Huwe1 Cas9-CKO Strategy

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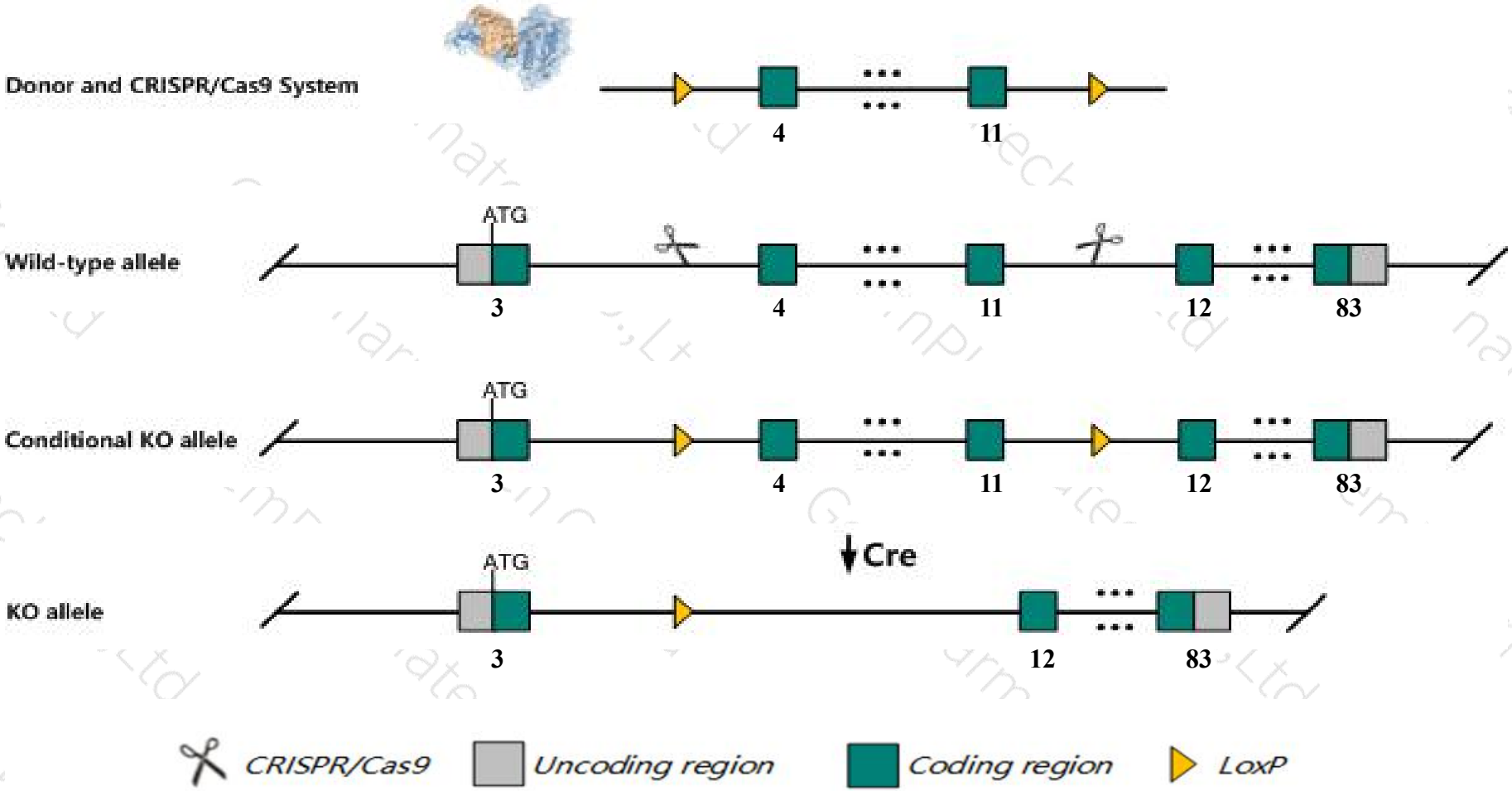
Design Date: 2019-8-15

Project Overview

Project Name	***Huwe1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Huwe1* gene. The schematic diagram is as follows:



Technical routes

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- The *Huwe1* gene has 23 transcripts. According to the structure of *Huwe1* gene, exon4-exon11 of *Huwe1-201* (ENSMUST00000026292.14) transcript is recommended as the knockout region.The region contains 817bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Huwe1* 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,Mice homozygous for a conditional allele activated in neurons results in neonatal lethality, poorly developed dentate gyrus, small cerebellum, increased cortex density, and increased neuronal precursor cell proliferation.

