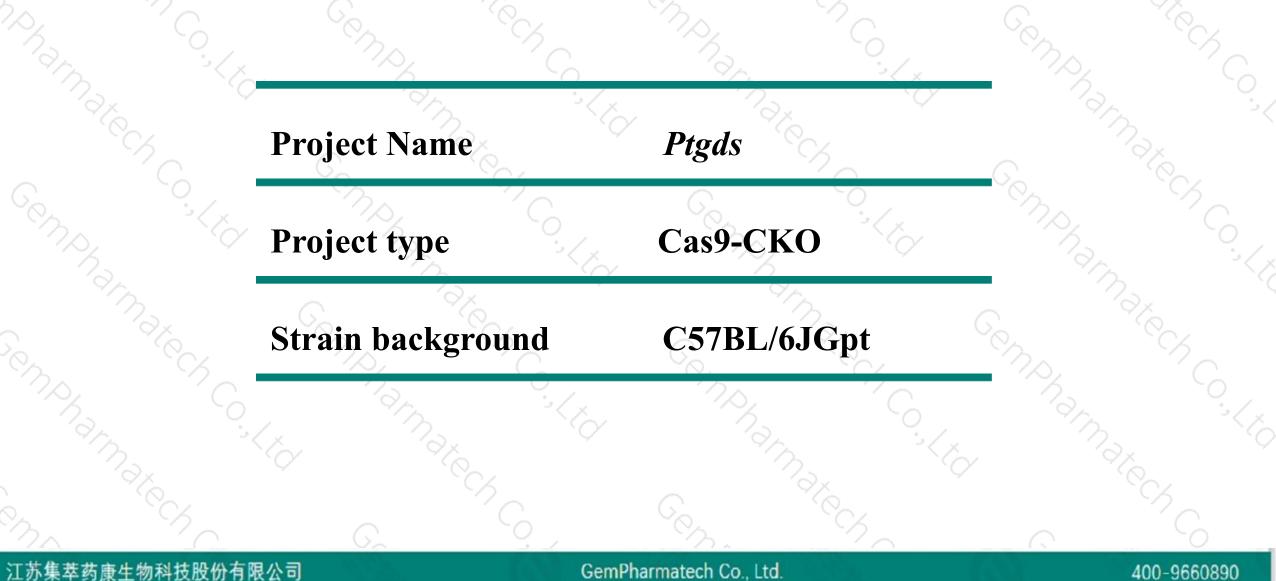


Ptgds Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-10-27

Project Overview





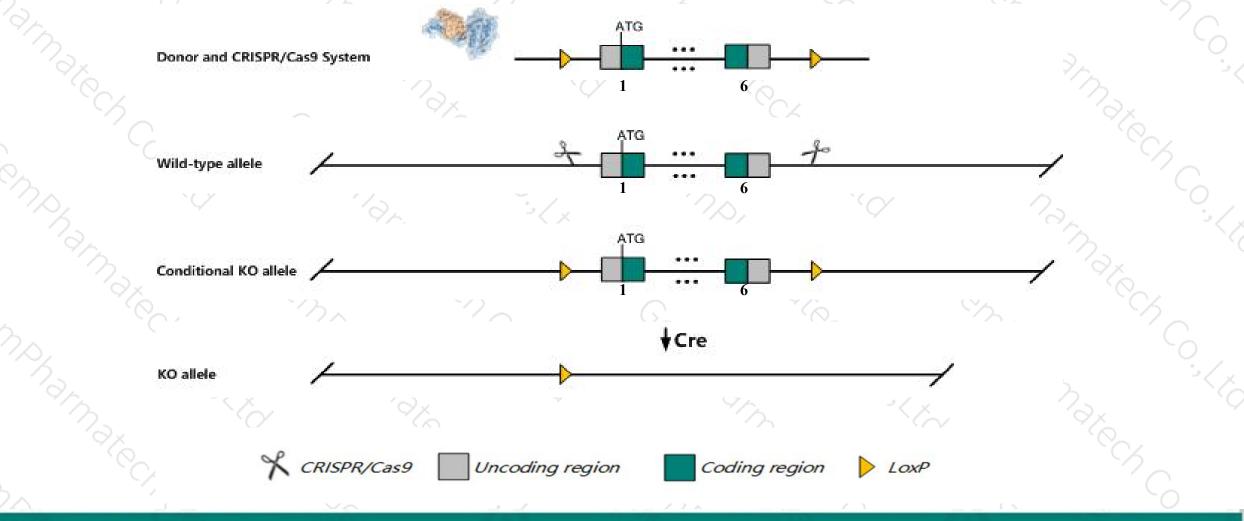
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Conditional Knockout strategy



