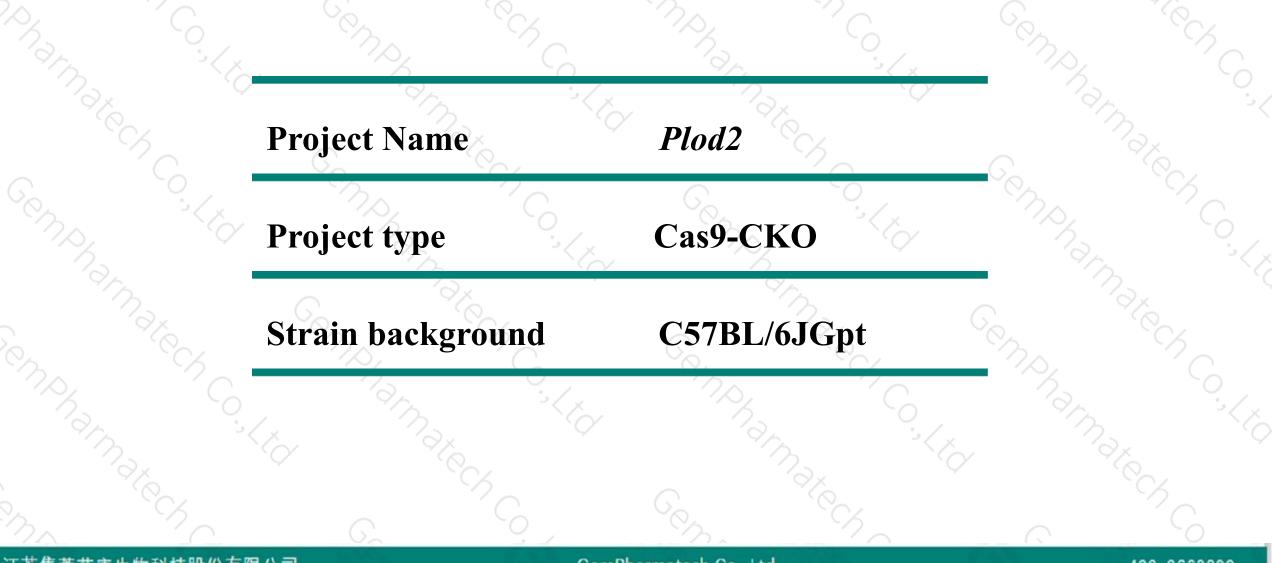


Plod2 Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-1-23

Project Overview





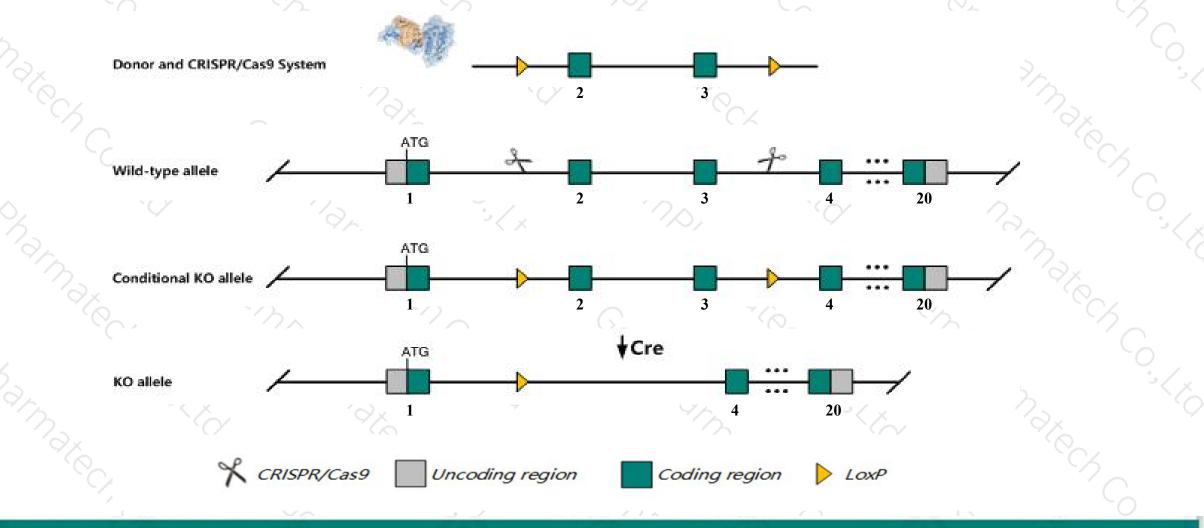
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Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Plod2* gene. The schematic diagram is as follows:



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The *Plod2* gene has 5 transcripts. According to the structure of *Plod2* gene, exon2-exon3 of *Plod2-203* (ENSMUST00000160359.1) transcript is recommended as the knockout region. The region contains 229bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Plod2* 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.



- The *Plod2* gene is located on the Chr9. 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)



\$?

