

Cdc42 Cas9-CKO Strategy

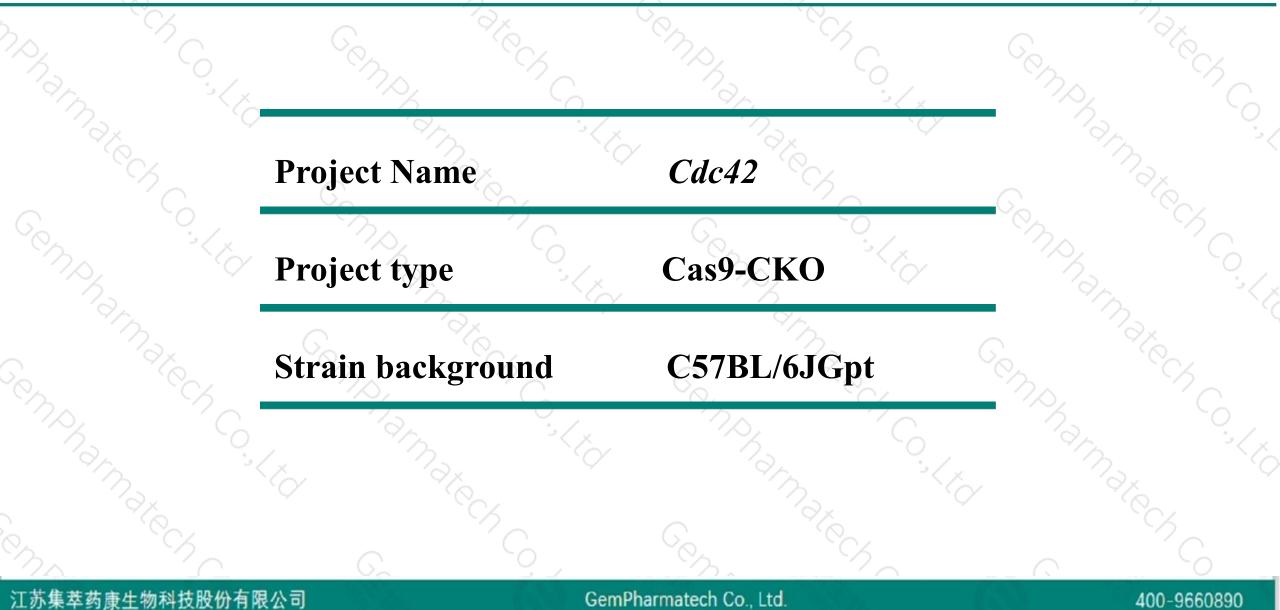
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-11-26

Project Overview



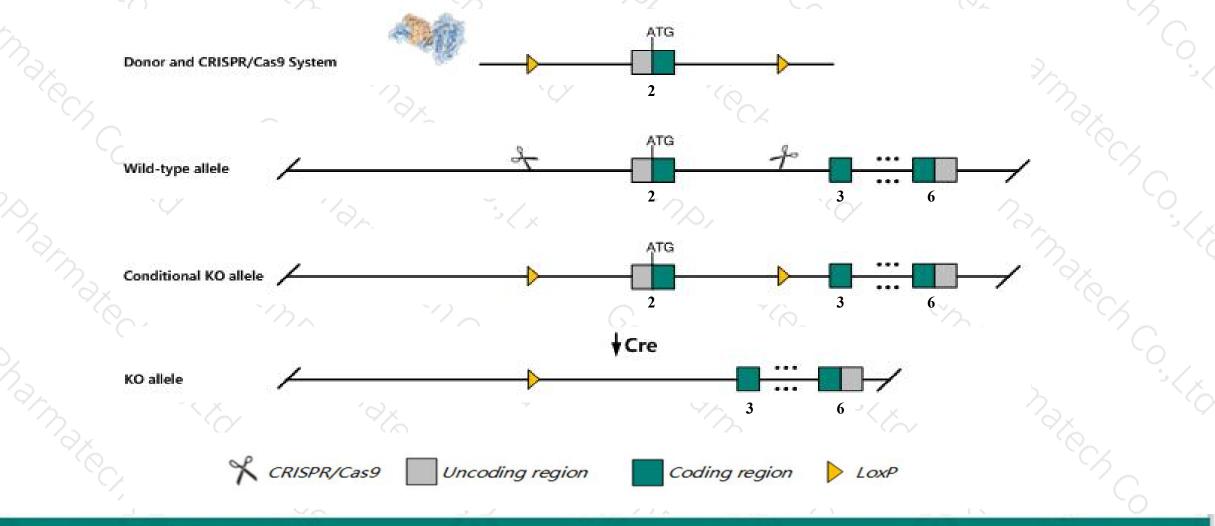


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Cdc42 gene. The schematic diagram is as follows:



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The Cdc42 gene has 2 transcripts. According to the structure of Cdc42 gene, exon2 of Cdc42-202 (ENSMUST00000051477.12) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Cdc42 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, Embryos homozygous for a knock-out allele are small, lack primary ectoderm, exhibit disorganized embryonic tissue and die before somite formation. Mice homozygous for a gene trapped allele die at E3.5-E7.5. Mice heterozygous for a targeted allele show reduced ventricle muscle contractility.
- The Cdc42 gene is located on the Chr4. 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)



