Optimization of nuclease digestion unit operation in a lentiviral vector process for improved reduction of DNA impurities

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ABSTRACT

As the Cell and Gene Therapy (C&GT) field expands, more and more new innovated therapies and treatments arise. To ensure the safety of these potentially life-changing treatments, one area of focus is around the process-related DNA impurity levels, such as host cell DNA (hcDNA) and plasmid DNA (pDNA), which are typically the concerning process-related impurities for viral vectors. However, due to the complex nature of viral vector production, many factors can inhibit proper reduction of these DNA impurities. Even with the addition of commonly used commercial endonucleases (e.g. Denarase®), the desired reduction in the DNA levels might not be sufficient to meet process or regulatory recommendations.

Here, we provide the statistical analysis results based on several DoE studies for reduction of hcDNA and pDNA levels in our Lentiviral Vector (LVV) platform process, LentiPeak™. Focusing on several critical parameters including nuclease concentration, supplement concentration, incubation time, and incubation conditions (pH and temperature), this data shows the optimal factors and ranges in the nuclease digestion step. In addition, our data characterizes the impact for each significant parameter to identify the relationship toward DNA impurities reduction for both in-process samples and in the final LVV pool to fully understand the carry through impact of the nuclease digestion step.

With the increasing need of viral vectors in the C&GT field, many upstream processes are evolving to meet production demands with higher cell densities and higher plasmid DNA concentrations. This factor, coupled with industry driven prioritization for patient safety, creates a need for processes, especially the nuclease digestion unit operation, to ensure the adequate clearance of DNA impurities. With this data, we hope to highlight the key parameters which support optimal nuclease digestion to be applied to all viral vector processes, and thus streamline the work needed in process development for clearance of the DNA impurities.

PROCESS BACKGROUND

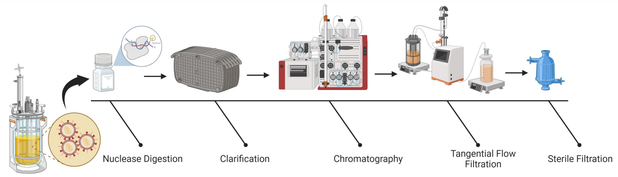


Figure 1. Lentiviral Vector Downstream Purification Process
The Lentiviral vector (LVV) downstream process utilizes 5 main unit operations to increase product concentration and reduce in-process impurities. The main unit operation for the reduction of DNA impurities is the nuclease digestion step, where the LVV harvest material is treated with an endonuclease enzyme.

No Significant DNA Reduction in Nuclease Digestion Step

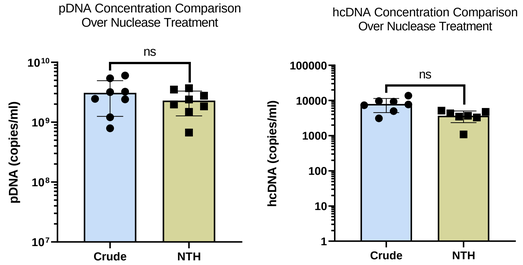


Figure 2. pDNA and hcDNA Concentration Post-Nuclease Digestion
The initial process development work for the nuclease treatment step showed no statistically significant (p-value < 0.05) difference of pDNA and hcDNA levels over the nuclease treated harvest (NTH) step.

Evaluation of Key Parameters for Nuclease Digestion

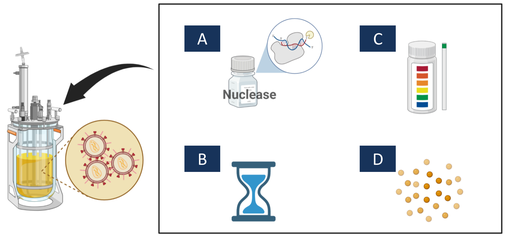
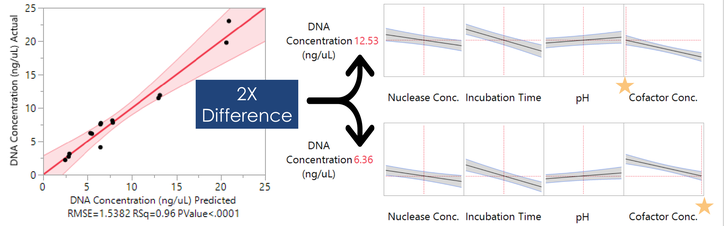


