Rab25 Cas9-KO Strategy

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Reviewer：	**Huan Fan**
Design Date:	**2020-5-15**

Project Overview

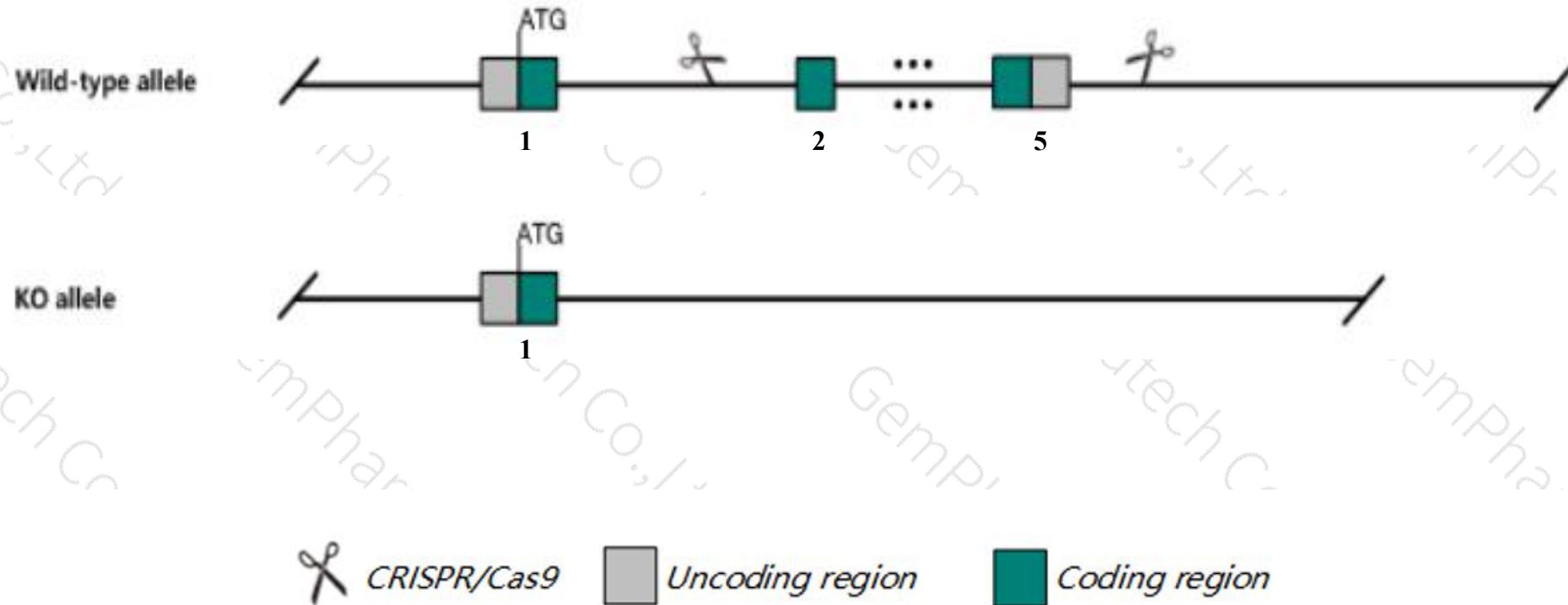|	
Project Name	*Rab25*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab25* gene. The schematic diagram is as follows:

Technical routes

➤ The *Rab25* gene has 4 transcripts. According to the structure of *Rab25* gene, exon2-exon5 of *Rab25-201* (ENSMUST00000008745.12) transcript is recommended as the knockout region. The region contains most coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Rab25* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

➢ According to the existing MGI data,on a 129 background, virgin female homozygotes older than 10 months of age develop vaginal cancer while both male and female homozygotes develop distal esophageal stricture due to squamous cell hyperplasia.

➢ The *Rab25* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

Rab25 RAB25, member RAS oncogene family [Mus musculus (house mouse)]
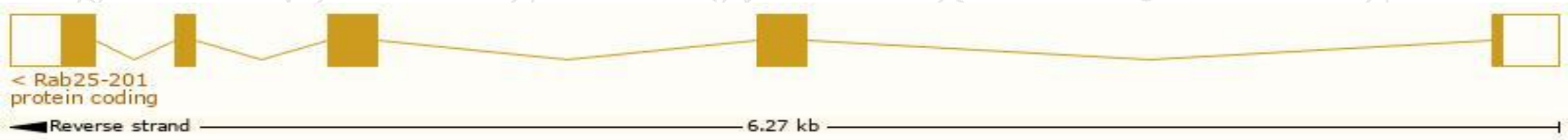
Gene ID: 53868, updated on 13-Mar-2020

▲ Summary

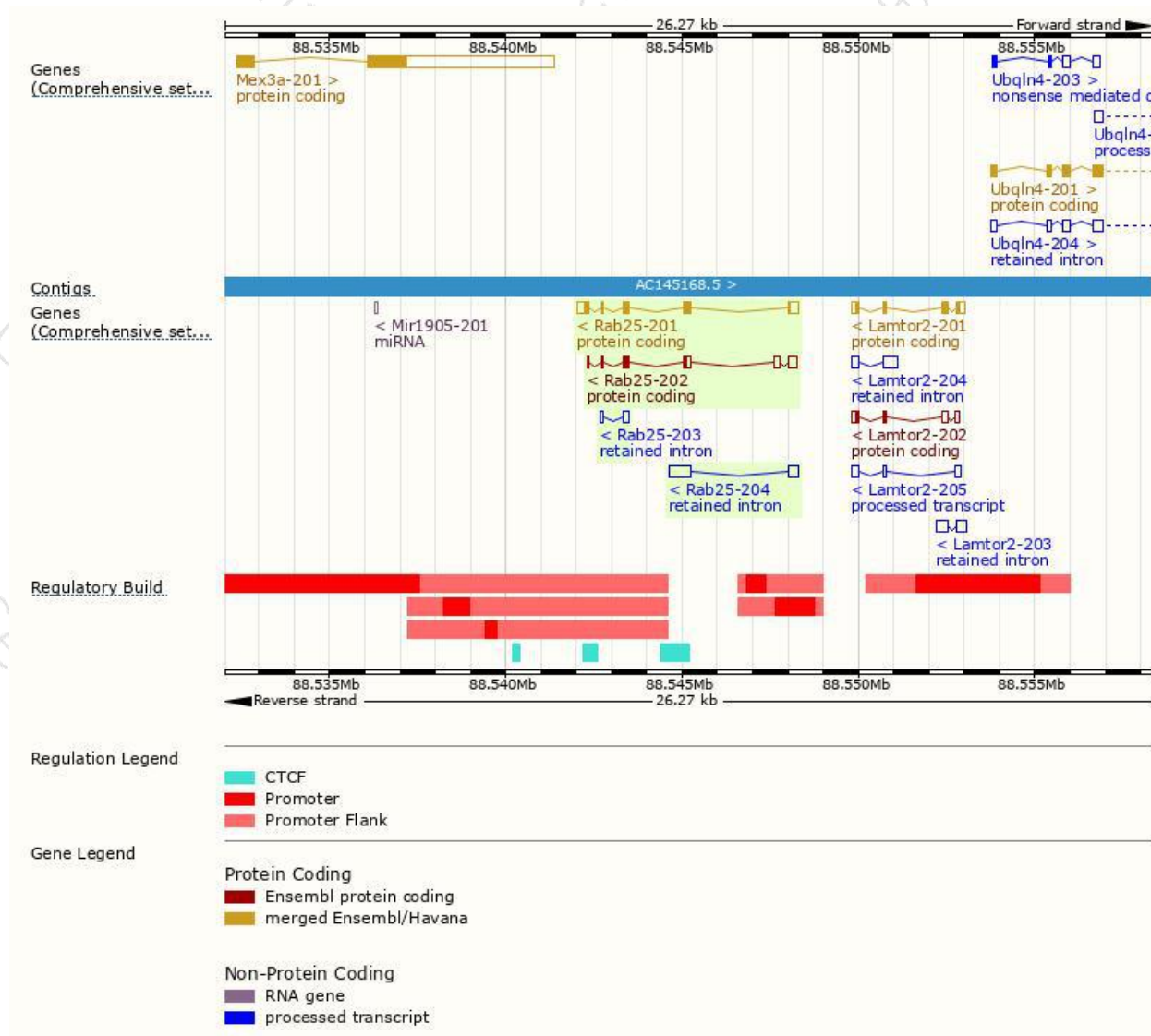|	
Official Symbol	Rab25 provided by MGI
Official Full Name	RAB25, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:1858203
See related	Ensembl:ENSMUSG00000008601
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AV012978
Expression	Biased expression in colon adult (RPKM 161.0), stomach adult (RPKM 130.1) and 10 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab25-201	ENSMUST00000008745.12	1081	213aa	Protein coding	CCDS17477	Q0PD30 Q9WTL2	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rab25-202	ENSMUST00000131775.1	897	138aa	Protein coding	-	D3YYB3	CDS 3' incomplete TSL:3
Rab25-204	ENSMUST00000149724.1	865	No protein	Retained intron	-	-	TSL:2
Rab25-203	ENSMUST00000141371.1	301	No protein	Retained intron	-	-	TSL:2

