

Adar Cas9-KO Strategy

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Overview

Target Gene Name

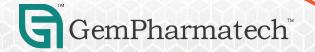
• Adar

Project Type

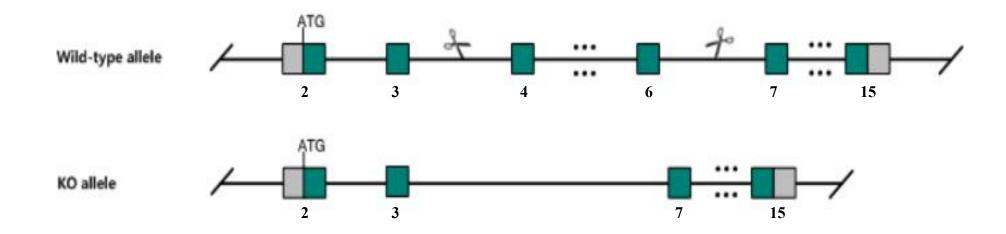
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy







Technical Information

- The *Adar* gene has 9 transcripts. According to the structure of *Adar* gene, exon4-exon6 of *Adar*-202 (ENSMUST00000098924.9) transcript is recommended as the knockout region. The region contains 473bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Adar* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and ontarget amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



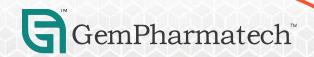
Gene Information

Adar adenosine deaminase, RNA-specific [Mus musculus (house mouse)]

Gene ID: 56417, updated on 28-Mar-2019



Source: https://www.ncbi.nlm.nih.gov/

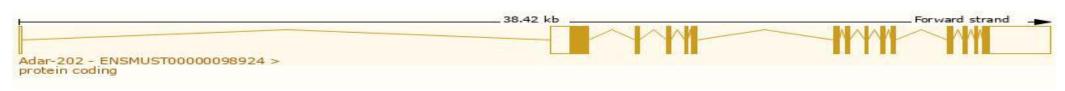


Transcript Information

The gene has 9 transcripts, all transcripts are shown below:

Transcript ID 🔷	Name A	bp 🌲	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000029563.14	Adar-201	3753	<u>1152aa</u>	Protein coding	CCDS17513 €	Q99MU3-2₽	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000098924.9	Adar-202	5909	930aa	Protein coding	CCDS17514 &	Q99MU3-5@	GENCODE basic TSL:1
ENSMUST00000107405.6	Adar-203	5871	<u>1178aa</u>	Protein coding	CCDS50963 ₺	Q99MU3-1@	Ensembl Canonical GENCODE basic TSL:1
ENSMUST00000118341.6	Adar-204	2399	660aa	Protein coding		Q99MU3-4₽	GENCODE basic TSL:1
ENSMUST00000121094.8	Adar-205	2321	634aa	Protein coding		Q99MU3-3₺	GENCODE basic TSL:1
ENSMUST00000123691.2	Adar-206	519	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000131030.2	Adar-207	481	No protein	Retained intron		-	TSL:2
ENSMUST00000150637.2	Adar-208	708	No protein	Retained intron		-	TSL:2
ENSMUST00000197253.2	Adar-209	1668	No protein	Retained intron		2	TSL:NA

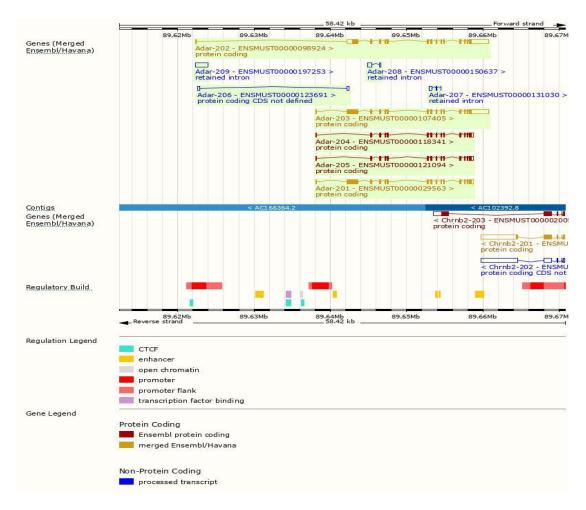
The strategy is based on the design of *Adar*-202 transcript, the transcription is shown below:



Source: https://www.ensembl.org



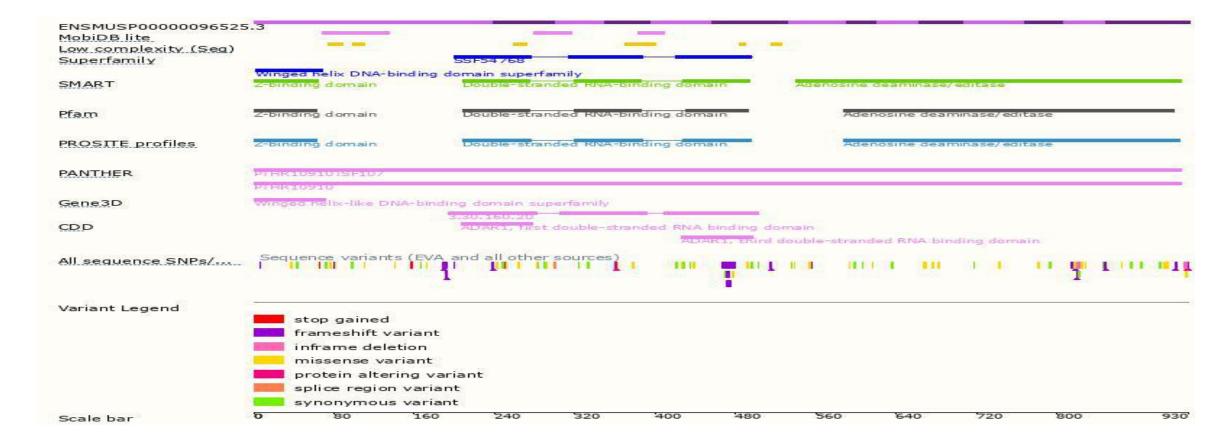
Genomic Information





Source: : https://www.ensembl.org

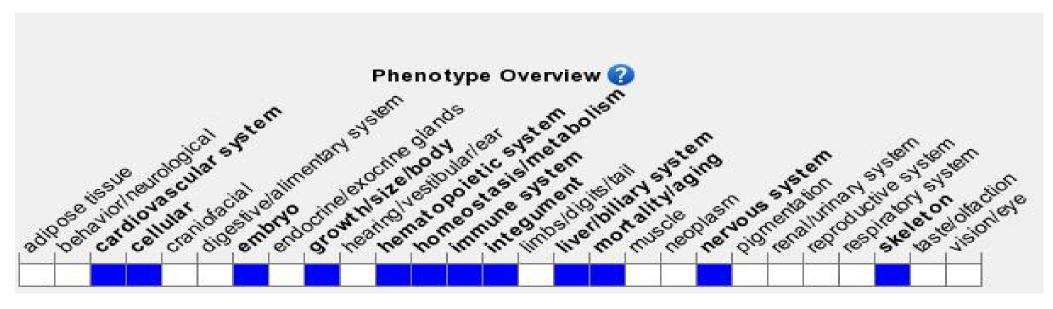
Protein Information



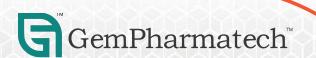


Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



• Homozygous null mice die during gestation. Inactivation of this locus has been associated with increased apoptosis and, in some lines, defects in both primitive and definitive hematopoiesis. Compound heterozygosity for the p.P195A mutation and KO affects liver, kidney and spleen, leading to low weight and severely shortening survival.



Source: https://www.informatics.jax.org

Important Information

- *Adar* is located on Chr3. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

