

***Phldb2* Cas9-CKO Strategy**

Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2020-9-7

Project Overview

Project Name

Phldb2

Project type

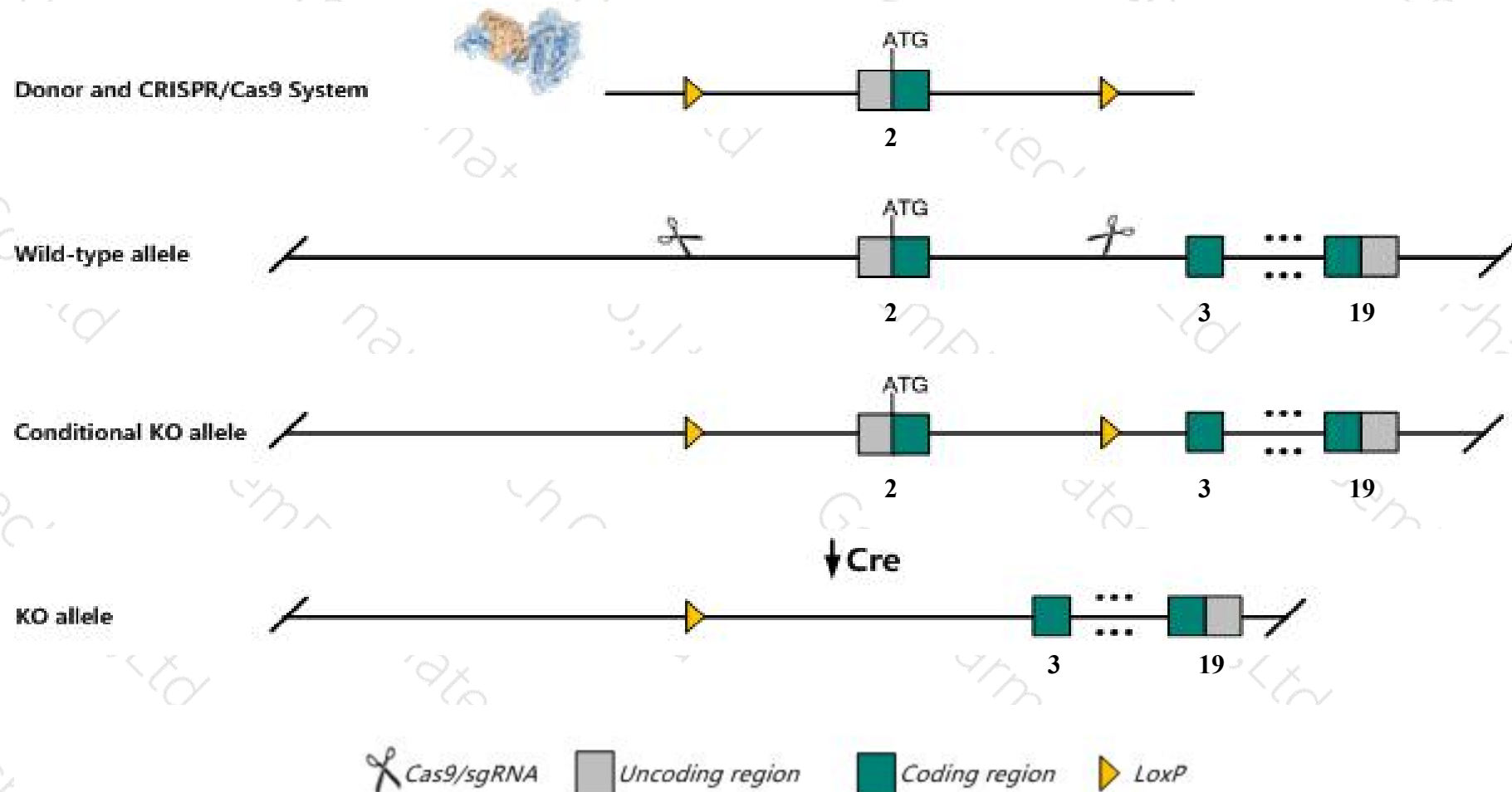
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Phldb2* gene. The schematic diagram is as follows:



- The *Phldb2* gene has 11 transcripts. According to the structure of *Phldb2* gene, exon2 of *Phldb2*-202(ENSMUST00000076333.11) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Phldb2* 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.

- According to the existing MGI data, mice homozygous for a conditional allele activated in neurons exhibit impaired LTP.
- Transcript *Phldb2*-204&206&208&210&211 may not be affected.
- The effect on transcript *Phldb2*-205&209 is unknown.
- The *Phldb2* gene is located on the Chr16. 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)

Phldb2 pleckstrin homology like domain, family B, member 2 [Mus musculus (house mouse)]

Gene ID: 208177, updated on 13-Mar-2020

Summary



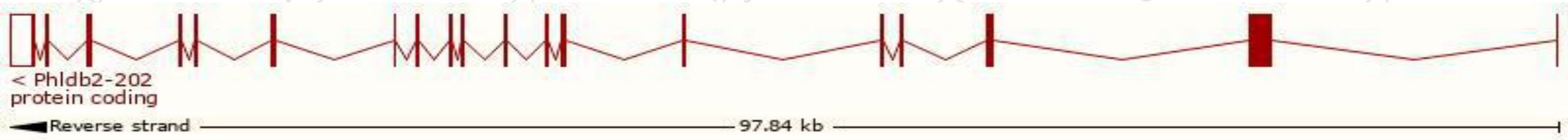
Official Symbol	Phldb2 provided by MGI
Official Full Name	pleckstrin homology like domain, family B, member 2 provided by MGI
Primary source	MGI:MGI:2444981
See related	Ensembl:ENSMUSG00000033149
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AV253284, C820004H04Rik, LL5b, LL5beta
Expression	Broad expression in bladder adult (RPKM 19.0), limb E14.5 (RPKM 13.1) and 20 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

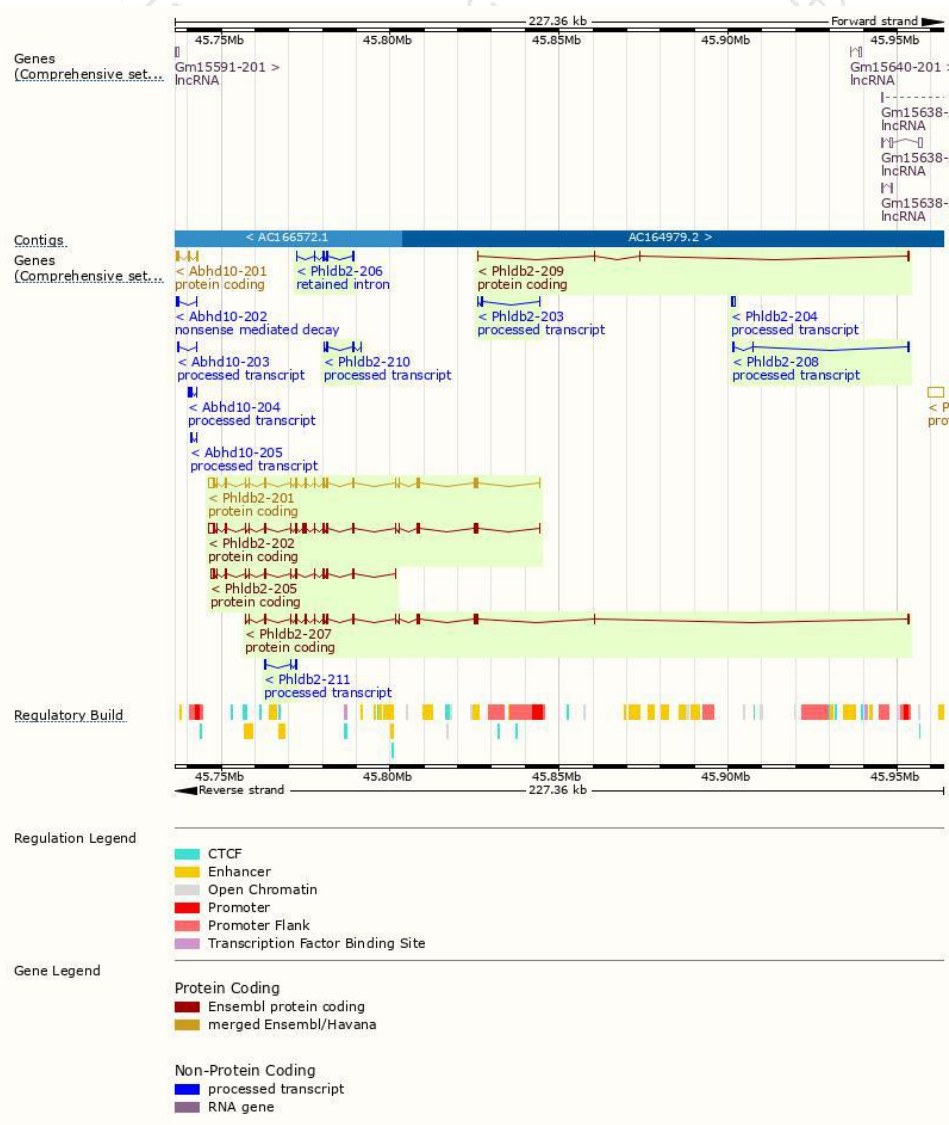
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Phldb2-201	ENSMUST00000036355.12	5483	1249aa	Protein coding	CCDS28202	Q8K1N2	TSL:1 GENCODE basic APPRIS P3
Phldb2-202	ENSMUST00000076333.11	5347	1302aa	Protein coding	CCDS57031	Q8K1N2	TSL:1 GENCODE basic APPRIS ALT2
Phldb2-207	ENSMUST00000134802.7	3612	1131aa	Protein coding	-	D3Z069	CDS 3' incomplete TSL:1
Phldb2-205	ENSMUST00000131003.7	2328	565aa	Protein coding	-	F6QU68	CDS 5' incomplete TSL:1
Phldb2-209	ENSMUST00000136405.1	595	133aa	Protein coding	-	D3Z231	CDS 3' incomplete TSL:3
Phldb2-204	ENSMUST00000128365.2	831	No protein	Processed transcript	-	-	TSL:5
Phldb2-211	ENSMUST00000151300.1	664	No protein	Processed transcript	-	-	TSL:3
Phldb2-208	ENSMUST00000136317.1	497	No protein	Processed transcript	-	-	TSL:2
Phldb2-210	ENSMUST00000142697.1	467	No protein	Processed transcript	-	-	TSL:5
Phldb2-203	ENSMUST00000125433.1	343	No protein	Processed transcript	-	-	TSL:3
Phldb2-206	ENSMUST00000133111.2	614	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Phldb2-202* transcript,the transcription is shown below:



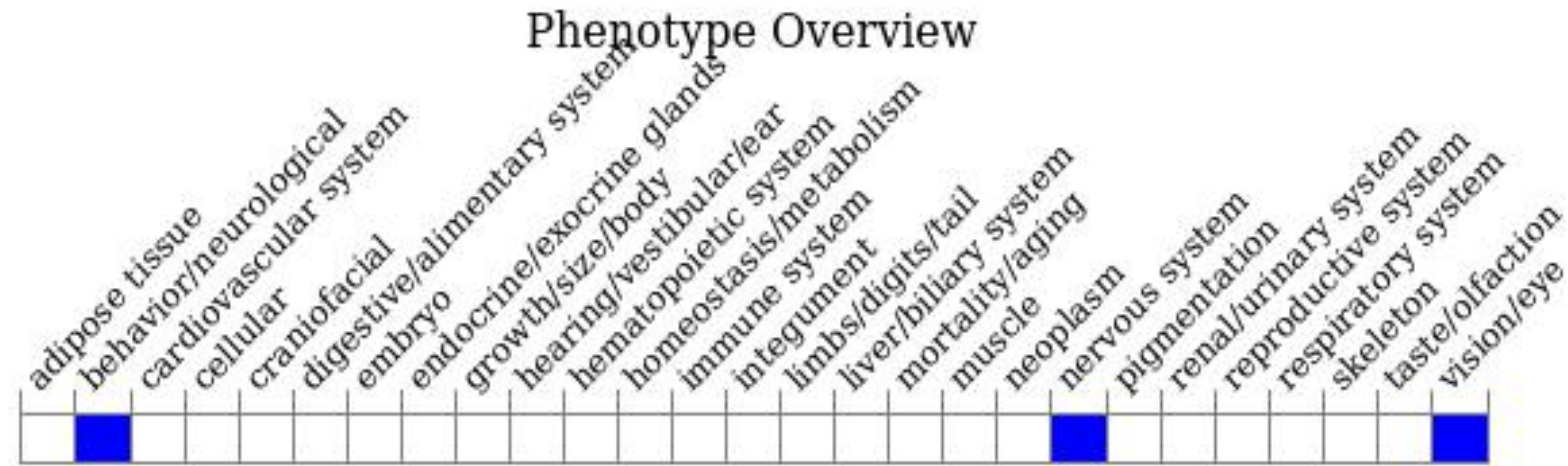
Genomic location distribution



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, mice homozygous for a conditional allele activated in neurons exhibit impaired LTP.

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

Tel: 025-5864 1534

