Nfat5 Cas9-CKO Strategy

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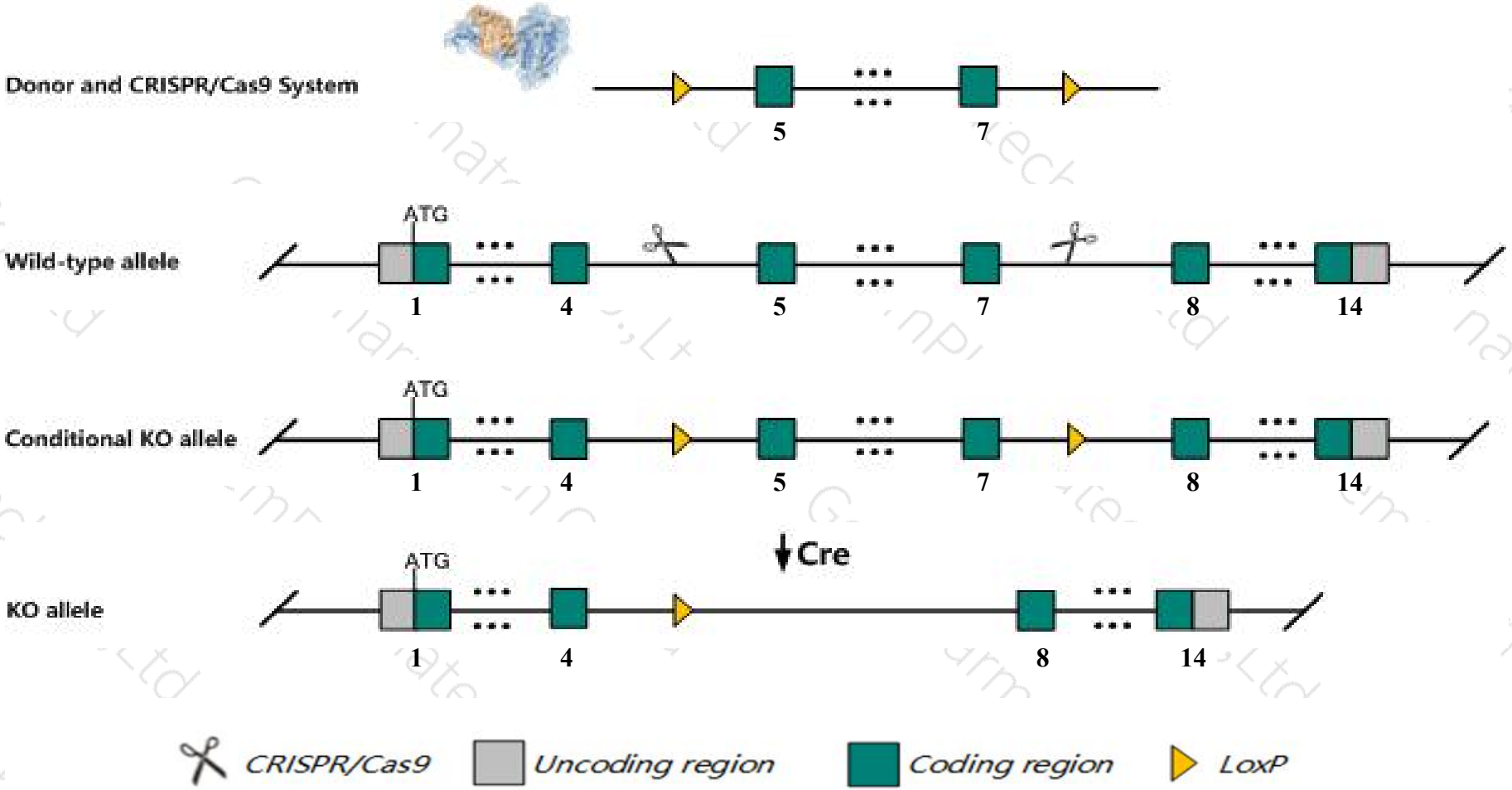
Project Overview

Project Name	***Nfat5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nfat5* gene. The schematic diagram is as follows:



Technical routes

- The *Nfat5* gene has 14 transcripts. According to the structure of *Nfat5* gene, exon5-exon7 of *Nfat5-214* (ENSMUST00000169453.7) transcript is recommended as the knockout region.The region contains 557bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nfat5* 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 one of several knock-out allele exhibit lethality between E14.5 and E17.5 as well as around P10 with kidney, cardiac or immune defects depending on the allele.

➢Transcript *Nfat5-207 and Nfat5-208* may not be affected.

