

# *Ank2* Cas9-CKO Strategy

**Designer:**

**Daohua Xu**

**Reviewer:**

**Huimin Su**

**Design Date:**

**2019-11-25**

# Project Overview

**Project Name**

*Ank2*

**Project type**

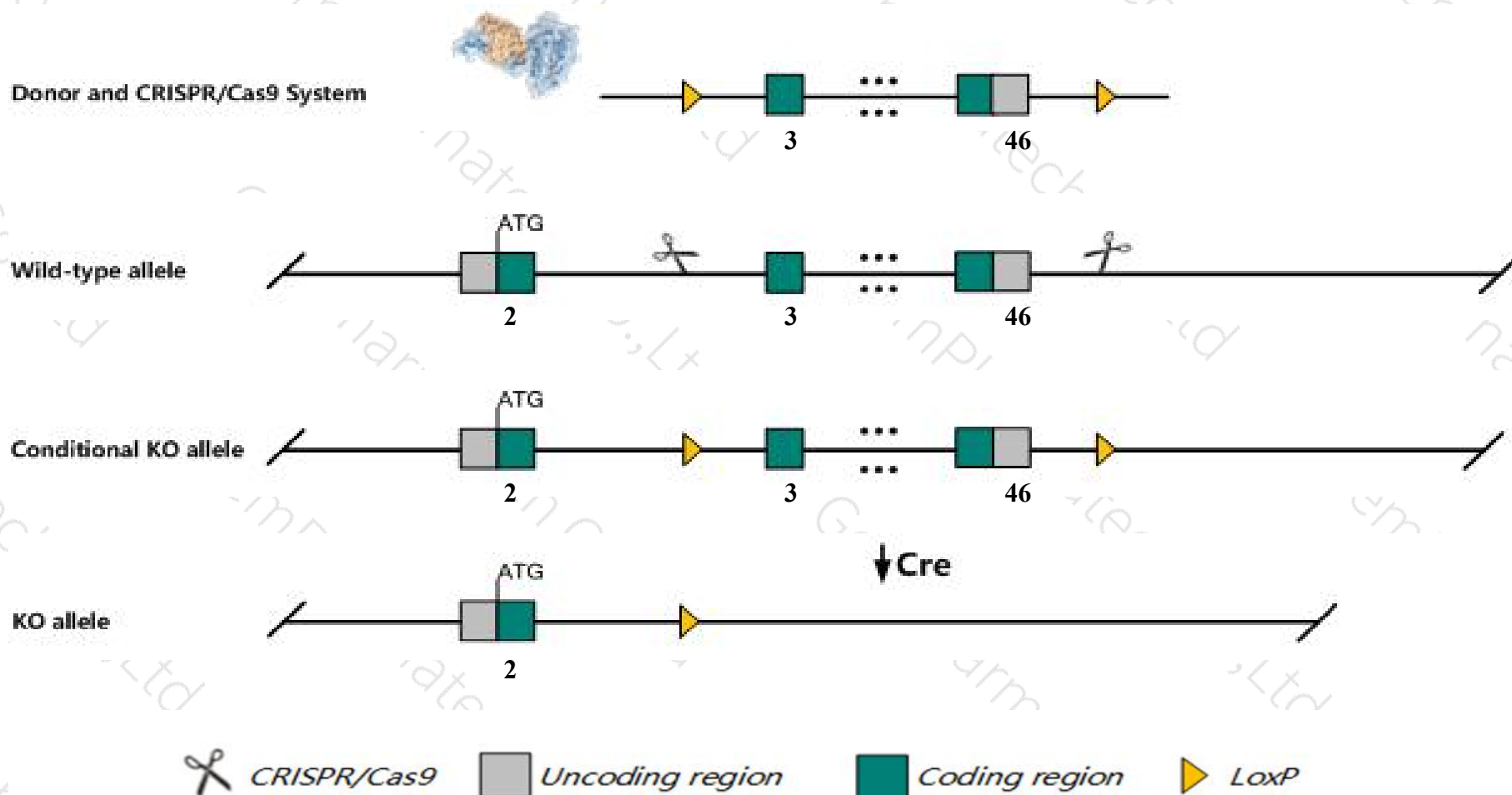
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ank2* gene. The schematic diagram is as follows:



