Advanced nuclease applications in Lentiviral vector bioprocessing for superior downstream recovery and vector product quality

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Introduction

Efficient and robust downstream processing of Lentiviral vectors (LV) is critical for producing high-quality gene therapy vectors. Traditional nucleases used in LV manufacturing often result in sub-optimal vector recovery and high residual DNA levels in the final drug product.

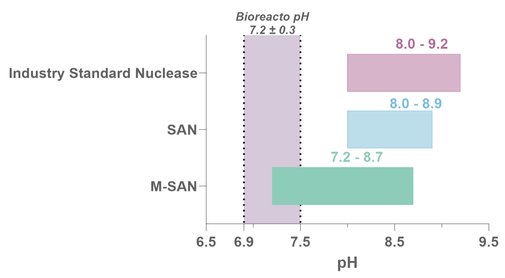
This project aimed to identify and integrate alternative nucleases, namely Salt Active Nuclease (SAN) and Medium-Salt Active Nuclease (M-SAN), into OXB's LV manufacturing workflows to enhance vector recovery and improve overall product quality. Key characteristics of alternative nucleases such as optimal pH (See Figure A) and salt buffer (See Figure B) conditions were evaluated and incorporated into downstream processes (See Figure C) and compared to traditional nuclease-based downstream processes.

Our findings demonstrate that the use of SAN and M-SAN exhibited superior activity under typical LV manufacturing conditions. Notably, the incorporation of alternative nucleases reduced vector aggregation during purification and improved around two-fold vector recovery during the challenging sterile filtration step of Drug Product processing. Most importantly, the incorporation of these nucleases resulted in markedly lower levels of residual DNA in the final drug product, addressing a critical quality attribute for gene therapy applications.

Methods

Three commercially available nucleases, each with distinct optimal enzymatic properties, were evaluated in OXB's LV suspension platform processes. The aim was to assess their impact on process performance, residual DNA clearance, and yield during downstream processing. Nuclease treatment was performed either within the bioreactor at the end of the LV production (Nuclease treatment 1) or after the LV elution from an anion exchange chromatography (AIEX) membrane (Nuclease treatment 2).

A



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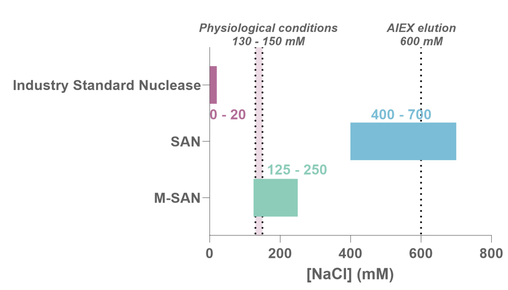

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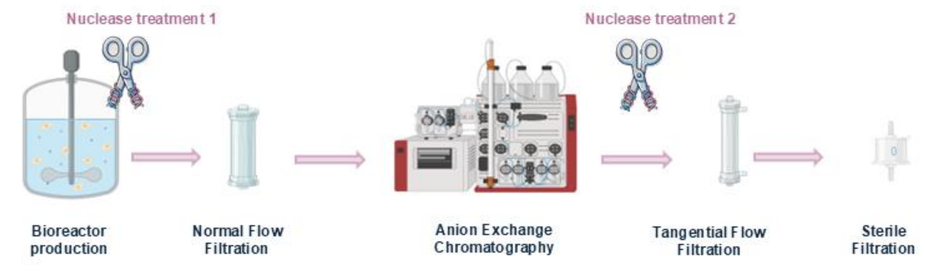
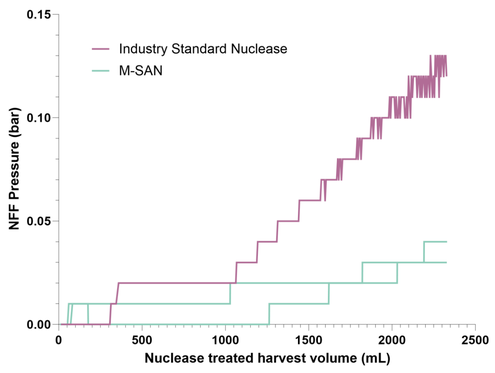


Evaluation of M-SAN in LV production

M-SAN demonstrated superior performance compared to the Industry Standard Nuclease when integrated into OXB's LV production process.

