Diaph1 Cas9-CKO Strategy

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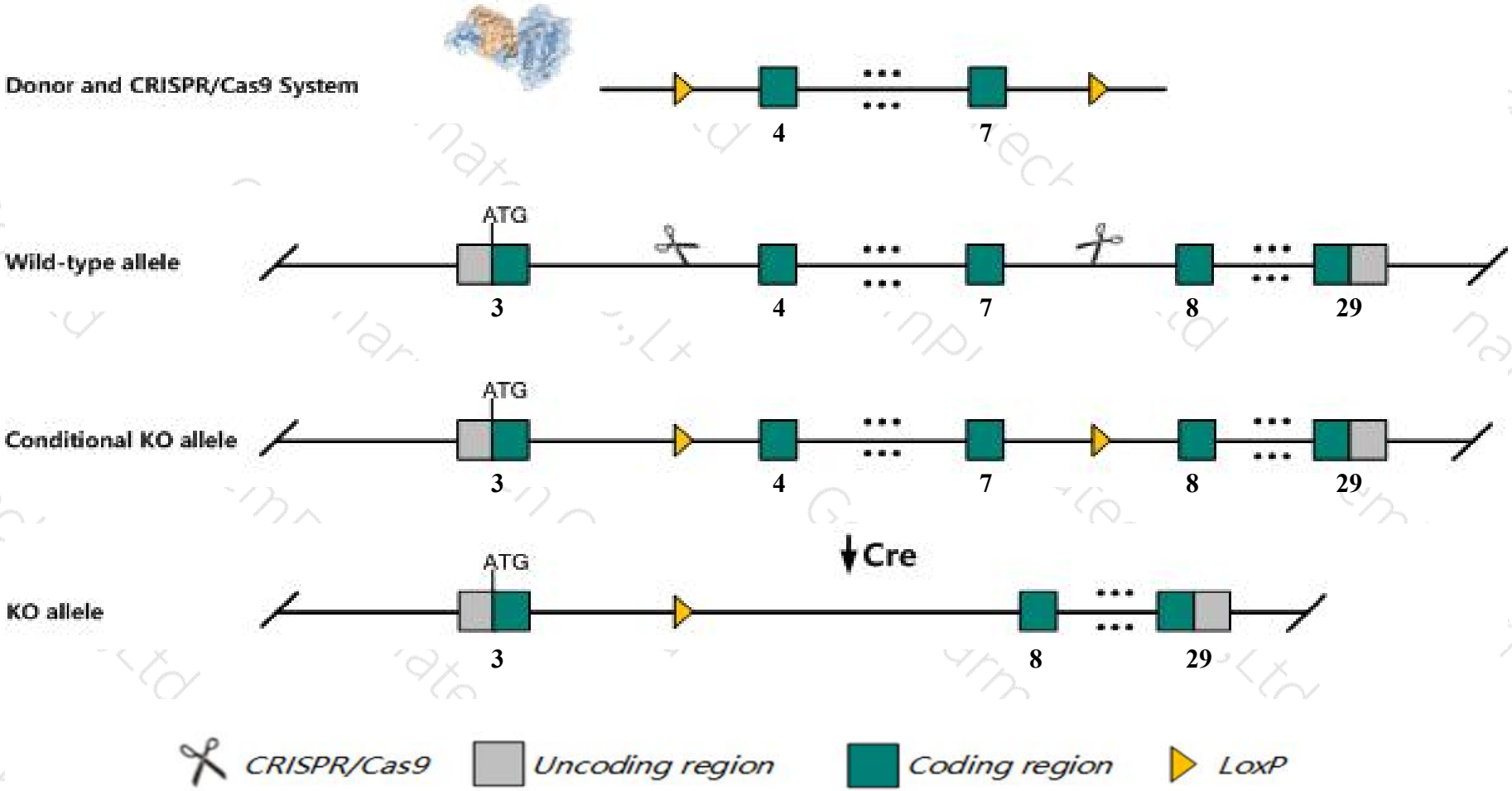
Project Overview

Project Name	***Diaph1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Diaph1* gene. The schematic diagram is as follows:



Technical routes

- The *Diaph1* gene has 8 transcripts. According to the structure of *Diaph1* gene, exon4-exon7 of *Diaph1-204* (ENSMUST00000115631.7) transcript is recommended as the knockout region.The region contains 476bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Diaph1* 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 null allele exhibit abnormal hematopoiesis, bone marrow cell morphology, spleen morphology, skin physiology, skull morphology, and postnatal growth.

