

***Calb2* Cas9-CKO Strategy**

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Project Overview

Project Name

Calb2

Project type

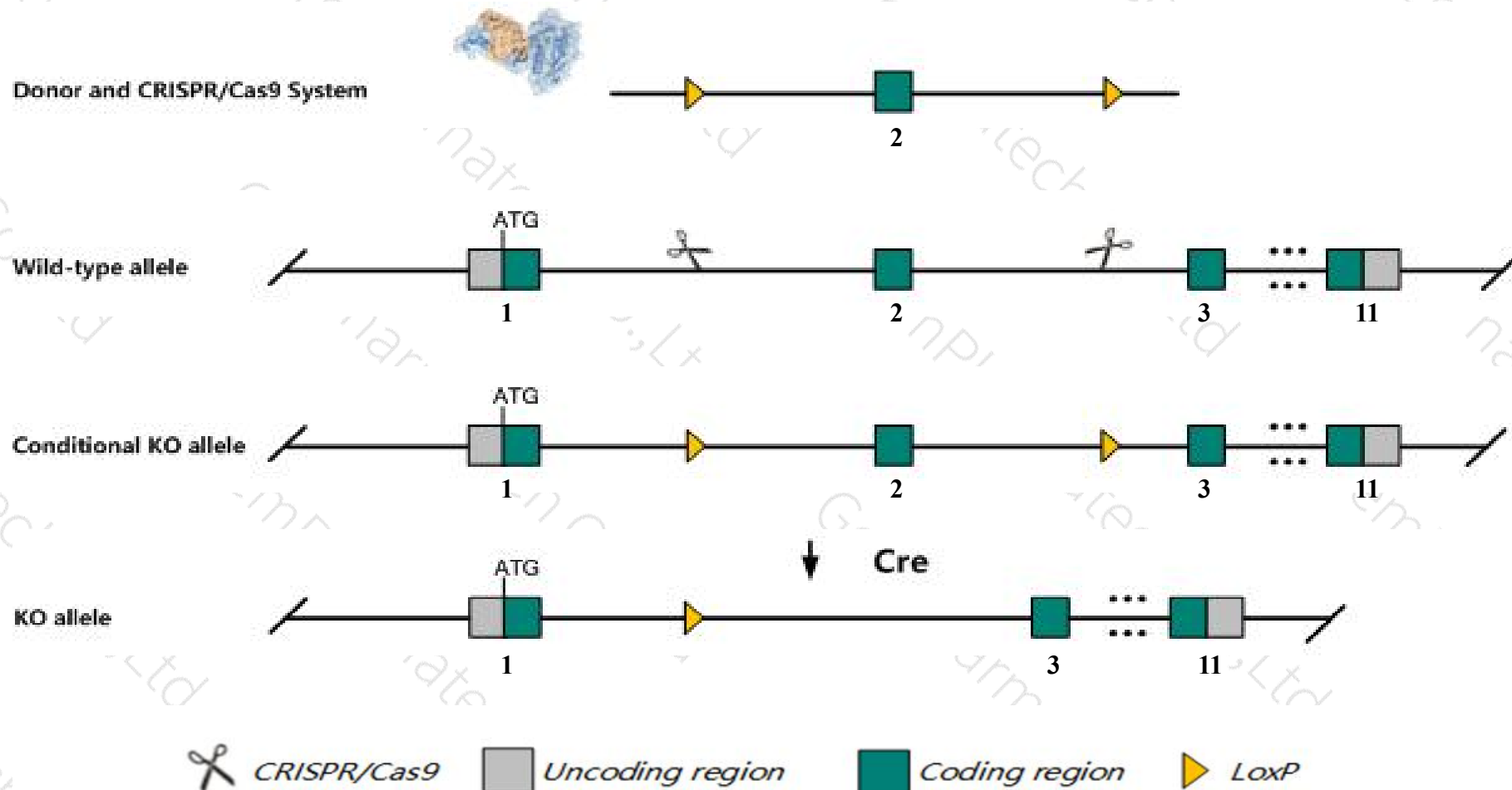
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Calb2* gene. The schematic diagram is as follows:



Technical routes

- The *Calb2* gene has 2 transcripts. According to the structure of *Calb2* gene, exon2 of *Calb2-201* (ENSMUST00000003754.7) transcript is recommended as the knockout region. The region contains 77bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Calb2* 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 targeted mutants showed normal growth, normal brain histology, and generally normal behavior. Impaired motor coordination was observed in wheel running in young and old mutant mice, and progressive impairment was seen on the runway and horizontal stationary rod tests in older mice. Abnormalities are observed in Purkinje cell firing, altering both simple and complex spikes.
- The *Calb2* gene is located on the Chr8. 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)

Calb2 calbindin 2 [Mus musculus (house mouse)]

Gene ID: 12308, updated on 26-Mar-2019

Summary



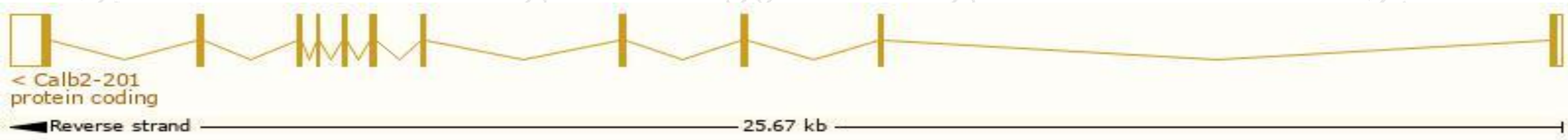
Official Symbol	Calb2 provided by MGI
Official Full Name	calbindin 2 provided by MGI
Primary source	MGI:MGI:101914
See related	Ensembl:ENSMUSG000000003657
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CR
Expression	Biased expression in cerebellum adult (RPKM 131.6), frontal lobe adult (RPKM 90.9) and 3 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

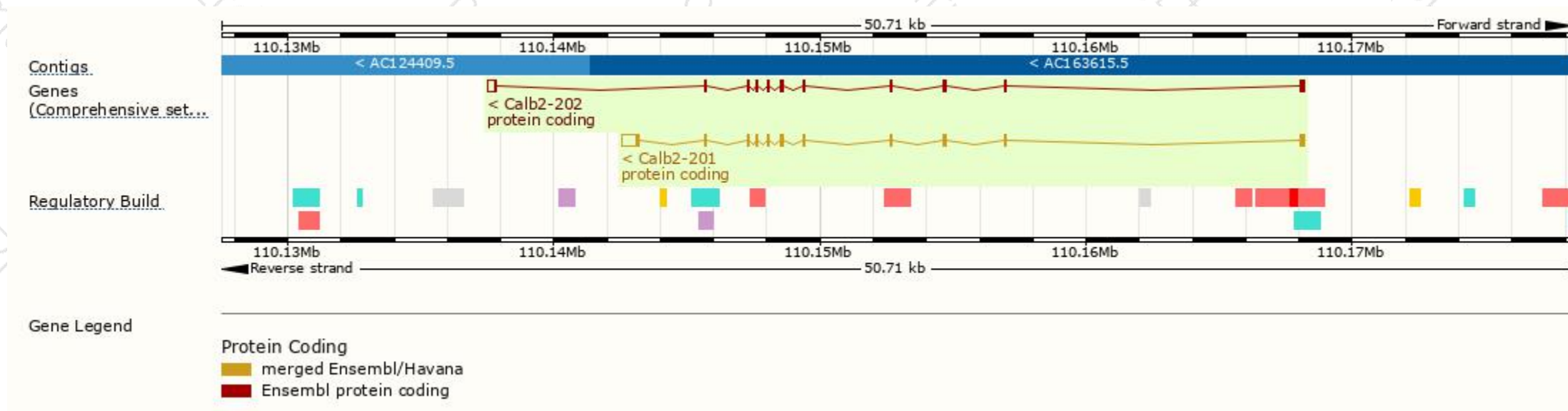
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Calb2-201	ENSMUST00000003754.7	1431	271aa	Protein coding	CCDS22661	Q08331	TSL:1 GENCODE basic APPRIS P1
Calb2-202	ENSMUST00000212297.1	1080	242aa	Protein coding	-	Q8CCS7	TSL:1 GENCODE basic

