

Col4a2 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Col4a2

Project type

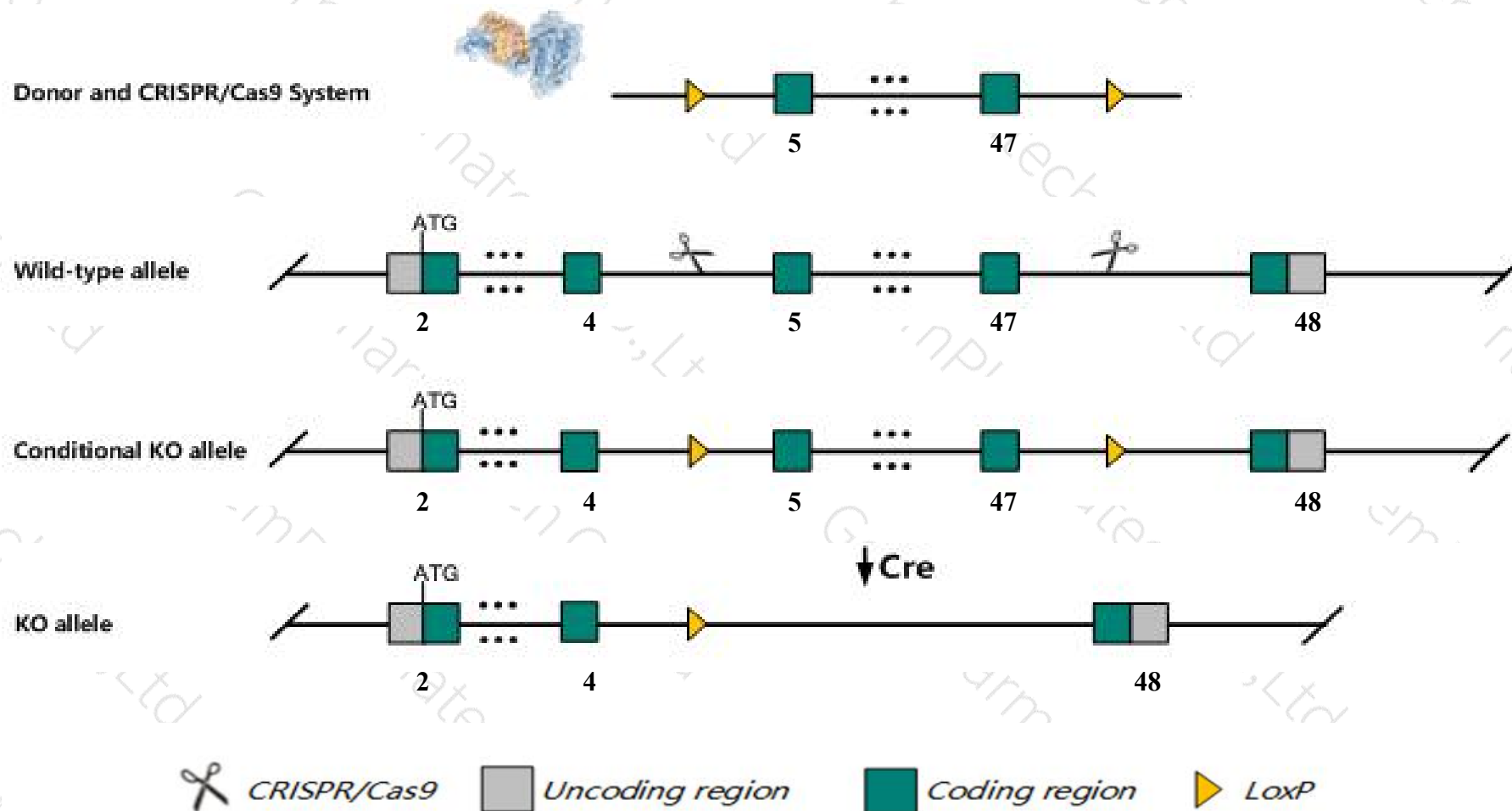
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col4a2* gene. The schematic diagram is as follows:



Technical routes

- The *Col4a2* gene has 3 transcripts. According to the structure of *Col4a2* gene, exon5-exon47 of *Col4a2-201* (ENSMUST00000033899.13) 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 *Col4a2* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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,ENU-induced missense mutations of this gene result in a variable phenotype affecting the eye, brain and vascular stability in heterozygotes, and fetal or postnatal survival in homozygotes.
- The KO region contains the *Gm15419* gene. Knockout the region may affect the function of *Gm15419* gene.
- The *Col4a2* 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)

Col4a2 collagen, type IV, alpha 2 [Mus musculus (house mouse)]

Gene ID: 12827, updated on 3-Feb-2019

Summary



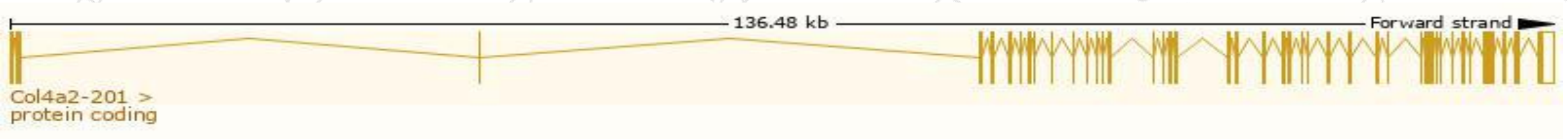
Official Symbol	Col4a2 provided by MGI
Official Full Name	collagen, type IV, alpha 2 provided by MGI
Primary source	MGI:MGI:88455
See related	Ensembl:ENSMUSG000000031503
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Col4a-2
Summary	This gene encodes the alpha-2 subunit of the type IV collagens, an essential component of basement membranes. The encoded protein forms a triple helical heterotrimer comprised of alpha-1 and alpha-2 subunits that assembles into a type IV collagen network. Canstatin, a peptide derived from the C-terminus of the collagen chain, is a matrikine that has been shown to inhibit angiogenesis. Homozygous knockout mice for this gene exhibit impaired basement membrane integrity and embryonic lethality. This gene shares a bi-directional promoter with a related gene on chromosome 8. [provided by RefSeq, Nov 2015]
Expression	Broad expression in subcutaneous fat pad adult (RPKM 132.8), lung adult (RPKM 102.4) and 22 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

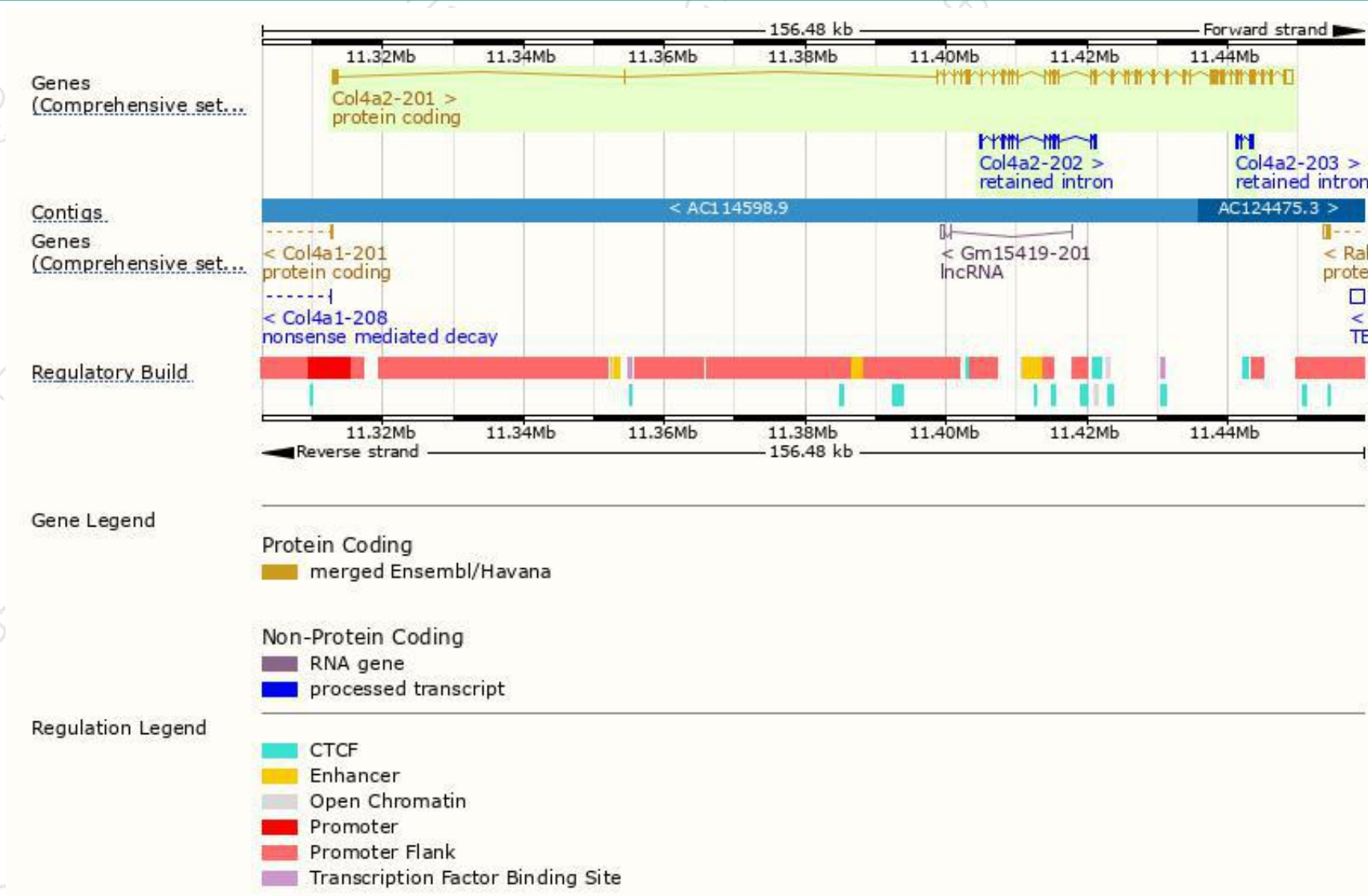
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col4a2-201	ENSMUST00000033899.13	6450	1707aa	Protein coding	CCDS40220	B2RQQ8 P08122	TSL:1 GENCODE basic APPRIS P1
Col4a2-202	ENSMUST00000145295.1	921	No protein	Retained intron	-	-	TSL:5
Col4a2-203	ENSMUST00000146219.1	764	No protein	Retained intron	-	-	TSL:2

