Denn2b Cas9-CKO Strategy

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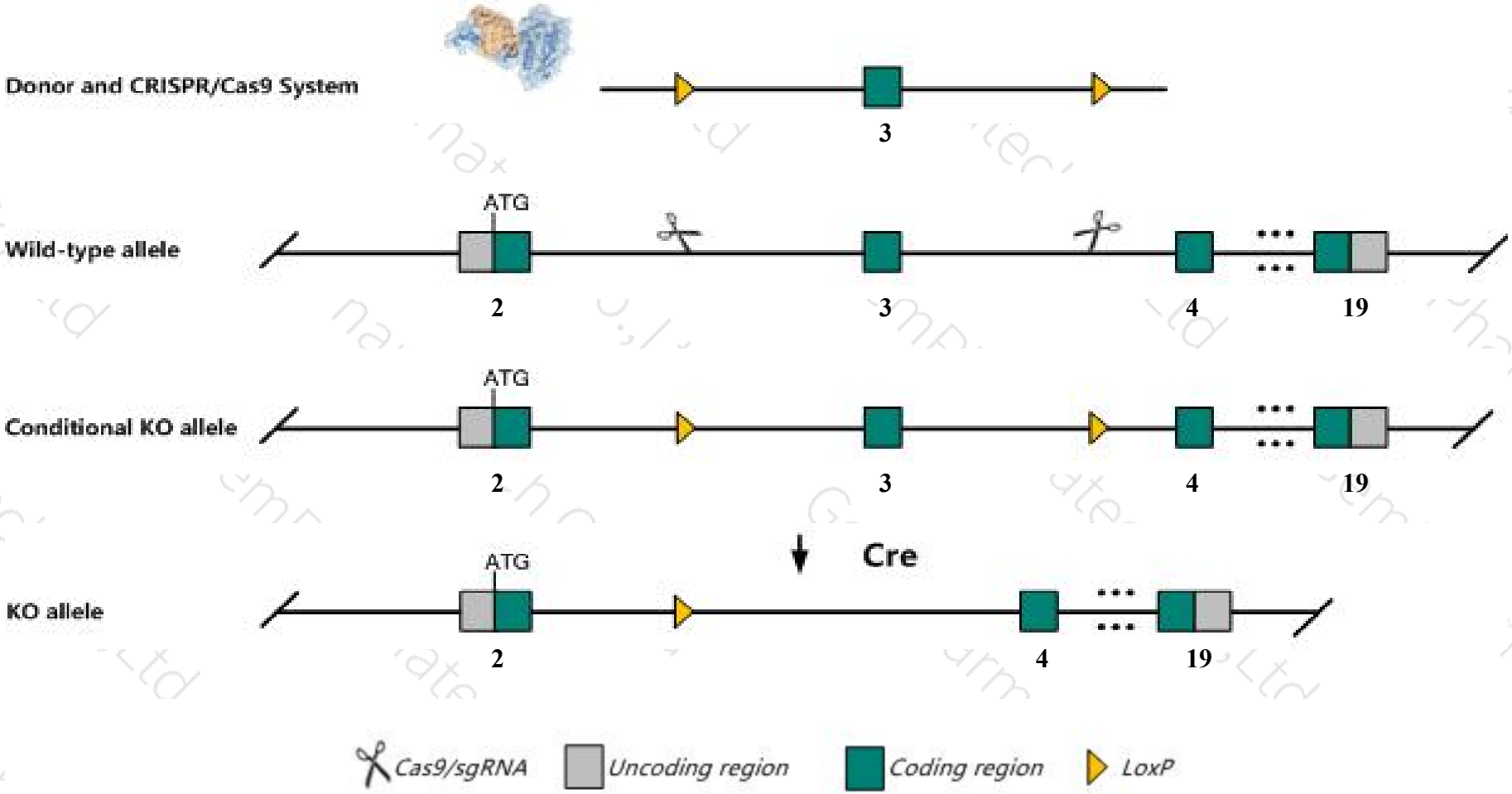
Project Overview

Project Name	***Denn2b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Denn2b* gene. The schematic diagram is as follows:



Technical routes

➢ The *Denn2b* gene has 11 transcripts. According to the structure of *Denn2b* gene, exon3 of *Denn2b-203*(ENSMUST00000084738.4) transcript is recommended as the knockout region.The region contains 137bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Denn2b* 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

➢ The *Denn2b* gene is located on the Chr7. 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.

➢ Transcript *Denn2b*-206 may not be affected.

➢ The effect on transcript *Denn2b*-205&207&209&210 is unknown.

