

# *Myo5a* Cas9-CKO Strategy

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**Reviewer: Lingyan Wu**

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# Project Overview

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**Project Name**

*Myo5a*

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**Project type**

**Cas9-CKO**

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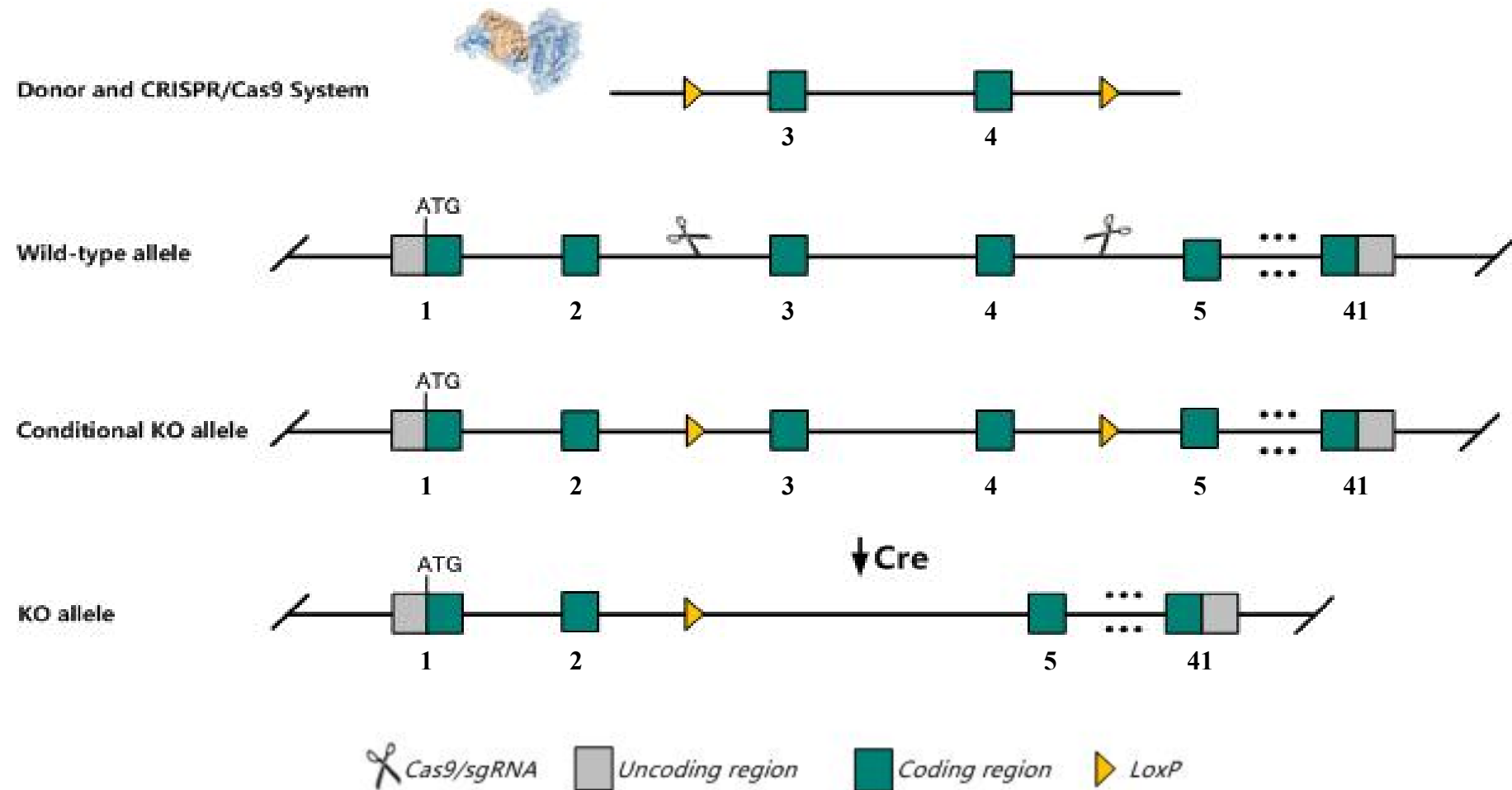
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myo5a* gene. The schematic diagram is as follows:



The *Myo5a* gene has 14 transcripts. According to the structure of *Myo5a* gene, exon3-exon4 of *Myo5a-201*(ENSMUST00000123128.8) transcript is recommended as the knockout region. The region contains 317bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Myo5a* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor was constructed. Cas9, sgRNA 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 was 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, mutations in this gene result in diluted coat color, behavioral deficits including opisthotonus, and postnatal or premature death.

Transcript *Myo5a*-203&204&210 may not be affected.

The *Myo5a* gene is located on the Chr9. 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.

