Tigit Cas9-CKO Strategy

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Design Date: **2019-11-22**

Project Overview

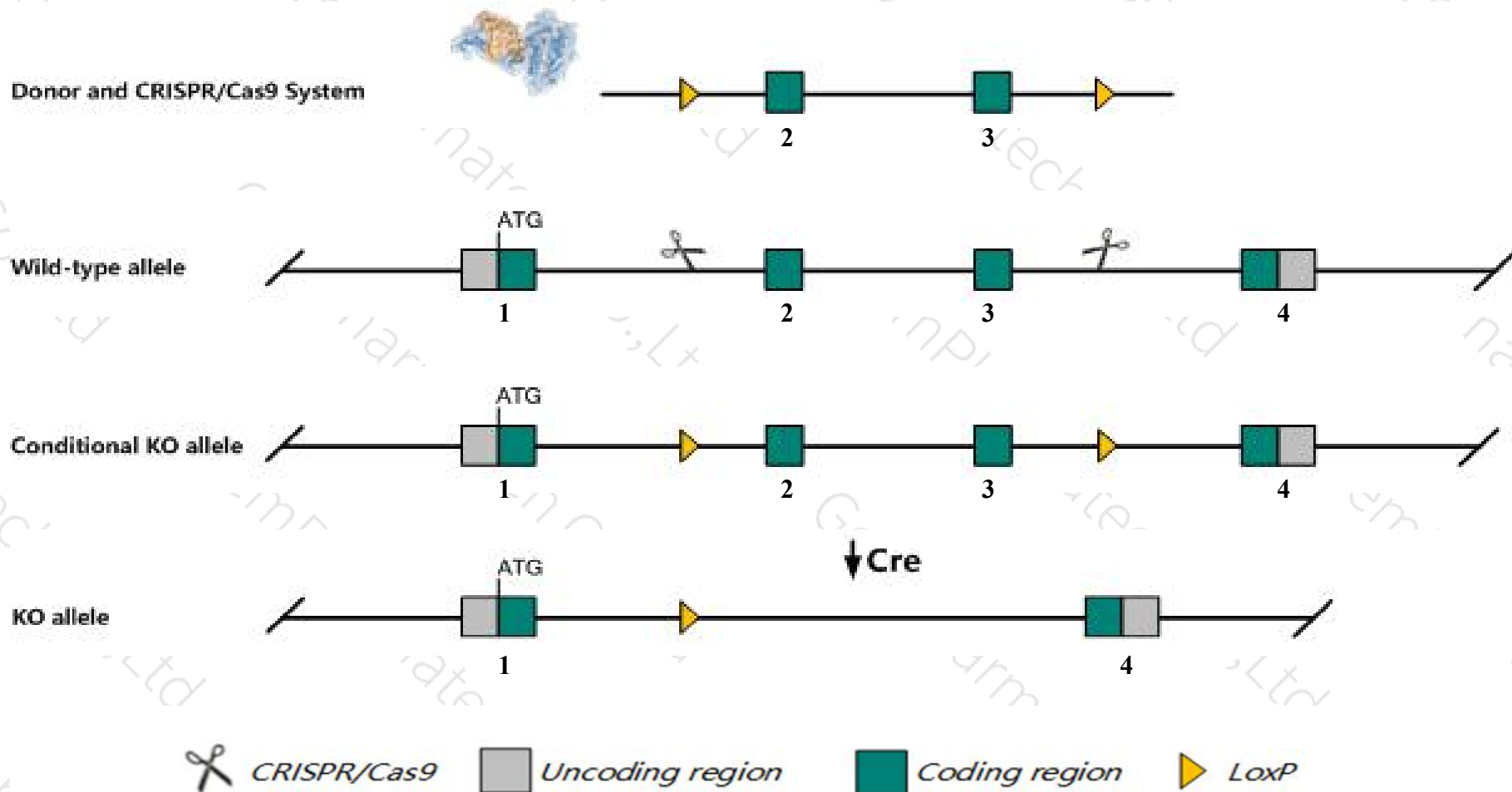|	
Project Name	*Tigit*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Technical routes

➢ The *Tigit* gene has 1 transcript. According to the structure of *Tigit* gene, exon2-exon3 of *Tigit-201* (ENSMUST00000096065.5) transcript is recommended as the knockout region.The region contains Ens 428bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tigit* 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 a knock-out allele exhibit increased T cell proliferation, antigen presenting cell stimuation of T cell proliferation, and susceptibility to EAE.

➢ The *Tigit* gene is located on the Chr16. 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）

Tigit T cell immunoreceptor with Ig and ITIM domains [Mus musculus (house mouse)]

Gene ID: 100043314, updated on 17-Feb-2019

Summary

|	
Official Symbol	Tigit provided by MGI
Official Full Name	T cell immunoreceptor with Ig and ITIM domains provided by MGI
Primary source	MGI:MGI:3642260
See related	Ensembl:ENSMUSG00000071552
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	Vstm3
Expression	Biased expression in large intestine adult (RPKM 1.9), small intestine adult (RPKM 1.8) and 8 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tigit-201	ENSMUST00000096065.5	919	241aa	Protein coding	CCDS49852	A0A0B4J1G6	TSL:1 GENCODE basic APPRIS P1

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

< Tigit-201
protein coding

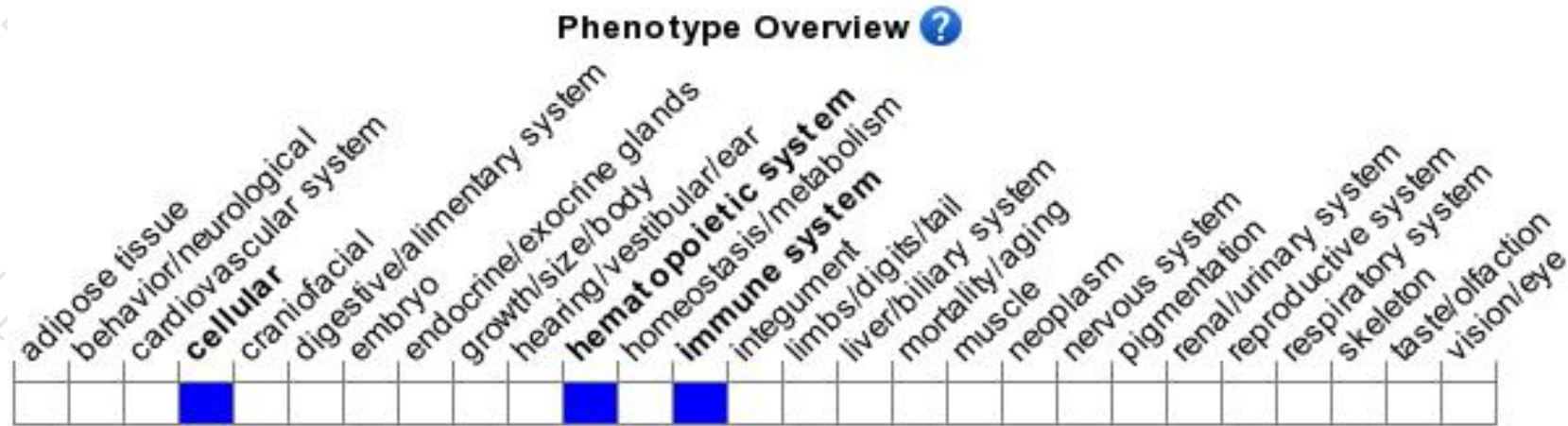◀─ Reverse strand

15.28 kb
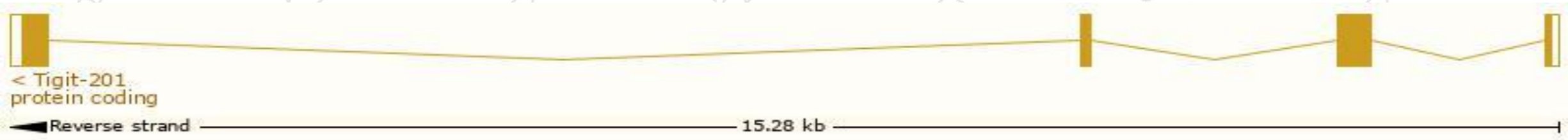
Genomic location distribution
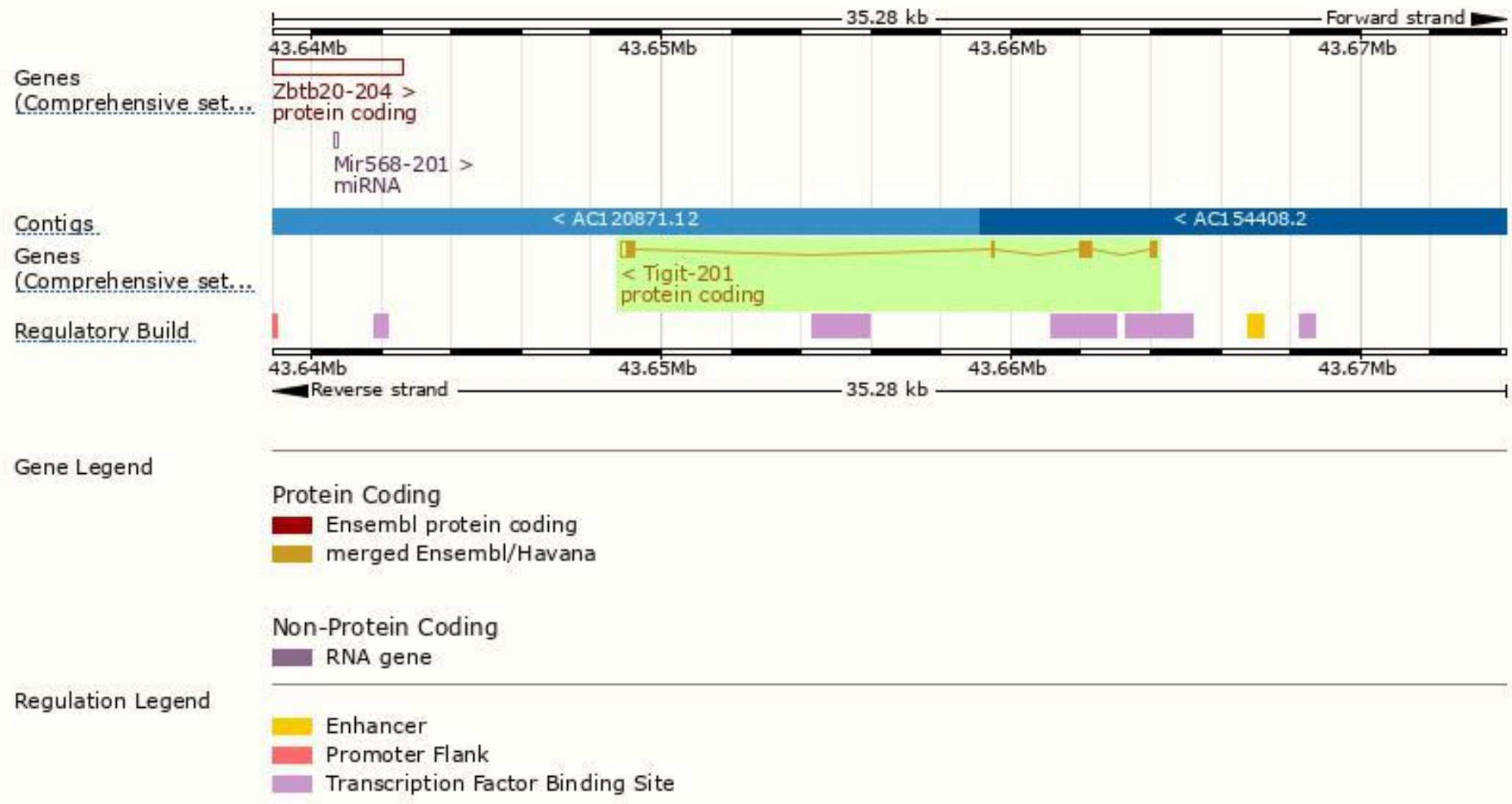
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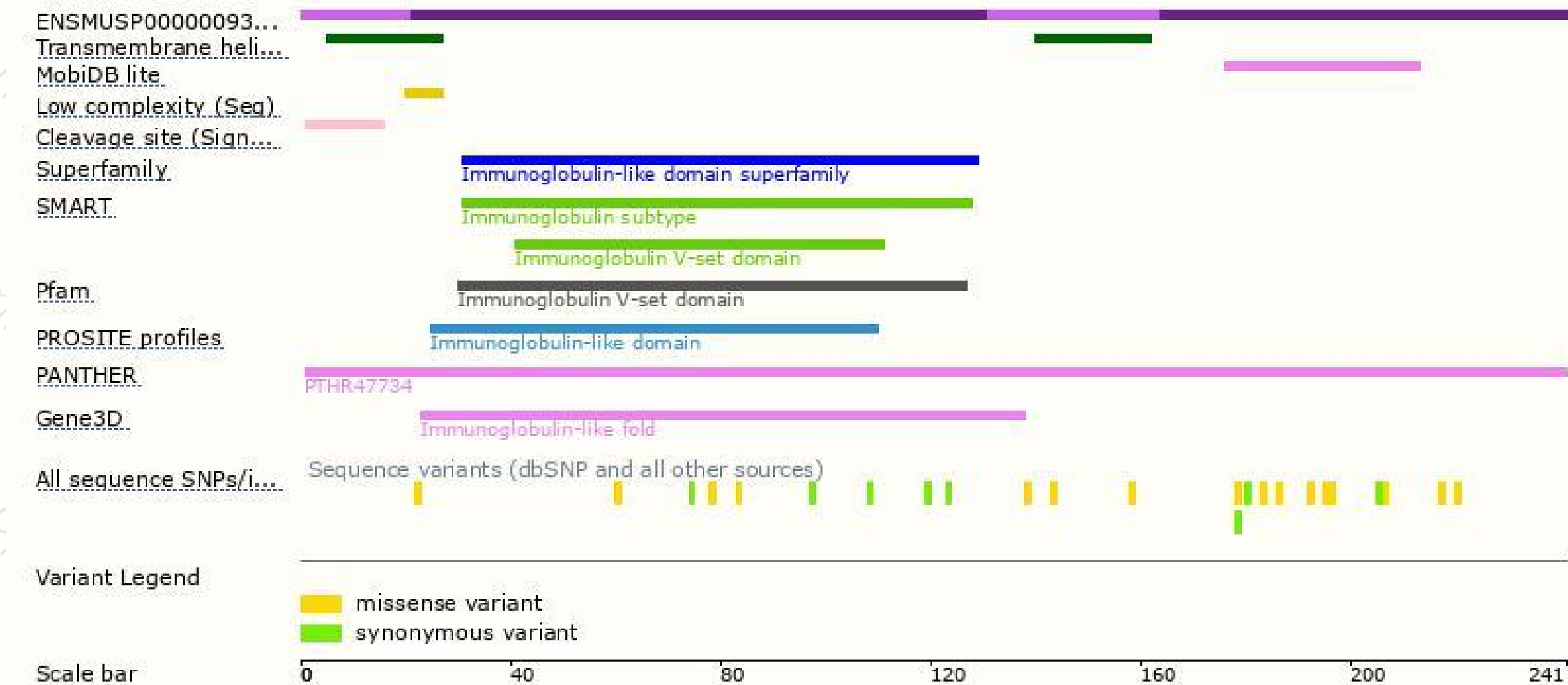
Mouse phenotype description(MGI)

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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