

Plxnd1 Cas9-CKO Strategy

Designer: Longyun Hu

Reviewer: Rui Xiong

Design Date: 2021-4-6

Project Overview

Project Name

Plxnd1

Project type

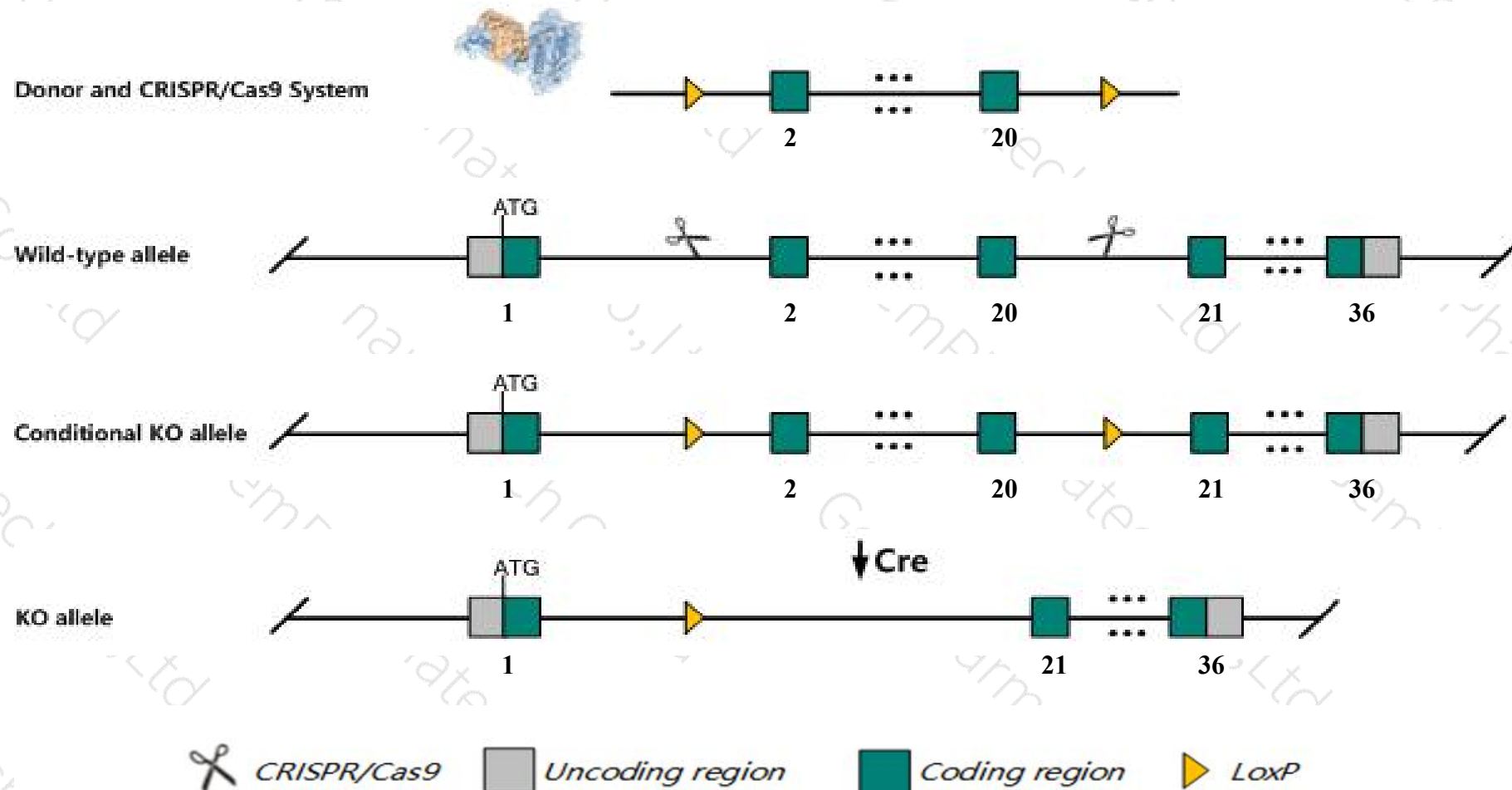
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Plxnd1* gene. The schematic diagram is as follows:



Technical routes

- The *Plxnd1* gene has 5 transcripts. According to the structure of *Plxnd1* gene, exon2-exon20 of *Plxnd1-201*(ENSMUST00000015511.14) transcript is recommended as the knockout region. The region contains 2548bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Plxnd1* 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 null mice display neonatal lethality, thin-walled atria, and vascular abnormalities including abnormal branchial arch artery development, cardiac outflow tract abnormalities, and reduced vascular smooth muscle around some vessels.
- The *Plxnd1* gene is located on the Chr6. 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)

Plxnd1 plexin D1 [Mus musculus (house mouse)]

Gene ID: 67784, updated on 13-Mar-2020

Summary



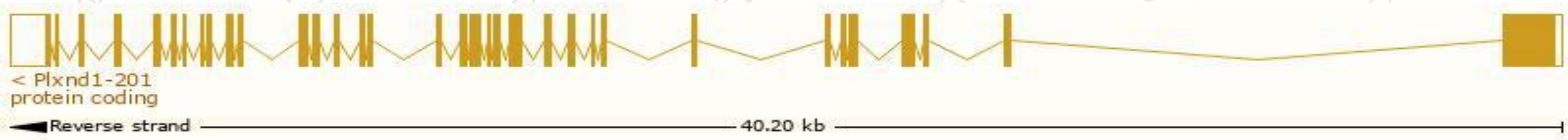
Official Symbol	Plxnd1 provided by MGI
Official Full Name	plexin D1 provided by MGI
Primary source	MGI:MGI:2154244
See related	Ensembl:ENSMUSG00000030123
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6230425C21Rik, b2b1863Clo, b2b553Clo
Expression	Broad expression in thymus adult (RPKM 164.2), adrenal adult (RPKM 135.5) and 20 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

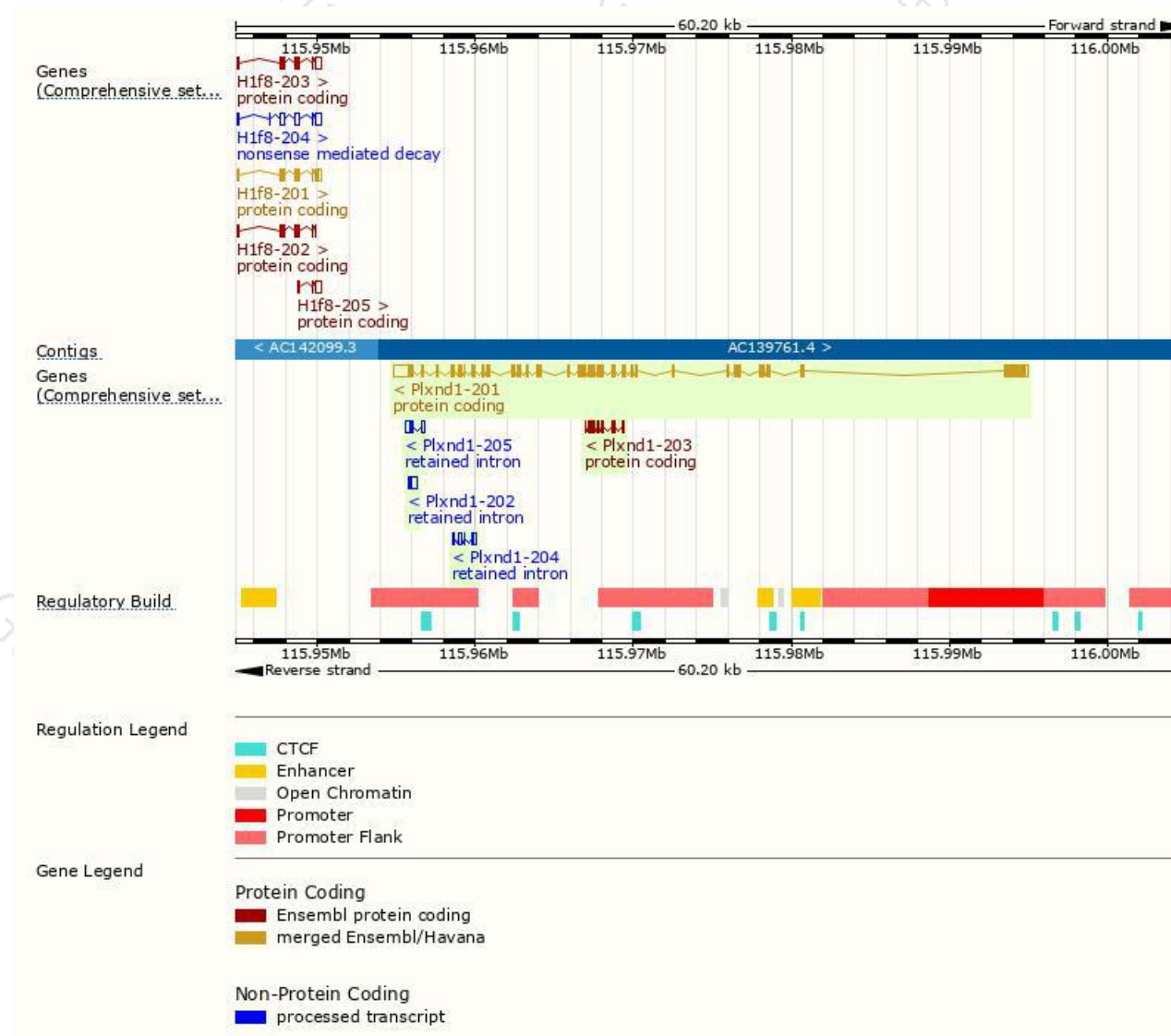
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plxnd1-201	ENSMUST00000015511.14	6907	1925aa	Protein coding	CCDS20448	Q3UH93	TSL:1 GENCODE basic APPRIS P1
Plxnd1-203	ENSMUST00000131590.1	700	233aa	Protein coding	-	F6T0L5	CDS 5' and 3' incomplete TSL:3
Plxnd1-205	ENSMUST00000205003.1	542	No protein	Retained intron	-	-	TSL:2
Plxnd1-204	ENSMUST00000203628.1	442	No protein	Retained intron	-	-	TSL:2
Plxnd1-202	ENSMUST00000123165.1	417	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Plxnd1-201* transcript,the transcription is shown below:



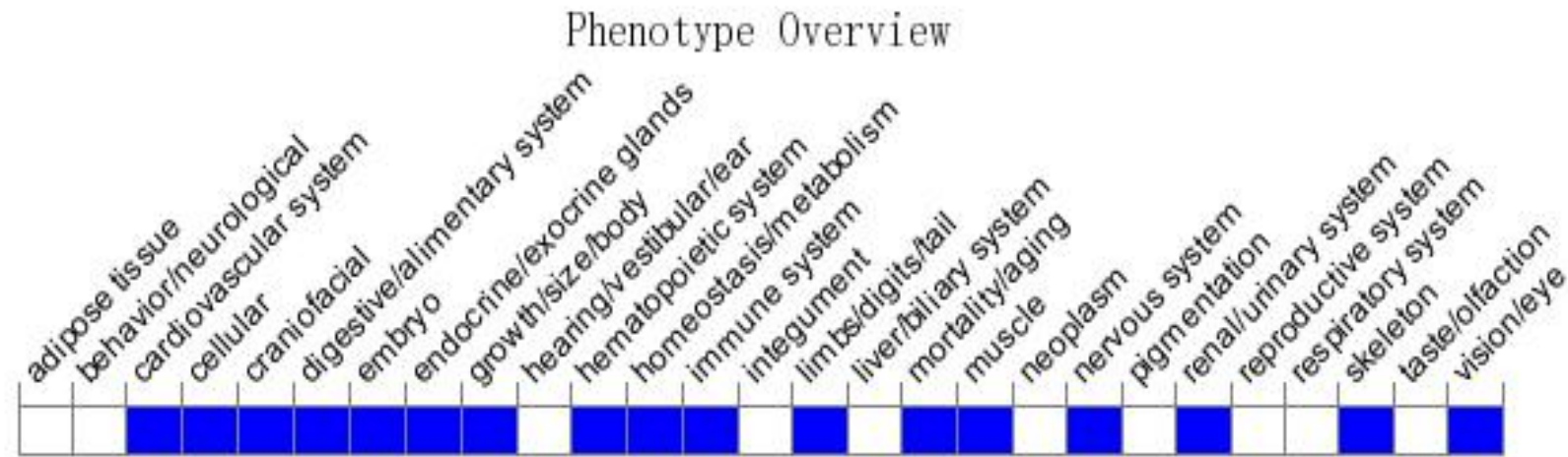
Genomic location distribution



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 null mice display neonatal lethality, thin-walled atria, and vascular abnormalities including abnormal branchial arch artery development, cardiac outflow tract abnormalities, and reduced vascular smooth muscle around some vessels.

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

Tel: 400-9660890

