

Rnf2 Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer :

Huimin Su

Design Date:

2019-11-25

Project Overview

Project Name

Rnf2

Project type

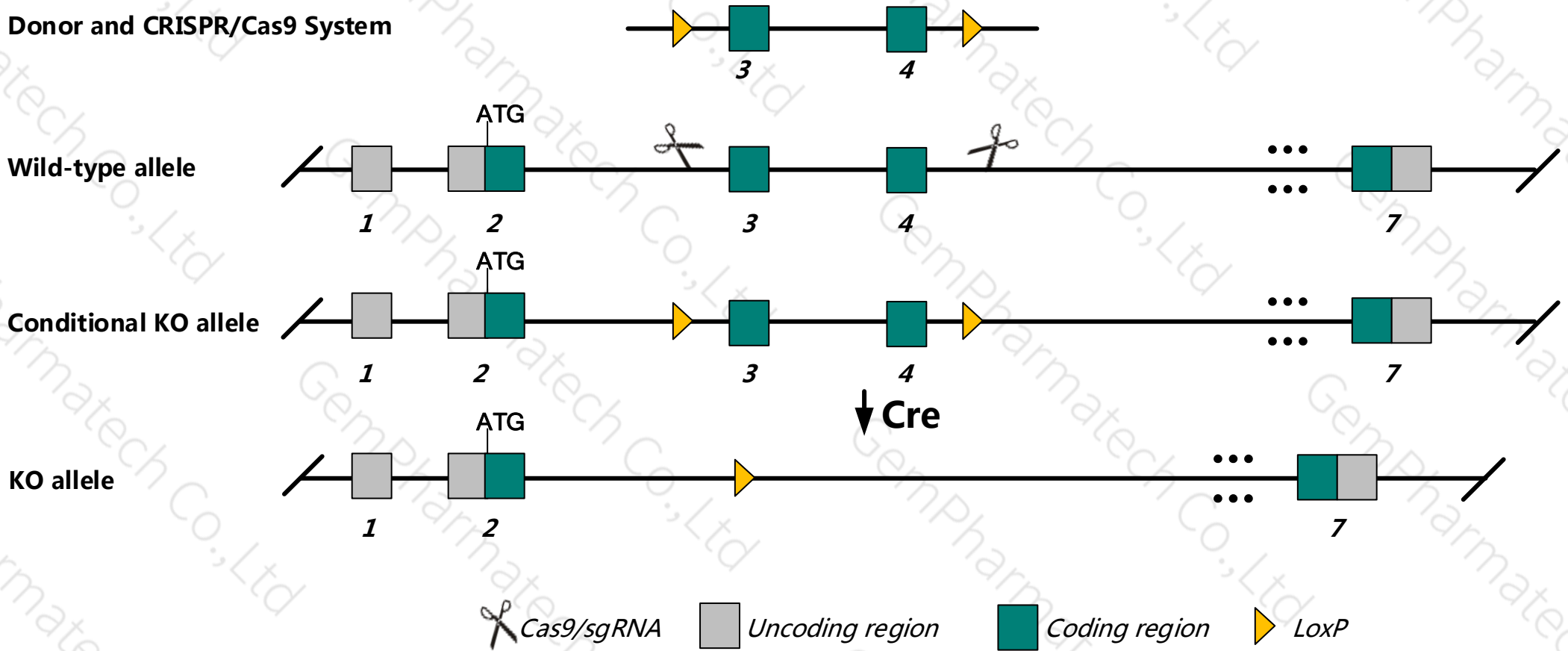
Cas9-CKO

Animal background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnf2* gene. The schematic diagram is as follows:



Technical routes

- The *Rnf2* gene has 7 transcripts, According to the structure of *Rnf2* gene, exon3-exon4 of *Rnf2*-201 transcript is recommended as the knockout region. The region contains the 377bp coding sequence. Knock out the region, result in destruction of protein.
- This project uses CRISPR/Cas9 technology to modify *Rnf2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed, Cas9, sgRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.
- 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 , Embryos homozygous for a null allele show an early growth arrest, failure to progress through gastrulation, impaired epiblast expansion, accumulation of posterior mesoderm and die before E10.5. Mice homozygous for a hypomorphic allele show posterior homeotic transformations of the axial skeleton.
- The *Rnf2* gene is located in the Chr1. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Rnf2 ring finger protein 2 [*Mus musculus* (house mouse)]

Gene ID: 19821, updated on 11-Dec-2018

Summary

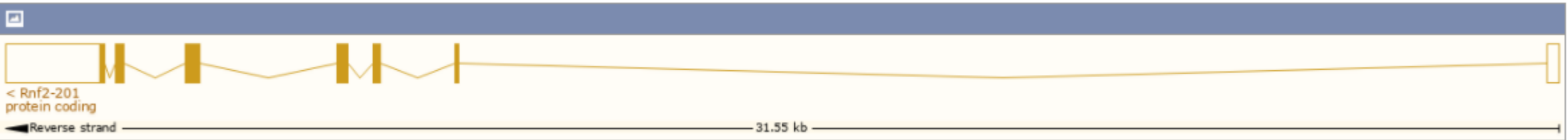
Official Symbol	Rnf2 provided by MGI
Official Full Name	ring finger protein 2 provided by MGI
Primary source	MGI:MGI:1101759
See related	Ensembl:ENSMUSG00000026484
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	dinG; Ring1B; AI326319; AI450156; AU019207
Expression	Broad expression in CNS E11.5 (RPKM 21.3), placenta adult (RPKM 21.2) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

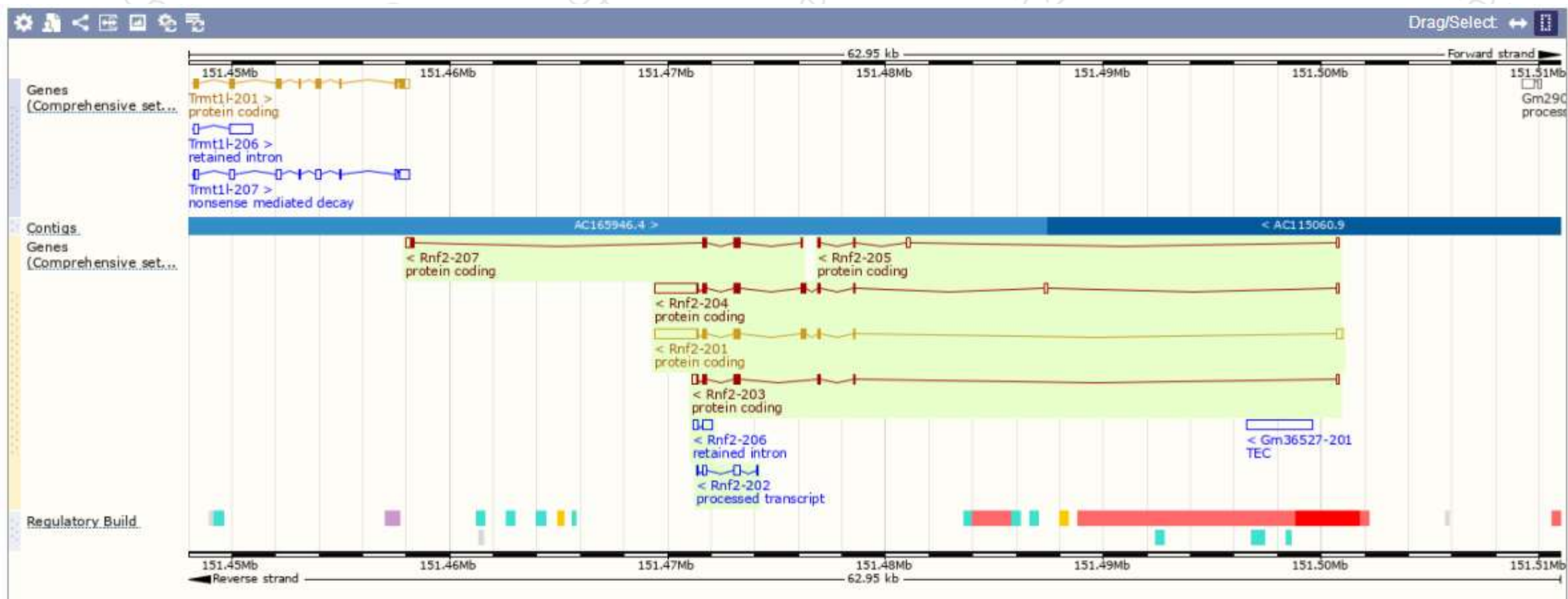
The gene has 7 transcripts, and all transcripts are shown below :

Show/hide columns (1 hidden)								Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags	
Rnf2-204	ENSMUST00000187048.6	3201	336aa	Protein coding	CCDS15362	Q9CQJ4	NM_001360847 NP_001347774 NP_001347776	TSL:1	GENCODE basic APPRIS P1
Rnf2-201	ENSMUST00000076110.10	3160	336aa	Protein coding	CCDS15362	Q9CQJ4	NM_011277 NP_001347774 NP_035407	TSL:1	GENCODE basic APPRIS P1
Rnf2-203	ENSMUST00000186415.6	1106	264aa	Protein coding	-	A0A087WRE9	-	TSL:5	GENCODE basic
Rnf2-207	ENSMUST00000190070.6	817	211aa	Protein coding	-	A0A087WP87	-	CDS 5' incomplete	TSL:5
Rnf2-205	ENSMUST00000187991.1	482	73aa	Protein coding	-	A0A087WQQ3	-	CDS 3' incomplete	TSL:3
Rnf2-202	ENSMUST00000185945.1	570	No protein	Processed transcript	-	-	-	TSL:3	
Rnf2-206	ENSMUST00000188871.1	658	No protein	Retained intron	-	-	-	TSL:2	

