

Lgals12 Cas9-CKO Strategy

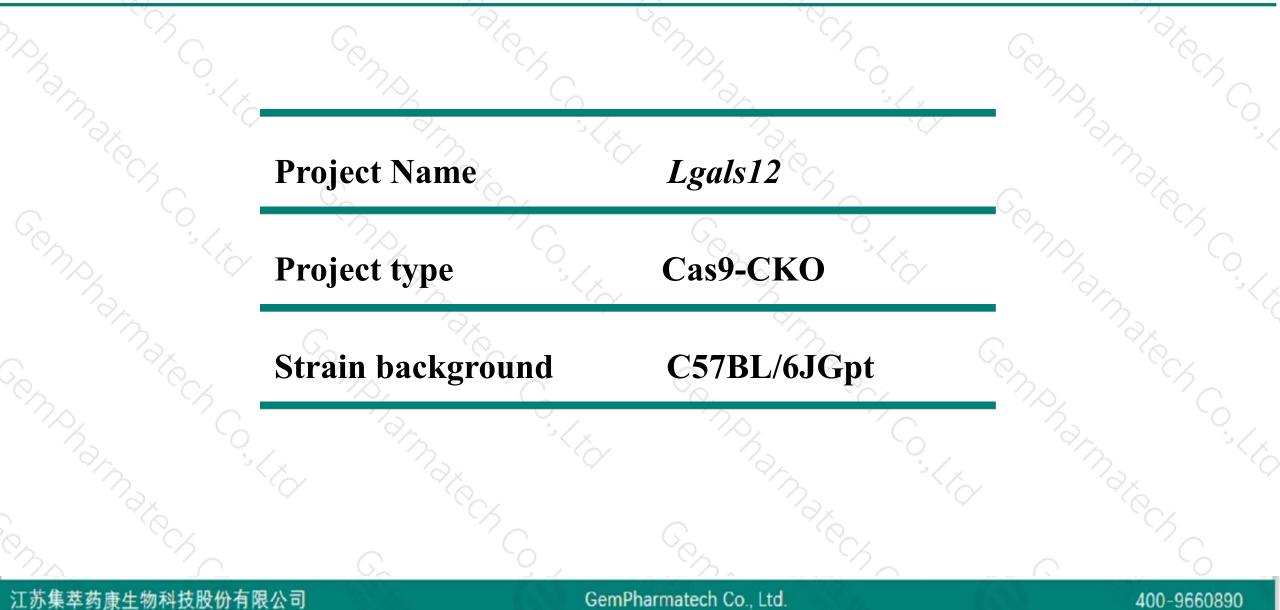
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Reviewer: Xiaojing Li

Design Date: 2020-8-14

Project Overview

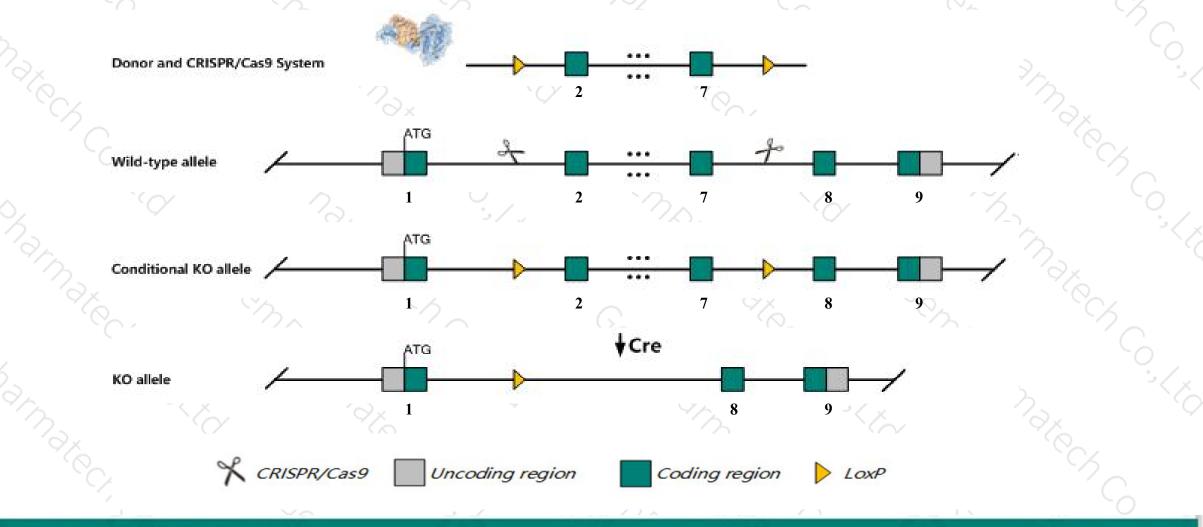




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Lgals12* gene. The schematic diagram is as follows:



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The Lgals12 gene has 3 transcripts. According to the structure of Lgals12 gene, exon2-exon7 of Lgals12-202(ENSMUST00000099729.9) transcript is recommended as the knockout region. The region contains 578bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify Lgals12 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 knock-out allele exhibit increased lipolysis, reduced adiposity, and enhanced insulin sensitivity.
- The Lgals12 gene is located on the Chr19. 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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Lgals12 lectin, galactose binding, soluble 12 [Mus musculus (house mouse)]

