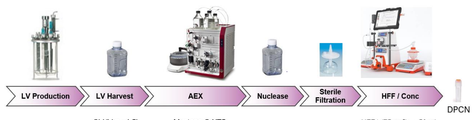
Multivariant analysis (Design of Experiment [DoE]) enabled the assessment of cell density, transfection reagent, use of proprietary LV enhancers 256U1 and I3A, mass of pGenome and timing of Dox induction.

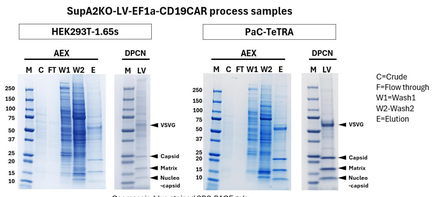
The DoE output provided insight into optimal parameter ranges for further study in AMBR-15 bioreactors allowing refinement of controlled upstream conditions.

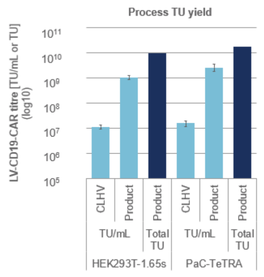
A more focussed AMBR-15 bioreactor study identified upstream conditions that enabled slightly higher output LV titres from PaC-TeTRA cells than the HEK293T-1.65s fully transient transfection process. These conditions formed the basis for scale-up into the 5L bioreactor and our model downstream process.

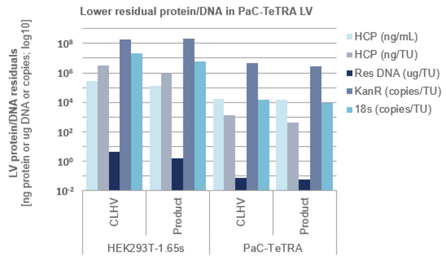


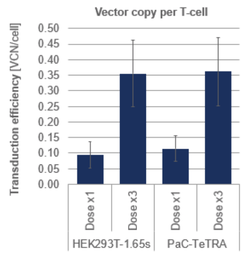
6 PaC-TeTRA process scale-up for LV-CD19-CAR production and T-cell transduction











LV-CAR was produced at 5L scale with the PaC-TeTRA cell line and the basal HEK293T-1.65s cell line in parallel, using the optimised upstream conditions identified. LV 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 transducing units (TUs) were slightly higher compared to the fully transient process.

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

Summary

- LV producer cell lines (such as LentiStable™) offer many advantages for LV supply, however commercial pressures typically dictate the pursuit of a fully transient transfection approach for early clinical use.
- Switching LV manufacture to stable cell lines after product approval becomes extremely challenging to due significant regulatory hurdles.
- We advocate the initial onboarding of LV process development using inducible packaging cell lines with transient transfection of pLV-GOI. Here, we demonstrate that this process can generate equivalent LV titres to basal HEK293T cells, with minimal optimisation.
- Our PaC-TeTRA packaging cell line utilises the TRiP System™, which suppresses GOI protein expression, to negate GOI effects such as the 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 later switch to stable LV production prior to commercialisation.

Some images were created with BioRender.com

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