Casp9 Cas9-CKO Strategy

Designer: Qiong Zhou
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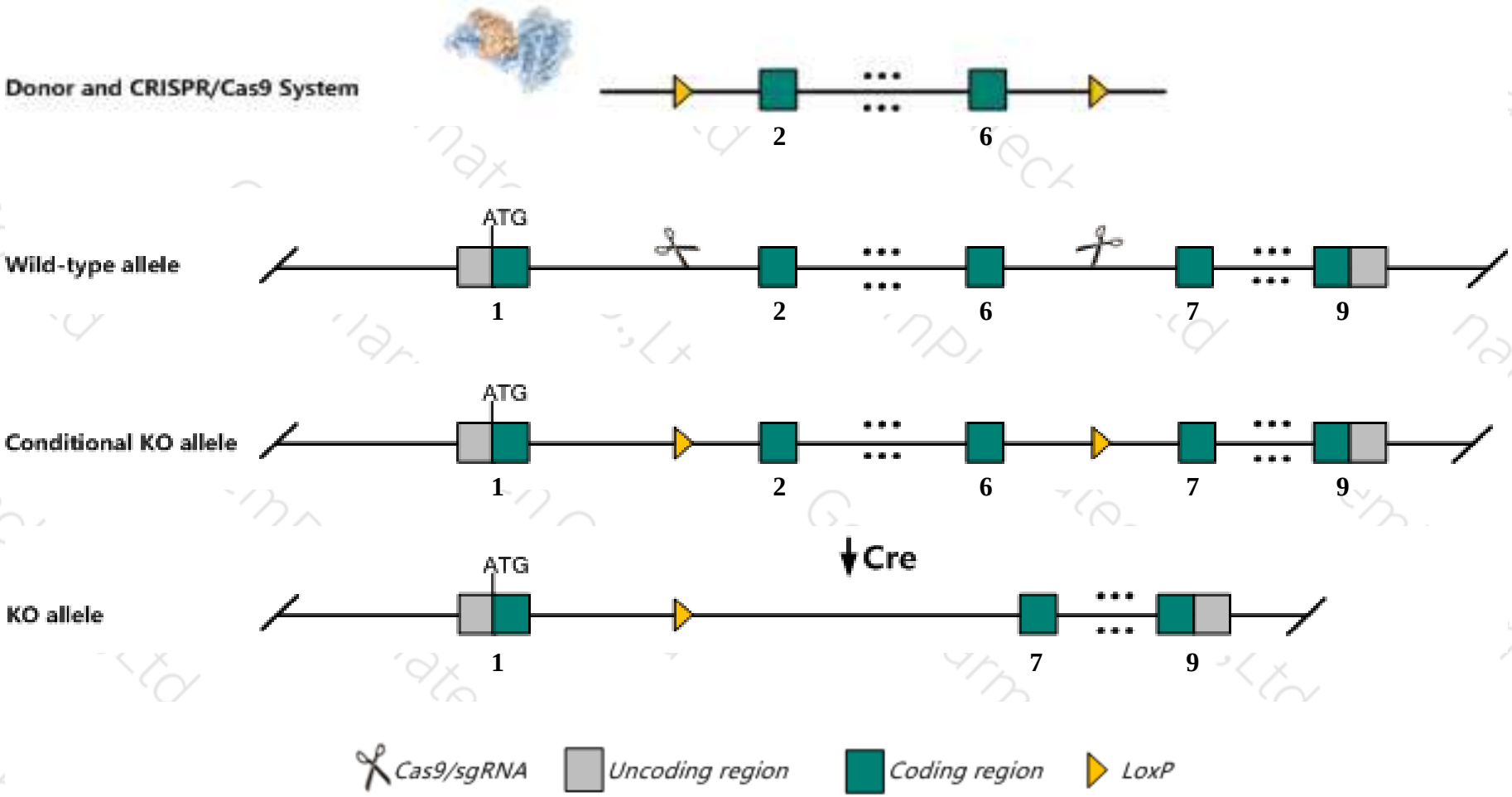
Project Overview

Project Name	***Casp9***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Casp9* gene. The schematic diagram is as follows:



Technical routes

- The *Casp9* gene has 6 transcripts. According to the structure of *Casp9* gene, exon2-exon6 of *Casp9-201* (ENSMUST00000030747.10) transcript is recommended as the knockout region.The region contains 850bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Casp9* 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.

Notice

- According to the existing MGI data,Homozygous targeted mutants die perinatally with enlarged and malformed cerebrums caused by reduced apoptosis during brain development. Broad system- and stimulus-dependent effects are seen on apoptosis.

- Transcript 203 may be unaffected.

- The *Casp9* gene is located on the Chr4. 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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Casp9 caspase 9 [Mus musculus (house mouse)]

Gene ID: 12371, updated on 19-Feb-2019

Summary

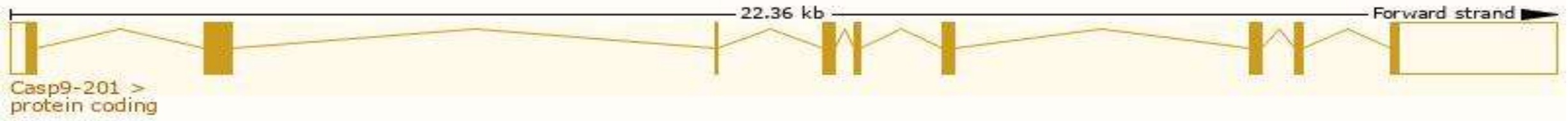
Official Symbol	Casp9 provided by MGI
Official Full Name	caspase 9 provided by MGI
Primary source	MGI:MGI:1277950
See related	Ensembl:ENSMUSG00000028914
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI115399, APAF-3, AW493809, CASP-9, Caspase-9, ICE-LAP6, Mch6
Summary	This gene is part of a family of caspases, aspartate-specific cysteine proteases well studied for their involvement in immune and apoptosis signaling. This protein, the initiator caspase, is activated after cytochrome c release from mitochondria and targets downstream effectors. In mouse, deficiency of this gene can cause perinatal lethality. This protein may have a role in normal brain development. Alternative splicing results in multiple transcript variants that encode different protein isoforms. [provided by RefSeq, Apr 2013]
Expression	Ubiquitous expression in small intestine adult (RPKM 37.5), spleen adult (RPKM 32.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

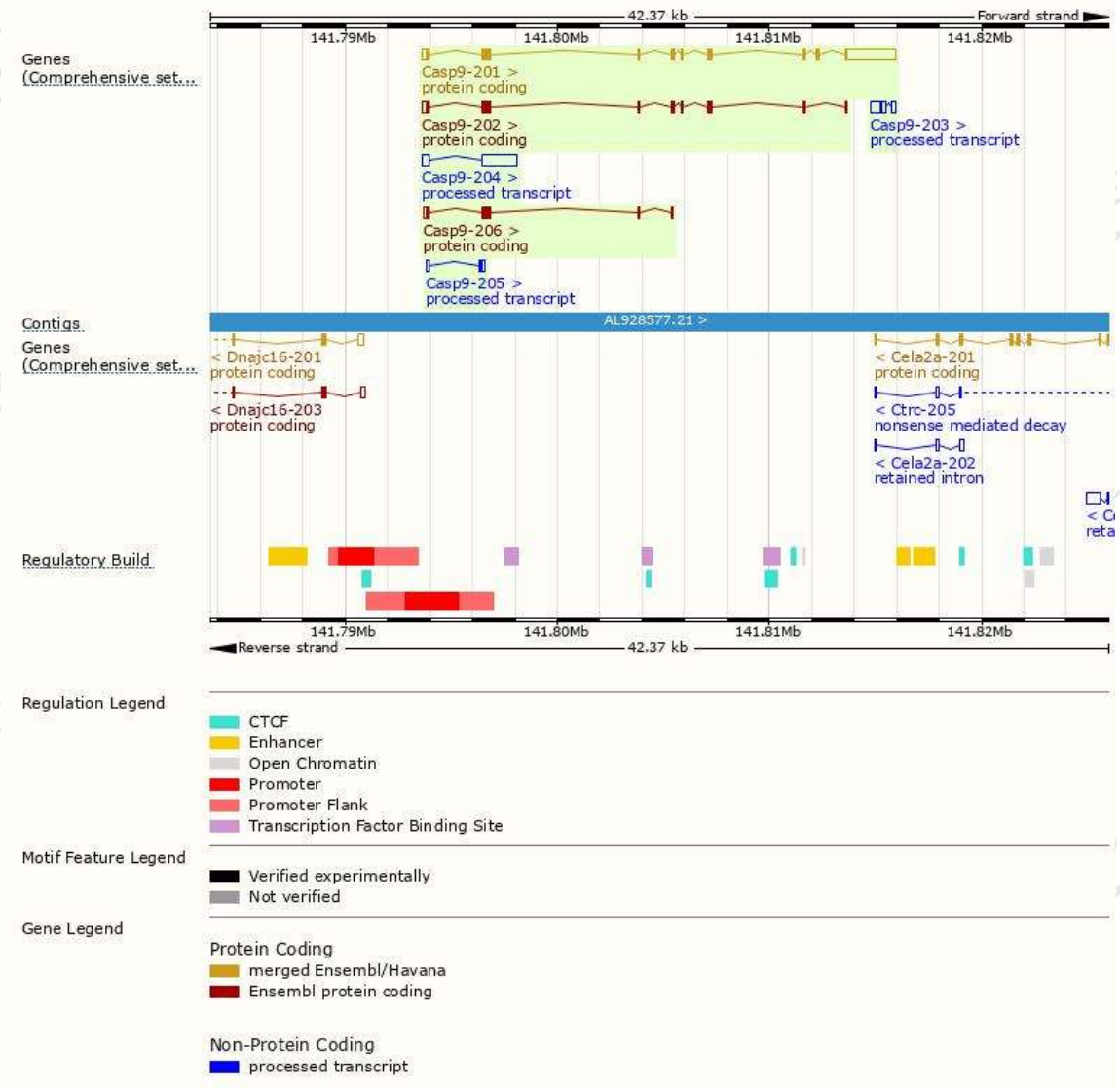
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Casp9-201	ENSMUST00000030747.10	3897	454aa	Protein coding	CCDS18883	Q8C3Q9	TSL:1 GENCODE basic APPRIS P1
Casp9-202	ENSMUST00000097805.10	1498	405aa	Protein coding	CCDS71510	A2AS93	TSL:1 GENCODE basic
Casp9-206	ENSMUST00000153094.1	790	215aa	Protein coding	-	A2AS92	CDS 3' incomplete TSL:3
Casp9-204	ENSMUST00000128660.1	2027	No protein	Processed transcript	-	-	TSL:1
Casp9-203	ENSMUST00000124161.1	854	No protein	Processed transcript	-	-	TSL:3
Casp9-205	ENSMUST00000138359.1	331	No protein	Processed transcript	-	-	TSL:5

The strategy is based on the design of *Casp9-201* transcript,The transcription is shown below

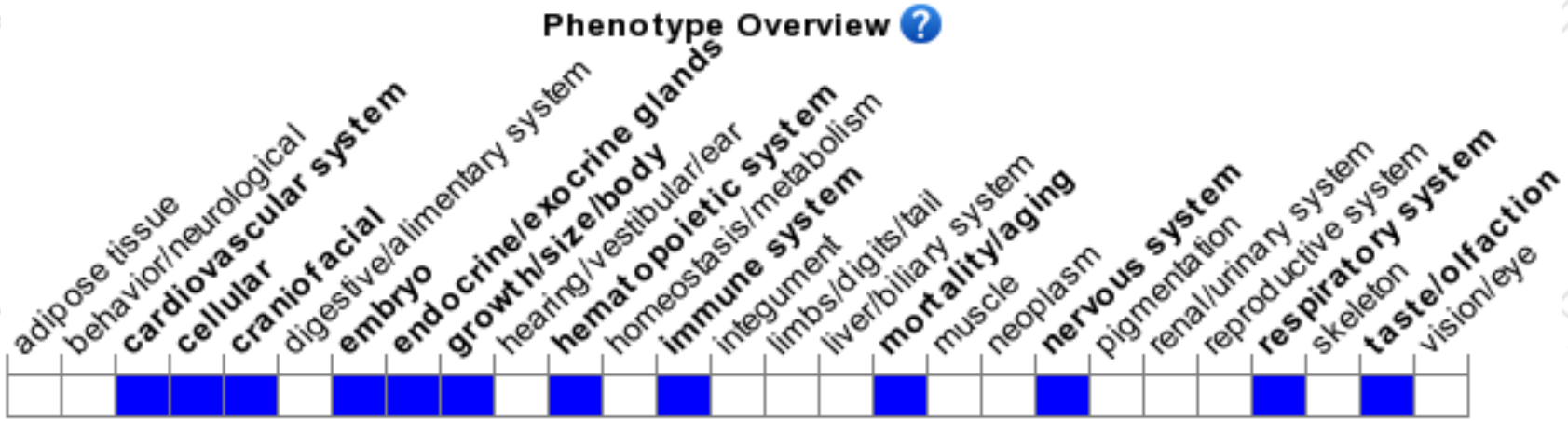


Genomic location distribution



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/).

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集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd