

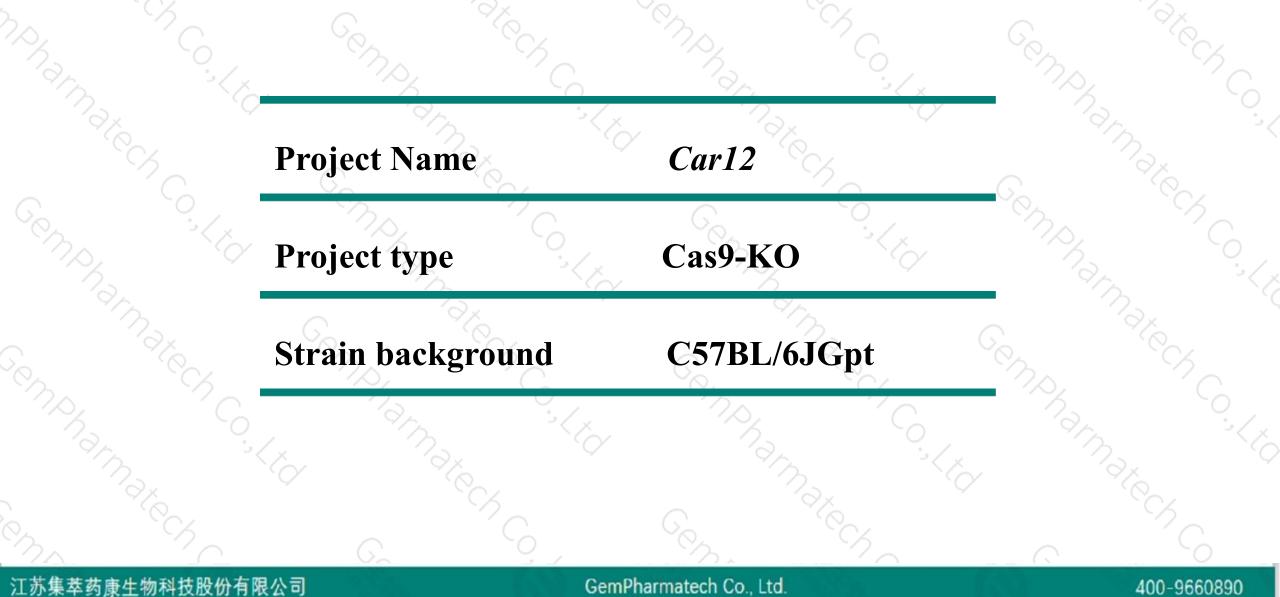
Car12 Cas9-KO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-02-12

0

Project Overview

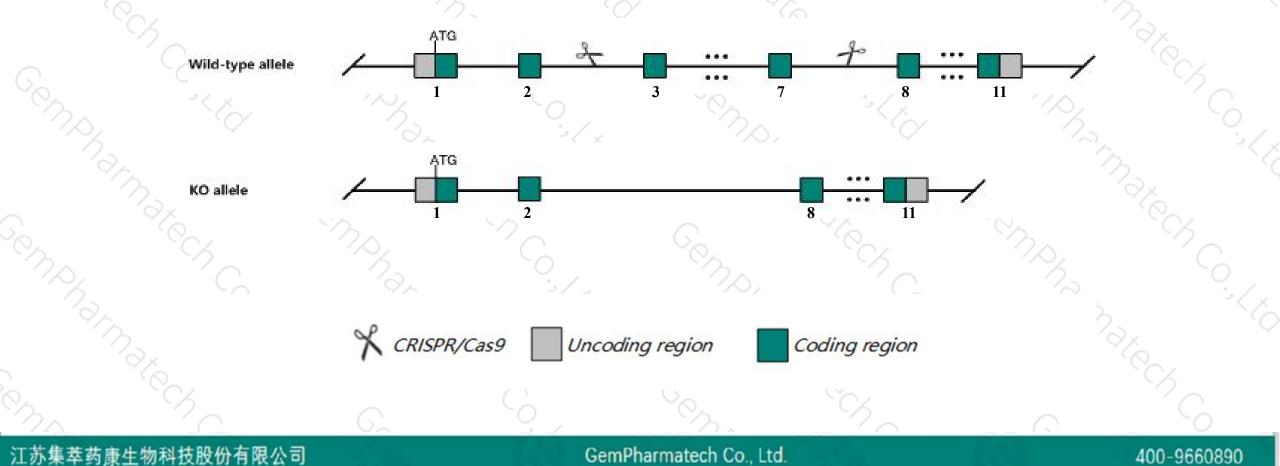




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Car12 gene. The schematic diagram is as follows:





- The Car12 gene has 6 transcripts. According to the structure of Car12 gene, exon3-exon7 of Car12-201 (ENSMUST00000071889.12) transcript is recommended as the knockout region. The region contains 644bp coding sequence. Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify Car12 gene. The brief process is as follows: CRISPR/Cas9 system

- According to the existing MGI data, Mice homozygous for a transposon-induced mutation that inactivates this gene display reduced fitness.
- ➤ Transcript *Car12*-206 may not be affected.
- > The effect on transcript Car12-204 is unknown.
- The Car12 gene is located on the Chr9. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

Gene information (NCBI)

限公司

江苏



400-9660890

 Summary 					* ?					
Official Symbol	Car12 provided by MGI									
Official Full Name	carbonic anhydrase 12 provided b	y <u>MGI</u>								
Primary source	MGI:MGI:1923709									
See related	Ensembl:ENSMUSG0000003237	<u>73</u>								
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									
	Ca12; CA-XII; AI314958; 2310047E01Rik									
	This gene encodes a membrane	bound member of the alpha carbonic anhydrase fami	and the second se							
	This gene encodes a membrane bicarbonate. These proteins parti	bound member of the alpha carbonic anhydrase fami biological processes, including re	espiration, calcifica	tion, acid-base balance, bone resorption, and the forr						
Summary	This gene encodes a membrane- bicarbonate. These proteins parti of aqueous humor, cerebrospinal	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the forr						
Summary Expression	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	bound member of the alpha carbonic anhydrase fami biological processes, including re	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the forr						
Summary	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the forr						
Summary Expression Orthologs	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the forr	mation					
Summary Expression Orthologs	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the forr						
Summary Expression	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the forr	mation					
Summary Expression Orthologs	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the form script variants. [provided by RefSeq, Apr 2015]	mation					
Summary Expression Orthologs Genomic context Location: 9; 9 C	This gene encodes a membrane bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adu	-bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re fluid, saliva, and gastric acid. Alternative splicing resu	espiration, calcifica ults in multiple tran	tion, acid-base balance, bone resorption, and the form script variants. [provided by RefSeq, Apr 2015]	mation					
Summary Expression Orthologs Genomic context Location: 9; 9 C Exon count: 11	This gene encodes a membrane- bicarbonate. These proteins parti of aqueous humor, cerebrospinal Biased expression in kidney adul human all	bound member of the alpha carbonic anhydrase fami icipate in a variety of biological processes, including re- fluid, saliva, and gastric acid. Alternative splicing resu (RPKM 57.2), limb E14.5 (RPKM 7.3) and 5 other tis	espiration, calcifica ults in multiple tran ssues <u>See more</u>	tion, acid-base balance, bone resorption, and the forr script variants. [provided by RefSeq, Apr 2015] See Car12 in <u>Genome D</u>	mation					

GemPharmatech Co., Ltd.

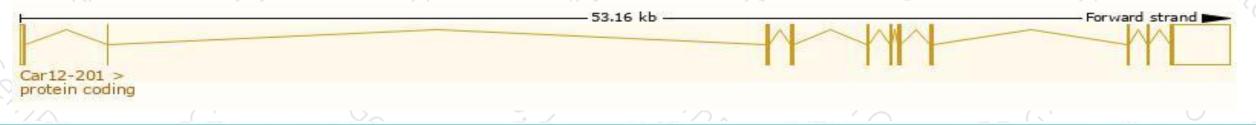
Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car12-201	ENSMUST00000071889.12	3716	<u>354aa</u>	Protein coding	CCDS23306	A0A0R4J0W4	TSL:1 GENCODE basic APPRIS P3
Car12-202	ENSMUST0000085420.11	3639	<u>344aa</u>	Protein coding	CCDS81026	<u>Q8K2J1</u>	TSL:1 GENCODE basic APPRIS ALT2
Car12-204	ENSMUST00000134829.1	614	<u>204aa</u>	Protein coding	0.20	F6W018	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:
Car12-206	ENSMUST00000217394.1	3263	No protein	Retained intron	1020	-	TSL:NA
Car12-205	ENSMUST00000152011.7	1652	No protein	Retained intron	(27)	5	TSL:2
Car12-203	ENSMUST00000123195.1	710	No protein	Retained intron		-	TSL:2

The strategy is based on the design of Car12-201 transcript, The transcription is shown below



