Improved enzymatic host-cell DNA removal in AAV manufacturing

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Introduction

Host cell DNA and other DNA species is a common process-related impurity encountered when manufacturing viral vectors for gene therapy. Due to safety issues, the maximum amount of DNA allowed in a final dose administrated to humans are set to 10 ng per dose (1). Furthermore, it has also been shown that presence of host-cell DNA in lysates from Adeno-Associated vector (AAV) manufacture increases the likelihood of AAV Aggregates (2). This is likely due to both the presence of chromatin and formation of DNA dependent aggregates based on charged interactions between DNA and proteins. One efficient manner to reduce these interactions is to increase the ionic strength of the solution by adding large amounts of salt, typically up to 0.5M NaCl (2).

Reduction of DNA impurities from AAV manufacturing is often achieved by enzymatic digestion early in the viral vector manufacturing process. The most commonly used nuclease for this purpose originate from *S. marcescens (Sm*.Nuclease*)*, which like most nucleases exhibit a low salt tolerance. Here, we show that increasing the salt concentration of the host-cell lysate while using a unique salt active nuclease with an optimum at ~0.5 M NaCl (SAN, ArcticZymes) results in superior DNA removal from AAV2 production cell lysates compared to using *Sm*.Nuclease. This implies a more efficient DNA removal and potential viral aggregate reduction, which consequently can result in a purer final product of higher yield.