**Myo5a myosin VA [Mus musculus (house mouse)]**

Gene ID: 17918, updated on 17-Dec-2020

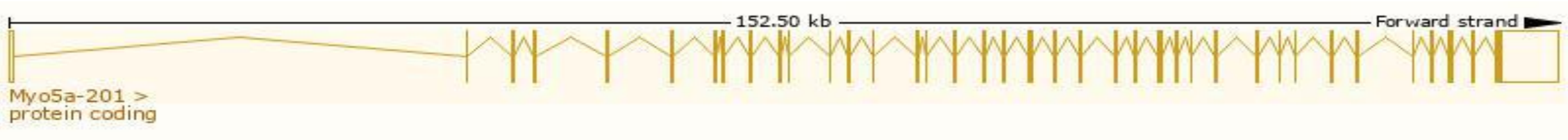
**Summary****Official Symbol** Myo5a provided by [MGI](#)**Official Full Name** myosin VA provided by [MGI](#)**Primary source** [MGI:MGI:105976](#)**See related** [Ensembl:ENSMUSG00000034593](#)**Gene type** protein coding**RefSeq status** VALIDATED**Organism** [Mus musculus](#)**Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus**Also known as** 9630007J19Rik, AI413174, AI661011, Db, Dbv, M, MVa, My, Myo, Myo5, MyoVA, Sev-1, d, d-120J, f, flail, flr**Expression** Broad expression in cortex adult (RPKM 22.9), frontal lobe adult (RPKM 21.0) and 18 other tissues [See more](#)**Orthologs** [human](#) [all](#)

# Transcript information      Ensembl

The gene has 14 transcripts,all transcripts are shown below:

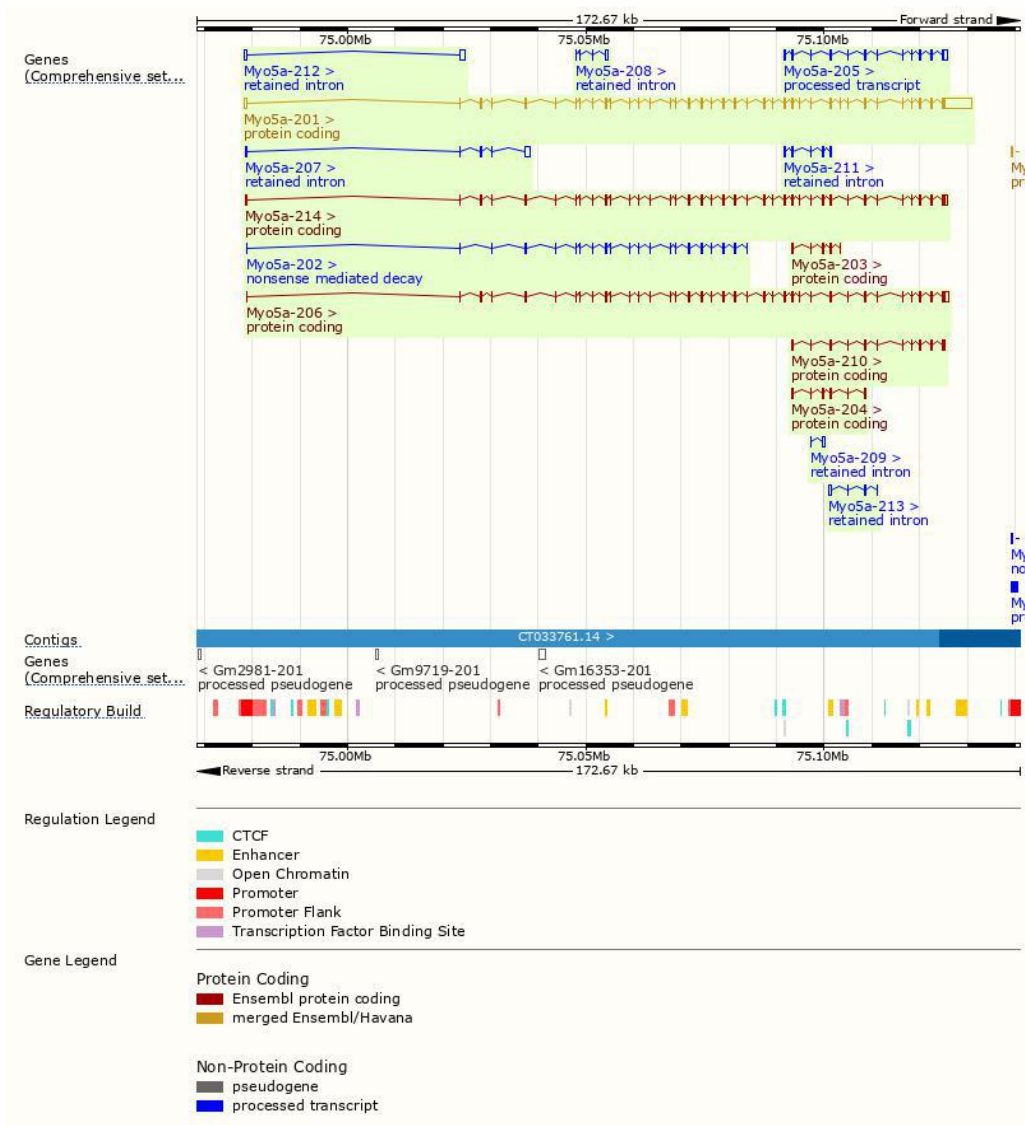
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myo5a-201	<a href="#">ENSMUST00000123128.8</a>	11702	<a href="#">1853aa</a>	Protein coding	<a href="#">CCDS52860</a>		TSL:5 , GENCODE basic ,
Myo5a-206	<a href="#">ENSMUST00000136731.8</a>	6480	<a href="#">1828aa</a>	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Myo5a-214	<a href="#">ENSMUST00000155282.9</a>	6372	<a href="#">1855aa</a>	Protein coding	-		TSL:5 , GENCODE basic , APPRIS P5 ,
