

# *Cars2* Cas9-CKO Strategy

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**Reviewer:**

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# Project Overview

**Project Name**

*Cars2*

**Project type**

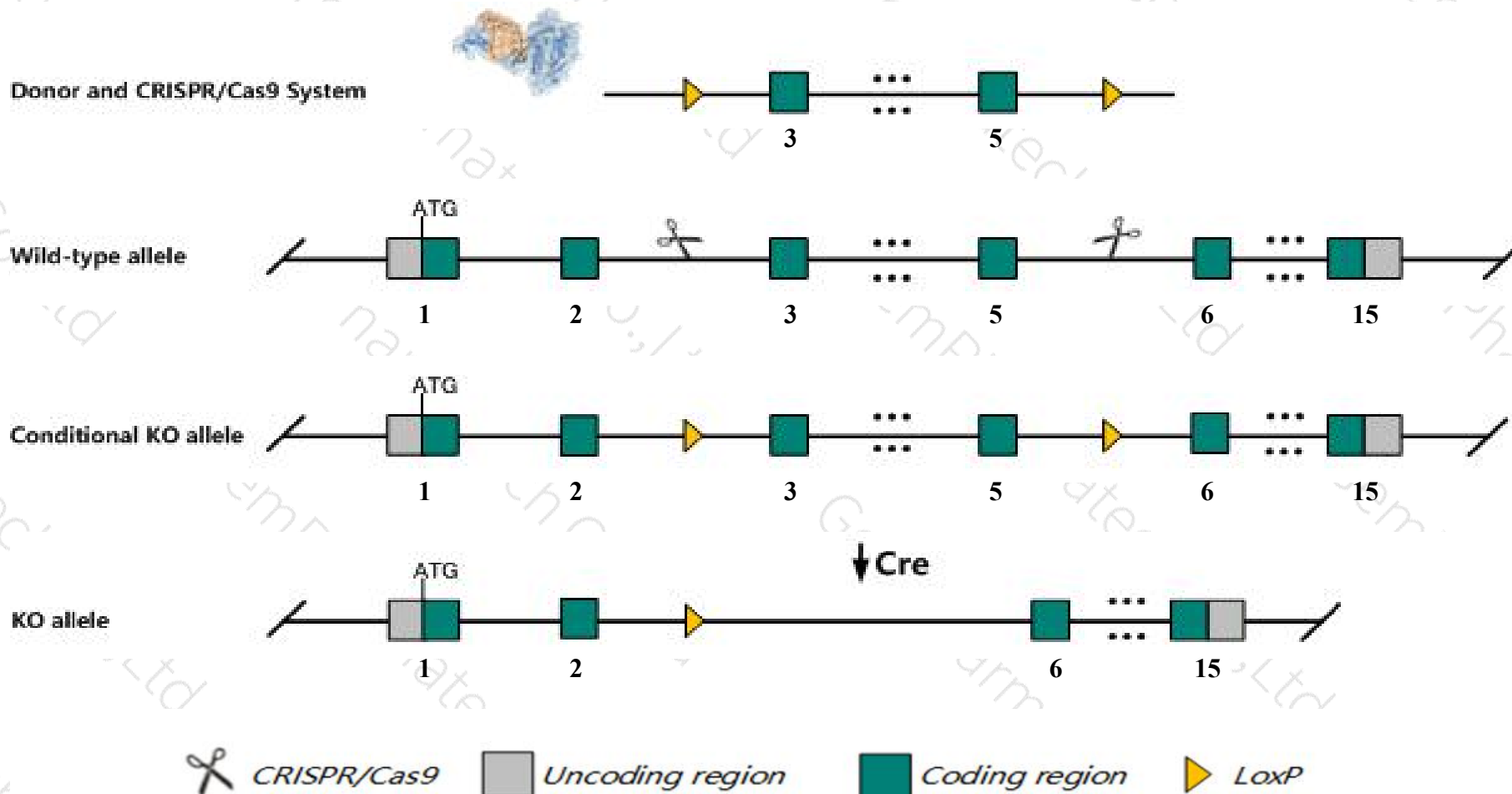
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cars2* gene. The schematic diagram is as follows:



- The *Cars2* gene has 12 transcripts. According to the structure of *Cars2* gene, exon3-exon5 of *Cars2-201* (ENSMUST00000049461.6) transcript is recommended as the knockout region. The region contains 296bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cars2* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

- According to the existing MGI data, mice homozygous for an enu-induced allele develop induced hyperactivity followed by head bobbing and tremors.
- Transcript 209 may not be affected. The effect of transcript 210 is unknown.
- Transcript 212 may be destroyed.
- The *Cars2* gene is located on the Chr8. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes 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 existing technological level.