Results and Discussion

SAN digests DNA optimally at high salt

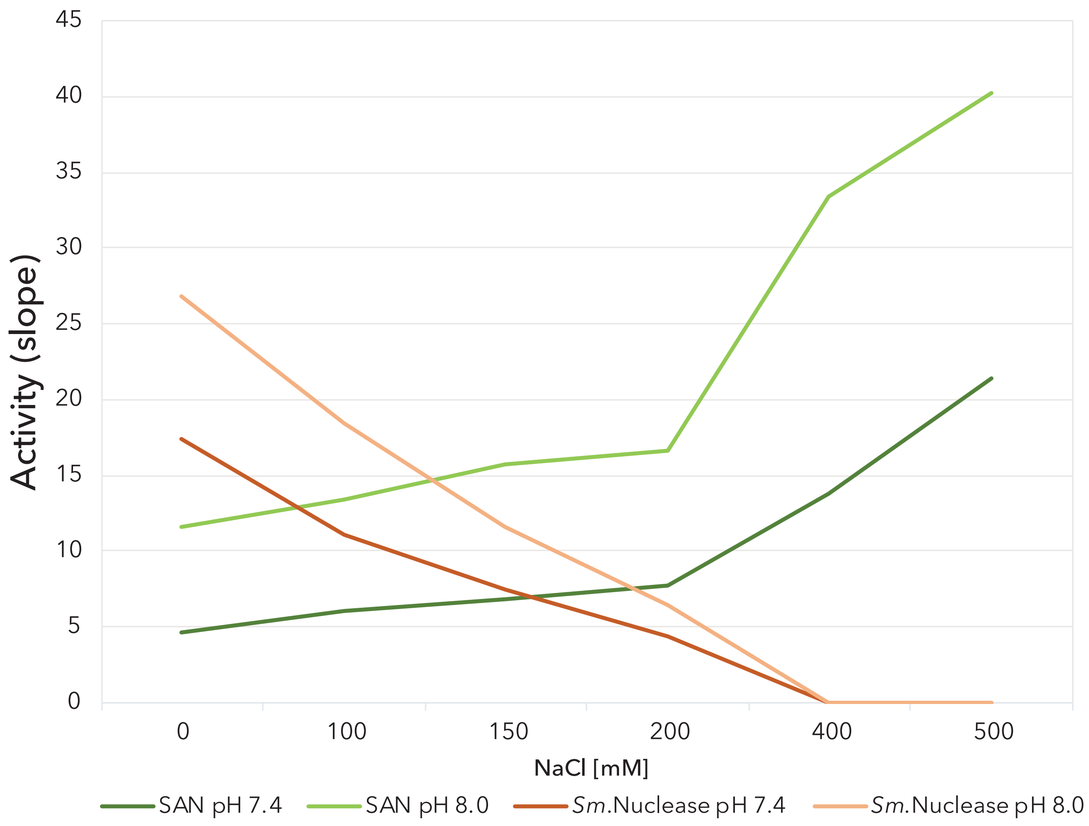

Figure 2: Nuclease activity at various NaCl concentrations using naked DNA. The activity of both nucleases was measured using calf thymus DNA as template. Equal Units were added of both nucleases (according to manufacturer Unit definition). The nucleases show opposite NaCl optimum profiles, while both nucleases have higher activity at more basic pH conditions. At the ionic strength indicated to reduce viral aggregates and chromatin structures (0.5 M NaCl), SAN exhibit optimum activity while *Sm*.Nuclease is almost completely inhibited.

