

Heatr6 Cas9-CKO Strategy

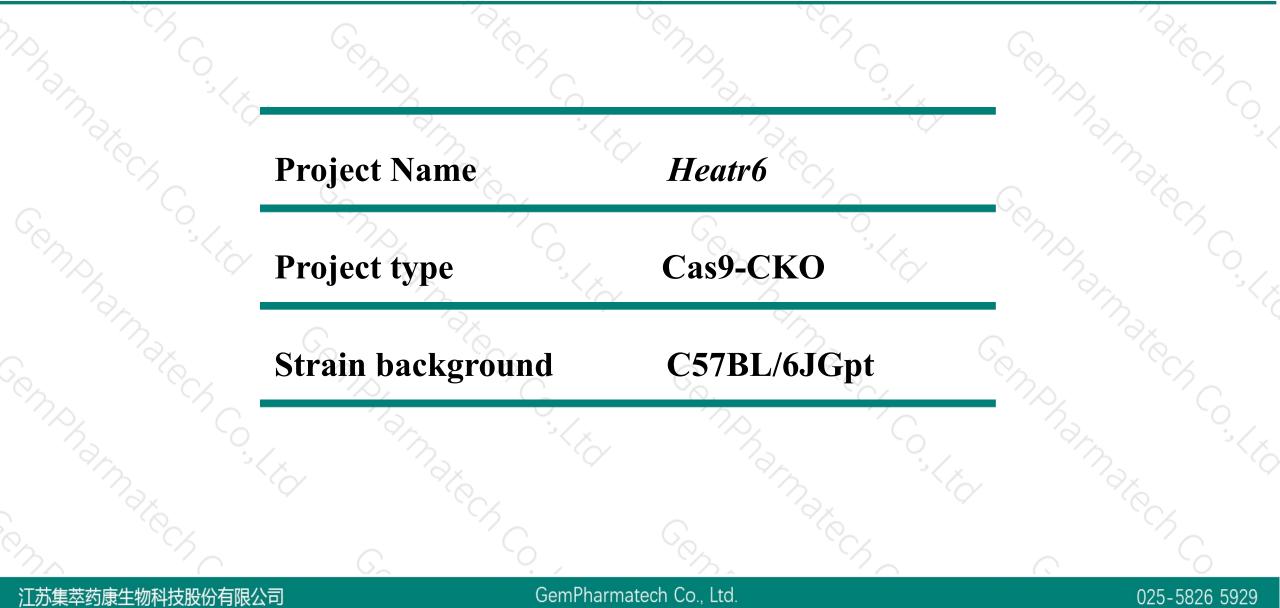
Designer: Jia Yu

Reviewer: Xiaojing Li

Design Date: 2020-11-12

Project Overview



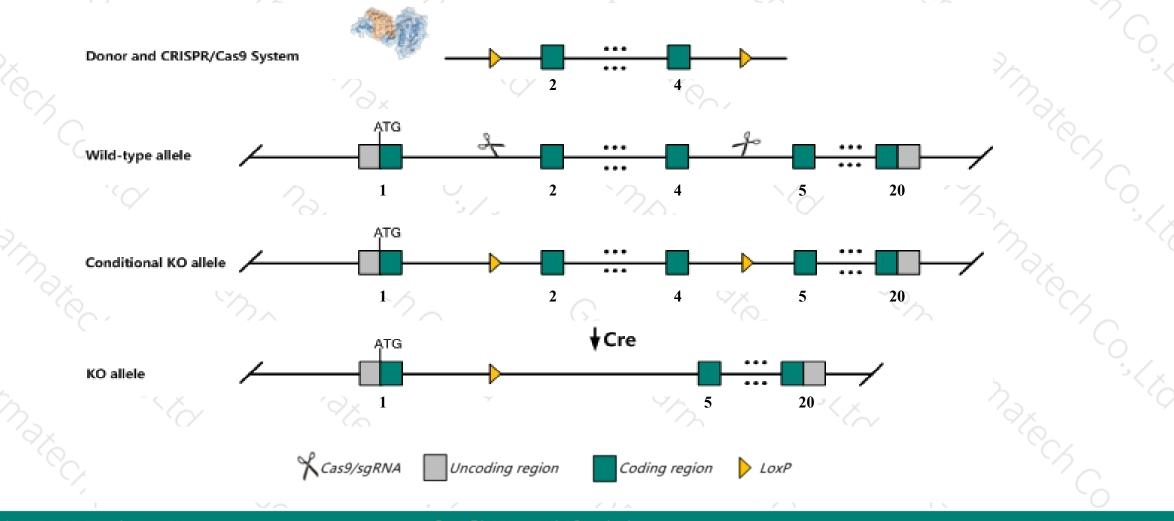


Conditional Knockout strategy



025-5826 5929

This model will use CRISPR/Cas9 technology to edit the *Heatr6* gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.



025-5826 5929

The *Heatr6* gene has 5 transcripts. According to the structure of *Heatr6* gene, exon2-exon4 of *Heatr6*-201(ENSMUST0000001002.7) transcript is recommended as the knockout region. The region contains 365bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Heatr6* 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.



- > The *Heatr6* gene is located on the Chr11. 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)



< ?

025-5826 5929

Heatr6 HEAT repeat containing 6 [Mus musculus (house mouse)]

Gene ID: 217026, updated on 13-Mar-2020

Summary

