

Cd28 Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer:

Huimin Su

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Project Overview

Project Name

Cd28

Project type

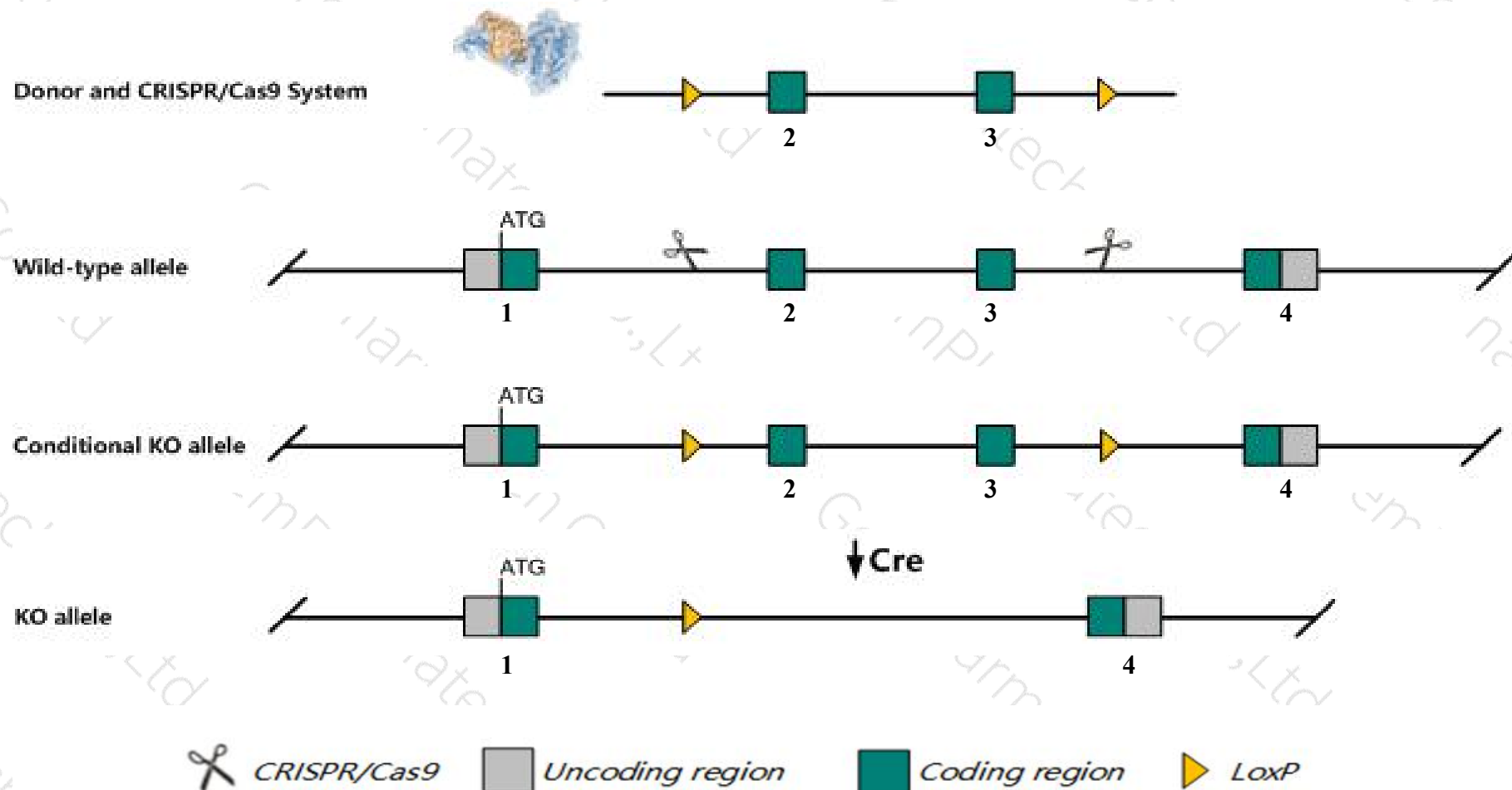
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cd28* gene. The schematic diagram is as follows:



Technical routes

- The *Cd28* gene has 3 transcripts. According to the structure of *Cd28* gene, exon2-exon3 of *Cd28-201* (ENSMUST00000027165.2) transcript is recommended as the knockout region. The region contains 473bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cd28* 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.

