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Molecular Characterization of Precise *In Vivo* Targeted Gene Editing in Human Cells using AAVHSC15, a New AAV Derived from Hematopoietic Stem Cells (AAVHSC)

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Targeted gene integration via precise homologous recombination (HR)-based gene editing has the potential to correct genetic diseases. AAV (adeno-associated virus) is being used to target the underlying cause of disease by nuclease-free gene integration at a disease-causing locus. Rapid advancements in the application of AAV-mediated, non-nuclease *in vivo* targeted integration as a novel therapeutic modality should require precise characterization of the efficiency and the nature of the changes being introduced to the genome at the molecular level. Here we describe a framework for assessing *in vivo* AAV-mediated non-nuclease targeted integration using two orthogonal methods to measure on-target integration frequencies: a method to detect *de novo* mutations, and long-read sequencing technologies to query inverted terminal repeats (ITR) integrations. Although these methods require optimization for each target genomic loci, these methods can be applied to fully characterize any targeted HR-based integration. Here we used AAVHSC15, a vector targeting integration into the human phenylalanine hydroxylase gene *(PAH),* the causal gene underlying the autosomal recessive disorder phenylketonuria (PKU). In a humanized liver model of PKU, we demonstrated that the hematopoietic stem-cell derived AAVHSC15 targeting vector achieved non-nuclease mediated gene integration at a frequency of 6% based on two independent assays measuring the integration into the PAH locus. Characterization with these molecular methods establishes *in vivo* non-nuclease mediated integration at levels higher than previously reported with AAV. In addition, no *de novo* mutations or ITR integrations at or above a lower limit of detection of 0.5%. Variant correction analysis aligns with previous findings demonstrating HR as the mechanism of AAV-mediated, targeted integration. These methods characterize targeted integration, or *in vivo* editing events, and measure editing precision at the molecular level, offering a more robust scientific approach for confirmation of editing and potential comparability across platforms.