

Plekho1 Cas9-CKO Strategy

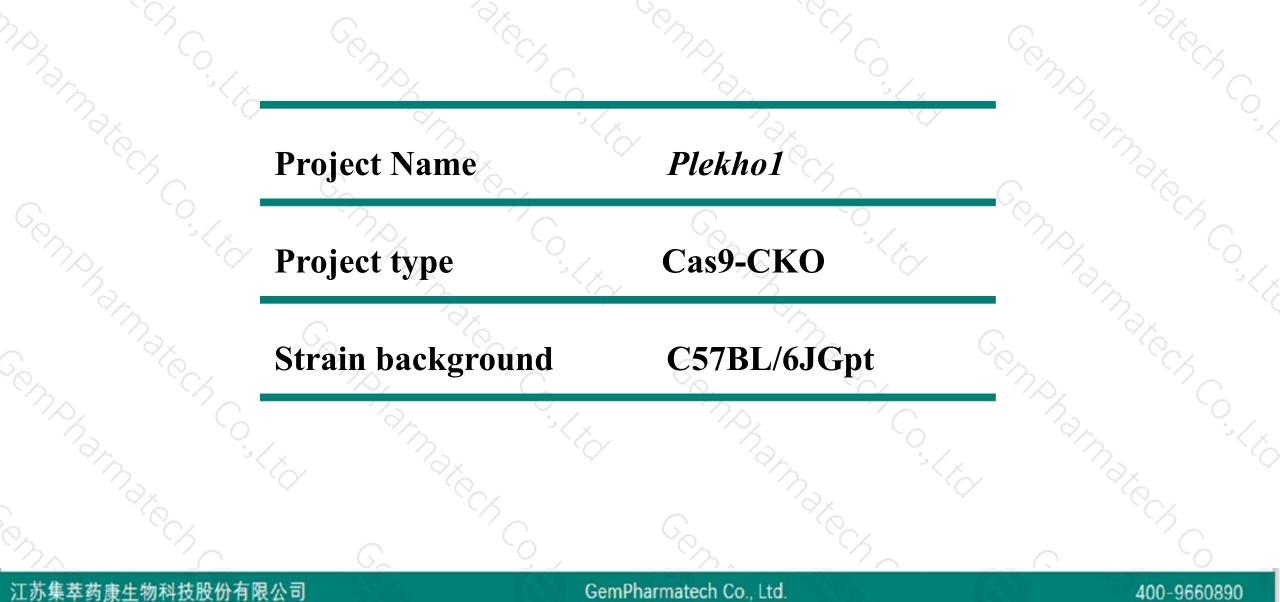
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2020-2-14

Project Overview



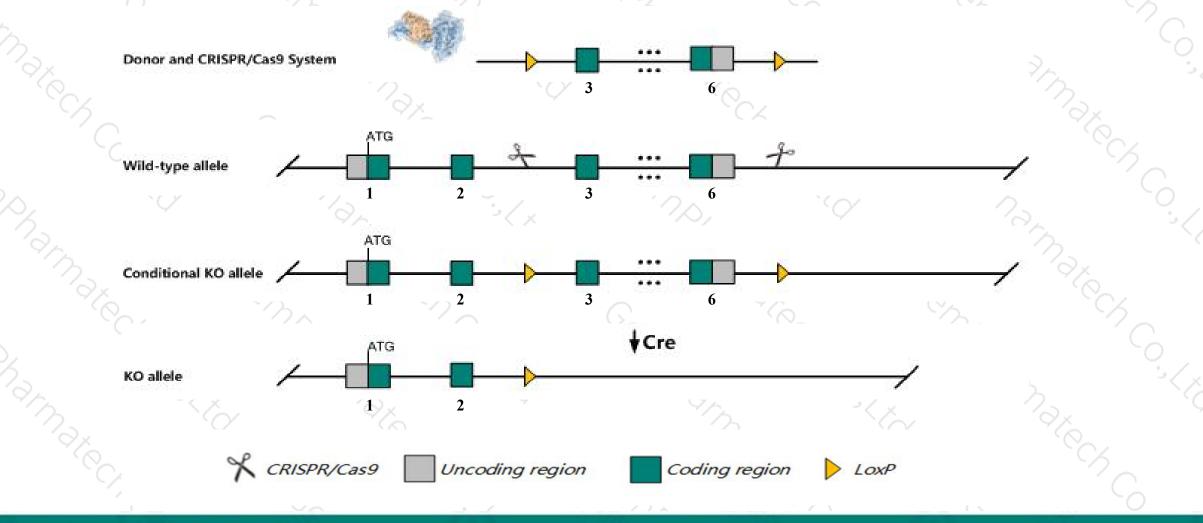


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Plekho1* 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.



The Plekho1 gene has 5 transcripts. According to the structure of Plekho1 gene, exon3-exon6 of Plekho1-201 (ENSMUST00000015889.9) transcript is recommended as the knockout region. The region contains most 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 *Plekho1* 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 a null allele exhibit age-dependent increase in bone volume and increased osteoblast activity.
- The *Plekho1* gene is located on the Chr3. 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)



\$?

