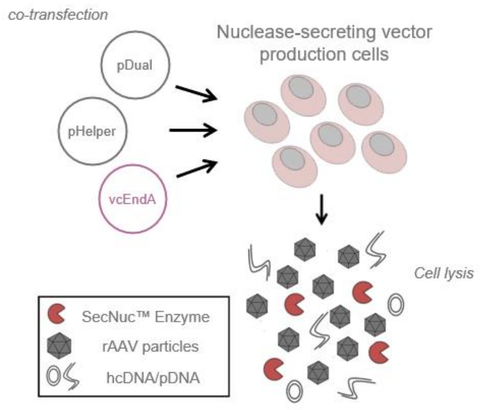
What's Upstream of Downstream Matters: Utilising High-Efficiency Secreted Nuclease (SecNuc™ Enzyme) and Transgene Repression (TRiP System™) to Improve rAAV Production

Alissa Bray[1], Antoine Cadiou[2], Emeline Pradel[2], Maria Lorena Martinez Quiles[1], Quentin Bazot[2], Dan Farley[1]

[1]Oxford Biomedica (UK) Ltd; [2]Oxford Biomedica (France)

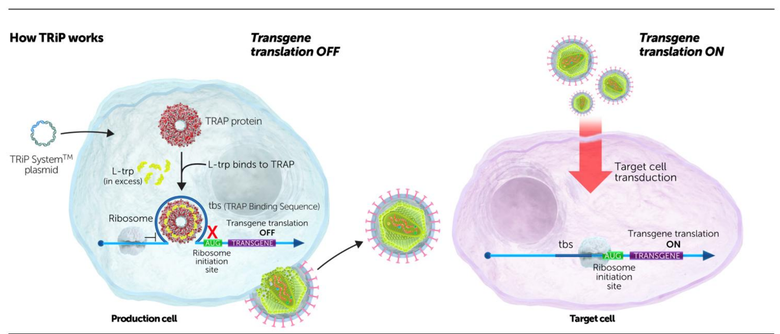
Introduction

rAAV manufacturing can be difficult for vectors with challenging transgenes – whether they be cytotoxic or otherwise detrimental during production. OXB has implemented the Transgene Repression in Vector Production (TRiP) system™ to prevent the expression of the transgene in production cells without impacting expression in the target cell, allowing maximum production yield universally. SecNuc™ incorporates a secreted nuclease into the production cell, allowing high active unit concentration during the crude harvest stage at physiological salt and a wide pH range. This provides an easily scalable, and cost-advantageous alternative to recombinant nucleases.



SecNuc™ enzyme during rAAV production

SecNuc™ enzyme vcEndA (*vibrio cholerae*) is co-transfected into the AAV production cells which become nuclease secreting cells. During the cell lysis step, SecNuc™ enzyme is released from the cells and degrades residual host-cell and plasmid DNA.



Adapted from Maunder et al. 2017

Transgene Repression In vector Production (TRiP System™)

TRiP system™ is a GOI translational repression system active during production. A 55 nucleotide TRAP-binding site (tbs) is placed in the 5'UTR of the transgene. The bacterial protein TRAP is expressed during vector production and binds to this tbs and blocks transgene translation. This prevents the transgene from eliciting deleterious effects on the production cell including cytotoxic, cytostatic and production interference – as well as preventing the transgene protein becoming itself a protein contaminant. When the vector is subsequently transduced into the target cell without TRAP present, transgene expression is permitted.

Methods

All rAAVs in this work were AAV9 capsid with either a standard GFP-Luciferase transgene or a TRiP transgene. Production in all cases comprised of the OXB Platform Process utilizing our dual plasmid system, plus additional SecNuc™ or TRAP plasmids, as necessary.

SecNuc™ production

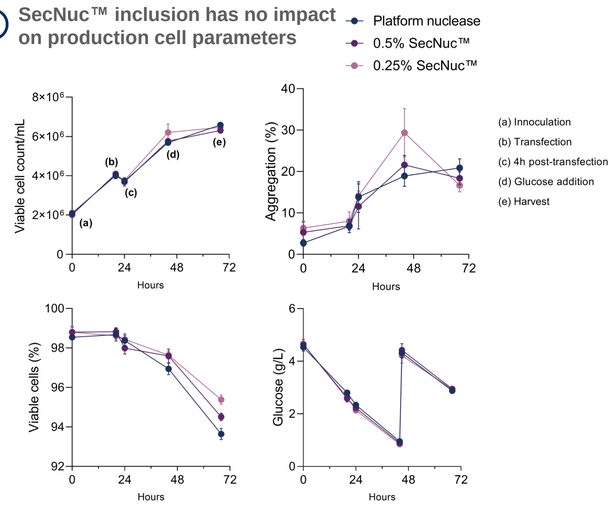
- SecNuc™ was tested in a head-to-head Platform Process production against our current platform recombinant nuclease in an AMBR250 bioreactor system.
- SecNuc™ plasmid was added as 0.25% or 0.5% of total pDNA mass at the transfection stage, and no additional nuclease was added at the lysis stage of harvest.
- Crude lysates were purified using POROS™ CaptureSelect™ AAV9 Affinity Resin.

Small-scale TRiP system™ rAAV production

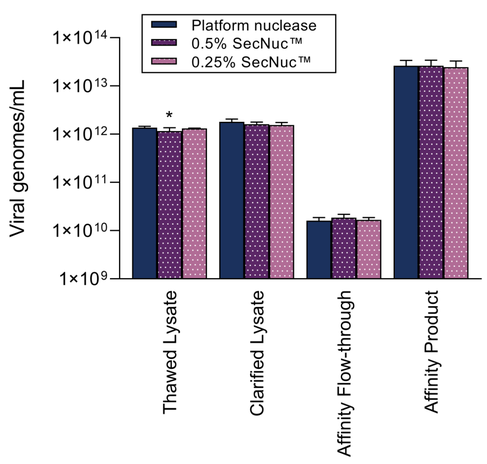
- TRiP dual plasmid genomes were tested at 10mL production scale, with the inclusion of TRAP plasmid or Bluescript as a negative control. Dual plasmid and Helper plasmids used are proprietary to OXB.
- Small-scale production was also carried out with or without the SecNuc™ enzyme.
- Small-scale production samples were analysed as crude samples only.

Figure 1: Comparing SecNuc™ to the current platform nuclease using the AMBR250

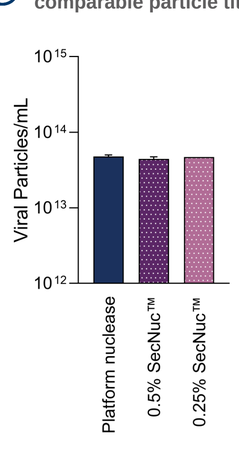
A SecNuc™ inclusion has no impact on production cell parameters



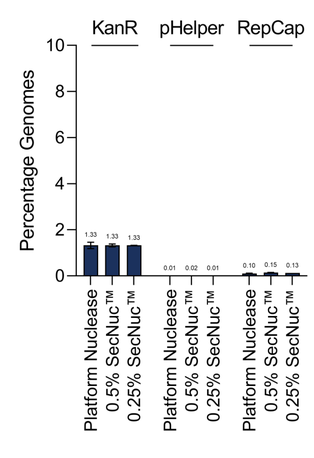
B SecNuc™ gives comparable genome titres



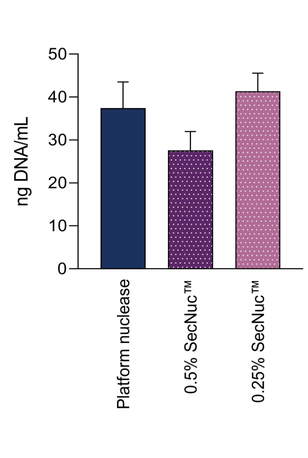
C SecNuc™ gives comparable particle titres



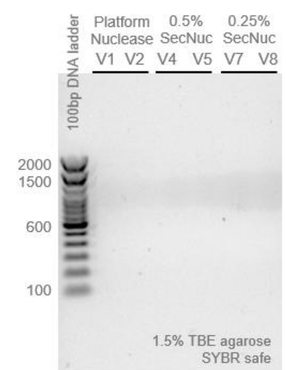
D Plasmid DNA residuals are not elevated by SecNuc™



