

Aars1 Cas9-CKO Strategy

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Overview

Target Gene Name

- Aars1

Project Type

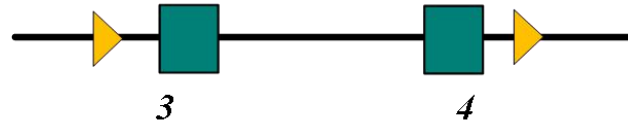
- Cas9-CKO

Genetic Background

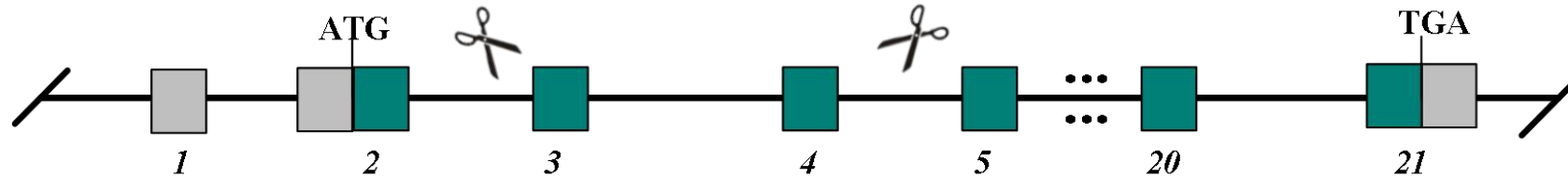
- C57BL/6JGpt

Strain Strategy

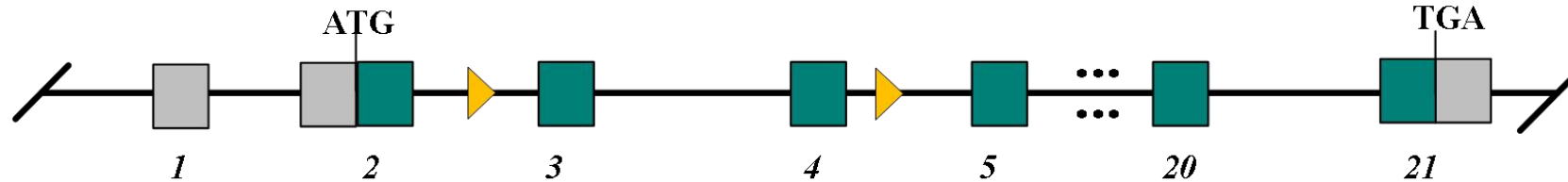
Donor and CRISPR-Cas9 System



Wild-type allele

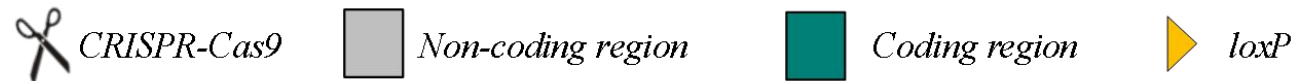
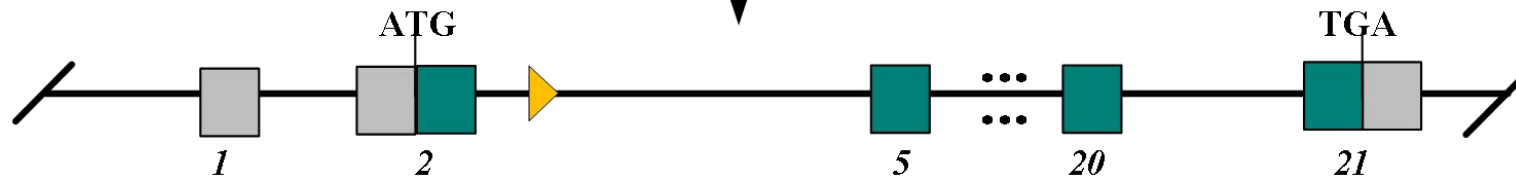


Conditional KO allele



↓ Cre

KO allele



Schematic representation of CRISPR-Cas9 engineering used to edit the *Aars1* gene.

Technical Information

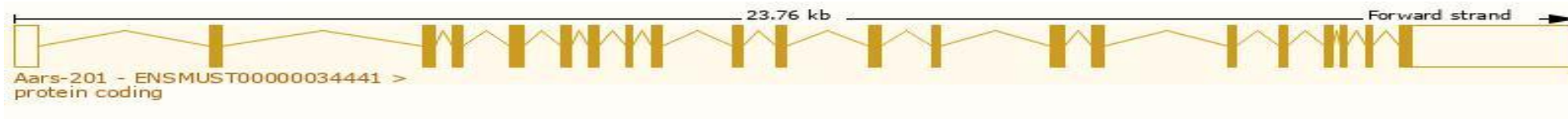
- The *Aars1* gene has 7 transcripts. According to the structure of *Aars1* gene, exon 3-exon 4 of *Aars-201* (ENSMUST00000034441.8) transcript is recommended as the knockout region. The region contains 335 bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Aars1* gene. The brief process is as follows: CRISPR-Cas9 system and Donor 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 on-target 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.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Transcript Information

The gene has 7 transcripts, all transcripts are shown below:

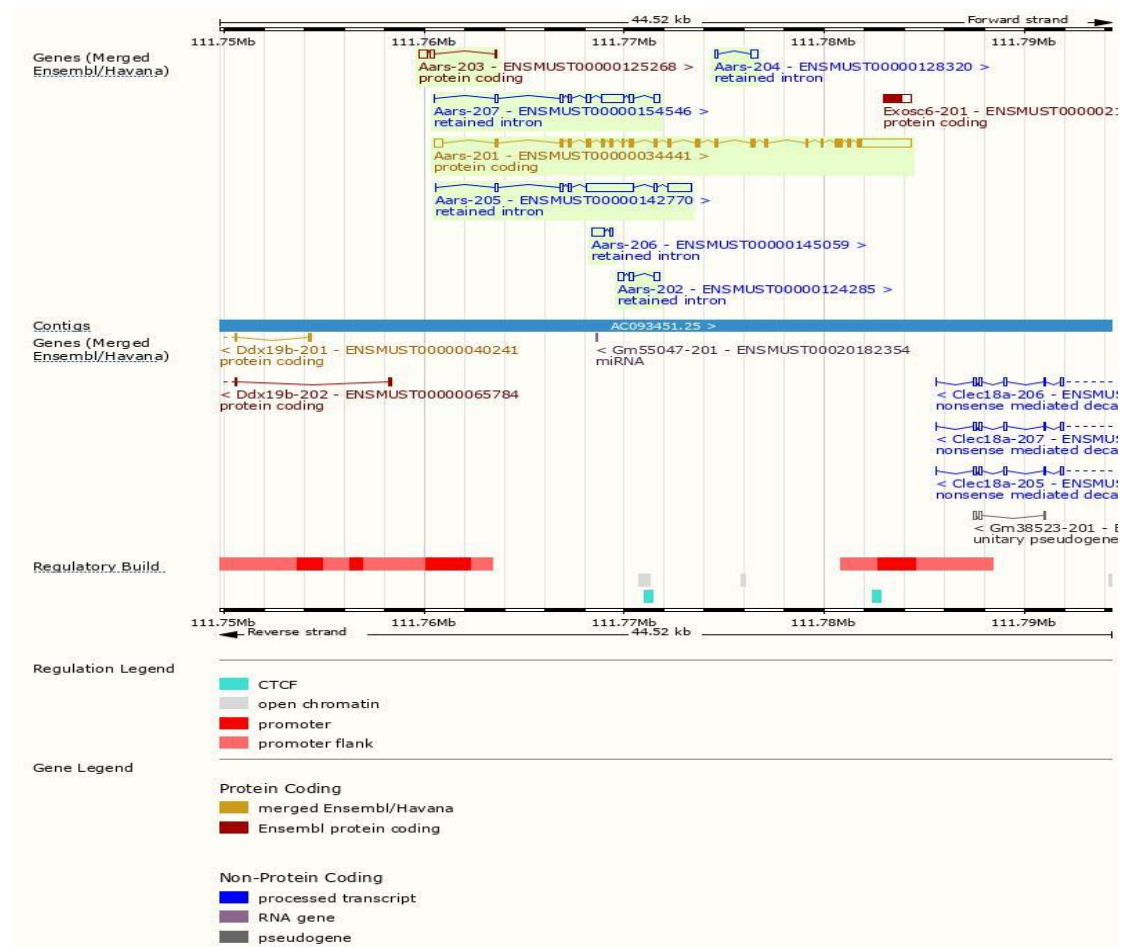
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000034441.8	Aars-201	5750	968aa	Protein coding	CCDS40478	Q8BGQ7	Ensembl Canonical Gencode basic APPRIS P1 TSL:1
ENSMUST00000125268.2	Aars-203	709	7aa	Protein coding		A0A1Y7VE86	TSL:3 CDS 3' incomplete
ENSMUST00000142770.2	Aars-205	4187	No protein	Retained intron		-	TSL:1
ENSMUST00000154546.8	Aars-207	2221	No protein	Retained intron		-	TSL:1
ENSMUST00000145059.2	Aars-206	807	No protein	Retained intron		-	TSL:1
ENSMUST00000124285.2	Aars-202	659	No protein	Retained intron		-	TSL:1
ENSMUST00000128320.2	Aars-204	426	No protein	Retained intron		-	TSL:1