Plekho1 pleckstrin homology domain containing, family O member 1 [Mus musculus (house mouse)]

Gene ID: 67220, updated on 17-Feb-2019

Summary

Official Symbol	Plekho1 provided by MGI
Official Full Name	pleckstrin homology domain containing, family O member 1 provided by MGI
Primary source	MGI:MGI:1914470
See related	Ensembl:ENSMUSG0000015745
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810052M02Rik, CKIP-1, Ckip1, JZA-20, Jza2
Expression	Ubiquitous expression in bladder adult (RPKM 51.0), CNS E11.5 (RPKM 47.2) and 27 other tissues See more
Orthologs	human all

江苏集萃药康生物科技股份有限公司

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
Plekho1-201	ENSMUST00000015889.9	1931	<u>408aa</u>	Protein coding	CCDS17627	Q9JIY0	TSL:1 GENCODE basic APPRIS P1
Plekho1-202	ENSMUST00000123006.7	1208	<u>365aa</u>	Protein coding	8-	F6XQM2	CDS 5' incomplete TSL:5
Plekho1-203	ENSMUST00000130043.7	788	<u>262aa</u>	Protein coding	3 4	F6VV25	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:2
Plekho1-204	ENSMUST00000143485.1	441	<u>124aa</u>	Protein coding	<u>8</u>	D3YVD1	CDS 3' incomplete TSL:3
Plekho1-205	ENSMUST00000157043.1	362	No protein	Retained intron	15	3	TSL:2

The strategy is based on the design of *Plekho1-201* transcript, The transcription is shown below

< Plekho1-201 protein coding

Reverse strand

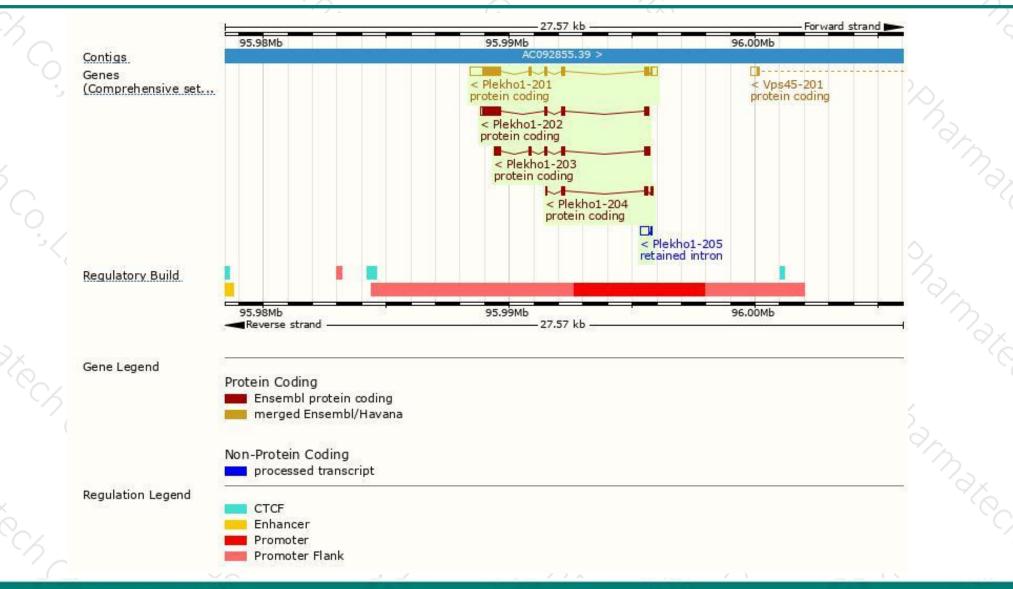
江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

7.57 kb

Genomic location distribution





<u>江苏集萃药康生物科技股份有限公司</u>

GemPharmatech Co., Ltd.

Protein domain



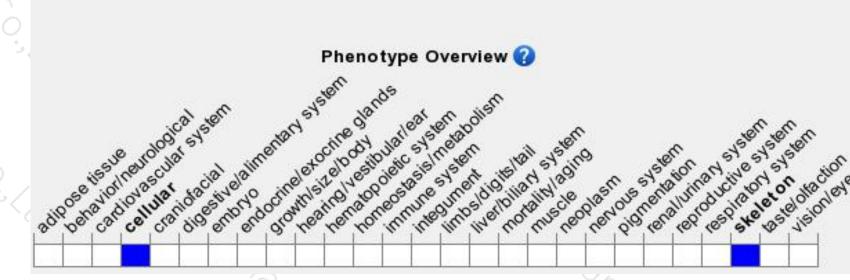
	20	<u> </u>						0		
₹,	ENSMUSP00000015 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils)		-			-				6.,
	Superfamily	SSF50729								
	SMART	Pleckstrin hom	nology domain							
	Pfam	Pleckstrin hor	nology domain							
	PROSITE profiles	Pleckstrin hom	ology domain						9	
	PANTHER	PTHR15871								ر بر / د
		Pleckstrin homology	domain-containin	g family O member 1						
	Gene3D	PH-like domain s	uperfamily							
	CDD	cd13317								
2	All sequence SNPs/i	Sequence variants	(dbSNP and all	l other sources)		11	ii.		0	
	Variant Legend	missense var							,	×č,
	Scale bar	0 40	80	120 160	200	240	280	320	360 408	
	5		0	No.		\sim				

江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

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 null allele exhibit age-dependent increase in bone volume and increased osteoblast activity.



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



