Capn5 Cas9-CKO Strategy

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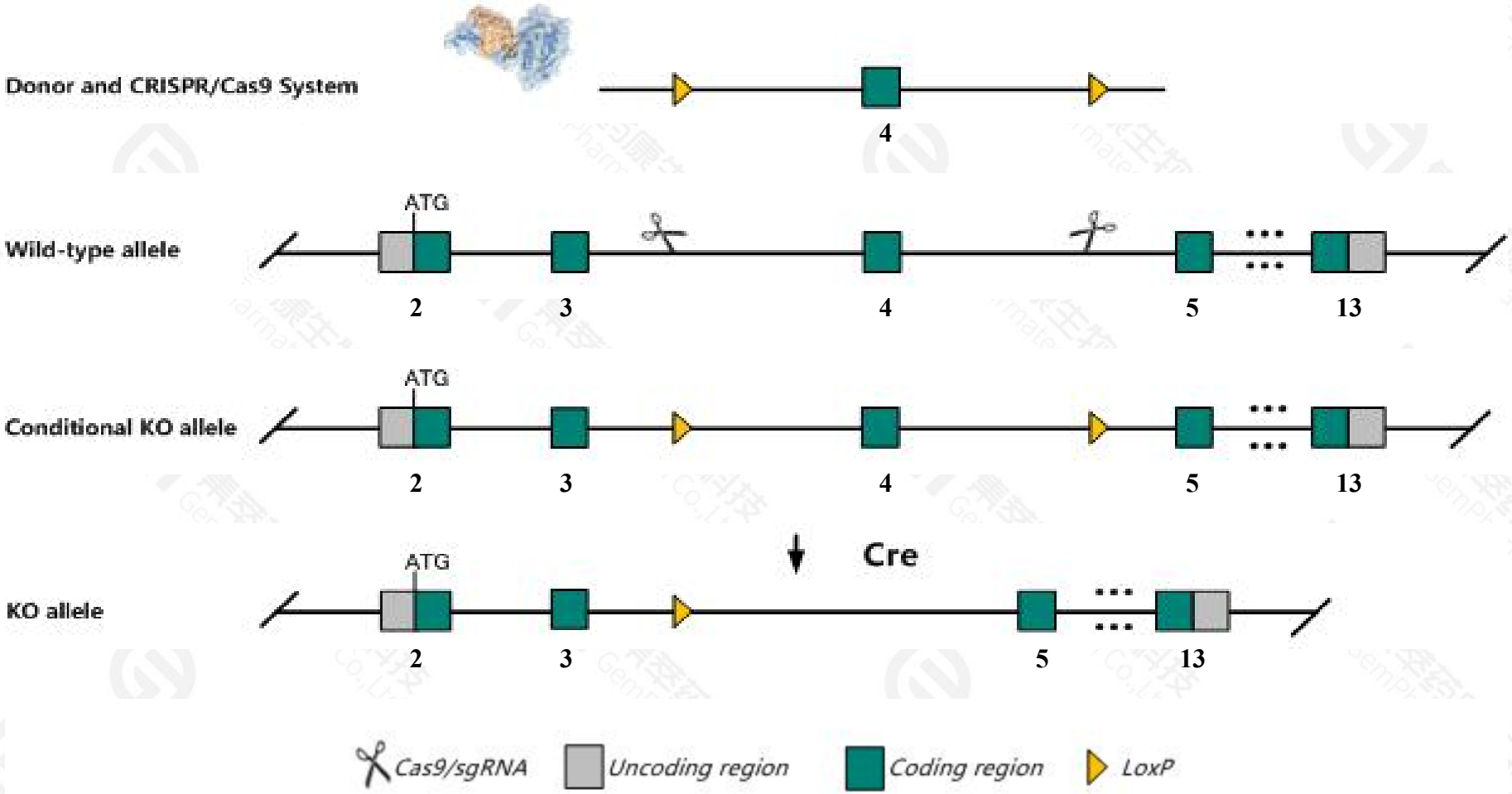
Project Overview

Project Name	***Capn5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Capn5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Capn5* gene has 5 transcripts. According to the structure of *Capn5* gene, exon4 of *Capn5-201*(ENSMUST00000040971.14) transcript is recommended as the knockout region.The region contains 209bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Capn5* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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,mice homozygous for one allele of this gene occasionally exhibit reduced viability but are usually normal. Homozygotes for another allele die as embryos.