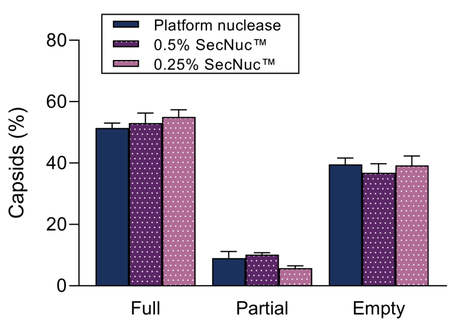
E Residual DNA is not significantly different across samples



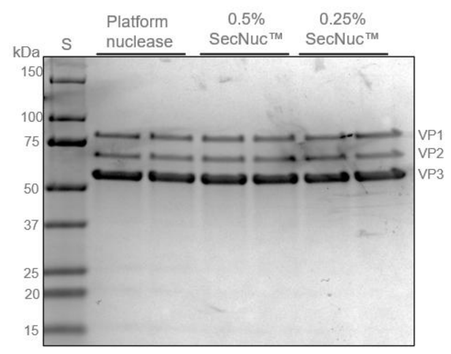
F No visible DNA in Affinity product



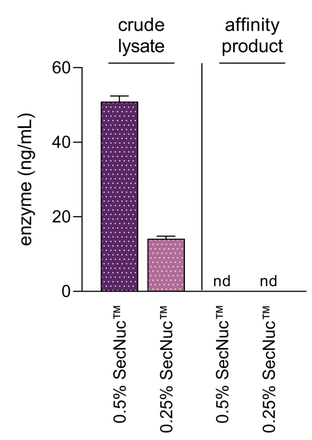
G SecNuc™ samples have comparable packaging efficiency



H VP protein ratio and affinity product purity comparable between samples



I ELISA shows SecNuc™ effectively removed through DSP



SecNuc™ Enzyme performance matches platform recombinant nuclease

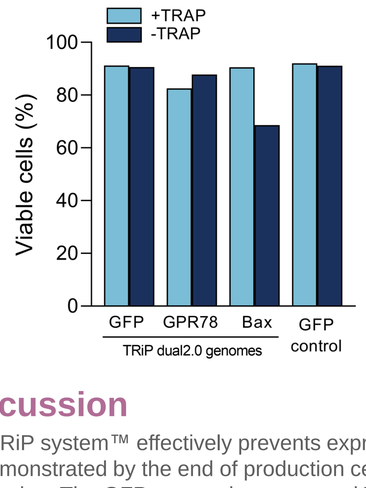
A – Cell data from Flex 2 (glucose) and NC-202 cell counter taken during upstream production.
B - Viral genome titre (qPCR). There is a small drop in measured viral genomes for 0.5% SecNuc™ at the thawed lysis stage (Unpaired two-tailed *t-test,* p=0.0463).
C – Viral particle titre of Affinity Product (ELISA).
D – Helper plasmid and KanR residuals measured by qPCR, given as a percentage of the total genomes
E – Residual DNA measured by PicoGreen assay of Affinity Product
F – 1.5% TBE agarose gel with neat affinity product in each lane, stained with SYBR safe DNA stain.
G – Mass photometry of Affinity Product (SamuxMP)
H – Coomassie blue stained SDS-PAGE gel of Affinity Product. 1.2ug of protein for replicate AMBR250 vessels were loaded per lane. S - Precision Plus Protein™ WesternC™ Blotting Standards
I – ELISA for SecNuc™ enzyme with both crude lysates and affinity product samples.

Discussion

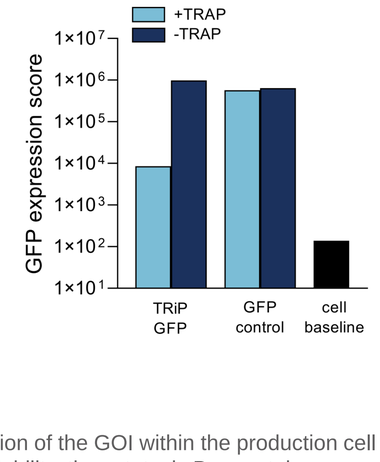
SecNuc™ has been shown to be a viable alternative to recombinant nucleases, with comparability across a multitude of analytical parameters. The benefit of the SecNuc™ enzyme when used in this way is that it removes the need to purchase expensive recombinant nucleases and can reduce the cost of the nuclease digestion step by up to 36-fold.