- The *Huwe1* gene is located on the ChrX. 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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Huwe1 HECT, UBA and WWE domain containing 1 [Mus musculus (house mouse)]

Gene ID: 59026, updated on 19-Mar-2019

Summary

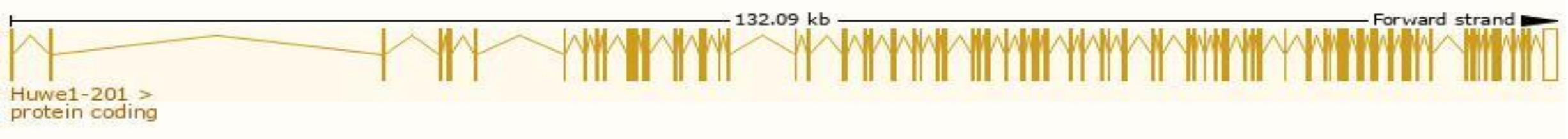
Official Symbol	Huwe1 provided by MGI
Official Full Name	HECT, UBA and WWE domain containing 1 provided by MGI
Primary source	MGI:MGI:1926884
See related	Ensembl:ENSMUSG00000025261
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5430439H10Rik, AU041296, Arf-bp1, C430014N20Rik, C80292, Gm1718, Ib772, LASU1, Mule, Ureb1
Expression	Ubiquitous expression in limb E14.5 (RPKM 24.1), CNS E14 (RPKM 22.4) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

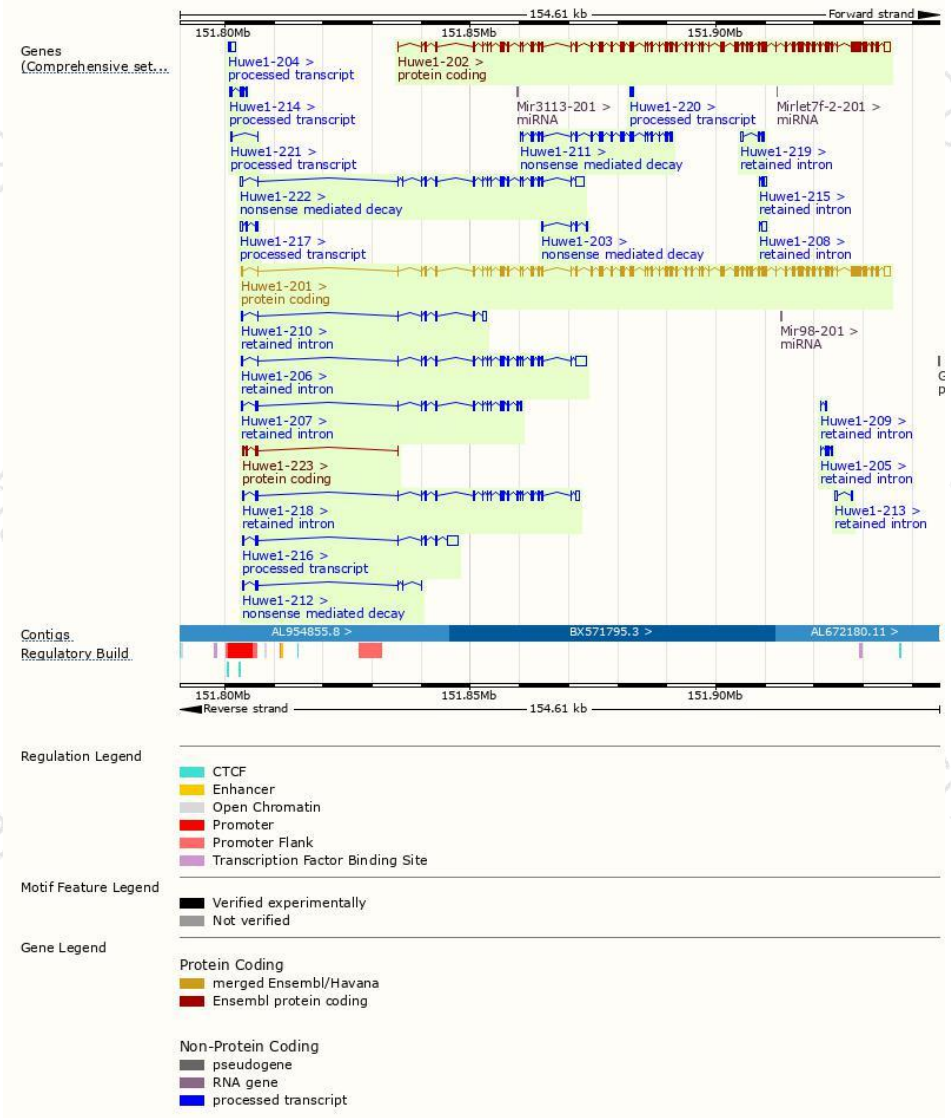
The gene has 23 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Huwe1-201	ENSMUST00000026292.14	14579	4378aa	Protein coding	CCDS41176	A2AFQ0	TSL:1 GENCODE basic APPRIS P2
Huwe1-202	ENSMUST00000112622.7	14168	4377aa	Protein coding	-	Q7TMY8	TSL:1 GENCODE basic APPRIS ALT2
Huwe1-223	ENSMUST00000156616.8	570	9aa	Protein coding	-	A0A0G2JD97	CDS 3' incomplete TSL:3
Huwe1-222	ENSMUST00000153687.7	4473	40aa	Nonsense mediated decay	-	D6RDF2	TSL:1
Huwe1-211	ENSMUST00000138023.7	3597	1176aa	Nonsense mediated decay	-	F6XP90	CDS 5' incomplete TSL:5
Huwe1-203	ENSMUST00000123306.1	673	150aa	Nonsense mediated decay	-	F6UYC1	CDS 5' incomplete TSL:3
Huwe1-212	ENSMUST00000138566.7	644	40aa	Nonsense mediated decay	-	D6RDF2	TSL:3
Huwe1-216	ENSMUST00000147666.7	3112	No protein	Processed transcript	-	-	TSL:1
Huwe1-204	ENSMUST00000129714.1	1250	No protein	Processed transcript	-	-	TSL:1
Huwe1-217	ENSMUST00000149344.7	681	No protein	Processed transcript	-	-	TSL:5
Huwe1-214	ENSMUST00000139386.1	665	No protein	Processed transcript	-	-	TSL:3
Huwe1-220	ENSMUST00000150882.1	252	No protein	Processed transcript	-	-	TSL:3
Huwe1-221	ENSMUST00000152757.7	194	No protein	Processed transcript	-	-	TSL:5
Huwe1-206	ENSMUST00000130243.7	5010	No protein	Retained intron	-	-	TSL:1
Huwe1-218	ENSMUST00000150020.1	3710	No protein	Retained intron	-	-	TSL:1
Huwe1-207	ENSMUST00000131786.7	2627	No protein	Retained intron	-	-	TSL:1
Huwe1-210	ENSMUST00000137816.7	1702	No protein	Retained intron	-	-	TSL:1
Huwe1-219	ENSMUST00000150426.7	901	No protein	Retained intron	-	-	TSL:5
Huwe1-208	ENSMUST00000132453.7	896	No protein	Retained intron	-	-	TSL:2
Huwe1-215	ENSMUST00000139909.1	821	No protein	Retained intron	-	-	TSL:2
Huwe1-205	ENSMUST00000130234.1	667	No protein	Retained intron	-	-	TSL:3
Huwe1-213	ENSMUST00000139245.1	621	No protein	Retained intron	-	-	TSL:3
Huwe1-209	ENSMUST00000133051.1	418	No protein	Retained intron	-	-	TSL:3

