Dlg1 Cas9-CKO Strategy

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Project Overview

Project Name	***Dlg1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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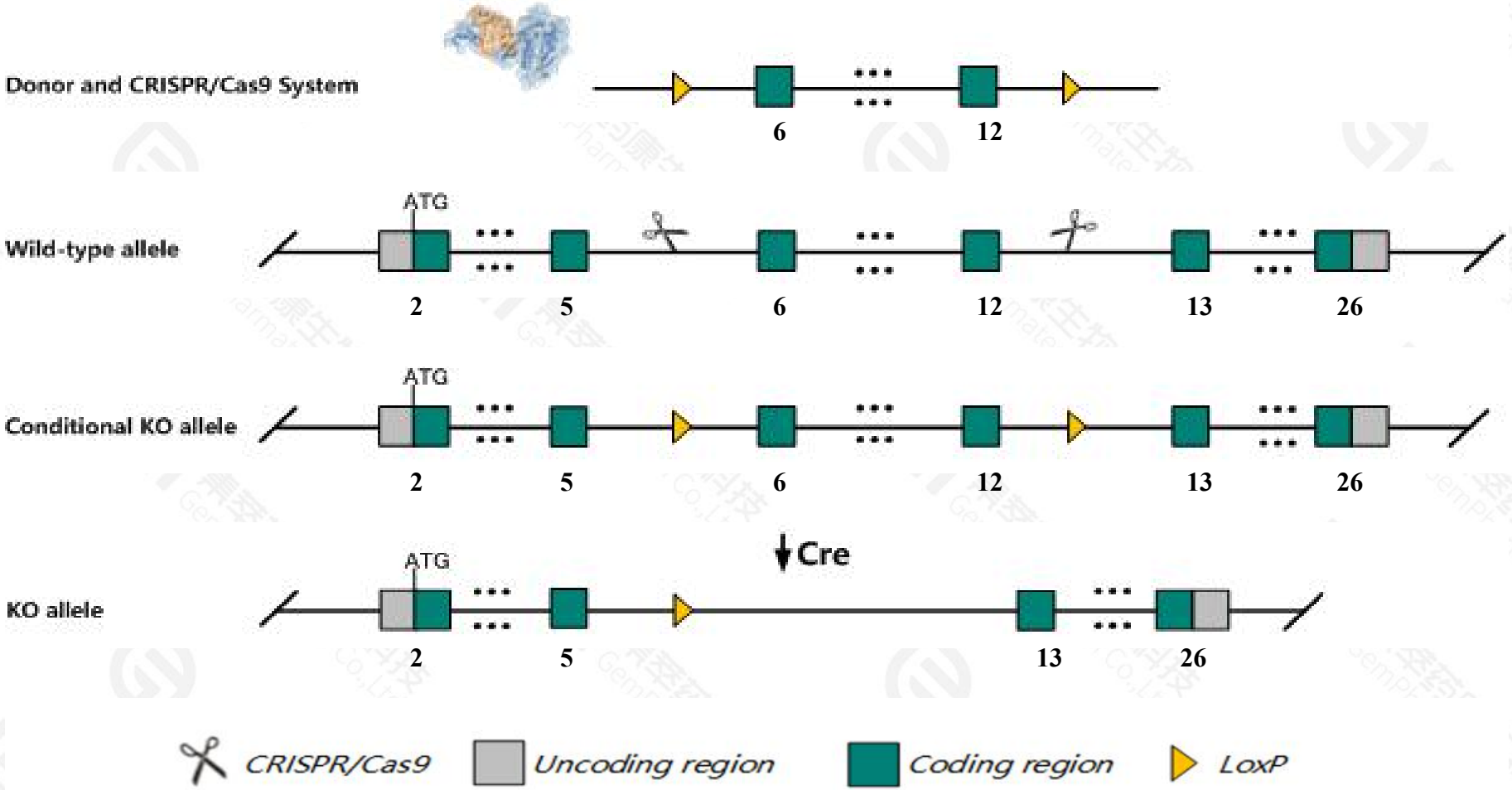
This model will use CRISPR/Cas9 technology to edit the *Dlg1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Dlg1* gene has 15 transcripts. According to the structure of *Dlg1* gene, exon6-exon12 of *Dlg1-202*(ENSMUST00000064477.14) transcript is recommended as the knockout region.The region contains 781bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dlg1* 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 a gene trap allele exhibit neonatal lethality, craniofacial defects, and abnormal eye morphology. Mice homozygous for knock-out alleles exhibit neonatal lethality, kidney defects, reproductive organ morphology, and cleft palate.