Plod2 procollagen lysine, 2-oxoglutarate 5-dioxygenase 2 [Mus musculus (house mouse)]

Gene ID: 26432, updated on 31-Jan-2019

Summary

Official Symbol	Plod2 provided by MGI					
Official Full Name	rocollagen lysine, 2-oxoglutarate 5-dioxygenase 2 provided by <u>MGI</u>					
Primary source	<u>//GI:///GI:1347007</u>					
See related	Ensembl:ENSMUSG0000032374					
Gene type	protein coding					
RefSeq status	VALIDATED					
Organism	Mus musculus					
Lineage	ineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;					
	Muroidea; Muridae; Murinae; Mus; Mus					
Also known as	n as D530025C14Rik, LH2, Plod-2					
Expression	pression Broad expression in limb E14.5 (RPKM 27.1), adrenal adult (RPKM 20.7) and 16 other tissues See more					
Orthologs	human all					

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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	
Plod2-203	ENSMUST00000160359.1	3663	<u>758aa</u>	Protein coding	CCDS52887	E9Q718	TSL:1 GENCODE basic APPRIS ALT2	
Plod2-201	ENSMUST00000070522.13	3649	<u>737aa</u>	Protein coding	CCDS23408	<u>Q9R0B9</u>	TSL:1 GENCODE basic APPRIS P3	
Plod2-205	ENSMUST00000190075.1	1276	No protein	Retained intron	9	1220	TSL:NA	
Plod2-202	ENSMUST00000160142.1	844	No protein	Retained intron	2	120	TSL:5	
Plod2-204 ENSMUST00000161906.1		596	No protein	Retained intron	1		TSL:3	

66.16 kb -

The strategy is based on the design of *Plod2-203* transcript, The transcription is shown below

Plod2-203 > protein coding

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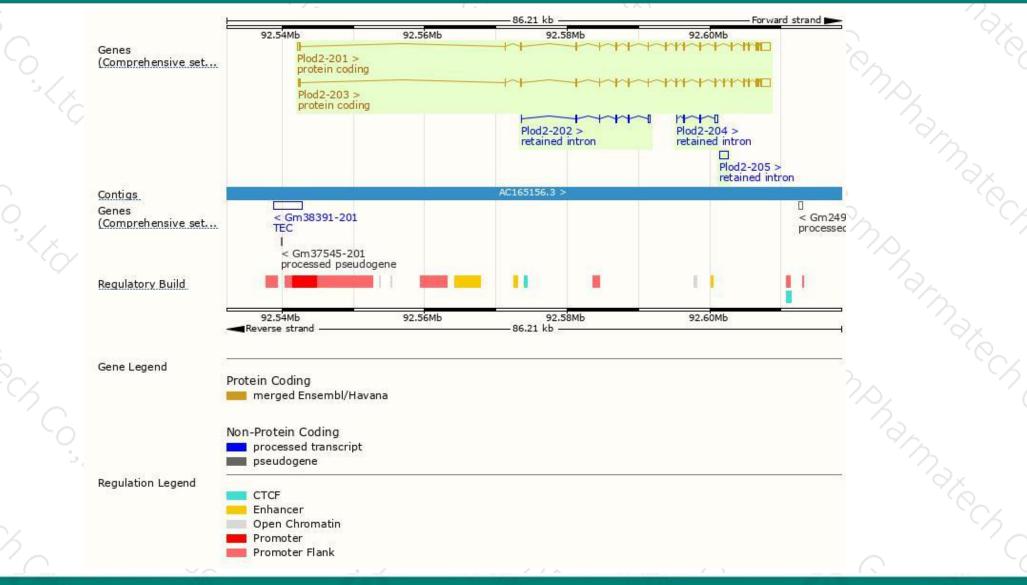
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400-9660890

Forward strand

Genomic location distribution





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Protein domain



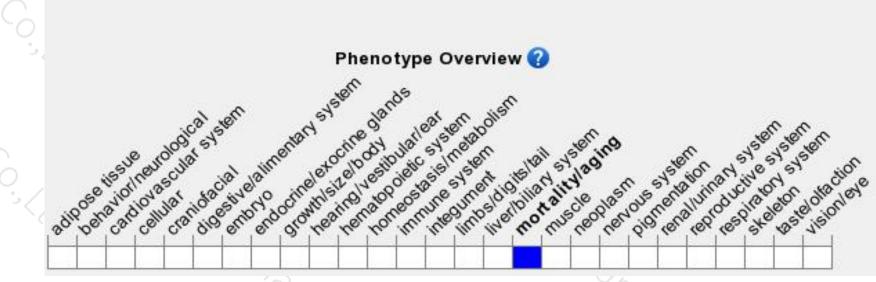
			°°C2	- ns.	10			
2	ENSMUSP00000125 Low complexity (Seg) Cleavage site (Sign Superfamily			Nucleotide	-diphospho-sugar tr	ansferases		
	SMART					Contraction of the local division of the loc	4-hydroxylase, alpha	sub
	Pfam.						Oxoglutarate	
	PROSITE profiles						Oxoglutarate/	
2	PROSITE patterns						Procollage	n-ly
	PANTHER	PTHR10730						- ~2
		PTHR10730:SF	6					
	Gene3D					2:60.3	120.620	
え	All sequence SNPs/i	Sequence variants	(dbSNP and all o	other sources)	1.01		1 0 1	6
	Variant Legend	missense var						
	Scale bar	0 80	160	240 320	400	480 560	640 7	58
	^C C/ _C		^C		, [°] °4			

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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/).



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



