Pvalb-IRES-iCre Cas9-KI Strategy

Designer: Design Date:

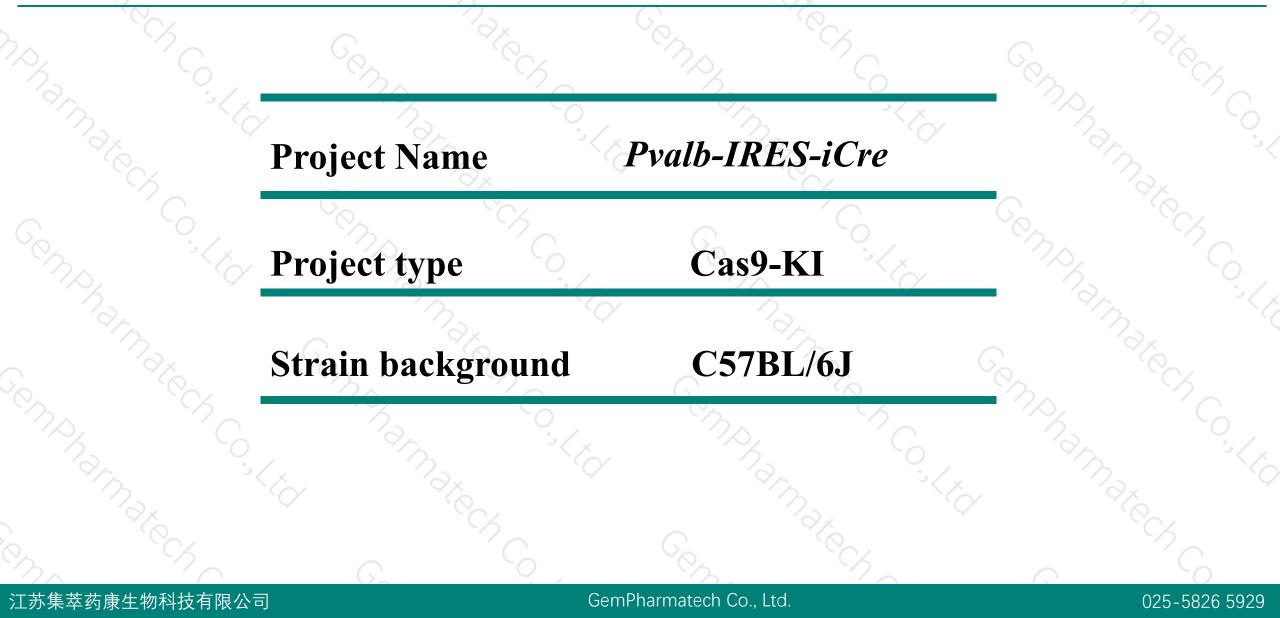
Reviewer

Xiaojing Li 2019-8-16

JiaYu

Project Overview

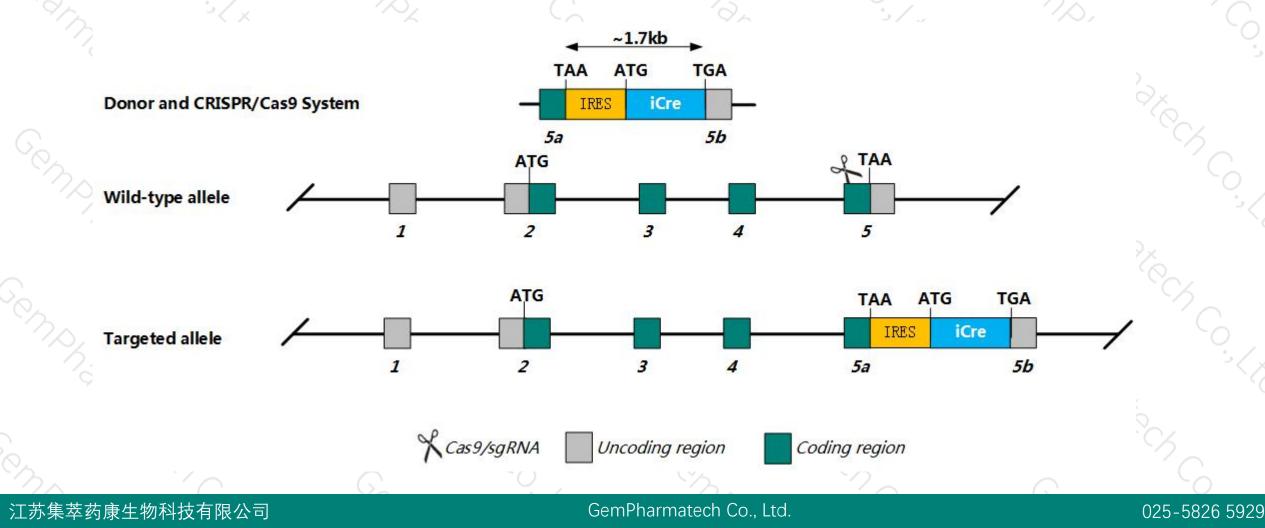




Knockin strategy



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



Technical routes



- The *Pvalb* gene has 2 transcripts. According to the structure of *Pvalb* gene, *Pvalb-202*(ENSMUST00000120592.1) is selected for presentation of the recommended strategy.
- > *Pvalb-202* gene has 5 exons, with the ATG start codon in exon2 and TAA stop codon in exon5.
- We make *Pvalb-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage at exon 5 near the stop codon TAA, and create a DSB(double-strand break). Such breaks will be repaired, and result in *IRES-iCre* inserted after the stop codon by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

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- According to the existing MGI data, Mice homozygous for deletion of this marker show slower cotraction-relaxation of fast twitch muscle and and increased force generation. Abnormalities are also reported in Purkinje cell morphology.
- Expression of Pvalb-Cre may be mainly expressed in parvalbumin-expressing neurons (such as interneurons in the brain and proprioceptive afferent sensory neurons in the dorsal root ganglia).
