Mitf Cas9-CKO Strategy

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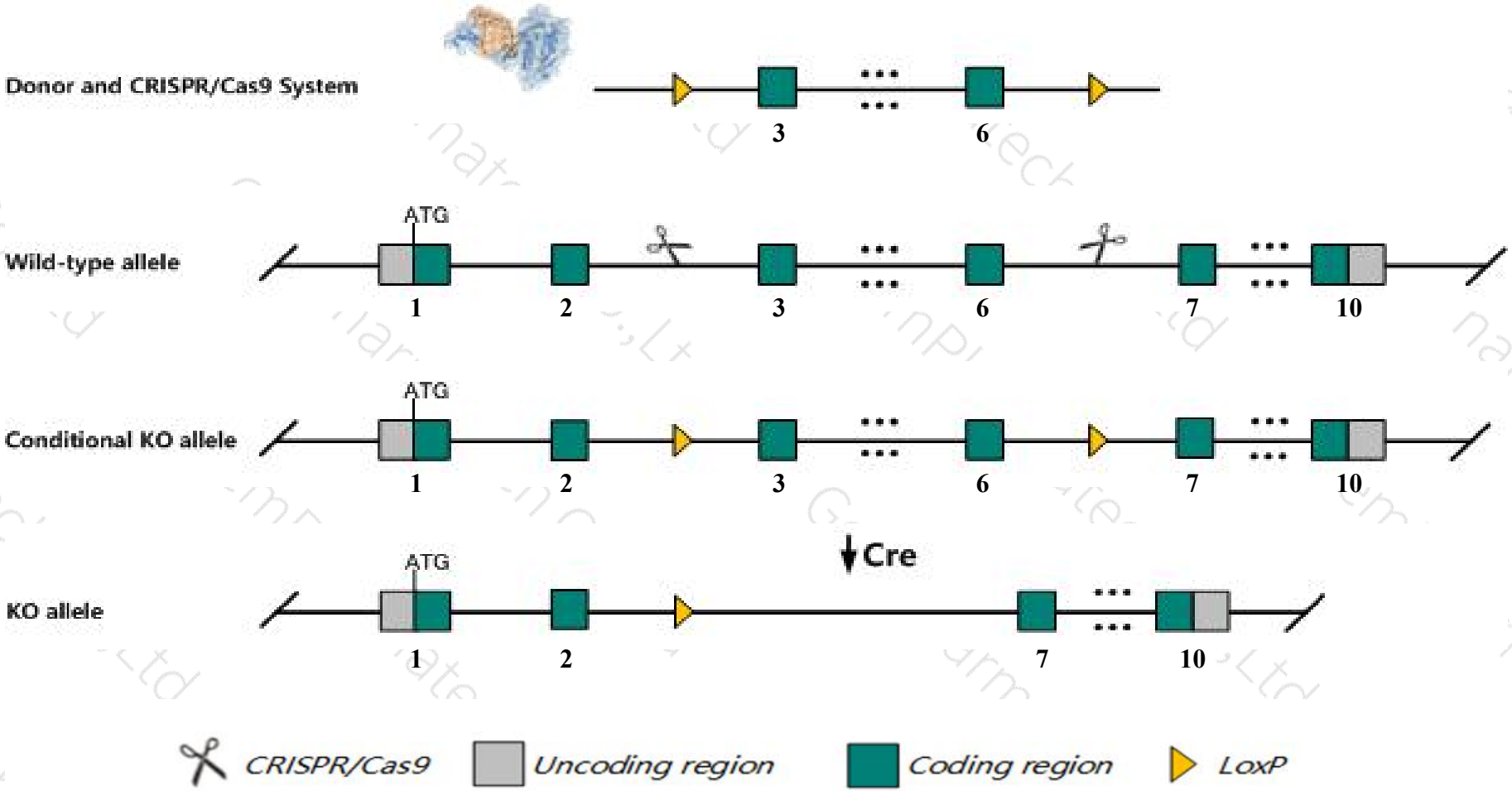
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Mitf* gene has 8 transcripts. According to the structure of *Mitf* gene, exon3-exon6 of *Mitf-202* (ENSMUST00000043637.13) transcript is recommended as the knockout region.The region contains 526bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mitf* 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,Mutations at this locus affect development of melanocytes, mast cells, osteoclasts and pigmented epithelium. Mutants variably display lack of pigment in coat and eye, microphthalmia, hearing loss, bone resorption anomalies, mast cell deficiency and lethality.

