

***Traf5* Cas9-CKO Strategy**

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Project Overview

Project Name

Traf5

Project type

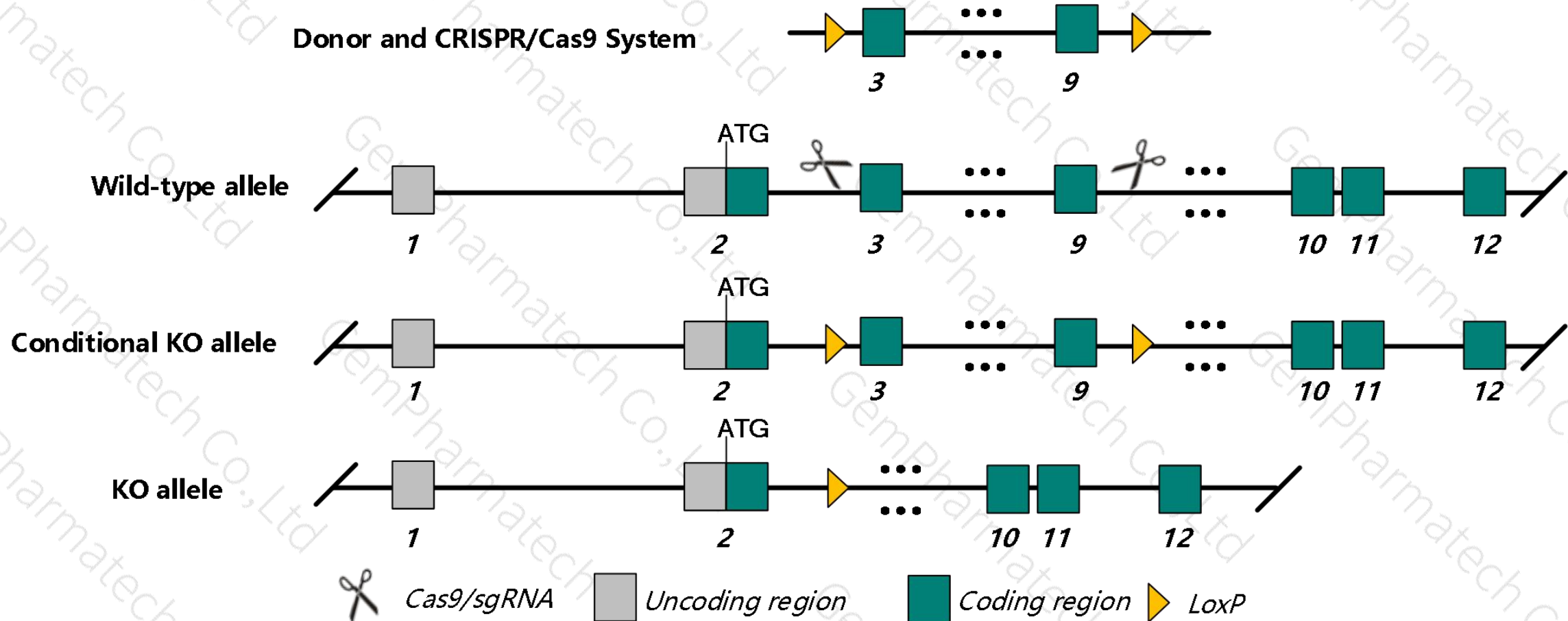
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Traf5* gene. The schematic diagram is as follows:



- The *Traf5* gene has 5 transcripts. According to the structure of *Traf5* gene, exon3-exon9 of *Traf5*-201 (ENSMUST00000085573.11) transcript is recommended as the knockout region. The region contains 586bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Traf5* 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.

