Cd86 Cas9-CKO Strategy

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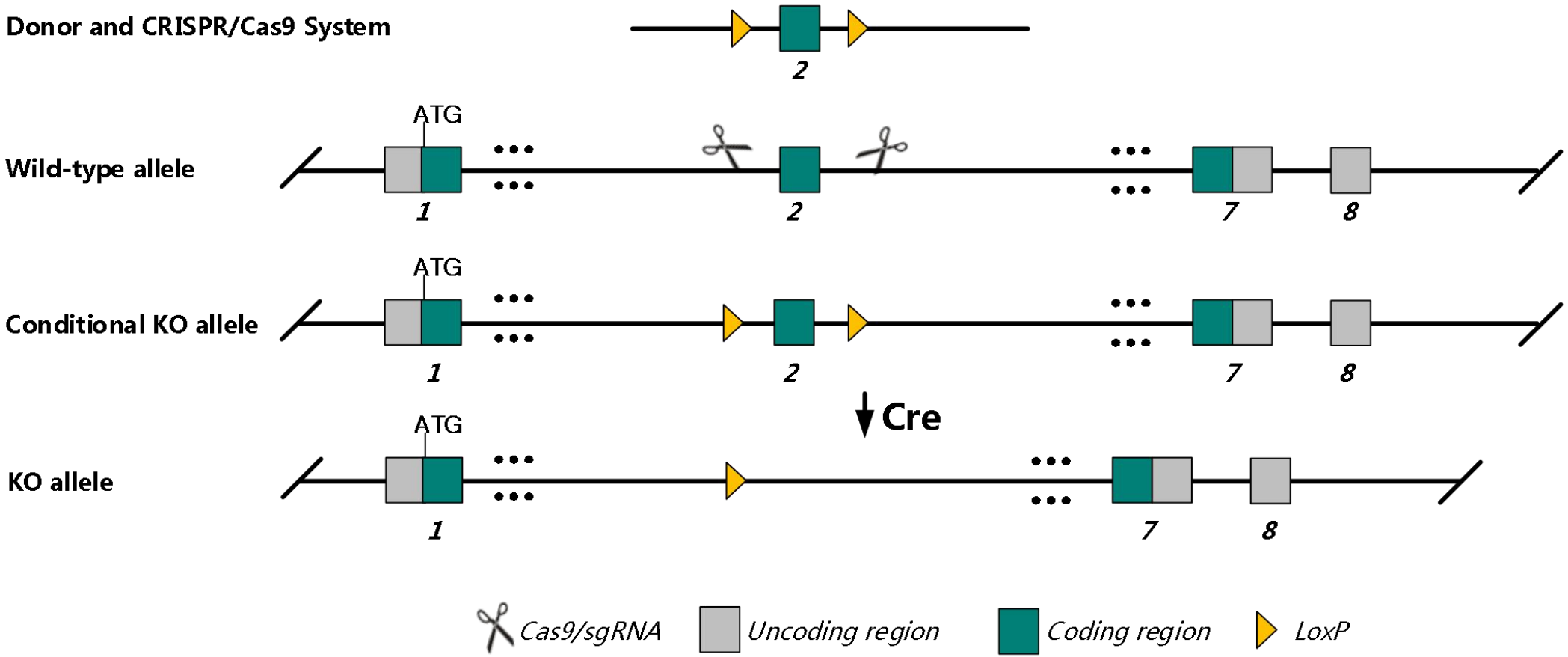
Project Overview

Project Name	***Cd86***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cd86* gene. The schematic diagram is as follows:



Technical routes

- The *Cd86* gene has 4 transcripts. According to the structure of *Cd86* gene, exon2 of *Cd86-201* (ENSMUST00000089620.10) transcript is recommended as the knockout region.The region contains 50bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cd86* 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,Homozygous null mice on an NOD background display a phenotype similar to human Guillain-Barre Syndrome, exhibiting severe peripheral nervous system inflammation, sciatic nerve demyelination, elevated auto-antibodies to myelin protein zero, hindlimb paralysis, and weak forelimb grip.