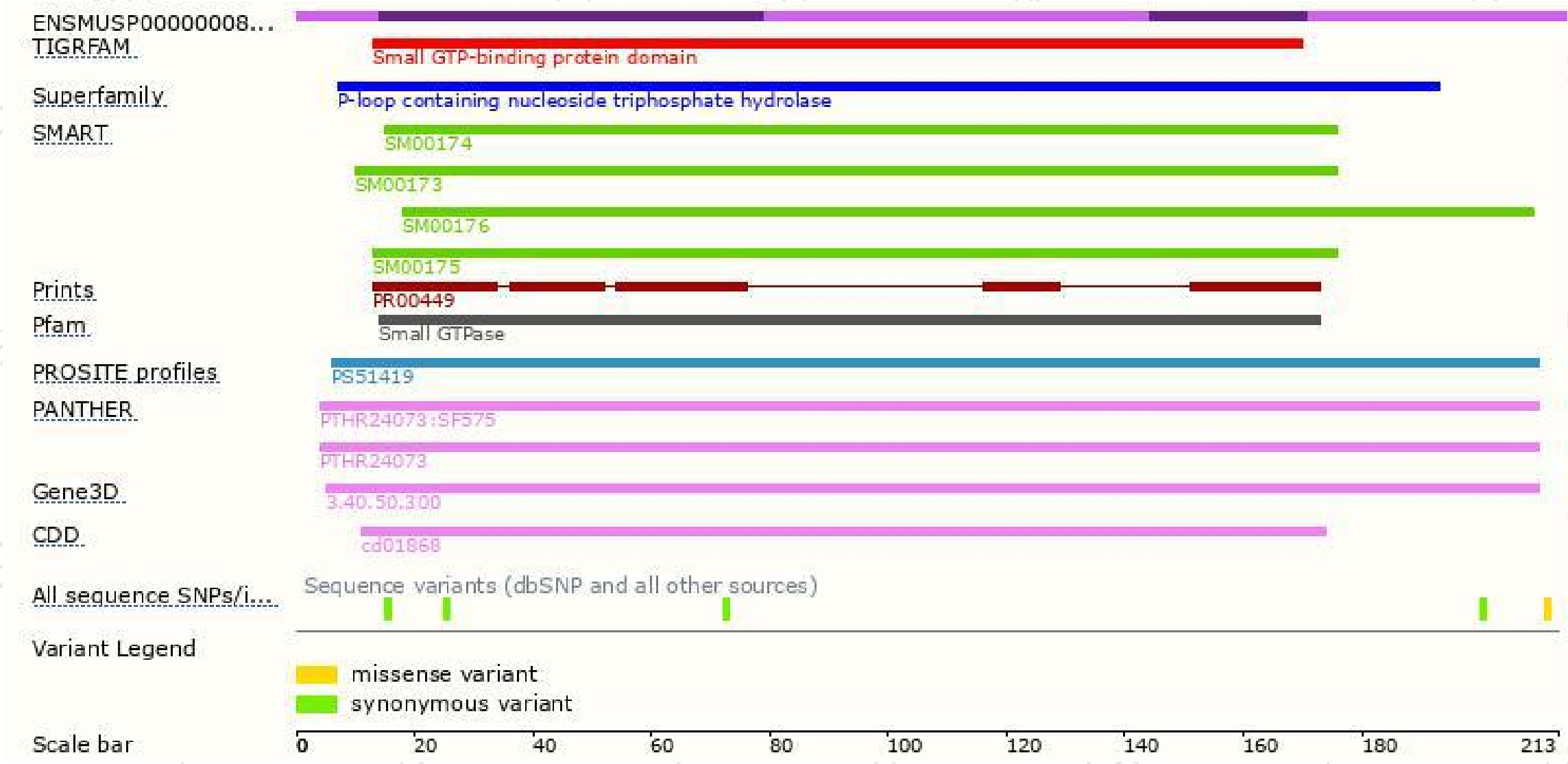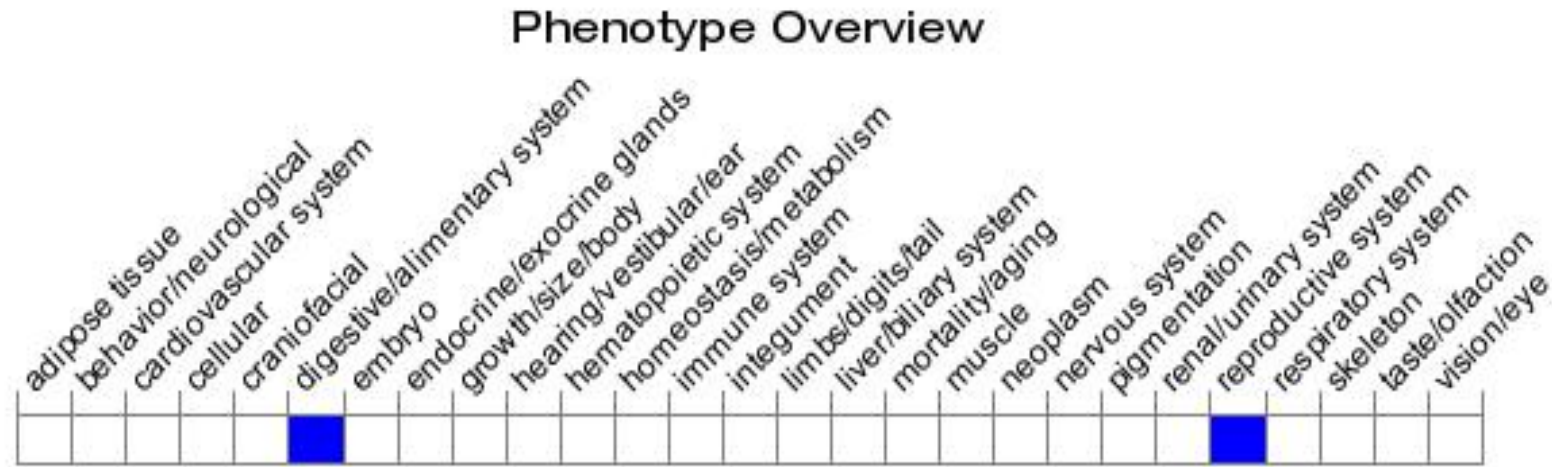The strategy is based on the design of *Rab25-201* transcript,the transcription is shown below

Genomic location distribution

Protein domain

ENSMUSP00000008...	
TIGRFAM	Small GTP-binding protein domain
Superfamily	P-loop containing nucleoside triphosphate hydrolase
SMART	SM00174
	SM00173
	SM00176
	SM00175
Prints	PR00449
Pfam	Small GTPase
PROSITE profiles	PS51419
PANTHER	PTHR24073:SF575
	PTHR24073
Gene3D	3.40.50.300
CDD	cd01868
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)
Variant Legend	▭ missense variant ▭ synonymous variant
Scale bar	0 20 40 60 80 100 120 140 160 180 213

Mouse phenotype description(MGI)

Phenotype Overview

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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