Col4a1 Cas9-CKO Strategy

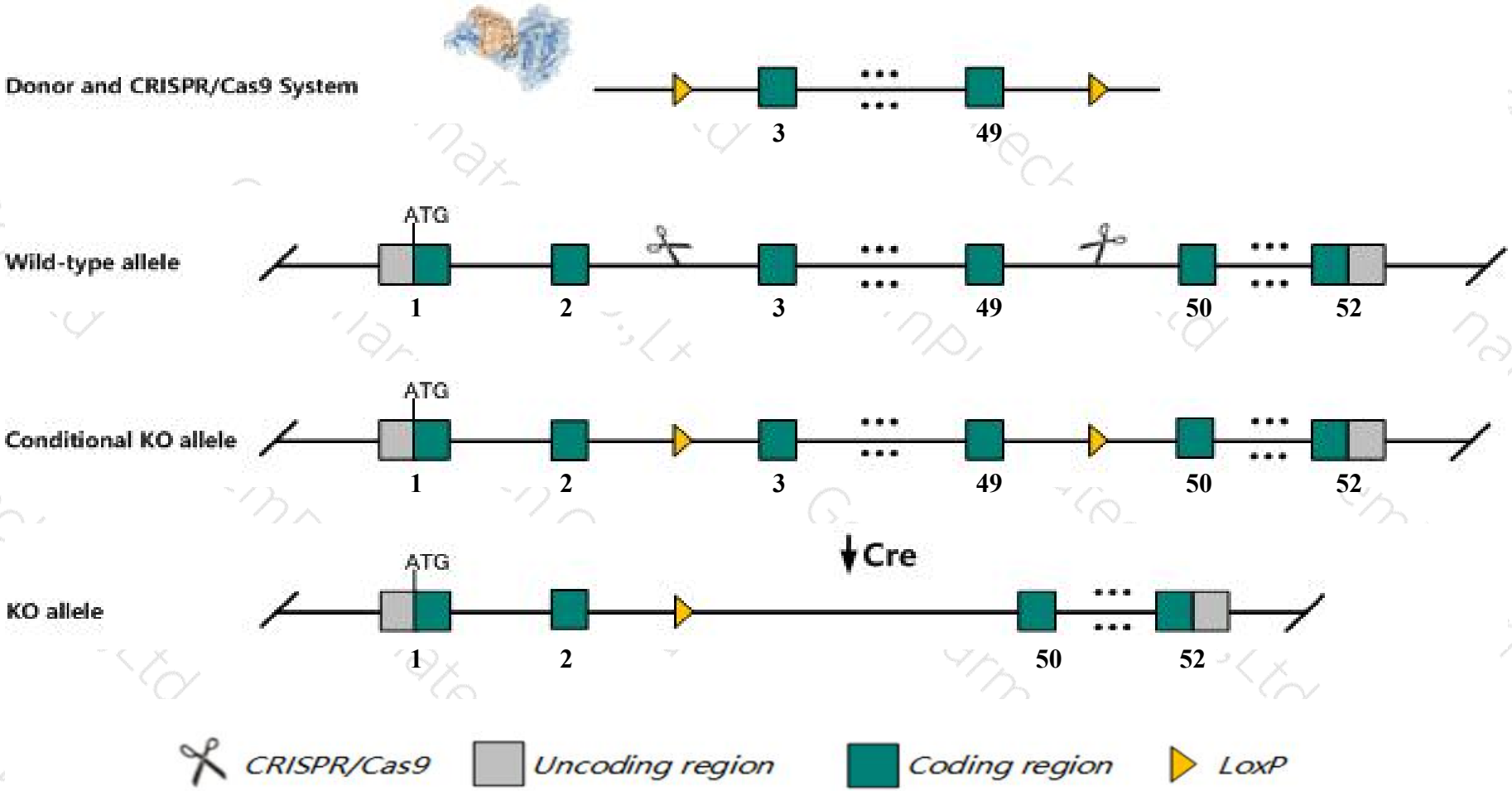
Designer: Shilei Zhu

Project Overview

Project Name	***Col4a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col4a1* gene. The schematic diagram is as follows:



Technical routes

- The *Col4a1* gene has 8 transcripts. According to the structure of *Col4a1* gene, exon3-exon49 of *Col4a1-201* (ENSMUST00000033898.9) transcript is recommended as the knockout region.The region contains 4496bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Col4a1* 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.