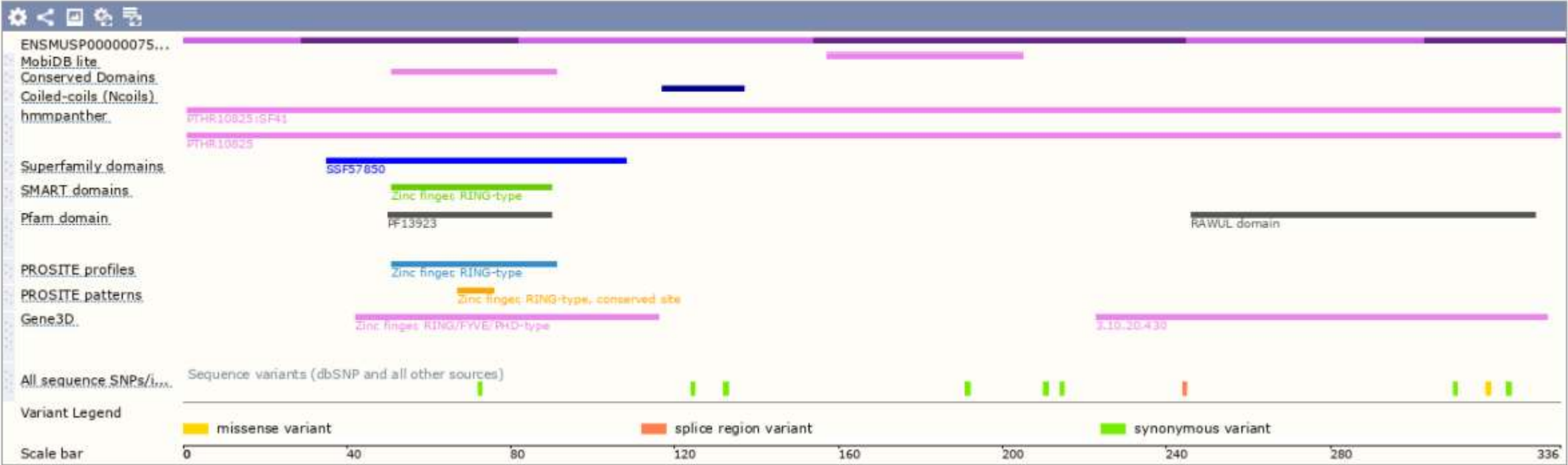
The strategy is based on the design of *Rnf2-201* transcript,The transcription is shown below :



Genomic location distribution

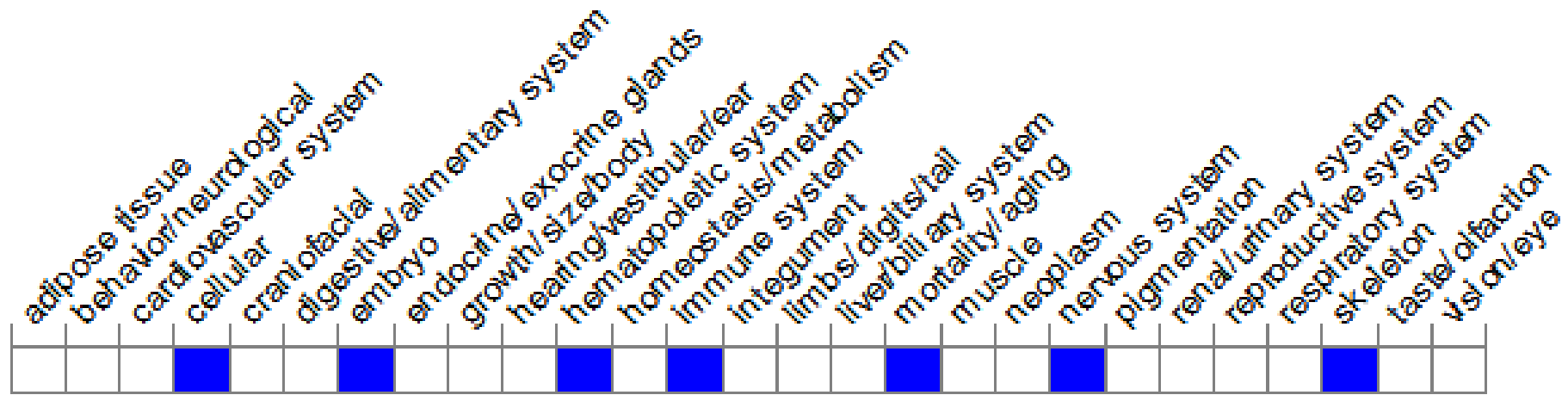


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



Click cells to view annotations.

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, Embryos homozygous for a null allele show an early growth arrest, failure to progress through gastrulation, impaired epiblast expansion, accumulation of posterior mesoderm and die before E10.5. Mice homozygous for a hypomorphic allele show posterior homeotic transformations of the axial skeleton.

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534

