Isl1 Cas9-CKO Strategy

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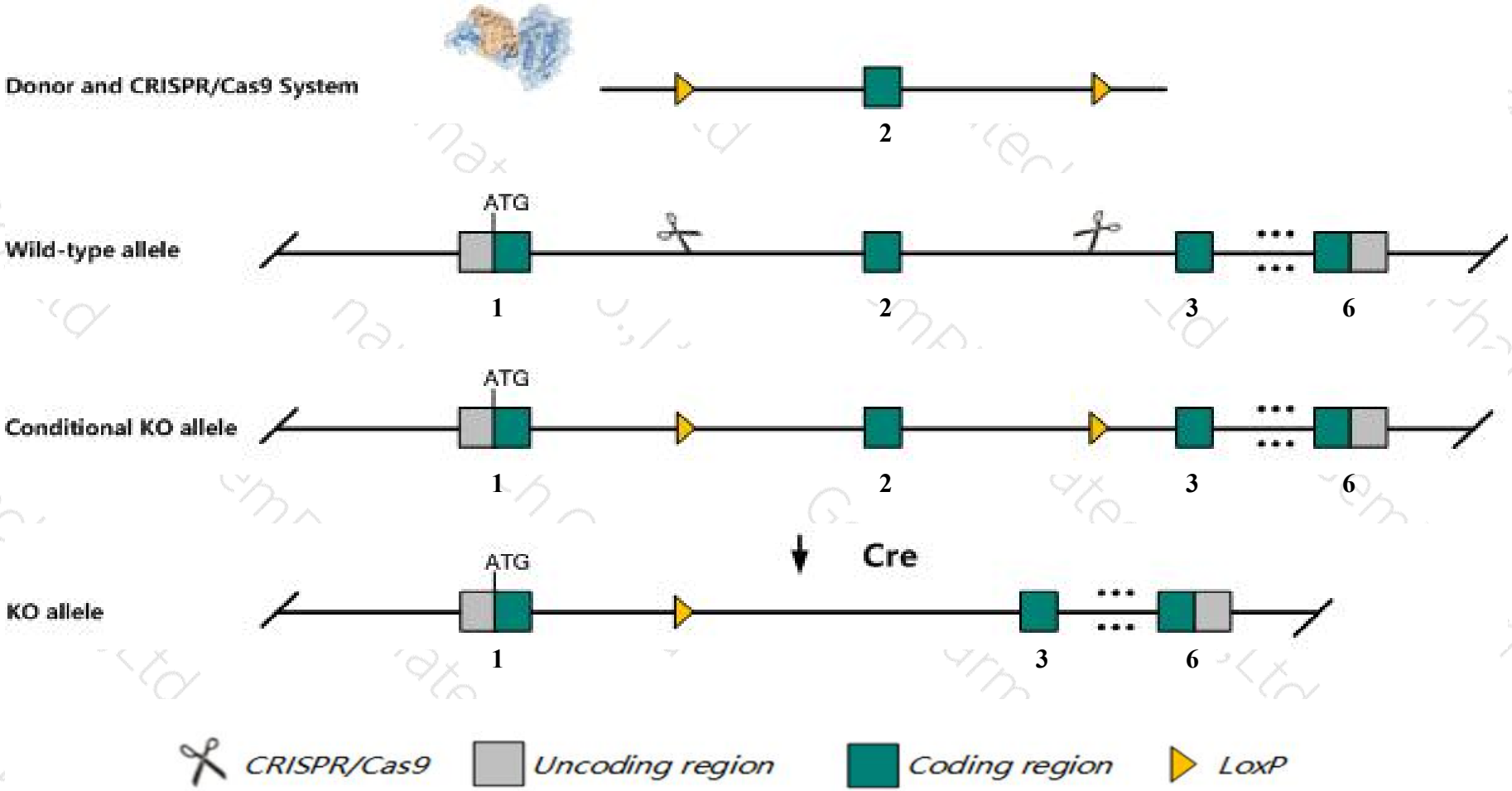
Project Overview

Project Name	***Isl1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Isl1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Isl1* gene has 5 transcripts. According to the structure of *Isl1* gene, exon2 of *Isl1-201*(ENSMUST00000036060.12) transcript is recommended as the knockout region.The region contains 190bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Isl1* 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,homozygotes for a targeted null mutation fail to develop motor neurons and die by embryonic day 11.5 with abnormal heart and pancreas development. Mice heterozygous for an ENU mutation exhibit chronic otitis media and hearing loss.

➢ The *Isl1* gene is located on the Chr13. 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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Isl1 ISL1 transcription factor, LIM/homeodomain [Mus musculus (house mouse)]

Gene ID: 16392, updated on 13-Mar-2020

Summary

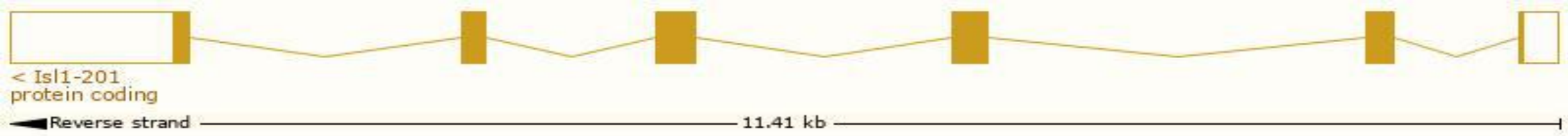
Official Symbol	Isl1 provided by MGI
Official Full Name	ISL1 transcription factor, LIM/homeodomain provided byMGI
Primary source	MGI:MGI:101791
See related	Ensembl:ENSMUSG00000042258
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in whole brain E14.5 (RPKM 10.4), CNS E14 (RPKM 9.7) and 9 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

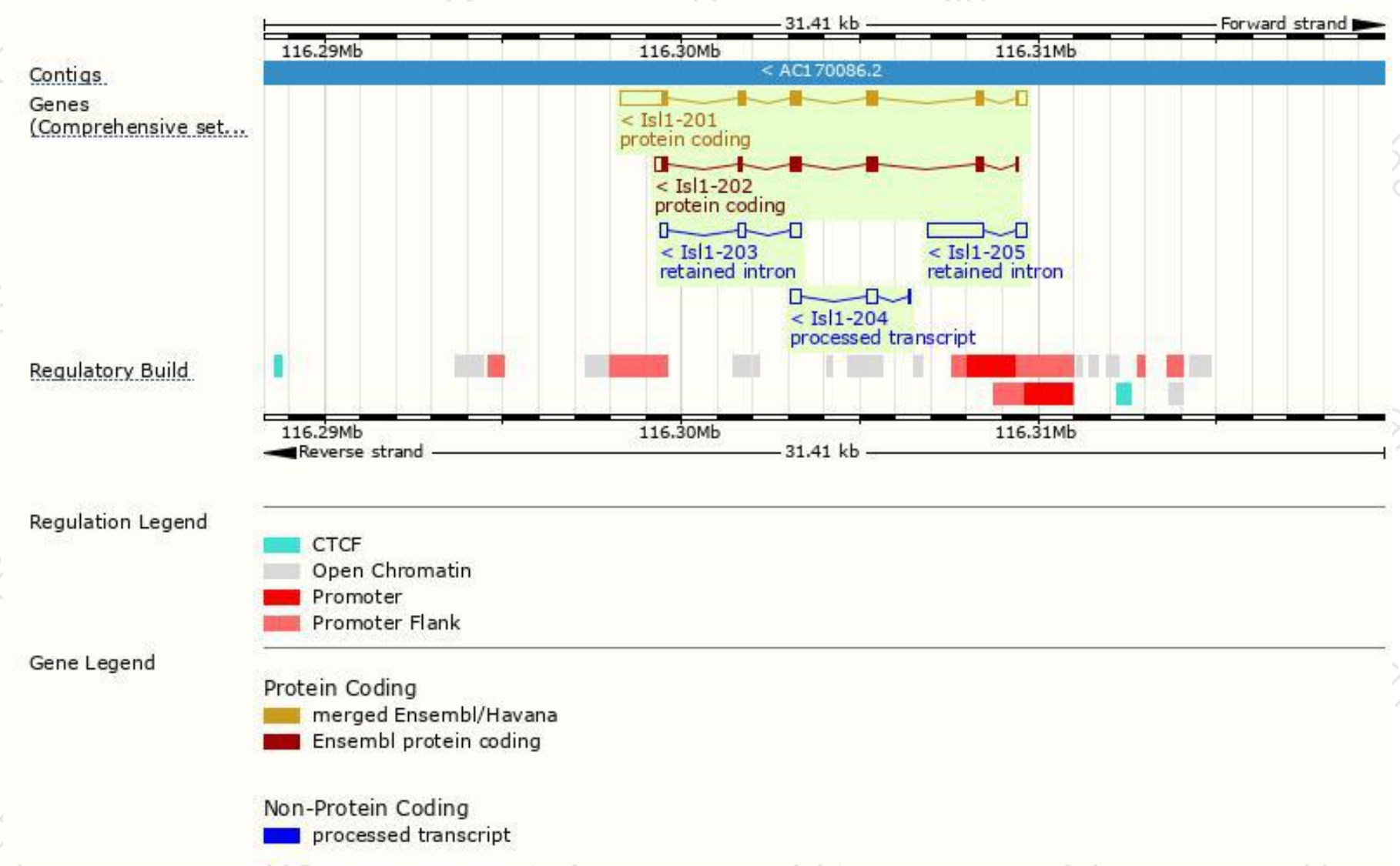
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Isl1-201	ENSMUST00000036060.12	2516	349aa	Protein coding	CCDS26790	A2RSV5 P61372	TSL:1 GENCODE basic APPRIS P1
Isl1-202	ENSMUST00000176044.2	1198	326aa	Protein coding	-	P61372	TSL:1 GENCODE basic
Isl1-204	ENSMUST00000176812.2	588	No protein	Processed transcript	-	-	TSL:3
Isl1-205	ENSMUST00000177469.2	1864	No protein	Retained intron	-	-	TSL:1
Isl1-203	ENSMUST00000176444.1	676	No protein	Retained intron	-	-	TSL:1

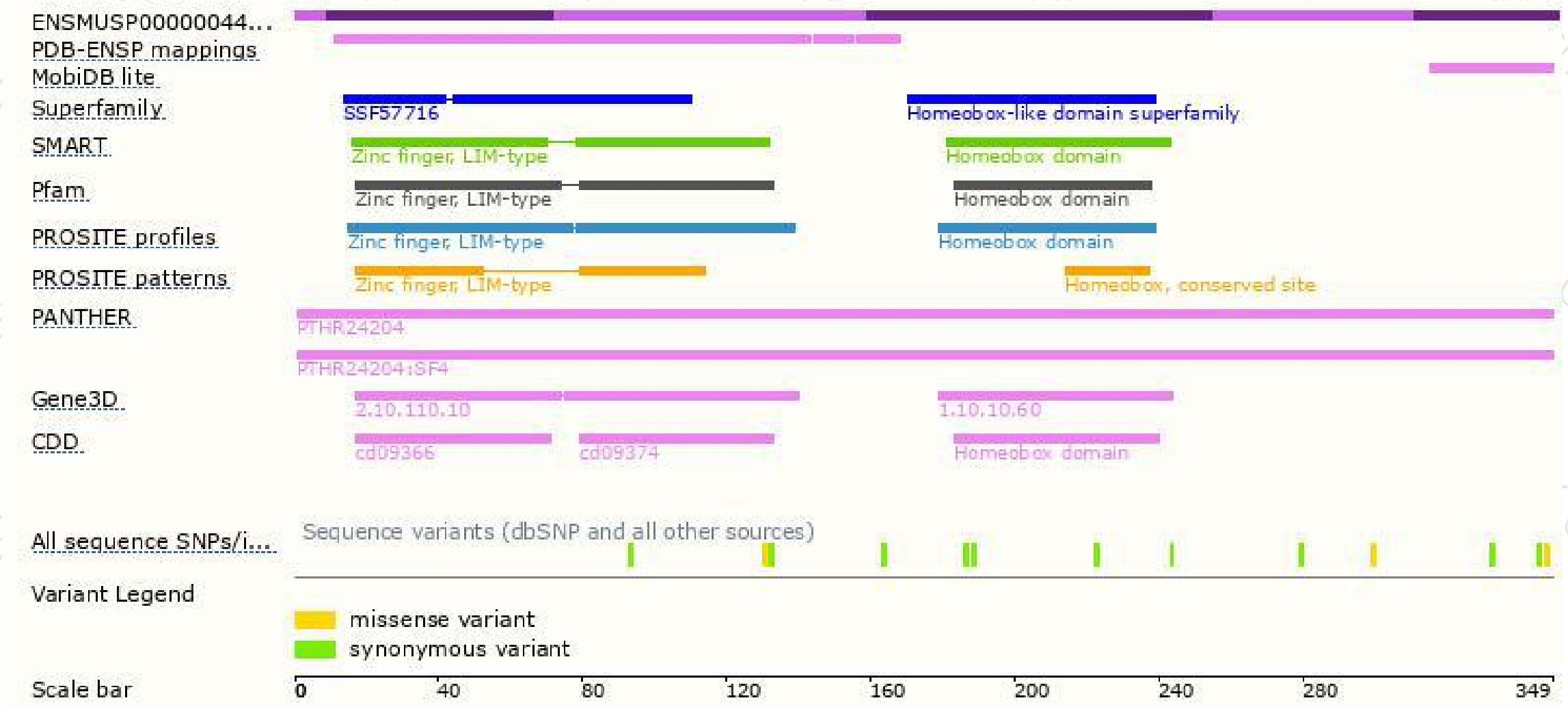
The strategy is based on the design of *Isl1-201* transcript,the transcription is shown below:



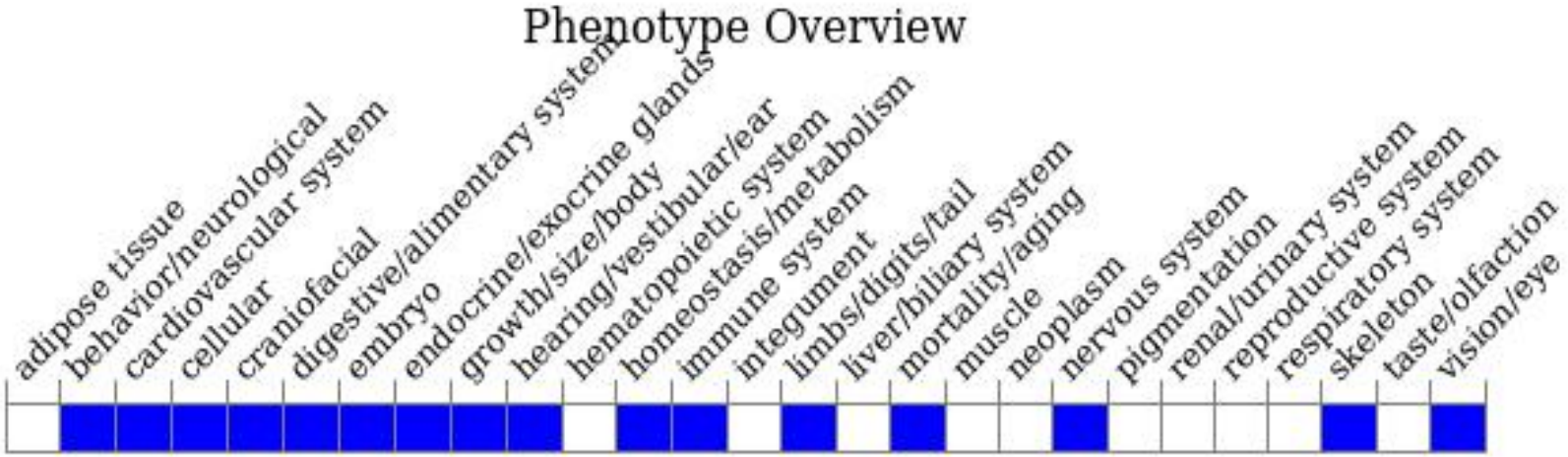
Genomic location distribution



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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