

***Dennd2a* Cas9-CKO Strategy**

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Design Date: 2020-10-13

Project Overview

Project Name

Dennd2a

Project type

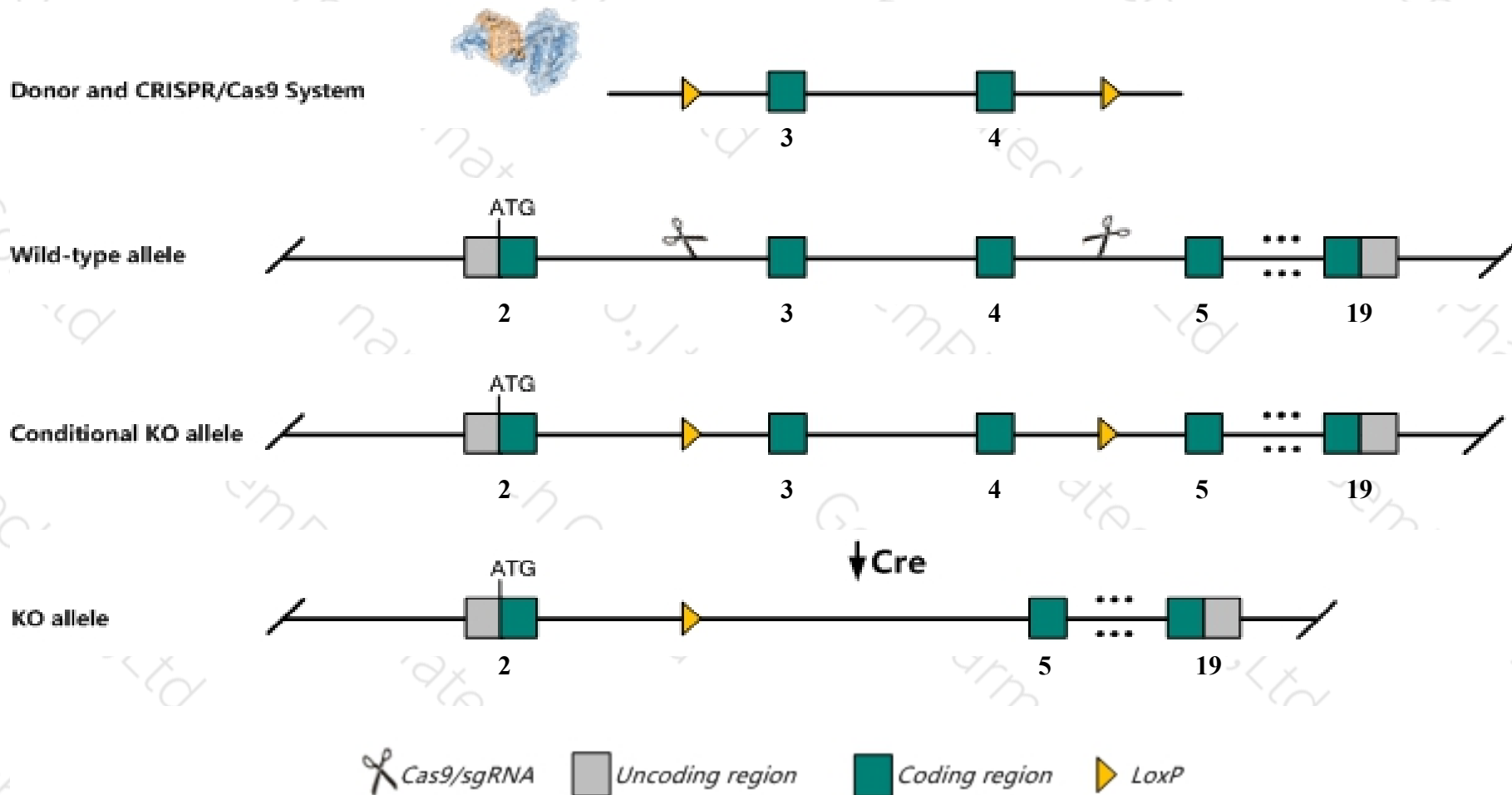
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dennd2a* gene. The schematic diagram is as follows:



- The *Dennd2a* gene has 5 transcripts. According to the structure of *Dennd2a* gene, exon3-exon4 of *Dennd2a-201*(ENSMUST00000036877.9) transcript is recommended as the knockout region. The region contains 250bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dennd2a* 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.

