Diaph3 Cas9-CKO Strategy

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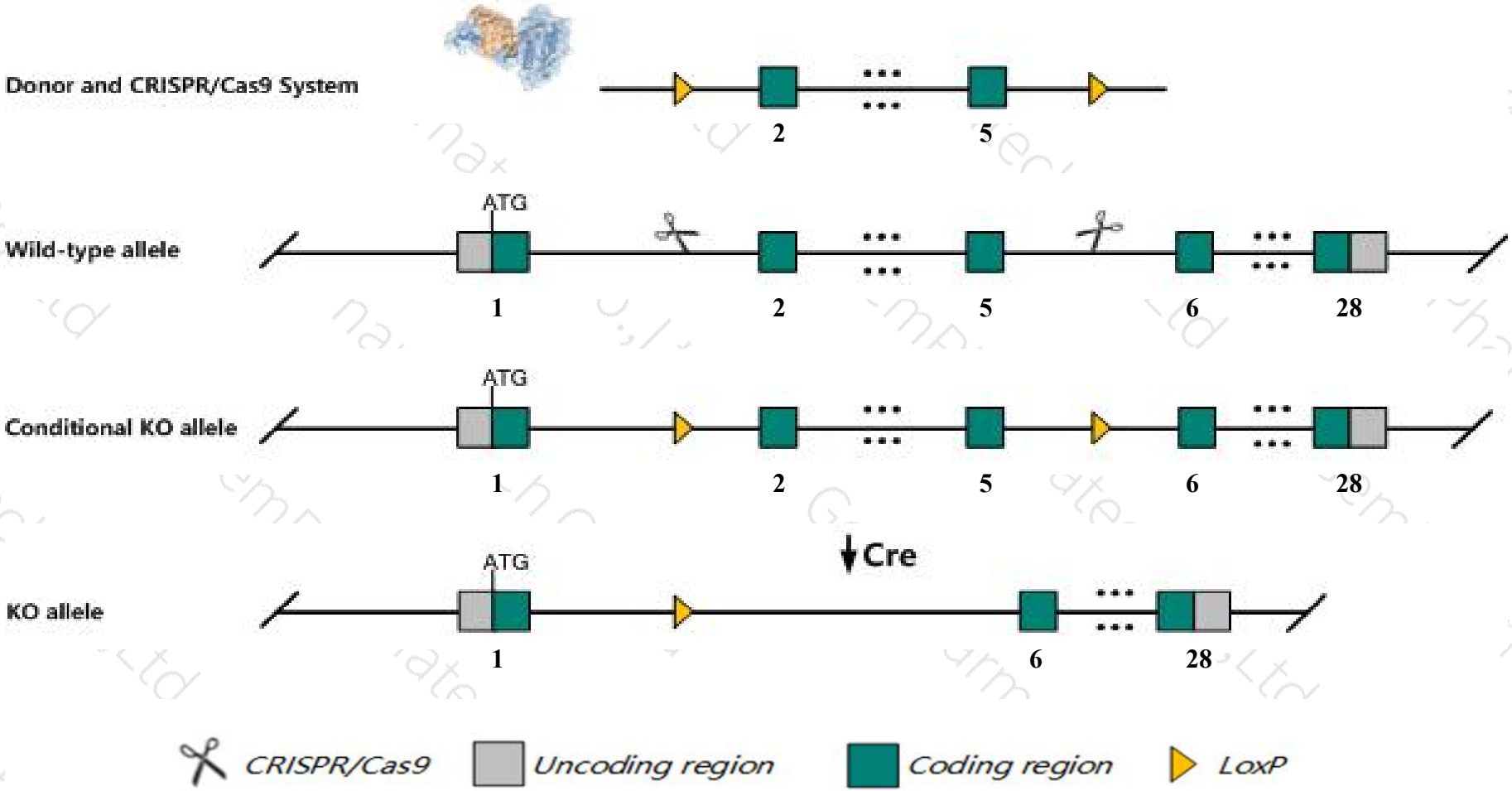
Project Overview

Project Name	***Diaph3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Diaph3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Diaph3* gene has 9 transcripts. According to the structure of *Diaph3* gene, exon2-exon5 of *Diaph3-202*(ENSMUST00000168889.2) transcript is recommended as the knockout region.The region contains 446bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Diaph3* 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 disruption of this gene display embryonic mortality and abnormal cytokinesis of RBC.