- The *Mitf* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Mitf melanogenesis associated transcription factor [Mus musculus (house mouse)]

Gene ID: 17342, updated on 5-Mar-2019

Summary

Official Symbol	Mitf provided by MGI
Official Full Name	melanogenesis associated transcription factor provided by MGI
Primary source	MGI:MGI:104554
See related	Ensembl:ENSMUSG00000035158
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	BCC2, Bhlhe32, Gsfbcc2, Vitiligo, Wh, bw, mi, vit
Summary	This transcription factor serves at a critical point between extracellular signaling and downstream targets in cell specification in early eye and neural crest development. Mutant alleles have been identified that generate distinct phenotypes. Some of these alleles are being used to model the human diseases Waardenburg syndrome IIa and Tietz syndrome. [provided by RefSeq, Jul 2008]
Expression	Biased expression in placenta adult (RPKM 9.0), heart adult (RPKM 4.3) and 12 other tissues See more
Orthologs	human all

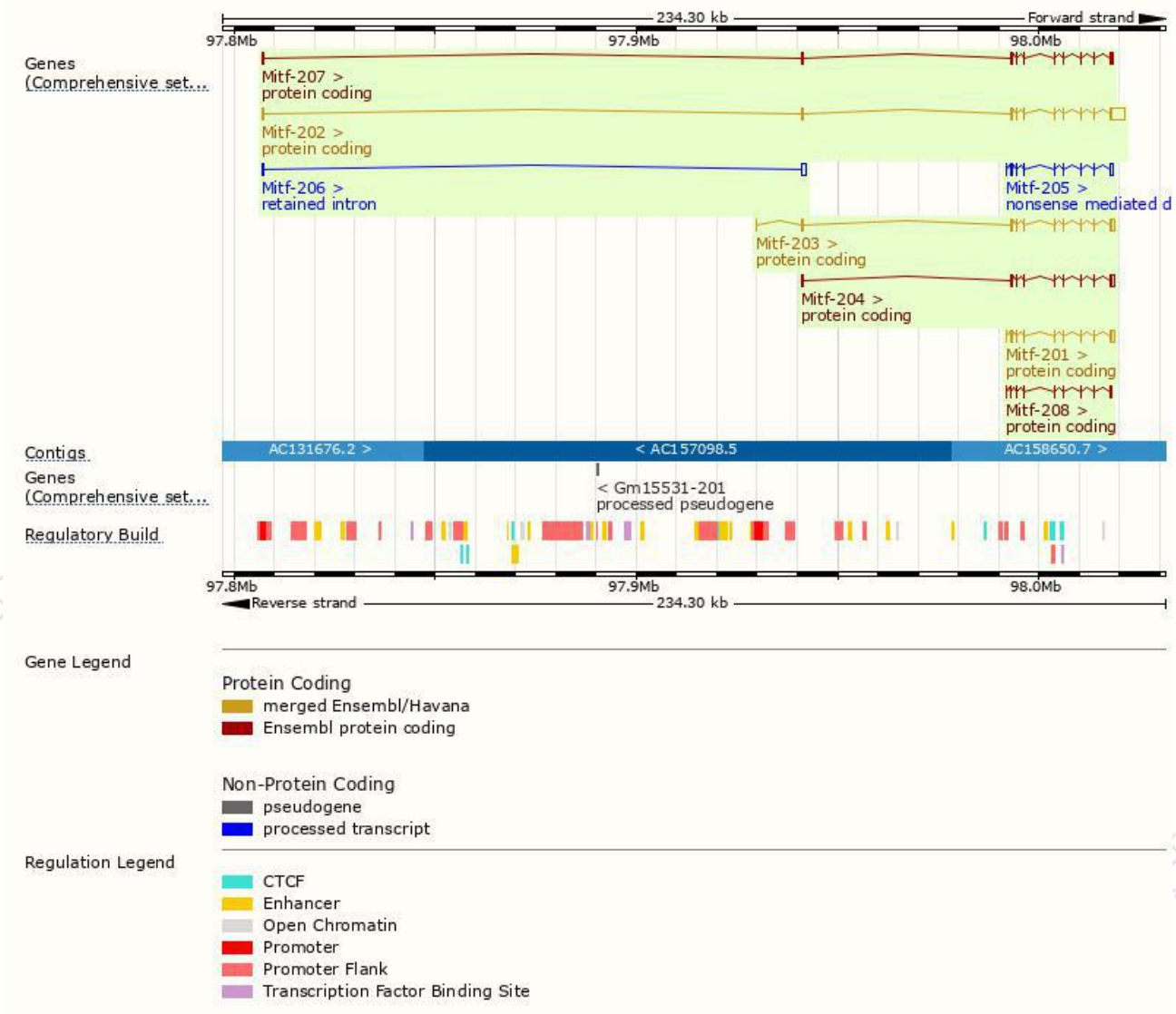
Transcript information（Ensembl）

The gene has 8 transcripts,all transcripts are shown below:

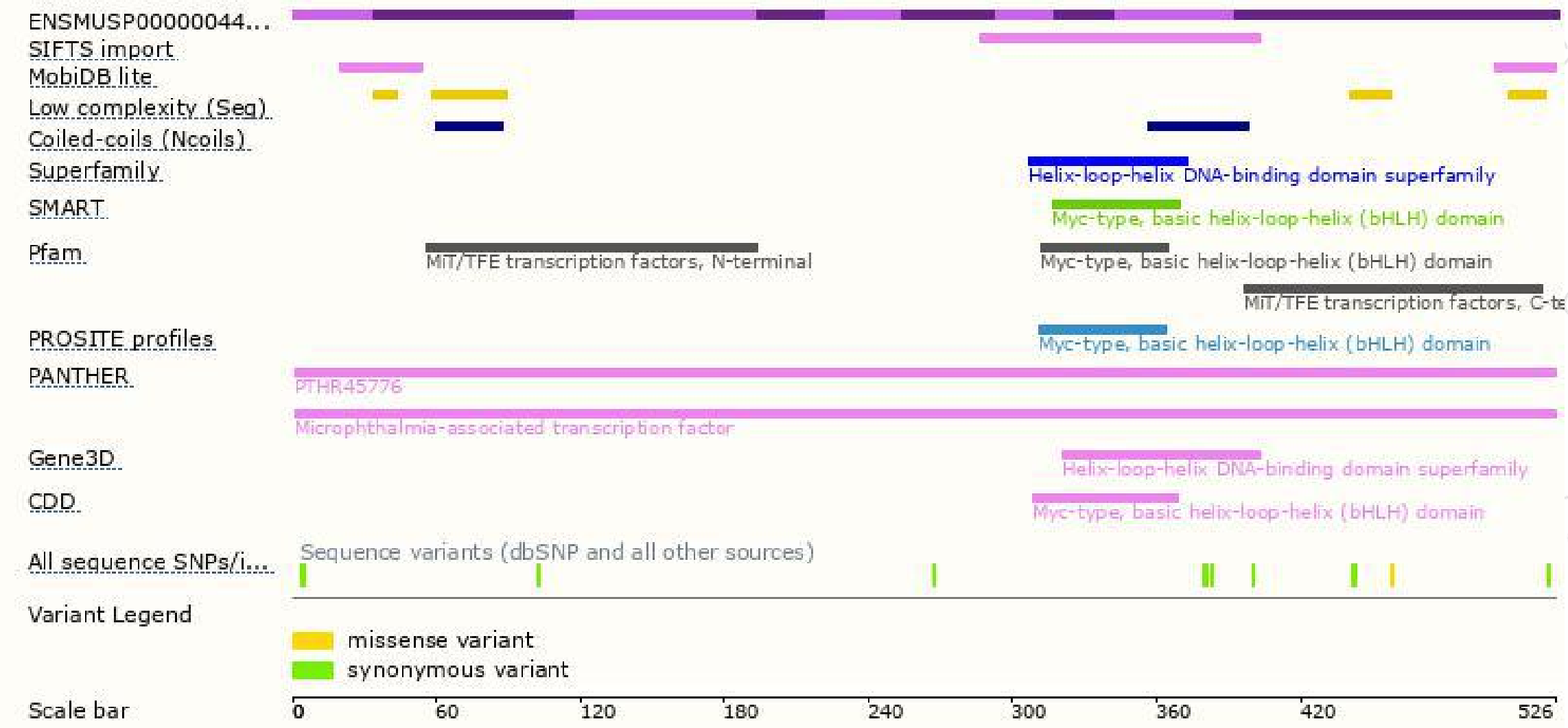
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mitf-202	ENSMUST00000043637.13	4881	526aa	Protein coding	CCDS51861	Q08874	TSL:1 GENCODE basic APPRIS P4
Mitf-203	ENSMUST00000101123.9	2144	510aa	Protein coding	CCDS51862	Q08874	TSL:5 GENCODE basic APPRIS ALT1
Mitf-201	ENSMUST00000043628.12	1921	419aa	Protein coding	CCDS20385	Q08874 Q32MU7	TSL:1 GENCODE basic
Mitf-204	ENSMUST00000113339.7	2144	501aa	Protein coding	-	E9PZ28	TSL:5 GENCODE basic
Mitf-207	ENSMUST00000203884.2	2113	520aa	Protein coding	-	-	TSL:5 GENCODE basic APPRIS ALT1
Mitf-208	ENSMUST00000203938.1	1074	357aa	Protein coding	-	A0A0N4SV79	TSL:5 GENCODE basic
Mitf-205	ENSMUST00000139462.1	1649	105aa	Nonsense mediated decay	-	D6RCW2	TSL:2
Mitf-206	ENSMUST00000148233.1	1360	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Mitf-202* transcript,The transcription is shown below