➢ The *Nfat5* gene is located on the Chr8. 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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Nfat5 nuclear factor of activated T cells 5 [Mus musculus (house mouse)]

Gene ID: 54446, updated on 5-Mar-2019

Summary

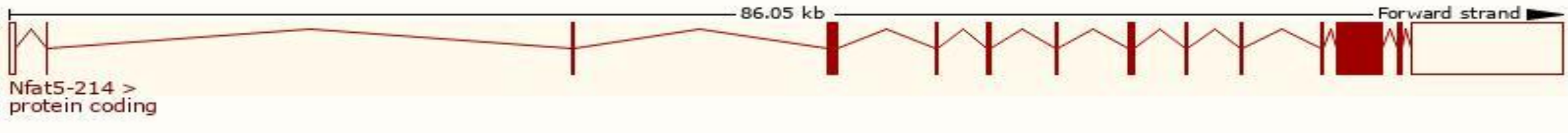
Official Symbol	Nfat5 provided by MGI
Official Full Name	nuclear factor of activated T cells 5 provided by MGI
Primary source	MGI:MGI:1859333
See related	Ensembl:ENSMUSG00000003847
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI225870, B130038B15Rik, CAG-8, CAG80, NFATL1, OREBP, TonEBP, mKIAA0827, nfatz
Expression	Ubiquitous expression in limb E14.5 (RPKM 18.9), bladder adult (RPKM 16.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

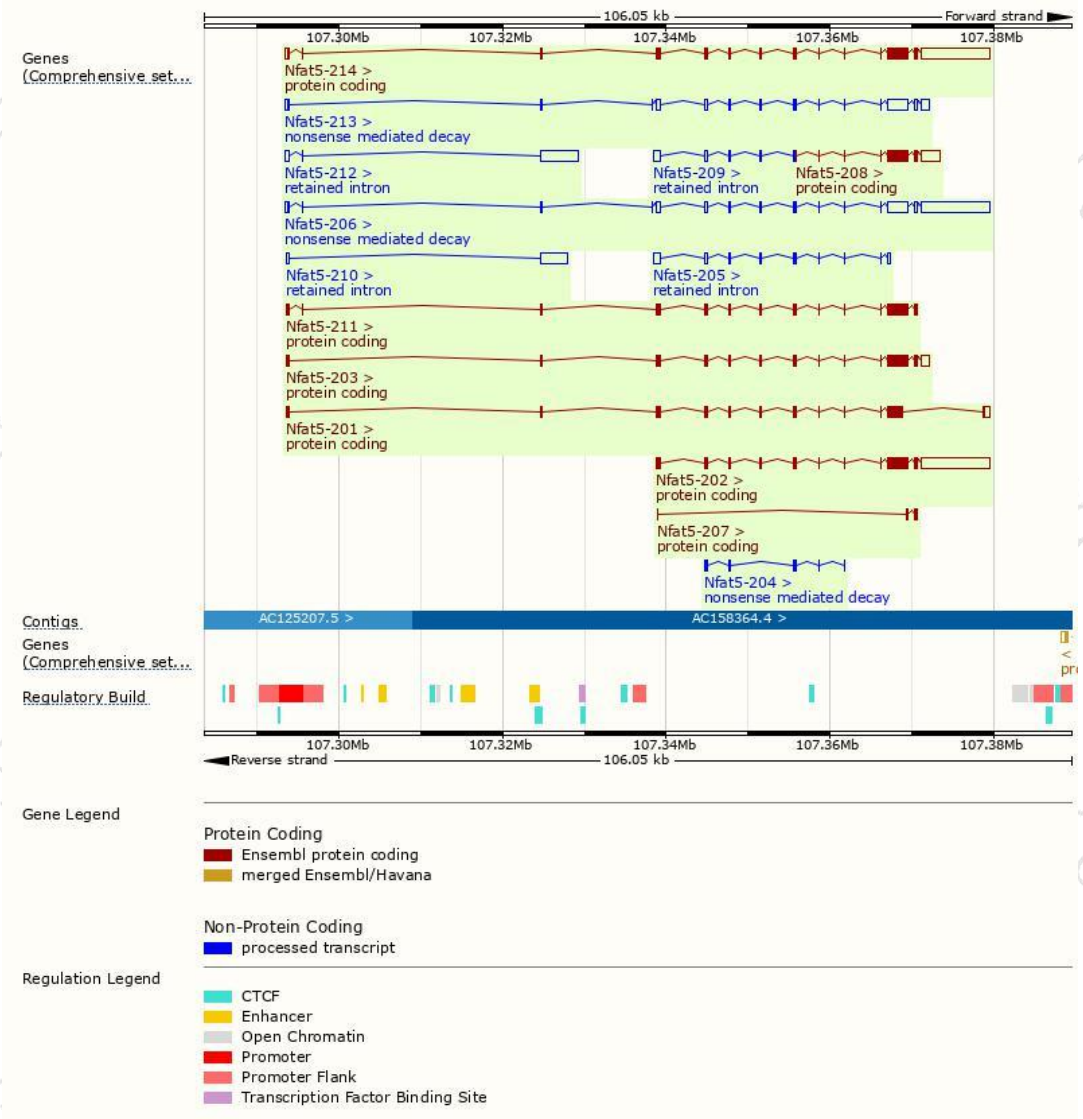
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfat5-214	ENSMUST00000169453.7	13380	1552aa	Protein coding	CCDS22648	Q9WV30	TSL:5 GENCODE basic APPRIS P3
Nfat5-202	ENSMUST00000077440.12	12773	1458aa	Protein coding	CCDS40464	A0A1U6URG8	TSL:5 GENCODE basic APPRIS ALT2
Nfat5-211	ENSMUST00000151114.7	4982	1552aa	Protein coding	CCDS22648	Q9WV30	TSL:1 GENCODE basic APPRIS P3
Nfat5-201	ENSMUST00000075922.10	4619	1225aa	Protein coding	CCDS72168	Q9WV30	TSL:1 GENCODE basic APPRIS ALT2
Nfat5-203	ENSMUST00000125721.7	5899	1534aa	Protein coding	-	Q9WV30	TSL:5 GENCODE basic APPRIS ALT2
Nfat5-208	ENSMUST00000147588.1	5481	1033aa	Protein coding	-	Q80TR0	CDS 5' incomplete TSL:5
Nfat5-207	ENSMUST00000144100.7	432	141aa	Protein coding	-	F6ZRF0	CDS 5' incomplete TSL:5
Nfat5-206	ENSMUST00000133026.7	13443	99aa	Nonsense mediated decay	-	D6RH97 Q9WV30	TSL:1
Nfat5-213	ENSMUST00000154474.7	6099	81aa	Nonsense mediated decay	-	D6RH40	TSL:5
Nfat5-204	ENSMUST00000126333.1	732	135aa	Nonsense mediated decay	-	F6VCW7	CDS 5' incomplete TSL:5
Nfat5-212	ENSMUST00000151582.1	5152	No protein	Retained intron	-	-	TSL:1
Nfat5-210	ENSMUST00000149972.1	3743	No protein	Retained intron	-	-	TSL:1
Nfat5-205	ENSMUST00000126397.7	2325	No protein	Retained intron	-	-	TSL:5
Nfat5-209	ENSMUST00000148006.1	1643	No protein	Retained intron	-	-	TSL:1