➢ Transcript *Diaph3-204* and *Diaph3-206* may not be affected.

➢ The *Diaph3* gene is located on the Chr14. 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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Diaph3 diaphanous related formin 3 [Mus musculus (house mouse)]

Gene ID: 56419, updated on 13-Mar-2020

Summary

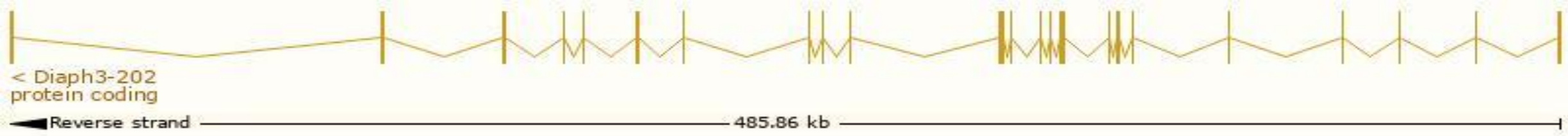
Official Symbol	Diaph3 provided by MGI
Official Full Name	diaphanous related formin 3 provided byMGI
Primary source	MGI:MGI:1927222
See related	Ensembl:ENSMUSG00000022021
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4930417P13Rik, Dia2, Diap3, Drf3, p134MDia2
Expression	Biased expression in liver E14 (RPKM 4.9), liver E14.5 (RPKM 3.7) and 10 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

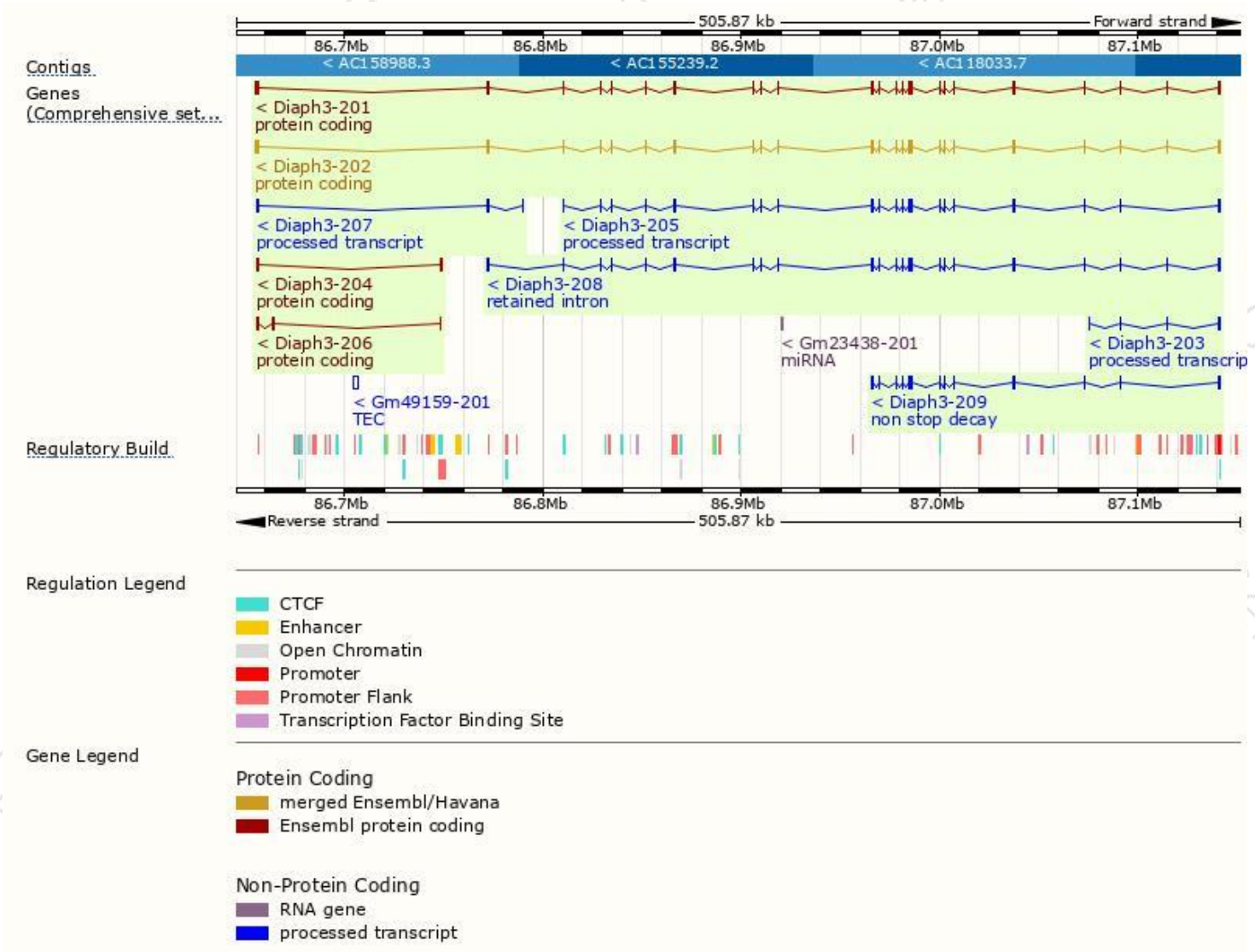
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Diaph3-202	ENSMUST00000168889.2	4582	1171aa	Protein coding	CCDS36990	Q9Z207	TSL:1 GENCODE basic APPRIS P2
Diaph3-201	ENSMUST00000022599.13	4582	1171aa	Protein coding	-	F8WIG5	TSL:5 GENCODE basic APPRIS ALT2
Diaph3-206	ENSMUST00000226745.1	713	82aa	Protein coding	-	A0A2I3BQ45	GENCODE basic
Diaph3-204	ENSMUST00000226254.1	682	82aa	Protein coding	-	A0A2I3BQ45	GENCODE basic
Diaph3-209	ENSMUST00000228000.1	2090	656aa	Non stop decay	-	Q3UVP0	
Diaph3-205	ENSMUST00000226492.1	3046	No protein	Processed transcript	-	-	
Diaph3-207	ENSMUST00000227638.1	678	No protein	Processed transcript	-	-	
Diaph3-203	ENSMUST00000226134.1	558	No protein	Processed transcript	-	-	
Diaph3-208	ENSMUST00000227666.1	3337	No protein	Retained intron	-	-	

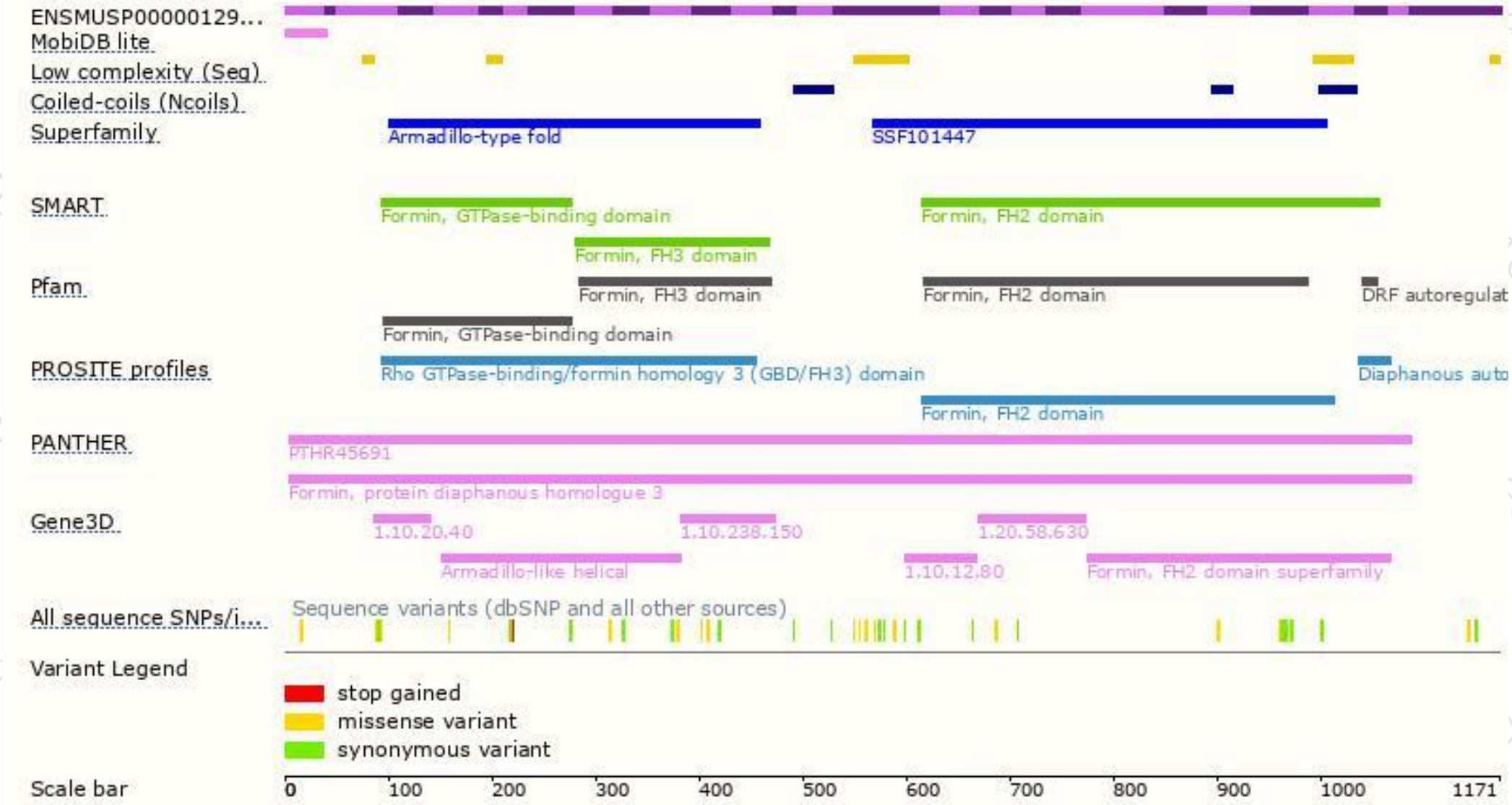
The strategy is based on the design of *Diaph3-202* transcript,the transcription is shown below:



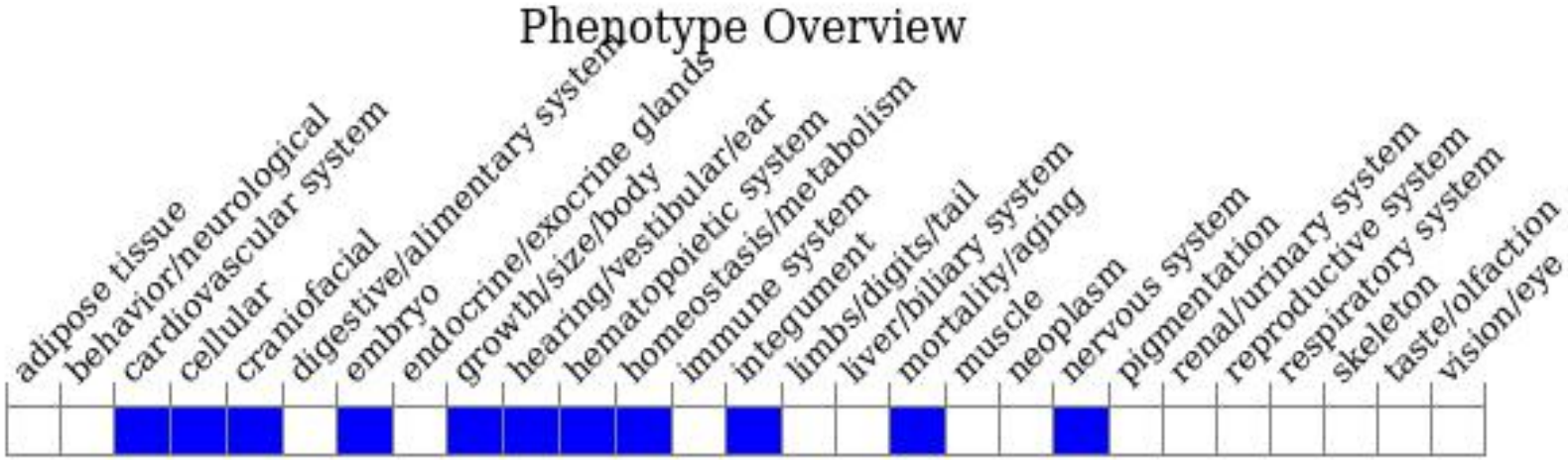
Genomic location distribution



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,mice homozygous for disruption of this gene display embryonic mortality and abnormal cytokinesis of RBC.

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

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