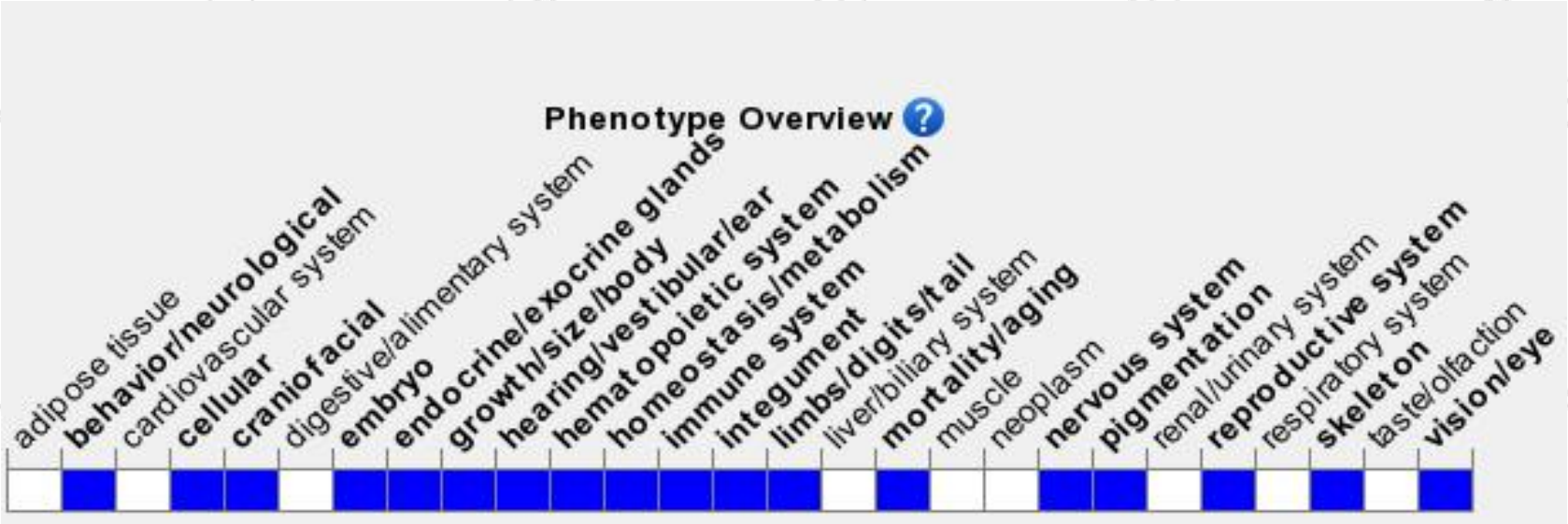
Genomic location distribution



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,Mutations at this locus affect development of melanocytes, mast cells, osteoclasts and pigmented epithelium. Mutants variably display lack of pigment in coat and eye, microphthalmia, hearing loss, bone resorption anomalies, mast cell deficiency and lethality.

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

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