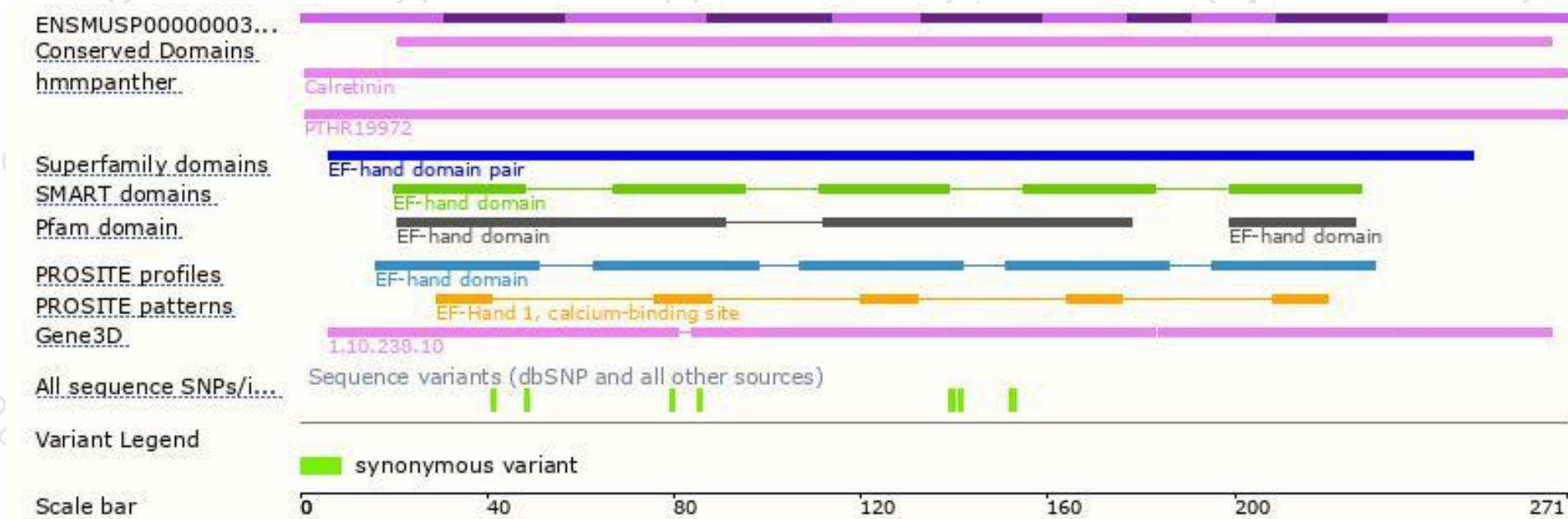
The strategy is based on the design of *Calb2-201* transcript,The transcription is shown below



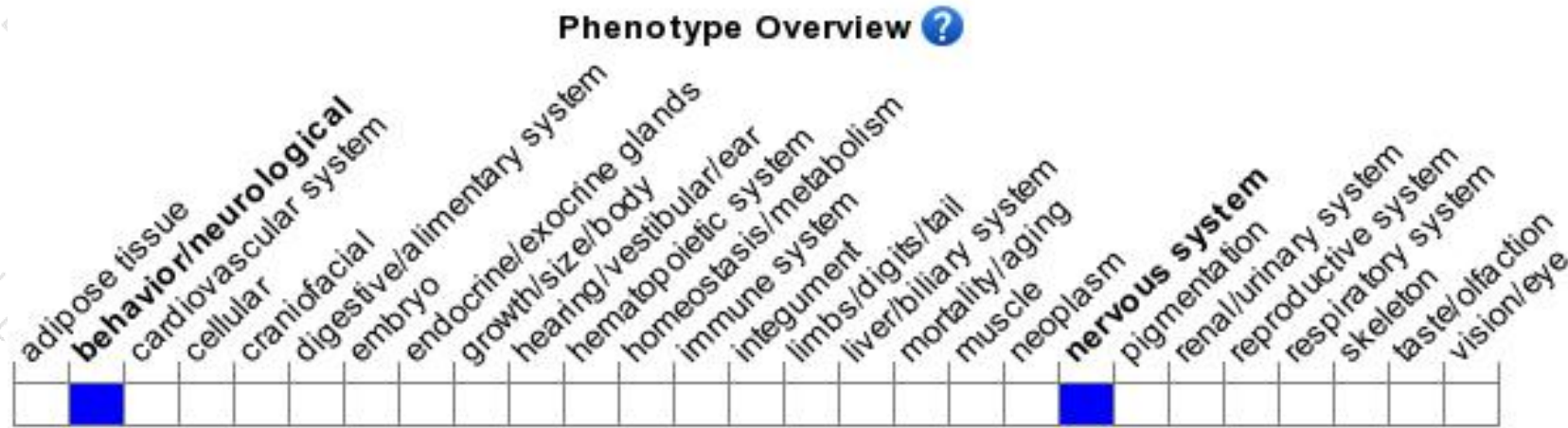
Genomic location distribution



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/>).

According to the existing MGI data, Homozygous targeted mutants showed normal growth, normal brain histology, and generally normal behavior. Impaired motor coordination was observed in wheel running in young and old mutant mice, and progressive impairment was seen on the runway and horizontal stationary rod tests in older mice. Abnormalities are observed in Purkinje cell firing, altering both simple and complex spikes.

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

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