- According to the existing MGI data, Homozygous null mice show defects in lymphocyte activation but are otherwise viable and develop normally.
- Transcript *Traf5*-202&204 may not be affected.
- The *Traf5* gene is located on the Chr1. 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)

Traf5 TNF receptor-associated factor 5 [*Mus musculus* (house mouse)]

Gene ID: 22033, updated on 15-Oct-2019

Summary

Official Symbol	Traf5 provided by MGI
Official Full Name	TNF receptor-associated factor 5 provided by MGI
Primary source	MGI:MGI:107548
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
Annotation information	Annotation category: suggests misassembly Annotation category: partial on reference assembly
Expression	Broad expression in spleen adult (RPKM 10.1), bladder adult (RPKM 8.7) and 25 other tissues See more
Orthologs	human all

Genomic context

Location: 1 H6; 1 97.11 cM

See Traf5 in [Genome Data Viewer](#)

Exon count: 12

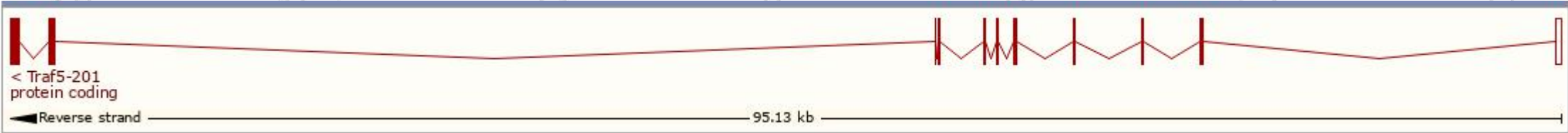
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (191997203..192092599, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC_000067.5 (193878227..193916445, complement) , (193821088..193823851, complement)

Transcript information (Ensembl)

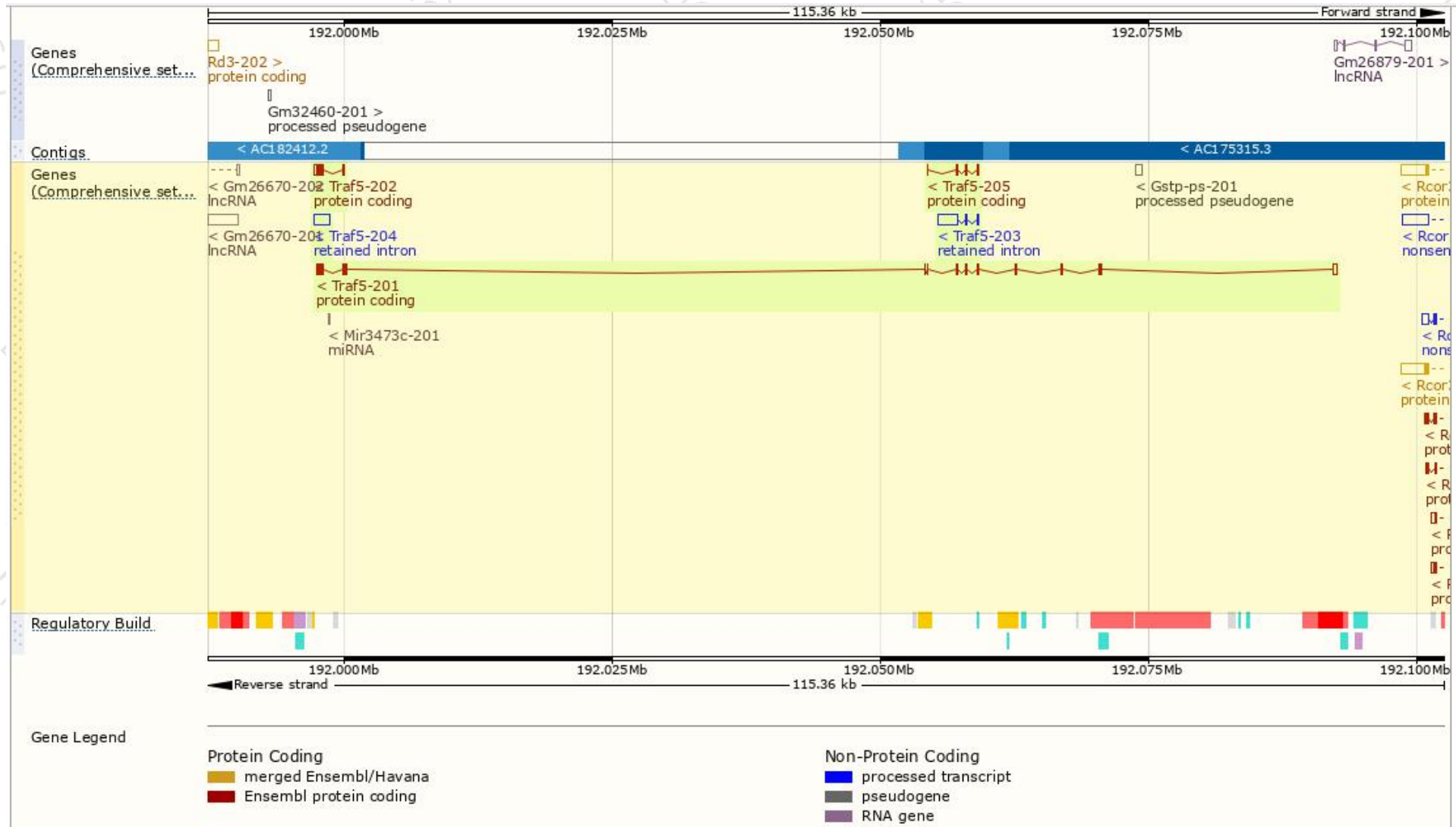
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Traf5-201	ENSMUST00000085573.11	1964	552aa	Protein coding	-	E9QMA6	TSL:5 Gencode basic APPRIS P1
Traf5-202	ENSMUST00000192628.1	976	250aa	Protein coding	-	A0A0A6YWK3	CDS 5' incomplete TSL:2
Traf5-205	ENSMUST00000195815.1	414	138aa	Protein coding	-	A0A0A6YXC4	CDS 5' and 3' incomplete TSL:5
Traf5-203	ENSMUST00000193355.1	2084	No protein	Retained intron	-	-	TSL:5
Traf5-204	ENSMUST00000194408.1	1414	No protein	Retained intron	-	-	TSL:NA