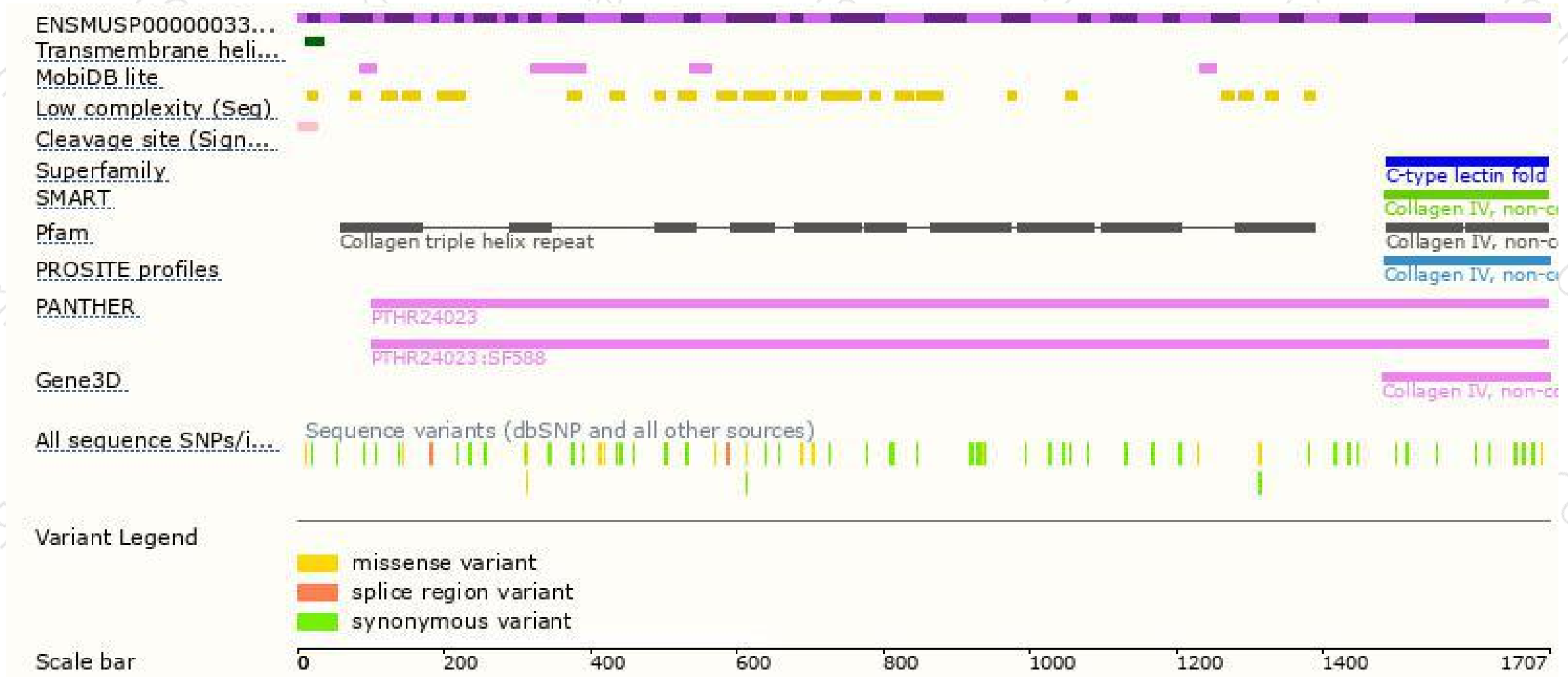
The strategy is based on the design of *Col4a2-201* transcript,The transcription is shown below