- The *Ank2* gene has 28 transcripts. According to the structure of *Ank2* gene, exon3-exon46 of *Ank2*-208 (ENSMUST00000182078.8) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ank2* 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, Homozygous mutation of this gene results in death by postnatal day 8, although some animals survive to P20. Mutant animals display reduced body size, impaired balance and locomotion, brain structure dysmorphologies, abnormal lens, and optic nerve degeneration.
- The *Ank2* gene is located on the Chr3. 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)

## Ank2 ankyrin 2, brain [Mus musculus (house mouse)]

Gene ID: 109676, updated on 7-Apr-2019

### Summary



<b>Official Symbol</b>	Ank2 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	ankyrin 2, brain provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:88025</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000032826</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>	100043364, AI835472, AW491075, Ank-2, Gm4392
<b>Expression</b>	Biased expression in cerebellum adult (RPKM 17.1), frontal lobe adult (RPKM 13.9) and 9 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

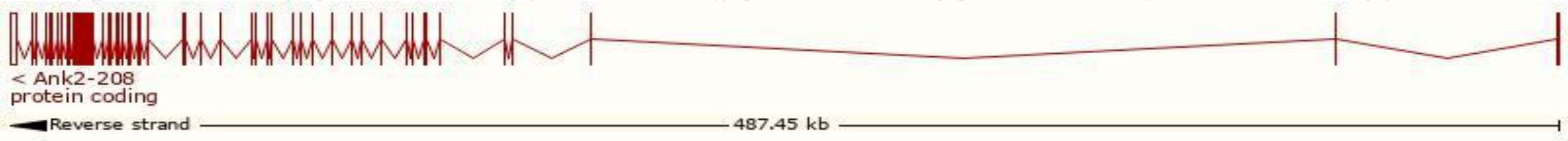
# Transcript information（Ensembl）



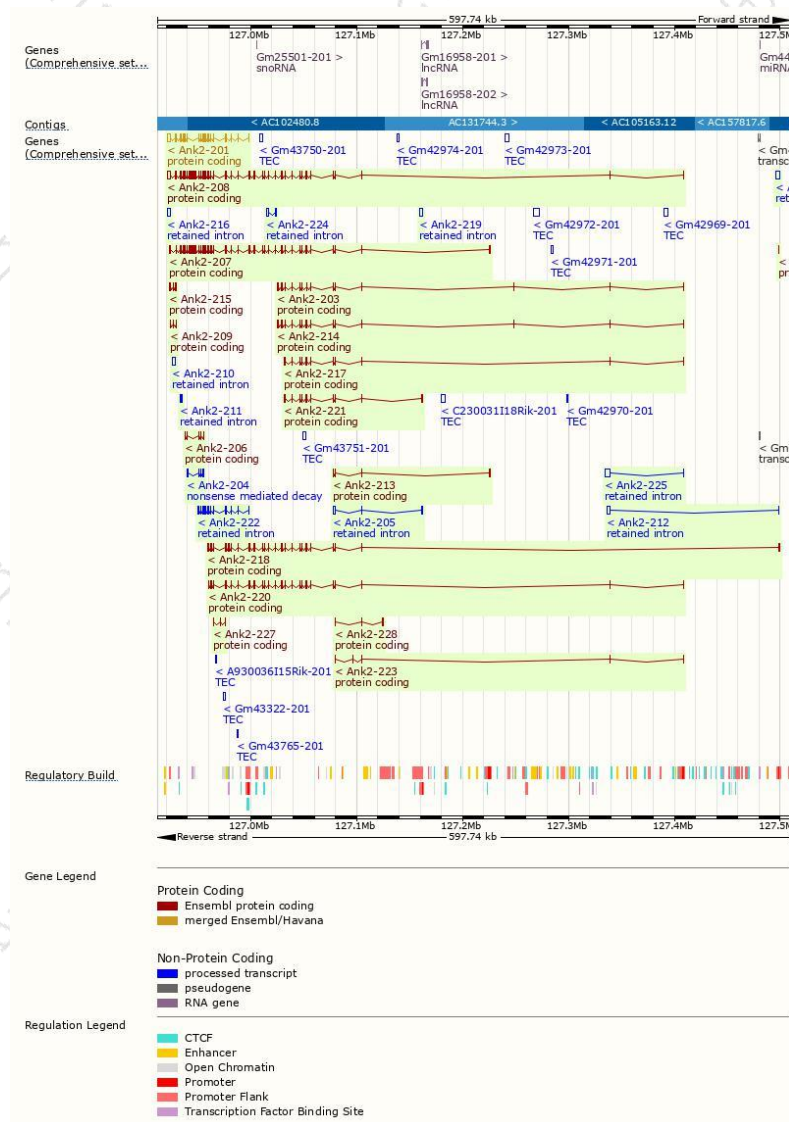
The gene has 28 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ank2-208	<a href="#">ENSMUST00000182078.8</a>	14305	<a href="#">3900aa</a>	Protein coding	<a href="#">CCDS84672</a>	<a href="#">S4R2B5</a>	TSL:5 GENCODE basic APPRIS P2
Ank2-201	<a href="#">ENSMUST0000044443.14</a>	5775	<a href="#">1050aa</a>	Protein coding	<a href="#">CCDS38625</a>	<a href="#">Q8C6R3</a>	TSL:1 GENCODE basic
Ank2-207	<a href="#">ENSMUST00000182064.8</a>	12413	<a href="#">3926aa</a>	Protein coding	-	<a href="#">S4R2F3</a>	TSL:5 GENCODE basic APPRIS ALT2
Ank2-218	<a href="#">ENSMUST00000182711.7</a>	4268	<a href="#">1219aa</a>	Protein coding	-	<a href="#">S4R2T7</a>	CDS 3' incomplete TSL:1
Ank2-220	<a href="#">ENSMUST00000182959.7</a>	3536	<a href="#">1114aa</a>	Protein coding	-	<a href="#">S4R1J9</a>	CDS 3' incomplete TSL:5
Ank2-221	<a href="#">ENSMUST00000182994.7</a>	2715	<a href="#">454aa</a>	Protein coding	-	<a href="#">Q8C6R3</a>	TSL:1 GENCODE basic
Ank2-203	<a href="#">ENSMUST00000182008.7</a>	2474	<a href="#">481aa</a>	Protein coding	-	<a href="#">Q8C6R3</a>	TSL:1 GENCODE basic