➢ The *Cd86* 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）

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Cd86 CD86 antigen [Mus musculus (house mouse)]

Gene ID: 12524, updated on 12-Feb-2019

Summary

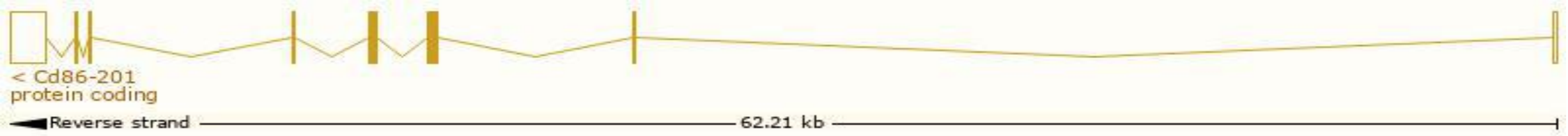
Official Symbol	Cd86 provided by MGI
Official Full Name	CD86 antigen provided by MGI
Primary source	MGI:MGI:101773
See related	Ensembl:ENSMUSG00000022901
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	B7, B7-2, B7.2, B70, CLS1, Cd28l2, ETC-1, Ly-58, Ly58, MB7, MB7-2, TS/A-2
Expression	Broad expression in spleen adult (RPKM 3.3), liver E18 (RPKM 2.8) and 21 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

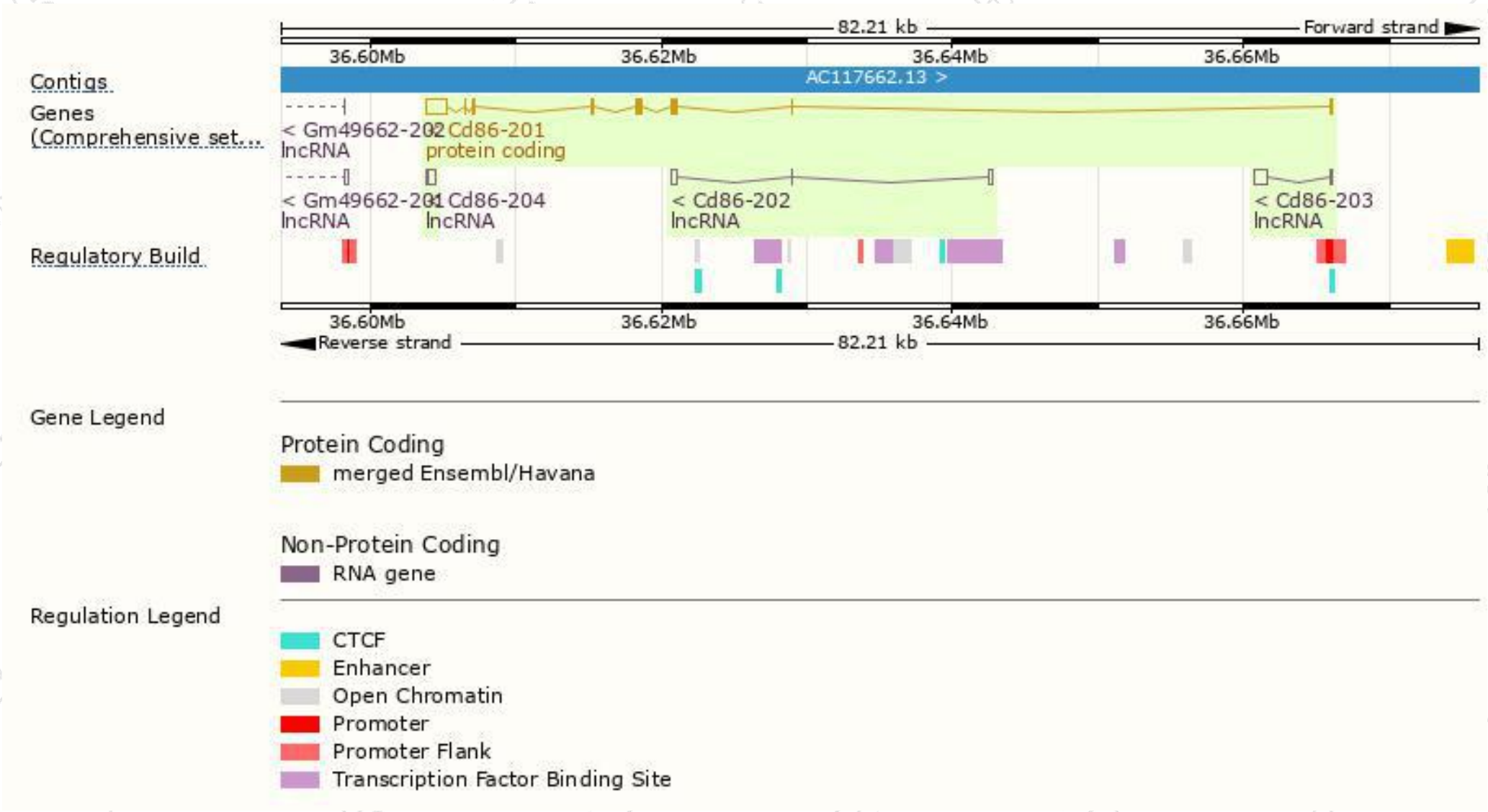
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd86-201	ENSMUST00000089620.10	2539	309aa	Protein coding	CCDS28155	P42082 Q549Q9	TSL:1 GENCODE basic APPRIS P1
Cd86-203	ENSMUST00000145506.1	975	No protein	lncRNA	-	-	TSL:1
Cd86-202	ENSMUST00000135280.1	654	No protein	lncRNA	-	-	TSL:3
Cd86-204	ENSMUST00000154485.1	548	No protein	lncRNA	-	-	TSL:3

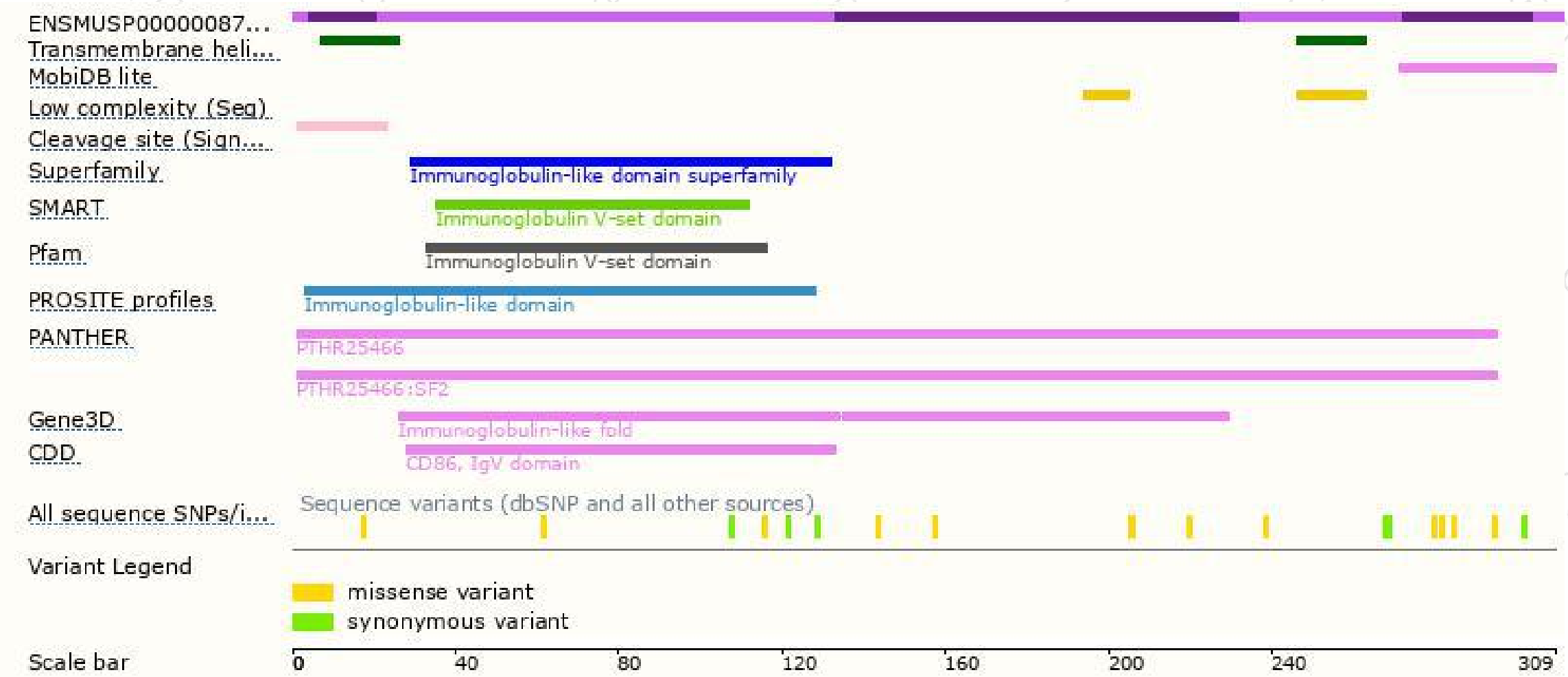
The strategy is based on the design of *Cd86-201* transcript,The transcription is shown below



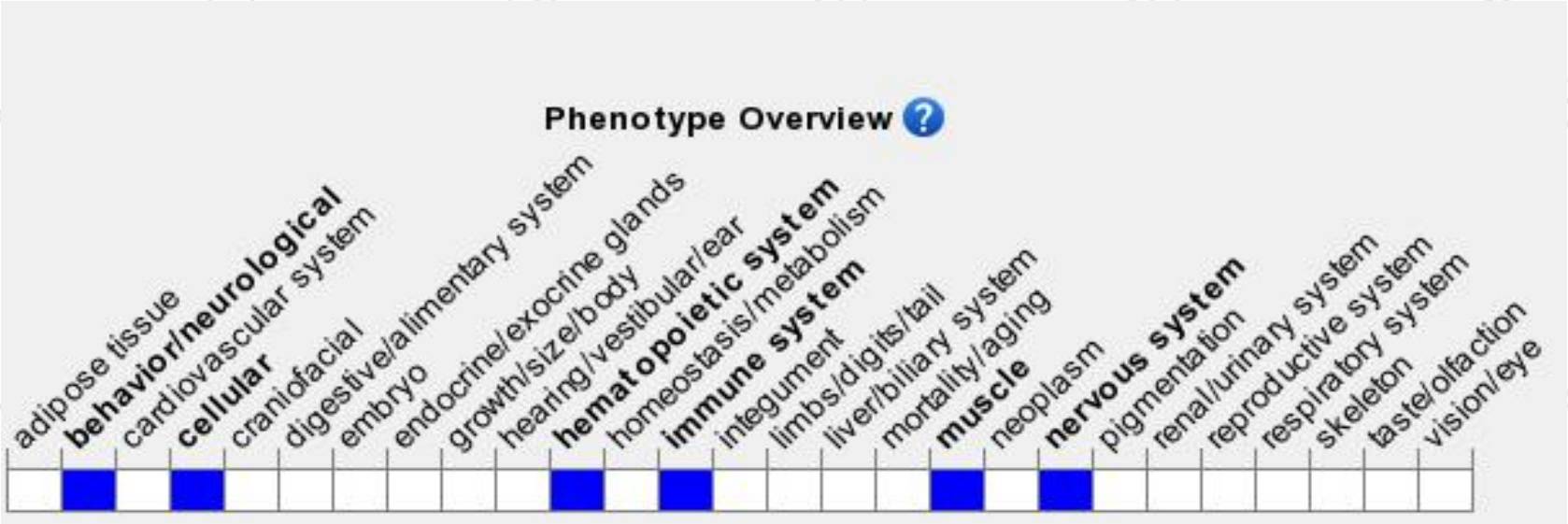
Genomic location distribution



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/).

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

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