# AAV vector integration into CRISPR-induced DNA breaks

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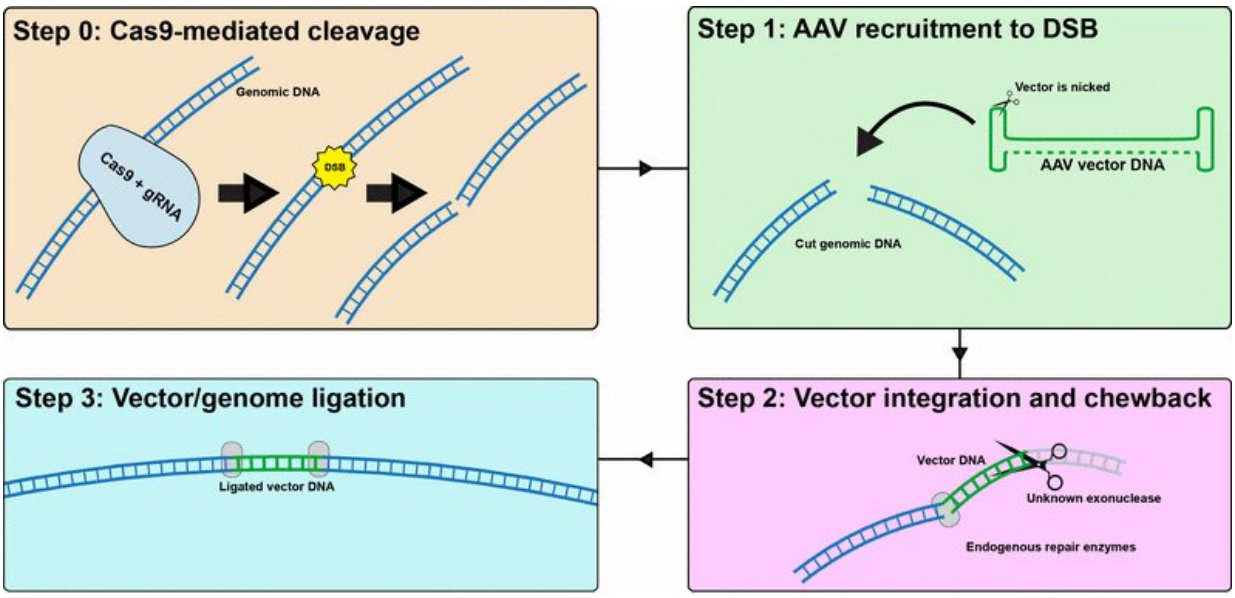


Figure 1 (Credit: Killian Hanlon) Hypothesis of AAV vector DNA integration after CRISPR-mediated DNA cleavage: after a CRISPR-induced DSB, a broken ITR gets captured by the genome; then, the vector is chewed back in some fashion, possibly by exonucleases; finally, repair enzymes facilitate the complete integration of the vector. Credit: Institute of Molecular and Clinical Ophthalmology Basel

To design safe clinical trials, it is crucial to better understand and predict gene editing outcomes in preclinical studies. Bence György and collaborators have shown that adeno-associated viruses (AAVs) can

stably integrate into CRISPR-Cas9-induced double-strand breaks, in up to almost half of the therapeutically targeted cells, in vitro and in vivo in mice. The team also showed that CRISPR did not cause an increase in genome-wide integration of AAV, but only at the CRISPR-cut site.

"This study helps to better understand the genomic consequences of therapeutic genome editing. It is currently not clear whether this site-specific AAV integration influences the safety profile of gene editing enzymes. We do not expect this, because as long as gRNAs are well-designed there is no increase of AAV integration at off-target sites. We will elaborate on these observations in further studies," says Bence György.

## Widely used tools for genome editing and gene therapy

In the seven years since CRISPR was brought to the forefront of molecular biology, it has emerged as one of the most widely used (and hotly anticipated) tools for genome editing and gene therapy, with human trials now beginning.

In order to deliver this powerful molecular machine to target cells, many researchers use AAVs. This small virus has become the most widely used vector for delivering gene therapy, with three AAV-based therapies now on the market. It allows for the safe and stable expression of therapeutic genes in a wide array of cells types by delivering a small payload of DNA into cells' nuclei.

## AAV vector integration after CRISPR intervention

AAV used for gene therapy lacks dedicated machinery to integrate into the genome. Therefore therapeutic AAV integrates at a very low

frequency of