400-9660890

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



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The Ptgds gene has 5 transcripts. According to the structure of Ptgds gene, exon1-exon6 of Ptgds-201 (ENSMUST00000015234.12) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ptgds* 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, Mice homozygous for one knock-out allele fail to exhibit PGE2- and bicuculline-induced allodynia and exhibit decreased susceptibility to IgE-induced PCA. Mice homozygous for another knock-out allele show normal induction of muscle injury after reperfusion of ischemic skeletal muscle.
 The floxed region is near to the N-terminal of *Paxx* gene, this strategy may influence the regulatory function of the N-terminal of *Paxx* gene.
- The *Ptgds* gene is located on the Chr2. 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)



Summary	
Official Symbol	Ptgds provided by MGI
Official Full Name	prostaglandin D2 synthase (brain) provided by MGI
Primary source	MGI:MGI:99261
See related	Ensembl:ENSMUSG0000015090
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
	PGD2; PGDS; 21kDa; PGDS2; Ptgs3; L-PGDS
	Biased expression in genital fat pad adult (RPKM 446.8), frontal lobe adult (RPKM 410.4) and 5 other tissues See more
Orthologs	human all
Genomic context	

Annotation release	Status	Assembly	Chr	Location	2
<u>108</u>	current	GRCm38.p6 (GCF_000001635.26)	2	NC_000068.7 (2546670925470110, complement)	- (
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	2	NC_000068.6 (2532223225325269, complement)	

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Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ptgds-201	ENSMUST0000015234.12	901	<u>189aa</u>	Protein coding	CCDS38074	009114	TSL:1 GENCODE basic APPRIS P1
Ptgds-202	ENSMUST00000114251.7	830	<u>189aa</u>	Protein coding	CCDS38074	009114	TSL:5 GENCODE basic APPRIS P1
Ptgds-203	ENSMUST00000114259.2	775	<u>189aa</u>	Protein coding	CCDS38074	009114	TSL:2 GENCODE basic APPRIS P1
Ptgds-205	ENSMUST00000144016.7	1327	No protein	Retained intron	1020	4	TSL:1
Ptgds-204	ENSMUST00000137417.1	645	No protein	IncRNA	1270		TSL:1

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

< Ptgds-201 protein coding

Reverse strand

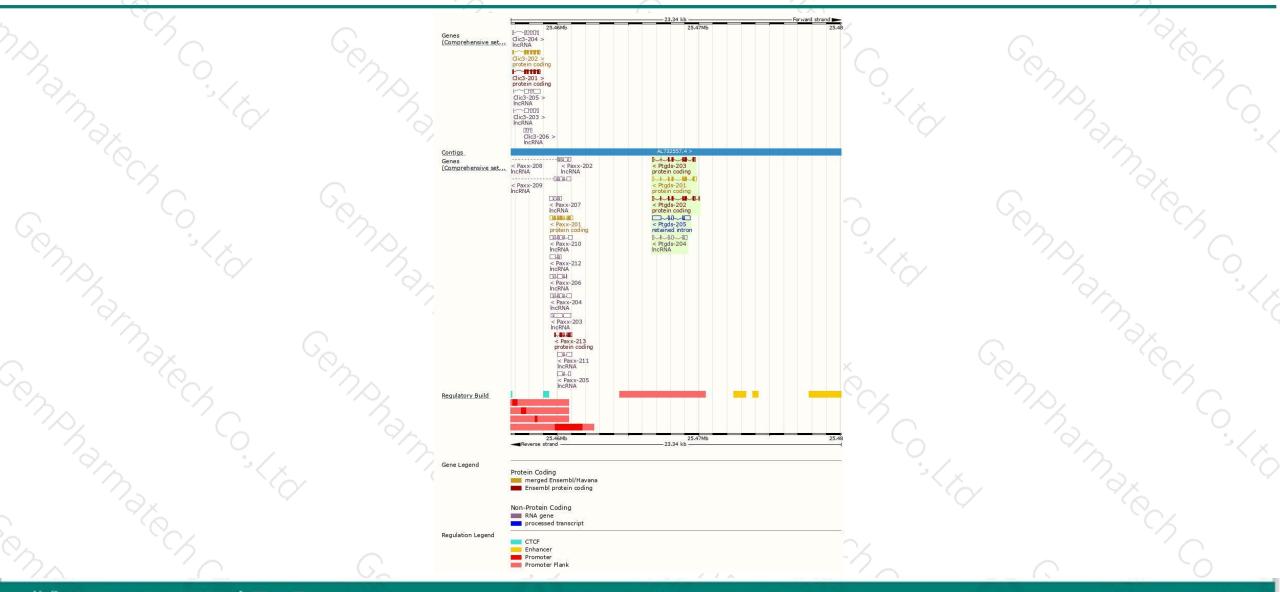
- 3.13 kb

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Genomic location distribution



