Dennd6b Cas9-CKO Strategy

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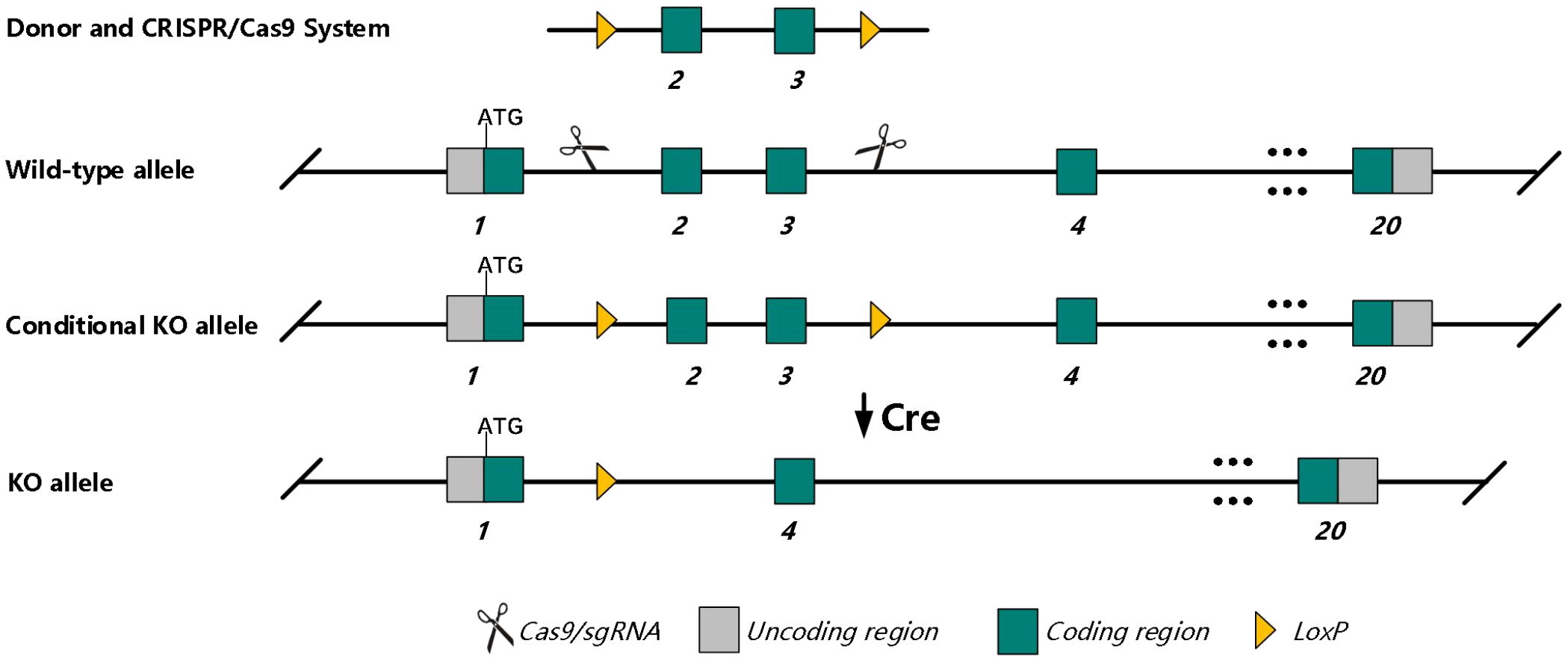
Project Overview

Project Name	***Dennd6b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dennd6b* gene. The schematic diagram is as follows:



Technical routes

- The *Dennd6b* gene has 6 transcripts. According to the structure of *Dennd6b* gene, exon2-exon3 of *Dennd6b-201* (ENSMUST00000078953.8) transcript is recommended as the knockout region.The region contains 82bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Dennd6b* 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

➢ The *Dennd6b* gene is located on the Chr15. 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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Dennd6b DENN/MADD domain containing 6B [*Mus musculus* (house mouse)]

Gene ID: 69440, updated on 13-Mar-2020

Summary

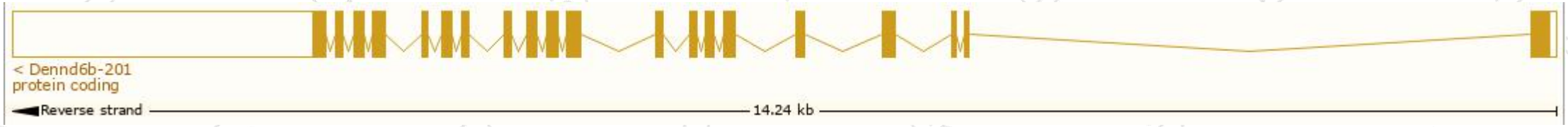
Official Symbol	Dennd6b provided by MGI
Official Full Name	DENN/MADD domain containing 6B provided by MGI
Primary source	MGI:MGI:1916690
See related	Ensembl:ENSMUSG00000015377
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Fam116b; AI414881; 1700027J05Rik
Expression	Broad expression in CNS E18 (RPKM 33.8), cerebellum adult (RPKM 32.4) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