Official Symbol	Heatr6 provided by MGI
Official Full Name	HEAT repeat containing 6 provided by MGI
Primary source	MGI:MGI:1919790
See related	Ensembl:ENSMUSG0000000976
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	2700008B19Rik, BB135312, BB249351
Expression	Ubiquitous expression in CNS E14 (RPKM 7.8), whole brain E14.5 (RPKM 7.8) and 28 other tissues <u>See more</u>
Orthologs	human all

GemPharmatech Co., Ltd.

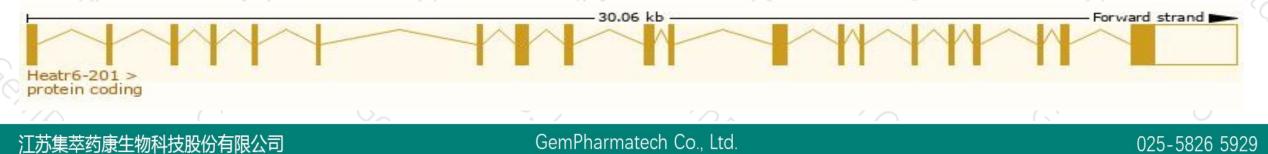
Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Heatr6-201	ENSMUST0000001002.7	5614	<u>1184aa</u>	Protein coding	CCDS36259	<u>Q6P1G0</u>	TSL:1 GENCODE basic APPRIS P1
Heatr6-203	ENSMUST00000125831.7	4067	No protein	Processed transcript	-	-	TSL:1
Heatr6-205	ENSMUST00000136572.1	522	No protein	Processed transcript	-	-	TSL:2
Heatr6-202	ENSMUST00000125602.1	357	No protein	Processed transcript	-	-	TSL:3
Heatr6-204	ENSMUST00000127428.1	709	No protein	Retained intron	-	-	TSL:2

