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Title: *Evaluation of Residual Host Cell DNA Clearance and Sizing During Production of a Lentiviral Vector*

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Viral vector-based gene therapy drug products can contain residual DNA derived from the production cells. Production typically occurs in continuous cell lines with tumorigenic potential and residual host cell DNA carries a theoretical risk of oncogene transmission to recipient cells. This risk decreases as a function of reduced concentration and size of residual DNA fragments. It is therefore important to monitor residual host cell DNA concentration and size in gene therapy products. We have evaluated methods for quantitation of residual host cell DNA concentration and size during production of an engineered lentiviral vector manufactured in HEK293T cells and used those methods to compare the performance of two commercially available nucleases in downstream processing steps.

We evaluated qPCR-based methods for quantitation of residual host cell DNA from multiple vendors, and multiple options were found to be accurate using the newly available ATCC/USP quantitative HEK293 genomic DNA reference standard. We find a substantial difference in performance between the two evaluated nucleases, DENARASE® and M-SAN HQ, across the downstream processing steps in our lentiviral production process, with material produced using M- SAN HQ having at least 10-fold lower residual DNA concentrations than the comparable material made with DENARASE®.

To assess the size of residual DNA fragments, we developed a method based on universal linker ligation followed by PCR amplification (LiLi-PCR) and size analysis by capillary electrophoresis. The method accurately reports on the size distribution of mixed synthetic DNA fragments in the range of 50-1000 base pairs in concentrations ranging from 10 ng/uL to 0.5 ng/uL, with some bias against larger fragments at lower concentrations. Further, the method faithfully recapitulates the size distribution of a smear of genomic DNA fragments produced by enzymatic digestion. We will report on progress in evaluating whether available nucleases result in differing distributions of residual DNA fragment size after digestion and downstream processing.