- According to the existing MGI data, Homozygous mutation of this gene results in impairment of some T cell responses and decreased basal immunoglobulin levels. Mutant animals have reduced T helper cell activity and impaired T cell response to lectins, but cytotoxic T cells can still be induced.
- The *Cd28* 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)

Cd28 CD28 antigen [Mus musculus (house mouse)]

Gene ID: 12487, updated on 9-Apr-2019

Summary



Official Symbol	Cd28 provided by MGI
Official Full Name	CD28 antigen provided by MGI
Primary source	MGI:MGI:88327
See related	Ensembl:ENSMUSG000000026012
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
Expression	Biased expression in thymus adult (RPKM 17.9), spleen adult (RPKM 1.8) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

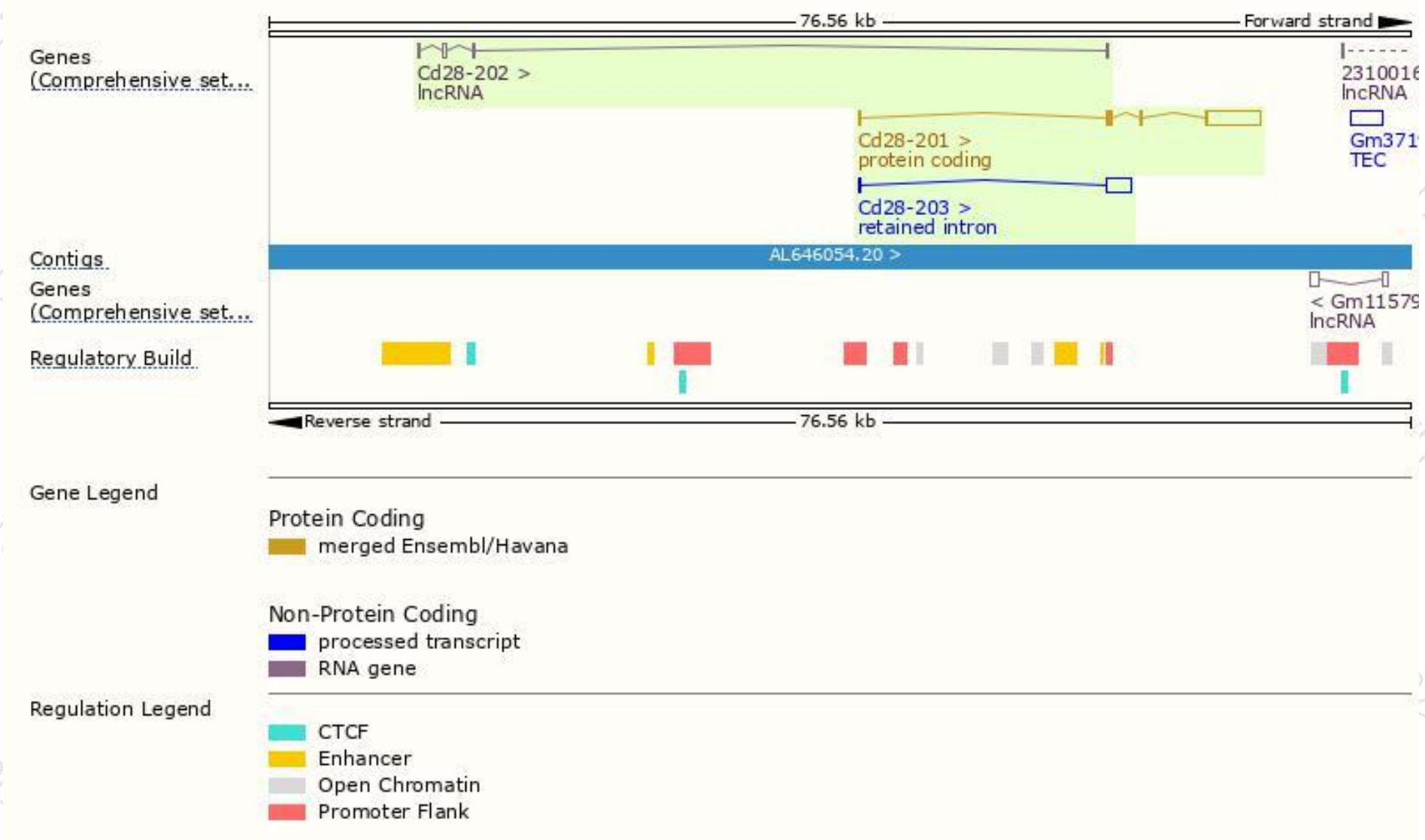
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd28-201	ENSMUST00000027165.2	4347	218aa	Protein coding	CCDS14992	P31041	TSL:1 GENCODE basic APPRIS P1
Cd28-202	ENSMUST00000132833.1	669	No protein	Processed transcript	-	-	TSL:5
Cd28-203	ENSMUST00000153207.1	1775	No protein	Retained intron	-	-	TSL:1

