Dennd4a Cas9-KO Strategy

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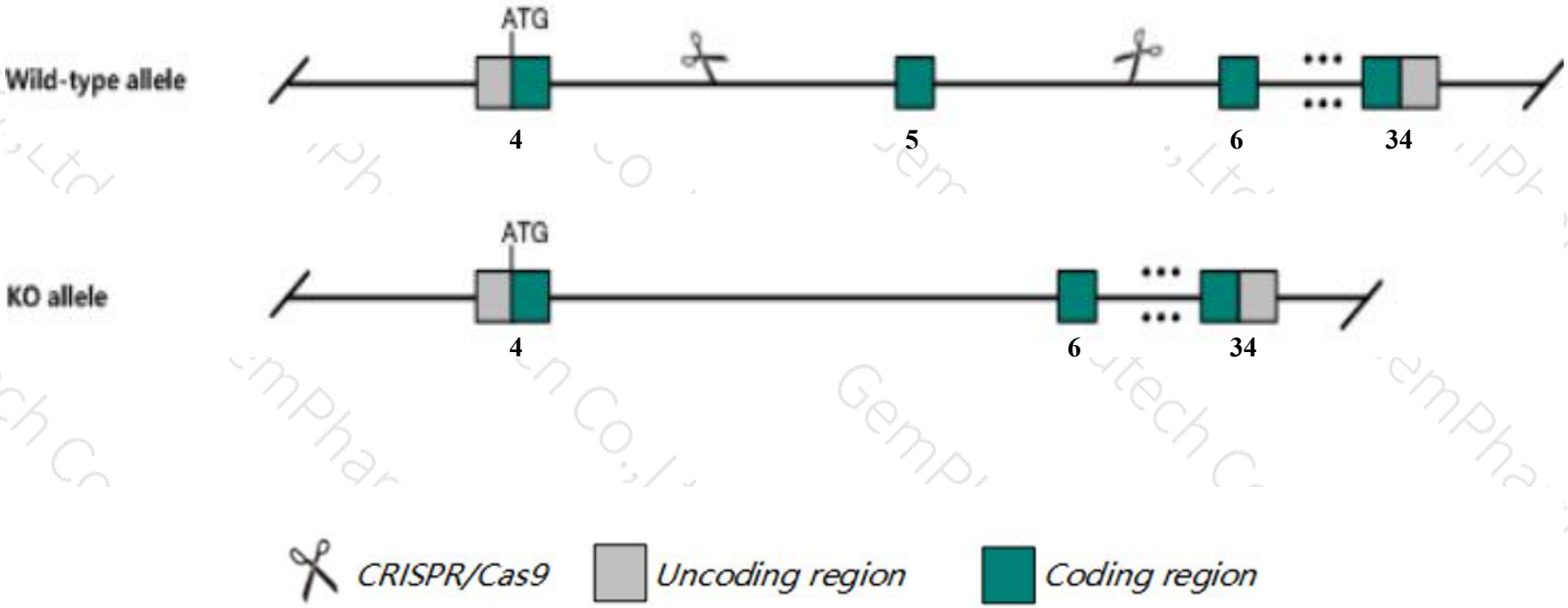
Design Date: 2020-12-16

Project Overview

Project Name	***Dennd4a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dennd4a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Dennd4a* gene has 9 transcripts. According to the structure of *Dennd4a* gene, exon5 of *Dennd4a-201*(ENSMUST00000038890.5) 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 *Dennd4a* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ The *Dennd4a* gene is located on the Chr9. 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 *Dennd4a*-203&205&206&208&209 may not be affected.

➢ The effect on transcript *Dennd4a*-204is unknown.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Dennd4a DENN/MADD domain containing 4A [Mus musculus (house mouse)]

Gene ID: 102442, updated on 13-Mar-2020

Summary

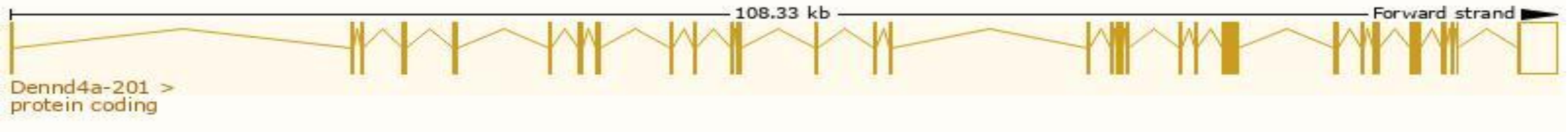
Official Symbol	Dennd4a provided by MGI
Official Full Name	DENN/MADD domain containing 4A provided byMGI
Primary source	MGI:MGI:2142979
See related	Ensembl:ENSMUSG00000053641
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI115600, AI851943, F730015K02Rik
Expression	Broad expression in liver E14 (RPKM 9.5), liver E14.5 (RPKM 9.1) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

