

# ***Col8a2* Cas9-CKO Strategy**

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# Project Overview

**Project Name**

*Col8a2*

**Project type**

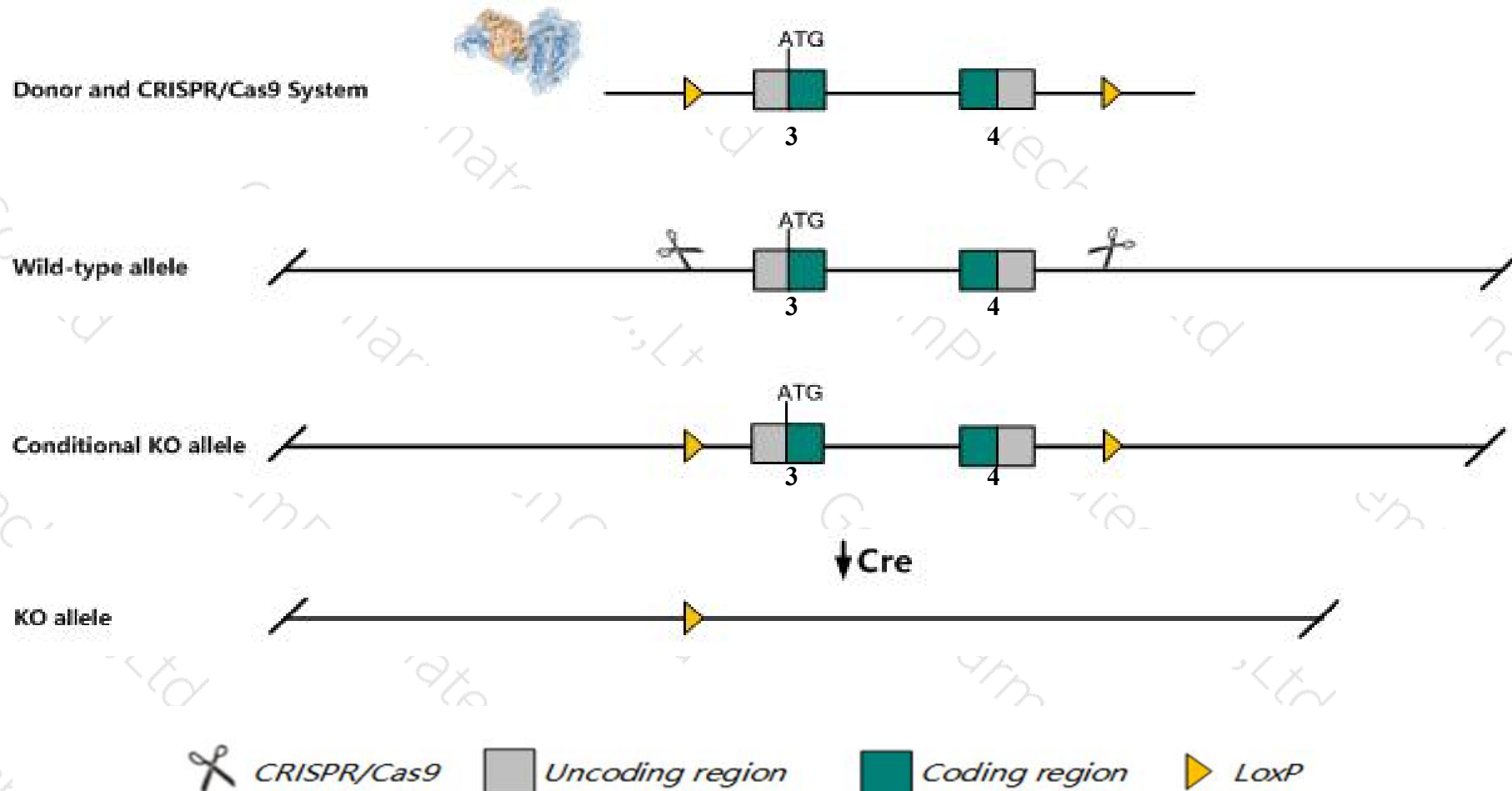
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col8a2* gene. The schematic diagram is as follows:



# Technical routes

- The *Col8a2* gene has 2 transcripts. According to the structure of *Col8a2* gene, exon3-exon4 of *Col8a2-201* (ENSMUST00000070132.6) transcript is recommended as the knockout region. The region contains all 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 *Col8a2* 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 exhibit a thinner Descemet's membrane of the cornea. Mice heterozygous or homozygous for an ENU-induced mutation exhibit thin cornea, corneal epithelium, stroma, and Descemet membrane, and enlarged anterior chamber.
- The flox region is about 1.4 kb away from the C-terminus of the *Adprhl2* gene, which may affect the regulation of the C-terminus of the gene.
- The *Col8a2* gene is located on the Chr4. 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)

## Col8a2 collagen, type VIII, alpha 2 [ *Mus musculus* (house mouse) ]

Gene ID: 329941, updated on 12-Aug-2019

### Summary

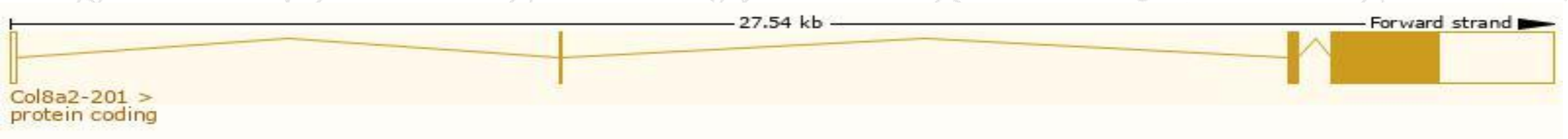
Official Symbol	Col8a2 provided by <a href="#">MGI</a>
Official Full Name	collagen, type VIII, alpha 2 provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:88464</a>
See related	<a href="#">Ensembl:ENSMUSG00000056174</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI429819
Expression	Biased expression in limb E14.5 (RPKM 16.0), bladder adult (RPKM 6.7) and 11 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

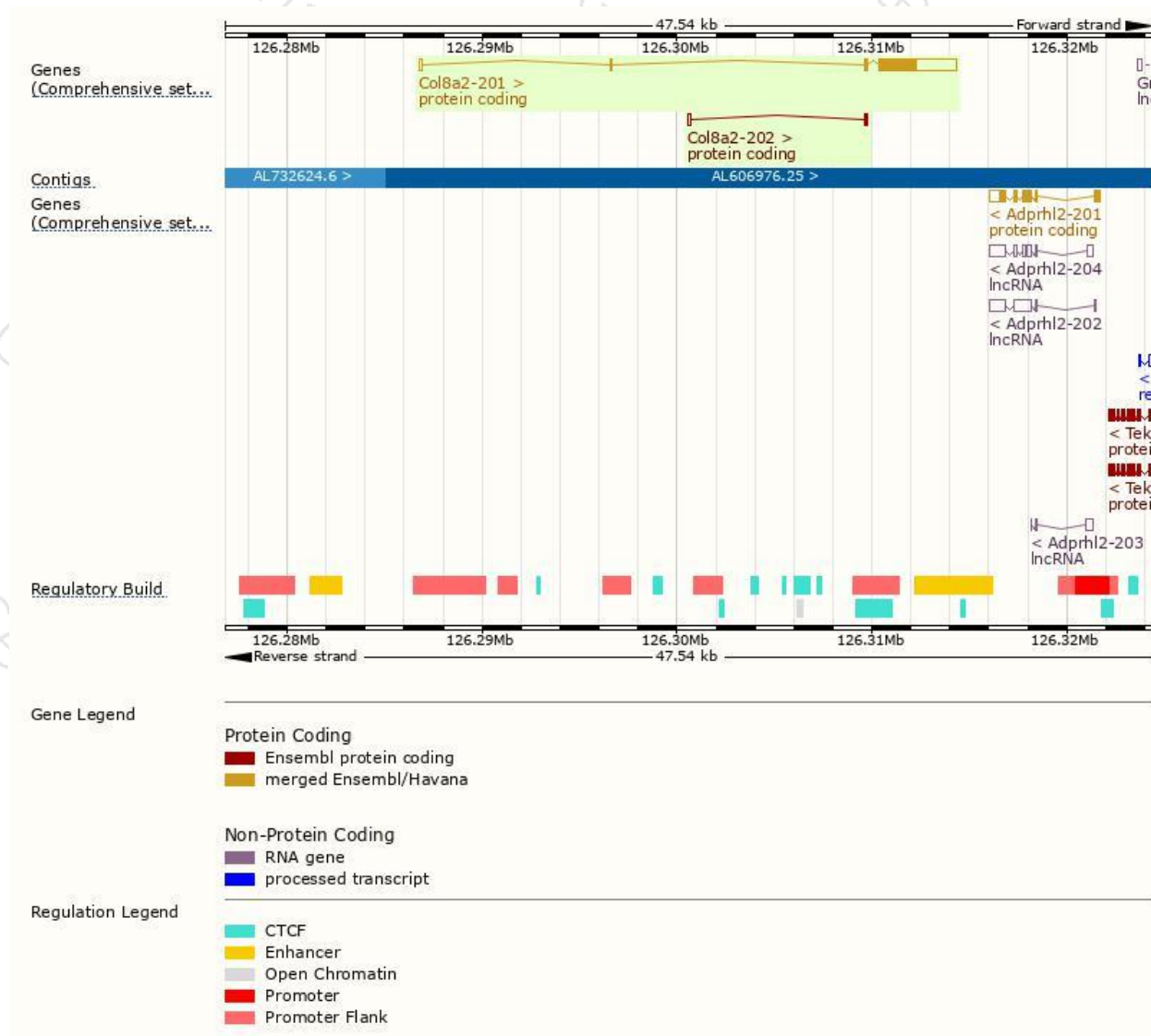
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col8a2-201	<a href="#">ENSMUST00000070132.6</a>	4332	<a href="#">699aa</a>	Protein coding	<a href="#">CCDS18649</a>	<a href="#">P25318</a>	TSL:1 GENCODE basic APPRIS P1
Col8a2-202	<a href="#">ENSMUST00000128435.1</a>	343	<a href="#">56aa</a>	Protein coding	-	<a href="#">A3KFY1</a>	CDS 3' incomplete TSL:2

