

**Intra-embryo Gene Cassette Knockin by CRISPR/Cas9-Mediated Genome Editing with Adeno-Associated Viral Vector.**

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**Public Summary:**

Efficient genetic editing of animal embryos for research has the potential to speed up scientific discovery and develop new models to study disease. Here, we developed an easy and efficient method to insert DNA sequences into the genome of animal embryos by combining CRISPR/Cas9 gene editing approaches with recombinant adeno-associated virus transduction.

**Scientific Abstract:**

Intra-embryo genome editing by CRISPR/Cas9 enables easy generation of gene-modified animals by non-homologous end joining (NHEJ)-mediated frameshift mutations or homology-directed repair (HDR)-mediated point mutations. However, large modifications, such as gene replacement or gene fusions, are still difficult to introduce in embryos without costly micromanipulators. Moreover, micromanipulation techniques for intra-embryo genome editing have been established in only a small set of animals. To overcome these issues, we developed a method of large-fragment DNA knockin without micromanipulation. In this study, we successfully delivered the knockin donor DNA into zygotes by adeno-associated virus (AAV) without removing the zona pellucida, and we succeeded in both large-DNA fragment knockin and whole exon exchange with electroporation of CRISPR/Cas9 ribonucleoprotein. By this method, we can exchange large DNA fragments conveniently in various animal species without micromanipulation.

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