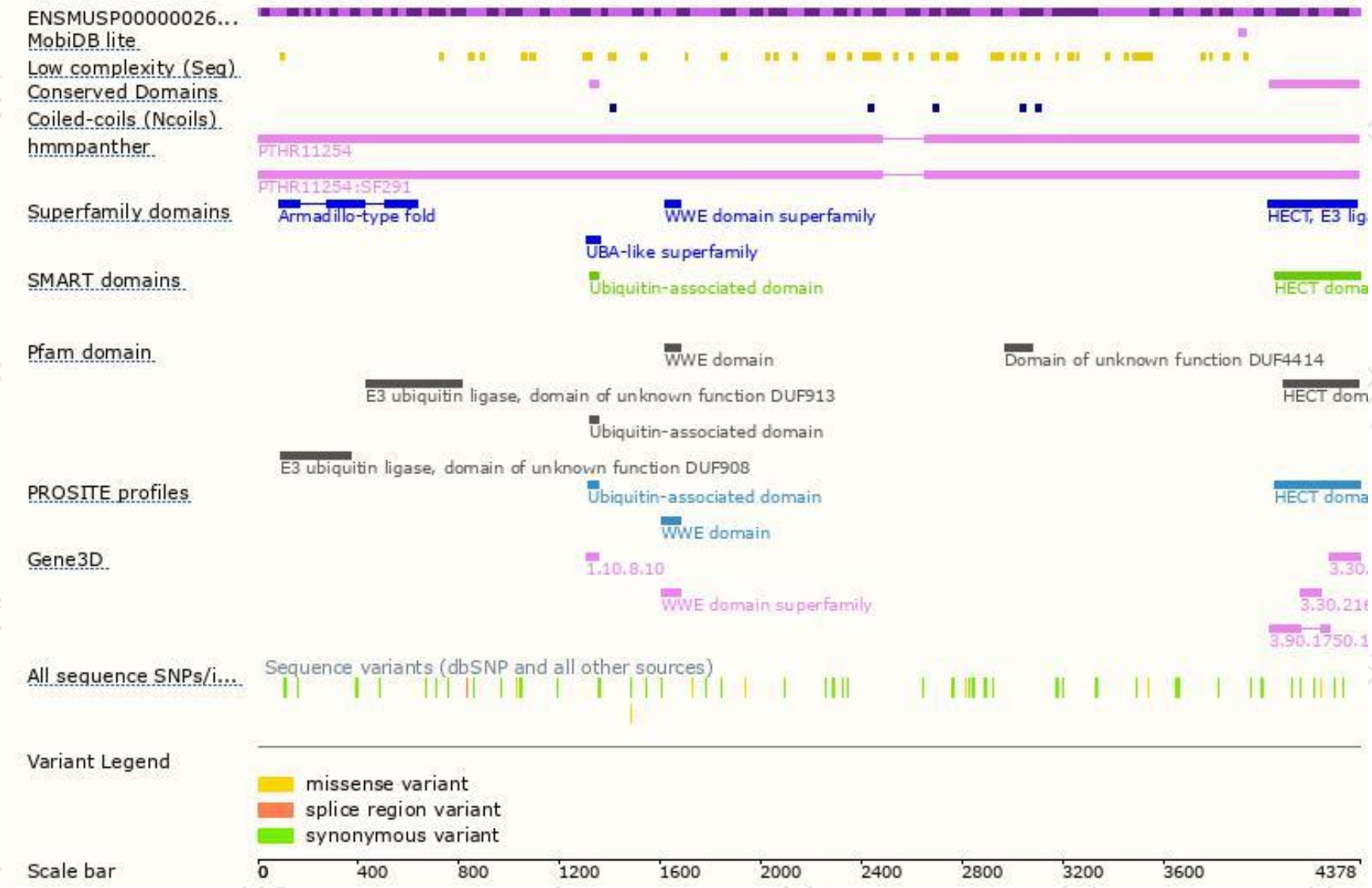
The strategy is based on the design of *Huwe1-201* transcript,The transcription is shown below