➢ Transcript *Dlg1*-207 may not be affected.

➢ The *Dlg1* 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）

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Dlg1 discs large MAGUK scaffold protein 1 [Mus musculus (house mouse)]

Gene ID: 13383, updated on 22-Mar-2020

Summary

Official Symbol	Dlg1 provided by MGI
Official Full Name	discs large MAGUK scaffold protein 1 provided by MGI
Primary source	MGI:MGI:107231
See related	Ensembl:ENSMUSG00000022770
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	B130052P05Rik, Dlgh1, E-dlg/SAP97, SAP-97, SAP97, mKIAA4187
Expression	Ubiquitous expression in cortex adult (RPKM 13.3), bladder adult (RPKM 13.0) and 28 other tissues See more
Orthologs	human all

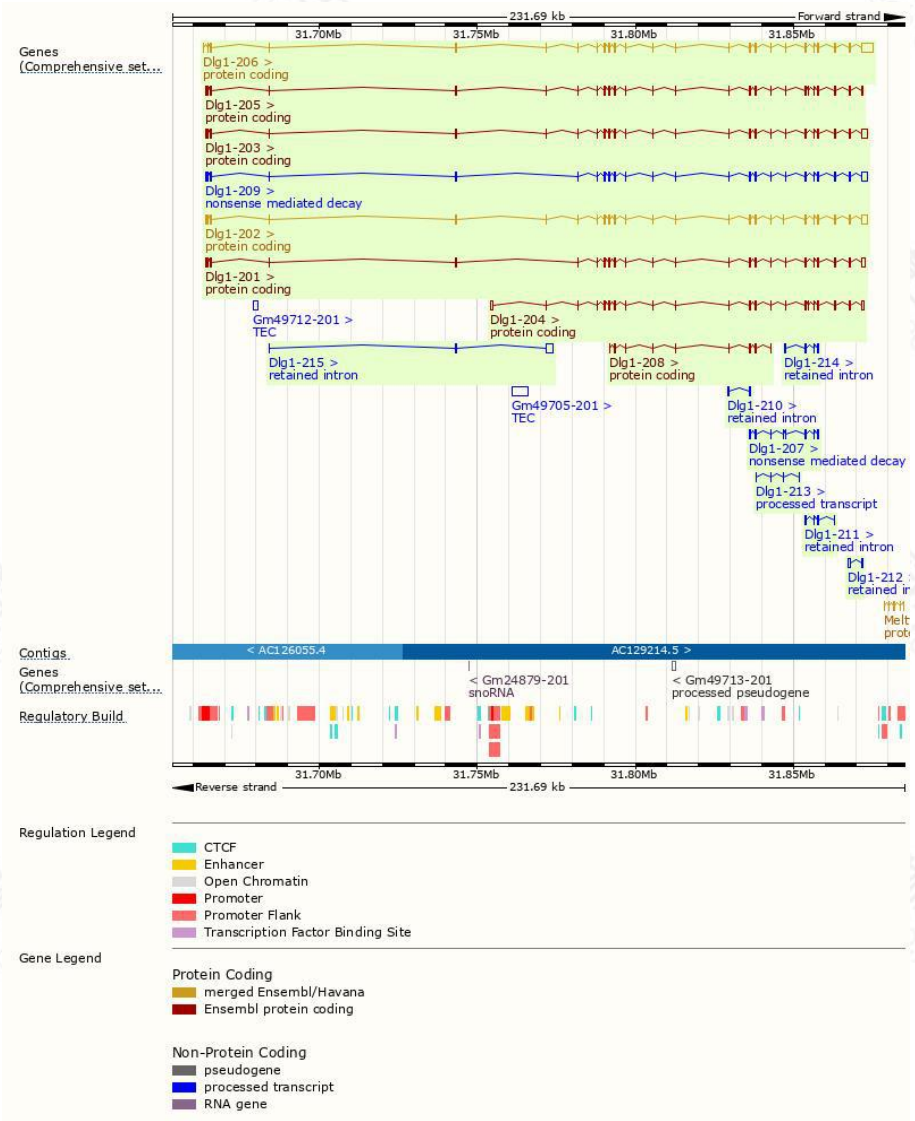
Transcript information（Ensembl）

The gene has 15 transcripts,all transcripts are shown below:

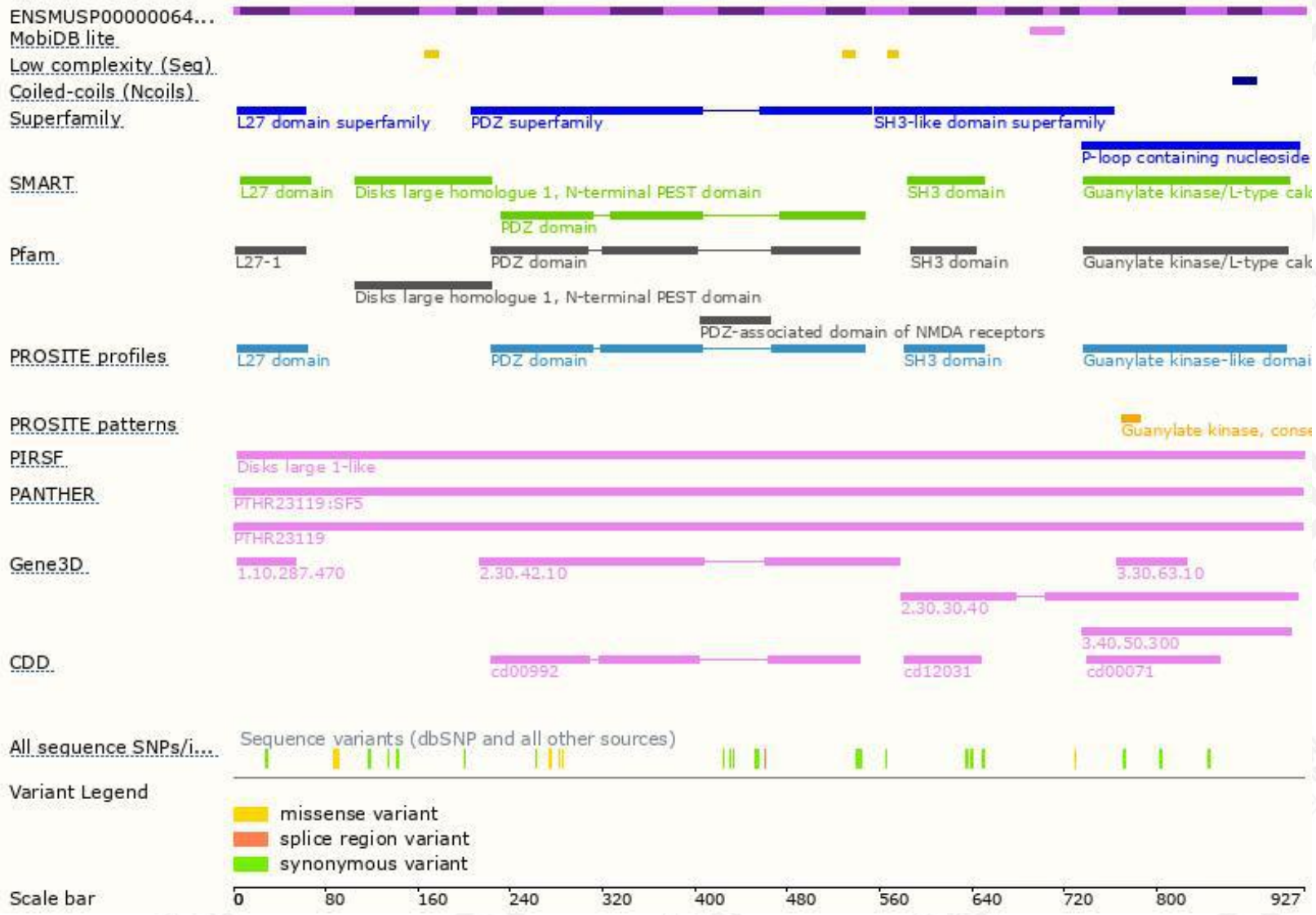
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dlg1-206	ENSMUST00000115205.8	6143	905aa	Protein coding	CCDS57025	Q811D0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Dlg1-203	ENSMUST00000100001.9	4711	905aa	Protein coding	CCDS57025	Q811D0	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Dlg1-202	ENSMUST00000064477.13	4663	927aa	Protein coding	CCDS28107	Q811D0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Dlg1-201	ENSMUST00000023454.11	3725	872aa	Protein coding	CCDS57026	H7BWY4	TSL:1 GENCODE basic
Dlg1-204	ENSMUST00000115196.7	3418	834aa	Protein coding	CCDS57027	D3Z3B8	TSL:1 GENCODE basic
Dlg1-205	ENSMUST00000115201.7	3248	912aa	Protein coding	-	E9Q9H0	TSL:5 GENCODE basic
Dlg1-208	ENSMUST00000131136.1	1073	358aa	Protein coding	-	F6UDT8	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:1
Dlg1-209	ENSMUST00000132176.7	4545	558aa	Nonsense mediated decay	-	S4R2T8	TSL:1
Dlg1-207	ENSMUST00000130920.7	678	30aa	Nonsense mediated decay	-	S4R2V5	CDS 5' incomplete TSL:3
Dlg1-213	ENSMUST00000147382.1	470	No protein	Processed transcript	-	-	TSL:5
Dlg1-215	ENSMUST00000155958.1	2225	No protein	Retained intron	-	-	TSL:2
Dlg1-212	ENSMUST00000140258.1	615	No protein	Retained intron	-	-	TSL:2
Dlg1-211	ENSMUST00000138213.1	596	No protein	Retained intron	-	-	TSL:2
Dlg1-210	ENSMUST00000137322.1	572	No protein	Retained intron	-	-	TSL:2
Dlg1-214	ENSMUST00000155847.7	567	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Dlg1-202* transcript,the transcription is shown below:

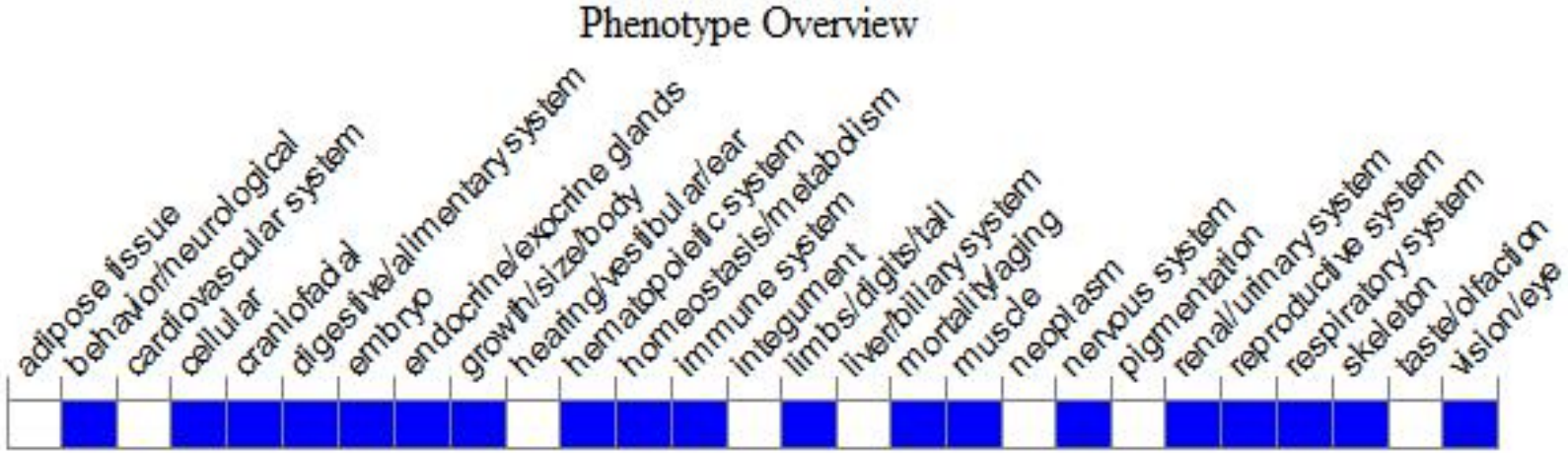
Genomic location distribution



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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