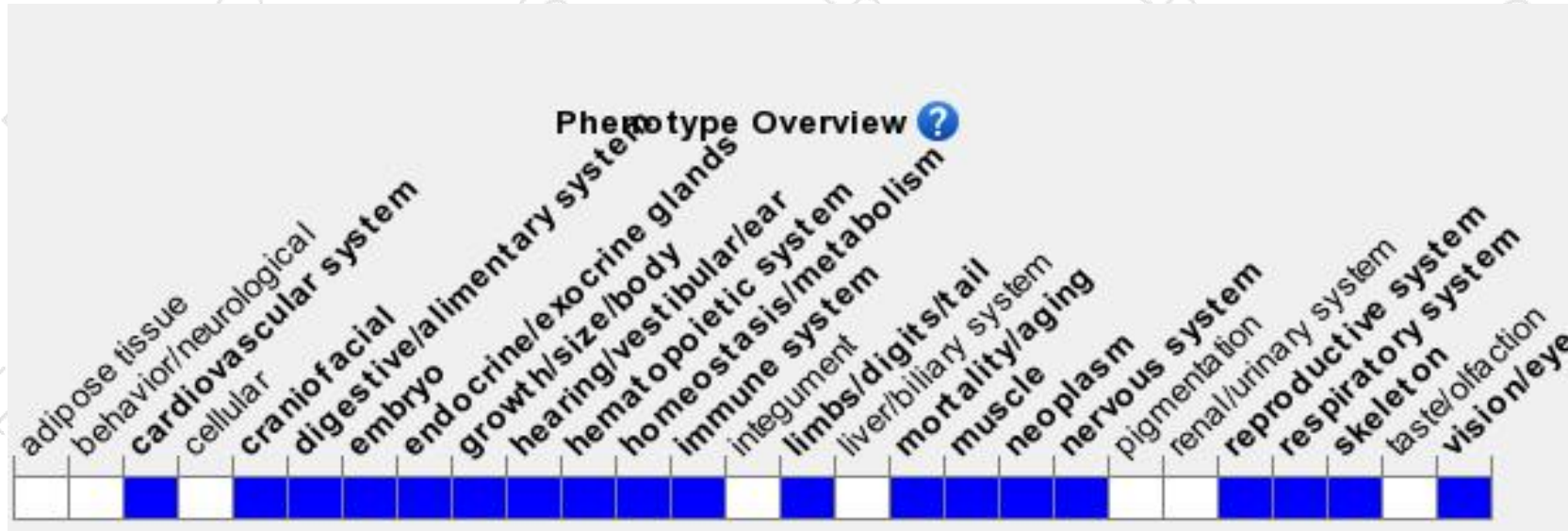
Genomic location distribution



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, ENU-induced missense mutations of this gene result in a variable phenotype affecting the eye, brain and vascular stability in heterozygotes, and fetal or postnatal survival in homozygotes.

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

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