

Klhl40 Cas9-CKO Strategy

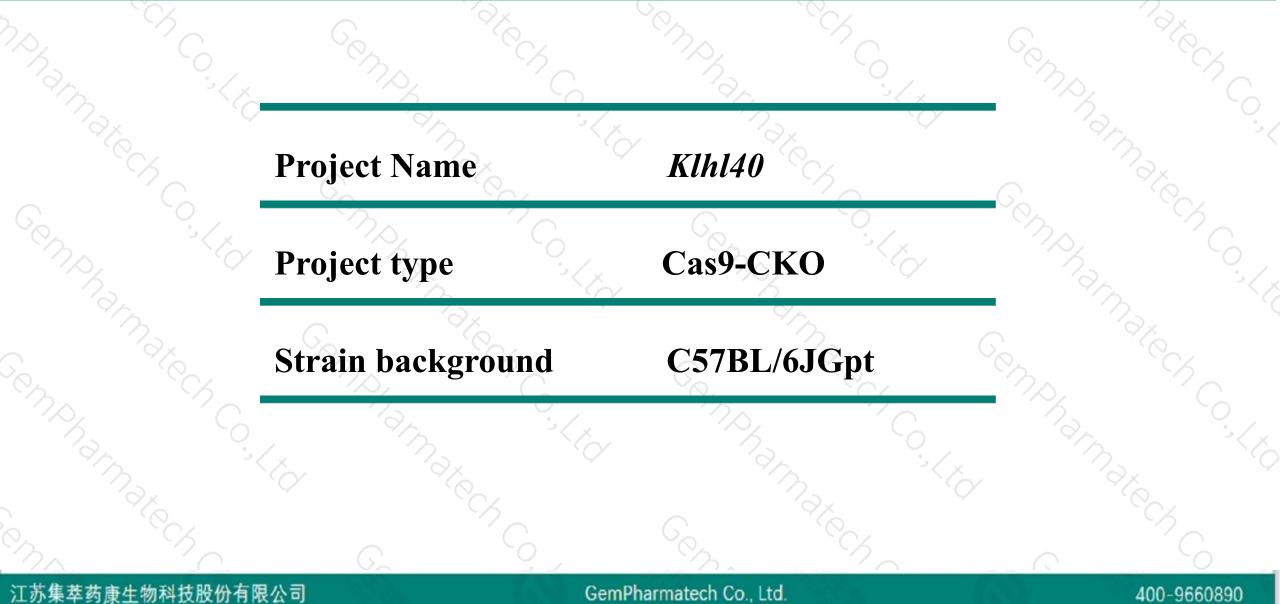
Designer: Reviewer:

Design Date:

Yanhua Shen Xueting Zhang 2019-12-18

Project Overview



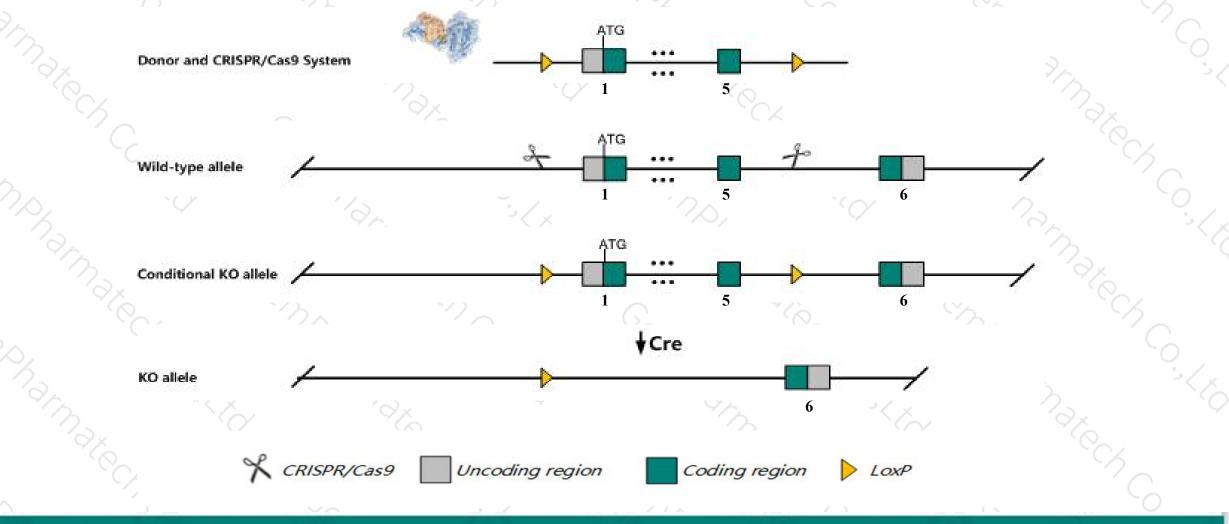


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Klhl40* gene. The schematic diagram is as follows:



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The Klhl40 gene has 2 transcripts. According to the structure of Klhl40 gene, exon1-exon5 of Klhl40-201 (ENSMUST00000098272.3) 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 *Klhl40* 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, Homozygous disruption of this gene results in postnatal growth retardation, abnormal sarcomere morphology, skeletal muscle dysfunction, and complete postnatal lethality. Homozygotes for a null allele develop a nemaline-like myopathy.

