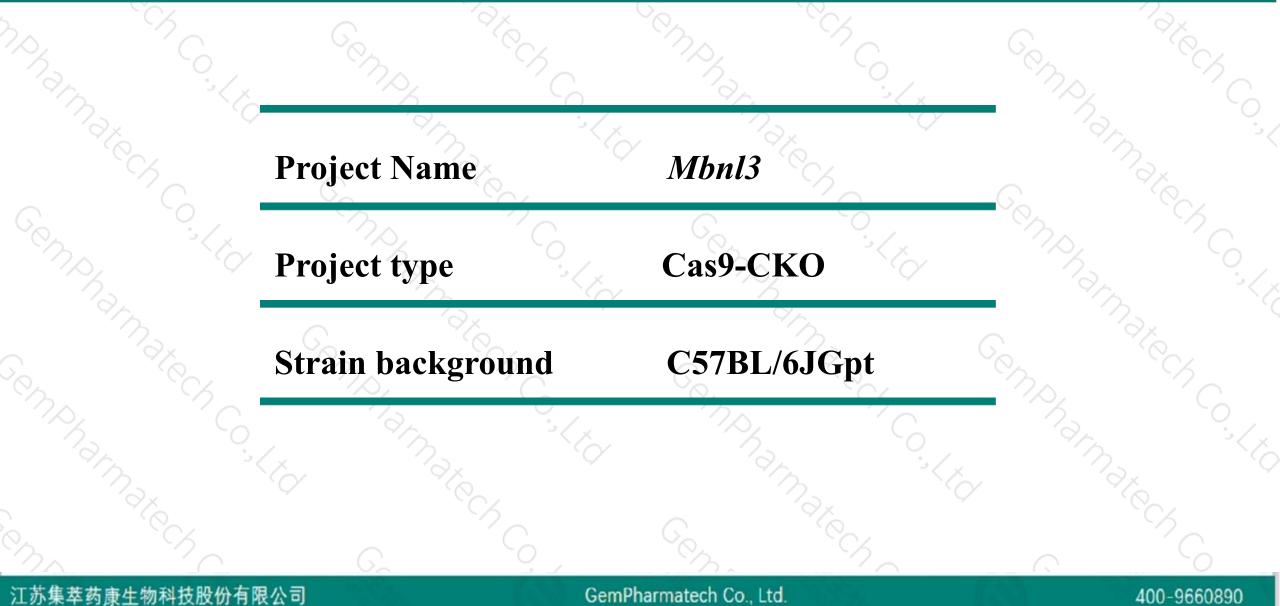


Mbnl3 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Huimin Su Ruirui Zhang 2020/2/12