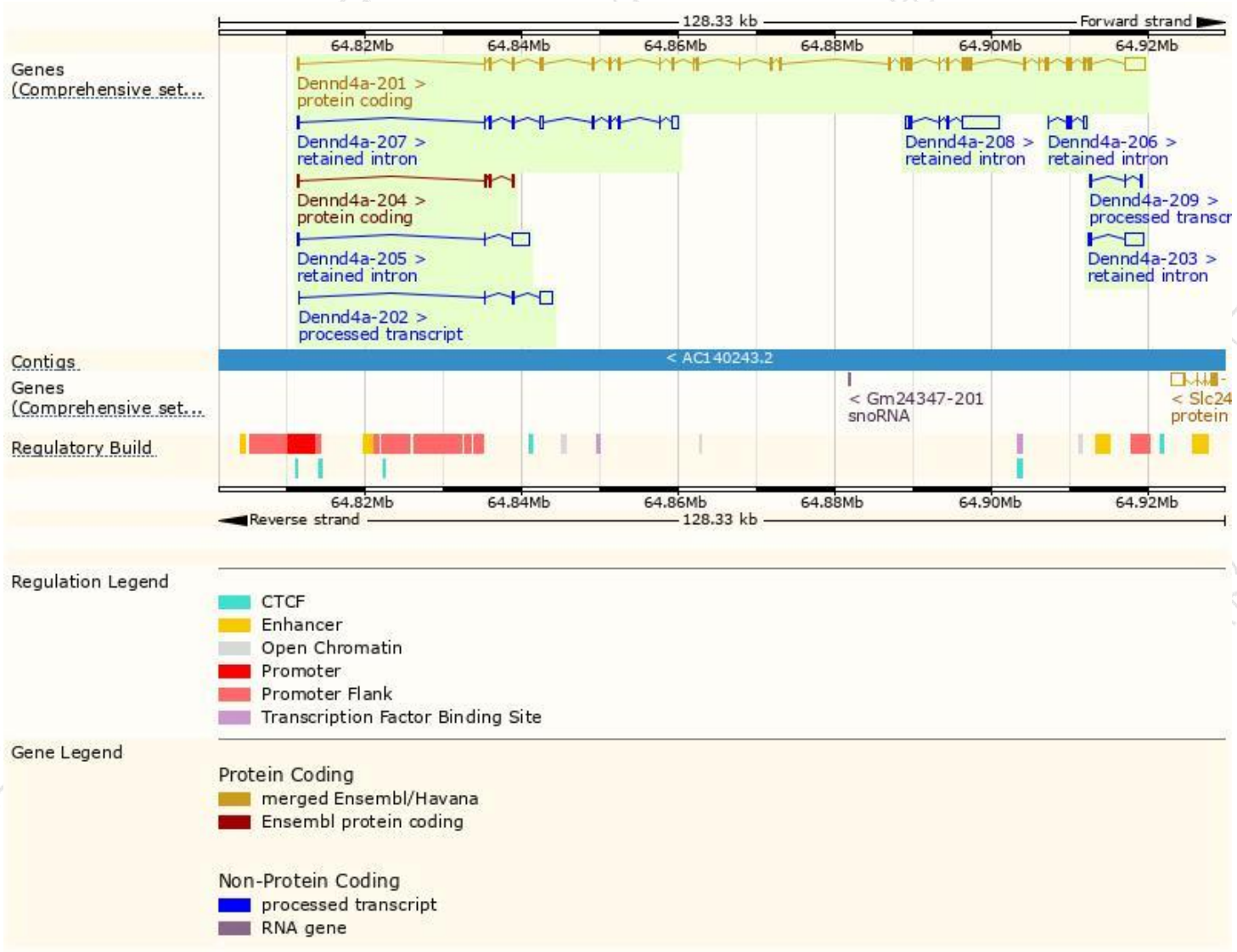
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dennd4a-201	ENSMUST00000038890.5	8645	1869aa	Protein coding	CCDS52834	E9Q8V6	TSL:5 GENCODE basic APPRIS P1
Dennd4a-204	ENSMUST00000213926.1	643	64aa	Protein coding	-	A0A1L1SUW5	CDS 3' incomplete TSL:3
Dennd4a-202	ENSMUST00000213437.1	1928	No protein	Processed transcript	-	-	TSL:1
Dennd4a-209	ENSMUST00000217307.1	426	No protein	Processed transcript	-	-	TSL:2
Dennd4a-208	ENSMUST00000216098.1	5418	No protein	Retained intron	-	-	TSL:1
Dennd4a-203	ENSMUST00000213744.1	2792	No protein	Retained intron	-	-	TSL:1
Dennd4a-205	ENSMUST00000214001.1	2491	No protein	Retained intron	-	-	TSL:1
Dennd4a-207	ENSMUST00000215025.1	2204	No protein	Retained intron	-	-	TSL:1
Dennd4a-206	ENSMUST00000214029.1	942	No protein	Retained intron	-	-	TSL:5

