The TetraVecta™ System: A new tool kit enhancing lentiviral vector production and performance for the next generation of gene therapies

Improving the quality, safety, capacity and production of lentiviral vectors (LVs) through vector engineering

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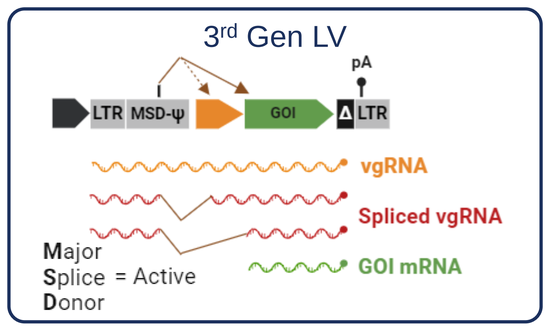
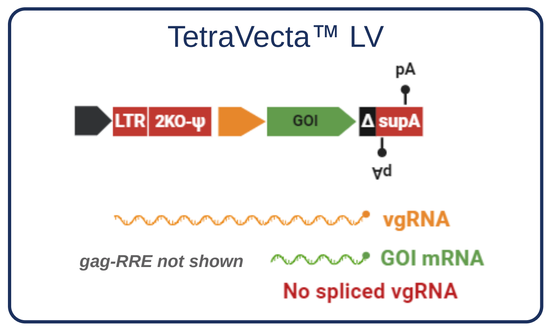
2KO genome™

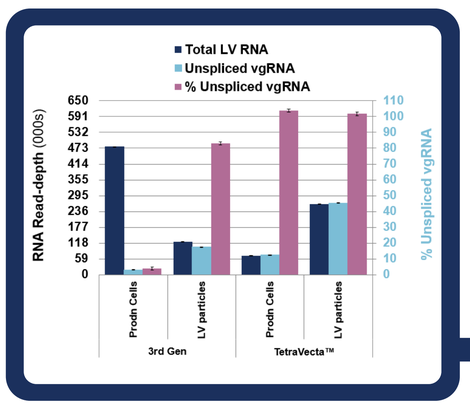
Stops aberrant splicing in LV backbone during production, eliminating vector RNA subspecies from LV product (safety/quality).



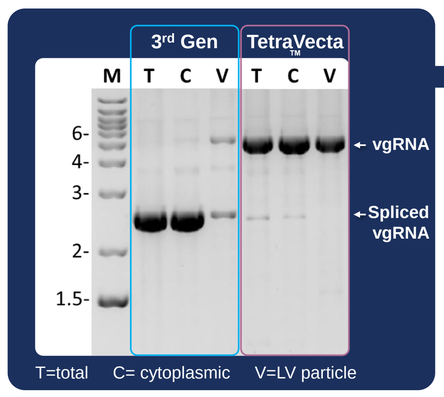
Aberrant splicing from major splice donor site in 3rd Gen LVs during production can lead to spliced vgRNA.



2KO-LVs have a mutated MSD and cannot mis-spice



2KO-LVs produced exclusively full length vgRNA



Detection of vector-specific RNA in production

As much as 95% of 3rd Gen LV RNA generated in production is spliced.

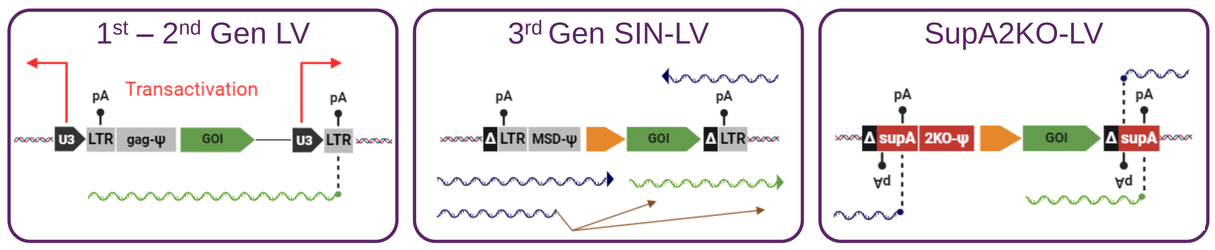
Spliced vgRNAs produced by 3rd Gen LVs can be detected in LV particles, and are converted to cDNA episomes (data not shown)

2KO-LVs only produce full length vgRNA and generate simplified LV particles.

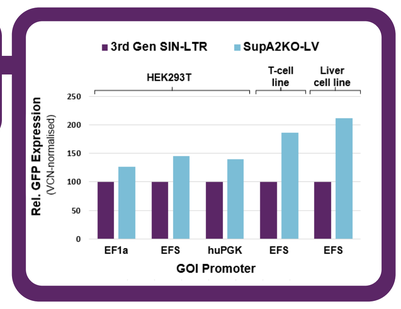
SupA-LTR™

Improved polyadenylation [pA] sequences provide minimised interaction with target cell transcriptome and enhance transgene expression (quality).

Evolution of LTR engineering over four generations

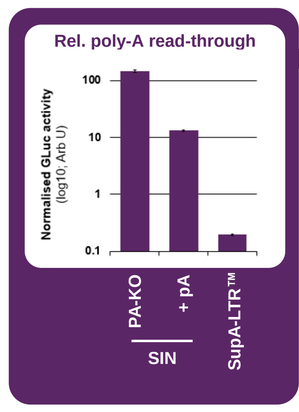


Improved transgene expression in cells

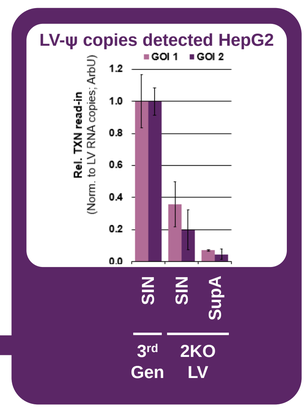


The native U3 promoter is deleted in 3rd Gen LVs to generate 'self-inactivating' (SIN)-LTRs to improve safety. Overlapping poly-A sequence enhancers are also deleted, resulting in transcriptional read-in/out from integrated LVs

Improved poly-A termination



Reduced transcription read-in



SupA-LTRs have been engineered to have strong poly-A sequences on top and bottom strands. They are subject to less transcription read-in/out, and have increase GOI expression.

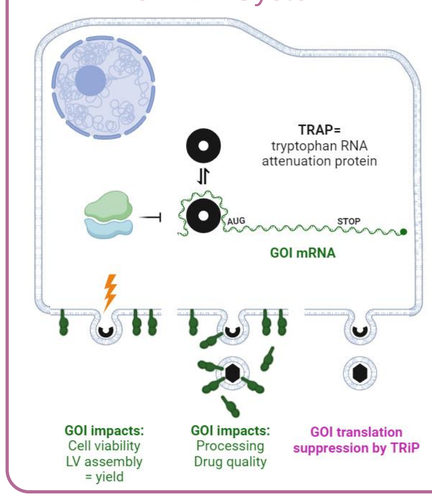
Integration site distribution of LVs bearing supA-LTRs and SIN-LTRs are the same (data not shown).

2KO genome™ | SupA-LTR™ | TRiP System™ | MaxPax™ | TetraVecta™

TRiP System™

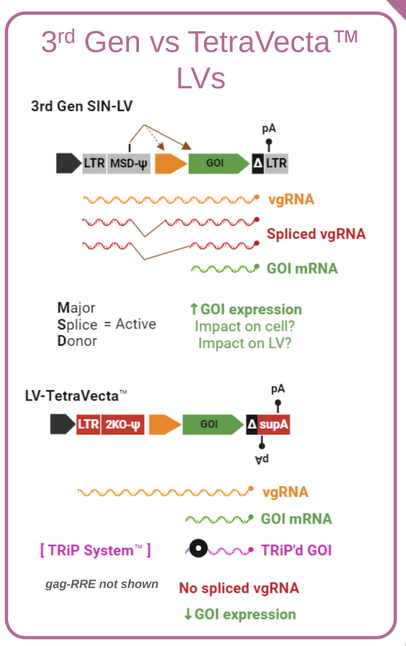
Suppression of transgene expression during LV production minimises impact of transgene protein on LV production titre and removes it from final product (quality/yield).

The TRiP System™

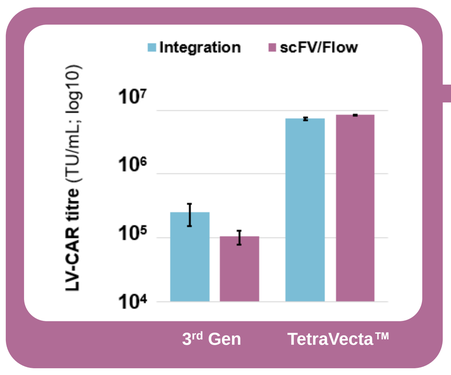


The bacterial protein TRAP binds to the transgene mRNA to stop translation during LV production. Side effects of transgene protein on titre and LV product are avoided.