Project Overview

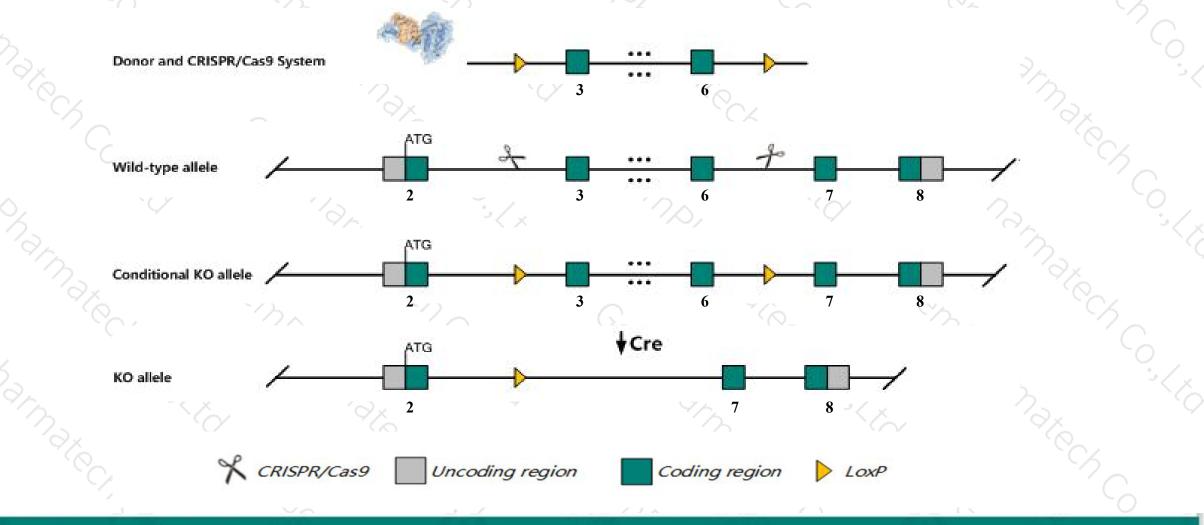




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Mbnl3* gene. The schematic diagram is as follows:



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The *Mbnl3* gene has 7 transcripts. According to the structure of *Mbnl3* gene, exon3-exon6 of *Mbnl3-203* (ENSMUST00000114876.8) transcript is recommended as the knockout region. The region contains 745bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Mbnl3* 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.

Notice



- According to the existing MGI data, Mice homozygous for an allele lacking exon 2 exhibit impaired muscle regeneration.
- The *Mbnl3* gene is located on the ChrX. 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)



\$?

Mbnl3 muscleblind like splicing factor 3 [Mus musculus (house mouse)]

Gene ID: 171170, updated on 12-Aug-2019

Summary

 Official Symbol
 Mbnl3 provided by MGI

 Official Full Name
 muscleblind like splicing factor 3 provided by MGI

 Primary source
 MGI:MGI:2444912

 See related
 Ensembl:ENSMUSG0000036109

 Gene type
 protein coding

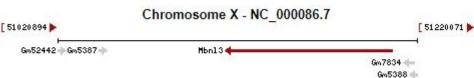
 RefSeq status
 VALIDATED

 Organism
 Mus musculus

 Lineage
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

 Also known as
 CHCR; MBLX; MBXL; MBLX39; Al661274; A530038J18Rik; E430034C16Rik

 Expression
 Biased expression in placenta adult (RPKM 12.8), genital fat pad adult (RPKM 8.0) and 9 other tissues See more orthologs human all



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The gene has 7 transcripts, all transcripts are shown below:

Name 🖕	Transcript ID	bp 💧	Protein 🖕	Biotype 💧	CCDS	UniProt 🖕	Flags		
Mbnl3-202	ENSMUST00000114875.7	4649	<u>246aa</u>	Protein coding	CCDS81126@	<u>Q3TJQ3</u> ₽	TSL:1 GENCODE basic		
Mbnl3-203	ENSMUST00000114876.8	2243	<u>342aa</u>	Protein coding	<u>CCDS40969</u> &	<u>Q542D8</u> & <u>Q8R003</u> &	TSL:1 GENCODE basic APPRIS P		
Mbnl3-201	ENSMUST00000041495.13	1803	<u>246aa</u>	Protein coding	<u>CCDS81126</u> 교	Q3TJQ3	TSL:1 GENCODE basic		
Mbnl3-204	ENSMUST00000136404.2	1632	<u>332aa</u>	Protein coding	2	<u>S4R267</u>	TSL:5 GENCODE basic		
Mbnl3-205	ENSMUST00000148116.1	1082	No protein	Processed transcript	2	2	TSL:5		
Mbnl3-207	ENSMUST00000156028.1	356	No protein	Processed transcript	2	2	TSL:3		
Mbnl3-206	ENSMUST00000150014.1	4121	No protein	Retained intron	0	2	TSL:1		

85.91 kl

The strategy is based on the design of Mbnl3-203 transcript, The transcription is shown below