Myo5a-210	<a href="#">ENSMUST00000148144.8</a>	1758	<a href="#">585aa</a>	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Myo5a-203	<a href="#">ENSMUST00000129281.8</a>	684	<a href="#">228aa</a>	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Myo5a-204	<a href="#">ENSMUST00000130384.2</a>	650	<a href="#">217aa</a>	Protein coding	-		CDS 5' and 3' incomplete , TSL:3 ,
Myo5a-202	<a href="#">ENSMUST00000123531.8</a>	3002	<a href="#">47aa</a>	Nonsense mediated decay	-		TSL:5 ,
Myo5a-205	<a href="#">ENSMUST00000136604.8</a>	2640	No protein	Processed transcript	-		TSL:1 ,
Myo5a-207	<a href="#">ENSMUST00000136871.2</a>	1919	No protein	Retained intron	-		TSL:1 ,
Myo5a-212	<a href="#">ENSMUST00000149823.2</a>	1768	No protein	Retained intron	-		TSL:1 ,
Myo5a-211	<a href="#">ENSMUST00000149032.2</a>	801	No protein	Retained intron	-		TSL:3 ,
Myo5a-208	<a href="#">ENSMUST00000142181.2</a>	679	No protein	Retained intron	-		TSL:3 ,
Myo5a-209	<a href="#">ENSMUST00000143355.2</a>	671	No protein	Retained intron	-		TSL:3 ,
Myo5a-213	<a href="#">ENSMUST00000153404.2</a>	651	No protein	Retained intron	-		TSL:3 ,

