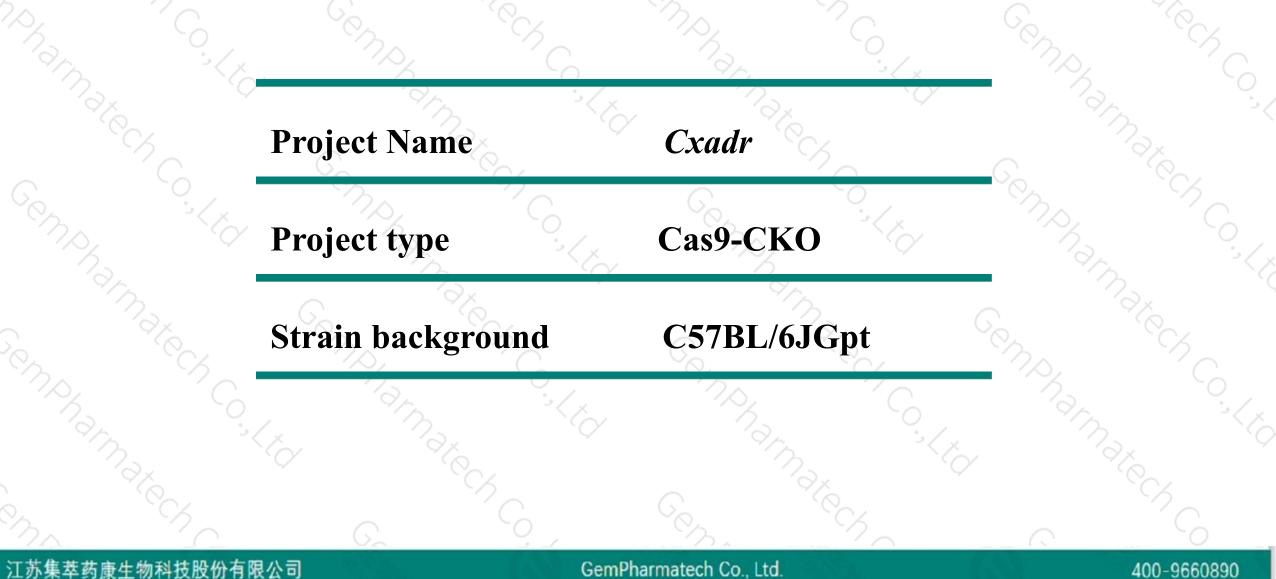


Cxadr Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2019-8-22

Project Overview



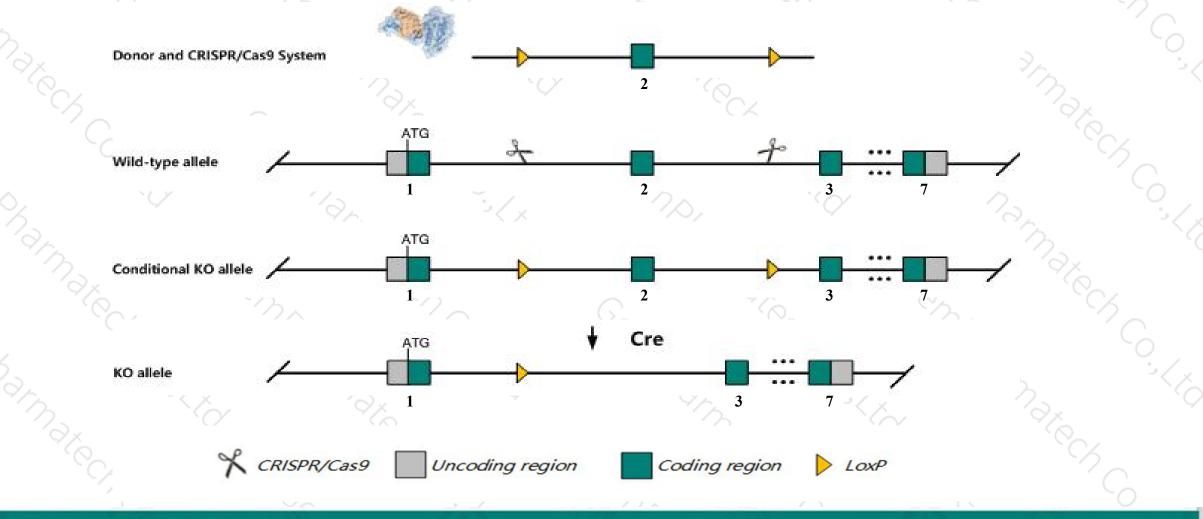


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Conditional Knockout strategy



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



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The Cxadr gene has 6 transcripts. According to the structure of Cxadr gene, exon2 of Cxadr-201 (ENSMUST00000023572.14) transcript is recommended as the knockout region. The region contains 167bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cxadr* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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.