High salt improves host-cell DNA removal

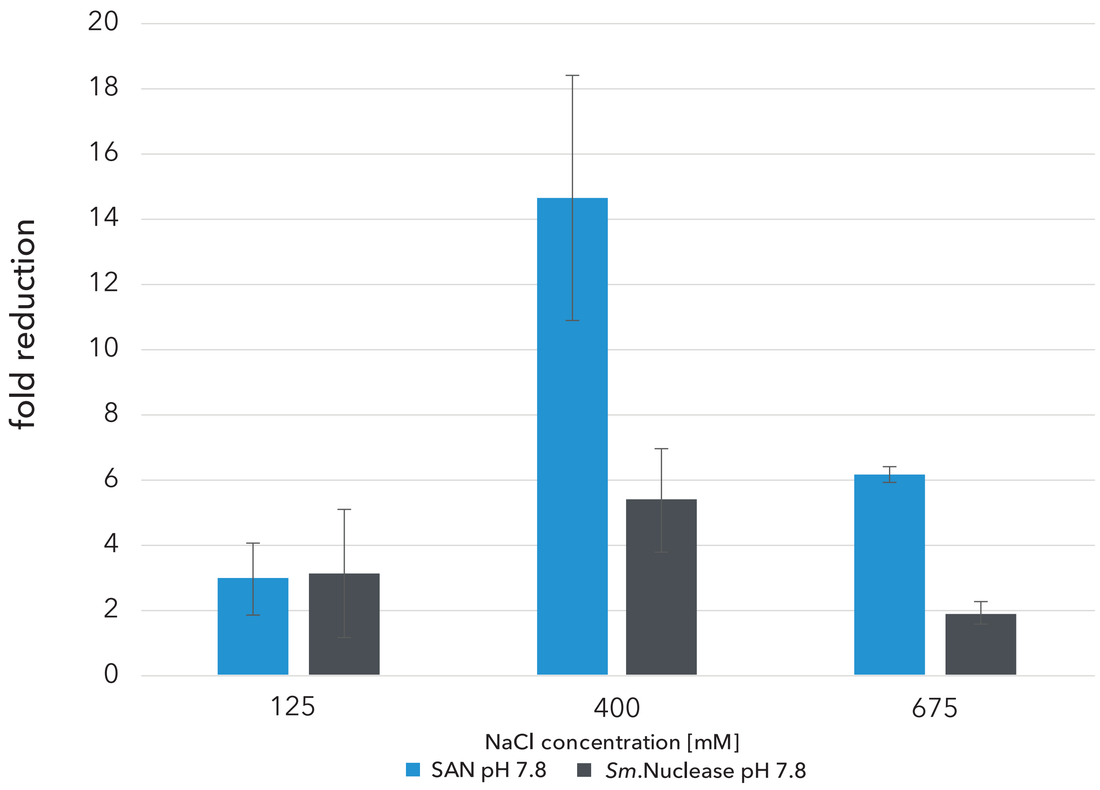

Figure 3. SAN and *Sm*.Nuclease digestion of host cell DNA impurities in cell lysates. SAN shows 3-fold more efficient DNA clearance compared to *Sm*.Nuclease at 400 mM NaCl, pH 7.8. This is similar to the pattern observed when digesting naked DNA (Figure 2). Interestingly, there is also slight indications of improved DNA clearance for the *Sm*.Nuclease at higher salt (400 mM) compared to low salt (125 mM), contrary to the results observed using naked DNA. For both nucleases, the highest fold reduction of DNA impurities is achieved at ~400 mM NaCl. Together, this indicates that higher ionic strength makes host cell DNA more accessible for nuclease digestion. High ionic strength has also been correlated with reduced formation of viral aggregates and hence higher yield (2). It is also likely that a cleaner lysate improves downstream capture and polishing steps, further contributing to a purer end product.

References

1: Guidance for Industry Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications, U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research (February 2010)

2: Wright, J. F. et al. Identification of factors that contribute to recombinant AAV2 particle aggregation and methods to prevent its occurrence during vector purification and formulation. Molecular Therapy 12, 171–178 (2005).

Methods

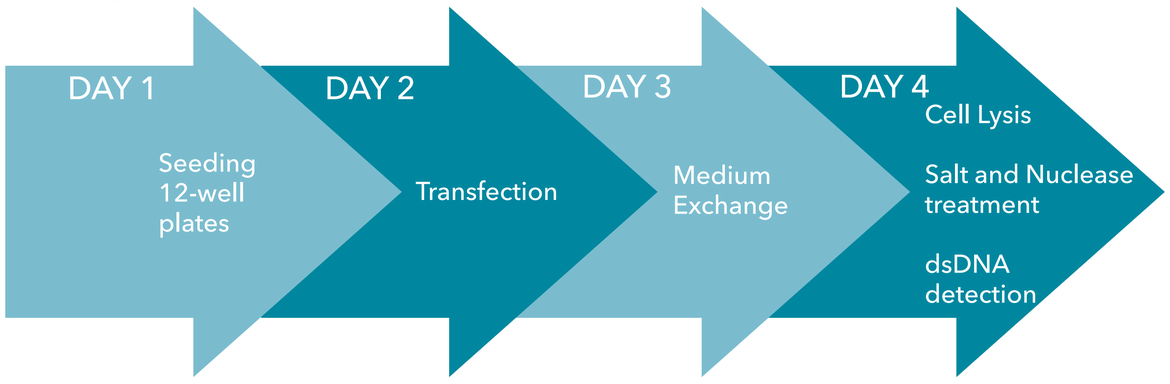

Figure 1: Workflow of AAV2 production including enzymatic digestion of DNA impurities at high salt.

Nuclease activity against naked DNA (Calf Thymus DNA) was measured using a modified Kunitz assay at the following buffer conditions: 25 mM Tris-HCl pH 7.4 and 8.0 @ 37°C, 5 mM $MgCl_2$, 50 µg/mL Calf thymus DNA and 0 – 500 mM NaCl. Activity was measured as an increase in A_{260}.

Design of experiment was used to setup different buffer conditions (pH and NaCl concentration) for nuclease treatment of AAV2 cell lysates. AAV2 was manufactured by triple transfection of Human Embryonic Kidney 293T cells in 12-well plates. 2 days post transfection, cells were lysed using Triton™ X-100 in 3 buffers (pH 6.6, 7.7 and 8.7) with various quantities of salt. Each of the nucleases were added directly to the buffers, mixed 1:1 with the cell media and incubated at 37°C for 1 hour at shaking for simultaneous lysis of cells and digestion of host DNA (25 U/ml nuclease). Following lysis, pH was measured to 6.4, 7.8 and 8.5. Salt from the lysis buffer contributed 50, 325 and 600 mM NaCl. Salt from the DMEM media was estimated to contribute additional ~75 mM salt, to a total approximate concentration of 125, 400 and 675 mM salt while digesting DNA in lysates. Remaining dsDNA was measured using QuantIT™ dsDNA HS reagent (Invitrogen). Controls (no nuclease added) were included for each condition. Results are presented in Figure 3 as fold change reduction of DNA impurities compared to the control samples.