Ank2-214	<a href="#">ENSMUST00000182547.7</a>	2428	<a href="#">489aa</a>	Protein coding	-	<a href="#">S4R2D6</a>	TSL:1 GENCODE basic
Ank2-217	<a href="#">ENSMUST00000182610.7</a>	2231	<a href="#">418aa</a>	Protein coding	-	<a href="#">S4R1I1</a>	TSL:1 GENCODE basic
Ank2-215	<a href="#">ENSMUST00000182571.2</a>	848	<a href="#">183aa</a>	Protein coding	-	<a href="#">S4R2Q9</a>	CDS 5' incomplete TSL:5
Ank2-206	<a href="#">ENSMUST00000182062.7</a>	561	<a href="#">187aa</a>	Protein coding	-	<a href="#">S4R1M4</a>	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Ank2-228	<a href="#">ENSMUST00000238781.1</a>	557	<a href="#">112aa</a>	Protein coding	-	-	CDS 3' incomplete
Ank2-213	<a href="#">ENSMUST00000182452.7</a>	458	<a href="#">124aa</a>	Protein coding	-	<a href="#">S4R1U2</a>	CDS 3' incomplete TSL:5
Ank2-223	<a href="#">ENSMUST00000183095.2</a>	420	<a href="#">90aa</a>	Protein coding	-	<a href="#">S4R241</a>	CDS 3' incomplete TSL:5
Ank2-209	<a href="#">ENSMUST00000182118.8</a>	367	<a href="#">122aa</a>	Protein coding	-	<a href="#">S4R1Q5</a>	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Ank2-227	<a href="#">ENSMUST00000211813.1</a>	365	<a href="#">121aa</a>	Protein coding	-	<a href="#">A0A1D5RLB2</a>	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Ank2-226	<a href="#">ENSMUST00000189369.1</a>	339	<a href="#">113aa</a>	Protein coding	-	<a href="#">A0A087WFM9</a>	TSL:NA GENCODE basic
Ank2-204	<a href="#">ENSMUST00000182025.5</a>	448	<a href="#">108aa</a>	Nonsense mediated decay	-	<a href="#">S4R1B7</a>	CDS 5' incomplete TSL:5
Ank2-225	<a href="#">ENSMUST00000183234.2</a>	4550	No protein	Retained intron	-	-	TSL:1
Ank2-202	<a href="#">ENSMUST00000104965.3</a>	3154	No protein	Retained intron	-	-	TSL:NA
Ank2-212	<a href="#">ENSMUST00000182316.2</a>	3134	No protein	Retained intron	-	-	TSL:1
Ank2-210	<a href="#">ENSMUST00000182212.2</a>	2781	No protein	Retained intron	-	-	TSL:NA
Ank2-224	<a href="#">ENSMUST00000183097.1</a>	2668	No protein	Retained intron	-	-	TSL:1
Ank2-222	<a href="#">ENSMUST00000183075.1</a>	2630	No protein	Retained intron	-	-	TSL:1
Ank2-205	<a href="#">ENSMUST00000182050.2</a>	2540	No protein	Retained intron	-	-	TSL:1
Ank2-219	<a href="#">ENSMUST00000182876.1</a>	2491	No protein	Retained intron	-	-	TSL:NA
Ank2-216	<a href="#">ENSMUST00000182588.4</a>	2359	No protein	Retained intron	-	-	TSL:NA
Ank2-211	<a href="#">ENSMUST00000182271.1</a>	623	No protein	Retained intron	-	-	TSL:3