# Gene information (NCBI)

## Cars2 cysteinyl-tRNA synthetase 2 (mitochondrial)(putative) [Mus musculus (house mouse)]

Gene ID: 71941, updated on 20-Mar-2020

### Summary



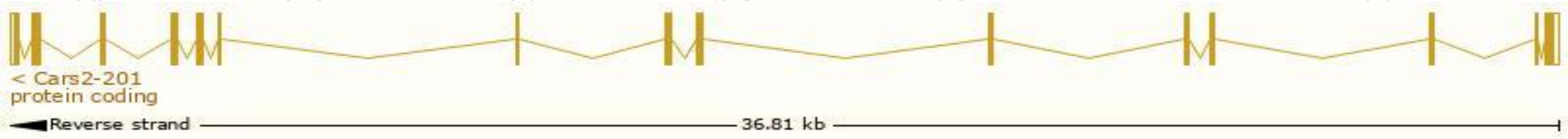
<b>Official Symbol</b>	Cars2 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	cysteinyl-tRNA synthetase 2 (mitochondrial)(putative) provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1919191</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000056228</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	2310051N18Rik, 2410044A07Rik, D530030H10Rik, cysRS
<b>Expression</b>	Ubiquitous expression in adrenal adult (RPKM 22.7), ovary adult (RPKM 18.5) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

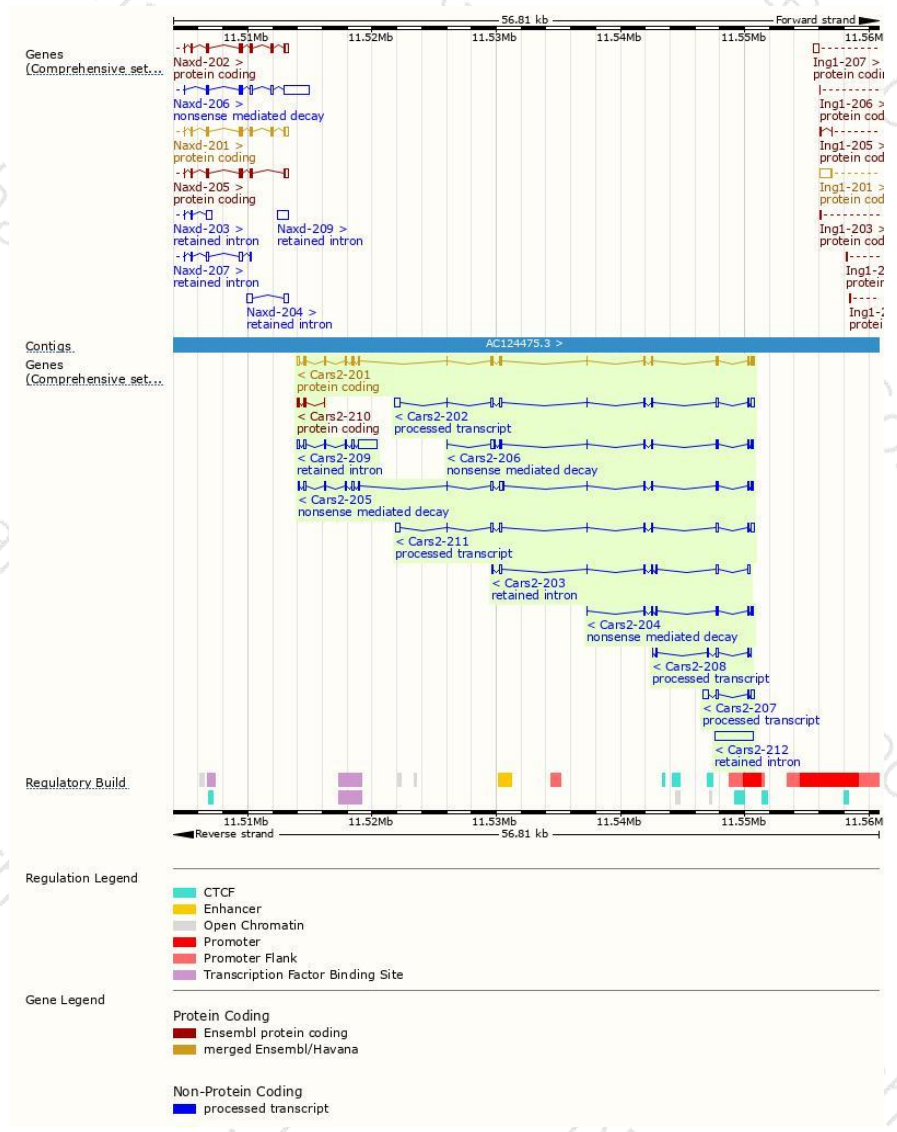
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cars2-201	<a href="#">ENSMUST00000049461.6</a>	1902	<a href="#">552aa</a>	Protein coding	<a href="#">CCDS52478</a>	<a href="#">G3X975</a>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Cars2-210	<a href="#">ENSMUST00000211395.1</a>	424	<a href="#">128aa</a>	Protein coding	-	<a href="#">A0A1B0GSP2</a>	CDS 5' incomplete TSL:5
Cars2-205	<a href="#">ENSMUST00000210478.1</a>	2007	<a href="#">226aa</a>	Nonsense mediated decay	-	<a href="#">A0A1B0GSW7</a>	TSL:1
Cars2-206	<a href="#">ENSMUST00000210599.1</a>	1107	<a href="#">270aa</a>	Nonsense mediated decay	-	<a href="#">A0A1B0GR87</a>	TSL:1
Cars2-204	<a href="#">ENSMUST00000209676.1</a>	704	<a href="#">135aa</a>	Nonsense mediated decay	-	<a href="#">A0A1B0GRF1</a>	TSL:3
Cars2-202	<a href="#">ENSMUST00000209218.1</a>	1375	No protein	Processed transcript	-	-	TSL:1
Cars2-211	<a href="#">ENSMUST00000211406.1</a>	1352	No protein	Processed transcript	-	-	TSL:1
Cars2-207	<a href="#">ENSMUST00000210845.1</a>	826	No protein	Processed transcript	-	-	TSL:1
Cars2-208	<a href="#">ENSMUST00000211161.1</a>	354	No protein	Processed transcript	-	-	TSL:5
Cars2-212	<a href="#">ENSMUST00000211734.1</a>	3135	No protein	Retained intron	-	-	TSL:NA
Cars2-209	<a href="#">ENSMUST00000211172.1</a>	2212	No protein	Retained intron	-	-	TSL:5
Cars2-203	<a href="#">ENSMUST00000209236.1</a>	821	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Cars2-201* transcript,the transcription is shown below:

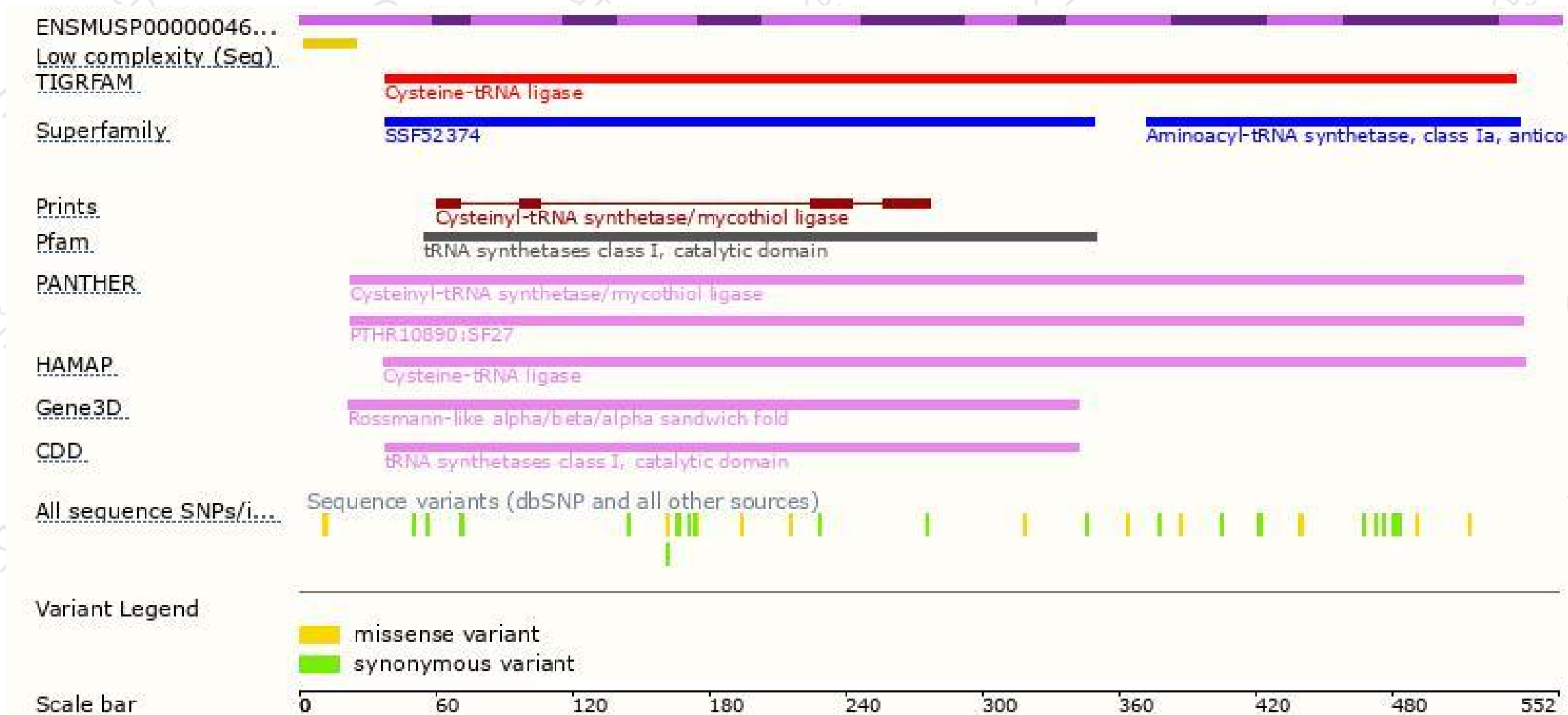


# Genomic location distribution



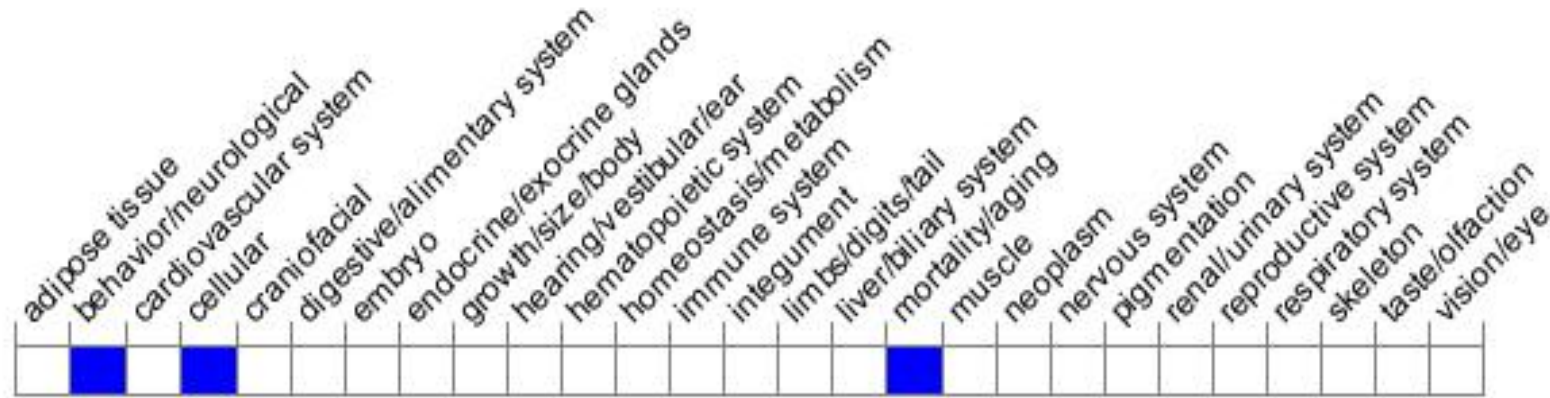


# Protein domain



# Mouse phenotype description(MGI)

Phenotype Overview



*Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).*

According to the existing MGI data, mice homozygous for an ENU-induced allele develop induced hyperactivity followed by head bobbing and tremors.

If you have any questions, you are welcome to inquire.

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