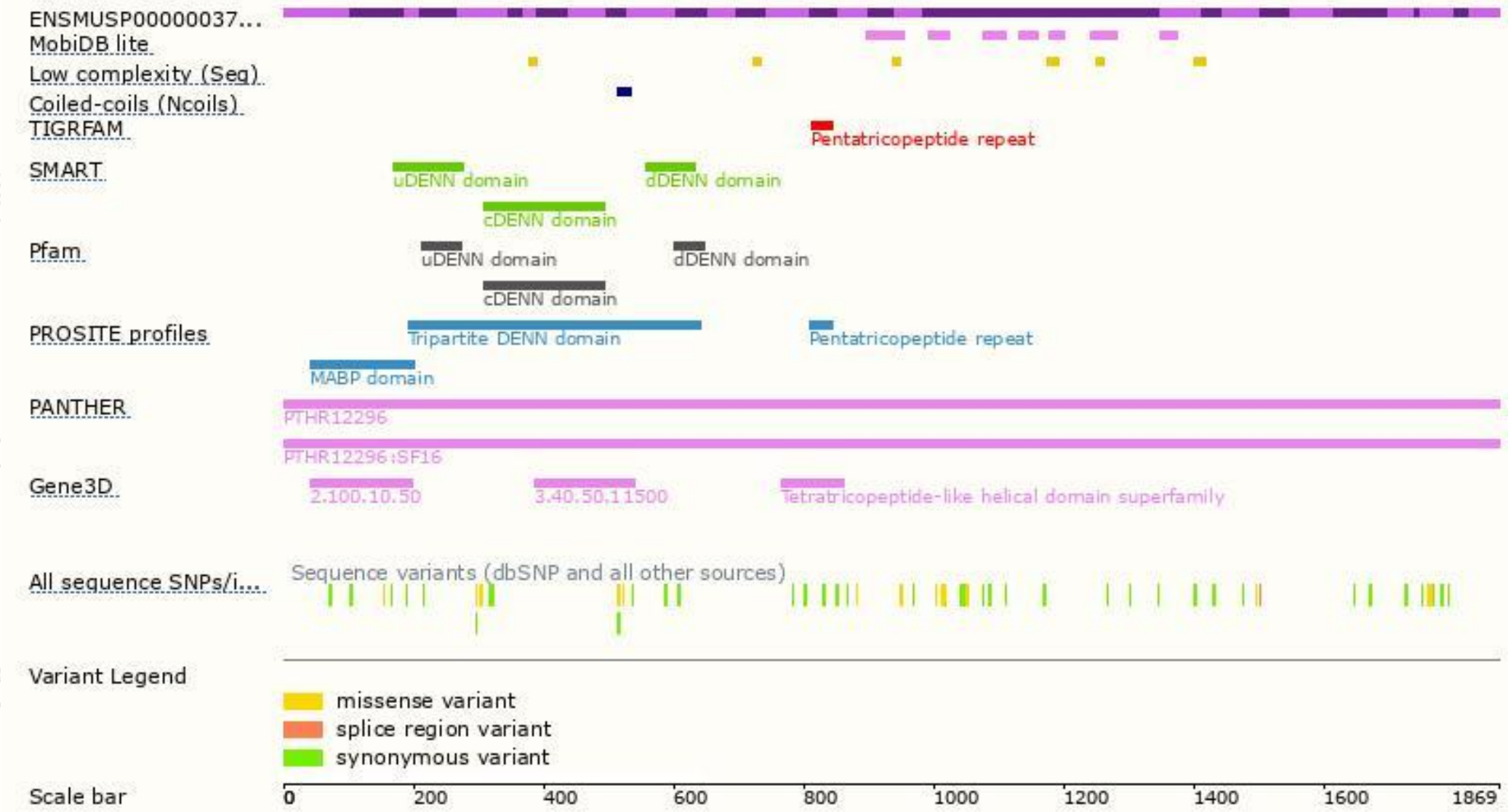
The strategy is based on the design of *Dennd4a-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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