➢ The *Capn5* 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）

集萃药康 GemPharmatech

Capn5 calpain 5 [Mus musculus (house mouse)]

Gene ID: 12337, updated on 17-Dec-2020

Summary

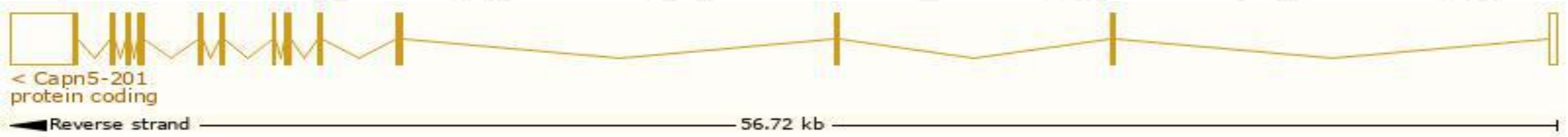
Official Symbol	Capn5 provided by MGI
Official Full Name	calpain 5 provided by MGI
Primary source	MGI:MGI:1100859
See related	Ensembl:ENSMUSG00000035547
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	nCL-3
Summary	The protein encoded by this gene is a member of the calpain family of proteins. Unlike many members of the calpain gene family, this gene lacks a calmodulin-like domain, required for calcium binding. Mouse models for Huntington's disease displayed increased levels of the protein encoded by this gene. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jul 2014]
Expression	Broad expression in colon adult (RPKM 41.6), genital fat pad adult (RPKM 35.6) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

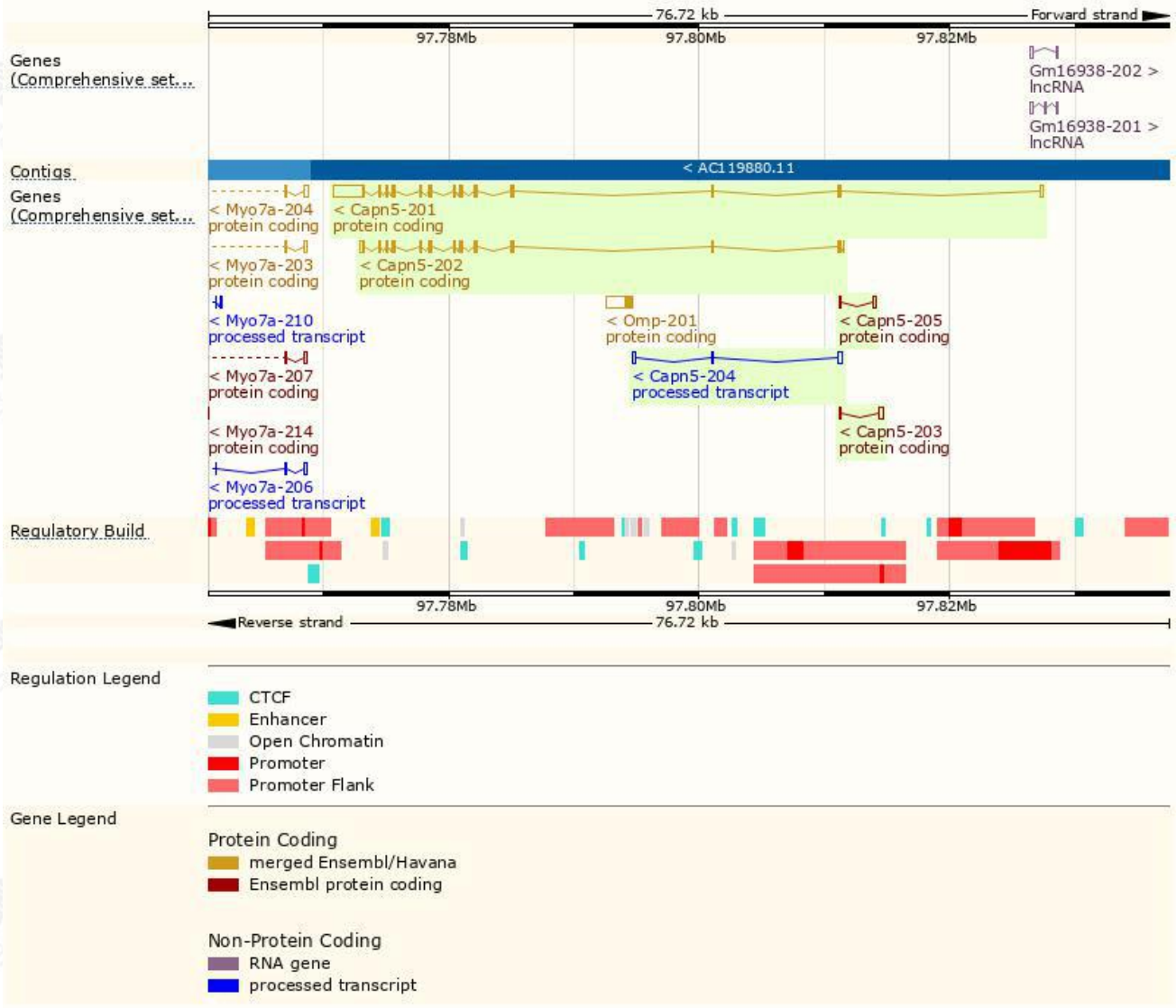
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Capn5-201	ENSMUST00000040971.14	4498	640aa	Protein coding	CCDS21465		TSL:1 , GENCODE basic , APPRIS P1 ,
Capn5-202	ENSMUST00000107112.2	2232	640aa	Protein coding	CCDS21465		TSL:1 , GENCODE basic , APPRIS P1 ,
Capn5-203	ENSMUST00000129430.2	440	27aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
Capn5-205	ENSMUST00000155056.2	382	46aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Capn5-204	ENSMUST00000134638.2	668	No protein	Processed transcript	-		TSL:3 ,