- According to the existing MGI data, Homozygous null mice display embryonic lethality with focal cardiomyocyte apoptosis and extensive thoracic hemorrhaging.
 - The *Cxadr* gene is located on the Chr16. 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)



Cxadr coxsackie virus and adenovirus receptor [Mus musculus (house mouse)]

Gene ID: 13052, updated on 19-Feb-2019

Summary

Official Symbol Cxadr provided by MGI Official Full Name coxsackie virus and adenovirus receptor provided by MGI Primary source MGI:MGI:1201679 See related Ensembl:ENSMUSG00000022865 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 2610206D03Rik, AU016810, AW553441, CAR, MCAR, MCVADR Summary This gene encodes a protein that is part of the Cortical Thymocyte marker in Xenopus (CTX) subfamily within the immunoglobulin superfamily. Members of this subfamily, predominantly expressed on the surface of endothelial and epithelial cells, help establish cell polarity and provide a barrier function, regulating migration of immune cells. This protein, first identified as the receptor for adenovirus subgroup C and coxsakieviruses group B, is developmentally regulated and plays an important role in cardiac development. Alternative splicing results in multiple transcript variants that encode different protein isoforms. [provided by RefSeq, Jan 2013] Expression Broad expression in CNS E18 (RPKM 24.6), whole brain E14.5 (RPKM 17.4) and 23 other tissuesSee more Orthologs human all

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

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cxadr-201	ENSMUST00000023572.14	5742	<u>365aa</u>	Protein coding	CCDS28276	P97792	TSL:1 GENCODE basic APPRIS P3
Cxadr-202	ENSMUST00000114229.3	1657	<u>352aa</u>	Protein coding	CCDS37379	<u>P97792</u>	TSL:1 GENCODE basic APPRIS ALT2
Cxadr-204	ENSMUST00000231356.1	1298	<u>164aa</u>	Protein coding	(44)	P97792	GENCODE basic
Cxadr-205	ENSMUST00000232148.1	650	<u>136aa</u>	Protein coding	825	A0A338P6N1	CDS 3' incomplete
Cxadr-203	ENSMUST00000231251.1	688	<u>52aa</u>	Nonsense mediated decay	(73)	A0A338P6U1	CDS 5' incomplete
Cxadr-206	ENSMUST00000232189.1	678	No protein	Retained intron	691		

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

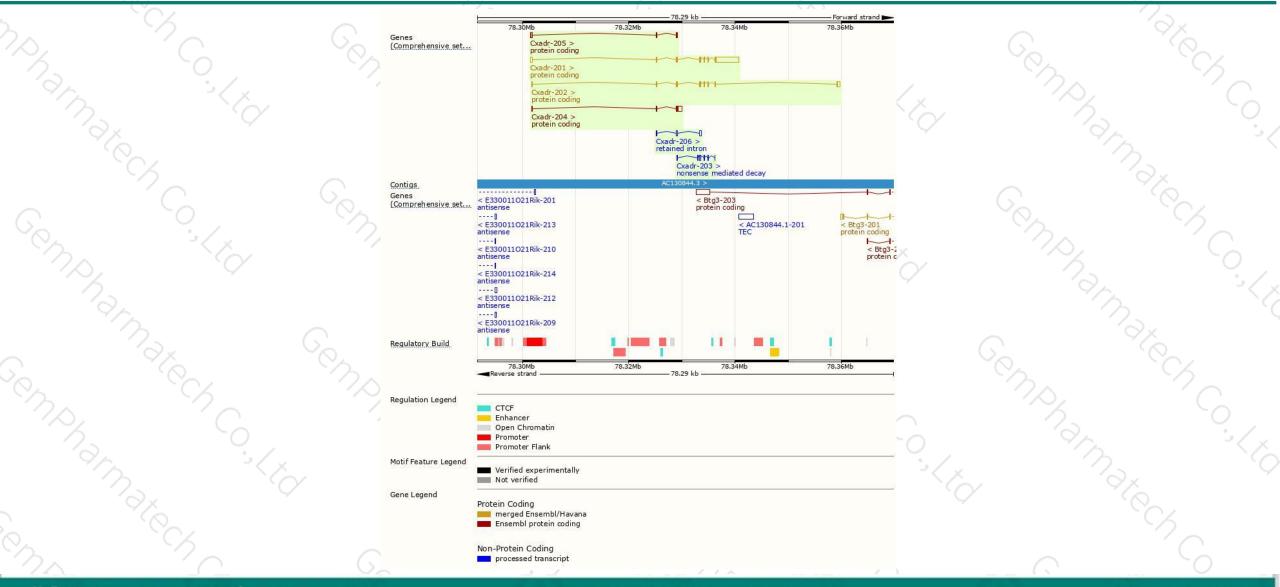


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Genomic location distribution



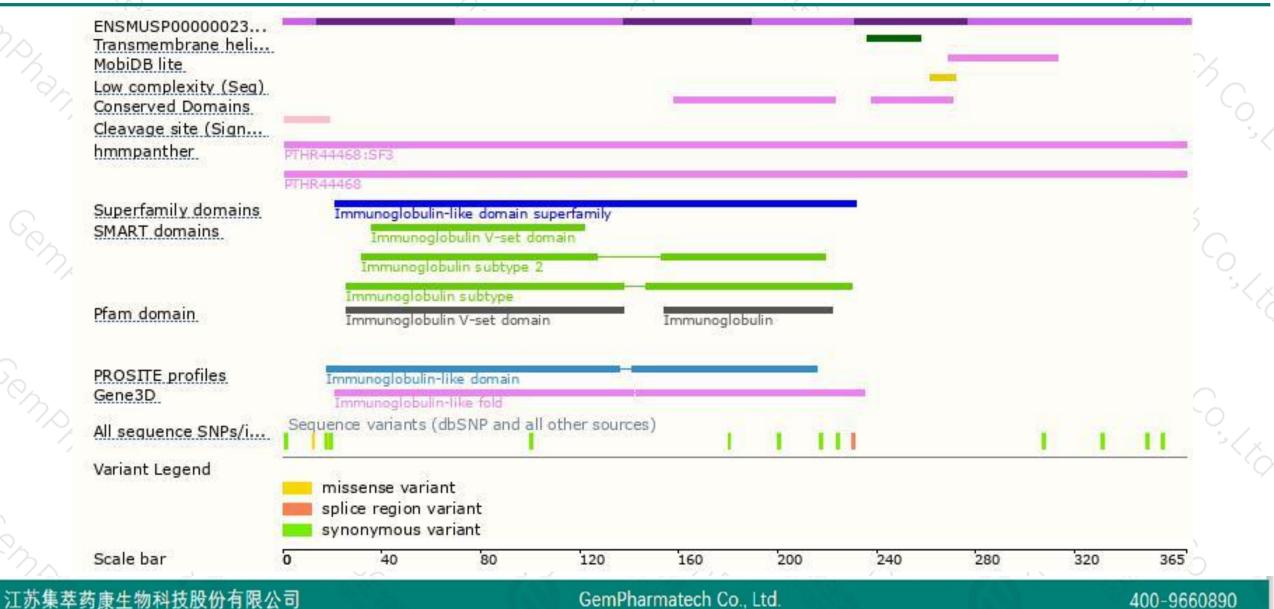


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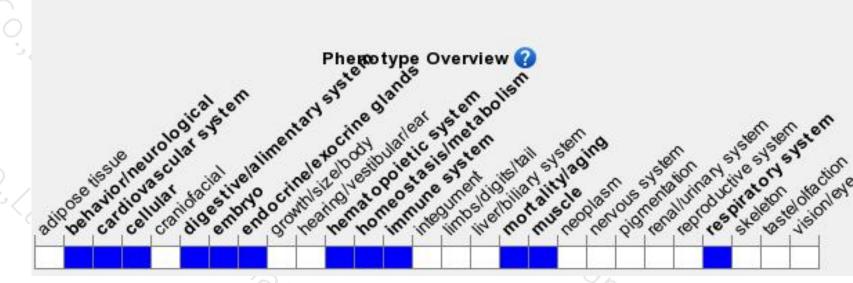
Protein domain





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, Homozygous null mice display embryonic lethality with focal cardiomyocyte apoptosis and extensive thoracic hemorrhaging.



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



