

C2cd3 Cas9-CKO Strategy

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Project Overview

Project Name

C2cd3

Project type

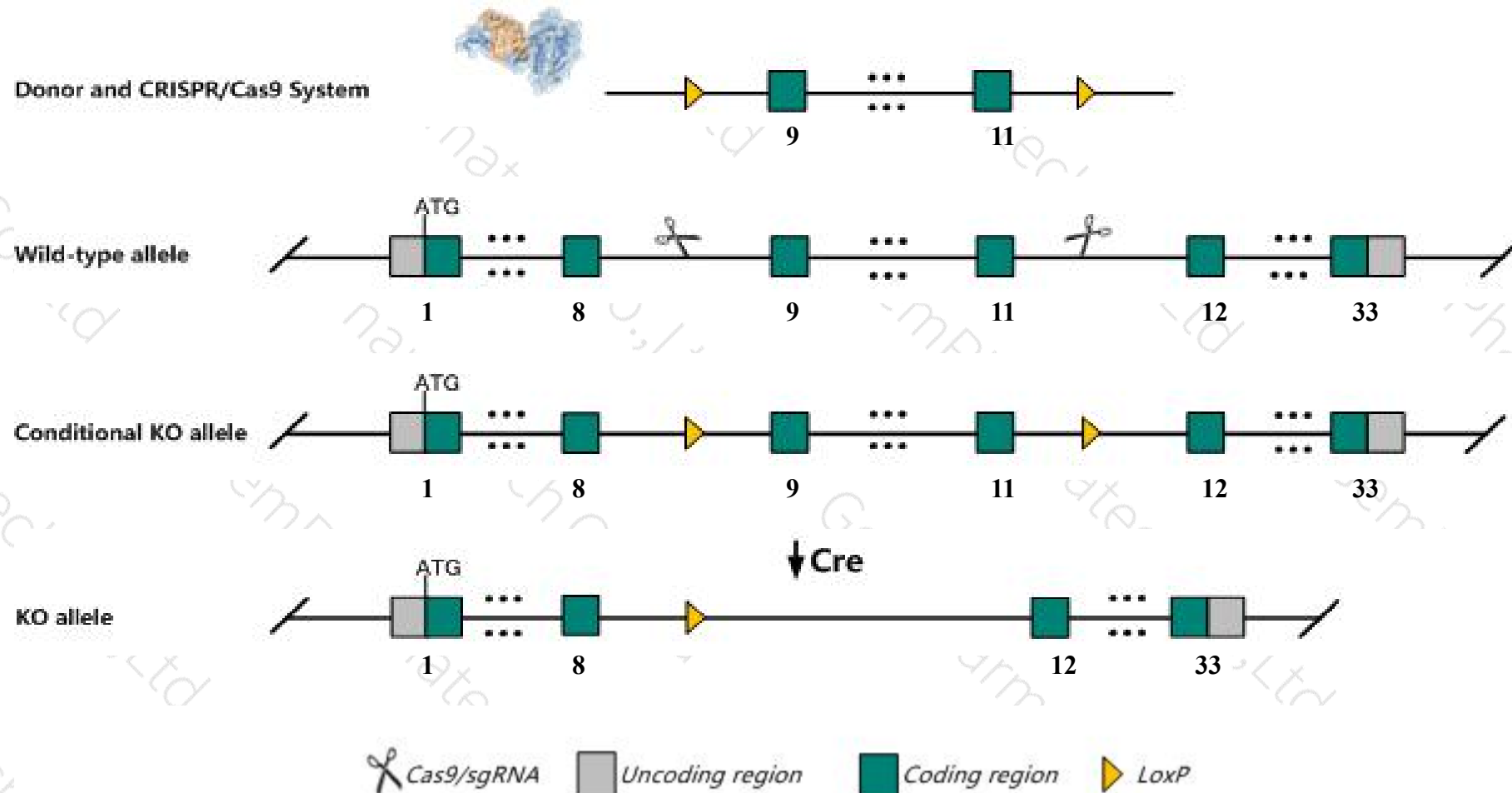
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *C2cd3* gene. The schematic diagram is as follows:



Technical routes

- The *C2cd3* gene has 14 transcripts. According to the structure of *C2cd3* gene, exon9-exon11 of *C2cd3-201*(ENSMUST00000051777.14) transcript is recommended as the knockout region. The region contains 472bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *C2cd3* 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, homozygotes inactivating allele are embryonic lethal with pericardial edema and twisted body axis, abnormal patterning of brain and open neural tube defect.
- The *C2cd3* gene is located on the Chr7. 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)

C2cd3 C2 calcium-dependent domain containing 3 [Mus musculus (house mouse)]

Gene ID: 277939, updated on 13-Mar-2020

Summary



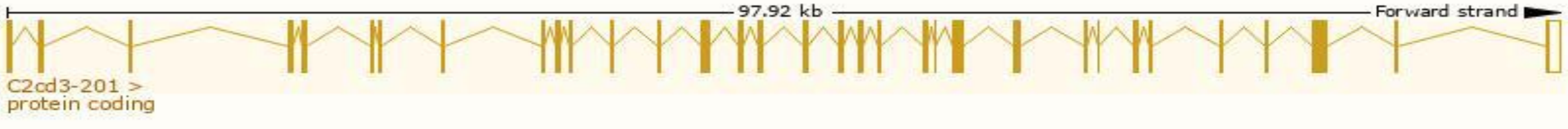
Official Symbol	C2cd3 provided by MGI
Official Full Name	C2 calcium-dependent domain containing 3 provided by MGI
Primary source	MGI:MGI:2142166
See related	Ensembl:ENSMUSG00000047248
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AU020772
Expression	Ubiquitous expression in testis adult (RPKM 8.4), thymus adult (RPKM 7.8) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

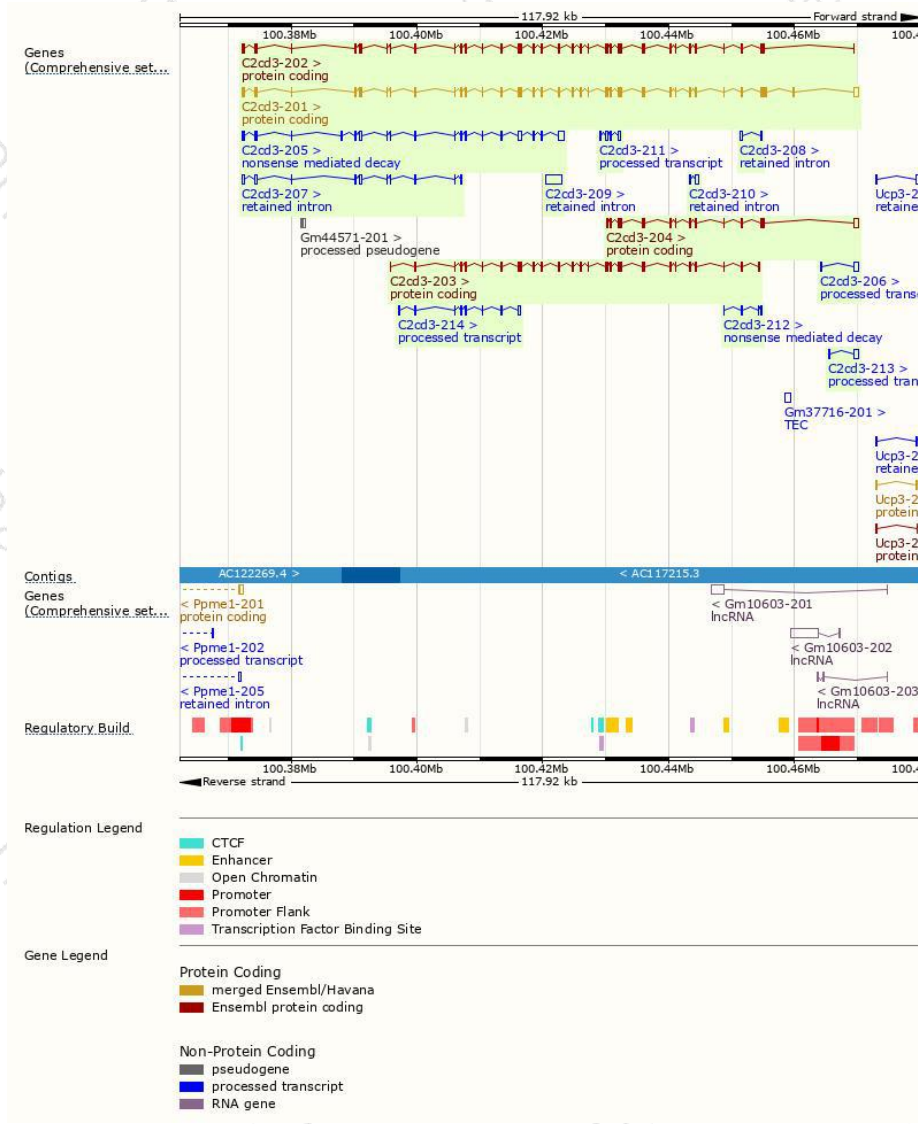
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
C2cd3-201	ENSMUST00000051777.14	7803	2323aa	Protein coding	CCDS57568	Q52KB6	TSL:5 GENCODE basic APPRIS P2
C2cd3-202	ENSMUST00000098259.10	6602	2130aa	Protein coding	-	E9Q526	TSL:5 GENCODE basic APPRIS ALT2
C2cd3-203	ENSMUST00000119647.8	4840	1566aa	Protein coding	-	D3Z640	CDS 5' incomplete TSL:1
C2cd3-204	ENSMUST00000120196.8	3391	899aa	Protein coding	-	D3Z4D9	CDS 5' incomplete TSL:5
C2cd3-205	ENSMUST00000133464.7	4167	250aa	Nonsense mediated decay	-	D6RH58	TSL:1
C2cd3-212	ENSMUST00000185084.1	600	100aa	Nonsense mediated decay	-	V9GXB2	CDS 5' incomplete TSL:3
C2cd3-214	ENSMUST00000208753.1	1304	No protein	Processed transcript	-	-	TSL:5
C2cd3-213	ENSMUST00000208018.1	925	No protein	Processed transcript	-	-	TSL:5
C2cd3-206	ENSMUST00000151573.2	879	No protein	Processed transcript	-	-	TSL:1
C2cd3-211	ENSMUST00000185051.1	788	No protein	Processed transcript	-	-	TSL:3
C2cd3-209	ENSMUST00000184753.1	2576	No protein	Retained intron	-	-	TSL:NA
C2cd3-207	ENSMUST00000156559.7	1909	No protein	Retained intron	-	-	TSL:1
C2cd3-210	ENSMUST00000184779.1	660	No protein	Retained intron	-	-	TSL:2
C2cd3-208	ENSMUST00000184657.1	639	No protein	Retained intron	-	-	TSL:2

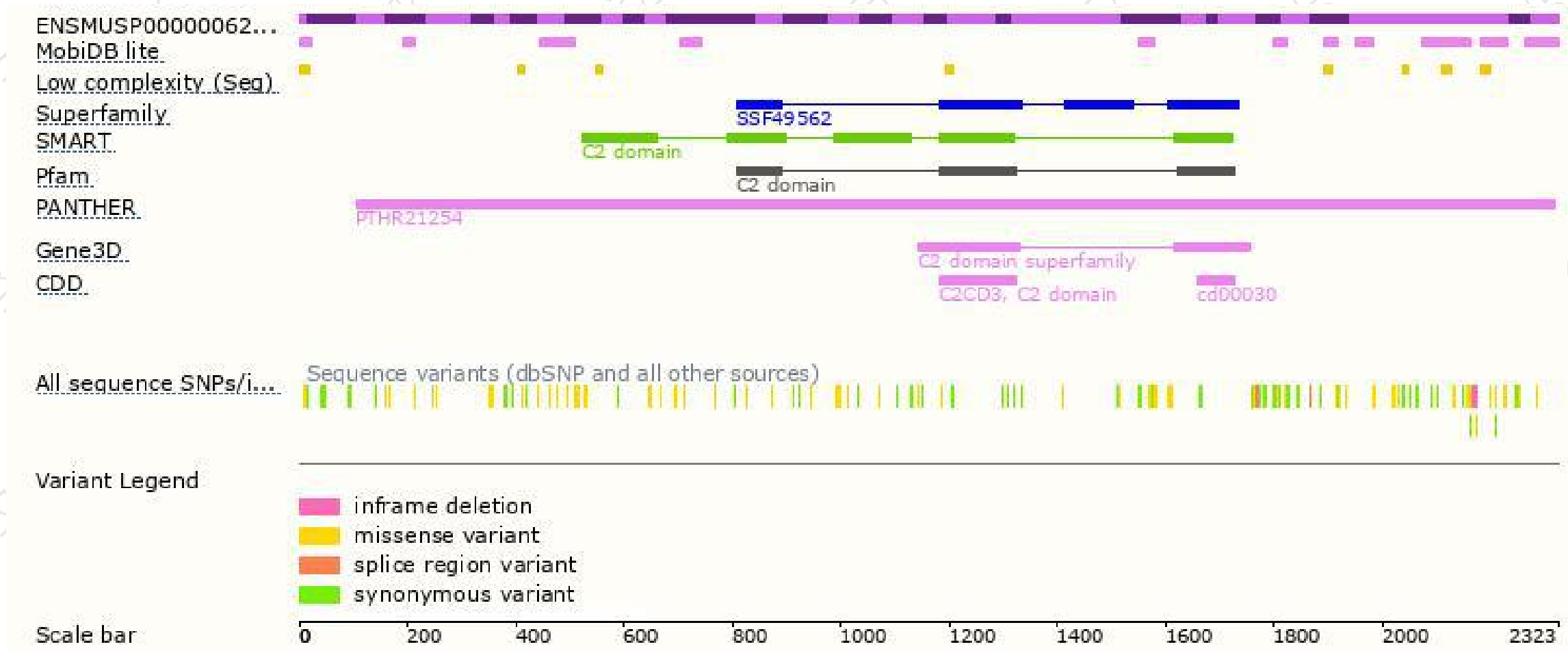
The strategy is based on the design of *C2cd3-201* transcript,the transcription is shown below:



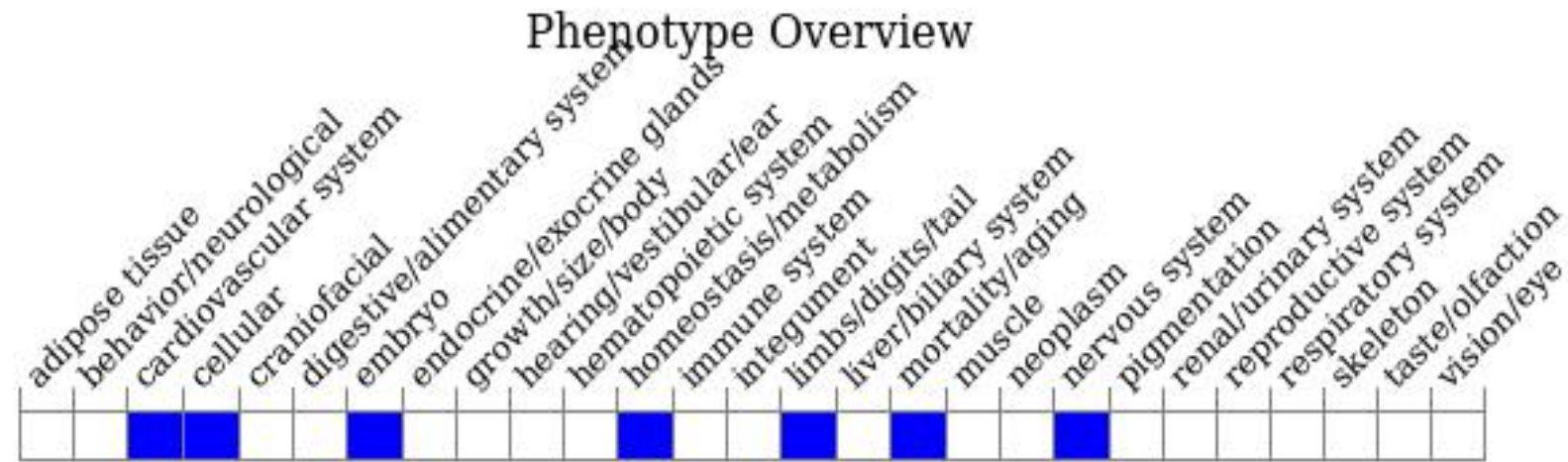
Genomic location distribution



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, homozygotes inactivating allele are embryonic lethal with pericardial edema and twisted body axis, abnormal patterning of brain and open neural tube defect.

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

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