江苏集萃药康生物科技股份有限公司

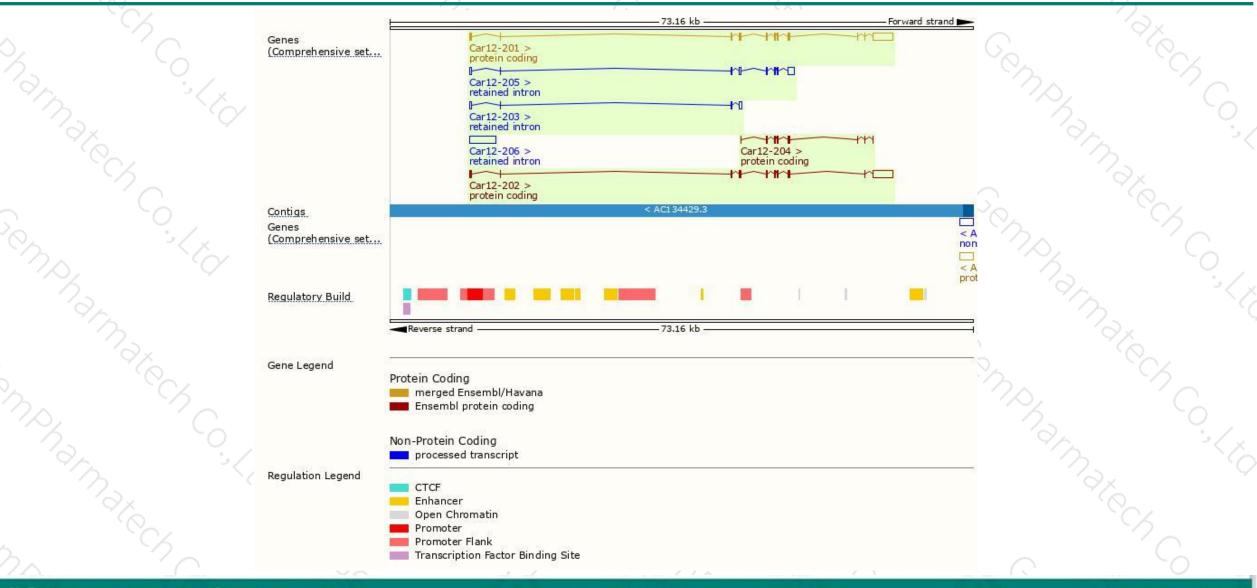
GemPharmatech Co., Ltd.

400-9660890

Genomic location distribution



400-9660890



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

Protein domain



						2	
ENSMUSP00000071		117 117	al.				
Transmembrane heli Low complexity (Seg)						-	– (
Cleavage site (Sign	()						
Superfamily		arbonic anhydrase dom	nain superfamily			-	
SMART	Alpha cr	arbonic anhydrase don	nain			-	
Pfam		arbonic anhydrase dom				-	
PROSITE profiles	Alpha ca	rbonic anhydrase dom	ain			-	C
PROSITE patterns	9.1			rase, alpha-class, cor	nserved site		0
PANTHER	Carbonic annyd	drase, CA-XII		and the second se			_
		drase, alpha-class					
Gene3D	and the second	rbonic anhydrase doma	ain superfamily				
All CMD=()	Wall School School School	ts (dbSNP and all oth					
All sequence SNPs/i							0
Variant Legend							و. ۲۰
	missense var						
Scale bar	0 40	80	120 160	200	240 24	180	354
Scare bai	0 10	C	120 100	YOZ	270	50	
10			5	· · · · · · · · · · · · · · · · · · ·			

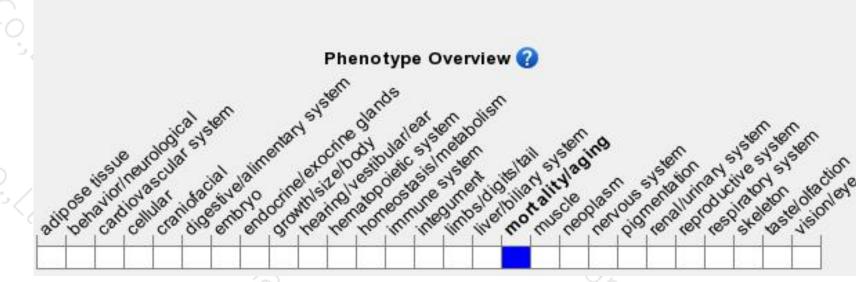
江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

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 a transposon-induced mutation that inactivates this gene display reduced fitness.



If you have any questions, you are welcome to inquire. Tel: 400-9660890