The strategy is based on the design of *Myo5a-201* transcript,the transcription is shown below:



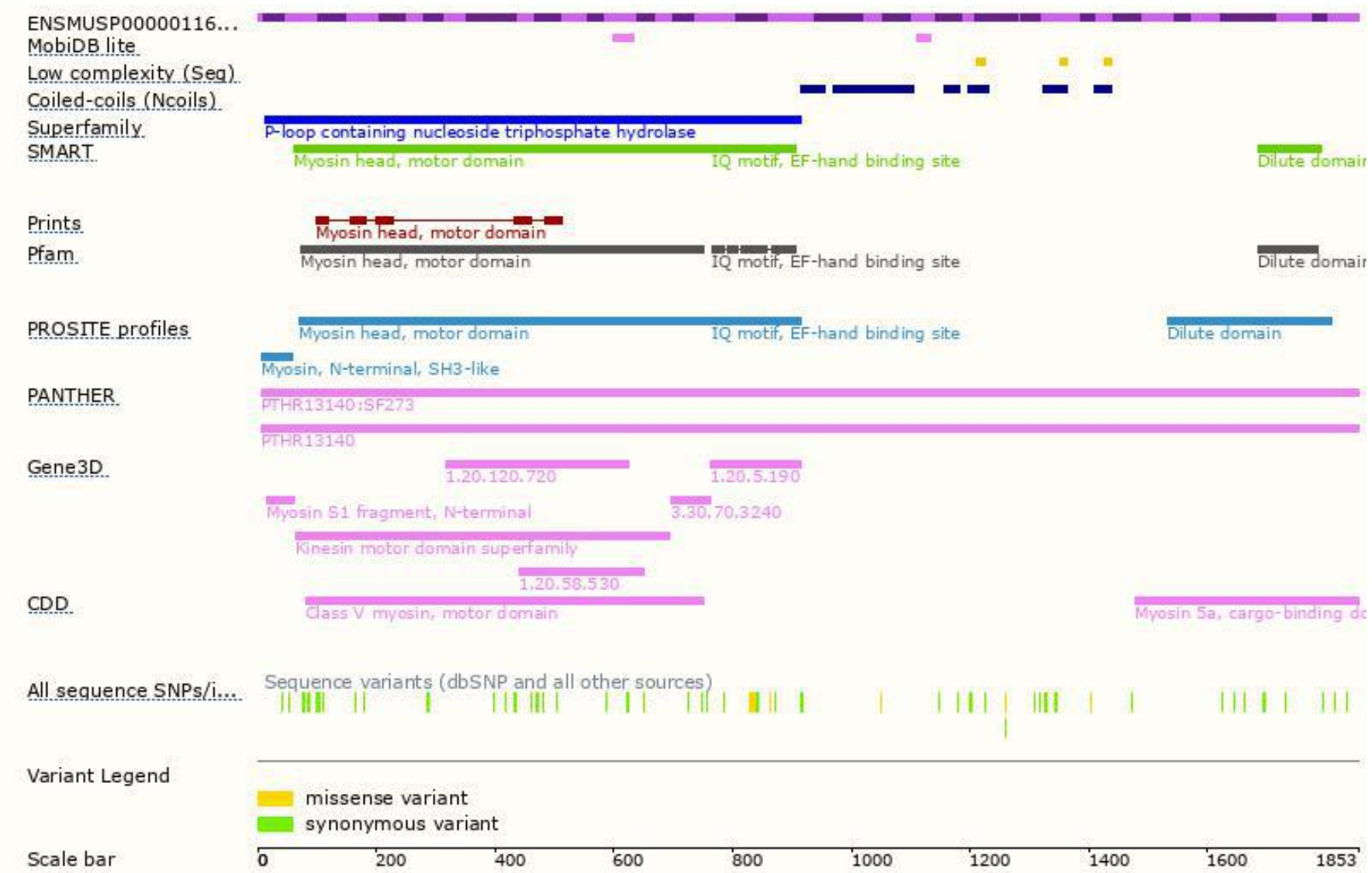


# Genomic location distribution

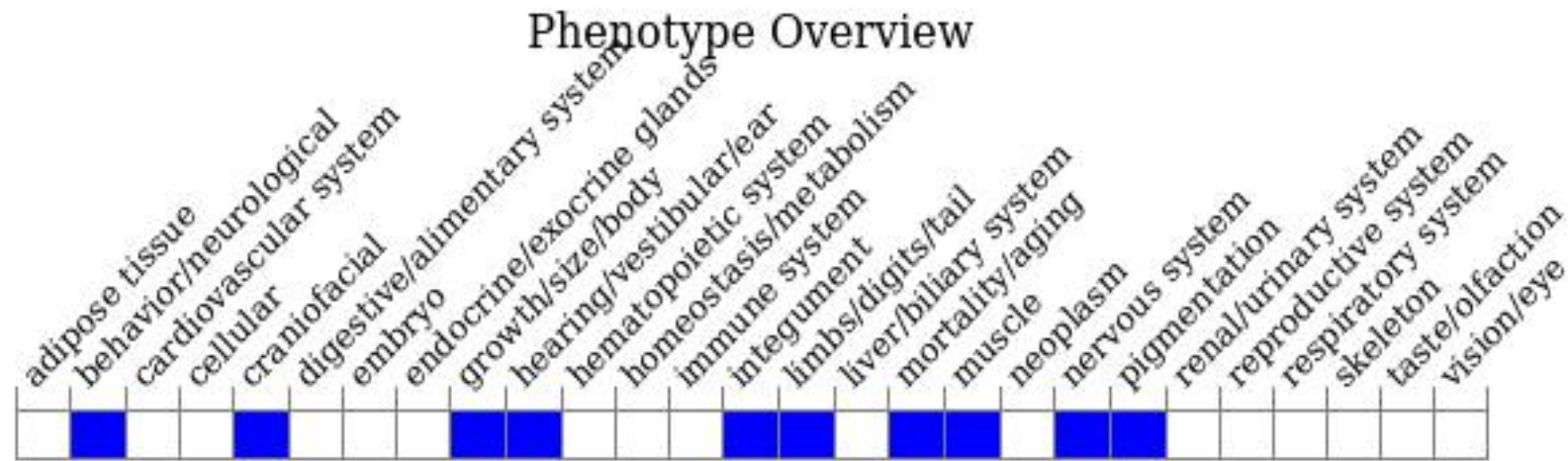




# Protein domain



# 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, mutations in this gene result in diluted coat color, behavioral deficits including opisthotonus, and postnatal or premature death.

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

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