

Analysis of the Adeno-Associated Virus (AAV) vector on the SeqStudio™ CE Platform

Genomics

Achim Karger (achim.karger@thermofisher.com), Thermo Fisher Scientific, **Stephen Williams**, Thermo Fisher Scientific, **Hannah Kim-Lindström**, Thermo Fisher Scientific, **Emily Bai**, Thermo Fisher Scientific

We describe the successful use of Applied Biosystems Genetic Analyzer capillary electrophoresis instrumentation for the DNA analysis of Adeno-associated virus (AAV). AAV constructs for gene therapy consist of single-stranded DNA up to 4.5kb in length terminated at both ends by notoriously difficult to sequence GC-rich, inverted tandem repeat regions (ITRs). Both ITRs were sequenced on an AAV vector control plasmid DNA using dGTP BigDye™ Terminator v3.0 chemistry. Based on the high-quality sequence data two variants were detected in the plasmid DNA, a 4 bp deletion near the left –ITR and one single nucleotide mutation. For a fast confirmation of the integrity of the viral vector genome we generated LongAmp™ PCR amplicons up to 3kb in length for multi-color restriction mapping using SNaPshot™ chemistry, a fast, potentially single-tube assay. A commercial AAV8 preparation was used to demonstrate BigDye™ Terminator sequencing of the transgene in AAV virus preparations. AAV viral DNA was extracted in good yield (80%) using the Dynabeads™ SILANE Viral NA kit.