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Single-Molecule, Modified Base Sequencing to identify frequency and cause of rAAV Vector Breakpoints

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There are approved gene therapies using recombinant adeno-associated virus (rAAV) as the vector for delivery of therapeutic genes, and the quality of the material is critical for safe and efficacious use. All AAV serotypes contain single-stranded DNA within each capsid that raises analytical challenges. Complementary genomic strands hybridize when capsids are lysed and standard sequencing library methods cause information from the individual genomes to be lost when inter-strand mismatches and gaps are repaired. This is especially important when gaps are repaired because that results in incomplete knowledge of whether genomes are partial or full-length. The presence of empty or partially full capsids is one factor that affects rAAV quality.

We developed a novel NGS library preparation method that allows us to identify and quantitate gaps by distinguishing pre-existing DNA from DNA added during NGS library preparation. During the repair process common to NGS library methods, the standard nucleotides dCTP and dATP are replaced with the modified nucleotides 4Me-dCTP and 6Me-dATP so that any new DNA includes stretches of modified bases while the pre-existing ssDNA consists of natural, unmodified bases. Using the Sequel II system, the modified bases can be distinguished from unmodified bases, enabling breakpoint identification at high resolution. This method was used on a vector known to break during packaging, a phenomenon that can occur in AAV vectors of all serotypes. The exact location and frequencies of breaks allowed us to alter nearby sequences and reduce the frequency of breakage. The resultant new designs provide a higher quality AAV vector that is less susceptible to partial genomes. Partially filled capsids have been an ongoing regulatory agency concern and this approach provides information that standard methods do not. This use of modified bases for localizing DNA breaks enables improved vector designs and provides better characterization metrics for AAV vectors, resulting in higher quality gene therapy vectors. The same approach can be used in other systems where knowledge of pre-existing sequence and structure is important and may be lost when DNA is repaired.