Rab23 Cas9-CKO Strategy

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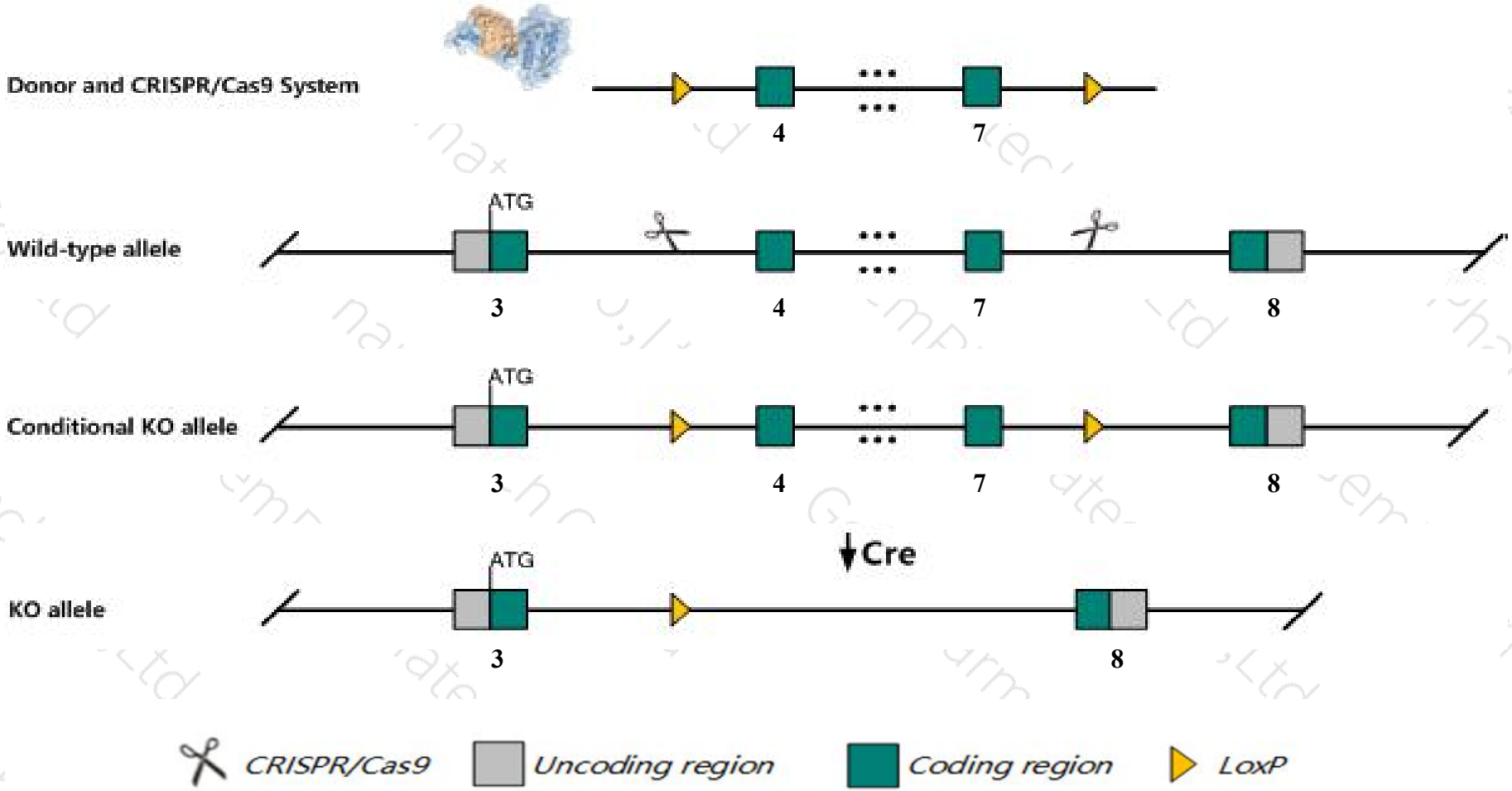
Project Overview

Project Name	***Rab23***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab23* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rab23* gene has 10 transcripts. According to the structure of *Rab23* gene, exon4-exon7 of *Rab23-201*(ENSMUST00000088287.9) transcript is recommended as the knockout region.The region contains 419bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rab23* 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.

Notice

➢ According to the existing MGI data,mice homozygous for a spontaneous allele show neural tube defects, exencephaly, spinal cord and dorsal root ganglia anomalies, malformed eyes and defects in the axial skeleton and developing limbs. Mice homozygous for an ENU-induced allele die in utero with exencephaly, polydactyly and eye defects.

➢ The *Rab23* gene is located on the Chr1. 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）

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Rab23 RAB23, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 19335, updated on 13-Mar-2020

Summary

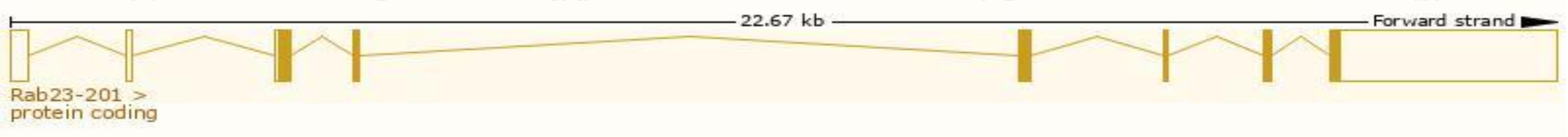
Official Symbol	Rab23 provided by MGI
Official Full Name	RAB23, member RAS oncogene family provided byMGI
Primary source	MGI:MGI:99833
See related	Ensembl:ENSMUSG00000004768
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW545388, opb, opb2
Expression	Ubiquitous expression in CNS E11.5 (RPKM 5.6), limb E14.5 (RPKM 5.2) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

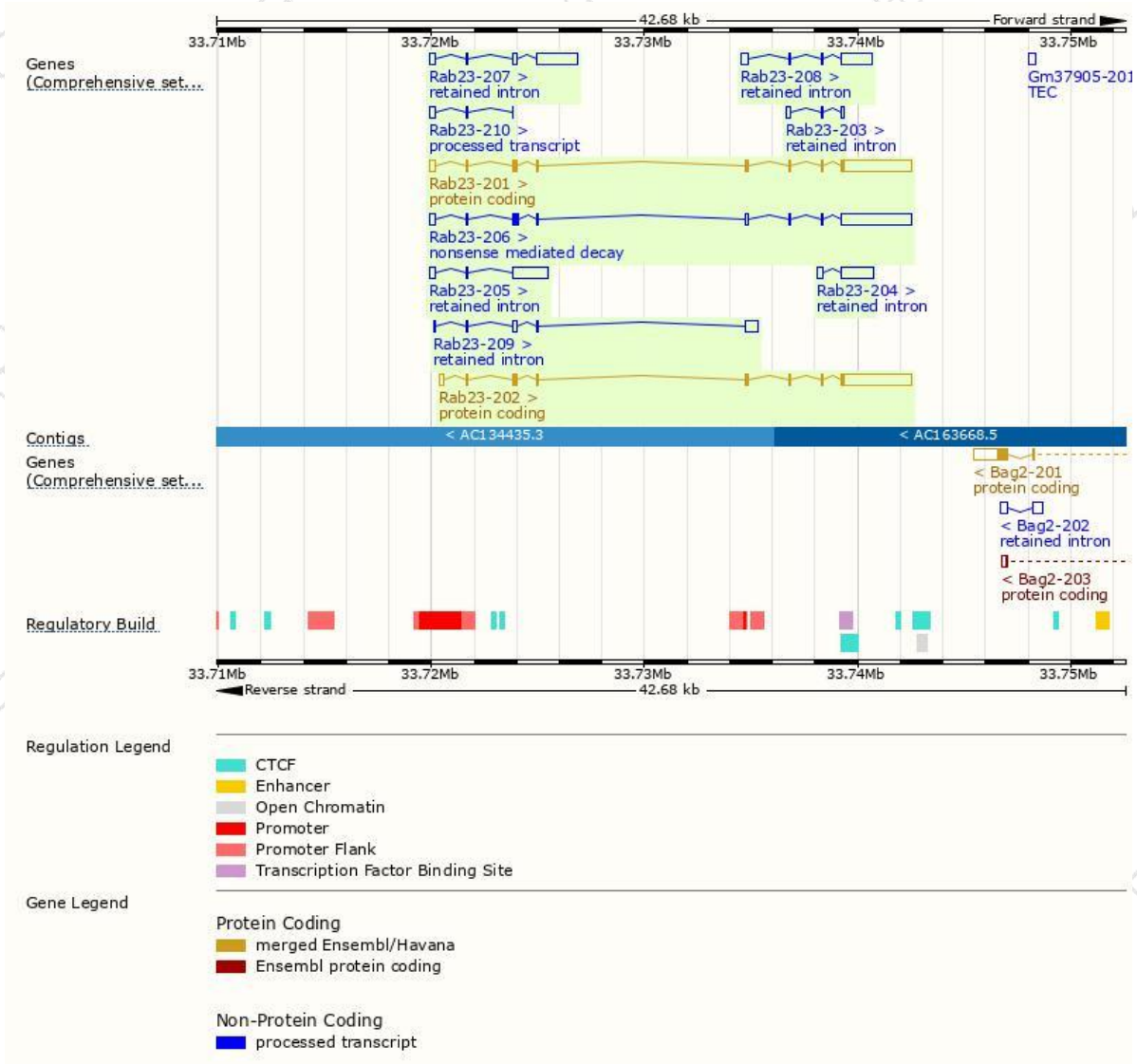
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab23-201	ENSMUST00000088287.9	4322	237aa	Protein coding	CCDS35532	Q9D4I9	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
Rab23-202	ENSMUST00000115174.3	4218	237aa	Protein coding	CCDS35532	Q9D4I9	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
Rab23-206	ENSMUST00000138024.7	4398	64aa	Nonsense mediated decay	-	M0QWL7	TSL:1
Rab23-210	ENSMUST00000195006.5	444	No protein	Processed transcript	-	-	TSL:3
Rab23-207	ENSMUST00000140354.7	2557	No protein	Retained intron	-	-	TSL:1
Rab23-205	ENSMUST00000135330.7	2047	No protein	Retained intron	-	-	TSL:1
Rab23-208	ENSMUST00000150593.7	1966	No protein	Retained intron	-	-	TSL:3
Rab23-204	ENSMUST00000132066.3	1764	No protein	Retained intron	-	-	TSL:2
Rab23-209	ENSMUST00000151482.7	1087	No protein	Retained intron	-	-	TSL:1
Rab23-203	ENSMUST00000122822.1	437	No protein	Retained intron	-	-	TSL:1

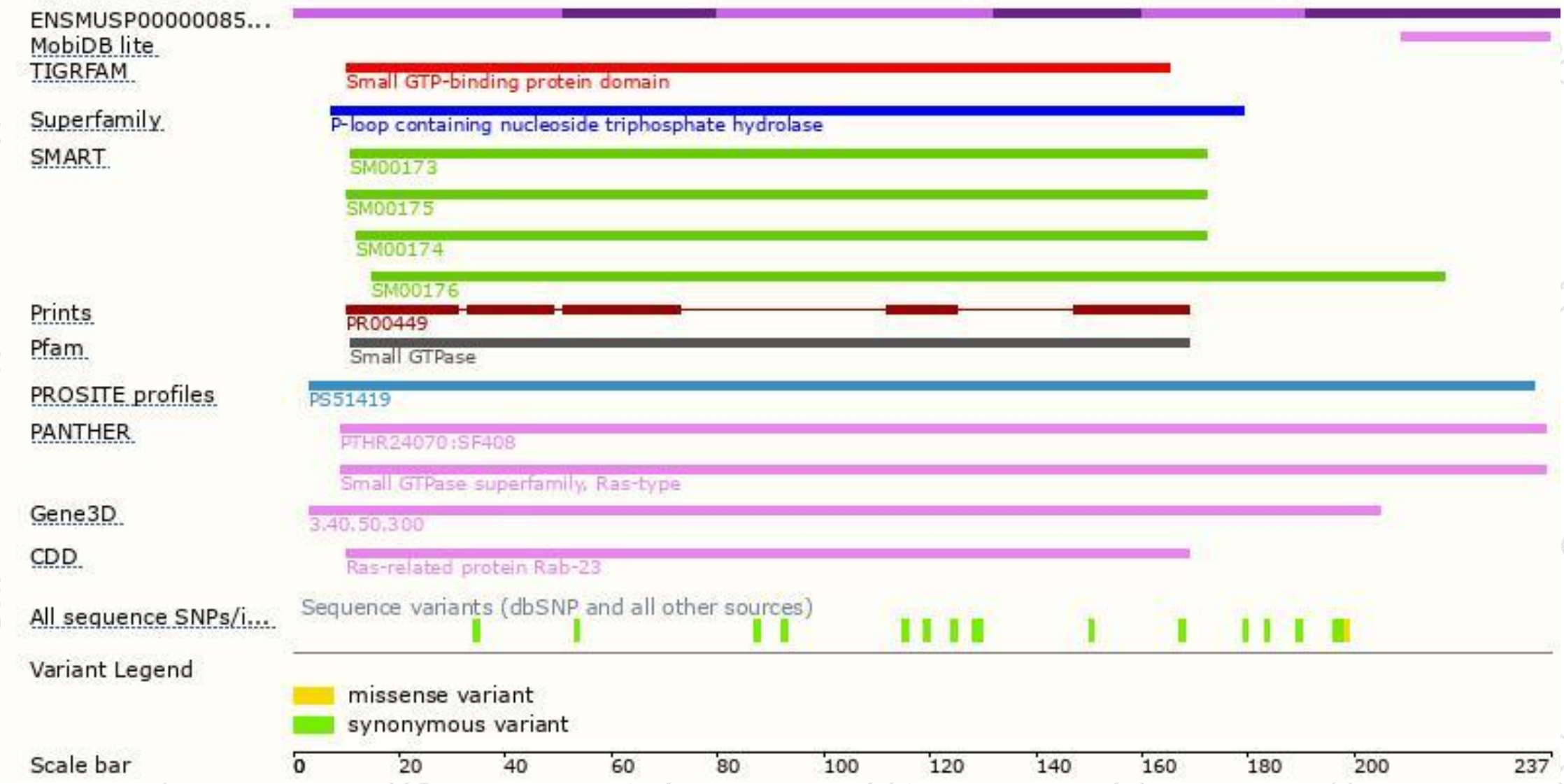
The strategy is based on the design of *Rab23-201* transcript,the transcription is shown below:



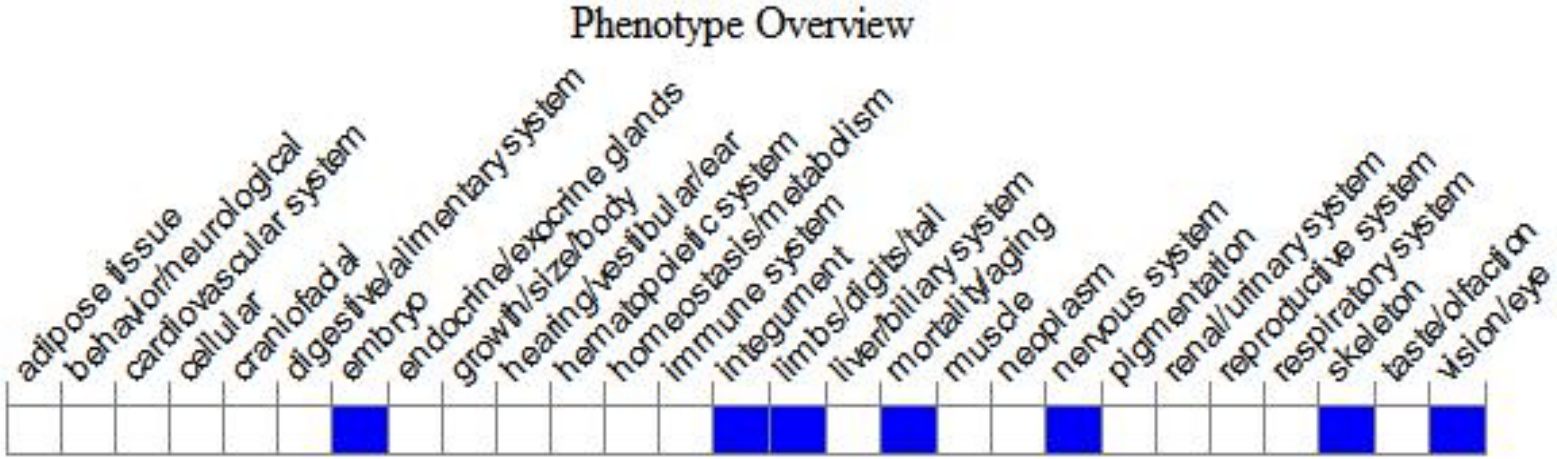
Genomic location distribution



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 spontaneous allele show neural tube defects, exencephaly, spinal cord and dorsal root ganglia anomalies, malformed eyes and defects in the axial skeleton and developing limbs. Mice homozygous for an ENU-induced allele die in utero with exencephaly, polydactyly and eye defects.

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

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