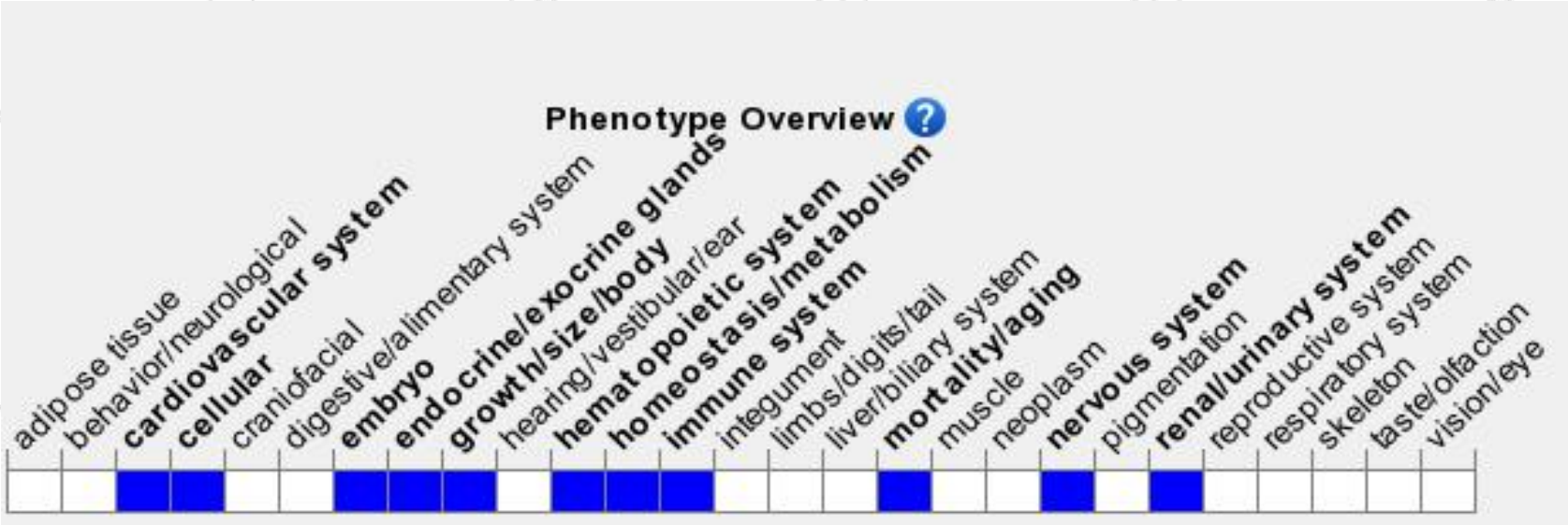
Genomic location distribution



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 conditional allele activated in neurons results in neonatal lethality, poorly developed dentate gyrus, small cerebellum, increased cortex density, and increased neuronal precursor cell proliferation.

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

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