Notice

- According to the existing MGI data,Mice with ENU induced alleles have various eye and vision defects and may show bruising at birth. Mice carrying the G498V mutation have renal glomerular defects that resolve within the first weeks of life, but show retinal tortuosity, muscular dystrophy, brain hemorrhages, and renal cysts as adults.

- The *Col4a1* 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）

集萃药康 GemPharmatech

Col4a1 collagen, type IV, alpha 1 [Mus musculus (house mouse)]

Gene ID: 12826, updated on 27-Mar-2019

Summary

Official Symbol	Col4a1 provided by MGI
Official Full Name	collagen, type IV, alpha 1 provided byMGI
Primary source	MGI:MGI:88454
See related	Ensembl:ENSMUSG00000031502
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	Bru, Col4a-1, Raw, Svc
Summary	This gene encodes the alpha-1 subunit of the type IV collagens, an essential component of basement membranes. The encoded protein forms a triple helical heterotrimer comprised of two alpha-1 and one alpha-2 subunits that assembles into a type IV collagen network. This gene is located adjacent to the gene encoding alpha-2 subunit. Mice lacking both the alpha-1 and alpha-2 subunits of collagen IV die in utero due to structural deficiencies in the basement membranes and certain mutations in this gene cause perinatal cerebral hemorrhage and porencephaly. Alternative splicing of this gene results in multiple transcript variants. [provided by RefSeq, Nov 2015]
Expression	Broad expression in subcutaneous fat pad adult (RPKM 160.4), lung adult (RPKM 107.4) and 21 other tissuesSee more
Orthologs	human all