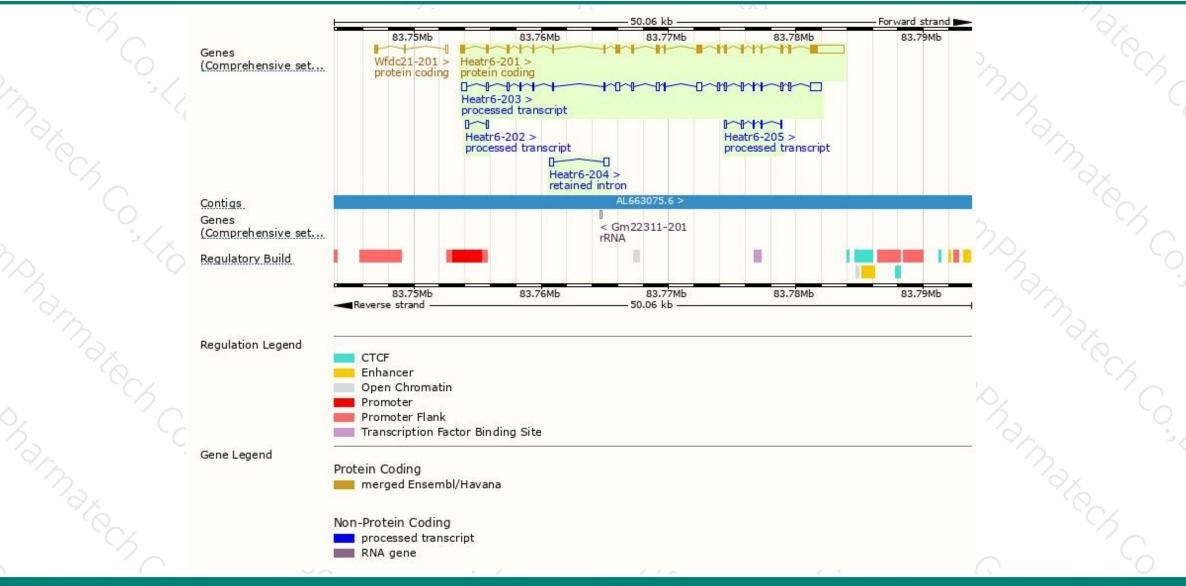
The strategy is based on the design of *Heatr6-201* transcript, the transcription is shown below:



Genomic location distribution



025-5826 5929

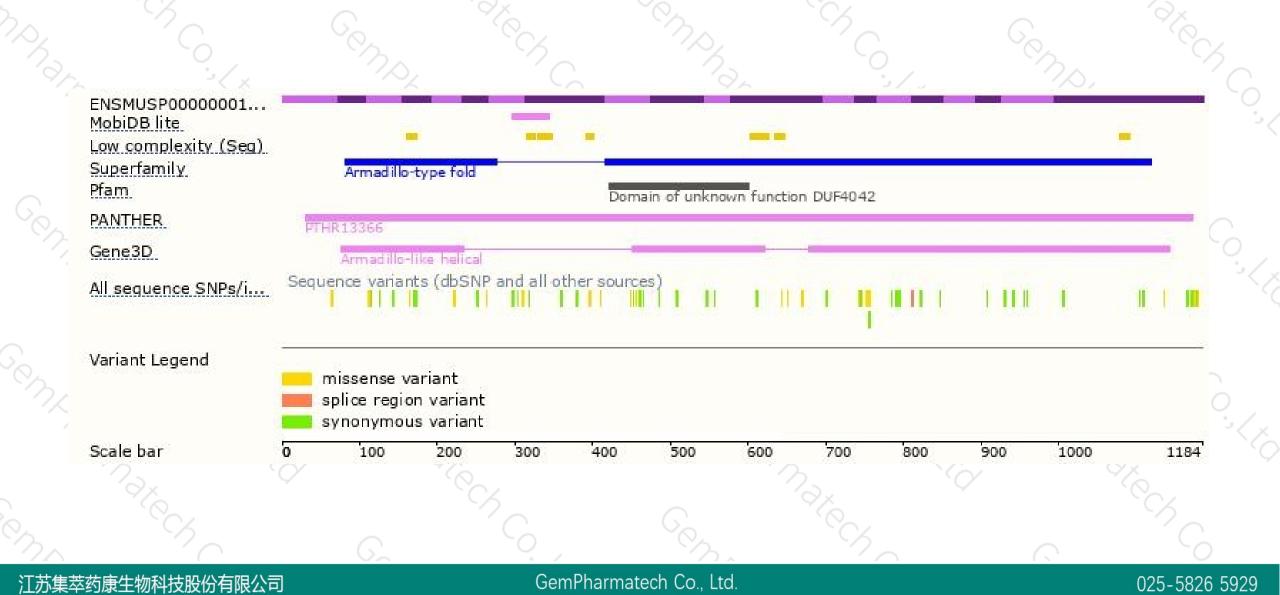


江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

Protein domain







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