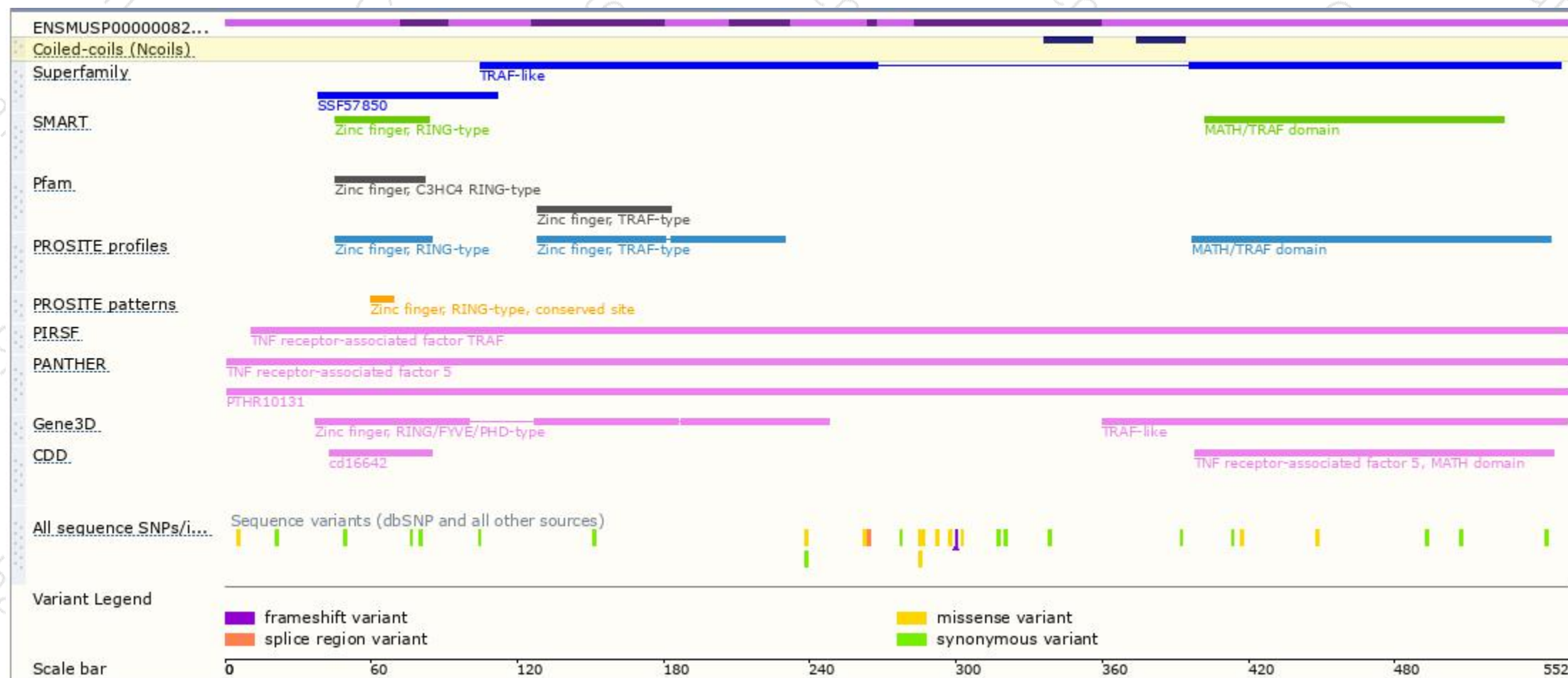
The strategy is based on the design of *Traf5-201* transcript,The transcription is shown below



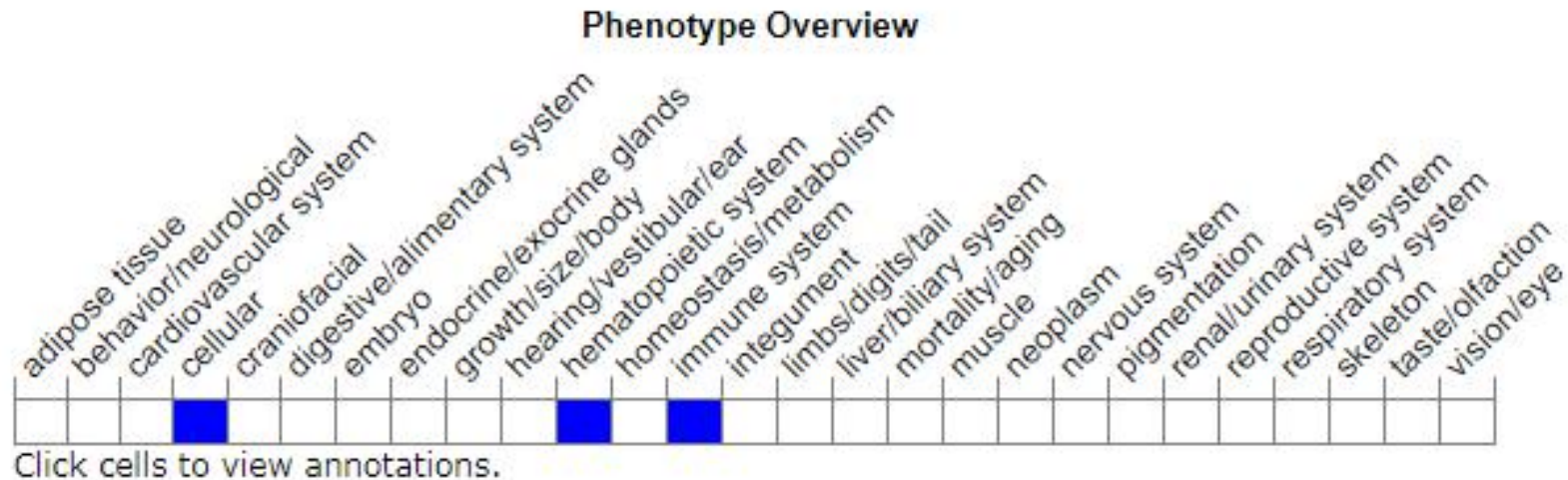
Genomic location distribution



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, Homozygous null mice show defects in lymphocyte activation but are otherwise viable and develop normally.

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

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