> *Gm*47108-201 will be knocked out at the same time.

The Klhl40 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.

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Gene information (NCBI)



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KIhl40 kelch-like 40 [Mus musculus (house mouse)]

Gene ID: 72330, updated on 12-Aug-2019

Summary

 Official Symbol
 Khl40 provided by MGI

 Official Full Name
 kelch-like 40 provided by MGI

 Primary source
 MGI:MGI:1919580

 See related
 Ensembl:ENSMUSG00000074001

 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
 Kbtbd5; 2310024D23Rik

 Expression
 Biased expression in mammary gland adult (RPKM 4.8), heart adult (RPKM 3.3) and 7 other tissues See more or human all

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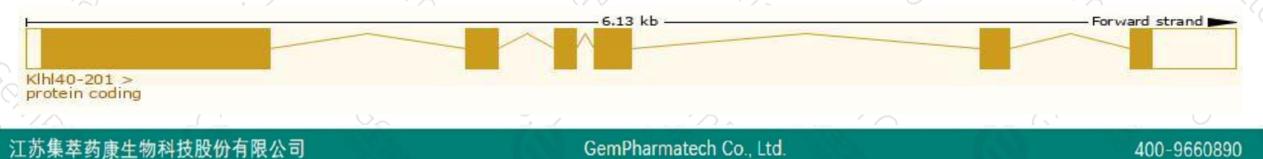
Transcript information (Ensembl)



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

Name 💧	Transcript ID	bp 🛔	Protein 🛔	Biotype 🍦	CCDS	UniProt 💧	Flags 🍦		
KIhl40-201	ENSMUST0000098272.3	2370	<u>621aa</u>	Protein coding	<u>CCDS23637</u> &	A0A0R4J166	TSL:1	GENCODE basic	APPRIS P1
Klhl40-202	ENSMUST00000216358.1	1368	No protein	Retained intron	8	-		TSL:NA	

The strategy is based on the design of Klhl40-201 transcript, The transcription is shown below



Genomic location distribution



26.21 kb Forward strand 121.79Mb 121.78Mb 121.77Mb Genes Klhl40-202 > Zfp651-201 > protein coding (Comprehensive set... retained intron 111 Zfp651-202 > retained intron Klhl40-201 > protein coding < AC165080.4 Contigs Genes < Gm47108-201 TEC < Hhatl-201 protein coding (Comprehensive set... -----.... < Hhatl-202 protein coding < Hhatl-203 protein coding MM < Hhatl-204 protein coding MA < Hhatl-205 protein coding MO < Hhatl-206 protein coding Regulatory Build 121.78Mb 121,77Mb 121.79Mb - 26.21 kb Reverse strand Gene Legend Protein Coding Ensembl protein coding merged Ensembl/Havana Non-Protein Coding processed transcript Regulation Legend CTCF Open Chromatin Promoter Promoter Flank

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Protein domain

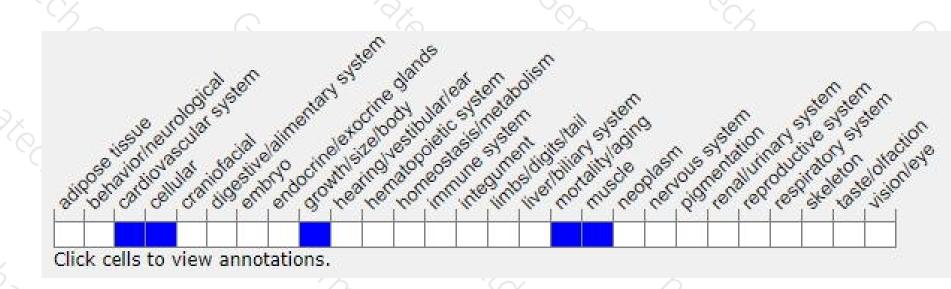
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1	All sequence SNPs/i Variant Legend	Sequence variants (d	IbSNP and all other sources)	T THEFT	IN COUNT	5
1	All sequence SNPs/i	Sequence variants (d		I HIIII	10 E BD 11	
0	100-100 ST					*
3	CDD		cd14735			
	Gene3D	3,30,710,10	1.25.40.420	Kelch-type beta propeller		3
		Kelch-like protein 40				
	PIRSF PANTHER	BTB-kelch protein				
	PROSITE profiles	BTB/POZ domain				í C
	Pfam.	BTB/POZ domain	BTB/Kelch-associated		Kelch repeat type 1	
	SMART	BTB/POZ domain	BTB/Kelch-associated	Kelch repeat typ	pe 1	
	Low complexity (Seg) Superfamily	SKP1/BTB/POZ doma	ain superfamily	Kelch-type beta prope	- `%	

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, Homozygous disruption of this gene results in postnatal growth retardation, abnormal sarcomere morphology, skeletal muscle dysfunction, and complete postnatal lethality. Homozygotes for a null allele develop a nemaline-like myopathy.

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



