

Id3 Cas9-CKO Strategy

Designer:

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Design Date:

2019-7-25

Project Overview

Project Name

Id3

Project type

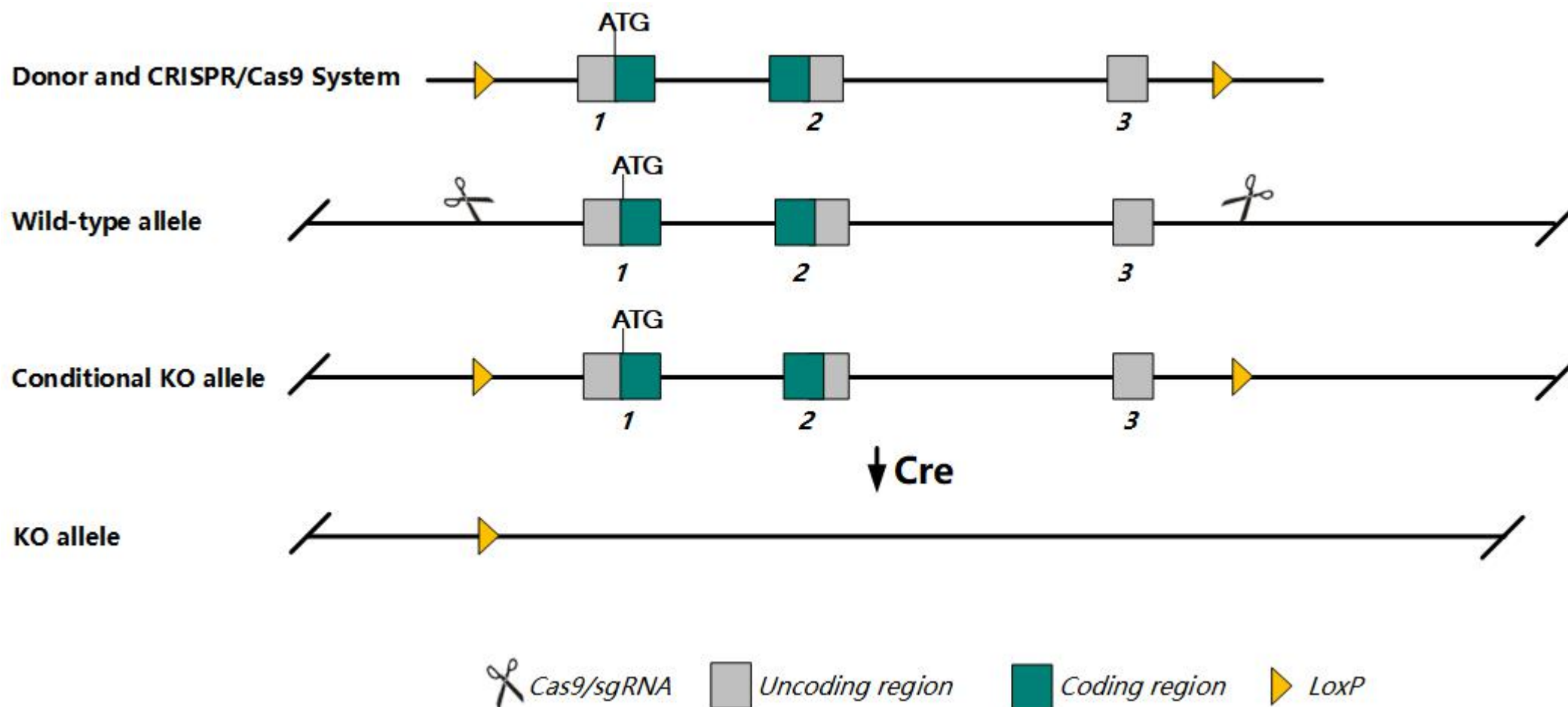
Cas9-CKO

Strain background

C57BL/6J

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Id3* gene. The schematic diagram is as follows:



Technical routes

- The *Id3* gene has 3 transcripts. According to the structure of *Id3* gene, exon1-exon3 of *Id3-201* (ENSMUST00000008016.2) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Id3* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.
- 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, Homozygotes for a targeted null mutation exhibit compromised humoral immunity. Homozygotes for knockout alleles of both *Id1* and *Id3* die by embryonic day 13.5 with vascular malformations of the forebrain.
- The KO region contains functional region of the *Gm55764-201* and *Gm57159-201* gene. Knockout the region will affect the function of *Gm55764-201* and *Gm57159-201* gene.
- The *Id3* gene is located on the Chr4. 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)

Id3 inhibitor of DNA binding 3 [Mus musculus (house mouse)]

Gene ID: 15903, updated on 31-Jan-2019

Summary



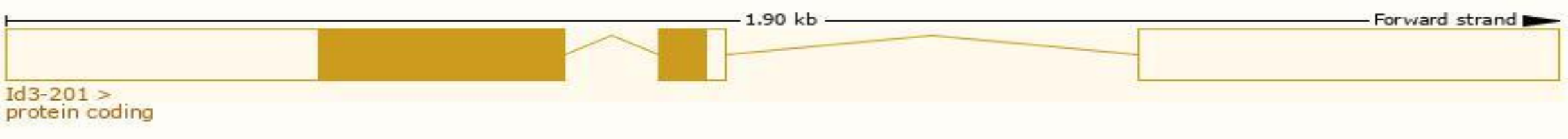
Official Symbol	Id3 provided by MGI
Official Full Name	inhibitor of DNA binding 3 provided by MGI
Primary source	MGI:MGI:96398
See related	Ensembl:ENSMUSG00000007872
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Hlh462, Idb3, bHLHb25
Expression	Ubiquitous expression in ovary adult (RPKM 274.3), stomach adult (RPKM 262.7) and 26 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

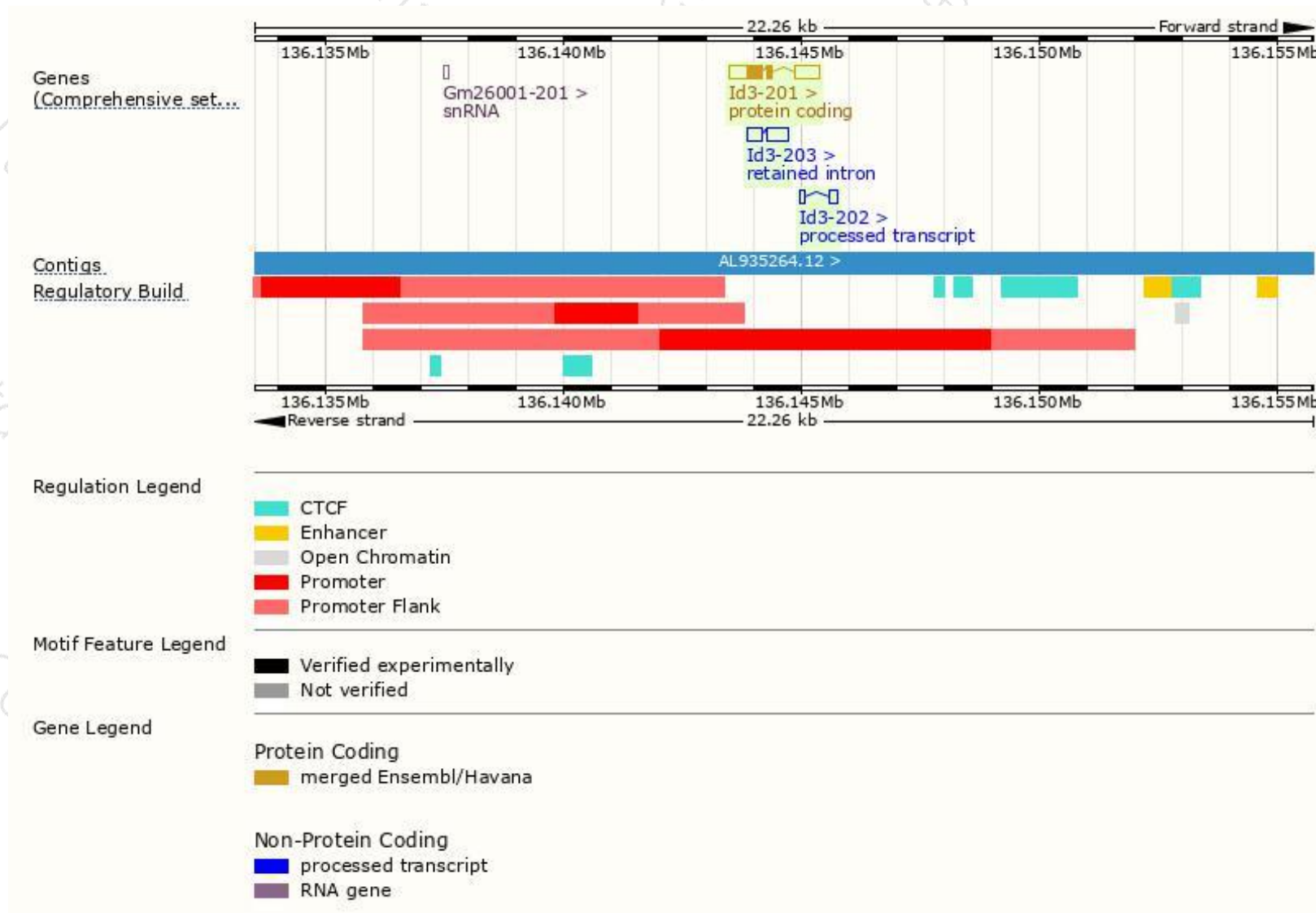
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Id3-201	ENSMUST00000008016.2	1278	119aa	Protein coding	CCDS18800	P41133 Q545W1	NM_008321 NP_032347	TSL:1 GENCODE basic APPRIS P1
Id3-202	ENSMUST00000133946.1	277	No protein	Processed transcript	-	-	-	TSL:3
Id3-203	ENSMUST00000151001.1	738	No protein	Retained intron	-	-	-	TSL:2