- Lower pressure during clarification after M-SAN treatment in the bioreactor (See Figure D).
- Higher Tangential Flow Filtration (TFF) flux rates (see Figure E).
- Comparable functional titre through downstream processing (See Figure F).
- Effective removal of total, host cell and plasmid DNA following M-SAN treatment in the bioreactor (See Figure G).
- Reduced DNA contaminants in the drug substance (See Figure H).
- Similar particle size in the drug substance after M-SAN incorporation (See Figure I).

D



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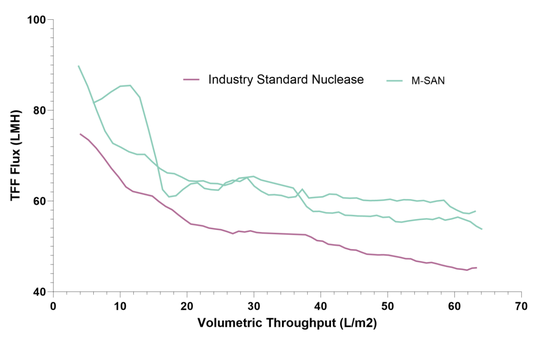

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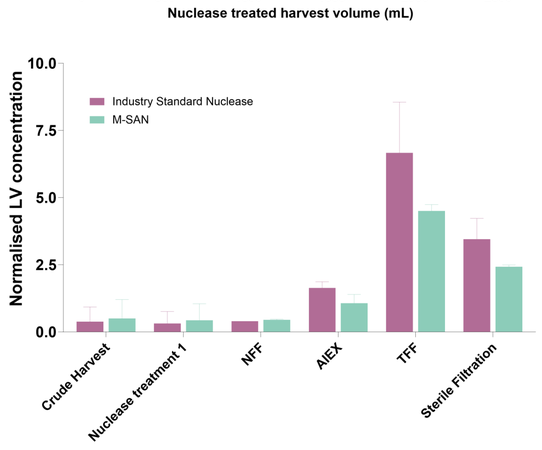

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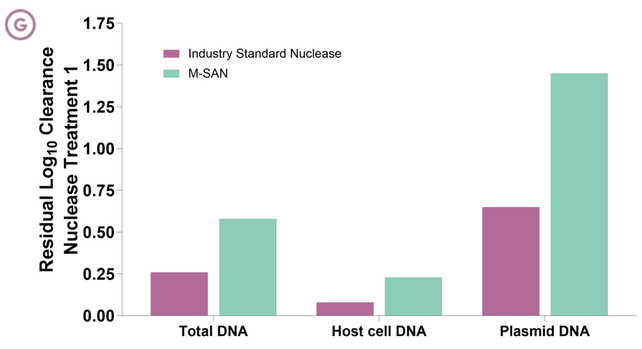

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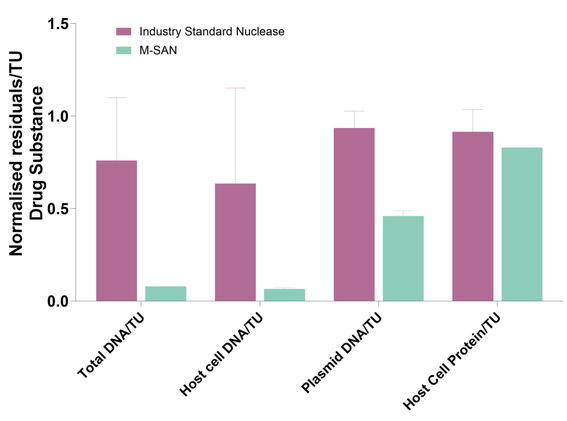

