Nup98 Cas9-CKO Strategy

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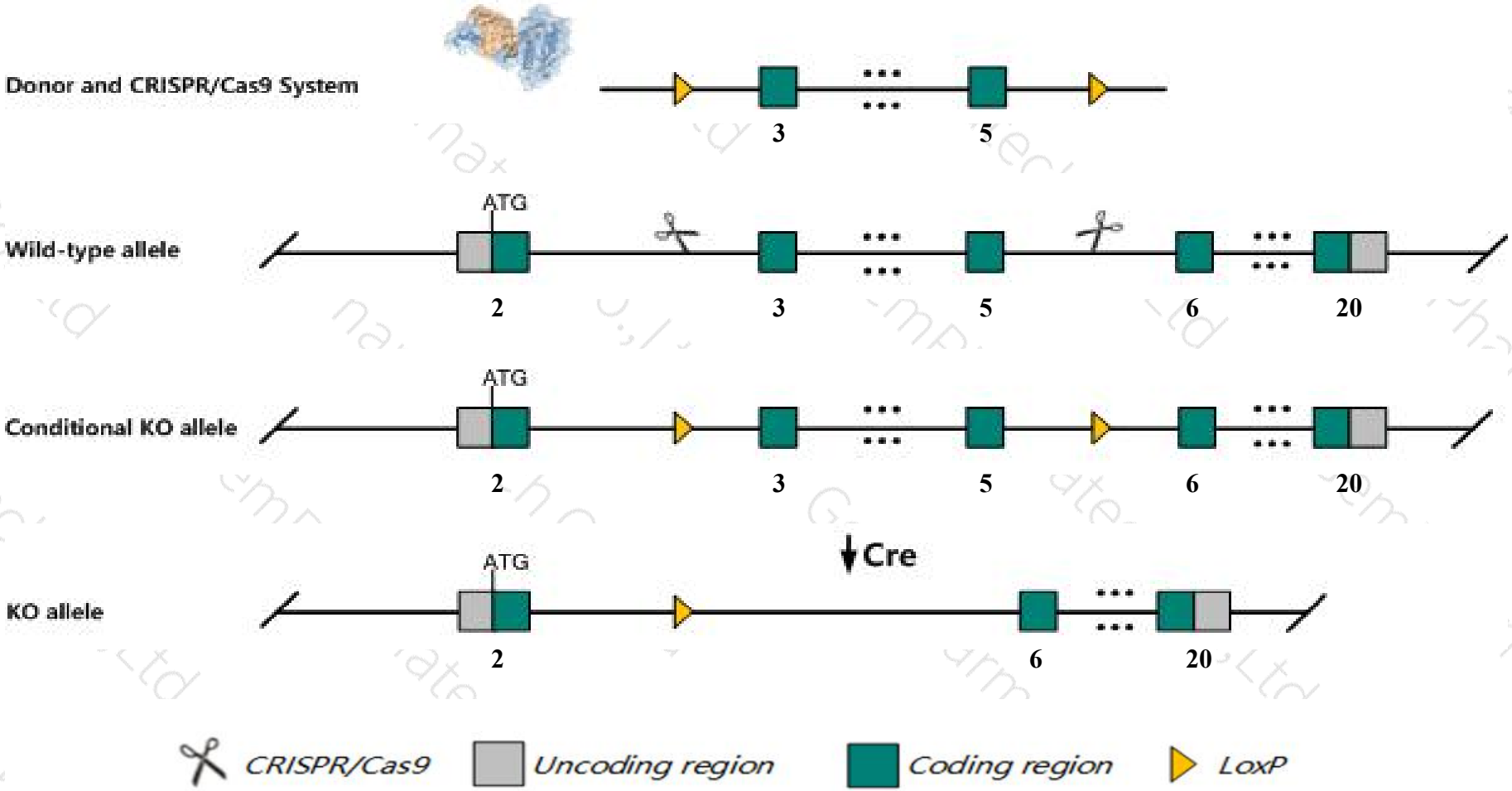
Design Date: 2020-8-1

Project Overview

Project Name	***Nup98***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nup98* gene. The schematic diagram is as follows:



Technical routes

➢ The *Nup98* gene has 8 transcripts. According to the structure of *Nup98* gene, exon3-exon5 of *Nup98-204*(ENSMUST00000211005.1) transcript is recommended as the knockout region.The region contains 419bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Nup98* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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

➢*Nup98-208* may not be affected.