- The Pvalb gene and iCre gene linked by IRES are expressed by the same promoter. The transcription level is consistent but translated independently. Usually the translation level of the latter gene is lower than that of the former gene.
- ➤ Insertion of iCre may affect the regulation of the 3' end of the *Pvalb* gene.
- > There will be 1 to 2 amino acid synonymous mutation in exon5 of *Pvalb* gene in this strategy.
- The *Pvalb* gene is located on the Chr15. 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.

Notice

Gene information



Pvalb parvalbumin [Mus musculus (house mouse)]

Gene ID: 19293, updated on 13-Aug-2019

Summary

Official Symbol Pvalb provided by MGI Official Full Name parvalbumin provided by MGI Primary source MGI:MGI:97821 See related Ensembl:ENSMUSG0000005716 Gene type protein coding RefSeg status VALIDATED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Lineage Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as PV; Pva; Parv Biased expression in cerebellum adult (RPKM 192.6), mammary gland adult (RPKM 147.4) and 2 other tissues See more Expression Orthologs human all

(NCBI)

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Transcript information (Ensembl)

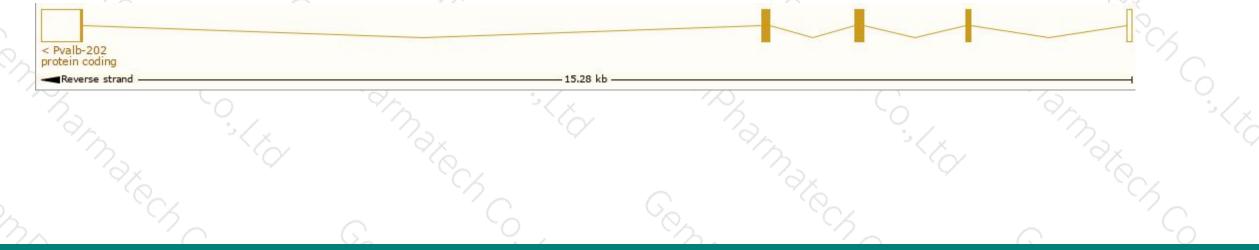


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The gene has 2 transcripts, and all transcripts are shown below :

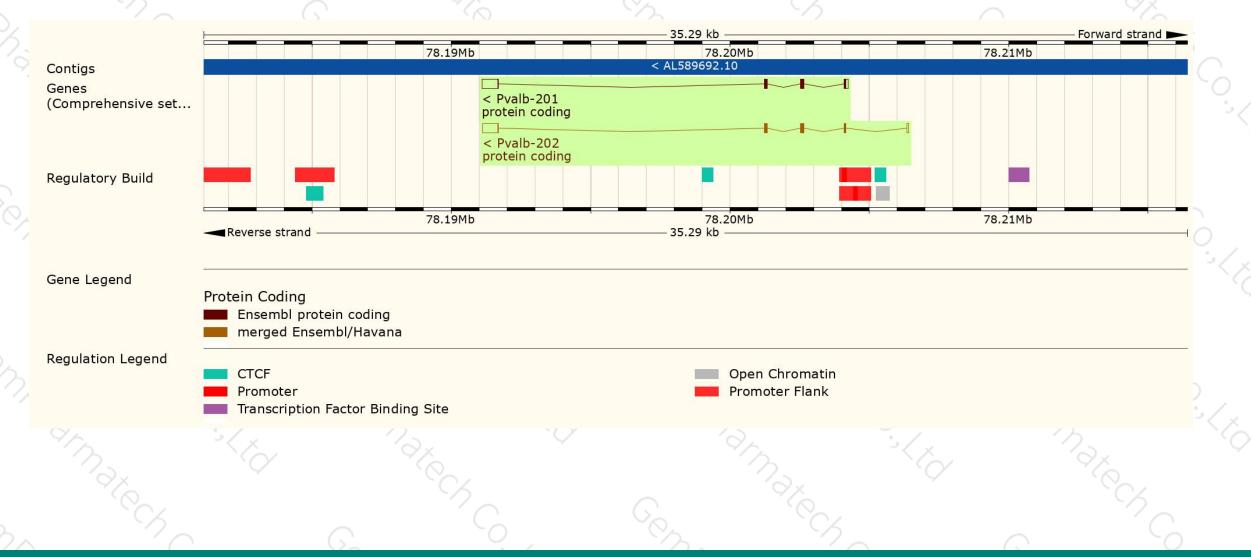
Name 🖕	Transcript ID	bp 🍦	Protein 🝦	Biotype 🖕	CCDS	UniProt 🖕	Flags		
Pvalb-201	ENSMUST0000005860.15	977	<u>110aa</u>	Protein coding	<u>CCDS27609</u> &	P32848& Q545M7&	TSL:1	GENCODE basic	APPRIS P1
Pvalb-202	ENSMUST00000120592.1	953	<u>110aa</u>	Protein coding	<u>CCDS27609</u> &	<u>P32848</u> & <u>Q545M7</u> &	TSL:1	GENCODE basic	APPRIS P1

The strategy is based on the design of Pvalb-202 transcript, The transcription is shown below



Genomic location distribution





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Protein domain

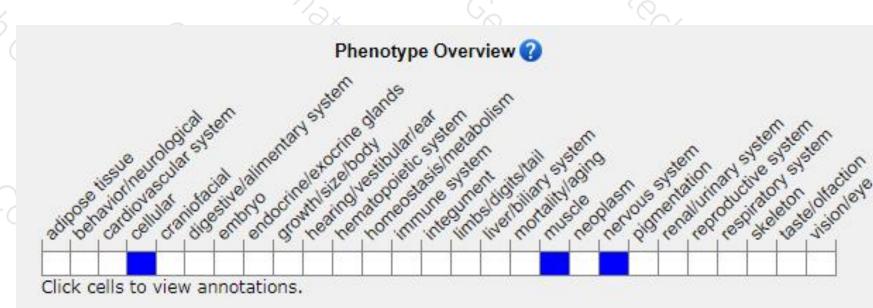


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	SMART Prints Pfam			PR01697	and domain							
	PROSITE patterns PANTHER	EF-hand domain EF-Hand 1, calcium-binding site										
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Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:97821).

Mice homozygous for deletion of this marker show slower cotraction-relaxation of fast twitch muscle and and increased force generation. Abnormalities are also reported in Purkinje cell morphology. If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