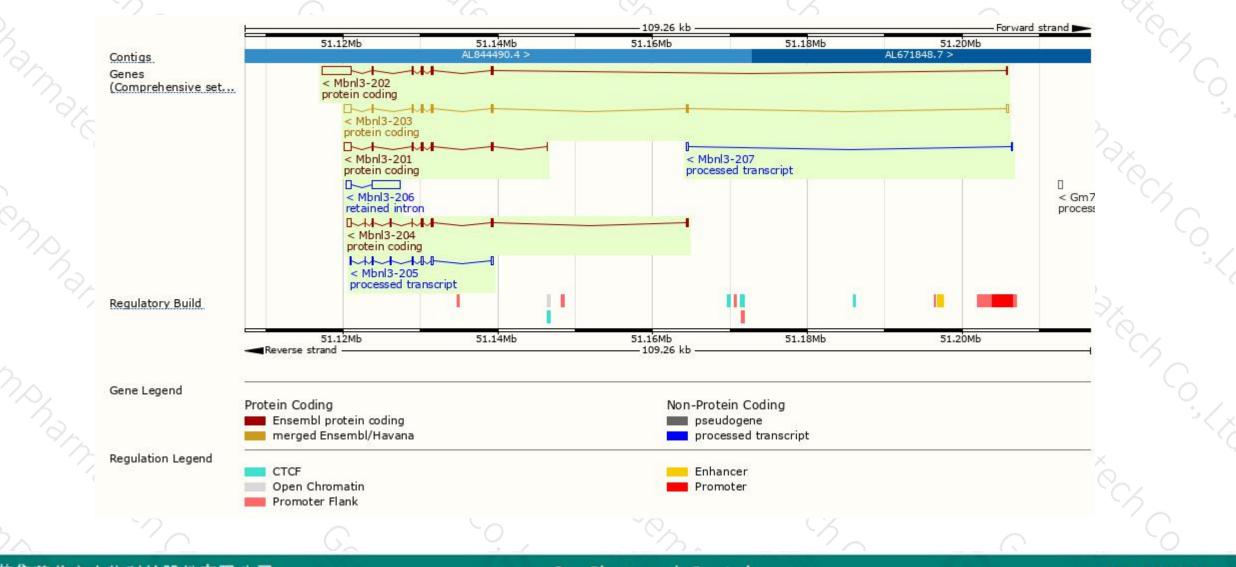
< Mbnl3-203 protein coding Reverse strand -

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Genomic location distribution





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Protein domain



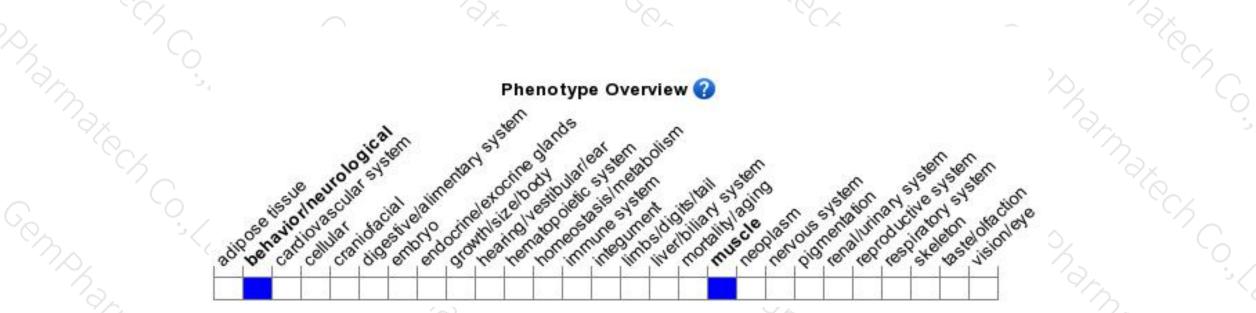
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	PROSITE profiles PANTHER	PF14608 Zinc finger, CCCH-type PTHR12675 PTHR12675 :SF3				_				
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	Variant Legend	inframe deletion synonymous variant		· ·						
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	20	G.	0	, Sp		3		· Co		

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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 an allele lacking exon 2 exhibit impaired muscle regeneration.



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