- The *Dennd2a* gene is located on the Chr6. 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)

Dennd2a DENN/MADD domain containing 2A [Mus musculus (house mouse)]

Gene ID: 209773, updated on 13-Mar-2020

Summary



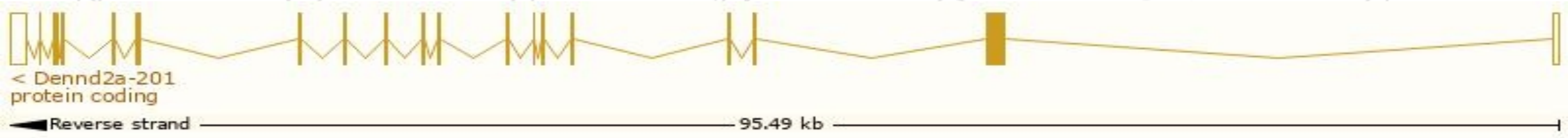
Official Symbol	Dennd2a provided by MGI
Official Full Name	DENN/MADD domain containing 2A provided by MGI
Primary source	MGI:MGI:2444961
See related	Ensembl:ENSMUSG00000038456
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	B930096L08Rik, C130031G22, mKIAA1277
Expression	Broad expression in CNS E11.5 (RPKM 15.8), limb E14.5 (RPKM 12.4) and 22 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

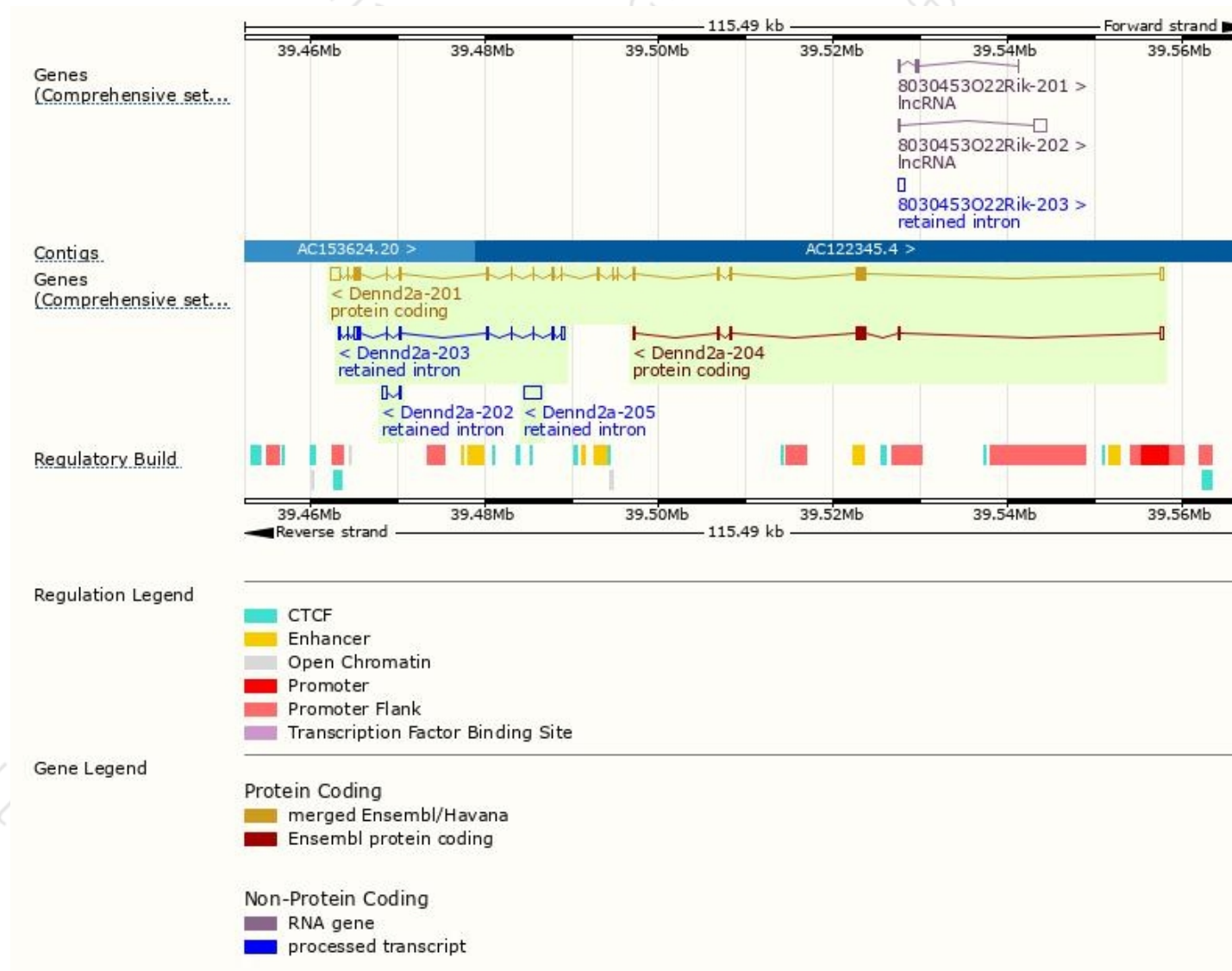
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dennd2a-201	ENSMUST00000036877.9	4468	1000aa	Protein coding	CCDS20023	Q8C4S8	TSL:1 GENCODE basic APPRIS P1
Dennd2a-204	ENSMUST00000154149.1	1787	420aa	Protein coding	-	D3Z1C6	CDS 3' incomplete TSL:1
Dennd2a-205	ENSMUST00000202707.1	1878	No protein	Retained intron	-	-	TSL:NA
Dennd2a-203	ENSMUST00000149162.1	1702	No protein	Retained intron	-	-	TSL:1
Dennd2a-202	ENSMUST00000145541.1	737	No protein	Retained intron	-	-	TSL:2

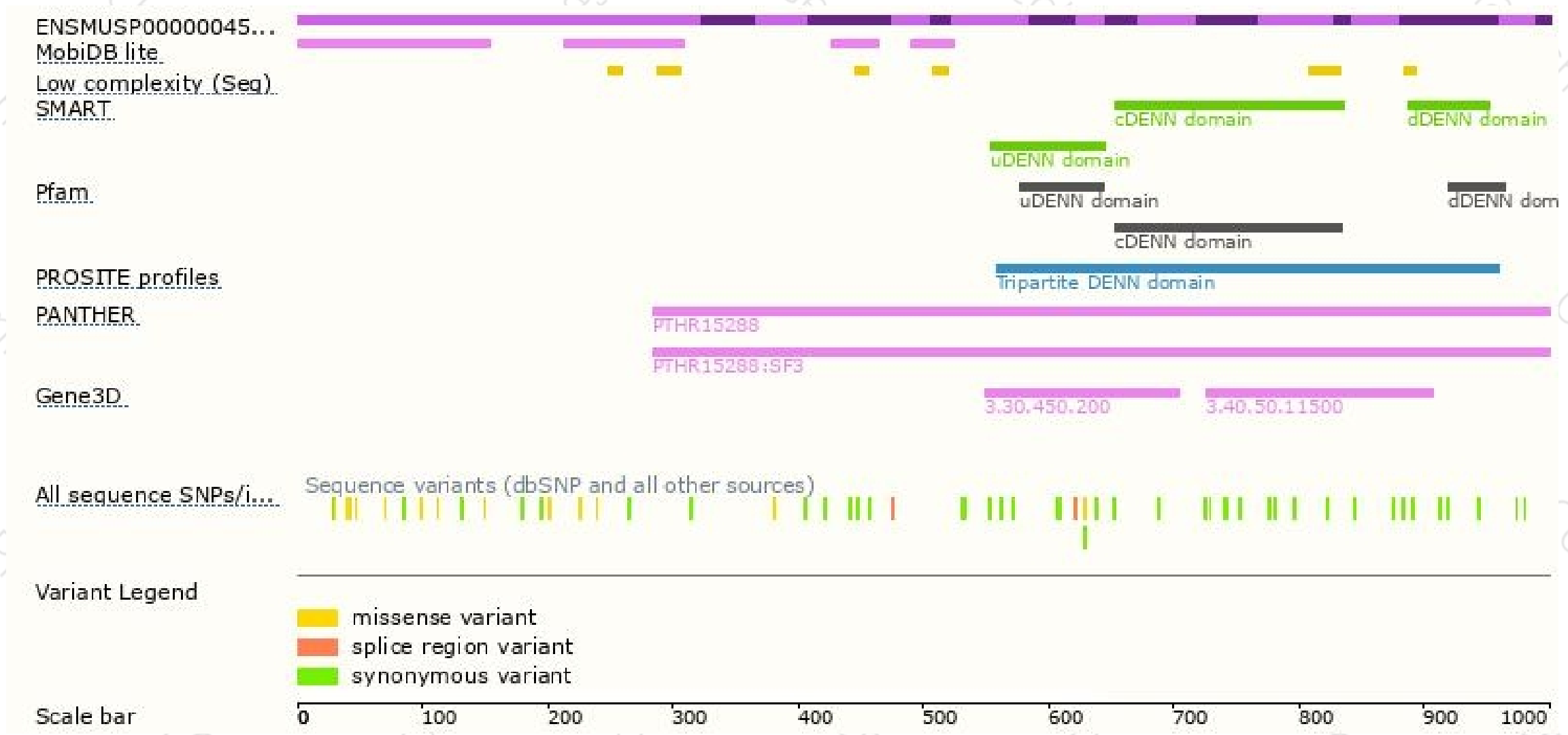
The strategy is based on the design of *Dennd2a-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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