3rd Gen vs TetraVecta™ LVs



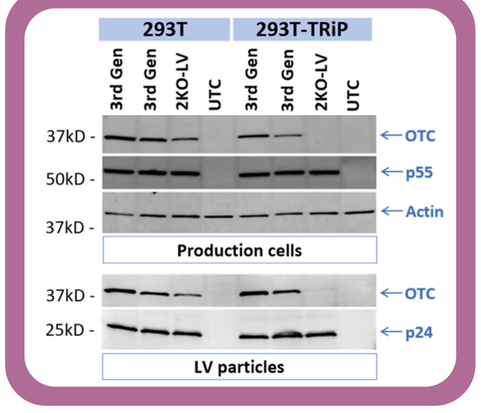
Rescue of problematic LV-CAR production



Aberrant splicing in 3rd Gen LVs leads to spliced vRNA that expresses the GOI.

2KO-LVs are optimal to host the TRiP System™

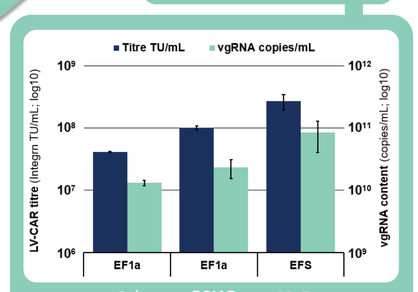
Absence of transgene protein in LV product (Liver GOI)

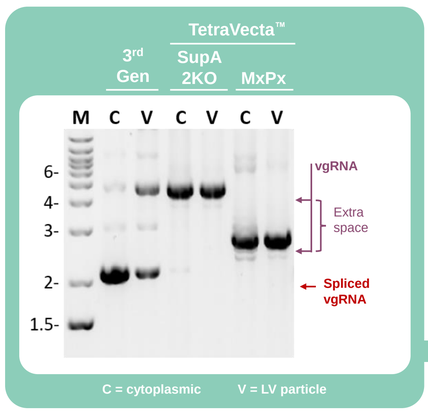


MaxPax™

2KO-LV genome with minimised backbone sequence liberates 1kb extra space for transgene sequences (capacity). Rev-independent, 3 plasmid system simplifies production.

MaxPax™ is the choice for intron-less transgene cassettes





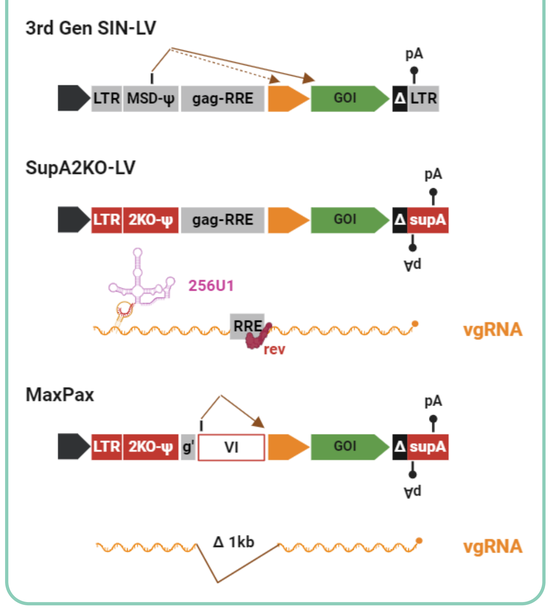
MaxPax™ has a larger space for transgene

MaxPax™ is the ideal vector backbone where introns are not required.

Ideal for large transgene payloads where space is premium.

Only pMaxPax, codon-optimised pGagPol and pEnv required; process development is simplified.

Increase capacity with MaxPax™



SupA2KO and MaxPax™ LVs are the two genome options within TetraVecta™.

SupA2KO-LVs are rev-dependent and require a modified U1 snRNA enhancer (256U1) to maximise titres.

MaxPax™ uses a 'Vector-Intron' (VI) instead of rev and 256U1, which doesn't contribute to vgRNA size; it has 1kb additional transgene space.

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Let's do something life-changing together

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