Tnfrsf25 Cas9-CKO Strategy

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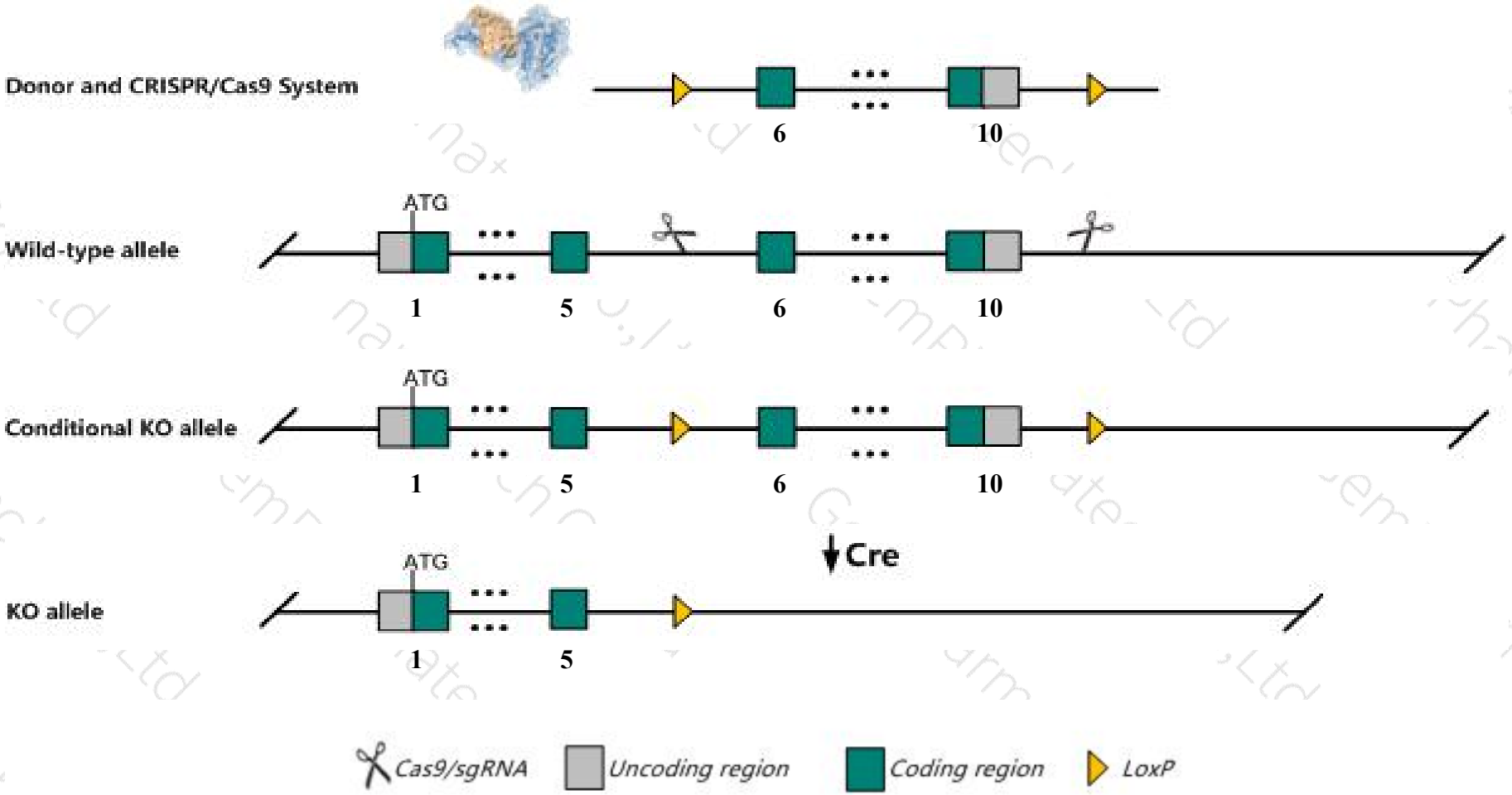
Project Overview

Project Name	***Tnfrsf25***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tnfrsf25* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tnfrsf25* gene has 4 transcripts. According to the structure of *Tnfrsf25* gene, exon6-exon10 of *Tnfrsf25-201*(ENSMUST00000025706.9) transcript is recommended as the knockout region.The region contains 706bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tnfrsf25* 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/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 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.

