

**Application of Next-Generation Sequencing in Viral Library Screening**

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Next-generation sequencing (NGS) is a breakthrough technology that allows for comprehensive information gathering about the composition and complexity of a sample. Moreover, NGS, applied to the analysis of barcoded DNA molecules allows for simultaneous, high-throughput screening of a multitude of samples. As such, NGS is, therefore, a highly applicable technology for screening of AAV libraries allowing a first pass screening of the biodistribution and the expression of selected variants in large animal models such as non-human primates (NHPs). In the current paper, we utilized Ion Proton NGS platform to investigate the biological distribution and expression mediated by 13 barcoded vectors each packaged in a unique AAV capsid. The experiments were performed in parallel in mouse and NHP utilizing the same pool of barcoded vectors. We found that barcode design and rAAV sample processing can significantly affect data interpretation related to the starting mixture composition. To interpret the data variability required inclusion of additional controls and AAV mixture testing for correction of per-processing biases and NGS data.