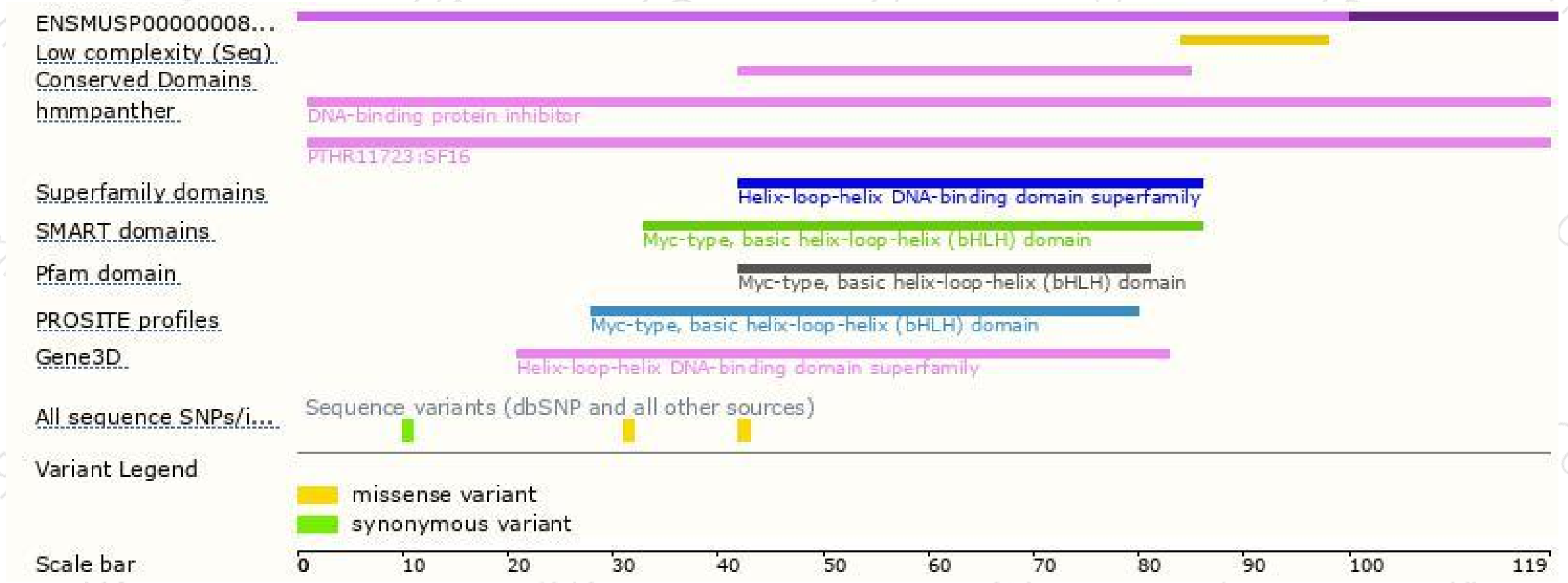
The strategy is based on the design of *Id3-201* transcript,The transcription is shown below



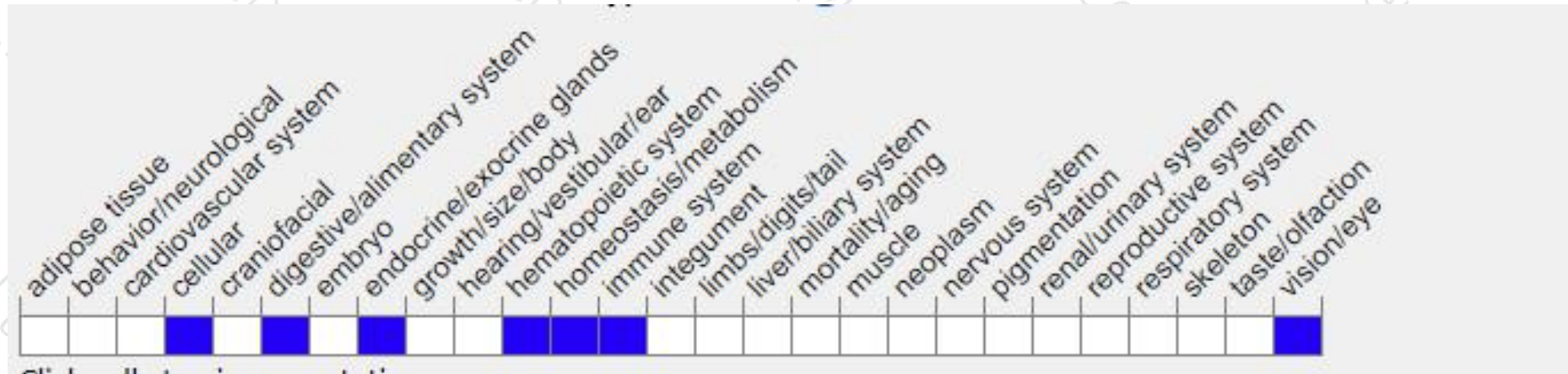
Genomic location distribution



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 targeted null mutation exhibit compromised humoral immunity.

Homozygotes for knockout alleles of both Id1 and Id3 die by embryonic day 13.5 with vascular malformations of the forebrain.

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

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