The strategy is based on the design of *Capn5-201* transcript,the transcription is shown below:

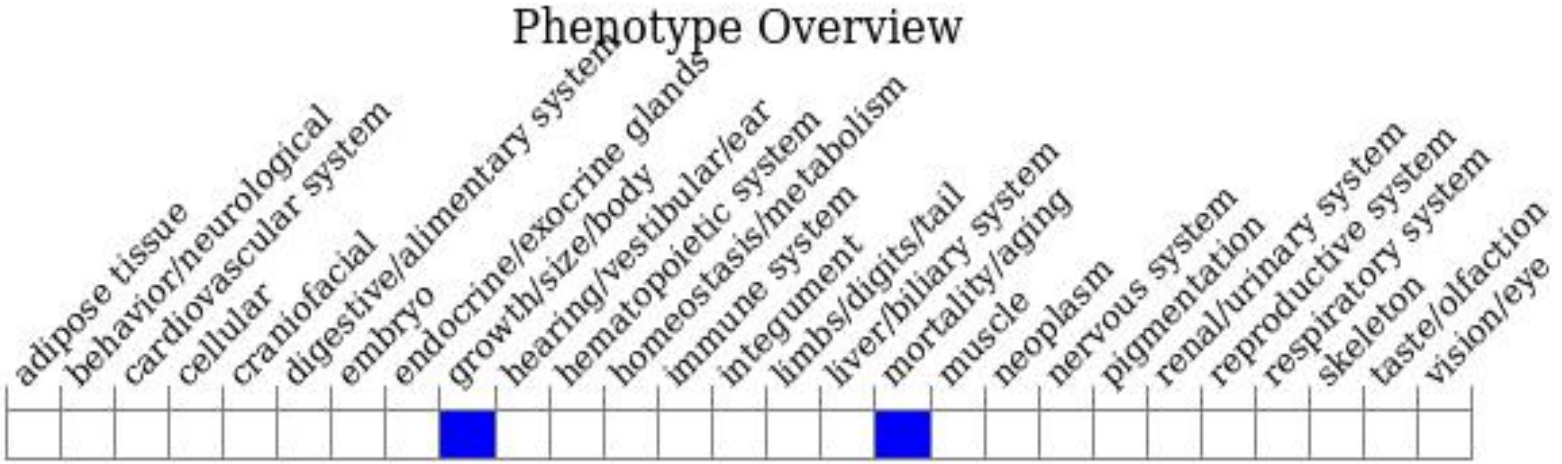


Genomic location distribution



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,mice homozygous for one allele of this gene occasionally exhibit reduced viability but are usually normal. Homozygotes for another allele die as embryos.

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

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