- The *Diaph1* gene is located on the Chr18. 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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Diaph1 diaphanous related formin 1 [*Mus musculus* (house mouse)]

Gene ID: 13367, updated on 24-Sep-2019

Summary

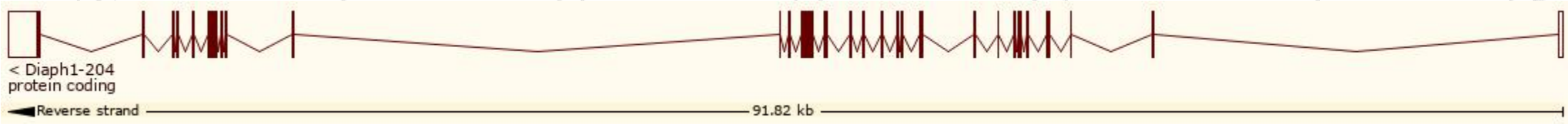
Official Symbol	Diaph1 provided by MGI
Official Full Name	diaphanous related formin 1 provided by MGI
Primary source	MGI:MGI:1194490
See related	Ensembl:ENSMUSG00000024456
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Dia1; Drf1; Diap1; p140mDia; D18Wsu154e
Summary	This gene encodes a member of the formin family of proteins that play important roles in cytoskeletal rearragnement by nucleation of actin filaments. Mice lacking the encoded protein develop age-dependent myeloproliferative defects resembling human myeloproliferative syndrome and myelodysplastic syndromes. Trafficking of T lymphocytes to secondary lymphoid organs and egression of thymocytes from the thymus are impaired in these animals. Lack of the encoded protein in T lymphocytes and thymocytes also reduces chemotaxis. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Sep 2016]
Expression	Ubiquitous expression in thymus adult (RPKM 20.8), lung adult (RPKM 15.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

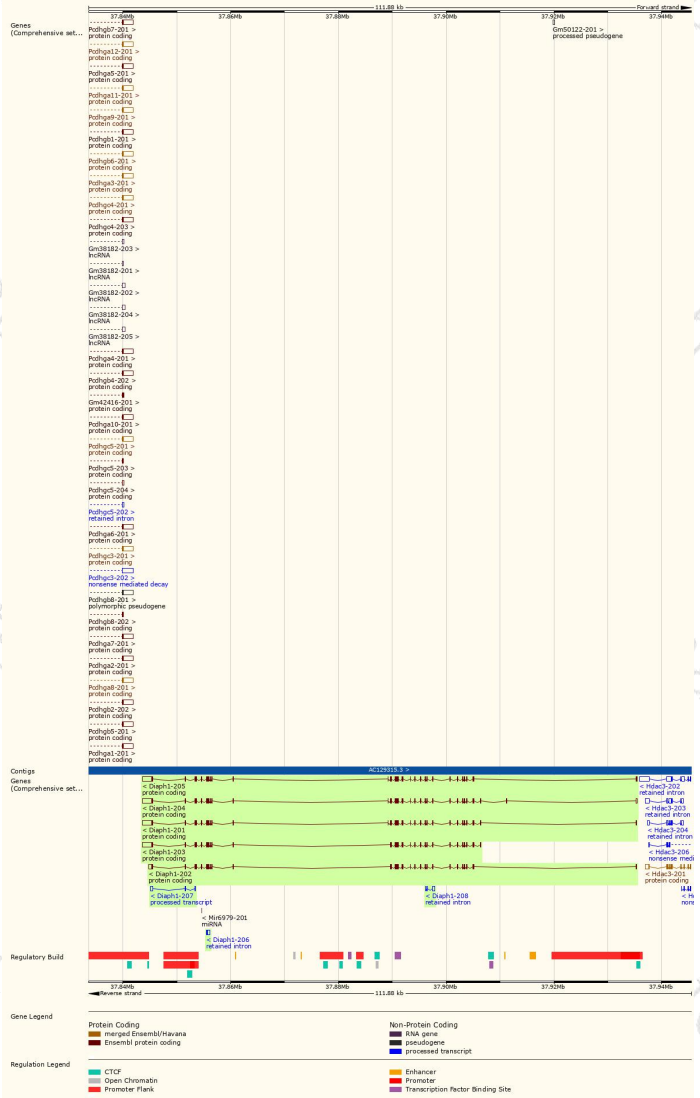
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Diaph1-201	ENSMUST00000025337.13	5596	1264aa	Protein coding	CCDS79635	E9PV41	TSL:5 GENCODE basic APPRIS ALT2
Diaph1-202	ENSMUST00000080033.6	4378	1255aa	Protein coding	-	E9PXV7	TSL:1 GENCODE basic APPRIS ALT2
Diaph1-203	ENSMUST00000115629.8	5416	1220aa	Protein coding	-	D3Z074	TSL:5 GENCODE basic APPRIS ALT2
Diaph1-204	ENSMUST00000115631.7	5741	1220aa	Protein coding	CCDS84377	F6XC54	TSL:5 GENCODE basic APPRIS ALT2
Diaph1-205	ENSMUST00000115634.7	5666	1255aa	Protein coding	CCDS57121	O08808	TSL:5 GENCODE basic APPRIS P3
Diaph1-206	ENSMUST00000124822.1	551	No protein	Retained intron	-	-	TSL:2
Diaph1-207	ENSMUST00000127346.1	584	No protein	Processed transcript	-	-	TSL:3
Diaph1-208	ENSMUST00000129688.1	617	No protein	Retained intron	-	-	TSL:2

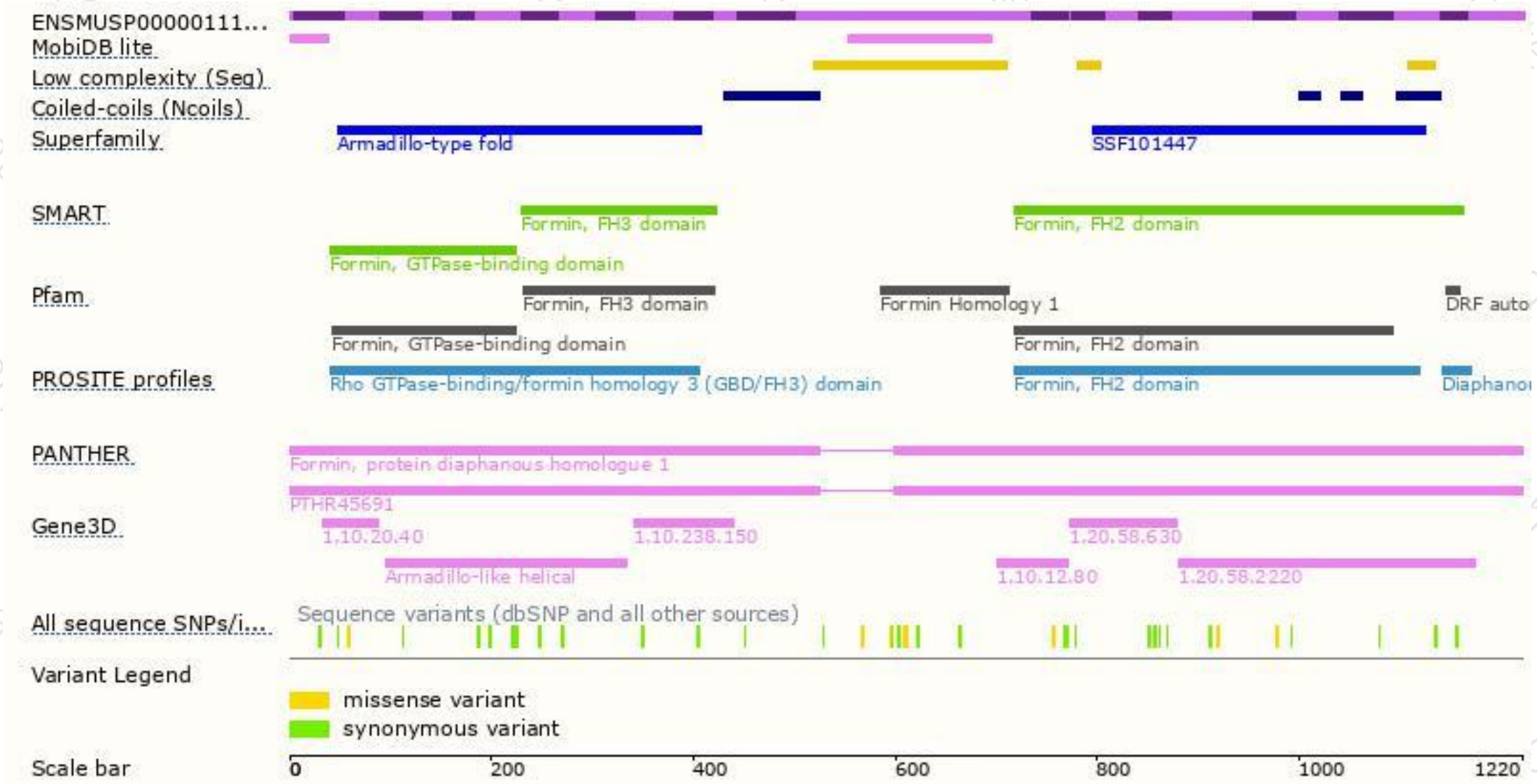
The strategy is based on the design of *Diaph1-204* transcript, the transcription is shown below:



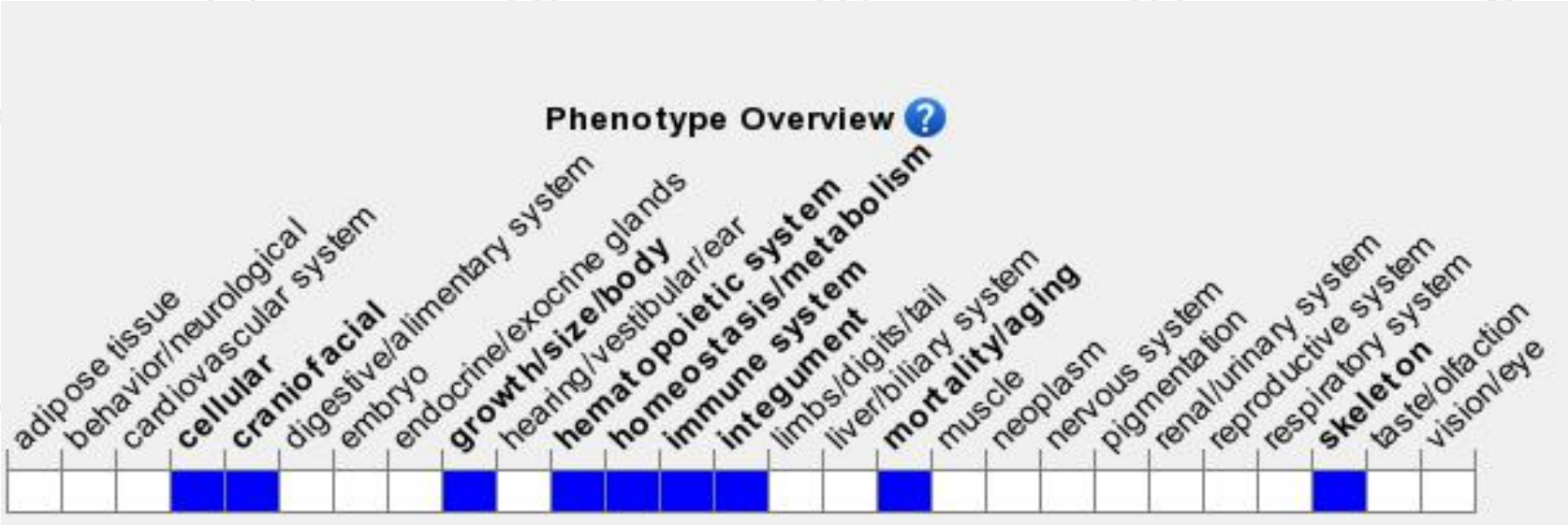
Genomic location distribution



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 null allele exhibit abnormal hematopoiesis, bone marrow cell morphology, spleen morphology, skin physiology, skull morphology, and postnatal growth.

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

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