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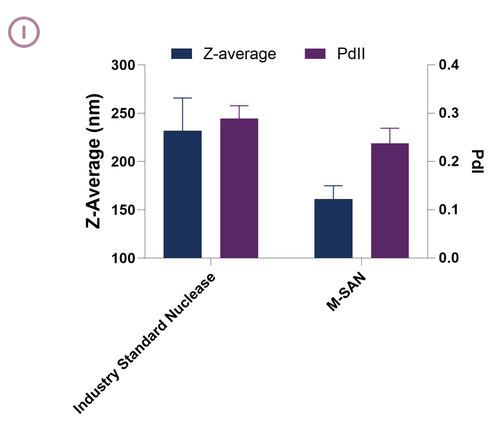
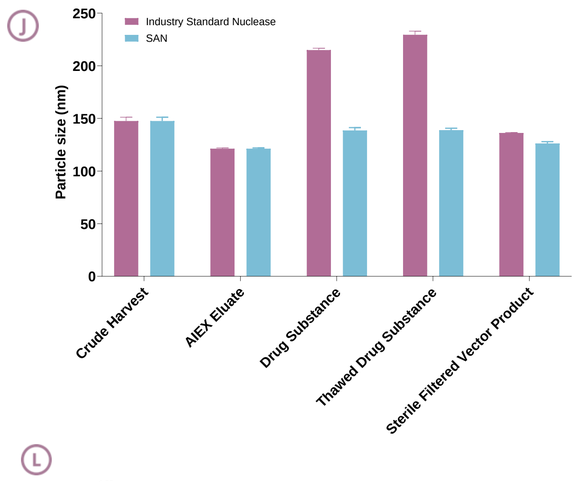


Evaluation of SAN in LV production

SAN also outperformed the Industry Standard Nuclease when integrated into OXB's LV production.

- Substantial reduction in vector aggregation during purification (See Figure J).
- Reduced aggregation improves filterability of SAN treated vector (See Figure K).
- Over 10-fold reduction in residual DNA compared to the Industry Standard Nuclease (See Figure L).
- Improved vector recovery during sterile filtration, leading to higher process yields (See Figure M).

J



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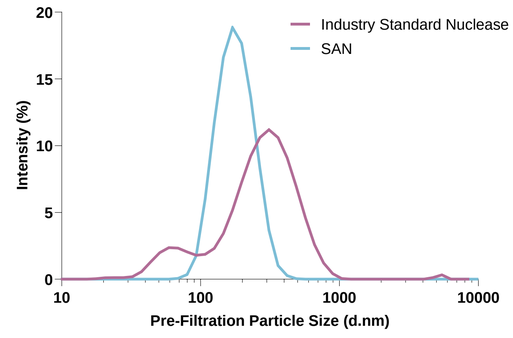

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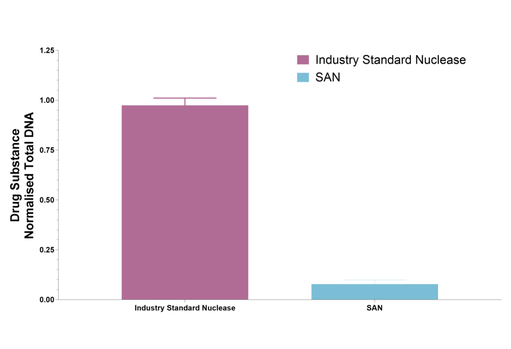

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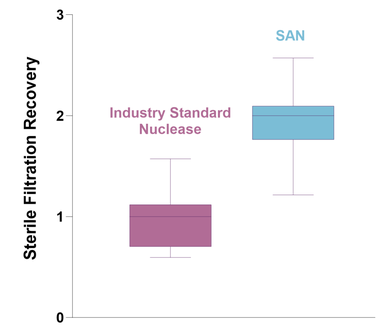

Conclusion

LVs offer significant promise for effective cell and gene therapy applications, and the adoption of new therapies will require efficient methodologies for their production and purification. This is particularly needful when considering use of LVs in an in vivo setting. The incorporation of alternative nucleases into the manufacturing process presents a viable strategy for the effective residual DNA removal and improved downstream processing. These advancements hold promise for enhancing the efficacy and safety of LV-based cell and gene therapies.

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