Gene ID: 56072, updated on 13-Mar-2020

Summary

Official Symbol	Lgals12 provided by MGI
Official Full Name	lectin, galactose binding, soluble 12 provided by MGI
Primary source	MGI:MGI:1929094
See related	Ensembl:ENSMUSG0000024972
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	Grip1, gal-12
Expression	Biased expression in subcutaneous fat pad adult (RPKM 28.5), mammary gland adult (RPKM 22.2) and 8 other tissues See
	more
Orthologs	human all

Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Lgals12-202	ENSMUST0000099729.9	2757	<u>314aa</u>	Protein coding	CCDS29529	<u>Q91VD1</u>	TSL:1 GENCODE basic APPRIS P1	
Lgals12-203	ENSMUST00000159983.7	2470	<u>314aa</u>	Protein coding	CCDS29529	<u>Q91VD1</u>	TSL:1 GENCODE basic APPRIS P1	
Lgals12-201	ENSMUST00000079902.5	2590	<u>301aa</u>	Protein coding	2	<u>Q91VD1</u>	TSL:1 GENCODE basic	

The strategy is based on the design of Lgals12-202 transcript, the transcription is shown below:

< Lgals12-202 protein coding

Reverse strand

– 10.52 kb –

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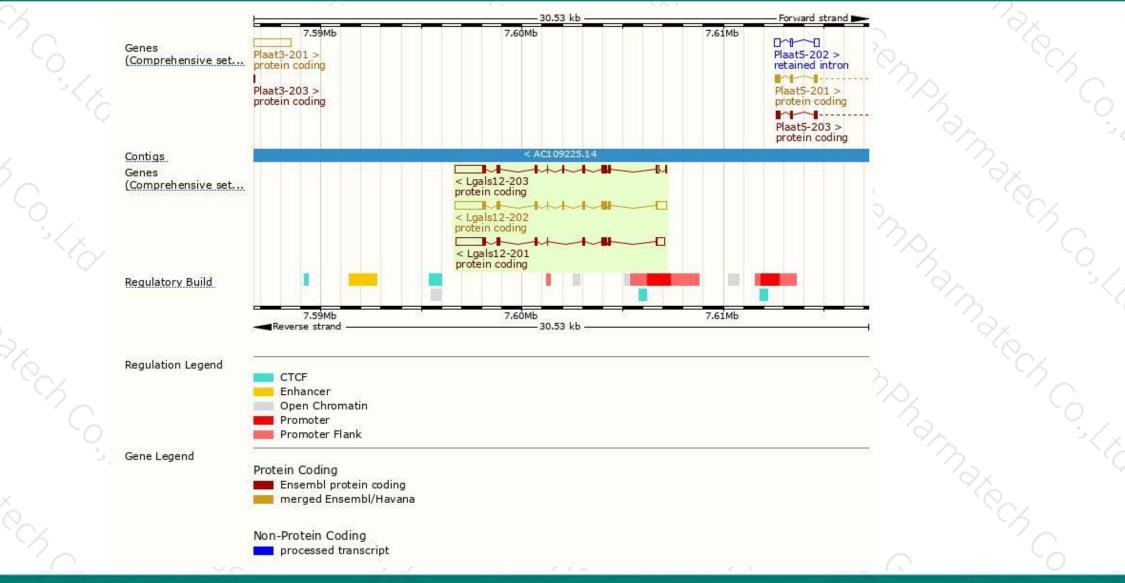
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Genomic location distribution



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Protein domain



ENSMUSP00000124	-		10	-	<u>).</u>	~0	· 2.	~?
Superfamily	C	oncanavalin A-I	ike lectin/glucanas	e domain superf	amily	-		
SMART	0	alectin, carbor	ydrate recognition	domain				
		2000	oohydrate recogniti					
<u>Pfam</u>		Galectin, carbo	ohydrate recognitio	n domain				
PROSITE profiles		Galectin, carbo	hydrate recognitio	n domain				
PANTHER	PTHR11346							
	Galectin-12							
Gene3D	2.6	0.120.200						
CDD_		and the second	nydrate recognition	n domain	0			
All sequence SNPs/i	Sequence	variants (db	SNP and all other	sources)	191	1	1	
Variant Legend		ense variant nymous varia	ant		-			0
Scale bar	0	40	80	120	160	200	240	314
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10				- V		20		

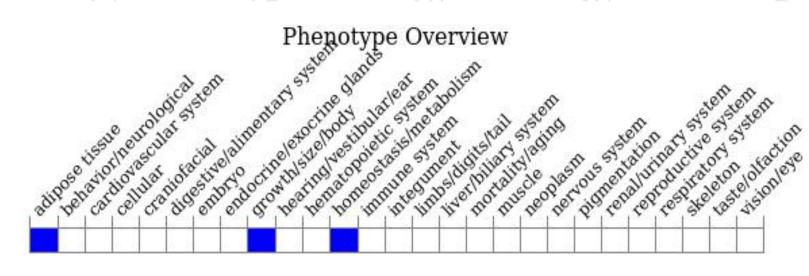
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Mouse phenotype description(MGI)





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 a knock-out allele exhibit increased lipolysis, reduced adiposity, and enhanced insulin sensitivity.



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