Figure 2: Demonstrating the power of the TRiP System™ to produce high titres with challenging transgenes in our Dual plasmid system

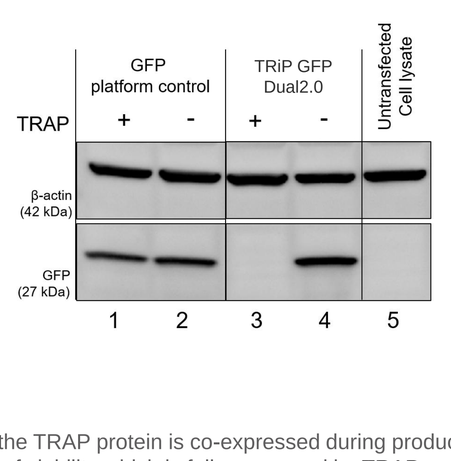
A TRiP system™ restores cell viability at harvest restored for cytotoxic proteins



B GFP expression reduced by 115-fold in production cell by TRiP system™



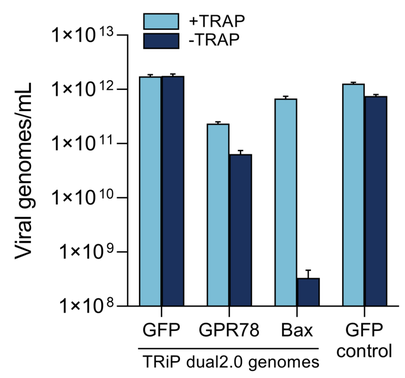
C Complete prevention of GFP expression during production by TRiP system™



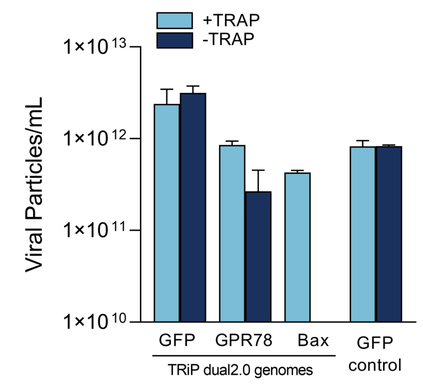
TRiP system™ allows strong transgene repression in production cells

A – Cell viability data from NC-202 cell counter taken immediately before harvest.
B – GFP expression score, as the MFI*%GFP positive cells by GFP-FACS.
C – Cell protein lysate western blot with anti-GFP and anti-beta-actin antibodies. 20µg protein loaded per lane.
D - Viral genome titre (qPCR).
E – Viral particle titre (ELISA).

D TRiP genomes allow restoration of genome titre for challenging transgenes



E Viral particle titre unaffected by TRiP system™ with non-challenging transgenes



Discussion

The TRiP system™ effectively prevents expression of the GOI within the production cell when the TRAP protein is co-expressed during production. As demonstrated by the end of production cell viability, the cytotoxic Bax protein causes a loss of viability which is fully recovered by TRAP co-expression. The GFP expression score and Western blot show the power of the TRiP system™ in preventing GFP expression within the production cell. Finally, the viral genome titres demonstrate a restoration in titre of up to 2000-fold (Bax) for challenging transgenes. TRAP protein and presence of the tbs has a negligible effect on capsid titres, viability or genome titres in cells with non-challenging transgenes or non-TRiP genomes – thus can be used as a universal element within any GOI for rAAV gene therapy.

References

Maunder, H. E., Wright, J., Kolli, B. R., Vieira, C. R., Mkandawire, T. T., Tatoris, S., Kennedy, V., Iqball, S., Devarajan, G., Ellis, S., Lad, Y., Clarkson, N. G., Mitrophanous, K. A., & Farley, D. C. (2017). Enhancing titres of therapeutic viral vectors using the transgene repression in vector production (TRiP) system. Nature communications, 8, 14834. https://doi.org/10.1038/ncomms14834

Oxford Biomedica (UK) Limited, Windrush Court, Transport Way, Oxford, OX4 6LT

Oxford Biomedica (France) 317 Av. Jean Jaurès, 69007 Lyon

UK | France | US
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