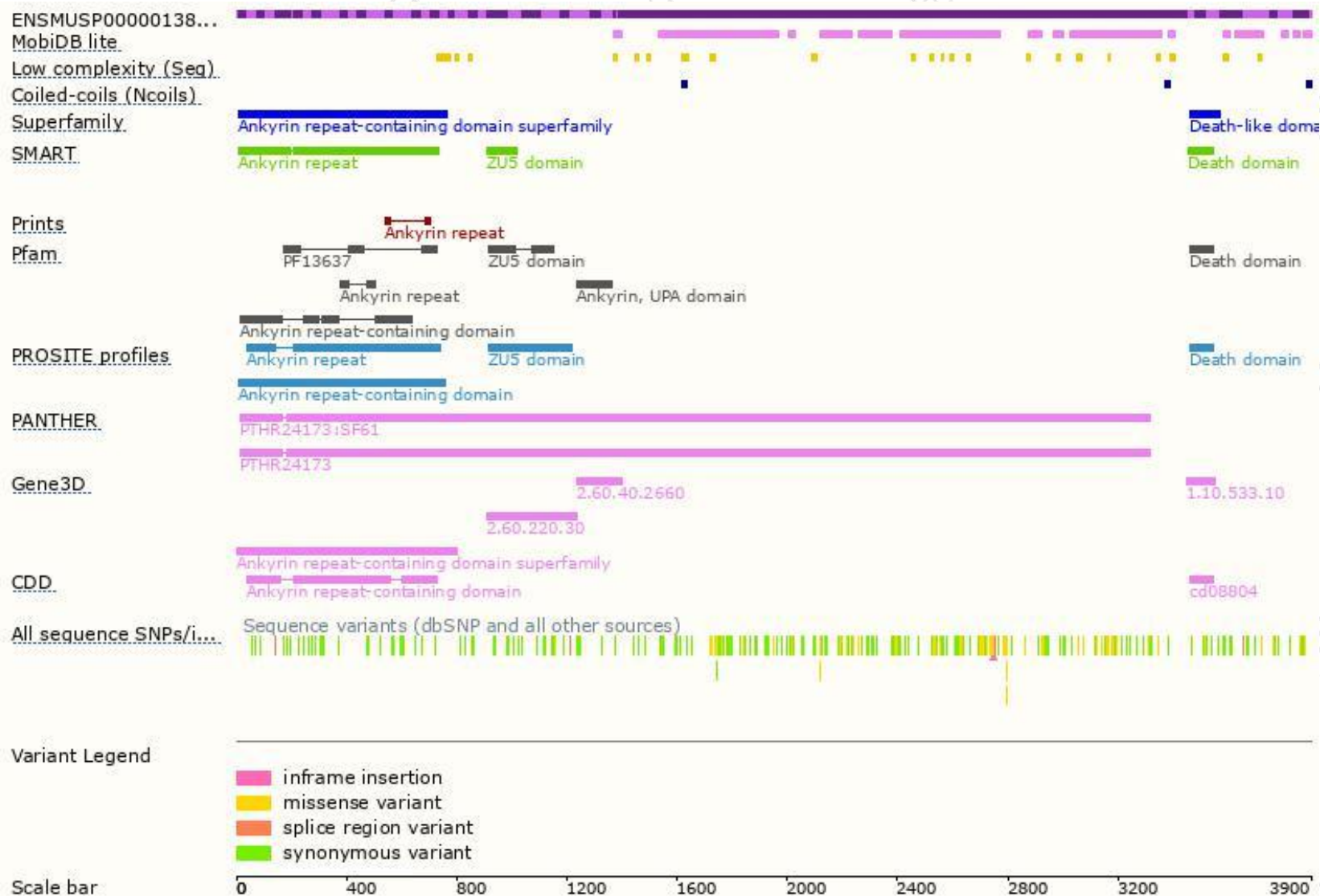
The strategy is based on the design of *Ank2-208* transcript,The transcription is shown below