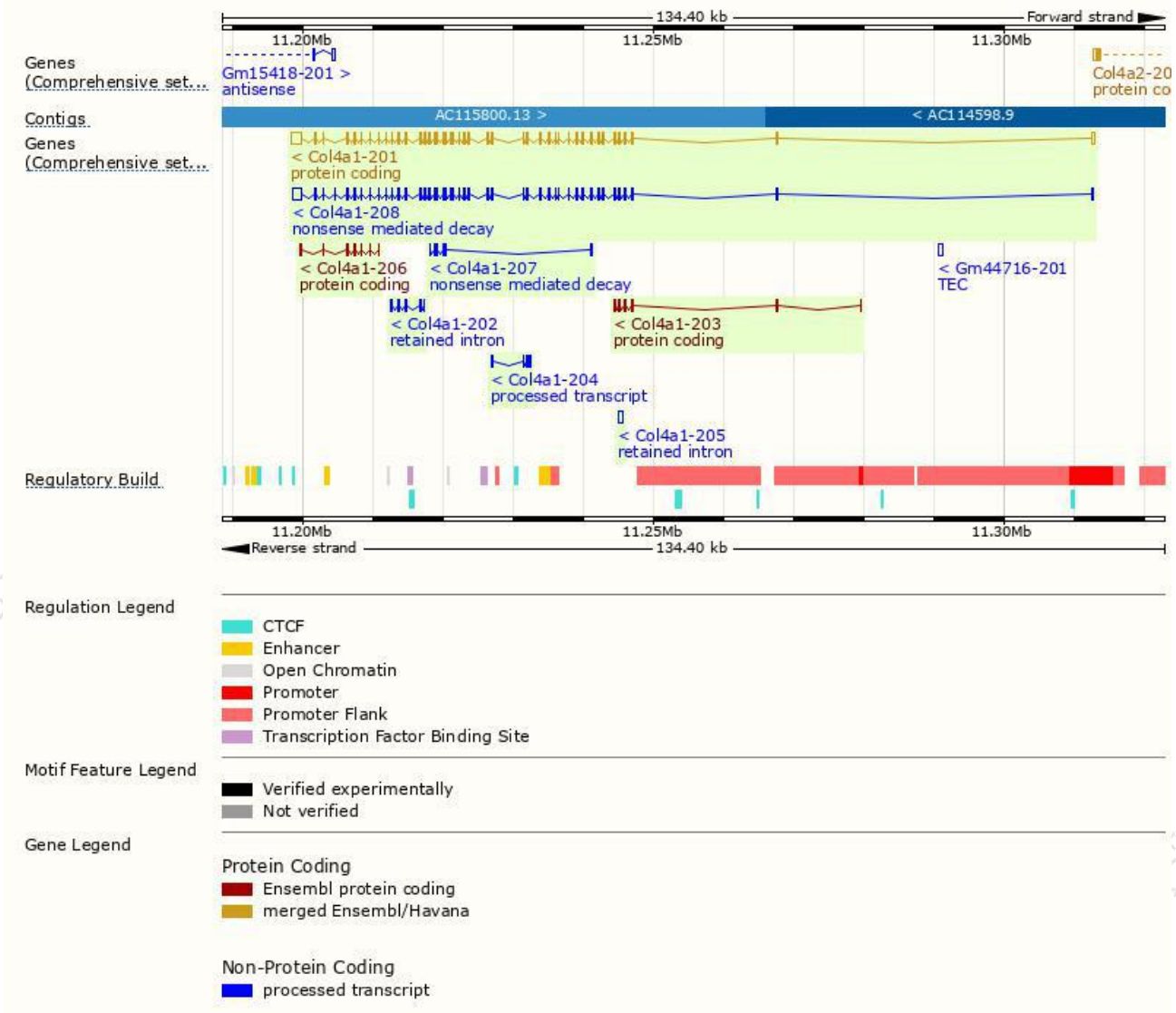
Transcript information（Ensembl）

The gene has 8 transcripts,all transcripts are shown below:

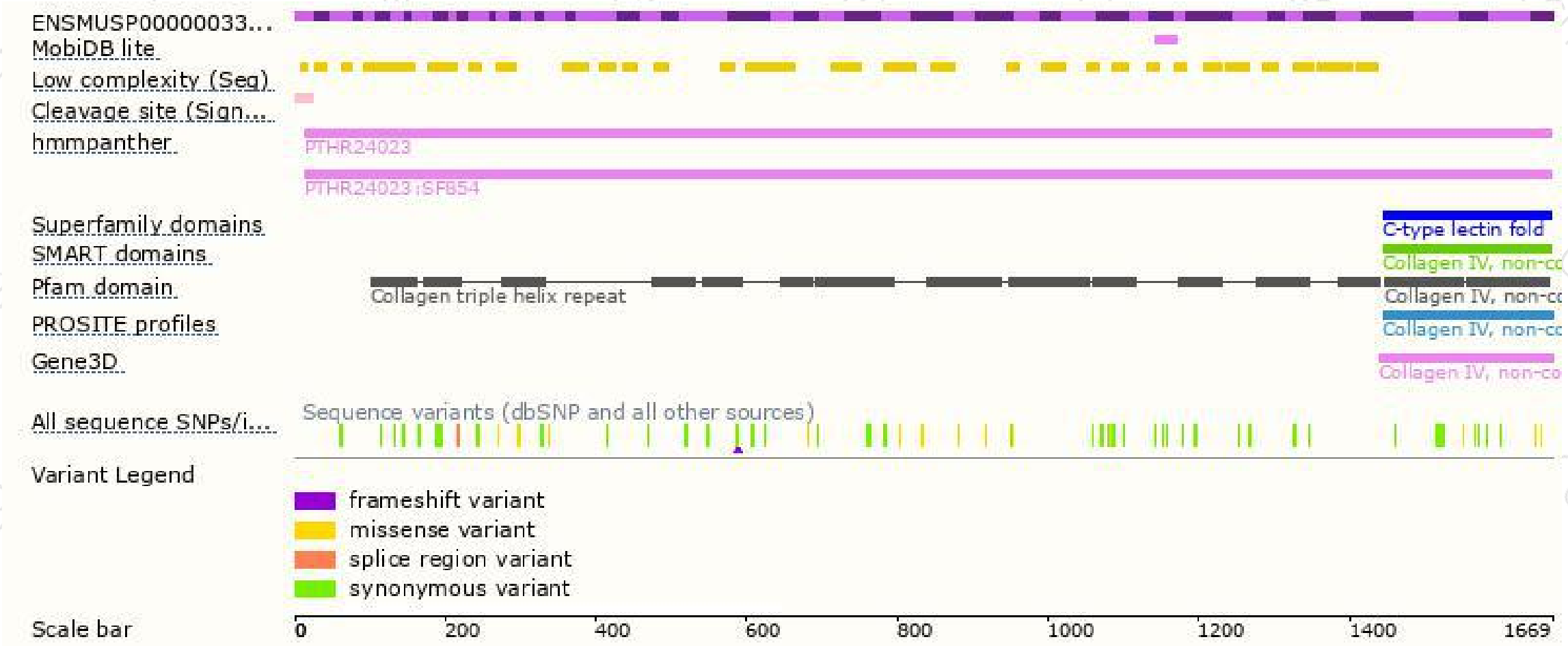
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col4a1-201	ENSMUST00000033898.9	6615	1669aa	Protein coding	CCDS40219	P02463	TSL:1 GENCODE basic APPRIS P1
Col4a1-206	ENSMUST00000209598.1	1170	328aa	Protein coding	-	A0A1B0GRC0	CDS 5' incomplete TSL:5
Col4a1-203	ENSMUST00000208095.1	545	133aa	Protein coding	-	A0A140LHU8	CDS 3' incomplete TSL:3
Col4a1-208	ENSMUST00000209735.1	6487	1562aa	Nonsense mediated decay	-	A0A1B0GSI7	TSL:2
Col4a1-207	ENSMUST00000209661.1	618	126aa	Nonsense mediated decay	-	A0A1B0GT69	CDS 5' incomplete TSL:5
Col4a1-204	ENSMUST00000208386.1	416	No protein	Processed transcript	-	-	TSL:3
Col4a1-202	ENSMUST00000130488.2	676	No protein	Retained intron	-	-	TSL:5
Col4a1-205	ENSMUST00000209000.1	604	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Col4a1-201* transcript,The transcription is shown below

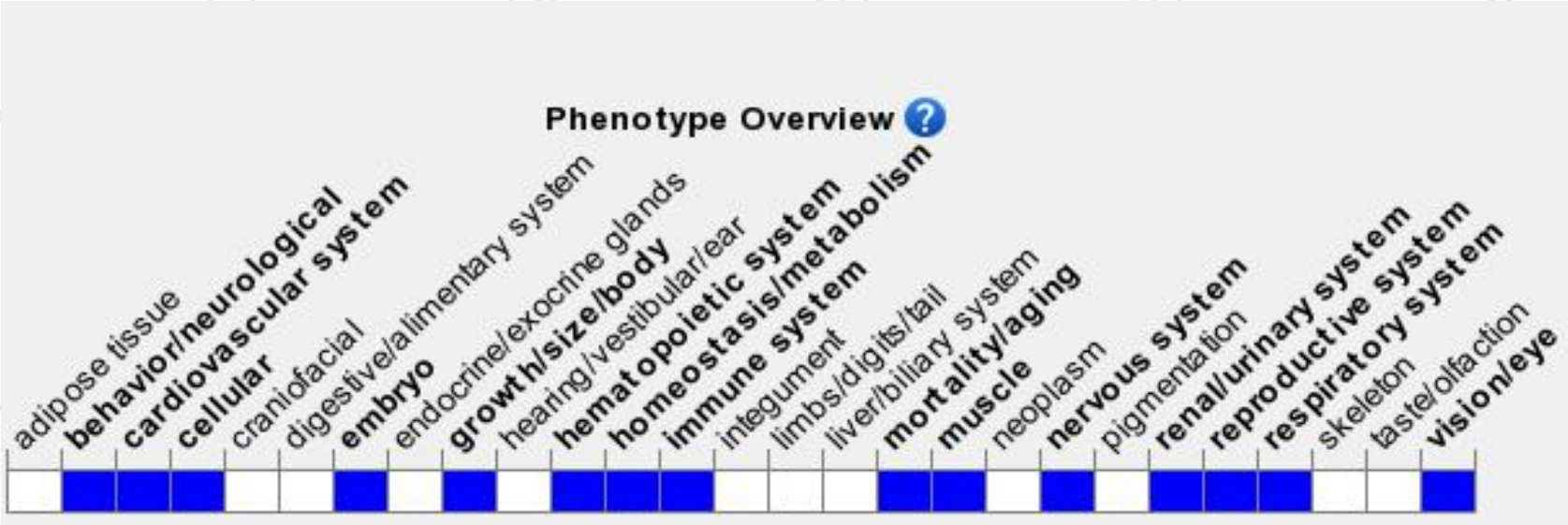
Genomic location distribution



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 with ENU induced alleles have various eye and vision defects and may show bruising at birth. Mice carrying the G498V mutation have renal glomerular defects that resolve within the first weeks of life, but show retinal tortuosity, muscular dystrophy, brain hemorrhages, and renal cysts as adults.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd