

Ptpn6 Cas9-CKO Strategy

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Reviewer :

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Design Date:

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Project Overview

Project Name

Ptpn6

Project type

Cas9-CKO

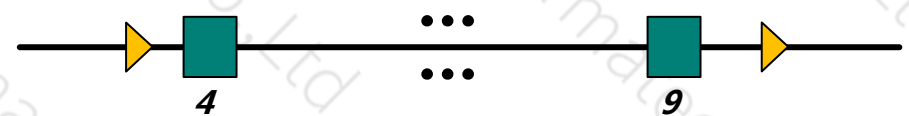
Animal background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ptpn6* gene. The schematic diagram is as follows:

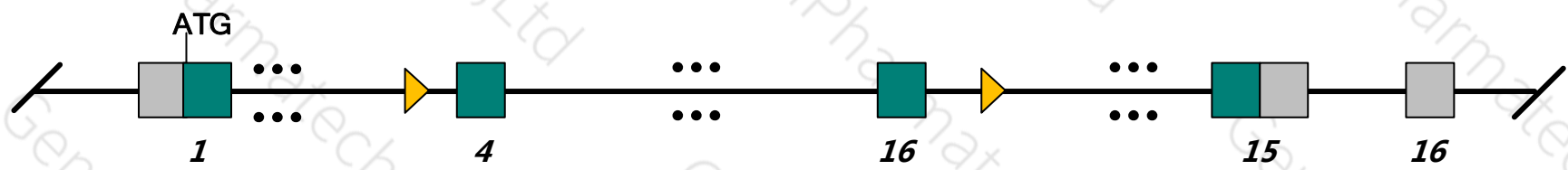
Donor and CRISPR/Cas9 System



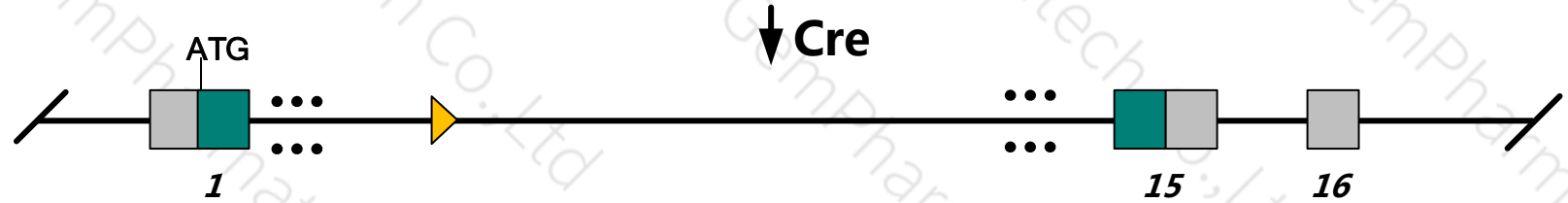
Wild-type allele



Conditional KO allele



KO allele



Technical routes

- The *Ptpn6* gene has 11 transcripts, According to the structure of *Ptpn6* gene, exon4-exon9 of *Ptpn6-203* transcript is recommended as the knockout region. The region contains the 748bp coding sequence. Knock out the region, result in destruction of protein.
- This project uses CRISPR/Cas9 technology to modify *Ptpn6* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed, Cas9, sgRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.
- The flox mice was 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.

- According to the existing MGI data , Homozygous mutants are immunodeficient and autoimmune and exhibit neutrophilic skin lesions that disrupt hair follicles and give the moth-eaten appearance. Alleles vary in severity, with death occurring at 6-9 weeks postnatally due to severe pneumonitis.
- Transcript *Ptpn6*-208 and *Ptpn6*-209 may not be affected.
- The *Ptpn6* gene is located in the Chr6. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Ptpn6 protein tyrosine phosphatase, non-receptor type 6 [*Mus musculus* (house mouse)]

Gene ID: 15170, updated on 5-Feb-2019

Summary

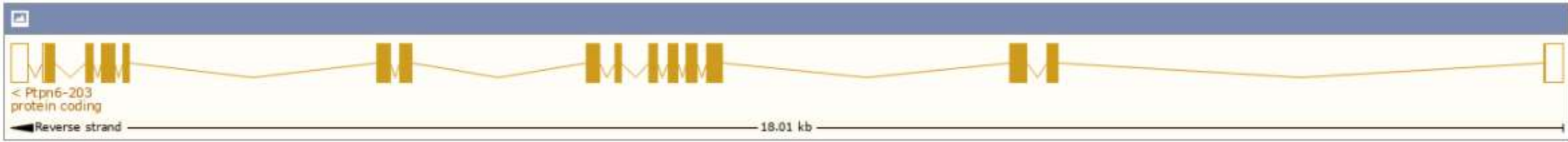
Official Symbol	Ptpn6 provided by MGI
Official Full Name	protein tyrosine phosphatase, non-receptor type 6 provided by MGI
Primary source	MGI:MGI:96055
See related	Ensembl:ENSMUSG000000004266
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	me; hcp; Hcph; Ptp1C; SHP-1; 70Z-SHP; SH-PTP1; PTPTY-42
Expression	Biased expression in spleen adult (RPKM 123.8), thymus adult (RPKM 59.8) and 14 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

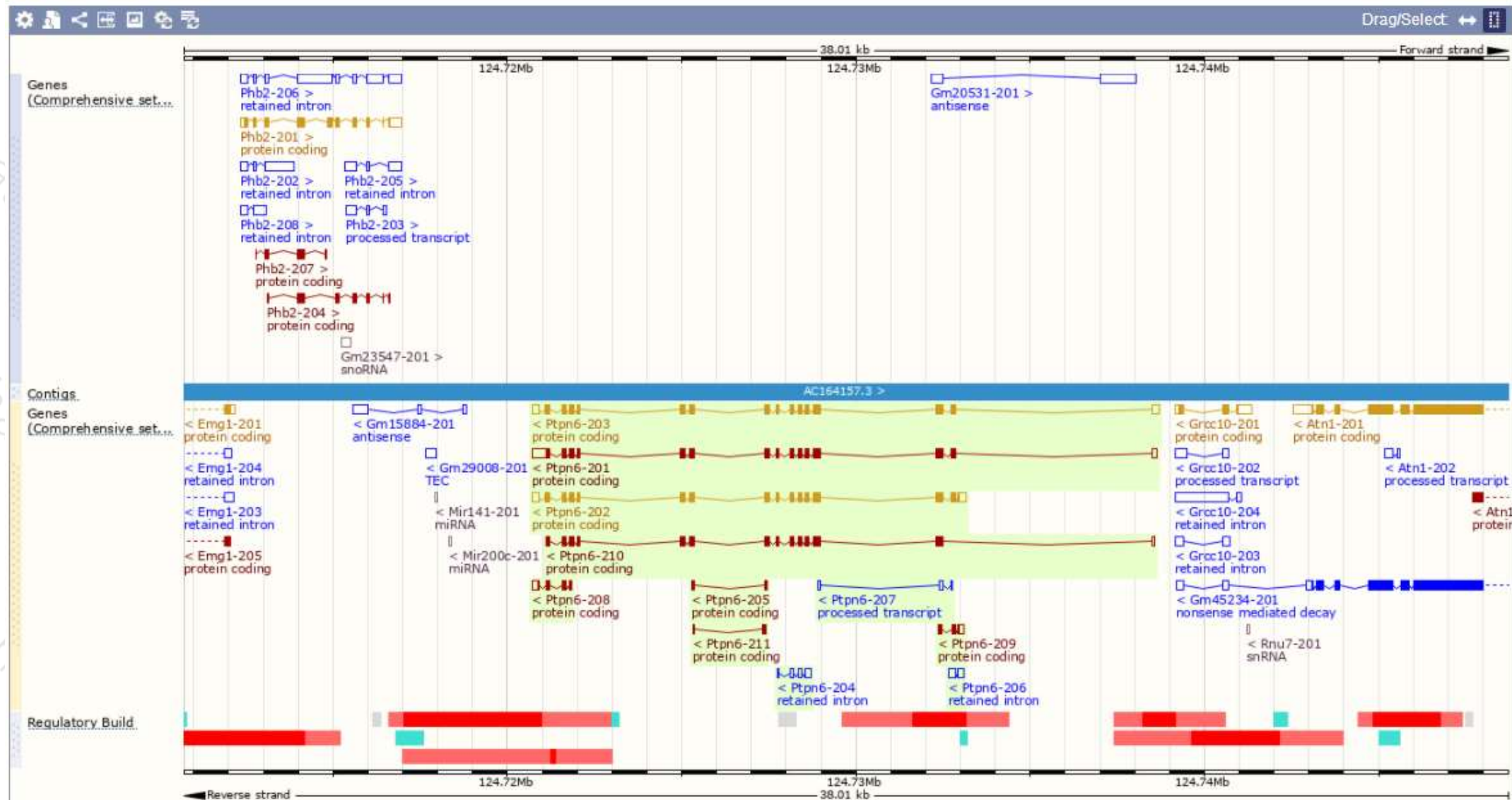
The gene has 11 transcripts, and all transcripts are shown below :

Show/hide columns (1 hidden)								Filter		
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags		
Ptpn6-201	ENSMUST00000004377.14	2308	597aa	Protein coding	CCDS51908	P29351 Q3UCJ0	-	TSL:1	GENCODE basic	APPRIS ALT1
Ptpn6-203	ENSMUST00000171549.8	2226	597aa	Protein coding	CCDS51908	P29351 Q3UCJ0	NM_001077705 NP_001071173	TSL:1	GENCODE basic	APPRIS ALT1
Ptpn6-202	ENSMUST00000112484.9	2219	595aa	Protein coding	CCDS39628	P29351	NM_013545 NP_038573	TSL:1	GENCODE basic	APPRIS P3
Ptpn6-210	ENSMUST00000174265.1	1743	556aa	Protein coding	-	P29351	-	TSL:5	GENCODE basic	
Ptpn6-208	ENSMUST00000173315.7	456	101aa	Protein coding	-	G3UYY5	-	CDS 5' incomplete	TSL:2	
Ptpn6-209	ENSMUST00000173647.1	330	67aa	Protein coding	-	G3UXM2	-	CDS 3' incomplete	TSL:5	
Ptpn6-205	ENSMUST00000172690.1	134	45aa	Protein coding	-	G3UZU6	-	CDS 5' and 3' incomplete	TSL:1	
Ptpn6-211	ENSMUST00000174787.1	120	40aa	Protein coding	-	G3UWU7	-	CDS 5' and 3' incomplete	TSL:1	
Ptpn6-207	ENSMUST00000173228.1	198	No protein	Processed transcript	-	-	-	TSL:1		
Ptpn6-204	ENSMUST00000172613.1	421	No protein	Retained intron	-	-	-	TSL:5		
Ptpn6-206	ENSMUST00000172770.1	371	No protein	Retained intron	-	-	-	TSL:3		

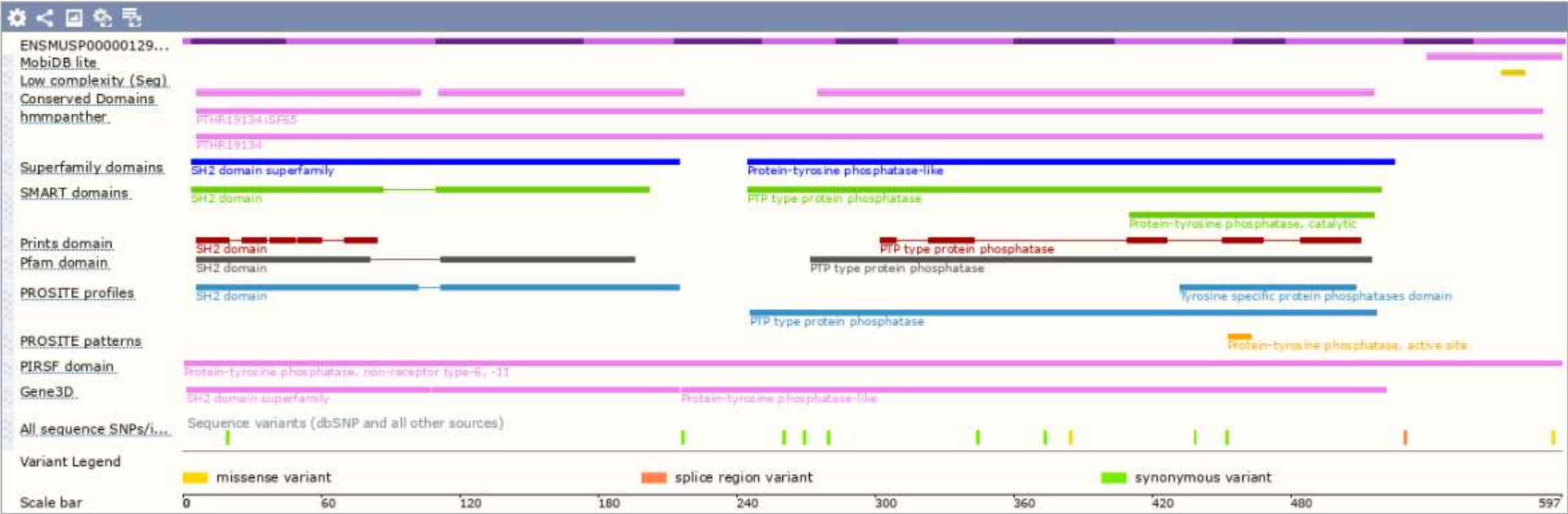
The strategy is based on the design of *Ptpn6-203* transcript,The transcription is shown below :



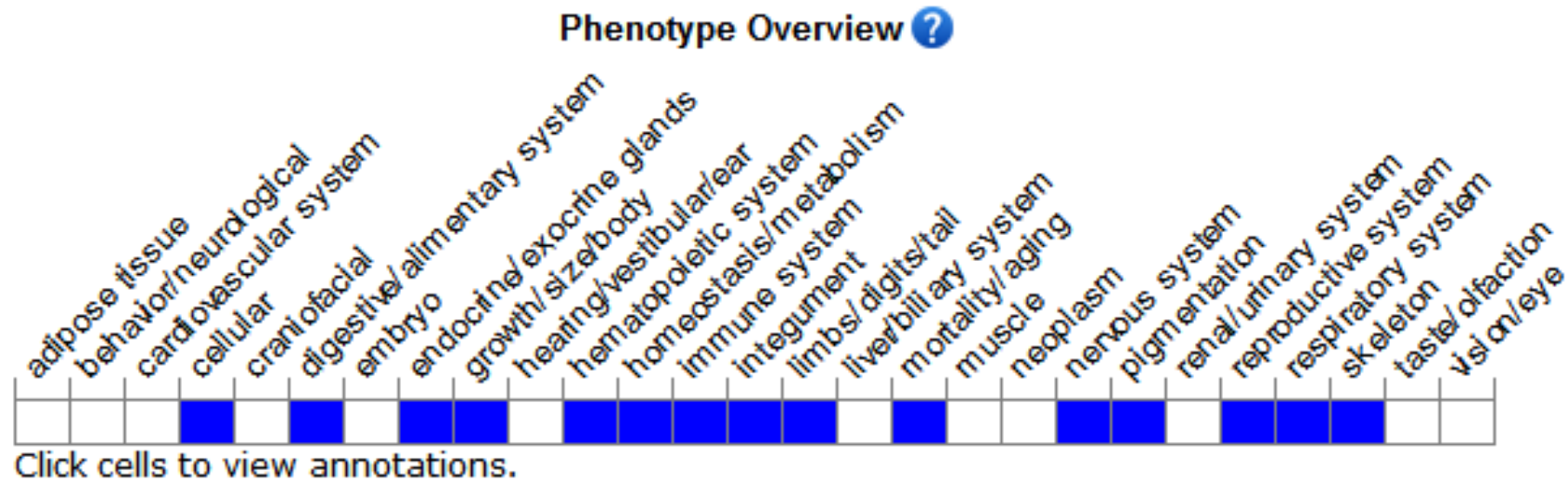
Genomic location distribution



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, Homozygous mutants are immunodeficient and autoimmune and exhibit neutrophilic skin lesions that disrupt hair follicles and give the moth-eaten appearance. Alleles vary in severity, with death occurring at 6-9 weeks postnatally due to severe pneumonitis.

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