

Lhfpl4 Cas9-CKO Strategy

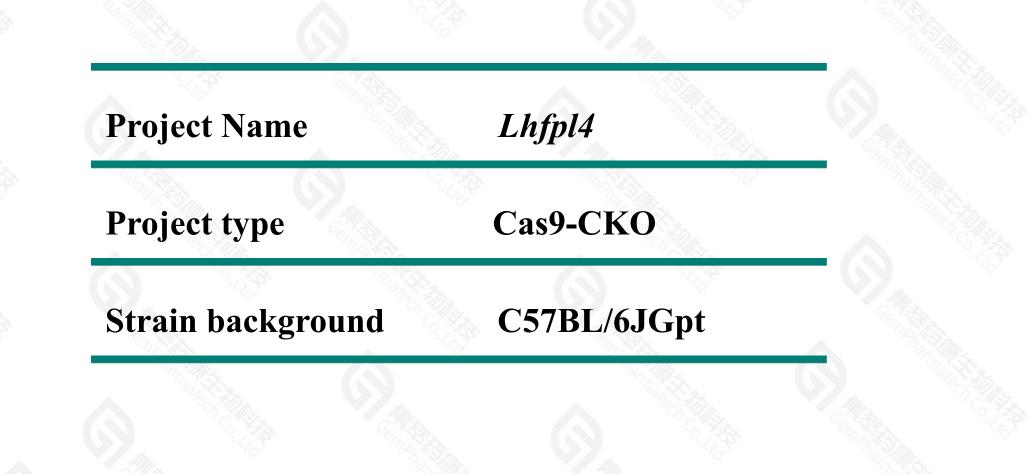
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Reviewer: Miaomiao Cui

Design Date: 2021-3-1

Project Overview



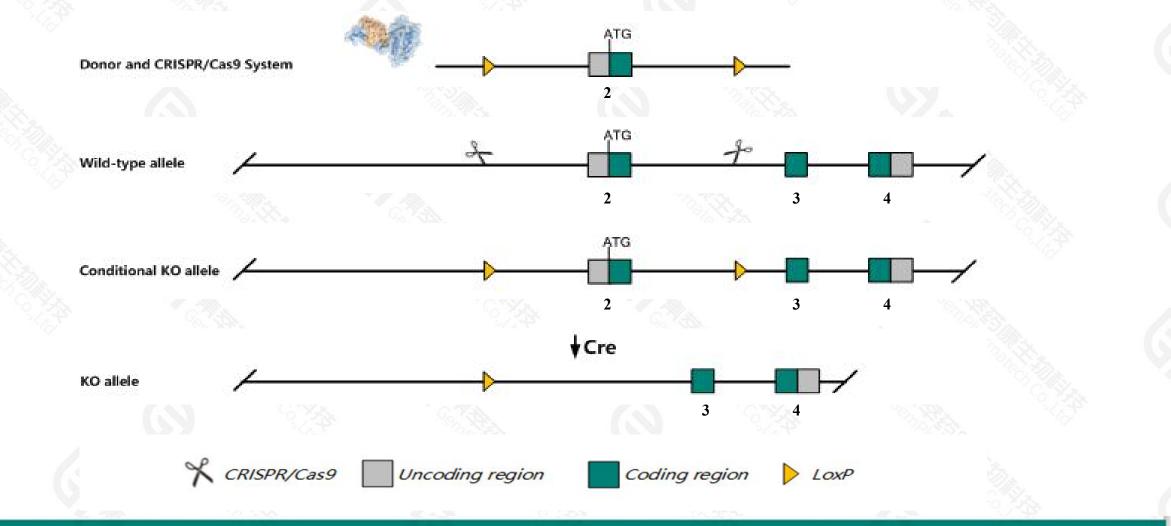


Conditional Knockout strategy

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This model will use CRISPR/Cas9 technology to edit the *Lhfpl4* gene. The schematic diagram is as follows:



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Technical routes



The Lhfpl4 gene has 2 transcripts. According to the structure of Lhfpl4 gene, exon2 of Lhfpl4-201(ENSMUST00000162280.1) 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 *Lhfpl4* 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 knockout affects inhibitory postsynaptic currents in the hippocampus.
 The *Lhfpl4* gene is located on the Chr6. 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)

Lhfpl4 lipoma HMGIC fusion partner-like protein 4 [Mus musculus (house mouse)]

Gene ID: 269788, updated on 13-Mar-2020

Summary

Official Symbol	Lhfpl4 provided by MGI
Official Full Name	lipoma HMGIC fusion partner-like protein 4 provided by MGI
Primary source	MGI:MGI:3057108
See related	Ensembl:ENSMUSG0000042873
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	1190004M23Rik, Al604880, B230384L07, mKIAA4027
Expression	Biased expression in whole brain E14.5 (RPKM 16.6), CNS E18 (RPKM 16.3) and 6 other tissues See more
Orthologs	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
Lhfpl4-201	ENSMUST00000162280.1	4762	<u>247aa</u>	Protein coding	CCDS39591	Q5U4E0	TSL:1 GENCODE basic APPRIS P1
Lhfpl4-202	ENSMUST00000203665.1	1414	<u>168aa</u>	Protein coding	-	A0A0N45V06	CDS 5' incomplete TSL:3

The strategy is based on the design of *Lhfpl4-201* transcript, the transcription is shown below:

< Lhfpl4-201 protein coding

Reverse strand

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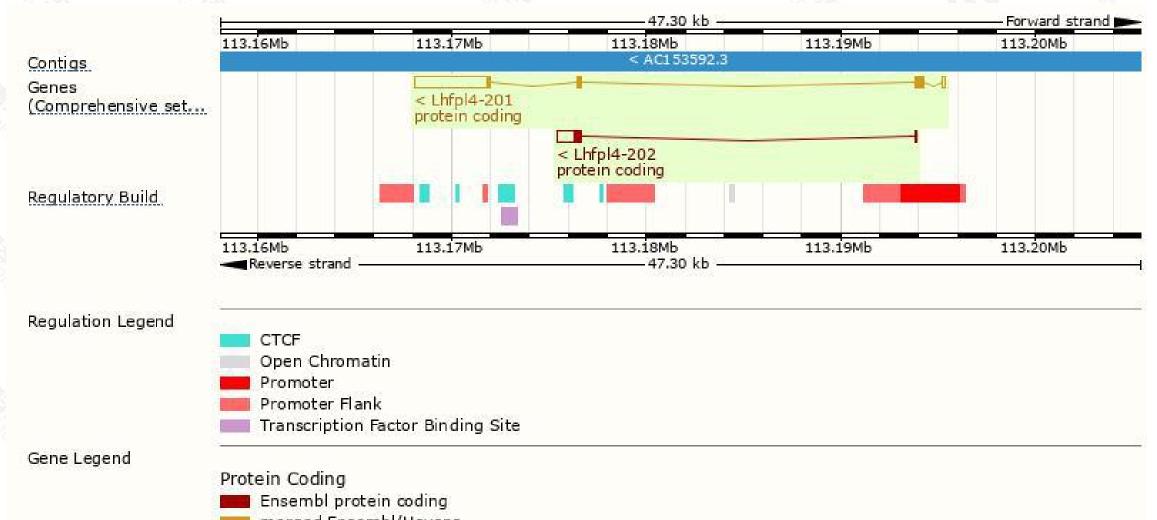
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27.30 kb

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Genomic location distribution





📕 merged Ensembl/Havana

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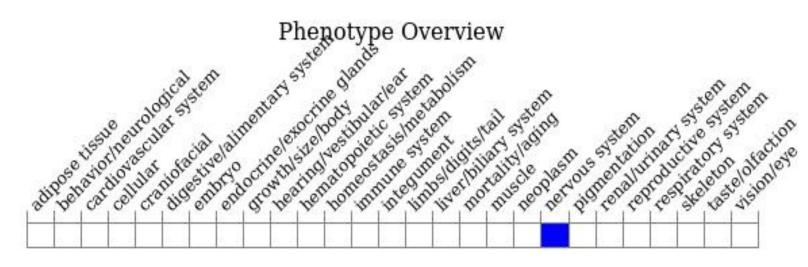
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Protein domain



а (2 —						
Lip	poma HMGIC fusion	n partner-like protein				
Upoma HMGI	C fusion partner-lik	ce protein				
PTHR12489;S	F14					
1,20,14	40.150					
Sequence v	ariants (dbSNP ar	nd all other sources))	$c \rightarrow m$	n n	1
synony	mous variant					
0	40	80	120	160	200	247
	Lipoma HMGI PTHR12489 (S 1,20,14 Sequence v synony	Lipoma HMGIC fusion Lipoma HMGIC fusion partner-lik PTHR12489:SF14 1,20,140,150 Sequence variants (dbSNP at synonymous variant	Lipoma HMGIC fusion partner-like protein Lipoma HMGIC fusion partner-like protein PTHR12489:SF14 1.20,140.150 Sequence variants (dbSNP and all other sources)	Lipoma HMGIC fusion partner-like protein Lipoma HMGIC fusion partner-like protein PTHR12489:SF14 1.20.140.150 Sequence variants (dbSNP and all other sources)	Lipoma HMGIC fusion partner-like protein Lipoma HMGIC fusion partner-like protein PTHR12489:SF14 1,20,140,150 Sequence variants (dbSNP and all other sources)	Lipoma HMGIC fusion partner-like protein Lipoma HMGIC fusion partner-like protein PTHR12489 :SF14 1.20,140.150 Sequence variants (dbSNP and all other sources)

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 knockout affects inhibitory postsynaptic currents in the hippocampus.

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