Catapult contributed to the data presented in Figure 1, 3 & 4. ArcticZymes contributed to the data presented in Figure 2.

SAN allows efficient removal of DNA directly in cell lysates

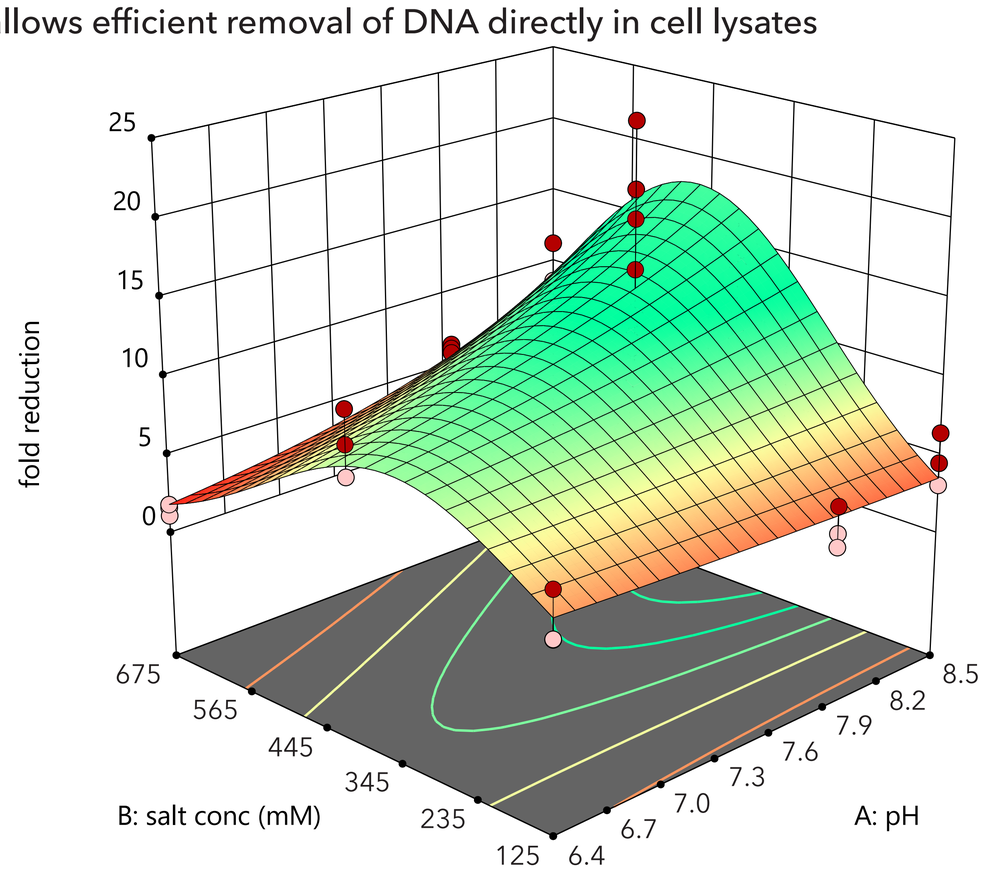

Figure 4. Response surface plot showing the mutual effect of pH and NaCl on DNA depletion activity by SAN. Data points represent replicates of at least biological triplicates. The center point is composed of 9 replicates. The p-value of the model is < 0.0001. The results are presented as fold change of DNA impurities relative to untreated controls. For SAN, optimal conditions for digestion of dsDNA resembles that of naked DNA (Figure 2); high salt (~0.400 mM) and pH (8.5). SAN with its optimum at 0.5 M salt provides the superior results for removing of DNA impurities directly in the cell lysate at high salt concentration.

Summary

- High salt (0.5M) makes host cell DNA more accessible for digestion
- Digestion with a salt active nuclease directly in high salt lysate improves DNA clearance
- No de-salting step needed before nuclease treatment
- High salt DNA digestion has shown to reduce AAV aggregation and could thus improve target yield

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