Optimization of Nuclease Treatment for Removal of Residual DNAs in Adeno-Associated Viruses

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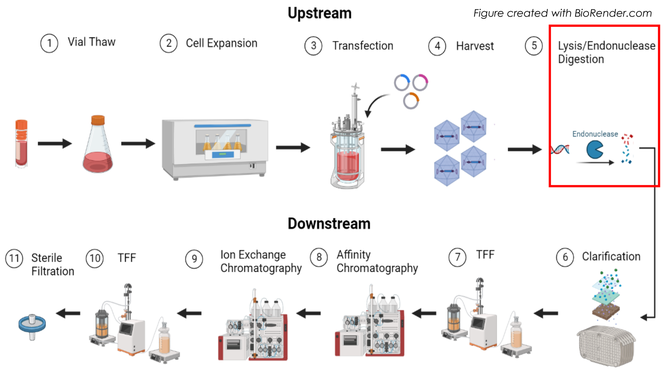
Introduction

The adeno-associated viral vector (AAV) has been developed as an efficient gene delivery tool, and the most prominent for *in vivo* gene therapy. The residual DNA impurities are a safety concern, and their removal is a primary challenge in process development for the *in vivo* application of AAV. In general, there are two sources of nucleic acids in the process, which are residual host cell DNA (hcDNA) and plasmid DNA from transfection (pDNA). Minimalization of residual DNA amounts has been demonstrated to increase virus yield in clarification and protect downstream chromatography and filter-based unit operations. In addition, decreasing impurities could potentially reduce immune responses *in vivo*.

In this study, we developed and optimized the nuclease-treatment condition to reduce the process-related nucleotide impurities in AAV materials. Firstly, the efficiency of nuclease digestion was compared, and the results showed that Nuclease B exhibited the best activity in AAV6 samples when compared to Nuclease A and C (Figure 1). To evaluate the conditions for the best Nuclease B activity, a DoE study was conducted, and the data showed the residual dsDNA was reduced through the increase of ion ($MgCl_2$) concentration and incubation time. However, the salt and nuclease concentration did not dramatically affect nuclease digestion efficiency (Figure 2), while the pH of treatment buffers showed significant impact on the Nuclease B digestion efficiency in AAV6 materials (Figure 3). Further experimentation showed the log reduction of dsDNA, including hcDNA and pDNA, and indicated that Nuclease B efficiently decreased DNA impurities over longer incubation times without negatively affecting AAV6 GC titers (Figure 4). Finally, a DoE study further confirmed treatment time was critical for efficient reduction of DNA impurities (Figure 5).

In conclusion, our results illustrated a well-optimized process that could reduce residual DNA impurities efficiently without impacting the vector genome titer as well as the other downstream unit operations.

AAV Upstream and Downstream Process Overview



Results

Nuclease B Showed Better Digestion Efficiency than Nuclease A & C

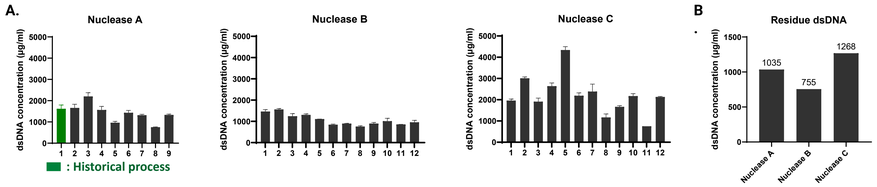


Fig 1. Comparison of Nuclease activity in AAV materials. (A) DoE design study to evaluate the nuclease digestion efficiency of Nuclease A, and two salt-tolerant nuclease (Nuclease B and C) in AAV6 samples. (B) The JMP model analysis predicted the minimal residual dsDNA (Picogreen) in nuclease treated materials.

DoE Design Study to Evaluate Conditions for Residual DNA Digestion

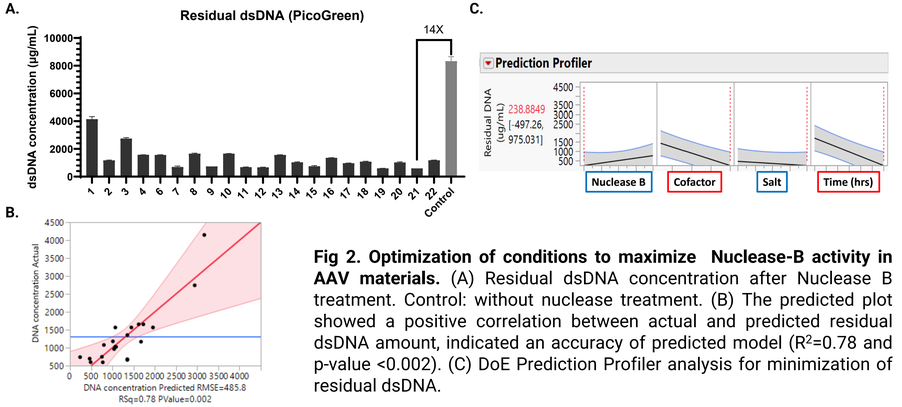


Fig 2. Optimization of conditions to maximize Nuclease-B activity in AAV materials. (A) Residual dsDNA concentration after Nuclease B treatment. Control: without nuclease treatment. (B) The predicted plot showed a positive correlation between actual and predicted residual dsDNA amount, indicated an accuracy of predicted model (R^2=0.78 and p-value <0.002). (C) DoE Prediction Profiler analysis for minimization of residual dsDNA.

