Rptor Cas9-CKO Strategy

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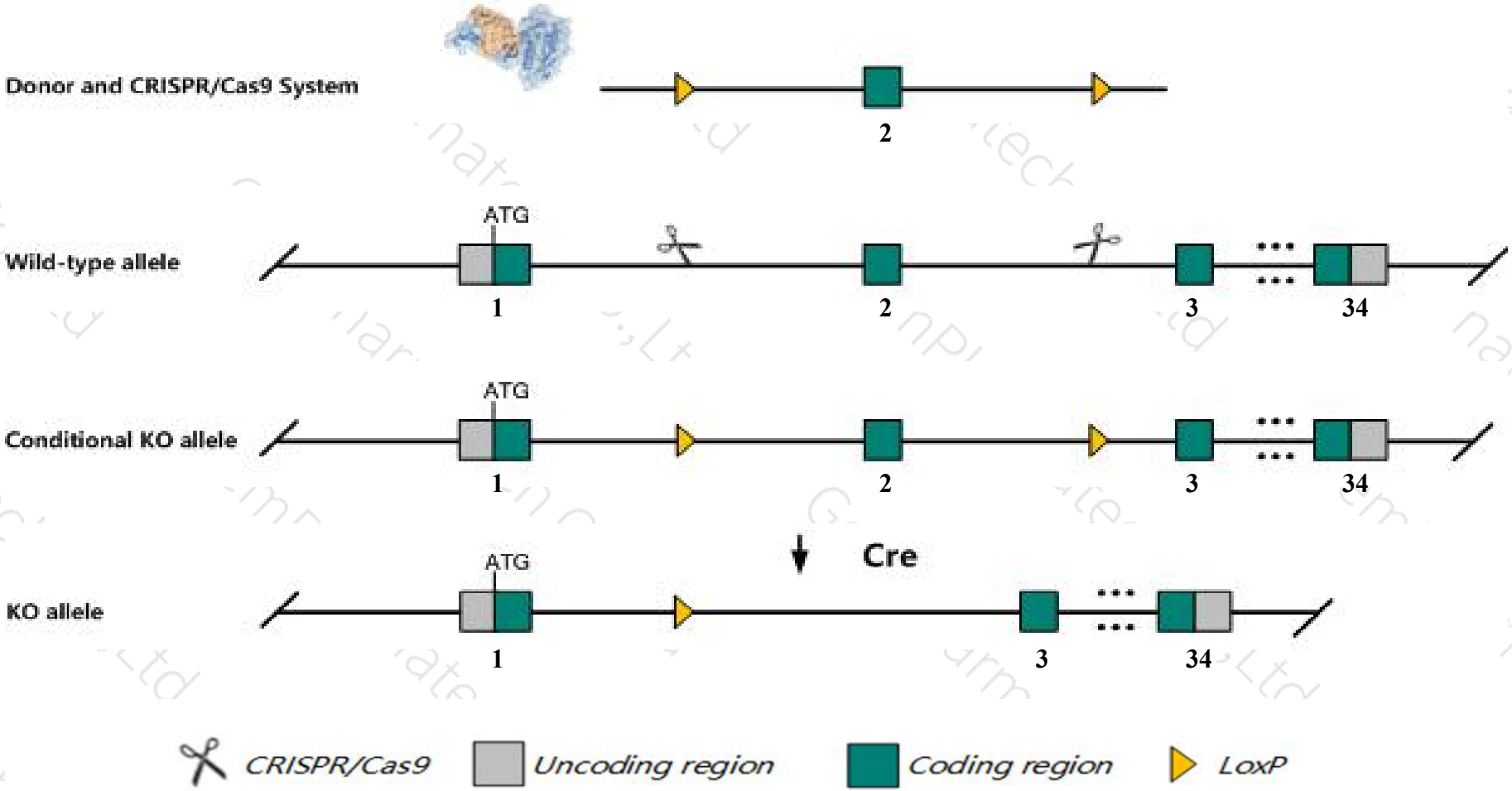
Project Overview

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

Conditional Knockout strategy

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



Technical routes

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- The *Rptor* gene has 12 transcripts. According to the structure of *Rptor* gene, exon2 of *Rptor-201* (ENSMUST00000026671.12) transcript is recommended as the knockout region.The region contains 103bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rptor* 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,Homozygous mutation of this gene results in lethality prior to somitogenesis. Mice homozygous for a conditional allele activated in dendritic cells exhibit increased susceptibility to induced colitis and expansion of certain populations of dendritic cells.

- The *Rptor* gene is located on the Chr11. 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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Rptor regulatory associated protein of MTOR, complex 1 [Mus musculus (house mouse)]

Gene ID: 74370, updated on 9-Apr-2019

Summary

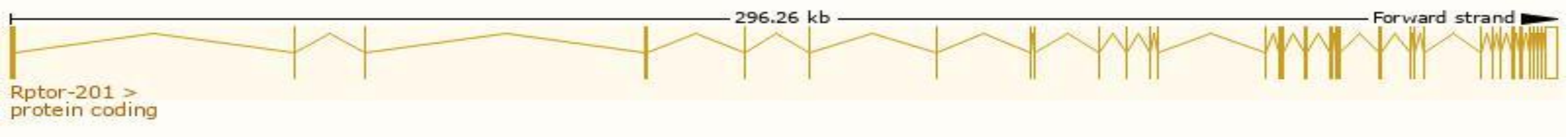
Official Symbol	Rptor provided by MGI
Official Full Name	regulatory associated protein of MTOR, complex 1 provided by MGI
Primary source	MGI:MGI:1921620
See related	Ensembl:ENSMUSG00000025583
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4932417H02Rik, Rap, Raptor, mKIAA1303
Summary	This gene encodes a subunit of mammalian target of rapamycin complex 1 (mTORC1), a component of the mTOR signaling pathway, which regulates cell growth in response to nutrient and energy levels. The encoded protein may regulate the assembly, localization, and substrate binding of the mTORC1 complex. Homozygous knockout mice for this gene exhibit embryonic lethality. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2015]
Expression	Ubiquitous expression in adrenal adult (RPKM 14.6), thymus adult (RPKM 13.0) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

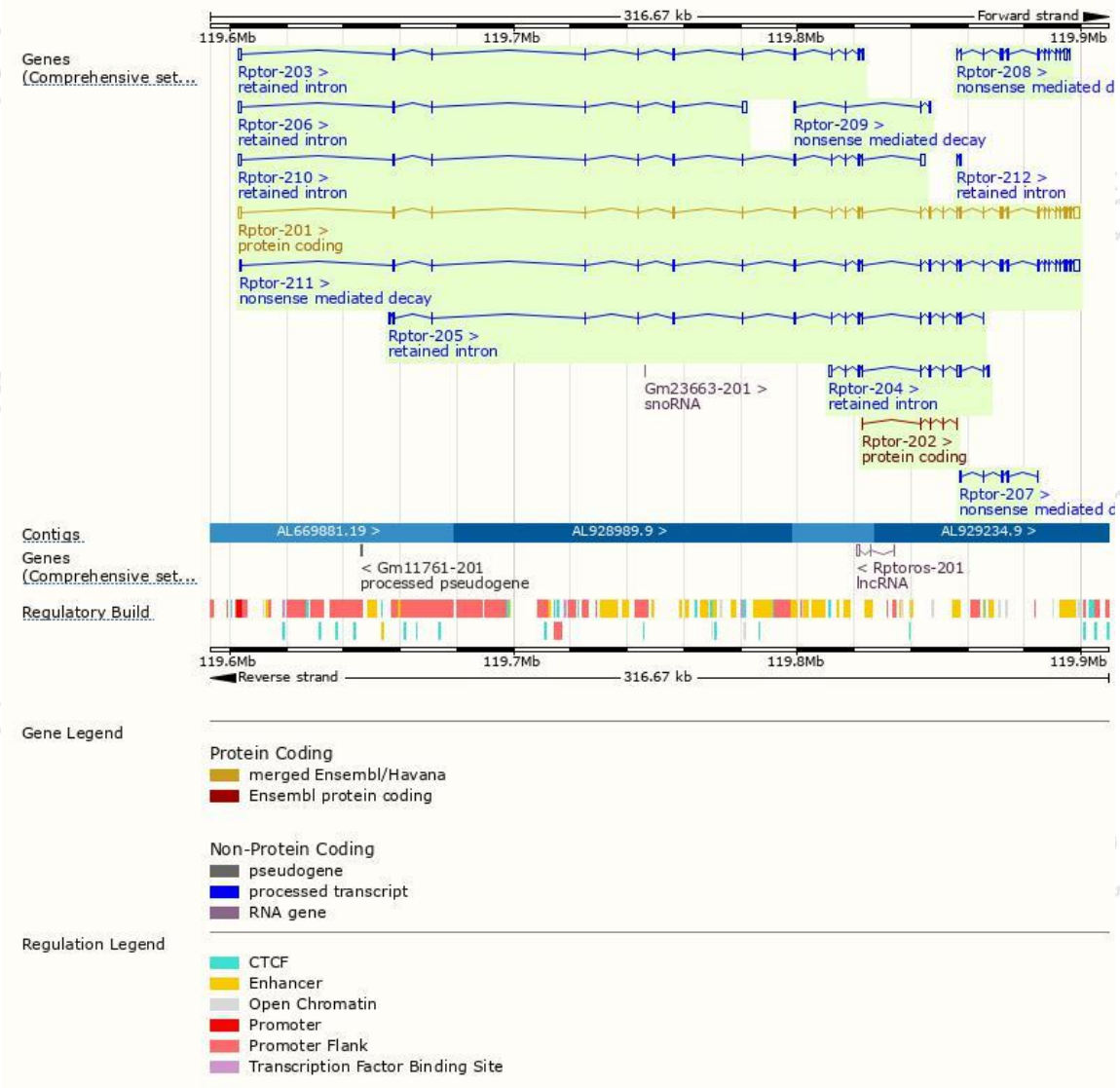
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rptor-201	ENSMUST00000026671.12	6594	1335aa	Protein coding	CCDS25720	A2ACM0	TSL:1 GENCODE basic APPRIS P1
Rptor-202	ENSMUST00000124401.1	515	171aa	Protein coding	-	F7BQS0	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Rptor-211	ENSMUST00000147781.7	6138	387aa	Nonsense mediated decay	-	E9PXZ5	TSL:1
Rptor-208	ENSMUST00000136662.7	2355	264aa	Nonsense mediated decay	-	F7CN67	CDS 5' incomplete TSL:2
Rptor-209	ENSMUST00000139728.7	688	89aa	Nonsense mediated decay	-	F6U5L3	CDS 5' incomplete TSL:3
Rptor-207	ENSMUST00000131217.1	627	102aa	Nonsense mediated decay	-	F7B6I3	CDS 5' incomplete TSL:5
Rptor-210	ENSMUST00000147772.7	3662	No protein	Retained intron	-	-	TSL:2
Rptor-206	ENSMUST00000130049.7	3384	No protein	Retained intron	-	-	TSL:2
Rptor-204	ENSMUST00000126802.1	3247	No protein	Retained intron	-	-	TSL:1
Rptor-205	ENSMUST00000127899.7	2805	No protein	Retained intron	-	-	TSL:2
Rptor-203	ENSMUST00000125583.7	2569	No protein	Retained intron	-	-	TSL:2
Rptor-212	ENSMUST00000148860.1	566	No protein	Retained intron	-	-	TSL:2

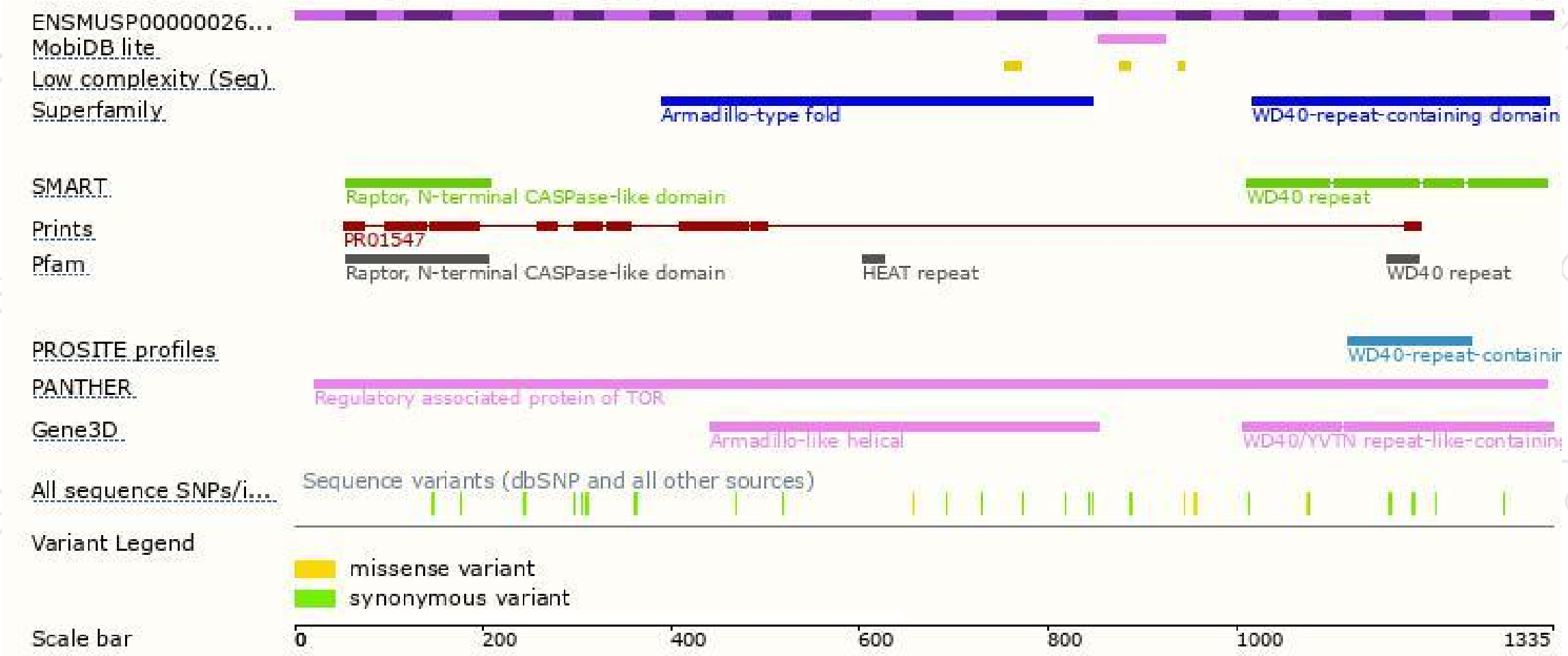
The strategy is based on the design of *Rptor-201* transcript,The transcription is shown below



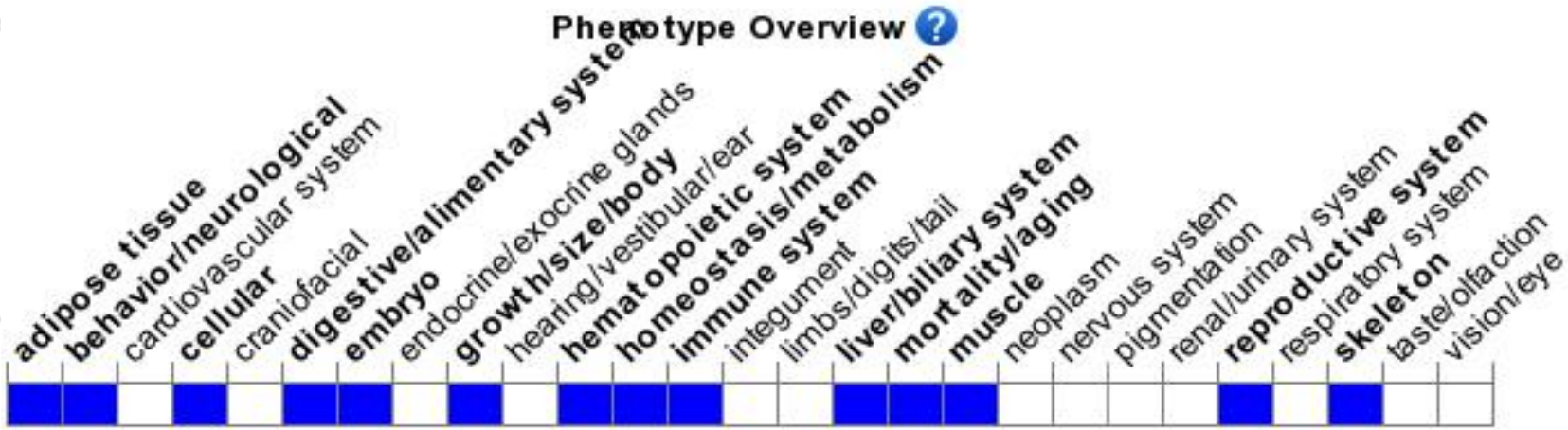
Genomic location distribution



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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