

Madd Cas9-CKO Strategy

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Project Overview

Project Name

Madd

Project type

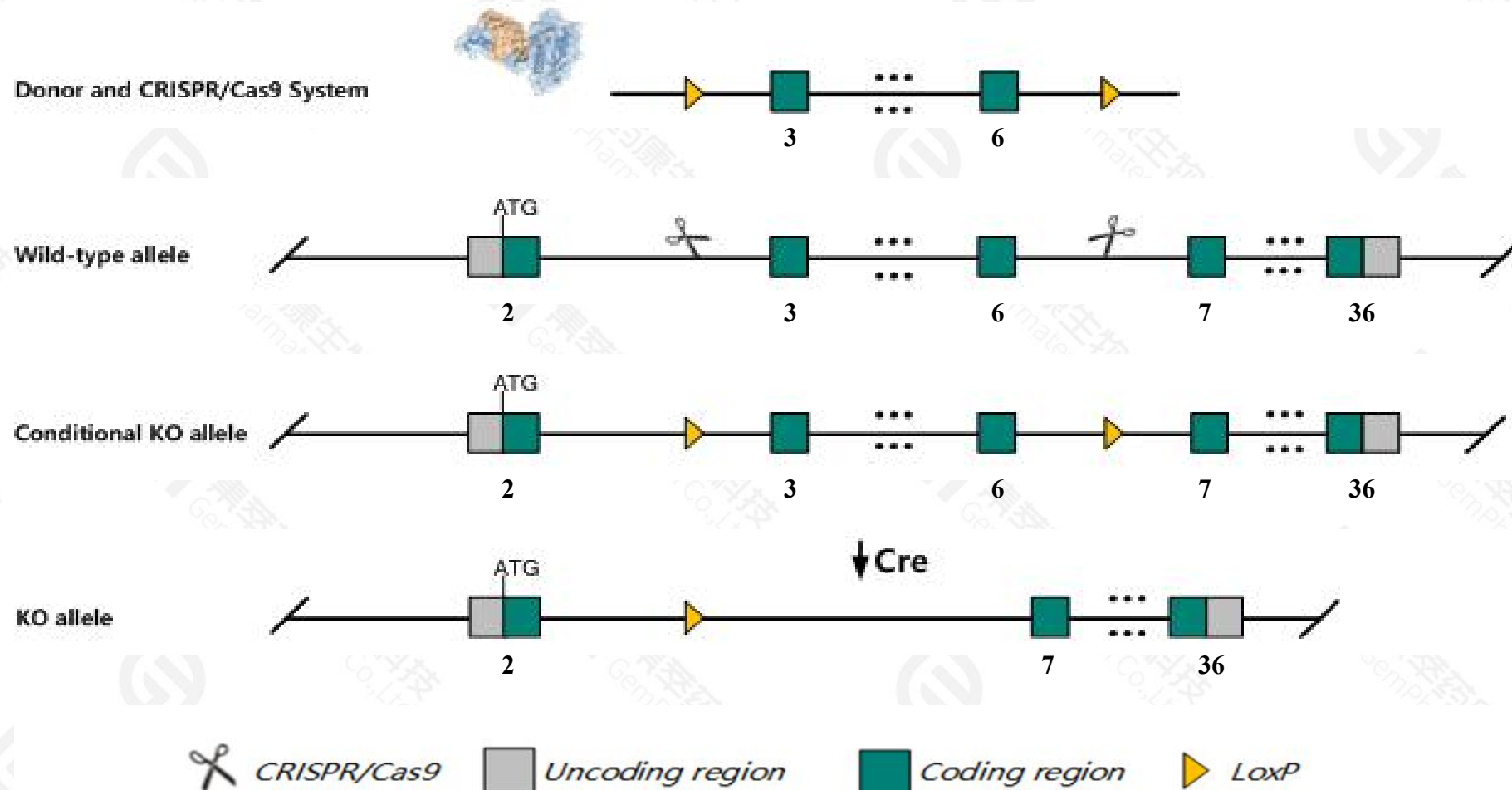
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Madd* gene has 28 transcripts. According to the structure of *Madd* gene, exon3-exon6 of *Madd*-202(ENSMUST00000066473.12) transcript is recommended as the knockout region. The region contains 1147bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Madd* 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 a knock-out allele die shortly after birth due to respiratory failure, are hyporesponsive to tactile stimuli, and exhibit defects in neurotransmitter release with impaired synaptic vesicle trafficking and depletion of synaptic vesicles at the neuromuscular junction.
- The *Madd* gene is located on the Chr2. 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)

Madd MAP-kinase activating death domain [Mus musculus (house mouse)]

Gene ID: 228355, updated on 17-Feb-2021

Summary



Official Symbol Madd provided by [MGI](#)

Official Full Name MAP-kinase activating death domain provided by [MGI](#)

Primary source [MGI:MGI:2444672](#)

See related [Ensembl:ENSMUSG00000040687](#)

Gene type protein coding

RefSeq status VALIDATED

Organism [Mus musculus](#)

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as 9630059K23Rik, IG20

Expression Broad expression in cerebellum adult (RPKM 20.7), cortex adult (RPKM 19.1) and 27 other tissues [See more](#)

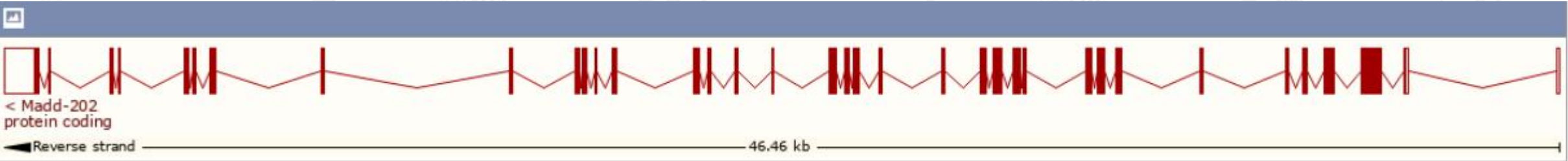
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

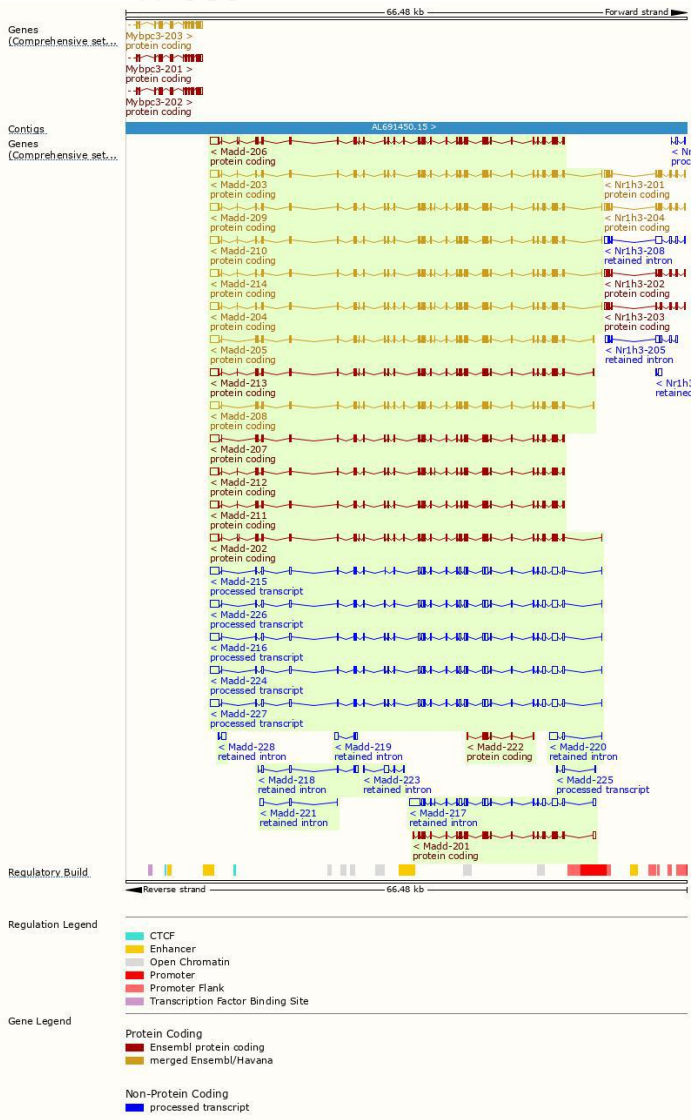
The gene has 28 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Madd-205	ENSMUST00000099723.9	5977	1577aa	Protein coding	CCDS50638		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Madd-202	ENSMUST00000066473.12	5974	1627aa	Protein coding	CCDS50635		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-206	ENSMUST00000099725.9	5905	1627aa	Protein coding	CCDS50635		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-208	ENSMUST00000111370.9	5879	1558aa	Protein coding	CCDS38178		TSL:1 , GENCODE basic , APPRIS P3 ,
Madd-204	ENSMUST00000077941.13	5990	1632aa	Protein coding	-		TSL:2 , GENCODE basic , APPRIS ALT2 ,
Madd-213	ENSMUST00000111376.8	5927	1602aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-214	ENSMUST00000111381.9	5873	1593aa	Protein coding	-		TSL:2 , GENCODE basic , APPRIS ALT2 ,
Madd-209	ENSMUST00000111371.8	5816	1574aa	Protein coding	-		TSL:2 , GENCODE basic , APPRIS ALT2 ,
Madd-210	ENSMUST00000111372.8	5813	1573aa	Protein coding	-		TSL:2 , GENCODE basic , APPRIS ALT2 ,
Madd-203	ENSMUST00000075269.10	5756	1554aa	Protein coding	-		TSL:2 , GENCODE basic , APPRIS ALT2 ,
Madd-212	ENSMUST00000111375.8	5706	1562aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-211	ENSMUST00000111373.8	5641	1541aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-207	ENSMUST00000111369.8	5582	1475aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-201	ENSMUST00000066420.12	3753	1115aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Madd-222	ENSMUST00000140600.2	791	263aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Madd-224	ENSMUST00000150461.8	5667	No protein	Processed transcript	-		TSL:2 ,
Madd-216	ENSMUST00000125321.8	5653	No protein	Processed transcript	-		TSL:5 ,
Madd-227	ENSMUST00000154028.8	5608	No protein	Processed transcript	-		TSL:5 ,
Madd-226	ENSMUST00000153688.8	5581	No protein	Processed transcript	-		TSL:5 ,
Madd-215	ENSMUST00000125227.8	5480	No protein	Processed transcript	-		TSL:5 ,
Madd-225	ENSMUST00000150517.2	260	No protein	Processed transcript	-		TSL:3 ,
Madd-217	ENSMUST00000130395.8	4655	No protein	Retained intron	-		TSL:1 ,
Madd-220	ENSMUST00000135715.8	1092	No protein	Retained intron	-		TSL:2 ,
Madd-218	ENSMUST00000130591.8	843	No protein	Retained intron	-		TSL:3 ,
Madd-223	ENSMUST00000146097.2	737	No protein	Retained intron	-		TSL:3 ,
Madd-219	ENSMUST00000132791.2	675	No protein	Retained intron	-		TSL:3 ,
Madd-228	ENSMUST00000156368.2	600	No protein	Retained intron	-		TSL:2 ,
Madd-221	ENSMUST00000135910.2	562	No protein	Retained intron	-		TSL:3 ,

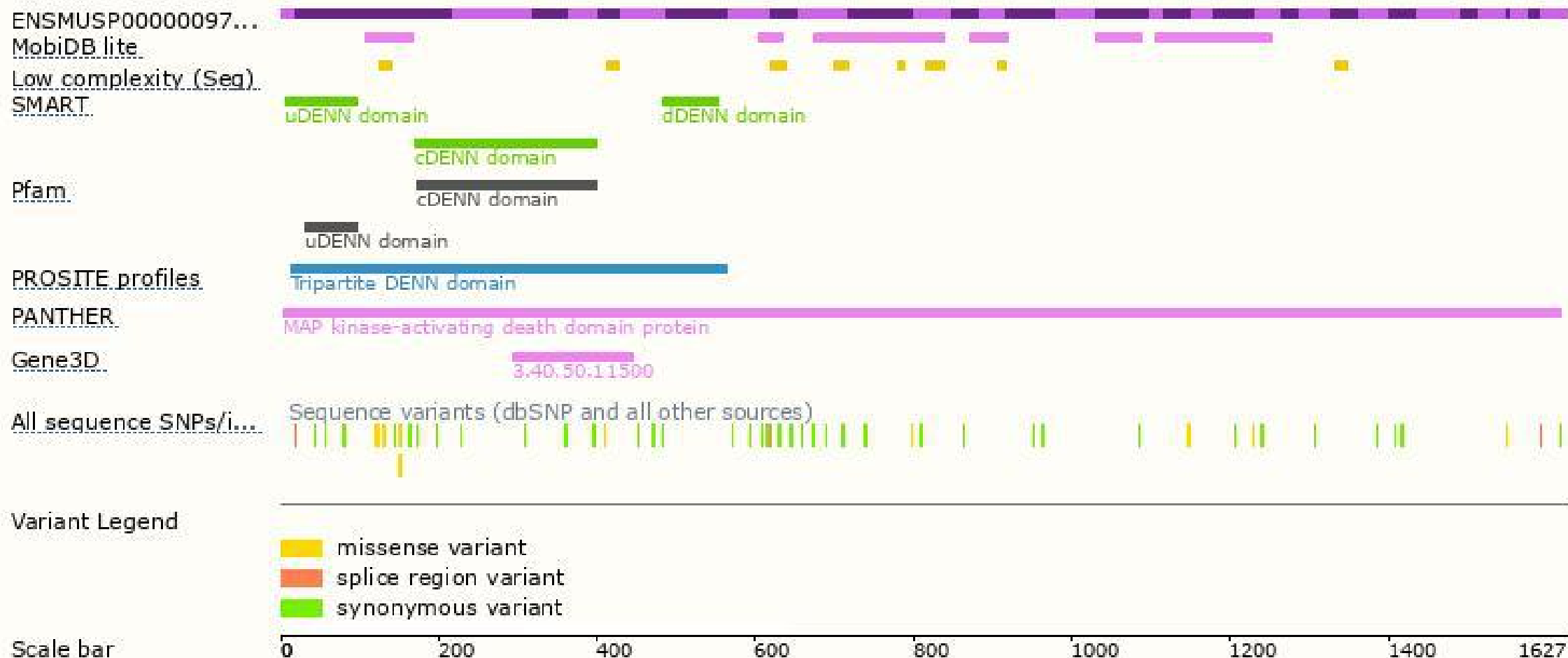
The strategy is based on the design of *Madd-202* transcript,the transcription is shown below:



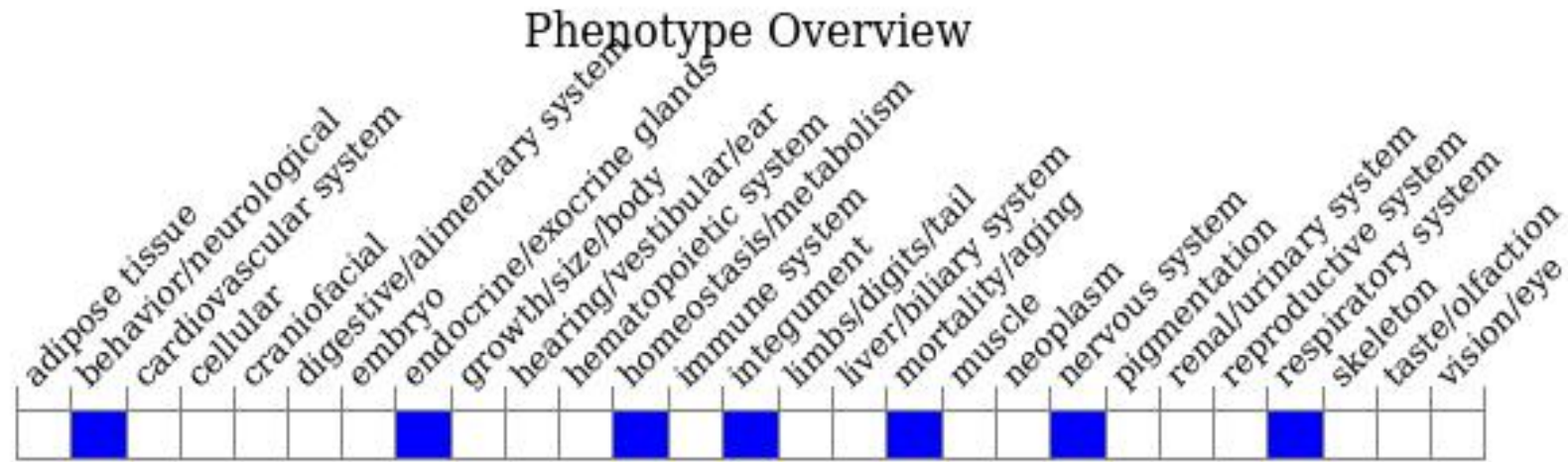
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



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 a knock-out allele die shortly after birth due to respiratory failure, are hyporesponsive to tactile stimuli, and exhibit defects in neurotransmitter release with impaired synaptic vesicle trafficking and depletion of synaptic vesicles at the neuromuscular junction.

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