pH Optimization for Nuclease B Digestion

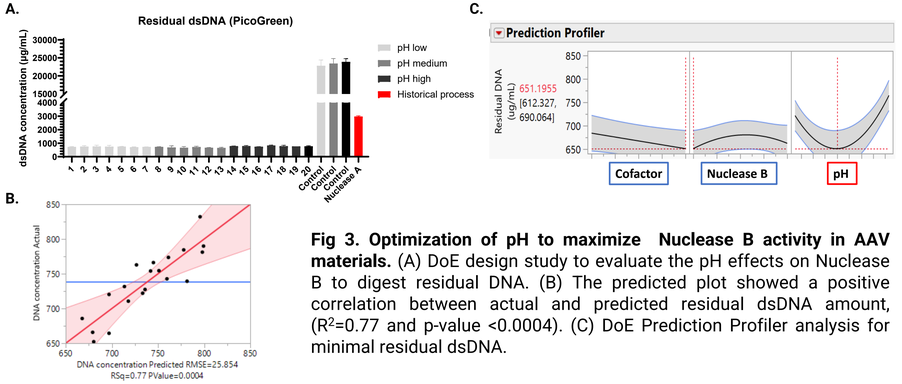


Fig 3. Optimization of pH to maximize Nuclease B activity in AAV materials. (A) DoE design study to evaluate the pH effects on Nuclease B to digest residual DNA. (B) The predicted plot showed a positive correlation between actual and predicted residual dsDNA amount, (R^2=0.77 and p-value <0.0004). (C) DoE Prediction Profiler analysis for minimal residual dsDNA.

Nuclease B Showed Better Digestion Activity Without Affecting AAV GC Titers

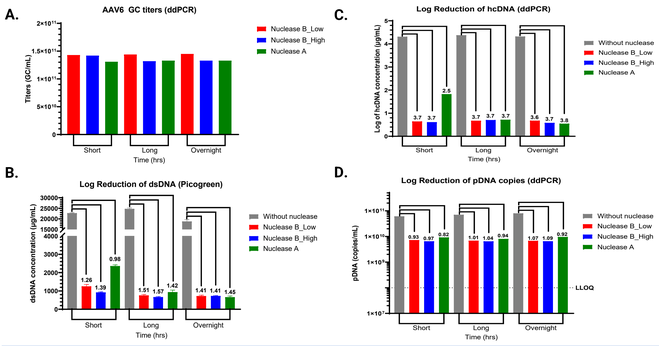


Fig 4. The impact of Nuclease B treatment time for the residual DNA removal in AAV materials. (A) Quantification of AAV6 GC titers post-nuclease treatment. (B) Log reduction of residual dsDNA. (C) Log reduction of residual genomic DNA (hcDNA). (D) Log reduction of residual plasmid DNA (pDNA) copies.

Nuclease B Showed the Maximum of Digestion within 3 Hours

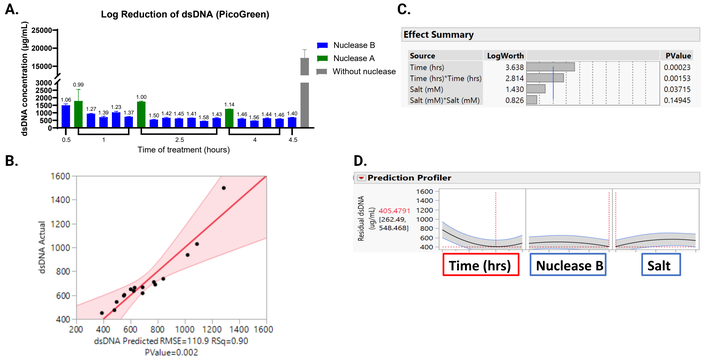


Fig 5. DoE optimization of nuclease treatment time in AAV materials. (A) Log reduction of residual dsDNA. (B) The predicted plot showed a positive correlation between actual and predicted residual dsDNA amount, indicated an accuracy of DoE model (R^2=0.90 and p-value <0.002). (C) DoE effect summary indicated the significancy of each factor. (D) DoE Prediction Profiler analysis for minimal residuals.

Conclusion

Our data illustrated the optimized nuclease treatment conditions for DNA impurity removal from AAV materials

- The results showed a novel nuclease had more efficiency to remove residual DNA in AAV6 materials when compared to the one in our current process.
- Through DoE studies, we optimized salt and ion concentration, pH, and nuclease treatment time to achieve 3~4X residual DNA removal compared to the historical treatment condition.
- With the optimized nuclease treatment condition, the residual DNA was effectively reduced without impact to AAV GC titers.