Pxdn Cas9-CKO Strategy

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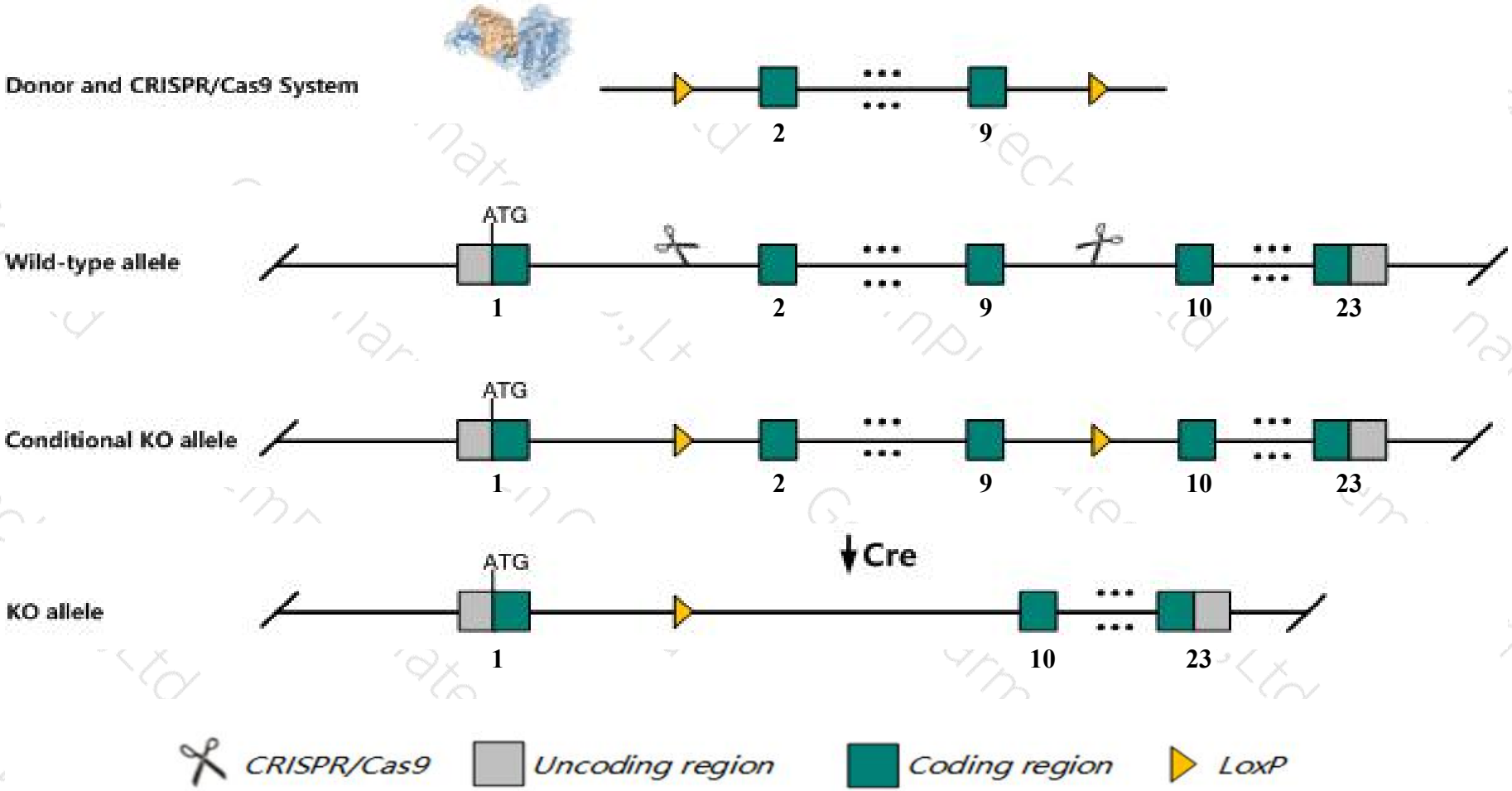
Project Overview

Project Name	***Pxdn***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Pxdn* gene has 8 transcripts. According to the structure of *Pxdn* gene, exon2-exon9 of *Pxdn-202* (ENSMUST00000122328.7) transcript is recommended as the knockout region.The region contains 818bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pxdn* 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.

Notice

➢ According to the existing MGI data,Mice homozygous for an ENU-induced allele exhibit abnormal eye development with early-onset glaucoma and progressive retinal dysgenesis.

➢ The *Pxdn* gene is located on the Chr12. 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）

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Pxdn peroxidasin [*Mus musculus* (house mouse)]

Gene ID: 69675, updated on 4-Dec-2019

Summary

Official Symbol	Pxdn provided by MGI
Official Full Name	peroxidasin provided by MGI
Primary source	MGI:MGI:1916925
See related	Ensembl:ENSMUSG00000020674
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	VPO1; C85409; mKIAA0230; E330004E07; 2310075M15Rik
Expression	Broad expression in subcutaneous fat pad adult (RPKM 54.0), limb E14.5 (RPKM 52.0) and 21 other tissues See more
Orthologs	human all

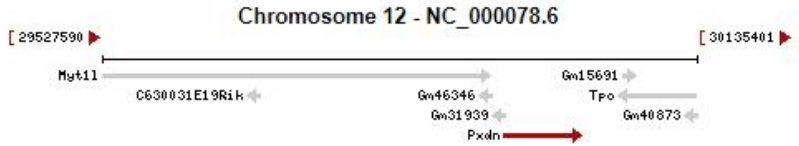
Genomic context

Location: 12; 12 A2

See Pxdn in Genome Data Viewer

Exon count: 24

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	12	NC_000078.6 (29936642..30017658)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	12	NC_000078.5 (30622901..30702523)



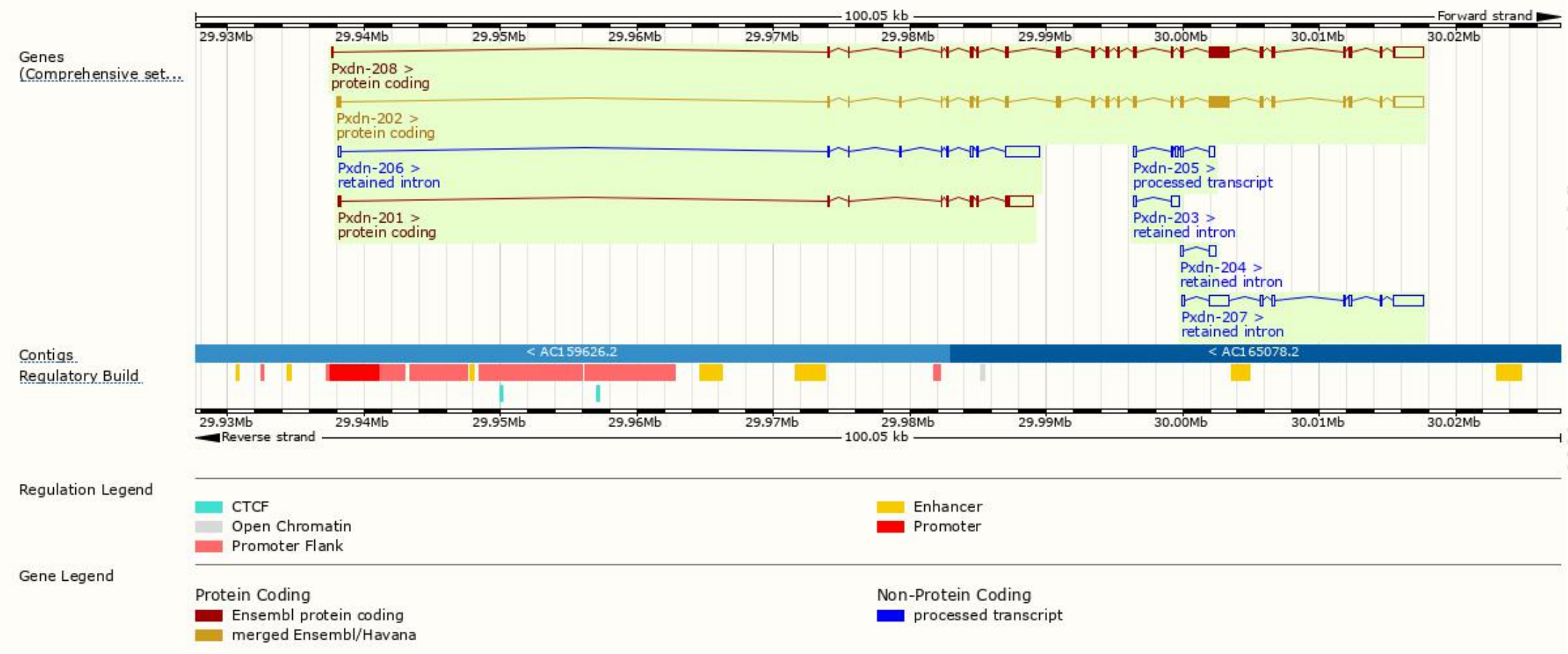
Transcript information（Ensembl）

The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pxdn-202	ENSMUST00000122328.7	6614	1475aa	Protein coding	CCDS25856	Q3UQ28	TSL:1 GENCODE basic APPRIS P1
Pxdn-208	ENSMUST00000220271.1	6377	1295aa	Protein coding	-	A0A1W2P6L9	TSL:5 GENCODE basic
Pxdn-201	ENSMUST00000118321.2	2698	307aa	Protein coding	-	D3Z5M7	TSL:1 GENCODE basic
Pxdn-205	ENSMUST00000155190.1	1024	No protein	Processed transcript	-	-	TSL:3
Pxdn-207	ENSMUST00000218620.1	4578	No protein	Retained intron	-	-	TSL:5
Pxdn-206	ENSMUST00000155318.1	3406	No protein	Retained intron	-	-	TSL:1
Pxdn-204	ENSMUST00000137316.1	787	No protein	Retained intron	-	-	TSL:2
Pxdn-203	ENSMUST00000126233.1	768	No protein	Retained intron	-	-	TSL:2

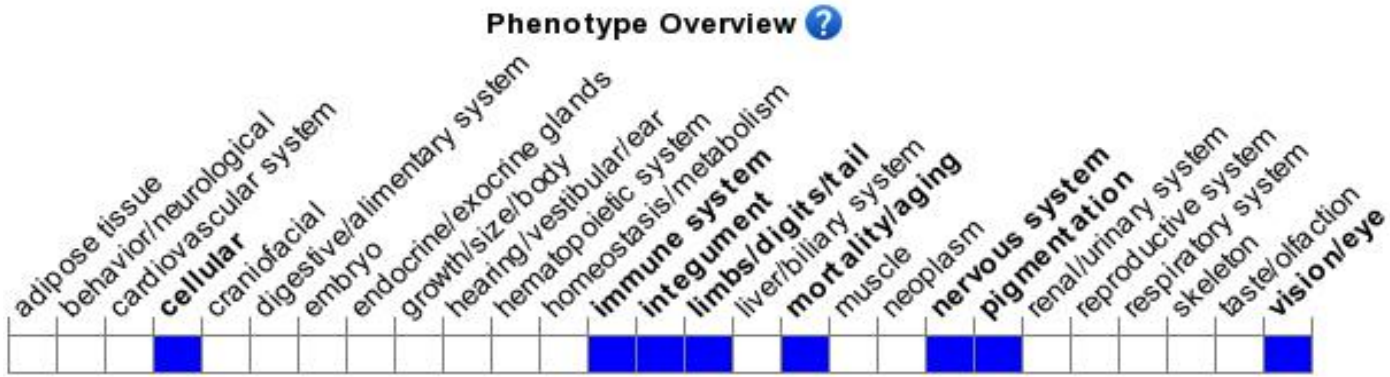
The strategy is based on the design of *Pxdn-202* transcript,The transcription is shown below

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/).

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

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