➢ According to the existing MGI data,homozygotes for a null allele die in utero with a severe growth delay and improper gastrulation and nuclear pore complex assembly/function. Heterozygotes for another null allele show impaired IFN-mediated responses, reduced T and B cell subsets in lymphoid organs and altered T and B cell functions.

➢ The *Nup98* gene is located on the Chr7. 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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Nup98 nucleoporin 98 [Mus musculus (house mouse)]

Gene ID: 269966, updated on 13-Mar-2020

Summary

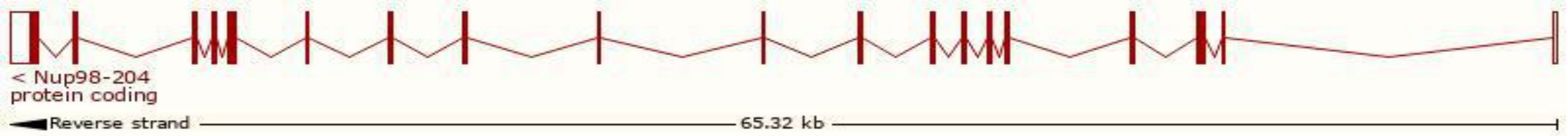
Official Symbol	Nup98 provided by MGI
Official Full Name	nucleoporin 98 provided byMGI
Primary source	MGI:MGI:109404
See related	Ensembl:ENSMUSG00000063550
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4732457F17, AI849286, Nup96
Expression	Broad expression in testis adult (RPKM 45.3), placenta adult (RPKM 7.7) and 27 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

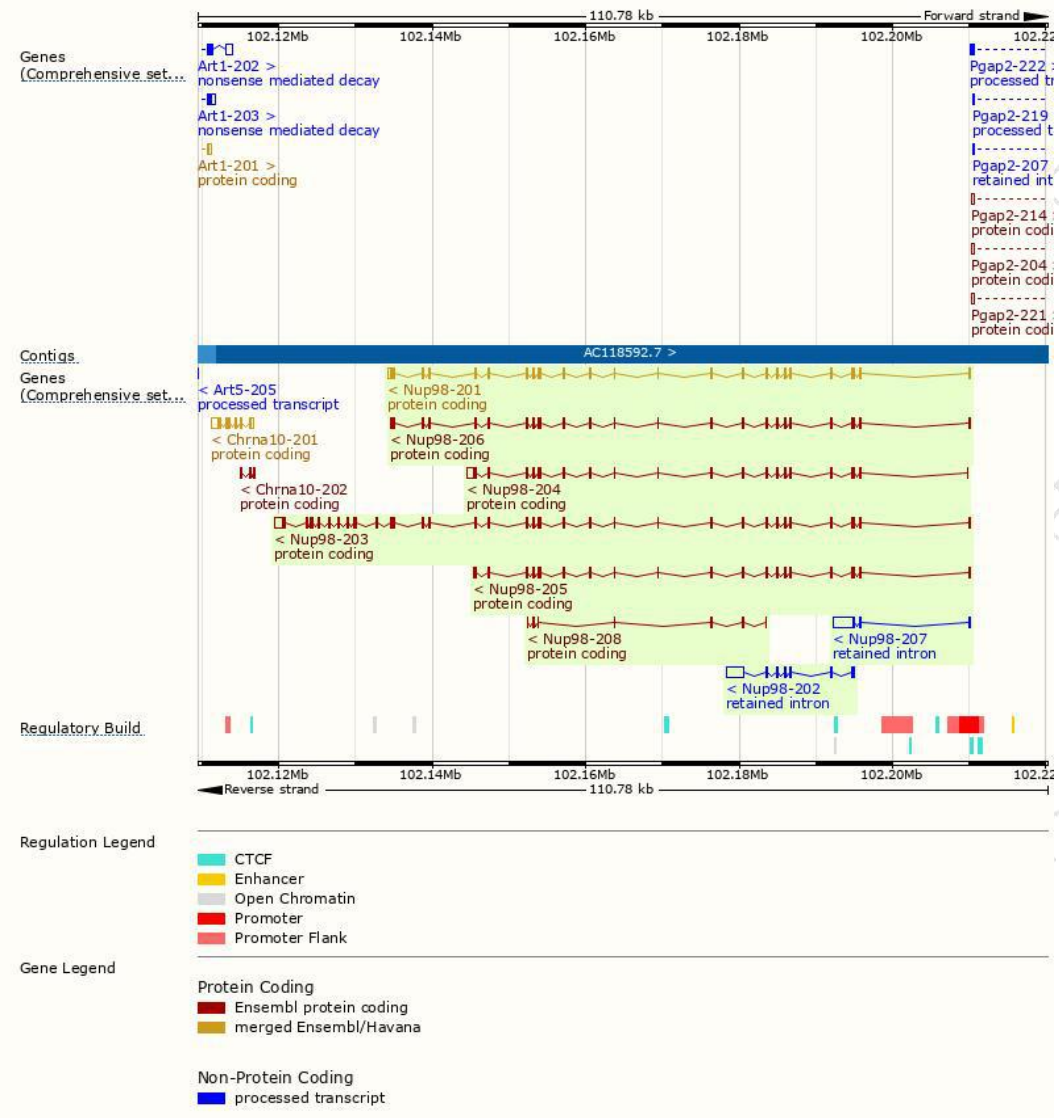
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nup98-203	ENSMUST00000210682.1	6694	1816aa	Protein coding	CCDS85358	A0A1B0GSX7	TSL:5 GENCODE basic APPRIS P1
Nup98-201	ENSMUST00000070165.6	4052	1187aa	Protein coding	CCDS21528	A0A140T8J8	TSL:1 GENCODE basic
Nup98-204	ENSMUST00000211005.1	3961	984aa	Protein coding	CCDS85357	A0A1B0GRA7	TSL:1 GENCODE basic
Nup98-206	ENSMUST00000211235.1	3748	1170aa	Protein coding	CCDS85360	B2RQL0	TSL:5 GENCODE basic
Nup98-205	ENSMUST00000211022.1	3132	967aa	Protein coding	CCDS85359	A0A1B0GRB5	TSL:2 GENCODE basic
Nup98-208	ENSMUST00000211764.1	689	230aa	Protein coding	-	A0A1B0GS62	CDS 5' and 3' incomplete TSL:3
Nup98-202	ENSMUST00000209242.1	3198	No protein	Retained intron	-	-	TSL:1
Nup98-207	ENSMUST00000211375.1	2823	No protein	Retained intron	-	-	TSL:1