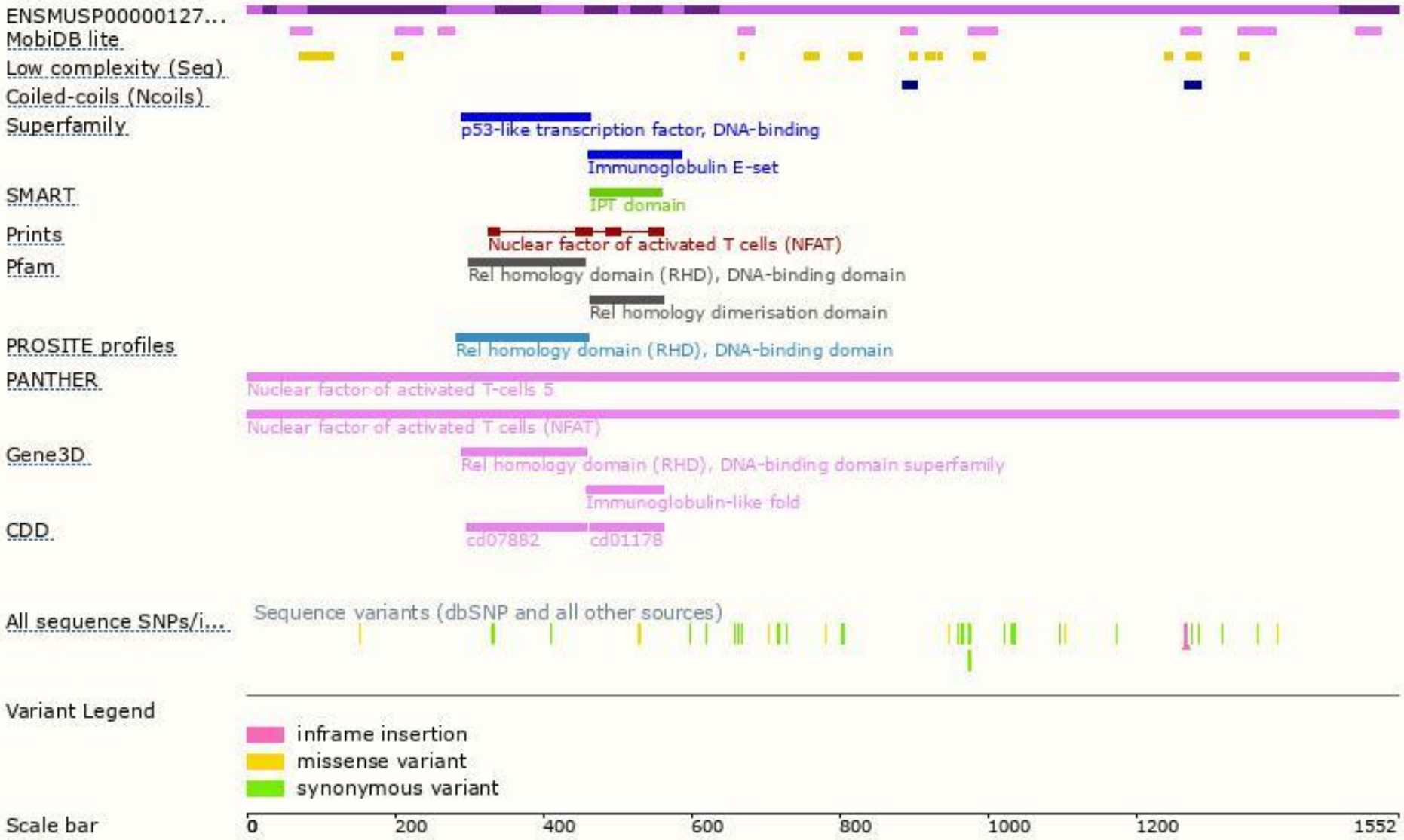
The strategy is based on the design of *Nfat5-214* transcript,The transcription is shown below



