Ica1 Cas9-KO Strategy

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Reviewer: Ruirui Zhang

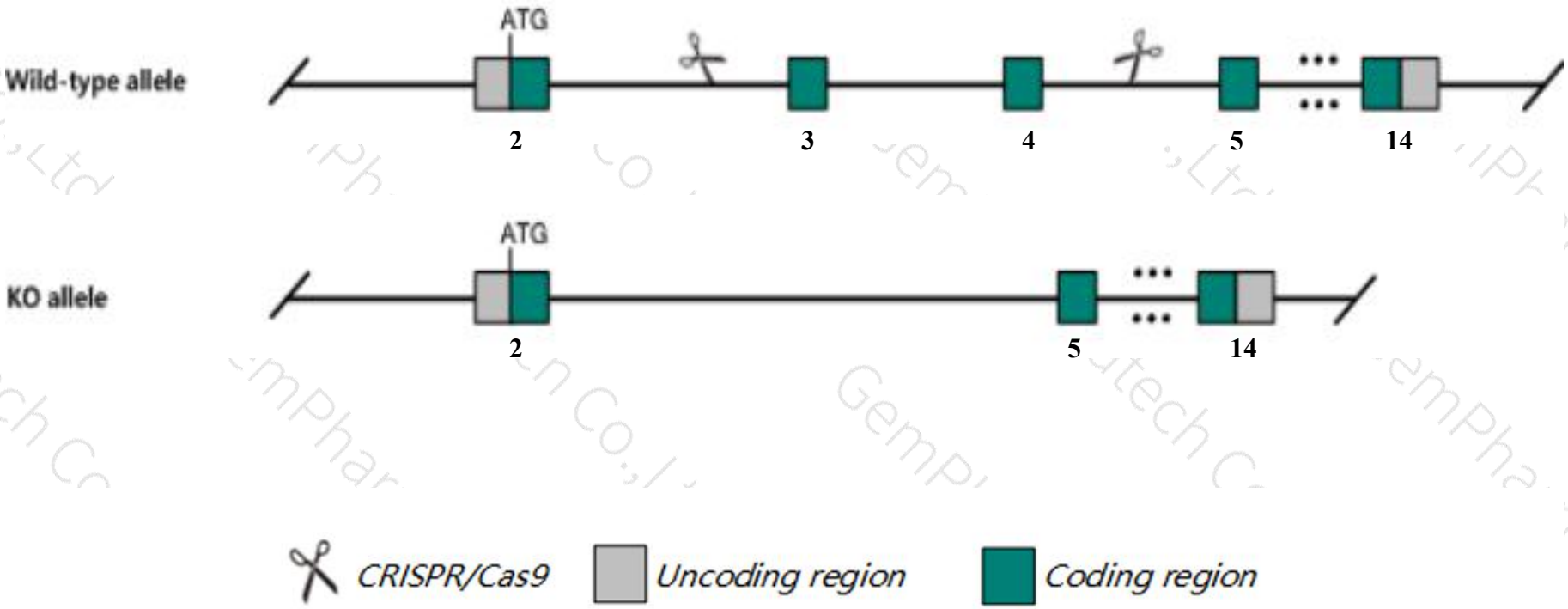
Design Date: 2020-7-24

Project Overview

Project Name	***Ica1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ica1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ica1* gene has 12 transcripts. According to the structure of *Ica1* gene, exon3-exon4 of *Ica1-201*(ENSMUST00000038403.11) transcript is recommended as the knockout region.The region contains 236bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ica1* 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.

Notice

➢ According to the existing MGI data,homozygous mutation of this gene results in diabetes and spontaneous lethality at 4-5 months of age on a NOD background, however mice on a 129/Sv background are normal. Onset of diabetes starts 4 weeks later than wild-type NOD mice and mutants are resistant to cyclophospamide-accelerated diabetes.

➢ Transcript *Ica1*-206 and *Ica1*-207 may not be affected.

➢ The *Ica1* gene is located on the Chr6. 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）

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Ica1 islet cell autoantigen 1 [Mus musculus (house mouse)]

Gene ID: 15893, updated on 13-Mar-2020

Summary

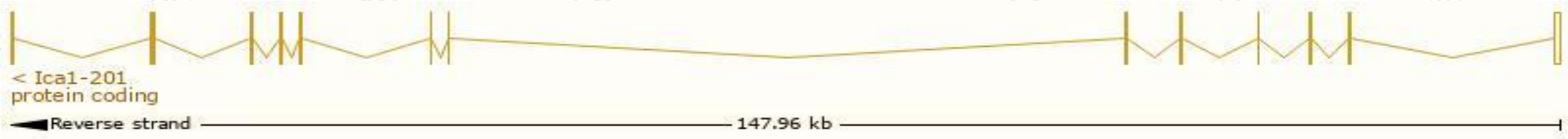
Official Symbol	Ica1 provided by MGI
Official Full Name	islet cell autoantigen 1 provided byMGI
Primary source	MGI:MGI:96391
See related	Ensembl:ENSMUSG00000062995
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	69kDa, ICA69
Expression	Broad expression in testis adult (RPKM 6.7), cortex adult (RPKM 5.0) and 19 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

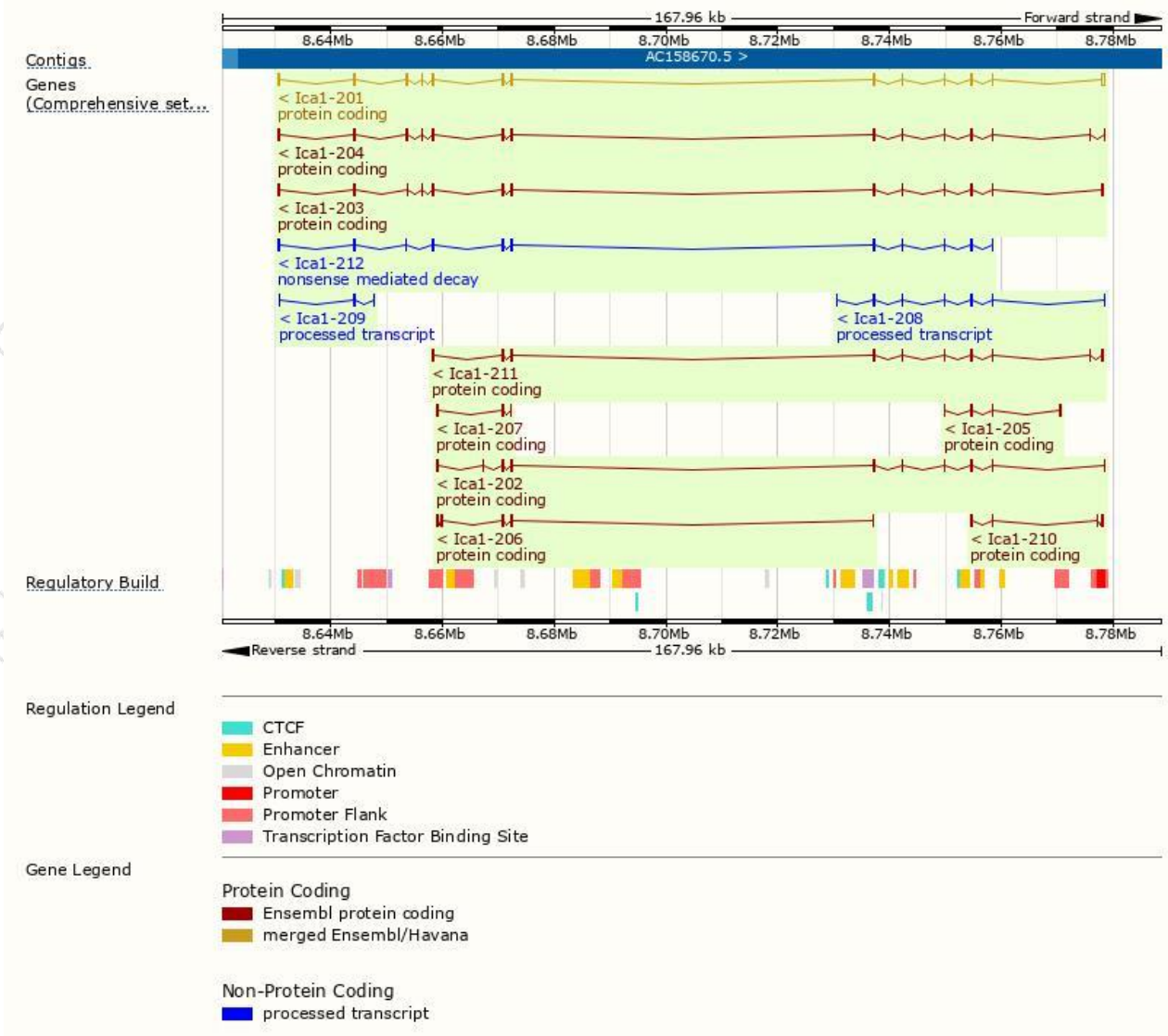
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ica1-201	ENSMUST00000038403.11	2075	478aa	Protein coding	CCDS19911	P97411	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 P2
Ica1-204	ENSMUST00000115520.7	1876	478aa	Protein coding	CCDS19911	P97411	TSL:5 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 P2
Ica1-203	ENSMUST00000115519.7	1706	465aa	Protein coding	-	D3Z118	TSL:5 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 ALT2
Ica1-202	ENSMUST00000115518.7	1256	310aa	Protein coding	-	D3Z119	TSL:1 GENCODE basic
Ica1-211	ENSMUST00000153390.7	1138	277aa	Protein coding	-	D3Z376	CDS 3' incomplete TSL:5
Ica1-206	ENSMUST00000126430.1	565	141aa	Protein coding	-	F7BG11	CDS 5' incomplete TSL:3
Ica1-210	ENSMUST00000151758.1	441	45aa	Protein coding	-	D3Z699	CDS 3' incomplete TSL:3
Ica1-205	ENSMUST00000126039.7	404	73aa	Protein coding	-	D3Z020	CDS 3' incomplete TSL:3
Ica1-207	ENSMUST00000127398.7	226	66aa	Protein coding	-	F6UY19	CDS 5' incomplete TSL:3
Ica1-212	ENSMUST00000156695.7	1493	302aa	Nonsense mediated decay	-	S4R217	TSL:5
Ica1-208	ENSMUST00000135113.1	960	No protein	Processed transcript	-	-	TSL:1
Ica1-209	ENSMUST00000145870.1	470	No protein	Processed transcript	-	-	TSL:2

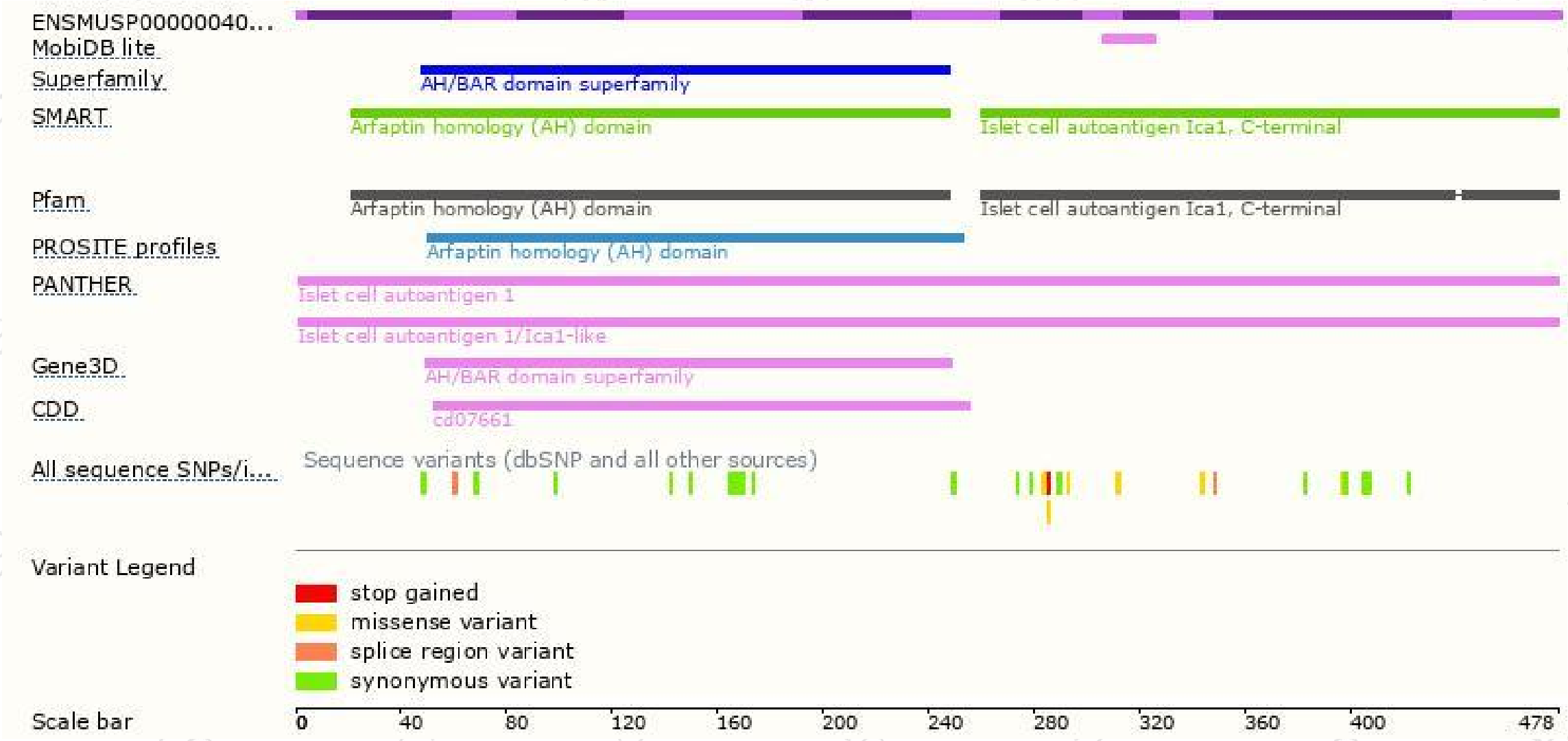
The strategy is based on the design of *Ica1-201* transcript,the transcription is shown below:



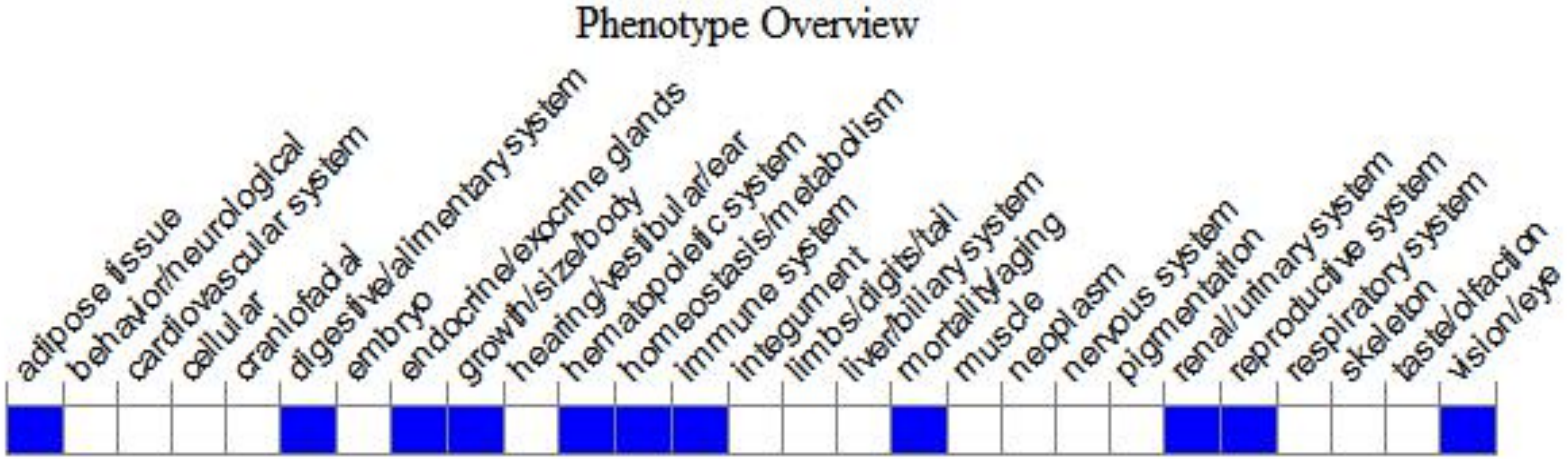
Genomic location distribution



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/).

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

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