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Cdc42 cell division cycle 42 [Mus musculus (house mouse)]

Gene ID: 12540, updated on 7-Apr-2019

Summary

Official Symbol	Cdc42 provided by MGI
Official Full Name	cell division cycle 42 provided byMGI
Primary source	MGI:MGI:106211
See related	Ensembl:ENSMUSG0000006699
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	AI747189, AU018915
Expression	Ubiquitous expression in CNS E18 (RPKM 107.5), placenta adult (RPKM 89.1) and 26 other tissues See more
Orthologs	human all

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The gene has 2 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdc42-202	ENSMUST00000051477.12	2108	<u>191aa</u>	Protein coding	CCDS18816	P60766	TSL:1 GENCODE basic APPRIS P3
Cdc42-201	ENSMUST0000030417.9	1448	<u>191aa</u>	Protein coding	CCDS57305	P60766	TSL:2 GENCODE basic APPRIS ALT1

The strategy is based on the design of Cdc42-202 transcript, The transcription is shown below

< Cdc42-202 protein coding

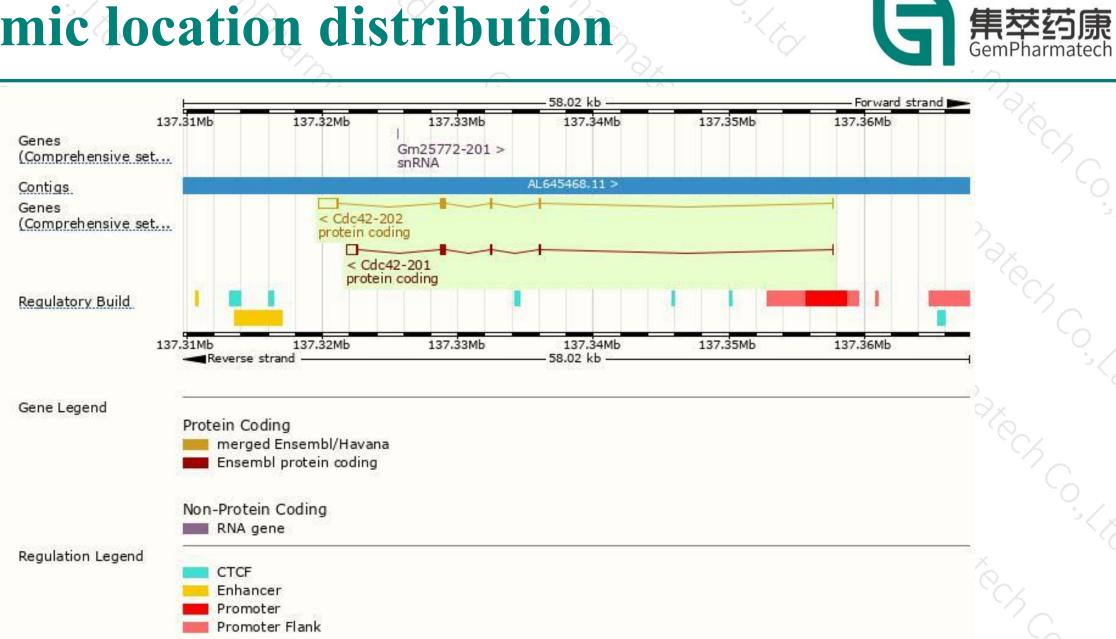
Reverse strand

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38.02 kb

Genomic location distribution



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Protein domain



ENSMUSP00000054.. SIFTS import TIGRFAM

Superfamily SMART

Prints Pfam PROSITE profiles PANTHER

Gene3D CDD

Scale bar

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All sequence SNPs/i...

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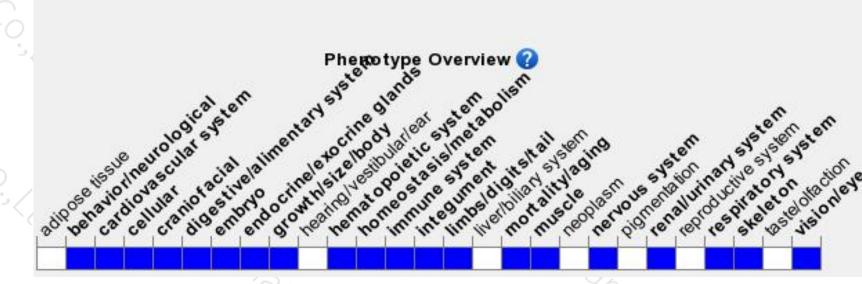
Variant Legend

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SM0017 PR004 Smal	and the second	ł						-		C
Small G	TPase Rho									0.3
PTHR.24	072:SF296								2	
PTHR24	1072									
3,40,50	300									
Cdc42										ò
Sequence variants (dbSNP and all other sources)										~
	ssense varia nonymous v									
SV										

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Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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



