

# Dolar Dake Ch Co. 1/4 Casp2 Cas9-CKO Strategy Romphamakech Co.

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# **Project Overview**



Project Name Casp2

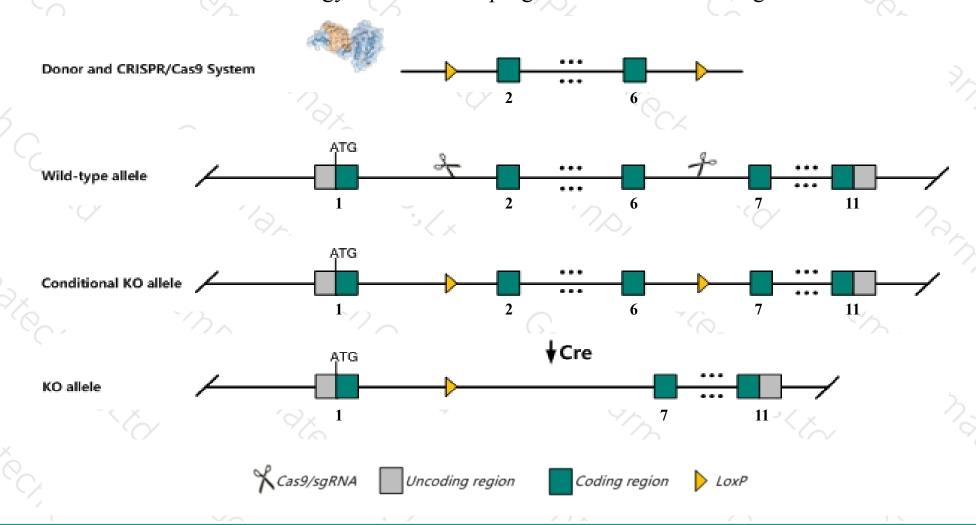
Project type Cas9-CKO

Strain background C57BL/6J

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Casp2 gene. The schematic diagram is as follows:



## **Technical routes**



- ➤ The *Casp2* gene has 10 transcripts. According to the structure of *Casp2* gene, exon2-exon6 of *Casp2-201* (ENSMUST00000031895.12) transcript is recommended as the knockout region. The region contains 673bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Casp2* 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/6J 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/6J 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.

### **Notice**



- ➤ According to the existing MGI data, Homozygous mutation of this gene results in abnormal apoptosis.

  Apoptosis is reduced in the female germline, but is increased in sympathetic neurons during development.
- > The Casp2 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)



#### Casp2 caspase 2 [Mus musculus (house mouse)]

Gene ID: 12366, updated on 9-Apr-2019

#### Summary

☆ [?

Official Symbol Casp2 provided by MGI

Official Full Name caspase 2 provided by MGI

Primary source MGI:MGI:97295

See related Ensembl:ENSMUSG00000029863

Gene type protein coding
RefSeq status REVIEWED

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as CASP-2, ICH-1, NEDD-2, Nedd2

Summary This gene encodes the evolutionarily ancient and most conserved member of the cysteine proteases that plays important role in stress-

induced apoptosis, DNA repair and tumor suppression. Mice lacking the encoded protein develop normally but display cell type-specific apoptotic defects. Germ cells and oocytes from such mice were found to be resistant to cell death after treatment with chemotherapeutic

drugs. [provided by RefSeq, Apr 2015]

Expression Ubiquitous expression in thymus adult (RPKM 27.3), limb E14.5 (RPKM 22.2) and 27 other tissuesSee more

Orthologs <u>human</u> all

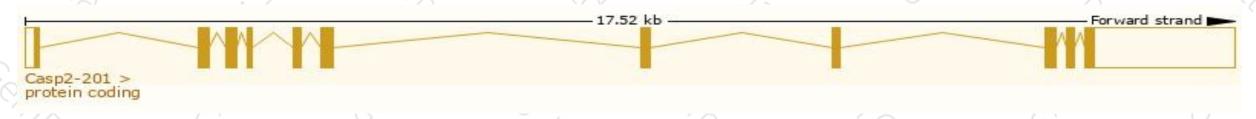
# Transcript information (Ensembl)



The gene has 10 transcripts, all transcripts are shown below:

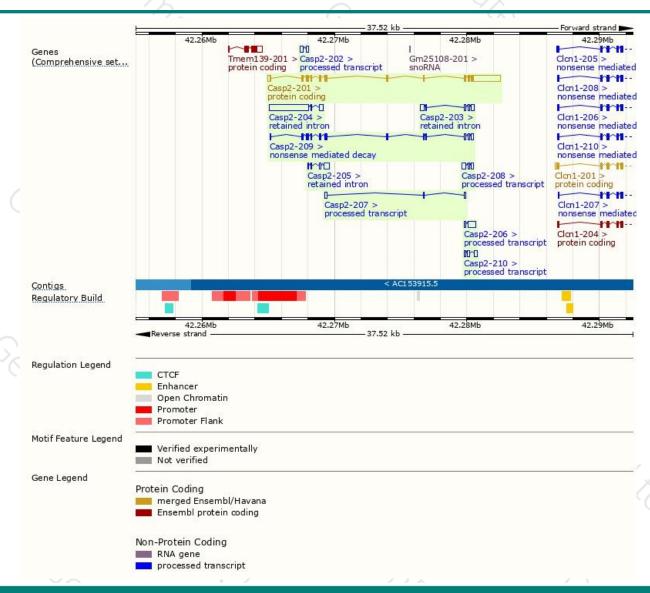
		3 /						
	Name	Transcript ID	bp 🛊	Protein	Biotype	CCDS	UniProt	Flags
	Casp2-201	ENSMUST00000031895.12	3529	<u>452aa</u>	Protein coding	CCDS20064₽	<u>P29594</u> €	TSL:1 GENCODE basic APPRIS P1
	Casp2-209	ENSMUST00000156829.7	1439	<u>343aa</u>	Nonsense mediated decay	-	<u>D6RFN6</u> ₽	TSL:5
2	Casp2-204	ENSMUST00000139930.7	3325	No protein	Retained intron	-	-	TSL:2
	Casp2-203	ENSMUST00000132398.7	808	No protein	Retained intron	-	-	TSL:3
	Casp2-205	ENSMUST00000141669.1	615	No protein	Retained intron	-	-	TSL:3
	Casp2-206	ENSMUST00000141727.1	704	No protein	IncRNA	-	-	TSL:2
	Casp2-208	ENSMUST00000152893.1	545	No protein	IncRNA	-	-	TSL:2
	Casp2-210	ENSMUST00000203089.1	494	No protein	IncRNA	-	-	TSL:5
	Casp2-202	ENSMUST00000132246.1	396	No protein	IncRNA	-	-	TSL:1
	Casp2-207	ENSMUST00000144821.1	384	No protein	IncRNA	-	-	TSL:3

The strategy is based on the design of Casp2-201 transcript, The transcription is shown below



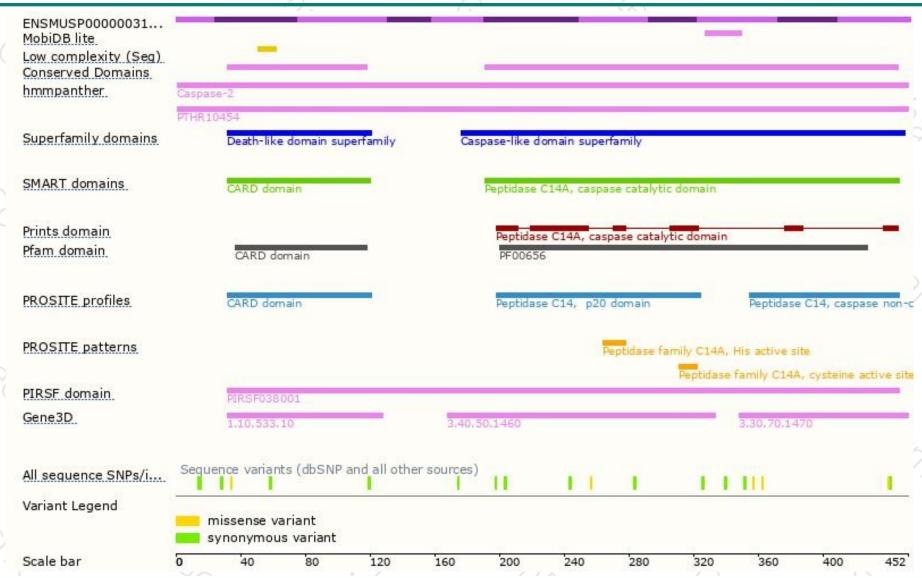
## Genomic location distribution





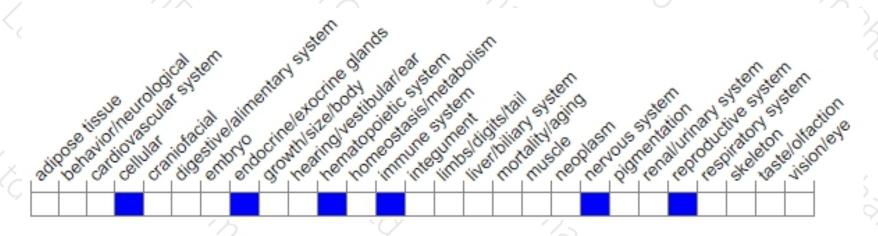
## Protein domain





# 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 mutation of this gene results in abnormal apoptosis. Apoptosis is reduced in the female germline, but is increased in sympathetic neurons during development.



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

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