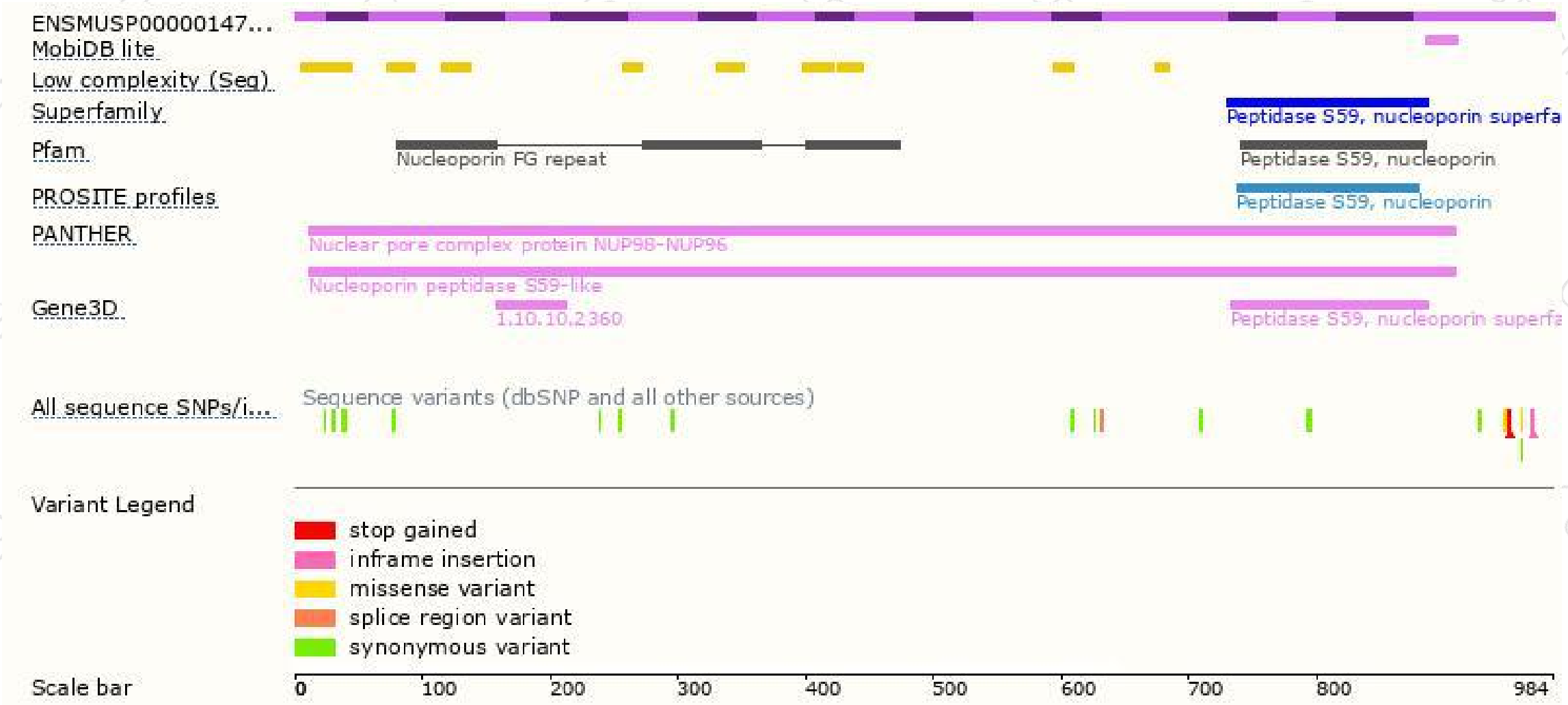
The strategy is based on the design of *Nup98-204* transcript,the transcription is shown below:



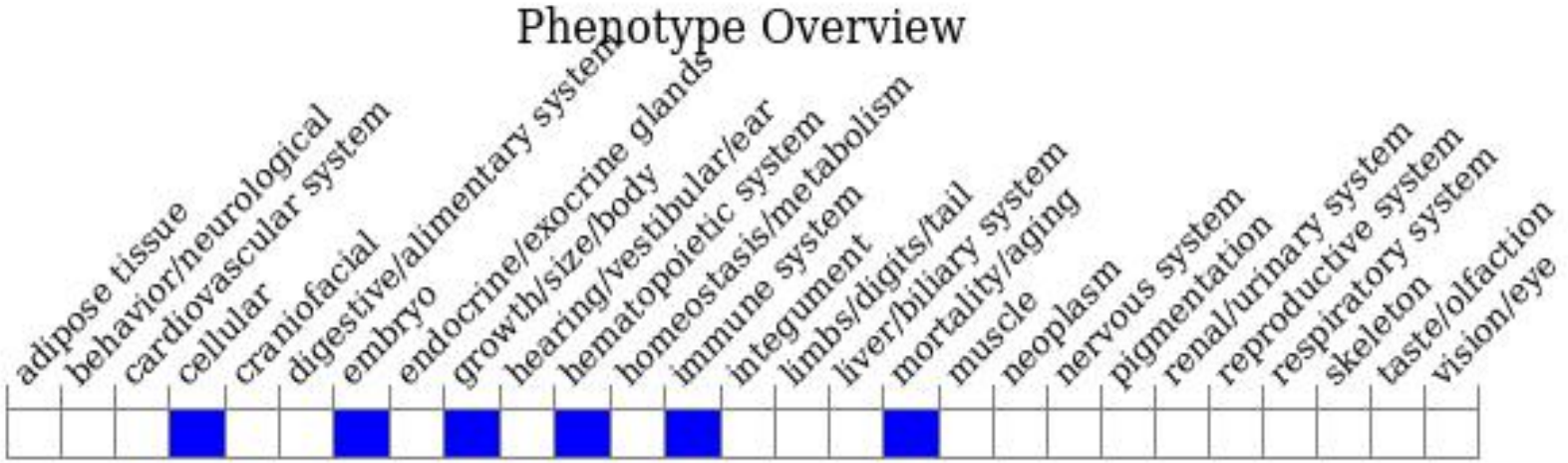
Genomic location distribution



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,homozygotes for a null allele die in utero with a severe growth delay and improper gastrulation and nuclear pore complex assembly/function. Heterozygotes for another null allele show impaired IFN-mediated responses, reduced T and B cell subsets in lymphoid organs and altered T and B cell functions.

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

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