Figure 3. Parameters for Further Development Work
To improve the nuclease digestion in the LVV harvest pool, four (4) parameters were tested to determine their impact on the DNA reduction: Nuclease concentration **(A)**, incubation time **(B)**, pH **(C)**, and cofactor concentration **(D)**.

Screening Study Shows Significant Impact on DNA Levels with All Parameters



Source	LogWorth	PValue
Incubation Time	4.301	0.00005
Cofactor Concentration	3.378	0.00042
Nuclease Concentration	2.301	0.00500
Incubation Time*Nuclease Concentration	2.207	0.00620
pH	1.403	0.03955

Figure 4. Nuclease Digestion Parameter Screen
The predicted model shows that all the parameters studied are statistically significant (p-value < 0.05). One of the most impactful parameters is the cofactor concentration, which shows a 2X difference in the DNA concentration (measured by spectrometer) post-nuclease digestion in the studied ranges.

Optimized Nuclease Digestion Parameters Shows Improved hcDNA Reduction Throughout the Process

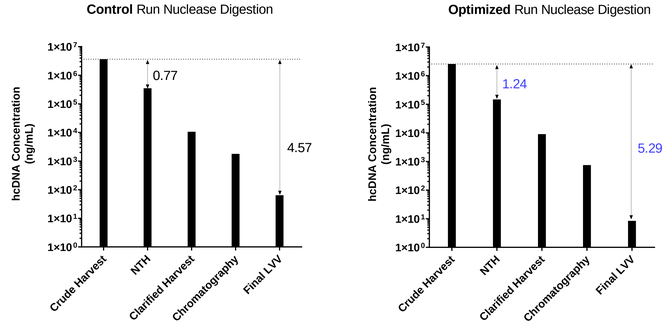


Figure 6. Optimized Nuclease Digestion Comparison Throughout the Process
The optimized nuclease digestion parameters were applied in the standard LVV process and showed an increase in hcDNA reduction in the nuclease treated harvest (NTH) pool (1.24 logs) and in the Final LVV material (5.29 logs) compared against the control process.

Cofactor Concentration and Addition Method Shows Increased Digestion in DNA

Run Number	Cofactor Condition
Run# 1	No Addition
Run# 2	Addition Method 1
Run# 3	Addition Method 2

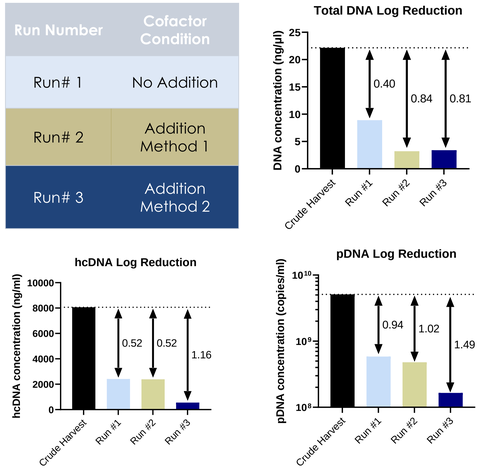


Figure 5. Cofactor Concentration and Addition Method Study
A cofactor parameter study using different concentrations and addition methods showed that the cofactor addition plays an important role in the nuclease activity, with Run# 3 showing the highest DNA reduction.

CONCLUSION

The studies completed here showed that many factors play into the optimal DNA digestion in the nuclease treatment step. The key takeaways are:

- All major parameters are statistically significant in the reduction of DNA
- The cofactor concentration and addition method improve the nuclease activity to further reduce the DNA levels
- Reduction in the DNA levels at the nuclease digestion step is carried throughout the process and into the final LVV pool

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