The strategy is based on the design of *Col8a2-201* transcript,The transcription is shown below

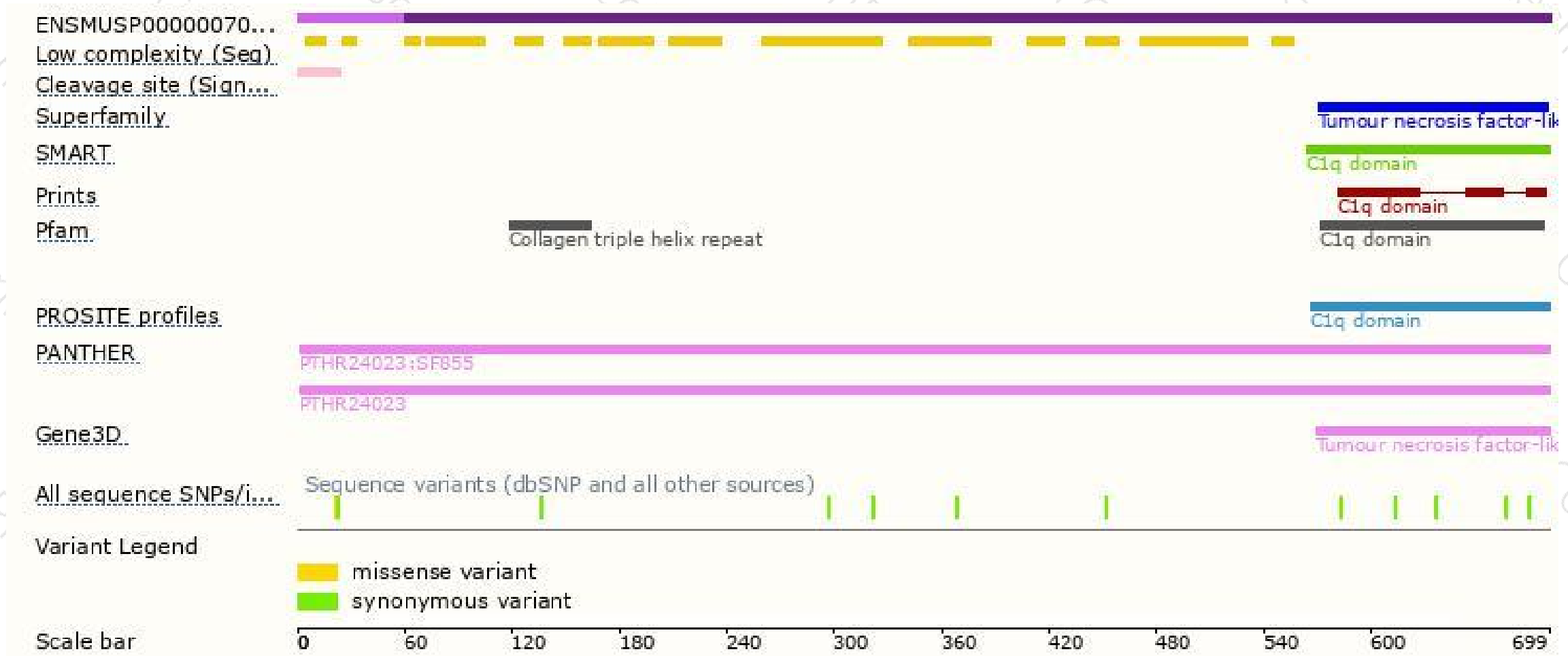


# Genomic location distribution

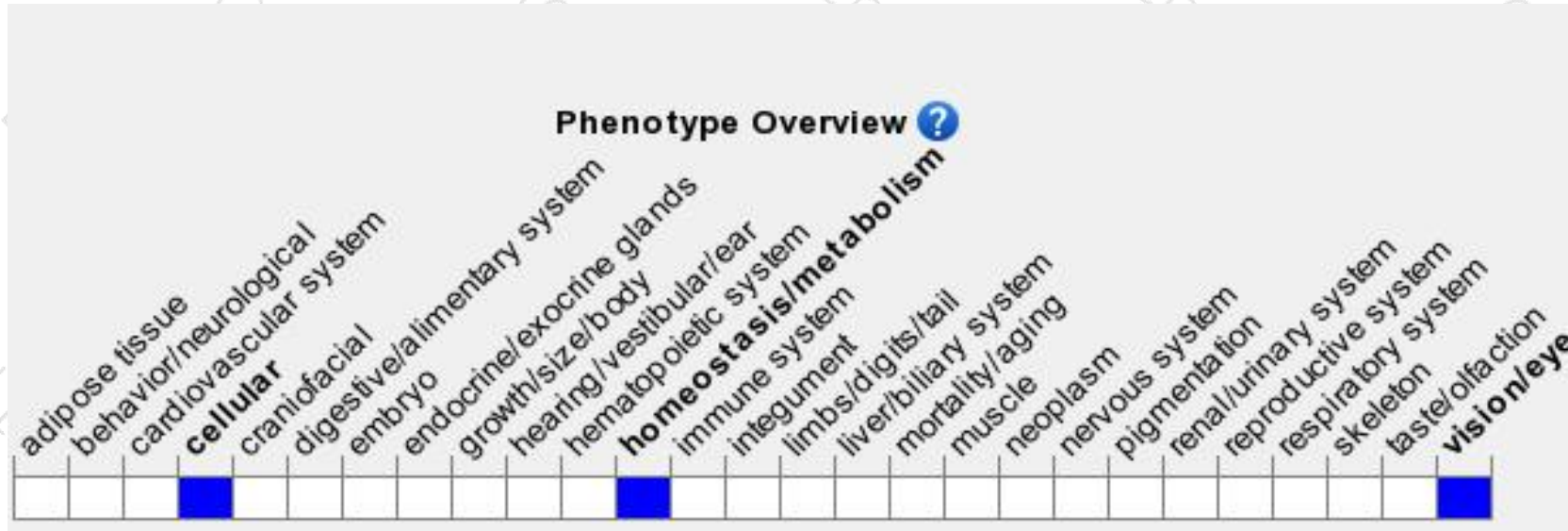




# Protein domain



# 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, Mice homozygous for a knock-out allele exhibit a thinner Descemet's membrane of the cornea. Mice heterozygous or homozygous for an ENU-induced mutation exhibit thin cornea, corneal epithelium, stroma, and Descemet membrane, and enlarged anterior chamber.

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

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