Evaluation of Salt-Tolerant Endonucleases for Lentiviral Vector Purification

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Introduction

The downstream purification of Lentivirus (LV) suspension culture typically starts with an endonuclease treatment, followed by a clarification step. The endonuclease treatment, carried out in the bioreactor, is designed to digest both free and chromatin-associated DNA. Residual DNA poses several challenges to the clarification step, as it can form large complexes with viral particles which may lead to aggregation and potential product recovery loss.

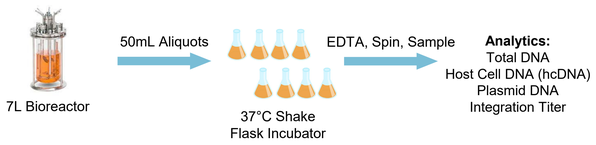
Despite its importance, traditional endonuclease treatment at physiological conditions (such as the LV bioreactor culture) often exhibit low DNA digestion efficiency. Thus, a second endonuclease step is required later in the downstream process to achieve higher log reduction values of residual DNA that meet regulatory requirements.

Recently, commercially available salt-tolerant endonucleases, such as M-SAN® HQ and Denarase® High Salt, have shown promise in efficiently removing DNA residuals at physiological conditions and near neutral pH. In this study, we compared these salt-tolerant endonucleases against a non-salt tolerant industry-standard endonuclease. Additionally, we looked at how salt, $MgCl_2$, and time impacted residual DNA digestion.

Methods

Three sets of experiments were carried out. All experiments utilized freshly generated LV culture from a 7L bioreactor. 50mL of culture was sampled into 250mL shake flasks and incubated with the endonuclease at 37°C for 1-, 2.5-, and 4-hours (**Figure A**). After incubation, samples were treated with EDTA, spun and frozen for analysis.

A Figure A: Experimental Design



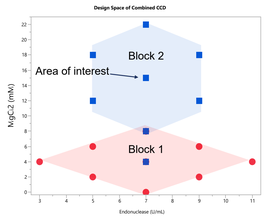
ENDONUCLEASE SCREENING

- Tested endonucleases: M-SAN® HQ, Denarase® High Salt and an industry-standard endonuclease (at 7 U/mL only).
- Salt-tolerant endonucleases tested at 3 concentrations.
- Assessed impact of NaCl and $MgCl_2$ to improve activity.

M-SAN® HQ 2-FACTOR DESIGN OF EXPERIMENT (2 BLOCKS)

- Block 1: CCD around M-SAN concentration (3-11 U/mL) and $MgCl_2$ concentration (0-8 mM) (**Figure B**).
- Block 1 consisted of 19 runs, with 2 conditions with "extreme" $MgCl_2$ and a control run, excluded from the CCD.
- Block 2: CCD around M-SAN concentration (5-9 U/mL) and $MgCl_2$ concentration (8-22 mM) (**Figure B**).
- Block 2 consisted of 9 runs, with 3 repeats from Block 1 (4mM, 8mM and 15mM of $MgCl_2$) for direct comparison.

B Figure B: Design Space of Each Experiment Block

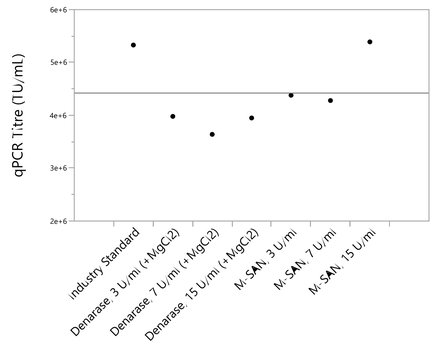


Results

ENDONUCLEASE SCREENING

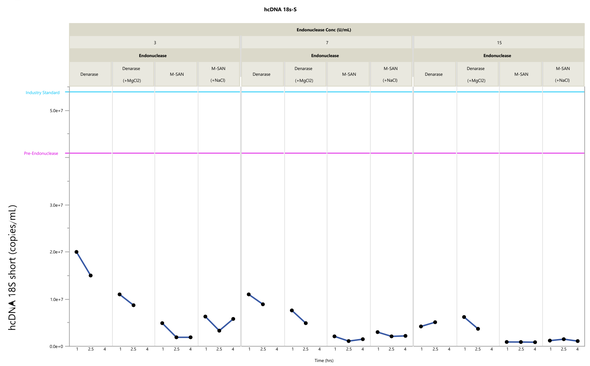
- No impact on the integration titer was observed with either M-SAN or Denarase HS after 4-hours and 2.5-hours incubation at 37°C, respectively (**Figure C**).

