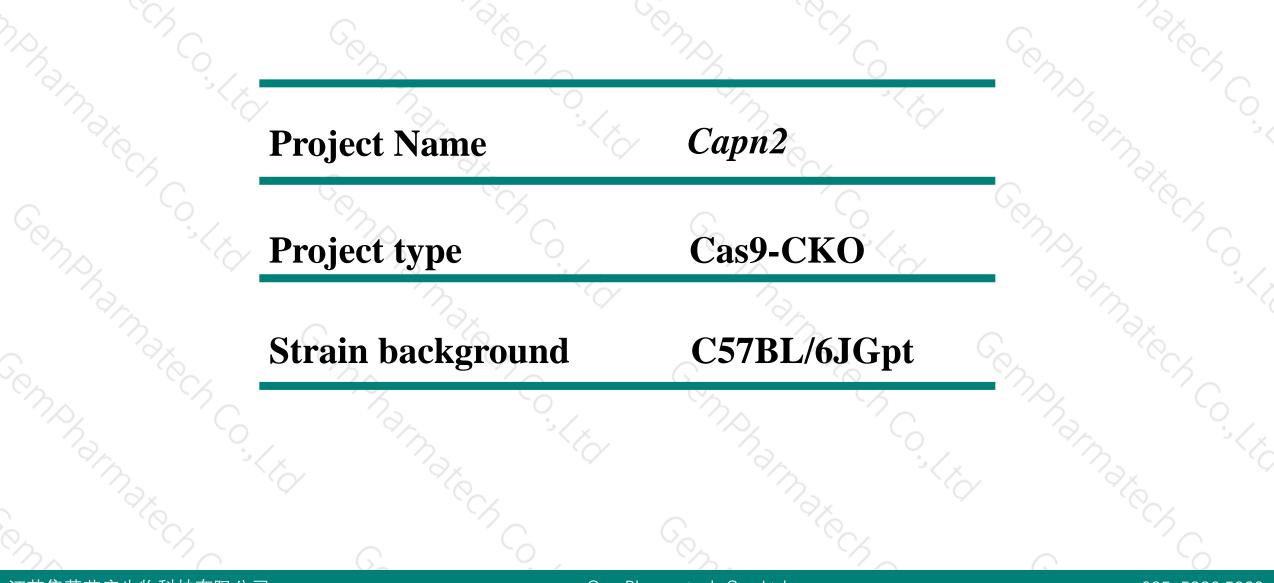
Capn2 Cas9-CKO Strategy

Designer: Design Date: Bingxuan Li 2019-9-5

Project Overview





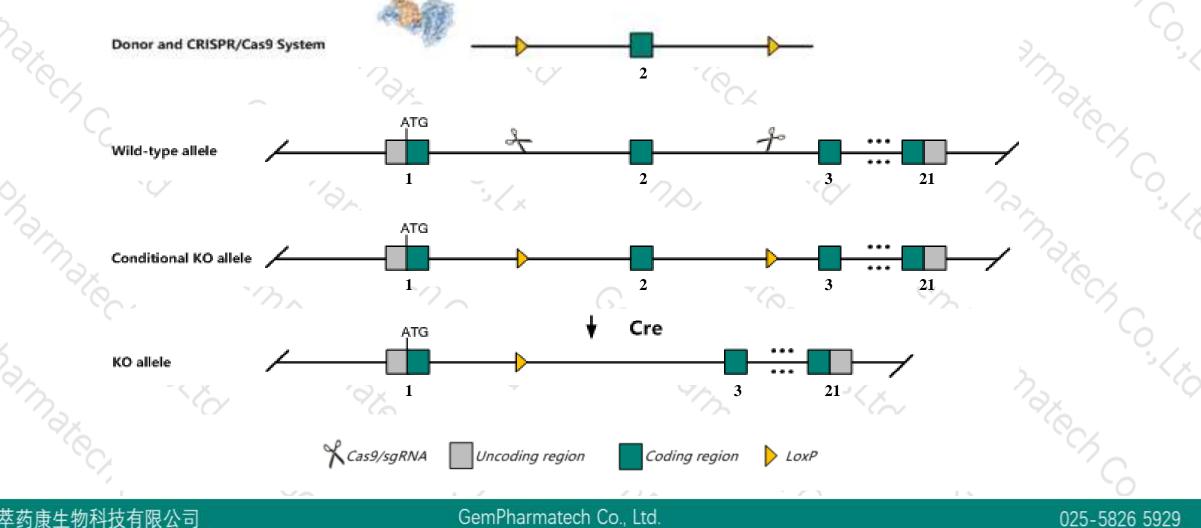
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Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Capn2* gene. The schematic diagram is as follows:



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- The Capn2 gene has 5 transcripts. According to the structure of Capn2 gene, exon2 of Capn2-201 (ENSMUST00000068505.9) transcript is recommended as the knockout region. The region contains 70bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Capn2* 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



025-5826 5929

- According to the existing MGI data, Homozygous inactivation of this gene leads to complete prenatal lethality. Mice homozygous for one null allele display placental dysfunction, thin ventricular walls, and peripheral vessel failure.
- The Capn2 gene is located on the Chr1. 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)



2 1

025-5826 5929

Capn2 calpain 2 [Mus musculus (house mouse)]

Gene ID: 12334, updated on 12-Aug-2019

🔺 Summary

Official Symbol	Capn2 provided by MGI										
Official Full Name	calpain 2 provided by <u>MGI</u>										
Primary source	MGI:MGI:88264										
See related	Ensembl:ENSMUSG0000026509										
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	Capa2; CALP80; Capa-2; Al326419; m-calpin; m-calpain										
Expression	Ubiquitous expression in bladder adult (RPKM 35.3), subcutaneous fat pad adult (RPKM 29.4) and 27 other tissues <u>See more</u>										
Orthologs	human all										
1 VX											

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Transcript information (Ensembl)

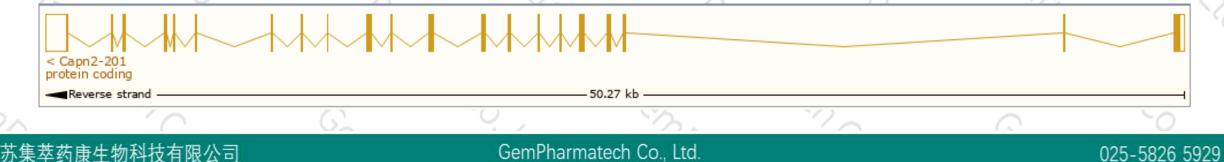


The gene has 5 transcripts, all transcripts are shown below:

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	Snownide	e columns (1 hidden)		Filter					
	Name 🍦	Transcript ID 🛛 🔺	bp 🌲	Protein 🖕	Biotype 🍦	CCDS 🍦	UniProt 🍦	Flags	*
	Capn2-201	ENSMUST0000068505.9	3225	<u>700aa</u>	Protein coding	<u>CCDS35813</u> &	<u>008529</u> 🗗	TSL:1 GENCODE basic	APPRIS P1
	Capn2-202	ENSMUST00000192230.5	2366	No protein	Retained intron	-	-	TSL:1	
2	Capn2-203	ENSMUST00000192483.1	3017	No protein	Retained intron	-	-	TSL:1	
	Capn2-204	ENSMUST00000194940.5	580	No protein	Retained intron	-	-	TSL:3	
	Capn2-205	ENSMUST00000194961.1	728	No protein	Retained intron	-	-	TSL:1	

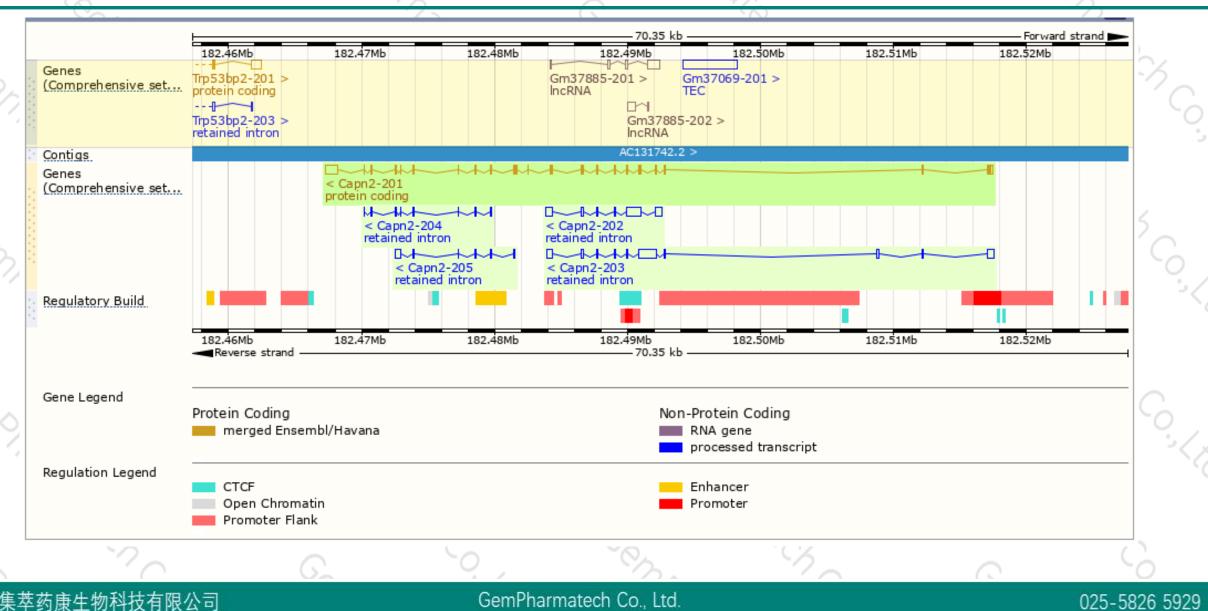
The strategy is based on the design of Capn2-201 transcript, The transcription is shown below



Genomic location distribution

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Protein domain



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	Prints	Peptidase C2, ca	nain family											
	Pfam		, calpain, catal	ytic domain			Peptid	ase C2, calpain	, large subunit	t, domain III				2
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		Calpain-2												
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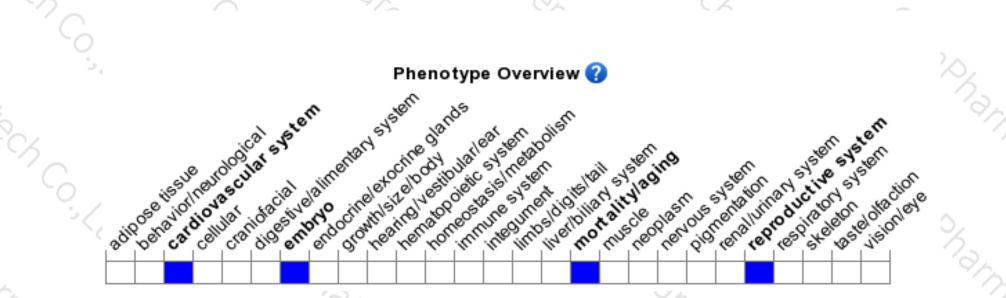
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025-5826 5929

Mouse phenotype description(MGI)



025-5826 5929



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 inactivation of this gene leads to complete prenatal lethality. Mice homozygous for one null allele display placental dysfunction, thin ventricular walls, and peripheral vessel failure.



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



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