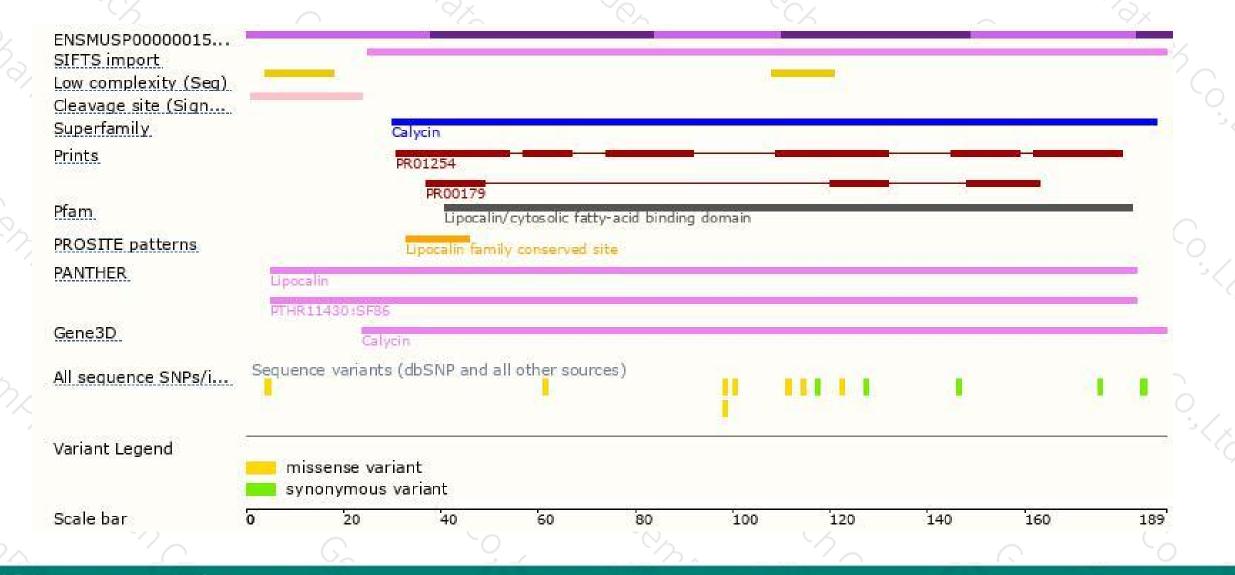


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Protein domain



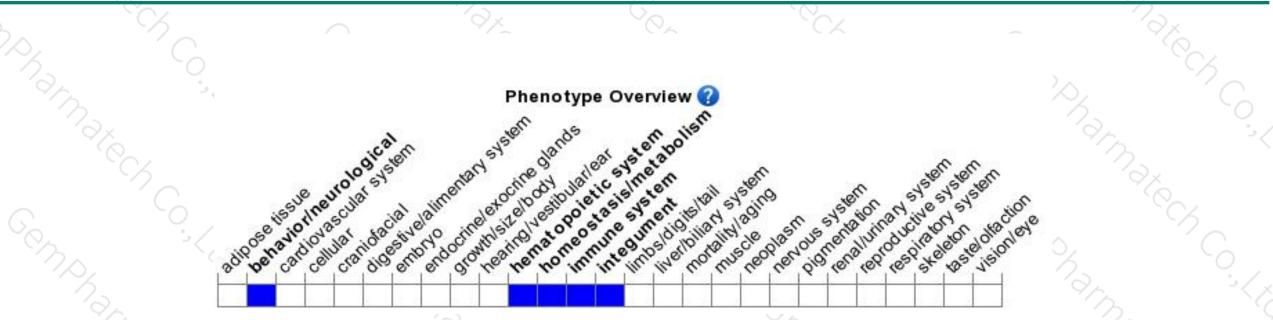


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Mouse phenotype description(MGI)





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

According to the existing MGI data, Mice homozygous for one knock-out allele fail to exhibit PGE2- and bicuculline-induced allodynia and exhibit decreased susceptibility to IgE-induced PCA. Mice homozygous for another knock-normal induction of muscle injury after reperfusion of ischemic skeletal muscle.

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