➢ 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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Denn2b DENN domain containing 2B [Mus musculus (house mouse)]

Gene ID: 76954, updated on 13-Mar-2020

Summary

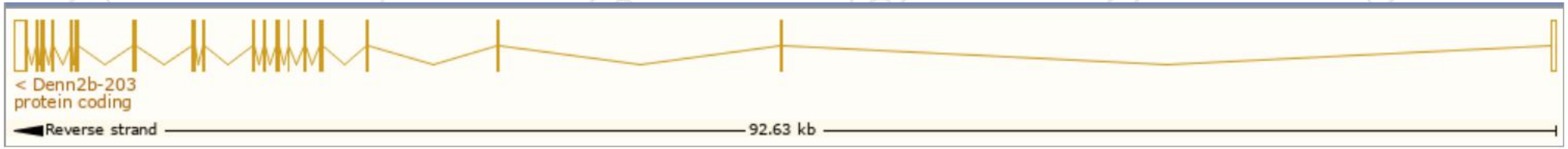
Official Symbol	Denn2b provided by MGI
Official Full Name	DENN domain containing 2B provided byMGI
Primary source	MGI:MGI:108517
See related	Ensembl:ENSMUSG00000031024
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010004M01Rik, 2610305K15Rik, St5
Expression	Ubiquitous expression in lung adult (RPKM 22.8), ovary adult (RPKM 12.9) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

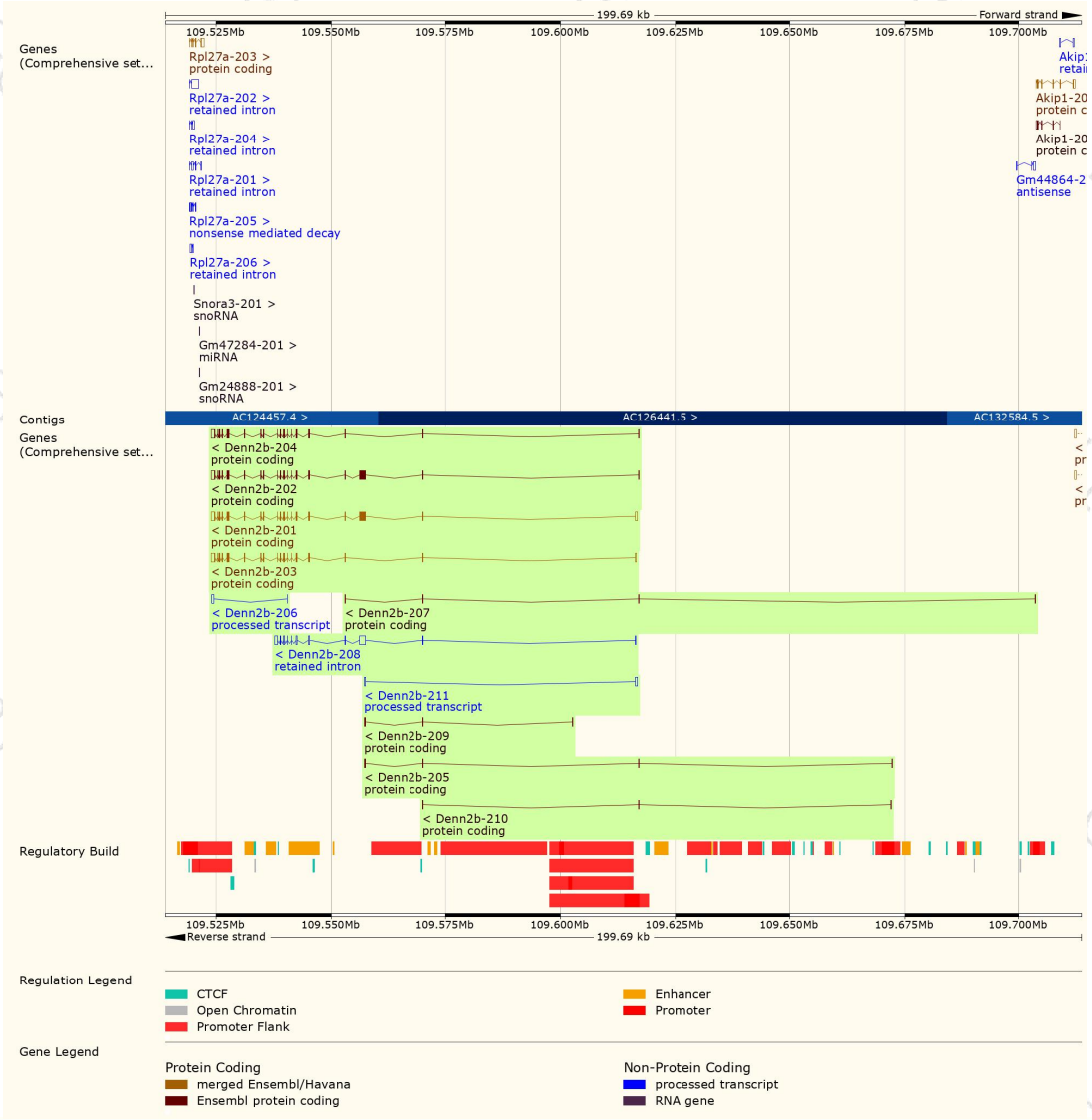
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Denn2b-201	ENSMUST00000077909.8	4612	1134aa	Protein coding	CCDS21734	Q924W7	TSL:1 GENCODE basic APPRIS P4
Denn2b-202	ENSMUST00000079282.7	4255	1134aa	Protein coding	CCDS21734	Q924W7	TSL:5 GENCODE basic APPRIS P4
Denn2b-203	ENSMUST00000084738.4	3161	717aa	Protein coding	CCDS21735	Q924W7	TSL:5 GENCODE basic APPRIS ALT2
Denn2b-204	ENSMUST00000168005.7	3004	717aa	Protein coding	CCDS21735	Q924W7	TSL:5 GENCODE basic APPRIS ALT2
Denn2b-205	ENSMUST00000207394.1	553	95aa	Protein coding	-	A0A140LHU4	CDS 3' incomplete TSL:2
Denn2b-209	ENSMUST00000208583.1	439	101aa	Protein coding	-	A0A140LIB1	CDS 3' incomplete TSL:3
Denn2b-207	ENSMUST00000207745.1	362	59aa	Protein coding	-	A0A140LIS1	CDS 3' incomplete TSL:3
Denn2b-210	ENSMUST00000208734.1	362	26aa	Protein coding	-	A0A140LII1	CDS 3' incomplete TSL:2
Denn2b-211	ENSMUST00000208981.1	737	No protein	Processed transcript	-	-	TSL:2
Denn2b-206	ENSMUST00000207664.1	514	No protein	Processed transcript	-	-	TSL:3
Denn2b-208	ENSMUST00000208557.1	2991	No protein	Retained intron	-	-	TSL:1

