Calb1-P2A-iCre Cas9-KI Strategy

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Design Date:	**2019-8-14**
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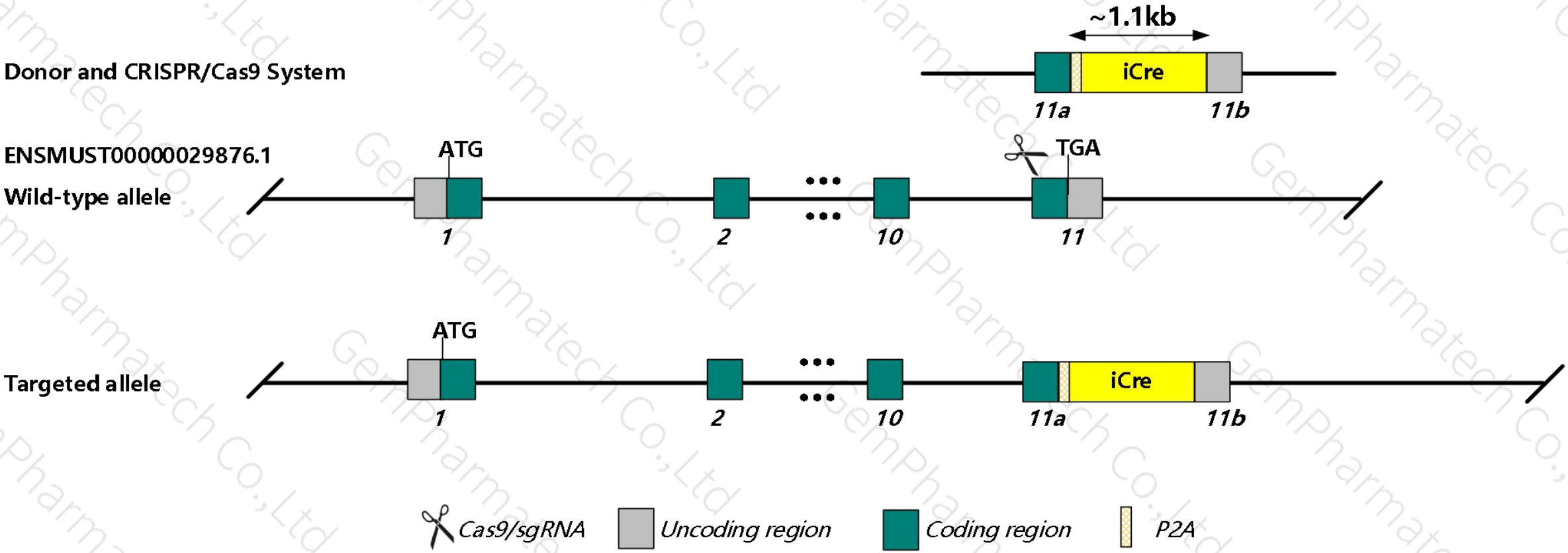
Project Overview

Project Name	***Calb1-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Calb1* gene. The schematic diagram is as follows:



Technical routes

- The *Calb1* gene has 3 transcripts.According to the structure of *Calb1* gene, *Calb1-201*(ENSMUST00000029876.1) is selected for presentation of the recommended strategy.

- *Calb1-201* gene has 11 exons, with the ATG start codon in exon1 and TGA stop codon in exon11.

- We make *Calb1-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TGA) of Calb1 gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre after stop coding(TGA) of Calb1 gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Homozygous targeted mutants show severely impairment in motor coordination and Purkinje cells in the cerebellum show changes of synaptically evoked postsynaptic calcium transients.
- According to the existing JAX data(023531： B6.Cg-Calb1tm1.1(folA/cre)Hze/J), Pattern correlates well with endogenous Calb1 expression: after trimethoprim induced Cre recombinase activity, Cre-inducible reporter allele expression is detected in scattered cells of the cortex, hippocampus, cerebellum and striatum, and restricted cell populations in thalamus and hypothalamus.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre may affect the regulation of the 3' end of the *Calb1* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon11 of *Calb1* gene in this strategy.
- The *Calb1* gene is located on the Chr4. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

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Calb1 calbindin 1 [*Mus musculus* (house mouse)]

Gene ID: 12307, updated on 13-Aug-2019

Summary

Official Symbol	Calb1 provided by MGI
Official Full Name	calbindin 1 provided by MGI
Primary source	MGI:MGI:88248
See related	Ensembl:ENSMUSG00000028222
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	CB; Calb; Calb-1; Brain-2
Expression	Biased expression in cerebellum adult (RPKM 80.7), kidney adult (RPKM 24.9) and 5 other tissues See more
Orthologs	human all

Genomic context

Location: 4 A2; 4 6.66 cM

See Calb1 in Genome Data Viewer

Exon count: 11

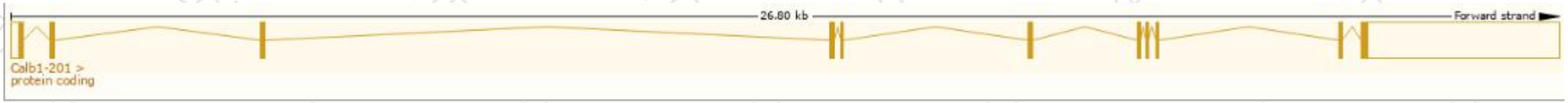
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	4	NC_000070.6 (15881264..15906709)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	4	NC_000070.5 (15808411..15833856)

Transcript information （Ensembl）

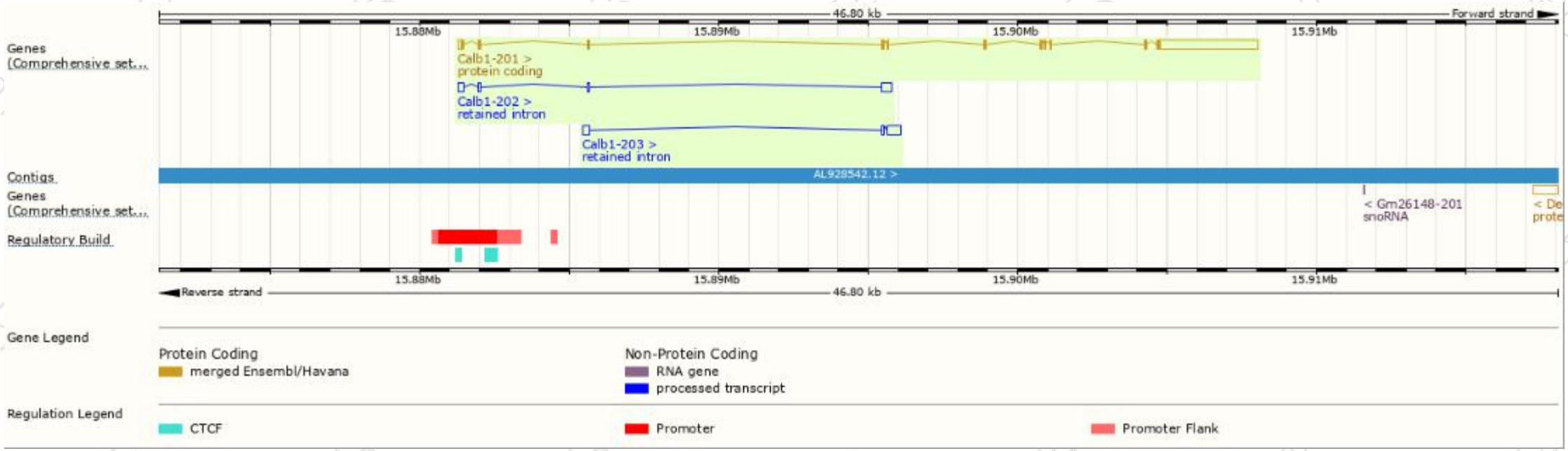
The gene has 3 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Calb1-201	ENSMUST00000029876.1	4222	261aa	Protein coding	CCDS17984	P12658	TSL:1 GENCODE basic APPRIS P1
Calb1-203	ENSMUST00000141336.1	736	No protein	Retained intron	-	-	TSL:3
Calb1-202	ENSMUST00000136266.1	701	No protein	Retained intron	-	-	TSL:2

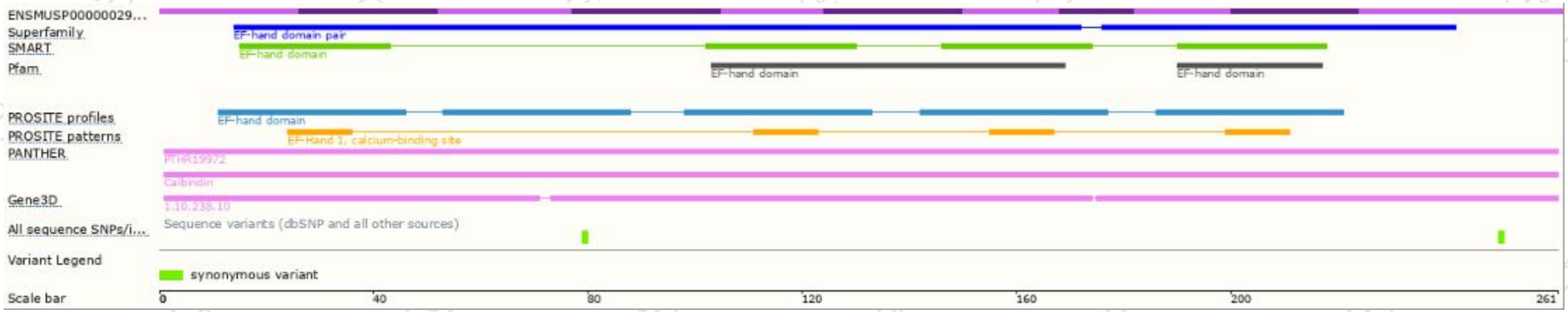
The strategy is based on the design of *Calb1-201* transcript,The transcription is shown below



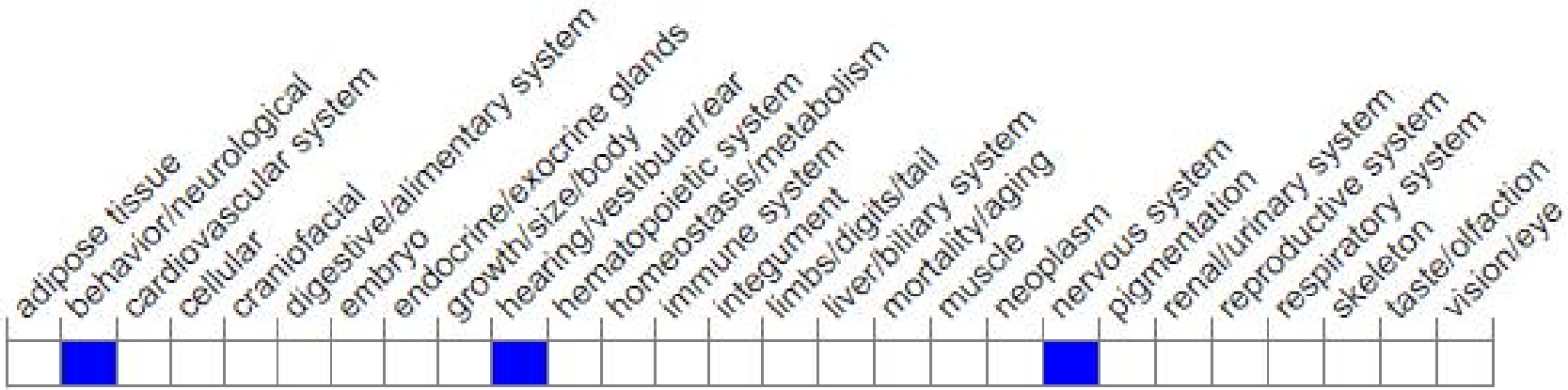
Genomic location distribution



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/marker/MGI:88248) .

Homozygous targeted mutants show severely impairment in motor coordination and Purkinje cells in the cerebellum show changes of synaptically evoked postsynaptic calcium transients

Targeted Progress (from Jax)

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The Jackson Laboratory

023531 - B6.Cg-Calb1$^{tm1.1(folA/cre)Hze}$/J

Mouse Datasheet

Calb1-2A-dgCre-D (or Calb1-T2A-dgCre-D) mice express a trimethoprim-inducible EGFP/Cre fusion gene directed by endogenous calbindin 1 promoter/enhancer elements. When induced, small-to-moderately increased Cre recombinase activity is directed at high levels to scattered cells of the cortex, hippocampus, cerebellum and striatum, and restricted cell populations in thalamus and hypothalamus.

Detailed Description

The Calb1-2A-dgCre-D (or Calb1-T2A-dgCre-D) targeted mutation has a viral 2A oligopeptide (T2A) that mediates ribosomal skipping and a destabilized EGFP/Cre fusion gene (dgCre) inserted downstream of the calbindin 1 translational STOP codon. This is designed to have both endogenous gene and dgCre expression directed to Calb1-expressing cells by the endogenous promoter/enhancer elements.

The ecDHFR$^{R12Y/Y100I}$ domain of dgCre leads to proteosomal degradation of the entire EGFP/Cre fusion protein, resulting in reduced overall Cre recombinase activity. Administration of the DHFR inhibitor, trimethoprim (TMP), prevents degradation of the dgCre fusion gene and results in increased Cre recombinase activity. The EGFP sequences included in the DHFR-Cre cassette contribute to the destabilization of the entire dgCre fusion protein in the absence of TMP.

https://www.jax.org/strain/023531

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

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