Notice

➢ According to the existing MGI data,homozygous mutant mice show no developmental defects or impairments of early thymocyte development. Negative selection and anti-CD3-induced apoptosis, however, are significantly impaired.

➢ The intron5 is only 552bp, loxp insertion may affect mRNA splicing.

➢ The 3-terminal loxp may affect the 3-terminal regulation of *Espn* gene.

➢There are more amino acids left at the N end of the *Tnfrsf25* gene, which may retain some functions.

➢ The *Tnfrsf25* 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）

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Tnfrsf25 tumor necrosis factor receptor superfamily, member 25 [Mus musculus (house mouse)]

Gene ID: 85030, updated on 13-Mar-2020

Summary

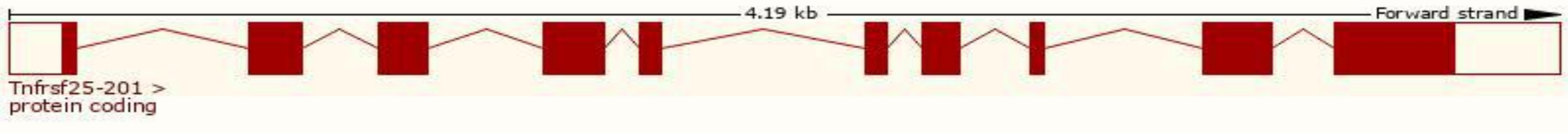
Official Symbol	Tnfrsf25 provided by MGI
Official Full Name	tumor necrosis factor receptor superfamily, member 25 provided by MGI
Primary source	MGI:MGI:1934667
See related	Ensembl:ENSMUSG00000024793
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	APO-3, DDR3, DR3, LARD, TR3, TRAMP, Tnfrsf12, WSL-1, WSL-LR, Wsl
Expression	Ubiquitous expression in mammary gland adult (RPKM 5.7), spleen adult (RPKM 5.0) and 25 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

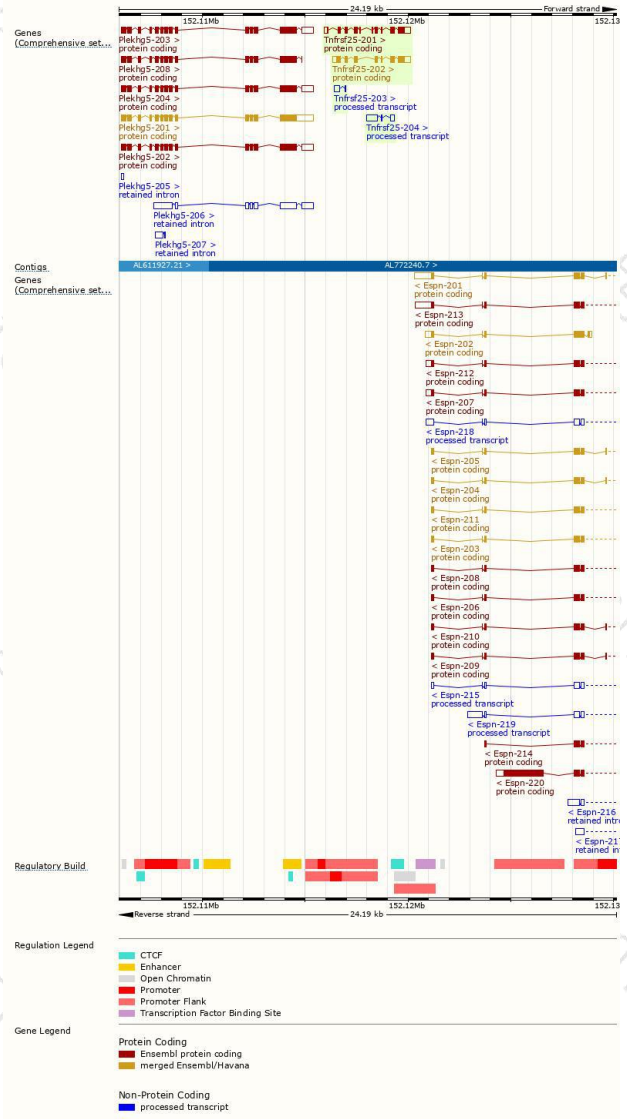
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tnfrsf25-201	ENSMUST00000025706.9	1661	411aa	Protein coding	CCDS71527	B1AWN9	TSL:1 GENCODE basic APPRIS ALT2
Tnfrsf25-202	ENSMUST00000035275.7	1603	387aa	Protein coding	CCDS38983	Q8VD70	TSL:1 GENCODE basic APPRIS P3
Tnfrsf25-204	ENSMUST00000153619.1	731	No protein	Processed transcript	-	-	TSL:3
Tnfrsf25-203	ENSMUST00000127111.1	346	No protein	Processed transcript	-	-	TSL:3

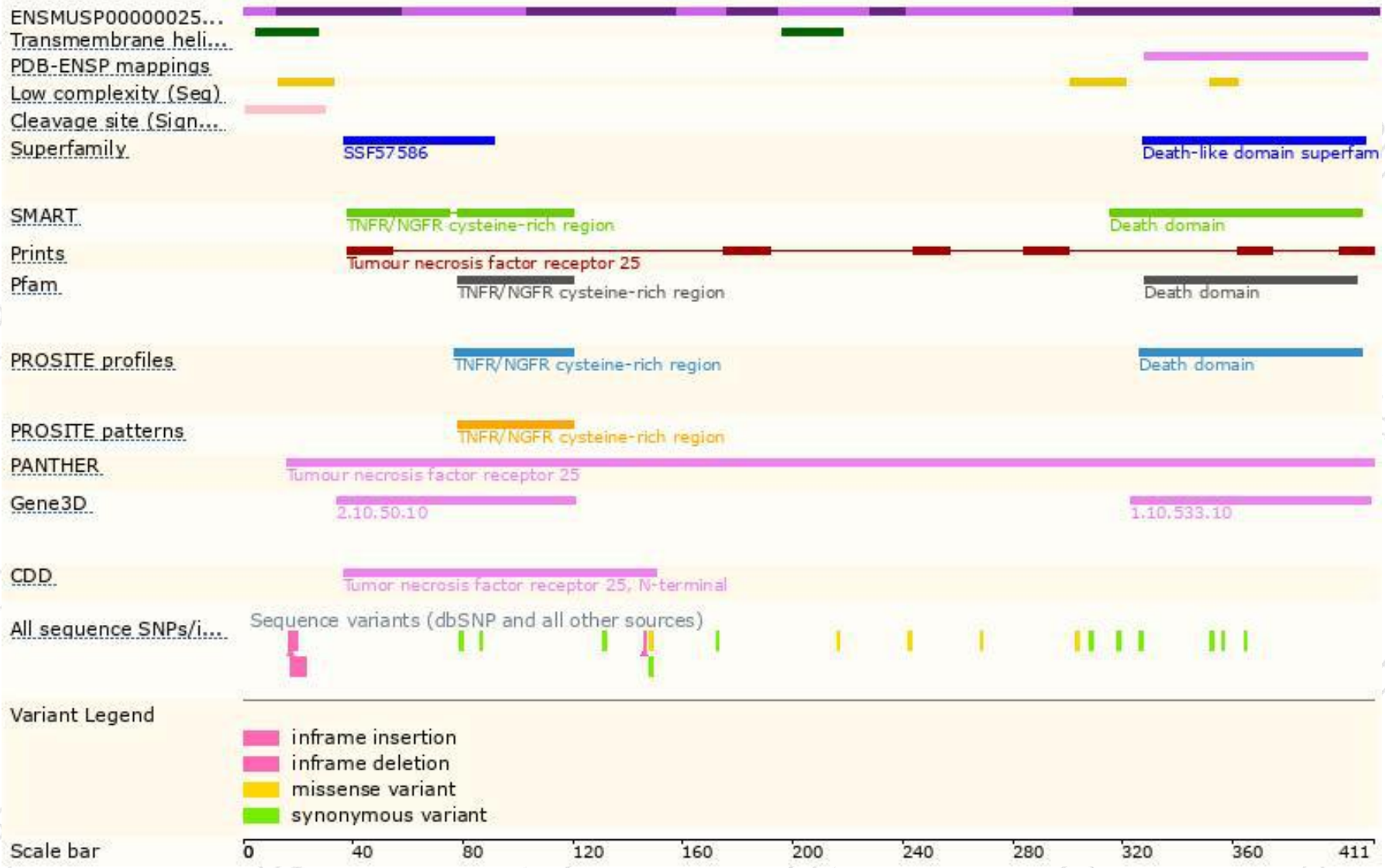
The strategy is based on the design of *Tnfrsf25-201* transcript,the transcription is shown below:



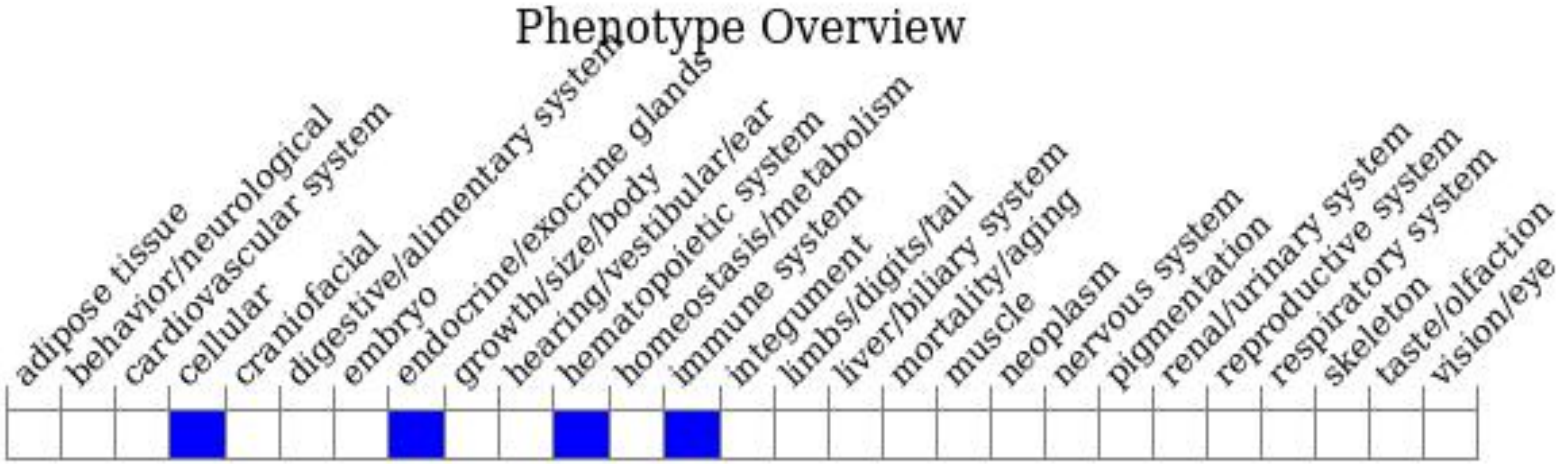
Genomic location distribution



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 mutant mice show no developmental defects or impairments of early thymocyte development. Negative selection and anti-CD3-induced apoptosis, however, are significantly impaired.

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

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