The strategy is based on the design of *Aars-201* transcript, the transcription is shown below:

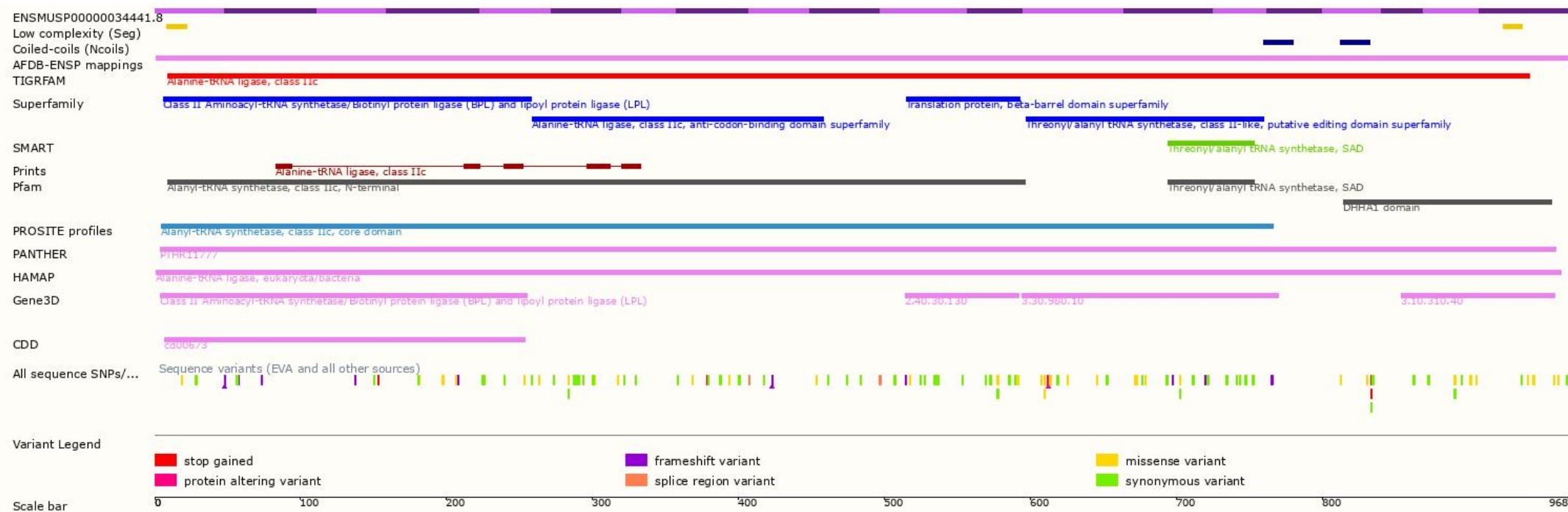


Source: <https://www.ensembl.org>

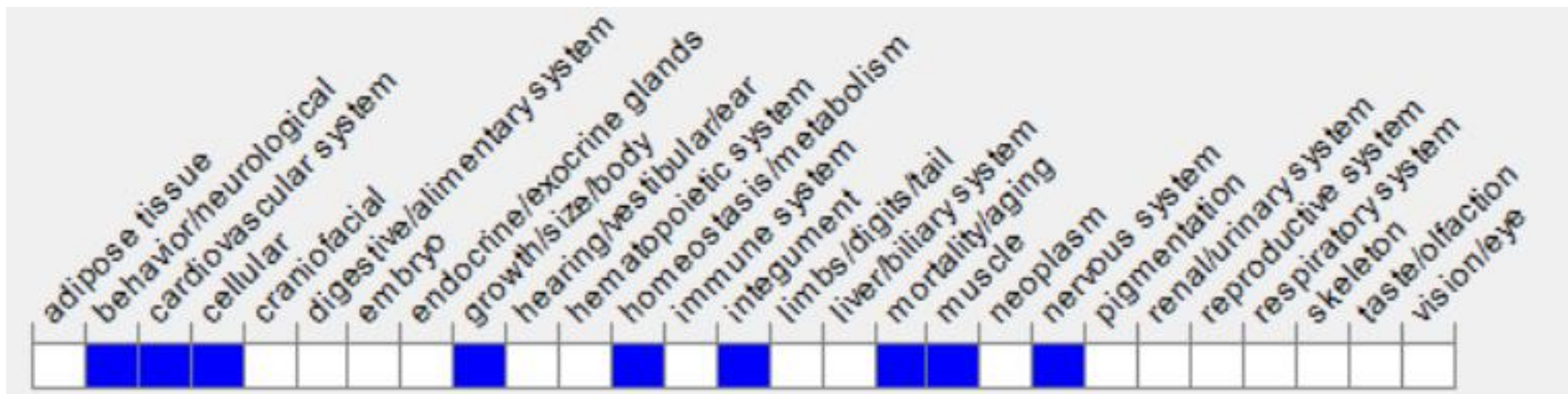
Genomic Information



Protein Information



Mouse Phenotype Information (MGI)



Homozygotes for a spontaneous point mutation (A734E) exhibit a rough sticky coat, follicular dystrophy, patchy hair loss, progressive ataxia, and Purkinje cell degeneration. Homozygotes for a targeted point mutation (C723A) die by mid-gestation, while heterozygotes show mild Purkinje cell loss.

Important Information

- According to the MGI information, homozygotes for a spontaneous point mutation (A734E) of *Aars1* exhibit a rough sticky coat, follicular dystrophy, patchy hair loss, progressive ataxia, and Purkinje cell degeneration. Homozygotes for a targeted point mutation (C723A) die by mid-gestation, while heterozygotes show mild Purkinje cell loss.
- The knockout region is located about 0.9 kb at the 3' end of the *Gm55047* miRNA and may affect its transcription regulation.
- The knockout region is located about 8 kb at the 5' end of the *Ddx19b* and may affect its transcription initiation.
- The effect of the knock-out region on the *Aars*-203 transcript is unknown.
- *Aars1* is located on Chr8. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.