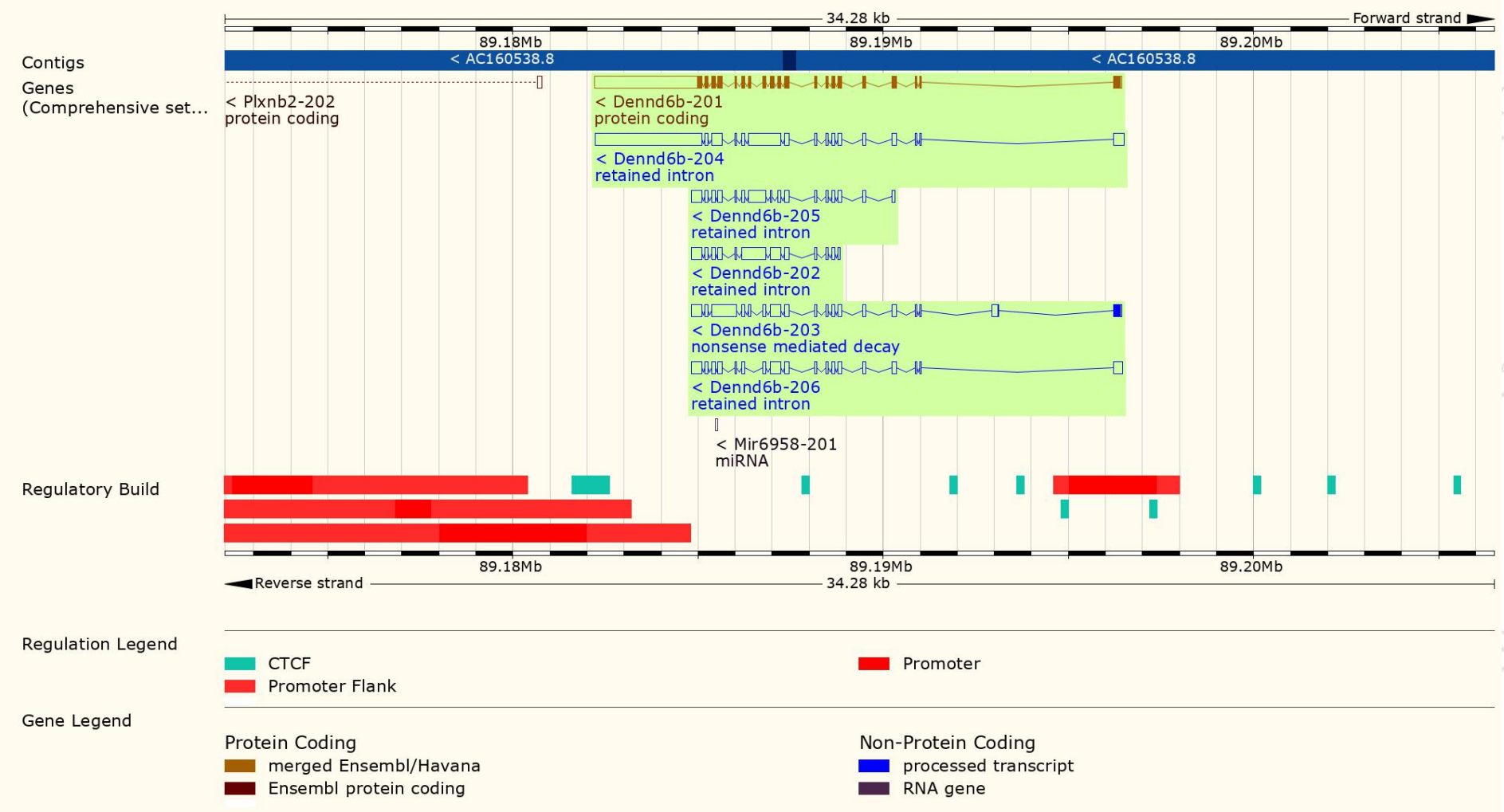
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dennd6b-201	ENSMUST00000078953.8	4585	585aa	Protein coding	CCDS57000	Q9D9V7	TSL:1 GENCODE basic APPRIS P1
Dennd6b-202	ENSMUST00000229416.1	1969	No protein	Retained intron	-	-	-
Dennd6b-203	ENSMUST00000229755.1	2677	64aa	Nonsense mediated decay	-	A0A2R8VKJ9	-
Dennd6b-204	ENSMUST00000229935.1	5261	No protein	Retained intron	-	-	-
Dennd6b-205	ENSMUST00000230111.1	1904	No protein	Retained intron	-	-	-
Dennd6b-206	ENSMUST00000230823.1	2028	No protein	Retained intron	-	-	-

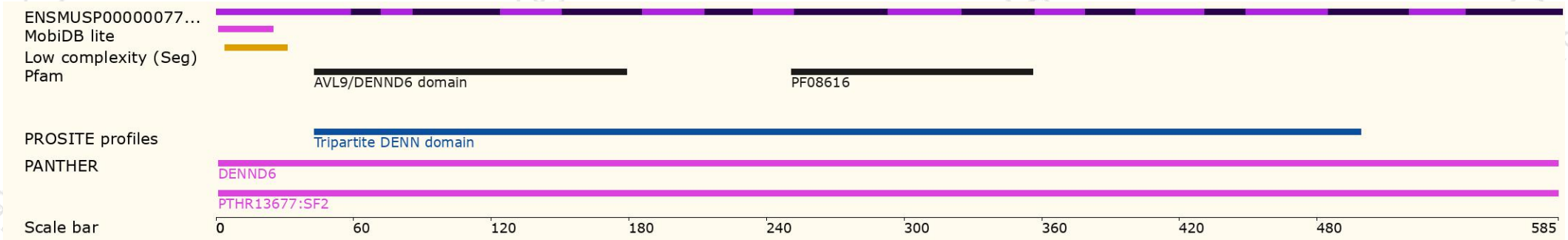
The strategy is based on the design of *Dennd6b-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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