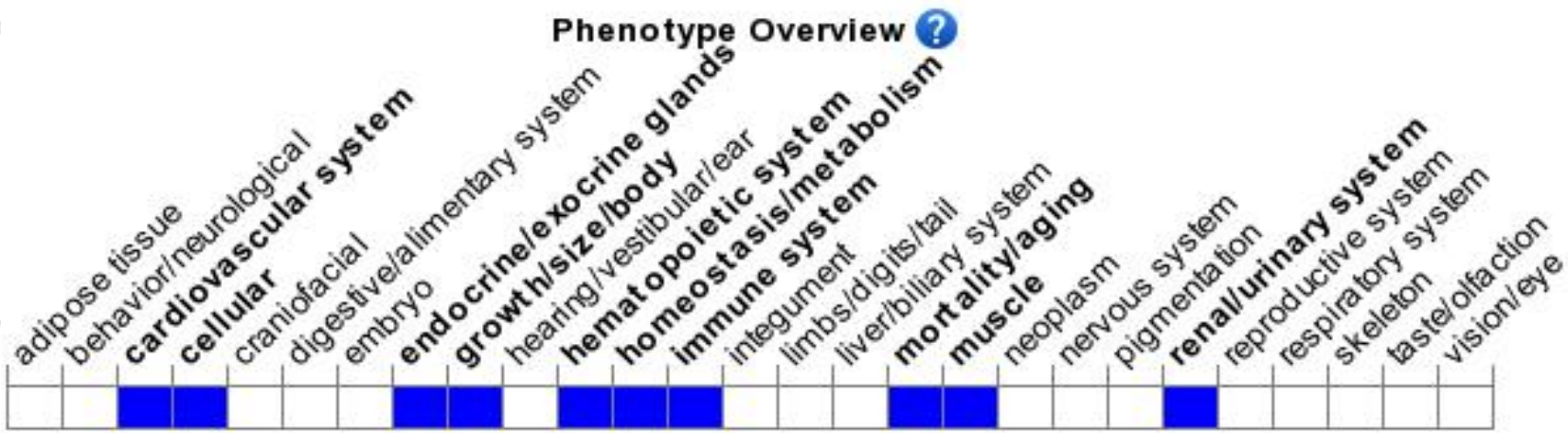
Genomic location distribution



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 one of several knock-out allele exhibit lethality between E14.5 and E17.5 as well as around P10 with kidney, cardiac or immune defects depending on the allele.

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

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