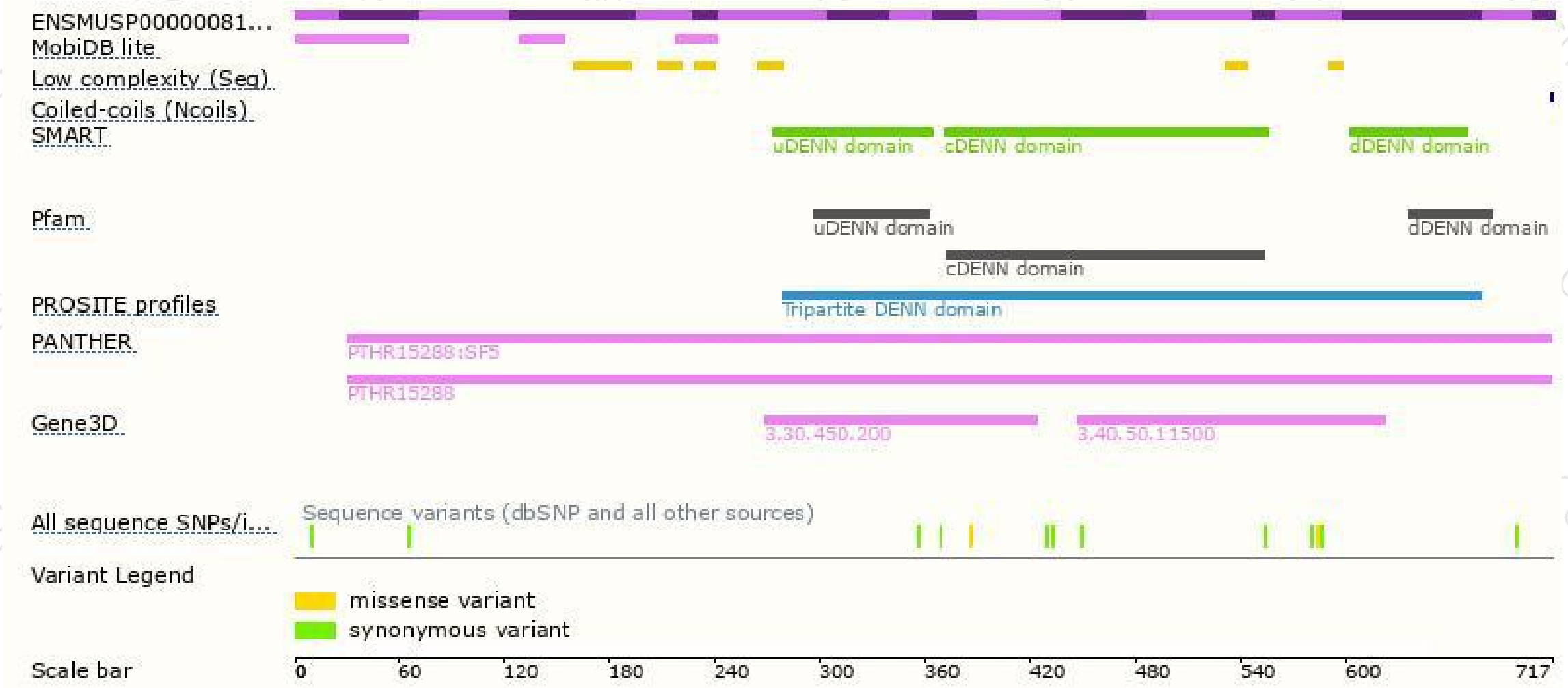
The strategy is based on the design of *Denn2b-203* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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