C Figure C: Integration Titers after Incubation at 37°C



- M-SAN® HQ and Denarase® High Salt outperformed the industry-standard endonuclease at all conditions (**Figure D**).
- Addition of NaCl had no significant effect on M-SAN activity.
- Addition of $MgCl_2$ improved the activity of Denarase HS.

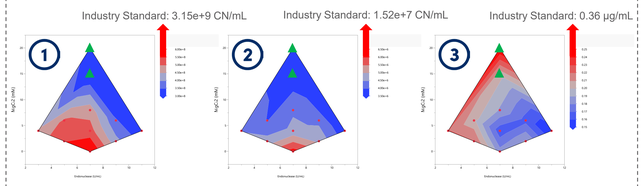
D Figure D: Residual Host Cell DNA Concentration 18s-Short



M-SAN® HQ 2-FACTOR DESIGN OF EXPERIMENT – BLOCK 1

- All M-SAN conditions showed lower hcDNA concentrations than the industry standard control run (**Figure E**).
- Integration titer of all conditions after 4-hours incubation at 37°C were comparable to the Pre-Endonuclease titer.
- Addition of "extreme" $MgCl_2$ (15 and 20 mM) suggests design space was too constrained. Second CCD executed to look at higher $MgCl_2$ concentrations (8-22 mM).

E Figure E: CCD Response Surface **(1)** Plasmid DNA (KanR), **(2)** Host Cell DNA and **(3)** Total DNA

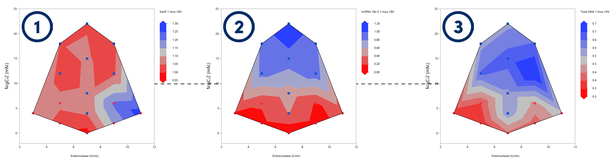


▲ "Extreme" $MgCl_2$ conditions, initially not a part of the Central Composite Design (CCD) Space

M-SAN® HQ 2-FACTOR DESIGN OF EXPERIMENT – BLOCK 2

- Block 2 crude lysate had higher residual DNA levels than Block 1 crude lysate.
- Log reductions values from pre-endonuclease were used to combine the CCD conditions of the 2 experiments, excluding Block 1 "Extremes" (**Figure F**).

F Figure F: LRV of Combined CCD Response Surface **(1)** Plasmid DNA (KanR), **(2)** Host Cell DNA and **(3)** Total DNA



- Reduced model of the combined CCDs showed that $MgCl_2$ and M-SAN concentration are significant factors in the reduction of residual DNA. But no statistically significant secondary interaction was found between these factors.
- More $MgCl_2$ can improve hcDNA and Total DNA removal. While Plasmid DNA is more sensitive to endonuclease concentration.

Conclusions

We demonstrated the potential of salt-tolerant endonucleases to enhance the efficiency of residual DNA digestion in the bioreactor during Lentiviral vector (LV) manufacturing, at physiological conditions and close to neutral pH.

- M-SAN® HQ and Denarase® High Salt **improves by 5-fold** hcDNA removal at the same endonuclease concentration.
- **No impact on integration titer** by qPCR was observed for any tested condition after 4-hours of incubation at 37°C.
- The reduced model from the combined CCD results predicts log reduction values of at least 1.0 for Plasmid DNA, 0.8 for hcDNA, and 0.5 for Total DNA after 1 hour of incubation at 37°C by using at least **5 U/mL M-SAN and 15 mM $MgCl_2$**.

References

1. Marc Struhalla, Svenja Michalek. *Innovator Insight.* DOI: 10.18609/cgti.2025.035.
2. Efficient Chromatin Removal in Viral Vector Manufacturing Using Salt-Active Nucleases [White paper]. 2024.

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