

# Dlg2 Cas9-CKO Strategy

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## Overview

### Target Gene Name

• Dlg2

# Project Type

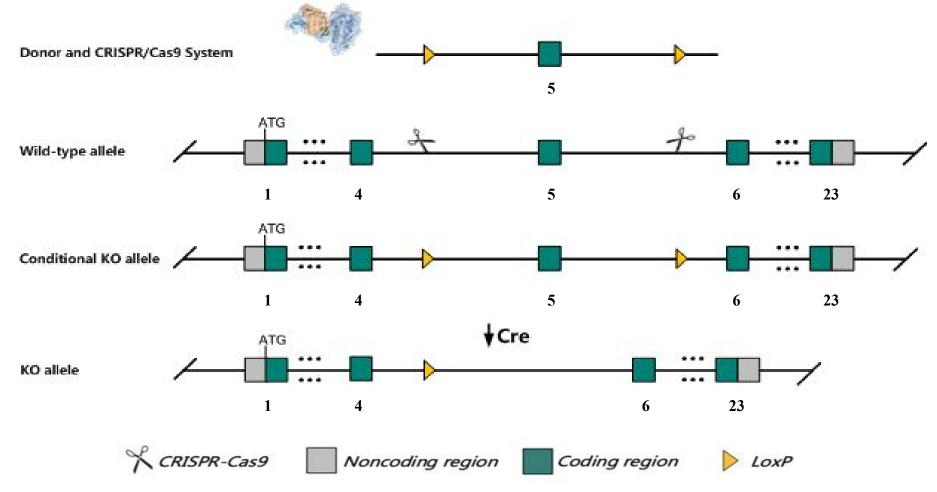
• Cas9-CKO

#### Genetic Background

• C57BL/6JGpt



# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Dlg2 gene.



### **Technical Information**

- The *Dlg2* gene has 22 transcripts. According to the structure of *Dlg2* gene, exon5 of *Dlg2*-204 (ENSMUST00000107196.10) transcript is recommended as the knockout region. The region contains 125bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Dlg2* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.



#### Gene Information

previous assembly

#### Dlg2 discs large MAGUK scaffold protein 2 [ Mus musculus (house mouse) ]

Gene ID: 23859, updated on 5-Mar-2024



NC 000073.6 (90476188..92449246)



GRCm38.p6 (GCF 000001635.26)

Source: https://www.ncbi.nlm.nih.gov/



108.20200622

# Transcript Information

The gene has 22 transcripts, all transcripts are shown below:

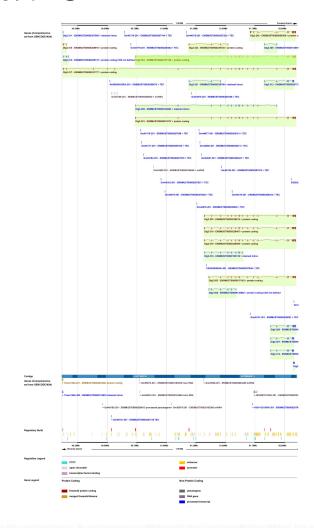
Transcript ID	Name	bp =	Protein	Biotype	CCDS 🛕	UniProt Match	Flags
ENSMUST00000231777.3	Dlg2-217	7801	<u>994aa</u>	Protein coding		A0A338P6J0 R	Ensembl Canonical GENCODE basic APPRIS Pt
ENSMUST00000238467.2	Dlg2-218	7573	<u>919aa</u>	Protein coding		A0A571BEZ4 <b>戊시</b>	GENCODE basic
ENSMUST00000238619.2	Dlg2-220	7559	887aa	Protein coding		<u>Q91XM9-4</u> اکہا	GENCODE basic
ENSMUST00000238608.2	Dlg2-219	7401	901aa	Protein coding		<u>A0A571BEK3</u> <b>₹</b> ₩	GENCODE basic
ENSMUST00000074273.10	Dlg2-201	5278	870aa	Protein coding		E902L2 戊시	GENCODE basic TSL:5
ENSMUST00000107193.8	Dlg2-203	4910	755aa	Protein coding		D3YUZ8 K	GENCODE basic TSL:5
ENSMUST00000239138.2	Dlg2-222	540	<u>78aa</u>	Protein coding		A0A5F8MQA2 ₹	CDS 3' incomplete
ENSMUST00000208919.2	Dlg2-215	396	<u>47aa</u>	Protein coding		A0A5F8MPY4 RN	TSL:3 CDS 3' incomplete
ENSMUST00000138389.8	Dlg2-208	3218	No protein	Protein coding CDS not defined		140	TSL:1
ENSMUST00000209170.2	Dlg2-216	1057	No protein	Protein coding CDS not defined		820	TSL:2
ENSMUST00000135581.3	Dlg2-207	964	No protein	Protein coding CDS not defined			TSL:3
ENSMUST00000128592.3	Dlg2-205	922	No protein	Protein coding CDS not defined		925	TSL:5
ENSMUST00000207798.2	Dlg2-212	842	No protein	Protein coding CDS not defined		898	TSL:5
ENSMUST00000208377.2	Dlg2-214	672	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000148583.9	Dlg2-209	4238	No protein	Retained intron		100	TSL:5
ENSMUST00000152139.8	Dlg2-210	2934	No protein	Retained intron		-	TSL:1
ENSMUST00000207095.2	Dlg2-211	2397	No protein	Retained intron			TSL:NA
ENSMUST00000129818.2	Dlg2-206	2218	No protein	Retained intron		150	TSL:1
ENSMUST00000238788.2	Dlg2-221	1216	No protein	Retained intron		-	15%
ENSMUST00000207891.2	Dlg2-213	448	No protein	Retained intron		150	TSL:3
ENSMUST00000107196.10	Dlg2-204	7490	852aa	Protein coding	CCDS40021 당사	<u> </u>	GENCODE basic TSL:1
ENSMUST00000098308.4	Dlg2-202	2329	481aa	Protein coding	CCDS57562 R	D3YWU0 RW	GENCODE basic TSL:1

The strategy is based on the design of Dlg2-204 transcript, the transcription is shown below:



Source: https://www.ensembl.org

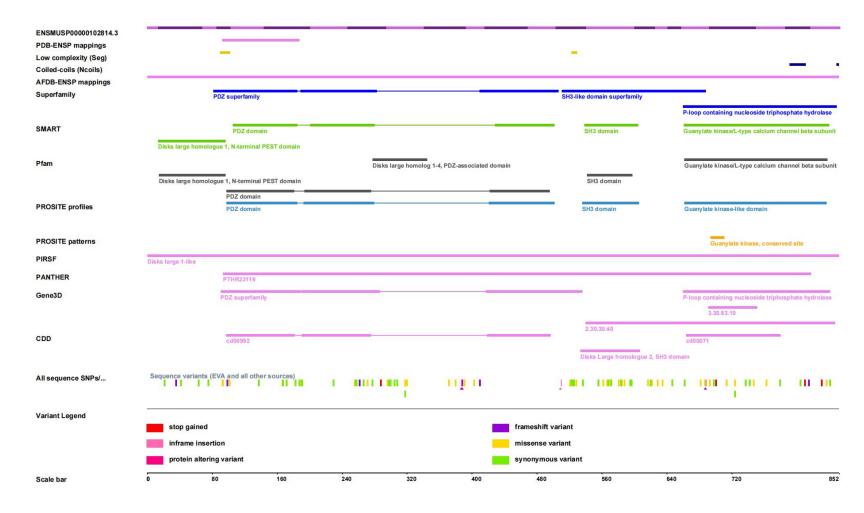
# Genomic Information





Source: : https://www.ensembl.org

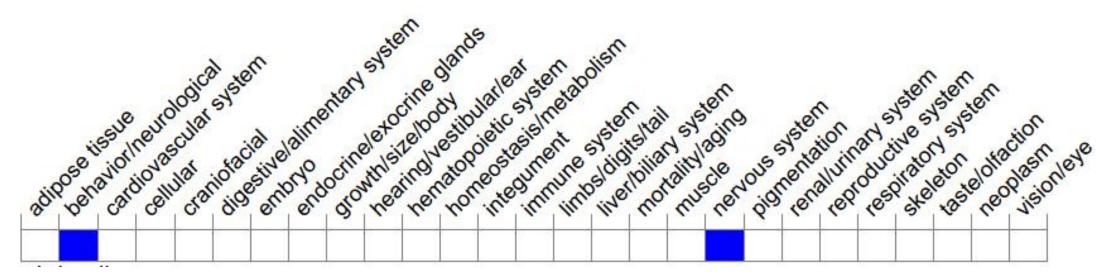
### Protein Information





Source: : https://www.ensembl.org

# Mouse Phenotype Information (MGI)



• Mice homozygous for a knock-out allele display lower surface expression of NMDA receptor (NMDAR) subunits NR2A and NR2B in dorsal horn neurons and significantly reduced NMDAR-mediated excitatory synaptic currents and NMDAR-dependent persistent inflammatory or nerve injury-induced neuropathic pain.



Source: https://www.informatics.jax.org

# Important Information

- This stratergy may not affect *Dlg2* -202 and *Dlg2* -205 coding transcript.
- A part of amino acid sequence (103 aa) will still remain at the N-terminal of *Dlg2* -204.
- *Dlg2* is located on Chr7. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