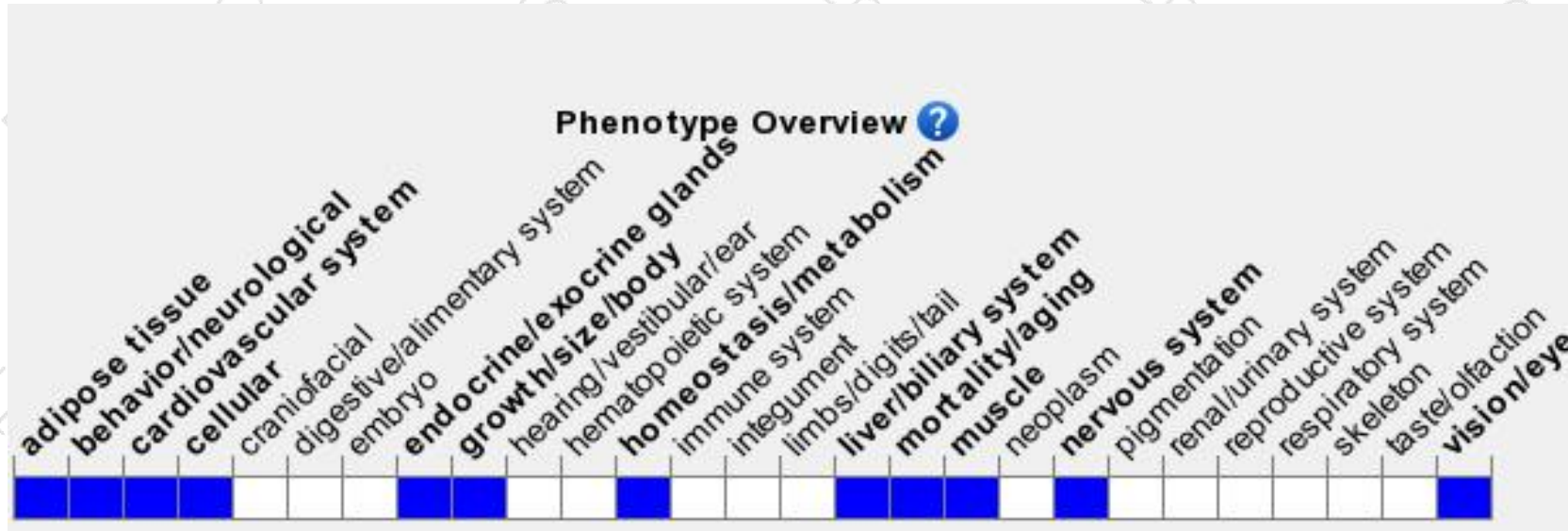
# Genomic location distribution



# 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, Homozygous mutation of this gene results in death by postnatal day 8, although some animals survive to P20. Mutant animals display reduced body size, impaired balance and locomotion, brain structure dysmorphologies, abnormal lens, and optic nerve degeneration.

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

Tel: 400-9660890