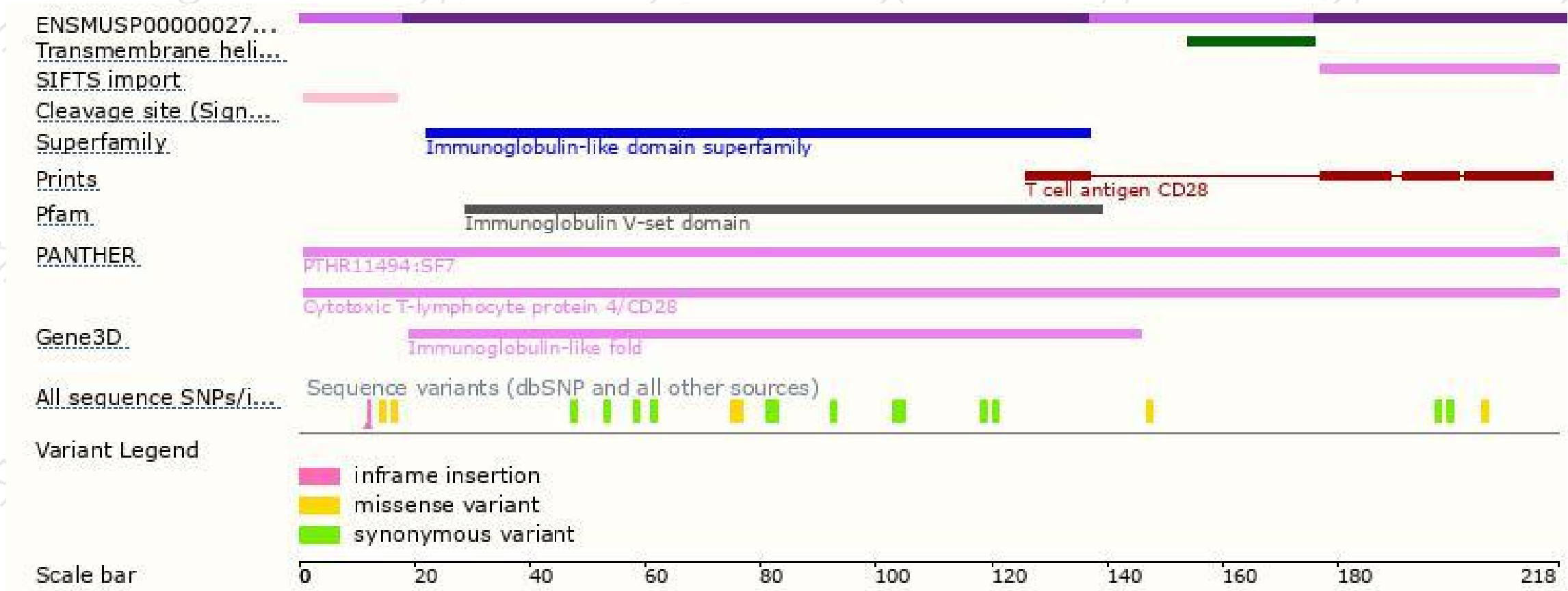
The strategy is based on the design of *Cd28-201* transcript,The transcription is shown below



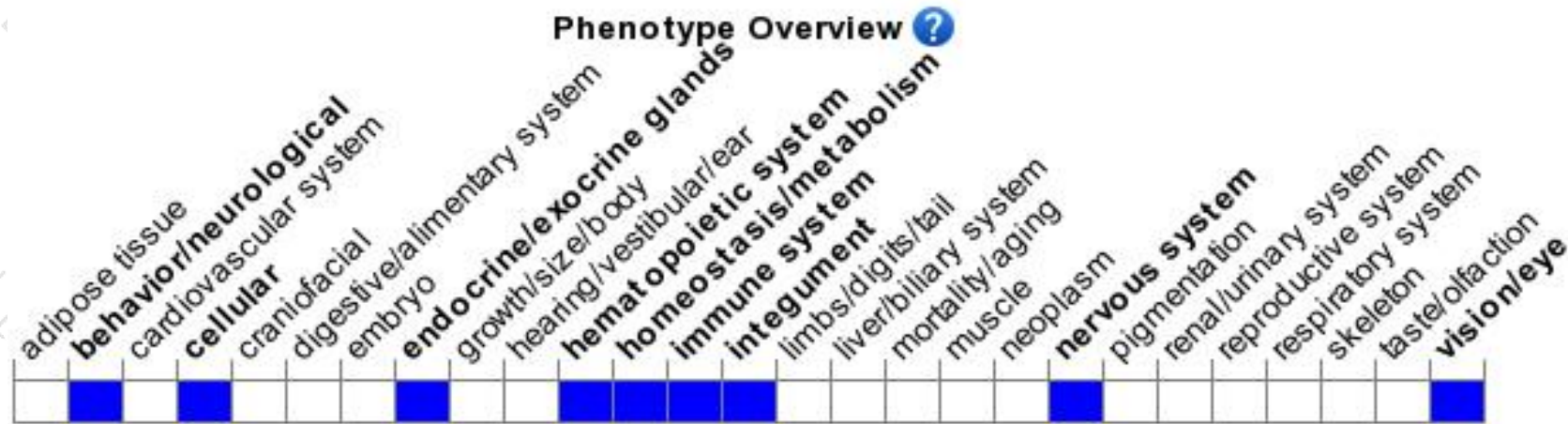
Genomic location distribution



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.

Tel: 400-9660890

